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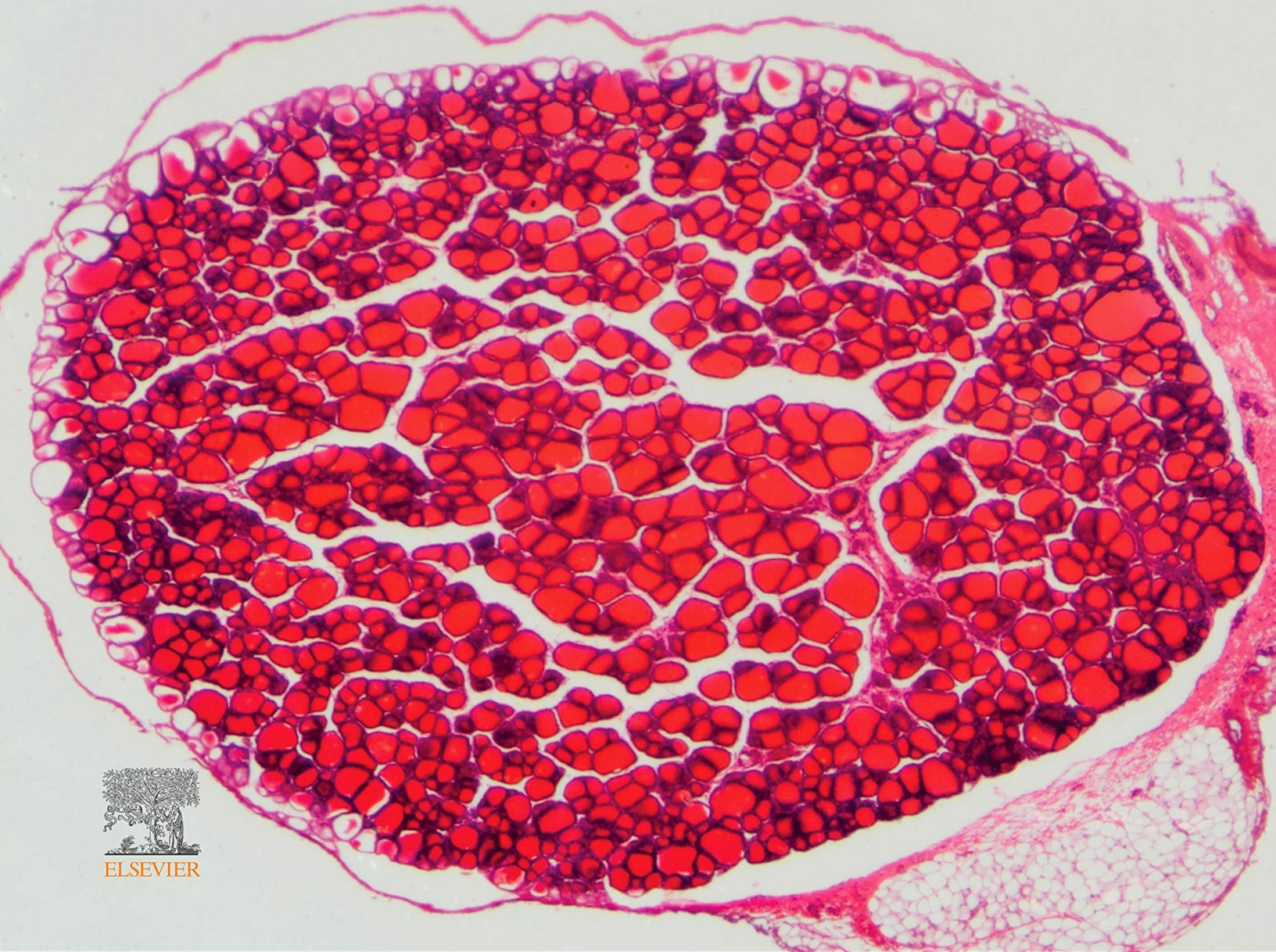
Williams *Textbook of* Endocrinology

14TH EDITION



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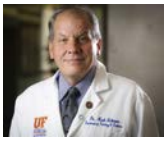

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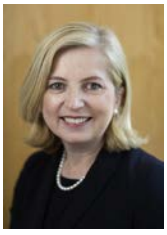

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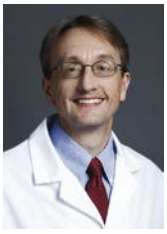

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Preface

The Editors are delighted to welcome you to the 69th anniversary 14th edition of *Williams Textbook of Endocrinology.* In this new edition, we have strived to maintain Robert Williams' original 1950 mandate to publish "a condensed and authoritative discussion of the management of clinical endocrinopathies based upon the application of fundamental information obtained from chemical and physiological investigation." With the passing of the decades, our scholarly goal has been enriched by the addition of genetic, molecular, cellular, and population sciences, together forming the basis of multiple new insights into both the pathogenesis and management of endocrine disorders. Editors of this textbook aim to provide a cogent navigation through the wealth of information emanating from novel medical discoveries that advance the field and bring new therapeutic approaches to patients with endocrine diseases. Our challenge remains to be both concise and didactic, while comprehensively covering relevant translational and clinical endocrine science in an accessible fashion.

With these goals in mind, we have once again assembled a team of outstanding authorities who each contribute their unique expertise to synthesize current knowledge in their respective topic area. For this edition, we have added new chapters on the global burden of endocrine disease and the navigation of the prolific expert endocrine guidelines, as well as chapters devoted to transgender endocrinology, and osteomalacia. The section on diabetes mellitus has been expanded with dedicated chapters on the physiology of insulin secretion, as well as a comprehensive update on therapeutics of type 2 diabetes mellitus. These new contributions reflect the changing emphasis of endocrine practice today and the availability of a wealth of new knowledge and therapeutic options that together affect clinical care. Each section has undergone significant revision and updating to bring the most current information to our readers.

We are deeply appreciative of the valued co-workers in our respective offices, including Shira Berman, and Grace Labrado for their dedicated efforts. We also thank our colleagues at Elsevier—Rae Robertson and Nancy Duffy—for shepherding the production process so professionally. The final product of this exemplary text is due to their skilled navigation of the medical publishing world. We are confident that our combined efforts have succeeded in achieving the high standards set by previous editions that have made Williams the classic "go to" book for all those interested in endocrinology.

1

Principles of Endocrinology

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CHAPTER OUTLINE

KEY POINTS

- Endocrinology is a scientific and medical discipline with a unique focus on hormones that features a multidisciplinary approach to understanding normal and pathologic hormone production and action, as well as diseases related to abnormal hormone signaling.
- Endocrine and paracrine systems differ in important respects that illustrate the evolutionary pressures on these distinct cell-signaling strategies.
- Differentiated hormone-secreting cells are designed to efficiently synthesize hormones and secrete them in a regulated way.
- Hormones in the bloodstream often are associated with binding proteins to enhance their solubility, protect them from degradation and renal excretion, and regulate their stability in the extracellular space.
- Hormones either act on receptors on the plasma membranes of target cells or move into cells to bind to intracellular receptors; in either case, the target cell is not a passive recipient of signals but rather has key roles in regulating hormonal responses.
- Control of hormone secretion involves integrated inputs from multiple distant targets, nervous system inputs, and local paracrine and autocrine factors, all leading to complex patterns of circadian secretion, pulsatile secretion, secretion driven by homeostatic stimuli, or stimuli that lead to secular changes over the lifespan.
- Endocrine diseases fall into broad categories of hormone overproduction or underproduction, altered tissue response to hormones, or tumors arising from endocrine tissue.
- Hormones and synthetic molecules designed to interact with hormone receptors are administered to diagnose and treat diseases.

About a hundred years ago, Starling coined the term *hormone* to describe secretin, a substance secreted by the small intestine into the bloodstream to stimulate pancreatic secretion. In his Croonian Lectures, Starling considered the endocrine and nervous systems as two distinct mechanisms for coordination and control of organ function. Thus, endocrinology found its first home in the discipline of mammalian physiology.

Over the next several decades, biochemists, physiologists, and clinical investigators characterized peptide and steroid hormones secreted into the bloodstream from discrete endocrine glands or other organs. Diseases such as hypothyroidism and diabetes could be treated successfully for the first time by replacing specific hormones. These initial triumphs of discovery formed the foundation of the clinical specialty of endocrinology.

Advances in cell biology, molecular biology, and genetics over the ensuing years began to reveal mechanisms underlying endocrine disease pathogenesis, hormone secretion, and action. Even though these advances have embedded endocrinology in the framework of molecular cell biology, they have not changed the essential subject of endocrinology—the signaling that coordinates

and controls functions of multiple organs and processes. Herein we survey general themes and principles that underpin diverse approaches used by clinicians, physiologists, biochemists, cell biologists, and geneticists to understand the endocrine system.

The Evolutionary Perspective

Hormones are broadly defined as chemical signals secreted into the bloodstream that act on distant tissues, usually in a regulatory fashion. Hormonal signaling represents a special case of the more general process of signaling between cells. Even unicellular organisms such as baker's yeast, *Saccharomyces cerevisiae,* secrete short peptide mating factors that act on receptors of other yeast cells to trigger mating between the two cells. These receptors resemble the ubiquitous family of seven membrane-spanning mammalian receptors that respond to diverse ligands such as photons and glycoprotein hormones. Because these yeast receptors trigger activation of heterotrimeric G proteins just as mammalian receptors do, this conserved signaling pathway was likely to have been present in the common ancestor of yeast and humans.

Signals from one cell to adjacent cells, termed *paracrine signals,* often use the same molecular pathways used by hormonal signals. For example, the sevenless receptor controls the differentiation of retinal cells in the *Drosophila* eye by responding to a membrane-anchored signal from an adjacent cell. Sevenless is a membrane-spanning receptor with an intracellular tyrosine kinase domain that closely resembles signaling by hormone receptors such as the insulin receptor tyrosine kinase. As paracrine factors and hormones can share signaling machinery, it is not surprising that hormones can, in some settings, act as paracrine factors. Testosterone, for example, is secreted into the bloodstream but also acts locally in the testes to control spermatogenesis. Insulin-like growth factor 1 (IGF1) is a polypeptide hormone secreted into the bloodstream from the liver and other tissues but is also a paracrine factor produced locally in most tissues to control cell proliferation. Furthermore, one receptor can mediate actions of a hormone, such as parathyroid hormone (PTH), and of a paracrine factor, such as parathyroid hormone–related protein. In some cases, the paracrine actions of "hormones" exhibit functions quite unrelated to the hormonal functions. For example, macrophages synthesize the active form of vitamin D, 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$), which can then bind to vitamin D receptors in the same cells and stimulate production of antimicrobial peptides.[1] This example illustrates that the vitamin D 1α-hydroxylase (P450 27B1) responsible for activating 25-hydroxyvitamin D is synthesized in tissues in which its function is unrelated to the calcium homeostatic actions of the $1,25(OH)_2D_3$ hormone.

Target cells respond similarly to signals that reach them from the bloodstream (hormones) or from adjacent cells (paracrine factors); the cellular response machinery does not distinguish between sites of origin of hormone signals. The shared final common pathways used by hormonal and paracrine signals should not, however, obscure important differences between hormonal and paracrine signaling systems (Fig. 1.1). Hormone synthesis occurs in specialized cells designed specifically for their production, and the hormone then travels in the bloodstream and diffuses in effective concentrations into tissues. Therefore, hormones must be produced in much larger amounts to act as hormones relative to the amounts needed to act as paracrine factors, which act at specific local locations. Hormones must be able to travel to and be protected from degradation in transit from the site of production to the distant site of action. Therefore, for example, lipophilic hormones bind to soluble proteins that allow them to travel in the aqueous media of blood at relatively high concentrations. The ability of hormones to diffuse through the extracellular space implies local hormone concentrations at target sites will rapidly decrease when glandular secretion of the hormone ceases. As hormones quickly diffuse throughout extracellular fluid, hormonal metabolism can occur in specialized organs, including liver and kidney, in a manner that determines the effective hormone concentration in other tissues.

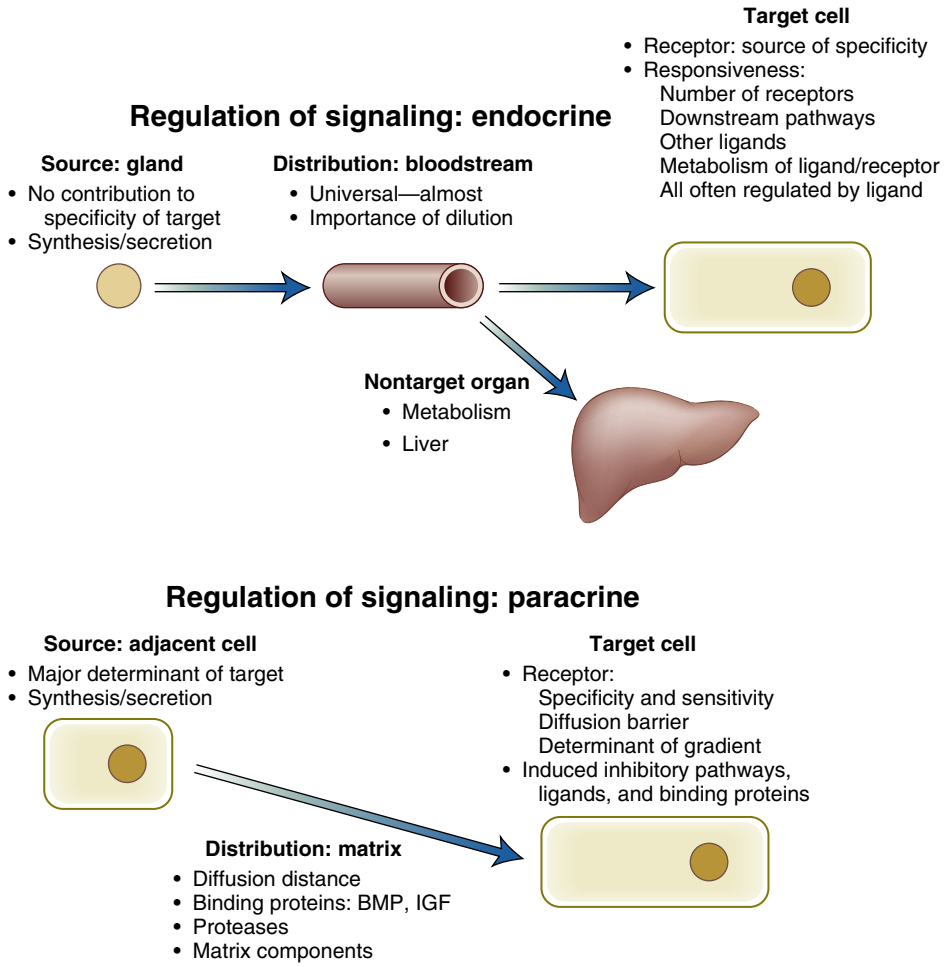
Paracrine factors have rather different constraints. Paracrine signals do not travel very far; consequently, the specific site of origin of a paracrine factor determines where it will act and provides specificity to that action. When the paracrine factor bone morphogenetic protein 4 (BMP4) is secreted by cells in the developing kidney, BMP4 regulates differentiation of renal cells; when the same factor is secreted by cells in bone, it regulates bone formation. Thus the site of origin of BMP4 determines its physiologic role. In contrast, because hormones are secreted into the bloodstream, their sites of origin are often divorced from their functions. Like BMP4, thyroid hormone, for example, acts in many tissues but the site of origin of thyroid hormone in a gland in the neck is not functionally relevant to the sites of action of the hormone.

Because specificity of paracrine factor action is so dependent on its precise site of origin, elaborate mechanisms have evolved to regulate and constrain the diffusion of paracrine factors. Paracrine factors of the hedgehog family, for example, are covalently bound to cholesterol to constrain diffusion of these molecules in the extracellular milieu. Most paracrine factors interact with binding proteins that block their action and control their diffusion. For example, chordin, noggin, and many other distinct proteins bind to various members of the BMP family to regulate their action. Proteases such as tolloid then destroy the binding proteins at specific sites to liberate BMPs so that they can act on appropriate target cells.

Thus, hormones and paracrine factors have several distinct strategies regulating biosynthesis, sites of action, transport, and metabolism. These differing strategies may partly explain why a hormone such as IGF1, unlike its close relative insulin, has multiple binding proteins to control its action in tissues. IGF1 exhibits a double life as both a hormone and a paracrine factor. Presumably the IGF1 actions mandate an elaborate binding protein apparatus to enable appropriate hormone signaling.

All the major hormonal signaling programs—G protein–coupled receptors, tyrosine kinase receptors, serine/threonine kinase receptors, ion channels, cytokine receptors, nuclear receptors—are also used by paracrine factors. In contrast, several paracrine signaling programs are used only by paracrine factors and not by hormones. For example, Notch receptors respond to membrane-based ligands to control cell fate, but no known blood-borne ligands use Notch-type signaling. Perhaps the complex intracellular strategy used by Notch, which involves cleavage of the receptor and subsequent nuclear actions of the receptor's cytoplasmic portion, is too inflexible to serve the purposes of hormones.

Analyses of the complete genomes of multiple bacterial species, the yeast *Saccharomyces cerevisiae,* the fruit fly *Drosophila melanogaster,* the worm *Caenorhabditis elegans,* the plant *Arabidopsis thaliana,* humans, and many other species have allowed a comprehensive view of the signaling machinery used by various forms of life. *S. cerevisiae* uses G protein–coupled receptors; this organism,



• **Fig. 1.1** Comparison of determinants of endocrine and paracrine signaling. *BMP,* bone morphogenetic protein; *IGF,* insulin-like growth factor.

however, lacks tyrosine kinases, used in the insulin signaling pathway, and nuclear receptors that resemble the estrogen/thyroid receptor family. In contrast, the worm and fly share with humans the use of each of these signaling pathways, although with substantial variation in numbers of genes committed to each pathway. For example, the *Drosophila* genome encodes 21 nuclear receptors, the *C. elegans* genome encodes about 284, and the human genome encodes 48 such receptors. These patterns suggest ancient multicellular animals must already have established the signaling systems that are the foundation of the endocrine system as we know it in mammals.

Our understanding of endocrine systems and novel physiologic biology continues to expand. Even before the sequencing of the human genome, sequence analyses had made clear that many receptor genes are found in mammalian genomes for which no clear ligand or function was known. Analyses of these "orphan" receptors broadened current understanding of hormonal signaling. For example, the liver X receptor (LXR) was one such orphan receptor found when searching for unknown putative nuclear receptors. Subsequent experiments found oxygenated derivatives of cholesterol are the ligands for LXR, which regulates genes involved in cholesterol and fatty acid metabolism.[2] The examples of LXR and many others raise the question of what constitutes a hormone. The classic view of hormones is that they are synthesized in discrete glands and have no function other than activating receptors on cell membranes or in the nucleus. Cholesterol, which is converted in cells to oxygenated derivatives that activate the LXR receptor, in contrast, uses a hormonal strategy to regulate its own metabolism. Other orphan nuclear receptors similarly respond to ligands such as bile acids and fatty acids. These "hormones" have important metabolic roles quite separate from their signaling properties, although hormone-like signaling permits regulation of the metabolic function. The calcium-sensing receptor is an example from the G protein–coupled receptor family that responds to a nonclassic ligand, ionic calcium. Calcium is released into the bloodstream from bone, kidney, and intestine and acts on the calcium-sensing receptor on parathyroid cells, renal tubular cells, and other cells to coordinate cellular responses to calcium. Thus, many important metabolic factors have hormonal properties as part of a regulatory strategy within complex organisms. Broadened understanding of these new metabolic factors is leading to new therapeutic approaches to treat or prevent human diseases.

Endocrine Glands

Hormone formation may occur either in the endocrine glands, which are localized collections of specific cells, or in cells that have additional roles. Many protein hormones, such as growth hormone (GH), PTH, prolactin (PRL), insulin, and glucagon, are

produced in dedicated cells by standard protein synthetic mechanisms common to all cells. These secretory cells contain specialized secretory granules designed to store large amounts of hormone and to release the hormones in response to specific signals. Hormones made in these glands and specialized cells are considered to be classic endocrine systems. Formation of small hormone molecules initiates with commonly found precursors, usually in specific glands such as the adrenals, gonads, or thyroid. In the case of the steroid hormones, the precursor is cholesterol, which is modified by various cytochrome P450–based hydroxylations and carbon-carbon bond cleavages and by specific oxidoreductases to form the glucocorticoids, androgens, estrogens, and their biologically active derivatives.

However, not all hormones are formed in dedicated and specialized endocrine glands; the adipose, enteroendocrine, and other systems are now also recognized to be complex endocrine systems. Thus with the discovery of novel peptides and amino acid or steroid-based molecules and their regulatory functions, the field of endocrinology and metabolism has recently been greatly expanded. For example, the protein hormone leptin, which regulates appetite and energy expenditure, is formed in adipocytes, providing a specific signal reflecting the nutritional state of the organism to the central nervous system. The enteroendocrine system comprises a unique hormonal system in which peptide hormones that regulate metabolic and other responses to oral nutrients are produced and secreted by specialized endocrine cells scattered throughout the intestinal epithelium. The cholesterol derivative, 7-dehydrocholesterol, the precursor of vitamin D_3, is converted in skin keratinocytes to previtamin D_3 by a photochemical reaction.

Thyroid hormone synthesis occurs via a unique pathway. The thyroid cell synthesizes a 660,000-kDa homodimer, thyroglobulin, which is then iodinated at specific tyrosines. Some iodotyrosines combine enzymatically to form the iodothyronine molecule within thyroglobulin, which is then stored in the lumen of the thyroid follicle. For tyrosine iodination to occur, the thyroid cell must concentrate trace quantities of iodide from the blood and oxidize it via a specific peroxidase. Release of thyroxine (T_4) from thyroglobulin requires phagocytosis and cathepsin-catalyzed digestion by the same cells.

Hormones are synthesized in response to biochemical signals generated by various modulating systems. Many of these systems are specific to the effects of the hormone product; for example, PTH synthesis is regulated by the concentration of ionized calcium, and insulin synthesis is regulated by the concentration of glucose. For others, such as gonadal, adrenal, and thyroid hormones, control of hormone synthesis is achieved by the homeostatic function of the hypothalamic-pituitary axis. Cells in the hypothalamus and pituitary monitor circulating hormone concentrations and secrete trophic hormones, which activate specific pathways for hormone synthesis and release. Typical examples are GH, luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and adrenocorticotropic hormone (ACTH).

These trophic hormones increase rates of hormone synthesis and secretion, and they may induce target cell division, thus causing enlargement of the various target glands. For example, in hypothyroid individuals living in iodine-deficient areas of the world, TSH secretion causes a marked hyperplasia of thyroid cells. In such regions, the thyroid gland may be 20 to 50 times its normal size. Adrenal hyperplasia occurs in patients with genetic deficiencies in cortisol formation. Hypertrophy and hyperplasia of parathyroid cells, initiated by an intrinsic response to the stress of hypocalcemia, occur in patients with renal insufficiency or calcium malabsorption.

Hormones may be fully active when released into the bloodstream (e.g., GH or insulin), or they may require activation in specific cells to produce biologic effects. These activation steps are often highly regulated. For example, T_4 released from the thyroid cell is a prohormone that must undergo specific deiodination to form the active 3,5,3'-triiodothyronine (T_3). This deiodination reaction can occur in target tissues, such as in the central nervous system; in thyrotrophs, where T_3 provides feedback regulation of TSH production; or in hepatic and renal cells, from which it is released into the circulation for uptake by all tissues. A similar postsecretory activation step catalyzed by a 5α-reductase causes tissue-specific activation of testosterone to dihydrotestosterone in target tissues, including the male urogenital tract, prostate, genital skin, and liver. Vitamin D undergoes hydroxylation at the 25 position in the liver and in the 1 position in the kidney. Both hydroxylations must occur to produce the active hormone, 1,25-dihydroxyvitamin D. Activity of 1α-hydroxylase, but not 25-hydroxylase, is stimulated by PTH and hypophosphatemia but is inhibited by calcium, 1,25-dihydroxyvitamin D, and fibroblast growth factor 23 (FGF23).

Most hormones are synthesized as required on a daily, hourly, or minute-to-minute basis with minimal storage, but there are significant exceptions. One is the thyroid gland, which contains enough stored hormone to last for about 2 months. This storage permits a constant supply of thyroid hormone despite significant variations in the availability of iodine. However, if iodine deficiency is prolonged, normal T_4 reservoirs can be depleted.

Feedback signaling systems exemplified earlier enable the hormonal *homeostasis* characteristic of virtually all endocrine systems. Regulation may include the central nervous system or local signal recogniton mechanisms in the glandular cells, such as the calcium-sensing receptor of the parathyroid cell. Disruption of hormonal homeostasis due to glandular or central regulatory system dysfunction has both clinical and laboratory consequences. Recognition and correction of disorders of these systems are the essence of clinical endocrinology.

Transport of Hormones in Blood

Protein hormones and some small molecules, such as catecholamines, are water soluble and readily transported via the circulatory system. Others are nearly insoluble in water (e.g., steroid and thyroid hormones), and their distribution presents special problems. Such molecules are tightly bound to 50-kDa to 60-kDa carrier plasma glycoproteins such as thyroxine-binding globulin (TBG), sex hormone–binding globulin (SHBG), and corticosteroid-binding globulin (CBG) or weakly bound to abundant albumin. Ligand-protein complexes serve as hormone reservoirs, ensure ubiquitous distribution of water-insoluble ligands, and protect small molecules from rapid inactivation or excretion in urine or bile. Protein-bound hormones exist in equilibria with the often-minute quantities of hormone in the aqueous plasma, with the "free" fraction of the circulating hormone taken up by the target cell. For example, if tracer thyroid hormone is injected into the portal vein in a protein-free solution, it binds to hepatocytes at the periphery of the hepatic sinusoid. When the same experiment is repeated with a protein-containing solution, uniform distribution of the tracer hormone occurs throughout the hepatic lobule.[3] Despite the very high affinity of some binding proteins for their

respective ligands, one specific protein may not be essential for hormone distribution. For example, in humans with congenital TBG deficiency, other proteins—transthyretin (TTR) and albumin—subsume its role. As the affinity of these secondary thyroid hormone transport proteins is several orders of magnitude lower than that of TBG, it is possible for the hypothalamic-pituitary feedback system to maintain free thyroid hormone in the normal range at a much lower total hormone concentration. The fact that the free hormone concentration is normal in individuals with TBG deficiency indicates that the hypothalamic-pituitary axis defends the free, active hormone.[4]

Availability of gene-targeting techniques allows specific assessments of the physiologic roles of hormone-binding proteins. For example, mice with targeted inactivation of the vitamin D–binding protein (DBP) have been generated.[5] Although the absence of DBP markedly reduces circulating concentrations of vitamin D, the mice are otherwise normal. However, they have enhanced susceptibility to a vitamin D–deficient diet due to the reduced reservoir of this sterol. In addition, the absence of DBP markedly reduces the half-life of 25-hydroxyvitamin D by accelerating its hepatic uptake, making the mice less susceptible to vitamin D intoxication.

Protein hormones and some small ligands (e.g., catecholamines) produce their effects by interacting with cell surface receptors. Others, such as steroid and thyroid hormones, must enter the cell to bind to cytosolic or nuclear receptors. In the past, it has been thought that much of the transmembrane transport of hormones was passive. Evidence now demonstrates specific transporters involved in cellular uptake of thyroid and some steroid hormones,[7] providing yet another mechanism for regulating the distribution of a hormone to its site of action. Studies in mice devoid of megalin, a large, cell surface protein in the low-density lipoprotein (LDL) receptor family, suggest estrogen and testosterone bound to SHBG use megalin to enter peripheral tissues while still bound to SHBG.[8] In this scenario, the hormone bound to SHBG, rather than "free" hormone, is the active moiety that enters cells. It is unclear how frequently this apparent exception to the "free hormone" hypothesis occurs.

MicroRNAs (miRNAs) have recently also been shown to elicit remote metabolic actions. For example, exosomal miRNA derived from adipose tissue regulates distant tissue gene expression, glucose tolerance, and circulating fibroblast growth factor 21 (FGF21) levels. MiRNAs may thus function as circulating adipokines.[9] Other small lipid signaling molecules are being discovered, especially for their role in activating or suppressing what were previously designated as orphan receptors.

Target Cells as Active Participants

Hormones determine cellular target actions by binding with high specificity to receptor proteins. Whether a peripheral cell is hormonally responsive depends to a large extent on the presence and function of specific and selective hormone receptors and the downstream signaling pathway molecules. Thus receptor expression and intracellular effector pathways activated by the hormone signal are key determinants for which cells will respond, and how. Receptor proteins may be localized to the cell membrane, cytoplasm, or nucleus. Broadly, polypeptide hormone receptors are cell membrane associated, but steroid hormones selectively bind soluble intracellular proteins (Fig. 1.2).

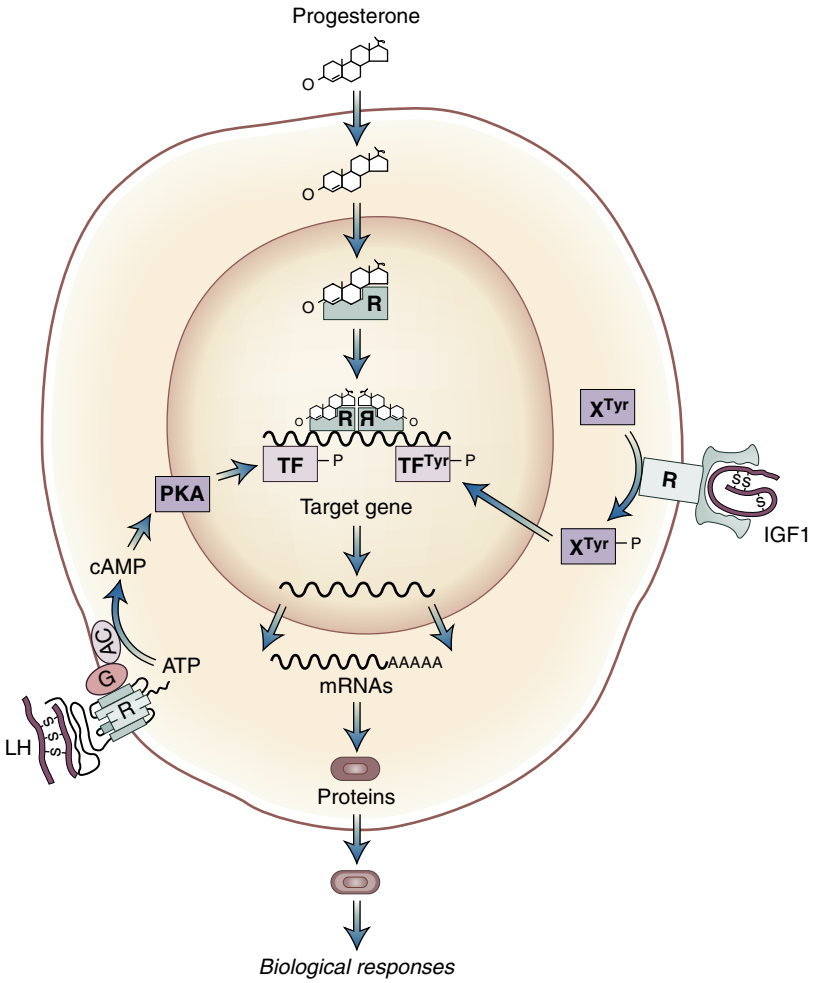
Membrane-associated receptor proteins usually consist of extracellular sequences that recognize and bind ligand, transmembrane-anchoring hydrophobic sequences, and intracellular sequences, which initiate intracellular signaling. Intracellular signaling is mediated by covalent modification and activation of intracellular signaling molecules (e.g., signal transducers and activators of transcription [STAT] proteins) or by generation of small molecule second messengers (e.g., cyclic adenosine monophosphate) through activation of heterotrimeric G proteins. Subunits of these G proteins (α-subunits, β-subunits, and γ-subunits) activate or suppress effector enzymes and ion channels that generate the second messengers. Some of these receptors (e.g., those for somatostatin) may in fact exhibit low constitutive activity and have been shown to signal in the absence of added ligand.

Several growth factors and hormone receptors (e.g., for insulin) behave as intrinsic tyrosine kinases or activate intracellular protein tyrosine kinases. Ligand activation may cause receptor dimerization (e.g., GH) or heterodimerization (e.g., interleukin 6), followed by activation of intracellular phosphorylation cascades. These activated proteins ultimately determine specific nuclear gene expression.

Both the number of receptors expressed per cell and their responses are regulated, thus providing a further level of control for hormone action. Several mechanisms account for altered receptor function. Receptor endocytosis causes internalization of cell surface receptors; the hormone-receptor complex is subsequently dissociated, resulting in abrogation of the hormone signal. Receptor trafficking may then result in recycling back to the cell surface (e.g., as for the insulin receptor) or the internalized receptor may undergo lysosomal degradation. Both these mechanisms triggered by activation of receptors effectively lead to impaired hormone signaling downregulation of the receptors. The hormone signaling pathway may also be downregulated by receptor desensitization (e.g., as for epinephrine); ligand-mediated receptor phosphorylation leads to a reversible deactivation of the receptor. Desensitization mechanisms can be activated by a receptor's ligand (homologous desensitization) or by another signal (heterologous desensitization), thereby attenuating receptor signaling in the continued presence of ligand. Receptor function may also be limited by action of specific phosphatases (e.g., Src homology phosphatase [SHP]) or by intracellular negative regulation of the signaling cascade (e.g., suppressor of cytokine signaling [SOCS] proteins inhibiting Janus kinase/signal transducers and activators of transcription [JAK-STAT] signaling). Certain ligand-receptor complexes may also translocate to the nucleus.

Mutational changes in receptor structure can also determine hormone action. Constitutive receptor activation may be induced by activating mutations (e.g., TSH receptor) leading to endocrine organ hyperfunction, even in the absence of ligand. Conversely, inactivating receptor mutations may lead to endocrine hypofunction (e.g., testosterone or vasopressin receptors). These syndromes are well characterized (Table 1.1) and are well described in subsequent chapters.

The functional diversity of receptor signaling results in overlapping or redundant intracellular pathways. For example, GH, PRL, and cytokines each activate JAK-STAT signaling, whereas the distal effects of these stimuli clearly differ. Thus, despite common upstream signaling pathways, hormones can elicit highly specific cellular effects. Tissue-type or cell-type genetic programs or receptor-receptor interactions at the cell surface (e.g., hetero-oligomerization of dopamine D2 with somatostatin receptor, or insulin with IGF1 receptor) may also confer a specific cellular response to a hormone and provide an additive cellular effect.[10] In addition, effector protein expression may differ in select cells to modulate



• **Fig. 1.2** Hormonal signaling by cell surface and intracellular receptors. The receptors for the water-soluble polypeptide hormones, luteinizing hormone (LH), and insulin-like growth factor 1 (IGF1) are integral membrane proteins located at the cell surface. They bind the hormone-utilizing extracellular sequences and transduce a signal by the generation of second messengers: cyclic adenosine monophosphate (cAMP) for the LH receptor and tyrosine-phosphorylated substrates for the IGF1 receptor. Although effects on gene expression are indicated, direct effects on cellular proteins (e.g., ion channels) are also observed. In contrast, the receptor for the lipophilic steroid hormone progesterone resides in the cell nucleus. It binds the hormone and becomes activated and capable of directly modulating target gene transcription. *AC,* adenylyl cyclase; *ATP,* adenosine triphosphate; *G,* heterotrimeric G protein; *mRNAs,* messenger RNAs; *PKA,* protein kinase A; *R,* receptor molecule; *TF,* transcription factor; *Tyr,* tyrosine found in protein X; *X,* unknown protein substrate. (From Mayo K. Receptors: molecular mediators of hormone action. In: Conn PM, Melmed S, eds. *Endocrinology: Basic and Clinical Principles.* Totowa, NJ: Humana Press; 1997:11.)

hormonal response. For example, the glucose transporter-4 protein, which leads to insulin-mediated glucose uptake, is most abundantly expressed in muscle, hepatic and adipose tissues, causing these tissues to be the most sensitive tissues for insulin-mediated glucose disposal.

A final mechanism of nuclear receptor modulation is prereceptor regulation via intracellular enzymes that convert the circulating molecules to more or less potent hormones. In addition to the activation of T_4 and testosterone described earlier, selective hormone inactivation occurs in some cells. In the distal nephron, the enzyme 11β-hydroxysteroid dehydrogenase type 2 converts the mineralocorticoid-receptor ligand cortisol to inactive cortisone, thus preventing receptor activation. This mechanism allows aldosterone, which is not a substrate for the enzyme, to regulate mineralocorticoid activity in the kidney despite circulating aldosterone concentrations 1000 times lower than those of cortisol.

Control of Hormone Secretion

Anatomically distinct endocrine glands are composed of highly differentiated cells that synthesize, store, and secrete hormones. Circulating hormone concentrations are a function of glandular secretory patterns and hormone clearance rates. Hormone secretion is tightly regulated to attain circulating levels that are most conducive to elicit the appropriate target tissue response. For example, longitudinal bone growth is initiated and maintained by exquisitely regulated levels of circulating GH, yet mild GH hypersecretion results in gigantism, and GH deficiency causes growth retardation. Ambient circulating hormone concentrations are not uniform, and secretion patterns determine appropriate physiologic function. Thus, insulin secretion occurs in short pulses elicited by nutrient and other signals; gonadotropin secretion is episodic, determined by a hypothalamic pulse generator; and PRL

TABLE 1.1 Diseases Caused by Mutations in G Protein–Coupled Receptors.

Condition[a]	Receptor	Inheritance	ΔFunction[b]
Retinitis pigmentosa	Rhodopsin	AD/AR	Loss
Nephrogenic diabetes insipidus	Vasopressin V2	X-linked	Loss
Familial glucocorticoid deficiency	ACTH	AR	Loss
Color blindness	Red/green opsins	X-linked	Loss
Familial precocious puberty	LH	AD (male)	Gain
Familial hypercalcemia	Ca^{2+} sensing	AD	Loss
Neonatal severe parathyroidism	Ca^{2+} sensing	AR	Loss
Dominant form hypocalcemia	Ca^{2+} sensing	AD	Gain
Congenital hyperthyroidism	TSH	AD	Gain
Hyperfunctioning thyroid adenoma	TSH	Somatic	Gain
Metaphyseal chondrodysplasia	PTH-PTHrP	Somatic	Gain
Hirschsprung disease	Endothelin-B	Multigenic	Loss
Coat color alteration (*E* locus, mice)	MSH	AD/AR	Loss and gain
Dwarfism (*little* locus, mice)	GHRH	AR	Loss

[a]All are human conditions with the exception of the final two entries, which refer to the mouse.

[b]Loss of function refers to inactivating mutations of the receptor, and gain of function to activating mutations.

ACTH, Adrenocorticotropic hormone; *AD,* autosomal dominant inheritance; *AR,* autosomal recessive inheritance; *FSH,* follicle-stimulating hormone; *GHRH,* growth hormone–releasing hormone; *LH,* luteinizing hormone; *MSH,* melanocyte-stimulating hormone; *PTH-PTHrP,* parathyroid hormone and parathyroid hormone–related peptide; *TSH,* thyroid-stimulating hormone.

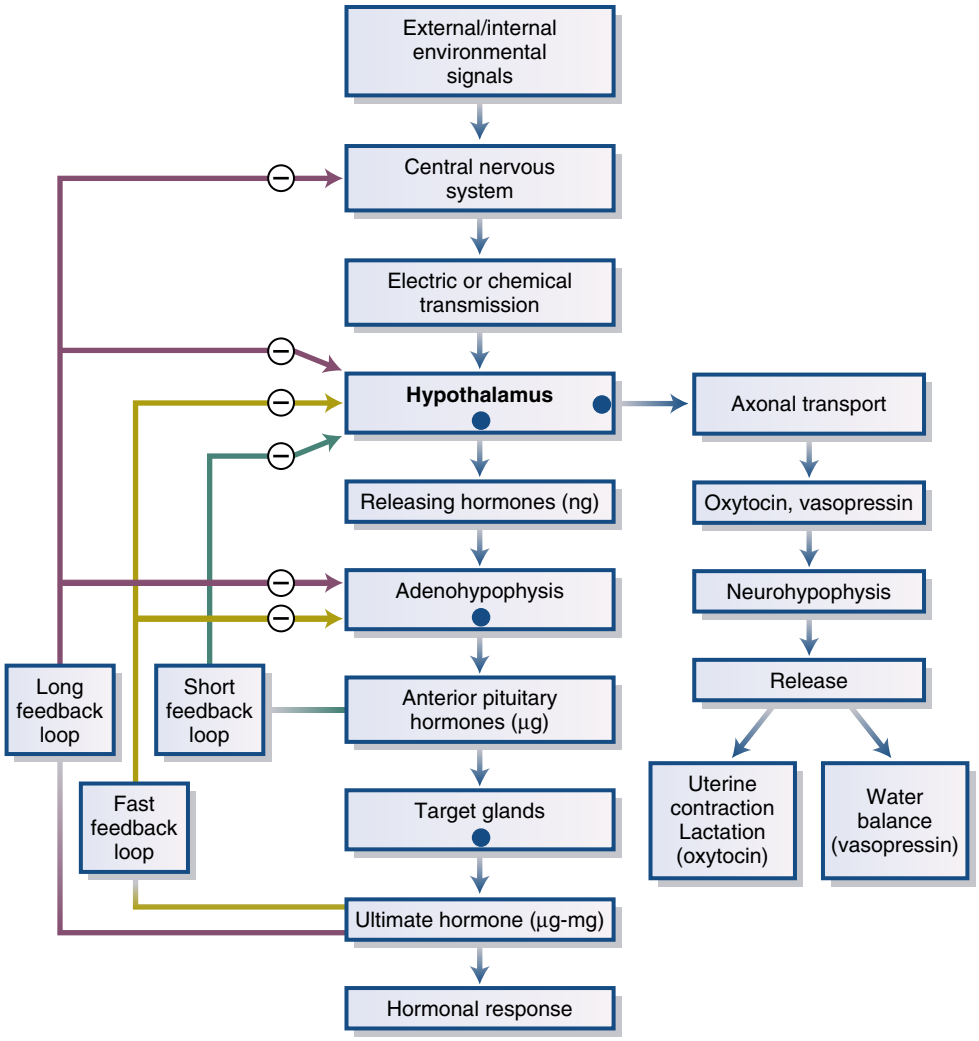
From Mayo K. Receptors: molecular mediators of hormone action. In: Conn PM, Melmed S, eds. *Endocrinology: Basic and Clinical Principles.* Totowa, NJ: Humana Press; 1997:27.

secretion appears to be relatively continuous, with secretory peaks elicited during suckling.

Hormone secretion also adheres to rhythmic patterns. Circadian rhythms serve as adaptive responses to environmental signals and are controlled by a circadian timing mechanism.[11] Light is the major environmental cue adjusting the endogenous clock. The retinohypothalamic tract entrains circadian pulse generators situated within hypothalamic suprachiasmatic nuclei. These signals subserve timing mechanisms for the sleep-wake cycle and determine patterns of hormone secretion and action. Disturbed circadian timing results in hormonal dysfunction and may also be reflective of entrainment or pulse generator lesions. For example, adult GH deficiency due to a damaged hypothalamus or pituitary is associated with elevations in integrated 24-hour leptin concentrations and decreased leptin pulsatility, yet preserved circadian rhythm of leptin. GH replacement restores leptin pulsatility, promoting loss of body fat mass.[12] Sleep is an important cue regulating hormone pulsatility. About 70% of overall GH secretion occurs during slow-wave sleep, and increasing age is associated with declining slow-wave sleep and concomitant decline in GH and elevation of cortisol secretion.[13] Most pituitary hormones are secreted in a circadian (day-night) rhythm, best exemplified by ACTH peaks before 9 AM, whereas ovarian steroids follow a 28-day menstrual rhythm. Disrupted episodic rhythms are often a hallmark of endocrine dysfunction. For example, loss of circadian ACTH secretion with high midnight cortisol levels is a feature of Cushing disease.

Hormone secretion is induced by multiple specific biochemical and neural signals. Integration of these stimuli results in the net temporal and quantitative secretion of the hormone (Fig. 1.3). Signals elicited by hypothalamic hormones (growth hormone–releasing hormone [GHRH], somatostatin), peripheral hormones (IGF1, sex steroids, thyroid hormone), nutrients, adrenergic pathways, stress, and other neuropeptides all converge on the somatotroph cell, resulting in the ultimate pattern and quantity of GH secretion. Networks of reciprocal interactions allow for dynamic adaptation and shifts in environmental signals. These regulatory systems involve the hypothalamic, pituitary, and target endocrine glands, as well as the adipocytes and lymphocytes. Peripheral inflammation and stress elicit cytokine signals that interface with the neuroendocrine system, resulting in hypothalamic-pituitary axis activation. Parathyroid and pancreatic secreting cells are less tightly controlled by the hypothalamus, but their functions are tightly regulated by the distal effects they elicit. For example, PTH secretion is induced when serum calcium levels fall and the signal for sustained PTH secretion is abrogated by rising calcium levels, whereas insulin secretion is induced when blood glucose rises but suppressed when glucose concentrations fall.

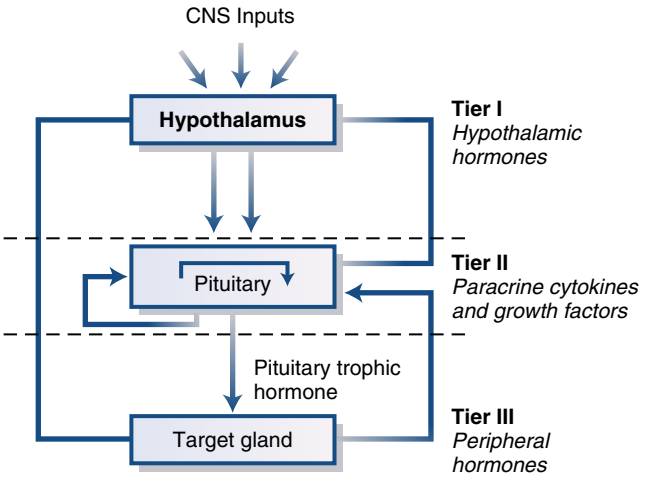
Several tiers of control subserve the ultimate net glandular secretion. First, central nervous system signals, including afferent stimuli, neuropeptides, and stress, signal the synthesis and secretion of hypothalamic hormones and neuropeptides (Fig. 1.4). Four hypothalamic-releasing hormones (GHRH, corticotropin-releasing hormone [CRH], thyrotropin-releasing hormone [TRH], and gonadotropin-releasing hormone [GnRH]) traverse the hypothalamic portal vessels and impinge upon their respective transmembrane trophic hormone-secreting cell receptors. These distinct cells express GH, ACTH, TSH, and gonadotropins, respectively. In contrast, hypothalamic somatostatin and dopamine suppress GH or PRL and TSH secretion, respectively. Trophic hormones



• **Fig. 1.3** Peripheral feedback mechanism and a millionfold amplifying cascade of hormonal signals. Environmental signals are transmitted to the central nervous system, which innervates the hypothalamus, which responds by secreting nanogram amounts of a specific releasing hormone. These are transported down a closed portal system, pass the blood-brain barrier at either end through fenestrations, and bind to specific anterior pituitary cell membrane receptors to elicit secretion of micrograms of specific anterior pituitary hormones. These hormones enter the venous circulation through fenestrated local capillaries, bind to specific target gland receptors, trigger release of micrograms to milligrams of daily hormone amounts, and elicit responses by binding to receptors in distal target tissues. Peripheral hormone receptors enable widespread cell signaling by a single initiating environmental signal, thus facilitating intimate homeostatic association with the external environment. Arrows with a large dot at their origin indicate a secretory process. (From Normal AW, Litwack G. *Hormones.* 2nd ed. New York: Academic Press; 1997:14.)

maintain the structural and functional integrity of endocrine organs, including the thyroid and adrenal glands and the gonads. Target hormones, in turn, serve as powerful negative feedback regulators of their respective trophic hormone, often also suppressing secretion of hypothalamic-releasing hormones. In certain circumstances (e.g., during puberty), peripheral sex steroids may positively induce the hypothalamic-pituitary-target gland axis. For example, LH induces ovarian estrogen secretion, which feeds back positively to induce further LH release. Pituitary hormones themselves, in a short feedback loop, also regulate their own respective hypothalamic-controlling hormone. Hypothalamic-releasing hormones are secreted in nanogram amounts and have short half-lives of a few minutes. Anterior pituitary hormones are produced in microgram amounts and have longer half-lives, but peripheral hormones can be produced in up to milligram amounts daily, with much longer half-lives.

A further level of secretion control occurs within the gland itself. Intraglandular paracrine or autocrine growth peptides serve to autoregulate pituitary hormone secretion, as exemplified by epidermal growth factor (EGF) control of PRL or IGF1 control of GH secretion. Molecules within the endocrine cell may also subserve an intracellular feedback loop. For example, corticotrope SOCS-3 induction by gp130-linked cytokines serves to abrogate the ligand-induced JAK-STAT cascade and block proopiomelanocortin (POMC) transcription and ACTH secretion. This rapid on-off regulation of ACTH secretion provides a plasticity for the endocrine response to changes in environmental signaling and serves to maintain homeostatic integrity.[14]



• **Fig. 1.4** Model for regulation of anterior pituitary hormone secretion by three tiers of control. Hypothalamic hormones impinge directly on their respective target cells. Intrapituitary cytokines and growth factors regulate trophic cell function by paracrine (and autocrine) control. Peripheral hormones exert negative feedback inhibition of respective pituitary trophic hormone synthesis and secretion. *CNS,* central nervous system. (From Ray D, Melmed S. Pituitary cytokine and growth factor expression and action. *Endocr Rev.* 1997;18:206–228.)

In addition to the central nervous system–neuroendocrine interface mediated by hypothalamic chemical signal transduction, the central nervous system directly controls several hormonal secretory processes. Posterior pituitary hormone secretion occurs as direct efferent neural extensions. Postganglionic sympathetic nerves also regulate rapid changes in renin, insulin, and glucagon secretion, while preganglionic sympathetic nerves signal to adrenal medullary cells eliciting epinephrine release.

Hormone Measurement

Endocrine function can be assessed by measuring levels of basal circulating hormone, evoked or suppressed hormone, or hormone-binding proteins. Alternatively, hormone function can be assessed. When a feedback loop exists between the hypothalamic-pituitary axis and a target gland, the circulating level of the pituitary trophic hormone, such as TSH or ACTH, is typically an exquisitely sensitive index of deficient or excessive function of the thyroid or the adrenal cortex, respectively. Meaningful strategies for timing hormonal measurements vary from system to system. In some cases, circulating hormone concentrations can be measured in randomly collected serum samples. This measurement, when standardized for fasting, environmental stress, age, and gender, is reflective of true hormone concentrations only when levels do not fluctuate appreciably. For example, thyroid hormone, PRL, and IGF1 levels can be accurately assessed in fasting morning serum samples. On the other hand, when hormone secretion is clearly episodic, timed samples may be required over a defined time course to reflect hormone bioavailability. Thus, early morning and late evening cortisol measurements are most appropriate. A 24-hour sampling for GH measurements, with samples collected every 2, 10, or 20 minutes, is expensive and cumbersome, yet may yield valuable diagnostic information. Random sampling may also reflect secretion peaks or nadirs, thus confounding adequate interpretation of results.

In general, confirmation of failed glandular function is made by attempting to evoke hormone secretion by recognized stimuli. Testing of pituitary hormone reserve may be accomplished by injecting appropriate hypothalamic-releasing hormones. Injection of trophic hormones, including TSH and ACTH, evokes specific target gland hormone secretion. Pharmacologic stimuli (e.g., metoclopramide for induction of PRL secretion) may also be useful tests of hormone reserve. In contrast, hormone hypersecretion can best be diagnosed by suppressing glandular function. Failure to appropriately suppress GH levels after a standardized glucose load implies inappropriate GH hypersecretion. Failure to suppress insulin secretion during hypoglycemia indicates inappropriate hypersecretion of insulin and should prompt a search for the cause, such as an insulin-secreting tumor.

Radioimmunoassays use highly specific antibodies that uniquely recognize the hormone, or a hormone fragment, to quantify hormone levels. Enzyme-linked immunosorbent assays (ELISAs) use enzyme-conjugated antibodies, and enzyme activity is reflective of hormone concentration. Immunometric assays use two antibodies directed to different epitopes of a polypeptide hormone: one "capture" antibody that isolates the hormone to a solid support and one "signal" antibody coupled to a signal-generating molecule such as acridinium ester or an enzyme. These sensitive techniques have allowed ultrasensitive measurements of physiologic hormone concentrations. Hormone-specific receptors may be used in place of the antibody in a radioreceptor assay. However, all antibody-based assays may be subject to artifacts, which should be kept in mind especially when the assay results are discordant with the clinical picture.

Endocrine Diseases

Endocrine diseases fall into four broad categories: (1) hormone overproduction, (2) hormone underproduction, (3) altered tissue responses to hormones, and (4) tumors of endocrine glands. An additional albeit atypical fifth category is exemplified by one kind of hypothyroidism in which overexpression of a hormone-inactivating enzyme in a tumor leads to thyroid hormone deficiency. Other disorders of inadequate hormone inactivation include apparent mineralocorticoid excess, vitamin D 24-hydroxylase deficiency, and X-linked hypophosphatemic rickets (PHEX deficiency).

Hormone Overproduction

Occasionally, hormones are secreted in increased amounts because of genetic abnormalities that cause abnormal regulation of hormone synthesis or release. For example, in glucocorticoid-remediable hyperaldosteronism, an abnormal chromosomal crossover event creates a fusion gene that encodes a protein with aldosterone synthase activity under the control of the ACTH-regulated 11β-hydroxylase promoter. More often, diseases of hormone overproduction are associated with an increase in the total number of hormone-producing cells. For example, hyperthyroidism associated with Graves disease, in which antibodies mimic TSH and activate the TSH receptors on thyroid cells, is accompanied by dramatic increase in thyroid cell proliferation and increased synthesis and release of thyroid hormone from each thyroid cell. In this example, the increase in thyroid cell number represents a polyclonal expansion of thyroid cells, in which large numbers of thyroid cells proliferate in response to an abnormal stimulus. However, most endocrine tumors are not polyclonal expansions but instead represent monoclonal expansions of a single mutated cell. Pituitary and parathyroid tumors, for example, are usually monoclonal expansions in which somatic mutations in multiple

tumor suppressor genes and proto-oncogenes occur. These mutations lead to an increase in proliferation or survival of the mutant cells. Sometimes this proliferation is associated with abnormal secretion of hormone from each tumor cell. For example, mutant $G_s\alpha$ proteins in somatotrophs can lead to both increased cellular proliferation and increased secretion of GH from each tumor cell.

Hormone Underproduction

Underproduction of hormone can result from a wide variety of processes, ranging from surgical removal of parathyroid glands during neck surgery, to tuberculous destruction of adrenal glands, to iron deposition in pancreatic beta cells of islets in hemochromatosis. A frequent cause of destruction of hormone-producing cells is autoimmunity. Autoimmune destruction of beta cells in type 1 diabetes mellitus or of thyroid cells in chronic lymphocytic (Hashimoto) thyroiditis are two of the most common disorders treated by endocrinologists. Recently a direct passage of insulin fragments by exocytosis from pancreatic islets to lymphoid tissue has been shown to trigger autoimmune diabetes in mice.[15] Multiple genetic abnormalities can also lead to decreased hormone production. These disorders can result from abnormal development of hormone-producing cells (e.g., hypogonadotropic hypogonadism caused by *KAL* gene mutations), from abnormal synthesis of hormones (e.g., deletion of the GH gene), or from abnormal regulation of hormone secretion (e.g., the hypoparathyroidism associated with activating mutations of the parathyroid cell's calcium-sensing receptor). Drugs are important causes of endocrine gland dysfunction as exemplified by immune checkpoint inhibitors leading to multiple endocrinopathies.

Altered Tissue Responses to Hormones

Resistance to hormones can be caused by a variety of genetic disorders. Examples include mutations in the GH receptor in Laron dwarfism and mutations in the $G_s\alpha$ gene in the hypocalcemia of pseudohypoparathyroidism type 1A. Insulin resistance in muscle and liver central to the cause of type 2 diabetes mellitus is complex in origin, resulting from inherited variations in many genes, as well as from theoretically reversible physiologic stresses. Type 2 diabetes is also an example of a disease in which end-organ insensitivity is worsened by signals from other organs, in this case by signals originating in fat cells. In other cases, the target organ of hormone action is more directly abnormal, as in PTH resistance occurring with renal failure.

Increased end-organ function can be caused by mutations in signal reception and propagation. For example, activating mutations in TSH, LH, and PTH receptors can cause increased activity of thyroid cells, Leydig cells, and osteoblasts, even in the absence of ligand. Similarly, activating mutations in the $G_s\alpha$ protein can cause precocious puberty, hyperthyroidism, and acromegaly in McCune-Albright syndrome.

Tumors of Endocrine Glands

Tumors of endocrine glands often result in hormone overproduction. Some endocrine gland tumors produce little if any hormone but cause disease by local, compressive symptoms or by metastatic spread. Examples include so-called nonfunctioning pituitary tumors, which are usually benign but can cause a variety of symptoms due to compression of adjacent structures, and thyroid cancer, which can metastasize without causing hyperthyroidism.

Excessive Hormone Inactivation or Destruction

Although most enzymes important for endocrine systems activate a prohormone or precursor protein, there are also those whose function is to inactivate the hormone in a physiologically regulated fashion. An example is the type 3 iodothyronine deiodinase (D3), which inactivates T_3 and T_4 by removing an inner ring iodine atom from the iodothyronine, blocking its nuclear receptor binding. Large infantile hepatic hemangiomas express high D3 levels, causing "consumptive hypothyroidism," because thyroid hormone is inactivated at a more rapid rate than it can be produced.[16,17] Furthermore, D3 may also be induced in other tumors by tyrosine kinase inhibitors. In theory, accelerated destruction of other hormones could occur from similar processes as yet to be determined.

Diagnostic and Therapeutic Uses of Hormones

In general, hormones are used pharmacologically for their replacement or suppressive effects. Hormones may also be used for diagnostic stimulatory effects (e.g., hypothalamic hormones) to evoke target organ responses or to diagnose endocrine hyperfunction by suppressing hormone hypersecretion (e.g., T_3). Ablation of endocrine gland function due to genetic or acquired causes can be restored by hormone replacement therapy. Thyroid hormones and some steroids can be replaced orally, whereas peptide hormones and analogues (e.g., insulin, PTH, GH) are administered parenterally or absorbed through mucous membranes (inhaled insulin, intranasal desmopressin). Gastrointestinal absorption and first-pass kinetics determine oral hormone dosage and availability. Physiologic replacement can achieve both appropriate hormone levels (e.g., thyroid) and approximate hormone secretory patterns (e.g., GnRH delivered intermittently via a pump). Hormones can also be used to treat diseases associated with glandular hyperfunction. Long-acting depot preparations of somatostatin receptor ligands suppress GH hypersecretion in acromegaly and hypersecretion of diarrhea-causing mediators from neuroendocrine tumors of the pancreas and small intestine. Estrogen receptor antagonists (e.g., tamoxifen) are useful for some patients with breast cancer, and GnRH analogues may downregulate the gonadotropin axis and benefit patients with prostate cancer.

Novel formulations of receptor-specific hormone ligands are now being clinically developed (e.g., estrogen agonists/antagonists, somatostatin receptor subtype-specific ligands, or peroxisome proliferator-activated receptor alpha [PPARα] ligands), resulting in more selective therapeutic targeting. Modes of hormone injection (e.g., for PTH) may also determine therapeutic specificity and efficacy. Improved hormone delivery systems, including computerized minipumps, intranasal sprays (e.g., for desmopressin), pulmonary inhalers, depot intramuscular injections, and orally bioavailable peptide formulations, will also enhance patient compliance and improve ease of administration. Cell-based therapies using the reprogramming of human cells to perform differentiated functions, either through differentiation of induced pluripotent stem cells or directed differentiation of one somatic cell type into another, are under active investigation.[18] Novel technologies offer promise of marked prolongation in the half-life of peptide hormones, thereby requiring infrequent administration. For example, a once weekly preparation of glucagon-like peptide-1 (GLP-1) analogues is used in the treatment of type 2 diabetes.

Important progress has been made in the therapeutic use of hormones. Although delivery of insulin usually still relies on frequent administration by injection and close monitoring by the patient, purity of the insulin preparations, as well as novel delivery devices, has enhanced patient compliance and quality of life. Preparations with differing pharmacokinetics allow the normal physiology of insulin secretion to be more closely mimicked. Continuous administration via subcutaneous pump infusion enhances therapeutic effectiveness in carefully selected patients. These include closed-loop systems, in which the dose of insulin is automatically adjusted depending on continuously monitored interstitial glucose concentrations. Implementation of such systems has the potential to substantially reduce the burden of this disease. However, hormones are biologically powerful molecules that exert therapeutic benefit and effectively replace pathologic deficits. They should not be prescribed without clear-cut indications and should not be administered without careful evaluation by an appropriately qualified medical practitioner.

Future Perspectives

An introduction to the principles underlying endocrinology should end by emphasizing the rapidly changing dynamics of discovery in this field and attempting to foresee what remains to be discovered. New hormones are continually being discovered, from the recent focus on major regulators of metabolism and phosphate homeostasis (FGF19, FGF21, and FGF23) to the continued quest to identify ligands for orphan nuclear and G protein–coupled receptors.[19] Presumably other equally important hormones remain to be discovered. The observation that nuclear receptors, like most transcription factors, bind to thousands of specific sites within the cell's nucleus stresses how little we understand about hormone action. Even the name "nuclear receptors" may be viewed in the future as misleading, since there is an increasing appreciation of extranuclear, rapid actions of nuclear receptors. Many of our diagnostic tests are severely limited by both technology and our inability to foresee novel diagnostic targets. For example, the "disappearance" of isolated GH deficiency when many children with that diagnosis achieve adulthood means either that we have little understanding of the etiology/pathogenesis of that childhood deficiency or that our diagnostic tools today yield many false-positive results. Although endocrinologists pride themselves with having logical treatments for many diseases, these treatments seldom address their underlying causes. We have no satisfactory tools for preventing autoimmune endocrine deficiencies or for preventing the benign tumors that underlie many diseases characterized by hormone excess. Treatments for diseases such as type 1 diabetes, although highly effective, are still very obtrusive in the lives of patients with this disease.

This new edition communicates major advances that have been made in our field over the past 5 years, yet gaps in our knowledge about endocrinology remain. Importantly, debilitating chronic endocrine illnesses with significant morbidity (e.g., diabetes and Cushing disease) still pose significant diagnostic and therapeutic challenges.

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2

Principles of Hormone Action

EVAN D. ROSEN AND CHRISTIN CARTER-SU

CHAPTER OUTLINE

KEY POINTS

- Hormones signal to target cells via receptors on the cell surface or in the cell nucleus.
- Polypeptide hormones act at the cell surface and trigger a cascade of events in the cytoplasm as well as in the nucleus that alter the function of their target cells.
- In addition to polypeptide hormones, many nonpolypeptide hormones such as catecholamines signal via cell surface receptors.
- There are multiple classes of cell surface receptors, including ligand-gated ion channel receptors, G protein–coupled receptors, receptors with intrinsic enzymatic activity, and receptors that associate with enzymes.
- Some of the cell surface receptors have intrinsic catalytic activity, whereas others depend on interaction with other signaling proteins to exert their actions.
- Ligand binding to the extracellular domain of cell surface receptors causes conformational changes in the receptors that activate enzymatic activity and recruitment of cytoplasmic signaling proteins.
- Steroid and thyroid hormones signal via nuclear receptors.
- Some nuclear receptors transduce signals from vitamins, metabolites, and drugs acting as ligands to regulate reproduction, growth, and metabolism.
- Nuclear receptors work directly in the cell nucleus to regulate gene transcription, acting at the genome and recruiting coregulator proteins called corepressors and coactivators.
- Hormone binding to nuclear receptors causes a conformational change in the receptor that favors the recruitment of coactivators to the specific genes that are regulated.
- Some nuclear receptors may work through additional pathways that involve nongenomic mechanisms.

Introduction to Hormone Signaling

The evolution of multicellularity enabled specialization of organs and tissues. As organs took on distinct functions, mechanisms were required to allow communication between tissues; this is the fundamental purpose of hormones. Hormones encode information about environmental or developmental conditions in one location and transmit that information to a separate location. This process ultimately requires that information move from outside of the target cell to its interior, so that cellular function can be altered to meet the needs of the organism. Specifically, the concentration of the substance must be detected by the target cell and converted into a change in cellular activity, a process known as *signal transduction*. The strategies used by hormones to affect cellular function are analogous and, in many cases, identical to those used by other extracellular agents such as neurotransmitters, drugs, and metabolites. However, classic endocrinology defines itself as the process by which extracellular signaling molecules use the bloodstream to travel from the organ of origin to the target tissue. By its nature, this process invariably results in dilution of the secreted molecule in the intravascular space, and thus, with rare exception, the target cell must be capable of detecting and responding to very low concentrations of hormone.

In spite of the vanishingly small concentrations of hormones present in the circulation, classic endocrine organs are usually uniquely equipped to secrete substantial amounts of hormone. Much of the history of endocrinology is defined by purification of hormones from these specialized secretory tissues. In the earliest days, the discovery of a hormone usually followed a stereotypical

course of events: (1) A syndrome, often resembling some human disease, was associated with removal of an endocrine gland; (2) the abnormal phenotype would be corrected by the reimplantation of the absent organ; (3) the same cure would be accomplished by administration of an extract from the organ of interest; and (4) the active agent would be purified from the organ. The discovery of insulin represents the prototype for this series of observations, but the same process led to the identification of other hormones such as thyroid hormone and cortisol.

Hormones can be divided into two groups on the basis of where they function in a target cell. The first group includes hormones that interact with receptors at the cell surface. All polypeptide hormones (e.g., growth hormone [GH], insulin), monoamines (e.g., serotonin), and prostaglandins (e.g., prostaglandin E_2) use cell surface receptors. The second group includes hormones that can enter cells. These hormones bind to intracellular receptors that function in the nucleus of the target cell to regulate gene expression. Classic hormones that use intracellular receptors include thyroid and steroid hormones.

It is worth noting that many molecules behave both as classical hormones that use the bloodstream to travel from their site of production to their site of action and as signaling molecules that do not meet that strict definition. For example, insulin-like growth factor 1 (IGF1) is produced and secreted by the liver under the positive influence of GH and circulates to target tissues like bone, but it is also produced locally by some tissues (e.g., chondrocytes at bone growth plates) to exert effects on neighboring cells. Similarly, norepinephrine is a neurotransmitter that is released at nerve endings and binds to cell surface receptors at postsynaptic membranes, but it is also secreted into the blood by the adrenal medulla, allowing it to act as a classic endocrine hormone. Testosterone is a nuclear receptor ligand that is produced by the Leydig cells of the testis; it can circulate as a hormone and act on muscle, bone, and other tissues, but it also acts as a paracrine agent on neighboring seminiferous tubules. Finally, many secreted molecules that are not regarded as classic hormones meet virtually all of the criteria used to define such agents. For example, cytokines are released by immune cells at the site of inflammation, but they also circulate in plasma and bind to cell surface receptors in the brain, evoking fever. In this sense, many circulating molecules, including those produced exogenously (i.e., obtained from the diet or synthesized by commensal bacteria), could be regarded as having hormonal properties. The key point is that a complete understanding of cell surface and nuclear receptor biology requires a more inclusive perspective than is typically achieved by adhering to a strict set of definitional criteria established decades ago. Having said that, in the interest of brevity, this chapter focuses primarily on receptors that bind classic hormonal ligands, with examples drawn from other systems as needed to provide a more comprehensive picture that reflects our current understanding of receptor biology.

Ligands That Act Through Cell Surface Receptors

The impermeability of the plasma membrane to peptides and small, water-soluble, charged molecules requires that receptors that recognize such substances be located on the outer surface of the cell. The limiting membrane of a typical eukaryotic cell is a 5-nm to 8-nm structure composed of proteins embedded in a bilayer of phospholipids and cholesterol, forming the fluid-mosaic membrane. The phospholipid polar head groups face outward from the membrane, interacting with the hydrophilic milieu that comprises the extracellular fluid and the cytoplasm. Buried between these two charged surfaces are the hydrophobic lipid tails of the phospholipids made up of acyl groups, which are long chains of hydrocarbons derived from fatty acids. The strongly nonpolar environment prevents the diffusion of water-soluble molecules, including many hormones, across the membrane. Thus surface proteins are needed to detect the presence of extracellular ligands that cannot diffuse and are not transported into the cell. Information from this hormone-binding process must then be transmitted across the plasma membrane so that intracellular signaling can commence.

TABLE 2.1 Hormones That Work on the Cell Surface

Peptides and Proteins

Adrenocorticotropic hormone (ACTH)
Antidiuretic hormone (ADH)
Atrial natriuretic peptide (ANP)
Calcitonin
Cholecystokinin
Corticotropin-releasing hormone (CRH)
Follicle-stimulating hormone (FSH)
Gastrin
Glucagon
Gonadotropin-releasing hormone (GnRH)
Growth hormone (GH)
Growth hormone–releasing hormone (GHRH)
Insulin
Insulin-like growth factor 1 (IGF1)
Luteinizing hormone (LH)
Oxytocin
Parathyroid hormone (PTH)
Prolactin (PRL)
Secretin
Somatostatin (SS)
Thyrotropin-releasing hormone (TRH)
Thyrotropin or thyroid-stimulating hormone (TSH)

Molecules Derived From Amino Acids

Dopamine (inhibits prolactin)
Epinephrine (also called adrenaline)
Norepinephrine (also called noradrenaline)
Serotonin

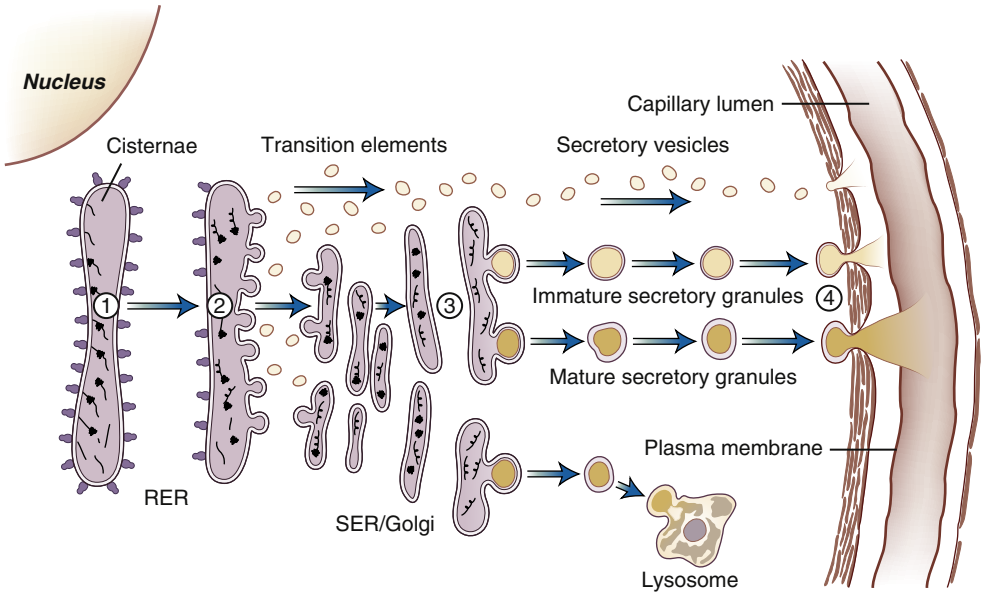
Eicosanoids

Prostaglandins: PGA_1, PGA_2, PGE_2

Classic Peptide Hormones

Most notable among the hormones that bind to cell surface receptors are the peptide hormones, which vary in size from a handful to hundreds of amino acids. Examples of peptide hormones include the glycoproteins and the GH family of proteins secreted by the pituitary, the pancreatic hormones glucagon and insulin, and numerous peptides secreted from nonglandular organs, such as leptin from adipocytes and atrial natriuretic peptide from the heart (Table 2.1).

A hormone's rate of secretion is closely tailored to its lifetime in the circulation and to its time course of action. In general, peptide hormones are released from endocrine glands quickly, as they are preformed and stored in secretory vesicles or granules. In the



• **Fig. 2.1** Subcellular organelles involved in transport and secretion of polypeptide hormones or other secreted proteins within a protein-secreting cell. (1) Synthesis of proteins on polyribosomes attached to rough endoplasmic reticulum (RER) and vectorial discharge of proteins through the membrane into the cisterna. (2) Formation of shuttling vesicles (transition elements) from endoplasmic reticulum followed by their transport to and incorporation by the Golgi complex. (3) Formation of secretory granules in the Golgi complex. (4) Transport of secretory granules to the plasma membrane, fusion with the plasma membrane, and exocytosis resulting in the release of granule contents into the extracellular space. Notice that secretion may occur by transport of secretory vesicles and immature granules or by transport of mature granules. Some granules are taken up and hydrolyzed by lysosomes (crinophagy). *Golgi,* Golgi complex; *SER,* smooth endoplasmic reticulum. (From Habener JF. Hormone biosynthesis and secretion. In: Felig P, Baxter JD, Broadus AE, et al, eds. *Endocrinology and Metabolism*. New York: McGraw-Hill; 1981:29–59.)

course of synthesis, peptide hormones are diverted to secretory vesicles via a regulated secretory pathway (Fig. 2.1). The cytoplasm of endocrine glands containing such secretory vesicles, such as the endocrine pancreas, the anterior pituitary, and the parathyroid glands, is filled with 200-nm electron-dense granules that represent the packaged hormone awaiting secretion. Just as secretion of hormones stored within vesicles can be evoked quickly, often within milliseconds, release can usually be terminated abruptly with great efficiency. Peptide hormones tend to have very short half-lives within the circulation, which allows blood levels to change rapidly in response to changes in secretion. Like the rapid changes in secretion and blood concentrations, initiation of signaling tends to be rapid, which is facilitated by high *on rates* for hormone binding to receptors. In contrast, the *off rate* is often slow, which results in a high equilibrium binding constant that enables the receptors to detect the relatively low levels of hormone in blood. However, a slow off rate is not compatible with the relatively rapid transient nature of peptide hormone–initiated signaling, suggesting mechanisms must exist for turning off the hormonal signal other than simple diffusion of the hormone off of the receptor.

A notable exception to the general rule that peptide hormones turn over quickly and have short durations of action is provided by IGF1. Unlike most peptide hormones, IGF1 circulates in the bloodstream bound to one or more binding proteins, which has two important consequences. First, the concentration of total IGF1 in blood is much greater than that of the unbound, biologically active hormone. Second, the lifetime of IGF1 is greatly extended, such that circulating levels of the hormone change slowly over the course of hours or days. As predicted by these properties, IGF1 primarily influences phenotypes that are modified over extended periods, such as growth and differentiation, and in marked contrast to its cousin insulin, most of the cellular targets of IGF1 are transcriptional.

Nonpeptide Hormones That Act at Cell Surface Receptors

In addition to peptide hormones, there are small, hydrophilic hormones related to monoamine neurotransmitters that bind cell surface receptors. These include adrenergic agents such as norepinephrine as well as other amino acid–derived water-soluble molecules such as melatonin, serotonin, and histamine. Like peptide hormones, these hormones can be stored in dense secretory vesicles, but they are more typically packaged into small, approximately 50-nm electron-lucent vesicles that are similar morphologically to vesicles in neural and neuroendocrine cells. The major difference is that in the presynaptic cleft, the vesicles are arranged in a tightly packed array at the membrane.

Interestingly, while most lipophilic molecules have intracellular receptors, there is at least one class of lipid that breaks this rule. The eicosanoids are a group of extracellular signaling molecules derived from 20-carbon fatty acids that includes the leukotrienes and prostaglandins. Many biologically active eicosanoids bind to cell surface receptors, which initiate their functions.[1]

The recent expansion of messenger types and the novel modes of interorgan communication have dramatically changed the traditional view of endocrinology such that all cell types can potentially both send and receive messages. One of the more interesting recent additions to the assortment of hormone-like molecules has

TABLE 2.2 Receptors for Metabolites and Ions

Metabolite	Receptor
Lactate	GPR81
Ketone bodies	GPR109A
3-Hydroxyoctanoate	GPR109B
Succinate	GPR91
α-Ketoglutarate	GPR80/99
Long-chain fatty acids	GPR40, GPR120
Medium-chain fatty acids	GPR84
Short-chain fatty acids	GPR41, GPR43
Calcium	CASR

been circulating metabolites such as lactate, ketone bodies, and succinate, as well as ions such as calcium[2] (Table 2.2). An even more distant modification of the original definition of *hormone* is the idea that metabolites produced by microbes in the gut, such as short-chain fatty acids, can signal by binding to cell surface receptors.[3]

Binding Properties of Cell Surface Receptors

When a hormone or hormone-like molecule arrives at a target cell, at least three critical components are required to induce the appropriate biologic response. First, there has to be recognition of the hormone as different from all other components of the extracellular milieu. This is an issue of specificity (the ability to distinguish the hormone from other structurally related molecules). Second, the receptor must be able to recognize the low concentration of hormone found in the blood, which is an issue of sufficient affinity of the receptor for the hormone. Third, the initial recognition step mediated by the receptor must be converted into a single action or a defined set of cellular events. Studies of the binding properties of hormones and neurotransmitters crystallized into a fundamental rule governing the action of extracellular agents: A biologic effect is directly proportional to the ligand occupancy of the receptor. A subtle but important modification to occupancy theory is the notion of *spare receptors,* which describes the situation in which a maximal biologic response is achieved by occupancy of only a portion of the available receptors. One consequence of the existence of spare receptors is that a decrease in the number of cellular receptors results in a change in the ED_{50} (effective dose for eliciting a 50% response) for a hormone but does not necessarily alter the maximal biologic response, as detailed later for insulin.[4,5]

As noted, a fundamental characteristic of a cell surface receptor is the ability to bind hormone with high specificity and high affinity. In addition, because there is a limited number of receptors, binding is saturable, such that adding additional ligand above a certain level results in no additional binding and no further increment in downstream biologic activity. Specificity, affinity, and saturability can be established experimentally by assessing the binding of ligands to receptors, using radioactive ligands in a variety of in vitro binding assays.[6] Authentic physiologic receptors for a given hormone will display a greater affinity for the cognate hormone than other potentially competing circulating molecules. In addition, the half maximal binding for a hormone to its real receptor will always be in the range of the circulating free concentration of that hormone.

Cell Surface Hormone Receptors

Cell surface receptors can be grouped conveniently into four classes: ligand-gated ion channel receptors, G protein–coupled receptors, receptors with intrinsic enzymatic activity, and receptors that associate with enzymes.

Ligand-Gated Ion Channels

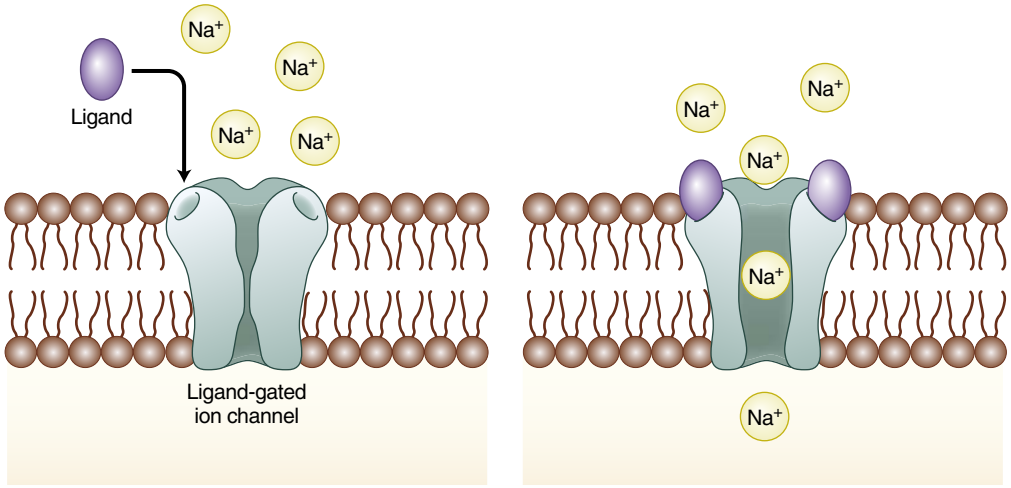
The simplest form of a cell surface signaling system is one in which both hormone-binding and signal-generating functions are provided by a single protein or complex of proteins. Ligand-gated ion channels fall into this category. They are made up of two key components: a ligand-binding domain accessible from the surface of the cell and a transmembrane domain containing a channel. Binding of ligand to the exofacial surface of the receptor generates a conformational change that results in the opening of a pore, allowing specific ions to travel through the channel across the plasma membrane (Fig. 2.2).

The prototype and founding member of the family of ligand-gated ion channels is the nicotinic acetylcholine receptor, which is present on some neurons and on the postsynaptic membrane of the neuromuscular junction.[7] When a nerve impulse arrives at the presynaptic terminal, depolarization leads to an increase in cytosolic calcium and secretion of acetylcholine. The secreted acetylcholine binds to its receptor on the muscle, which elicits a conformational change that opens the pore and allows sodium and potassium ions to pass in and out of the cell, respectively. This leads to depolarization and muscle contraction. The structure of the acetylcholine receptor, which is made up of four different peptides that constitute five subunits, defines a family of receptors that also includes the 5-hydroxytryptamine type 3 ($5HT_3R$), glycine, and inhibitory GABA type A receptors. Another shared characteristic of pentameric receptors is a conserved 15-amino acid dicysteine loop in the extracellular ligand-binding domain (LBD), giving this family its alternative name, the *cys-loop receptors*.[8]

Most ligand-gated ion channels, which when activated can elicit microsecond changes in signal transduction, serve as neurotransmitter receptors rather than receptors for classic hormones. A notable exception involves the receptors for some hypothalamic releasing factors, which are discharged from hypothalamic neurons into the portal circulation to regulate the secretion of hormones from the anterior pituitary. For example, it is thought that serotonin regulates release of prolactin by binding to the $5HT_3R$ receptor in lactotrophs of the anterior pituitary.[9] Glycine and GABA receptors are present in the pituitary gland, but their physiologic functions appear complex and remain imperfectly understood. Another class of ligand-gated ion channels, the purinergic cation receptors, are also expressed in the pituitary and most likely function in an autocrine/paracrine fashion in response to extracellular adenosine triphosphate (ATP).

G Protein–Coupled Receptors

The largest family of cell surface receptors is defined by their use of heterotrimeric G proteins for signaling, leading to their designation *G protein–coupled receptors* (GPCRs). These receptors



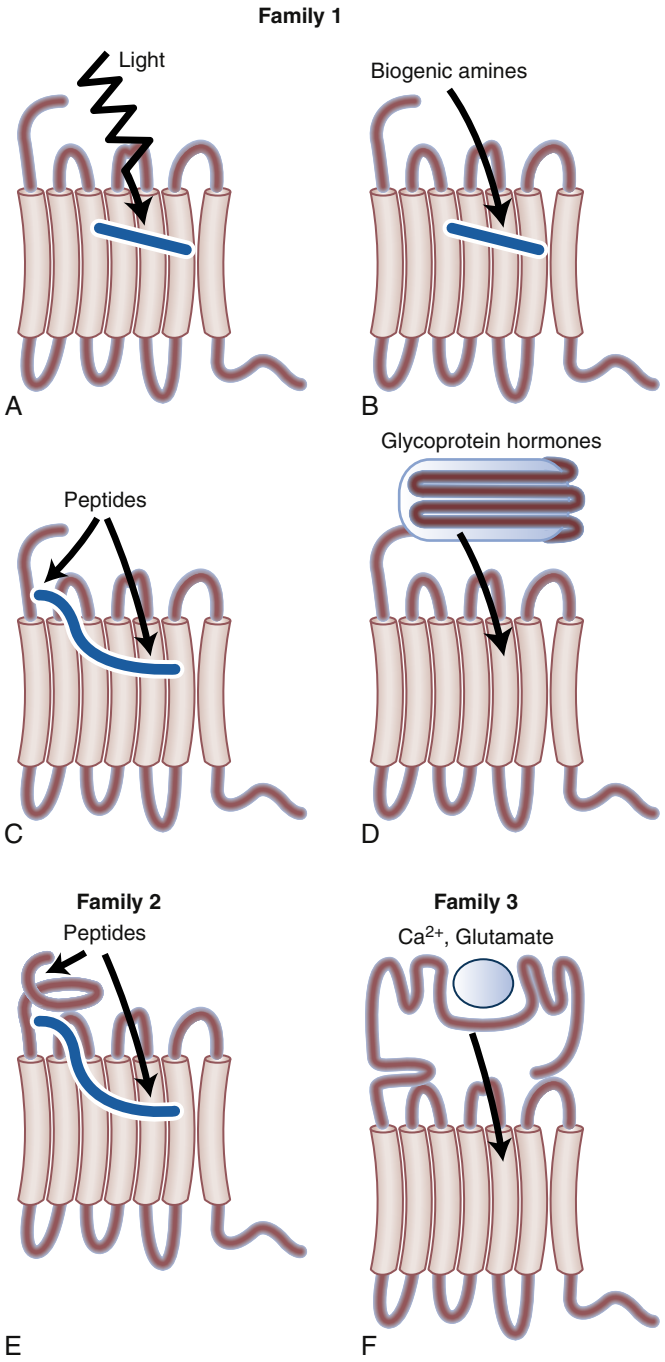
• **Fig. 2.2** Ligand-gated ion channels are transmembrane proteins that comprise at least two domains, a ligand-binding domain and a membrane-spanning domain capable of functioning as a pore. When a ligand binds, it induces a conformational change in the receptor such that the pore opens to the passage of ions, in this case sodium ions, down their electrochemical gradient.

have seven 25–amino acid α-helical segments that pass through the plasma membrane, with the amino (N)-terminus and carboxy (C)-terminus outside the cell and in the cytoplasm, respectively, leading to the name *seven transmembrane* (7TM) proteins.[10] There are more than 800 GPCR family members, with the vast majority being olfactory receptors. The diversity of ligands capable of binding to GPCRs is remarkable, ranging from a single photon to large proteins and including ions, odorants, amines, peptides, lipids, nucleotides, and metabolic intermediates. The smaller hormones, including catecholamines, bind to their GPCRs within the transmembrane-spanning region, oriented parallel to the cell surface; larger hormones bind to the extracellular N-terminus, which itself can range in size from 10 to 600 amino acids,[11] in addition to interacting with the transmembrane-spanning region (Fig. 2.3). The GPCR family has been divided into five subfamilies based on primary sequence and phylogeny, named the glutamate, rhodopsin, adhesion, frizzled/taste2, and secretin families.[12] Many hormones, including some hypothalamic releasing factors, the glycoprotein hormones secreted by the pituitary, and the amines, bind to members of the rhodopsin-like family. On the other hand, glucagon, parathyroid hormone (PTH), calcitonin, and some hypothalamic hormones, such as GH-releasing factor and corticotropin-releasing factor, bind to members of the secretin-like family. For many GPCRs, the endogenous ligand and its function are not known; these GPCRs are known as *orphan receptors*.

Signaling by Heterotrimeric G Proteins

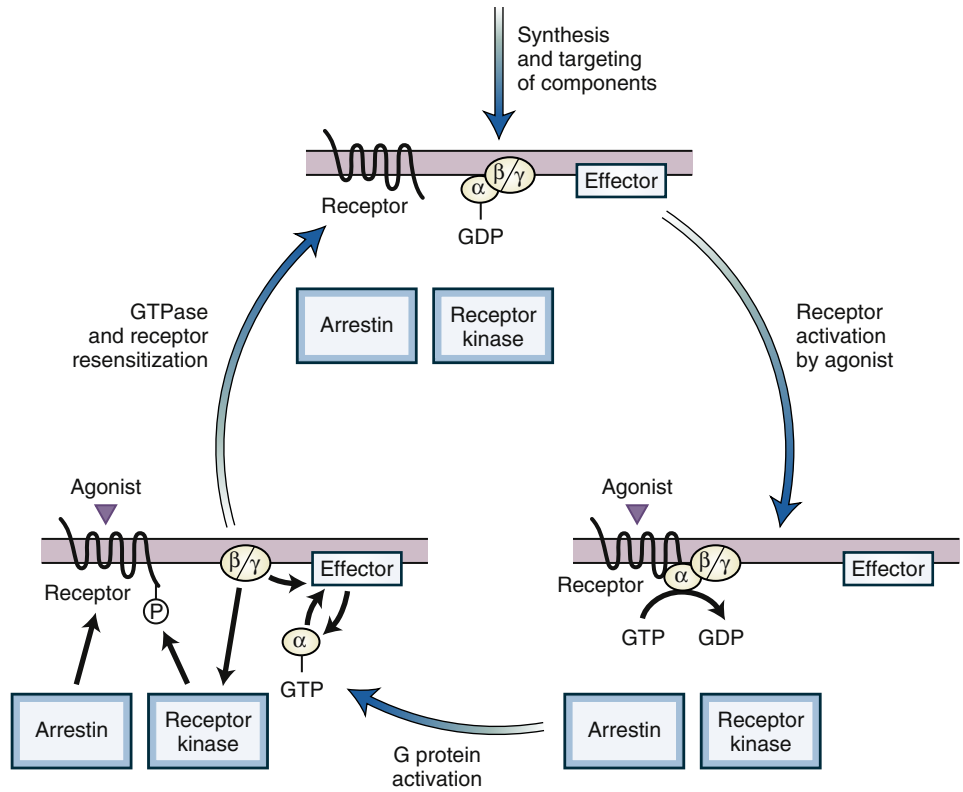
An important advance in the understanding of GPCRs occurred when Bourne and associates took advantage of the lethality of cyclic adenosine monophosphate (cAMP) toward lymphoma cells to select mutant lines resistant to the actions of the β-adrenergic agent isoproterenol.[13] Because the mutant cell lines lost responsiveness to a number of nonadrenergic agonists, it was clear that the genetic lesion did not reside in the β-adrenergic receptor but in a downstream component. When the signaling module that restored hormone responsiveness to the deficient membranes was purified, it turned out to be a heterotrimeric G protein complex, now known as G_s.[14] G_s binds a single guanosine triphosphate (GTP) to its α-subunit, which causes the α-subunit to dissociate from its other two (β, γ) subunits. The GTP-bound α-subunit of G_s is necessary and sufficient for activation of its downstream target, adenylyl cyclase. Like G_s, all G protein complexes are composed of one member each of the α-subunit, β-subunit, and γ-subunit families. Which exact subunit family member determines the downstream effector(s). Sixteen distinct genes encode about 20 different G protein α-subunits, which can be divided into four groups based on both structure and function: $G\alpha_s$, $G\alpha_i$, $G\alpha_{q/11}$, and $G\alpha_{12}$.[10] The $G\alpha_s$ family has only two members, $G\alpha_s$ and the G protein for the olfactory receptor, $G\alpha_{olf}$; both couple to activation of adenylyl cyclase. The $G\alpha_i$ group of eight includes three $G\alpha_i$ proteins, all of which inhibit adenylyl cyclase; two $G\alpha_0$ proteins that are abundant brain proteins whose multiple targets are still not completely defined; two $G\alpha_t$ proteins that couple photoreceptors to cAMP phosphodiesterase (PDE); and $G\alpha_z$, which inhibits potassium channels. The $G\alpha_{q/11}$ subfamily consists of six members, all of which activate the enzyme phospholipase C beta (PLCβ), generating the second messengers diacylglycerol (DAG) and inositol trisphosphate (IP_3). $G\alpha_{12}$ and $G\alpha_{13}$, which inhibit and activate the guanine nucleotide exchange factor, RhoGEF, respectively, form the final group. The combinational possibilities are complex, with 5 β-subunit isoforms and over 12 γ-subunit isoforms.

The key operational feature of G protein signaling is that the system behaves like a timed switch. Engagement of hormone with its cognate receptor promotes its association with a heterotrimeric G protein comprised of subunits Gα, Gβ, and Gγ (Fig. 2.4). This stimulates dissociation of guanosine diphosphate (GDP) from the α-subunit, allowing GTP to bind to the unoccupied site as a result of its greater intracellular concentration compared to GDP. The occupied receptor then detaches from the G protein. GTP loading of the G protein also induces the trimeric G protein complex to dissociate into the α-subunit and a dimeric β/γ-subunit, at least in vitro; it is not clear that dissociation actually occurs in an intact cell. In most cases, the α-subunit modulates an associated amplifier, which in the case of G_s is adenylyl cyclase, but other targets of α-subunits include those referred to previously. The β/γ-dimer can also interact with and regulate downstream signaling molecules. For example, the β/γ-dimer activates potassium channels following ligand binding to the muscarinic acetylcholine receptor.



• **Fig. 2.3** The G protein–coupled receptor (GPCR) superfamily: diversity in ligand binding and structure. Each panel depicts members of the GPCR superfamily. The seven-membrane-spanning α-helices are shown as cylinders, with the extracellular amino (N)-terminus and three extracellular loops above them and the intracellular carboxy (C)-terminus and three intracellular loops below. The superfamily can be divided into three subfamilies on the basis of amino acid sequence conservation within the transmembrane helices. Family 1 includes the opsins (A), in which light *(arrow)* causes isomerization of retinal covalently bound within the pocket created by the transmembrane helices *(bar)*; monoamine receptors (B), in which agonists *(arrow)* bind noncovalently within the pocket created by the transmembrane helices *(bar)*; receptors for peptides such as vasopressin (C), in which agonist binding *(arrow)* may involve parts of the extracellular N-terminus and loops and the transmembrane helices *(bar)*; and glycoprotein hormone receptors (D), in which agonists *(oval)* bind to the large extracellular N-terminus, activating the receptor through undefined interactions with the extracellular loops or transmembrane helices *(arrow)*. (E) Family 2 includes receptors for peptide hormones such as parathyroid hormone and secretin. Agonists *(arrow)* may bind to residues in the extracellular N-terminus and loops and to transmembrane helices *(bar)*. (F) Family 3 includes the extracellular Ca^{2+}-sensing receptor and metabotropic glutamate receptors. Agonists *(circle)* bind in a cleft of the Venus flytrap–like domain in the large extracellular N-terminus, activating the receptor through undefined interactions with the extracellular loops or transmembrane helices *(arrow)*.

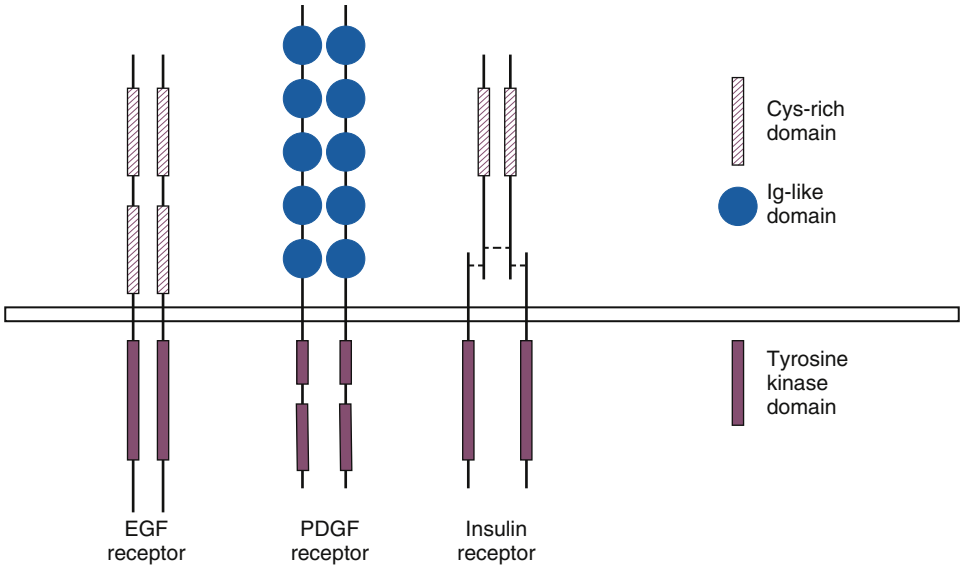
Critical to signal transduction by G proteins is that they remain active as long as GTP is bound. The rate of conversion of nucleotide GTP to GDP determines both the timing for inactivation of signaling and reassembly of subunits. Thus the G protein can exist in two distinct states: bound to GTP and active or bound to GDP and inactive; the time spent in each condition defines the strength of signaling. G protein α-subunits have low levels of intrinsic GTPase activity, but this can be enhanced by association with the regulators of G protein signaling (RGS) proteins.[15] Thus RGS proteins, which function as GTPase accelerating proteins



• **Fig. 2.4** The G protein guanosine triphosphatase (GTPase) and G protein–coupled receptor (GPCR) desensitization-resensitization cycle. In each panel, the *shaded area* denotes the plasma membrane, with the extracellular region above and the intracellular region below. In the basal state, the G protein is a heterotrimer with guanosine diphosphate (GDP) tightly bound to the α-subunit. The agonist-activated GPCR catalyzes release of GDP, which permits guanosine triphosphate (GTP) to bind. The GTP-bound α-subunit dissociates from the βγ-dimer. *Arrows* from the α-subunit to the effector and from the βγ-dimer to the effector indicate regulation of effector activity by the respective subunits. The *arrow* from effector to the α-subunit indicates regulation of its GTPase activity by effector interaction. Under physiologic conditions, effector regulation by G protein subunits is transient and is terminated by the GTPase activity of the α-subunit. The latter converts bound GTP to GDP, thereby returning the α-subunit to its inactivated state with high affinity for the βγ dimer, which reassociates to form the heterotrimer in the basal state. In the basal state, the receptor kinase and arrestin are shown as cytosolic proteins. Dissociation of the GTP-bound α-subunit from the βγ-dimer permits the dimer to facilitate binding of receptor kinase to the plasma membrane (*arrow* from βγ-dimer to receptor kinase). Plasma membrane binding permits the receptor kinase to phosphorylate the agonist-bound GPCR (*P,* depicted here as occurring on the carboxy-terminal tail of the GPCR, although sites on intracellular loops are also possible). GPCR phosphorylation facilitates arrestin binding to the GPCR, resulting in desensitization. Endocytic trafficking of arrestin-bound GPCR and recycling to the plasma membrane during resensitization are not shown.

(GAPs), serve to shorten the duration of signaling by G proteins, providing another important site of regulation. Many members of the large family of RGS proteins contain within their primary sequences canonical domains indicative of other functions and undergo complex post-translational modification. Modulation of the levels of RGS proteins affords a mechanism for signaling pathways to communicate with each other. For example, both thyroid-stimulating hormone (TSH, thyrotropin) and PTH signal through a G_s-cAMP pathway to increase expression of RGS2, which feeds back to inhibit G_s and to antagonize other pathways that depend on G_q.

Another GPCR regulatory system involves a family of proteins called arrestins (see Fig. 2.4). Two of the four arrestins (1 and 4) have been designated visual arrestins because they are expressed only in photoreceptor cells, while two arrestins (2 and 3) are expressed ubiquitously; the latter two are also called β-arrestins 1 and 2. Ligand binding to a GPCR not only signals the dissociation of the G protein complex as described earlier, but also promotes a conformational change in the GPCR that often leads to phosphorylation of the receptor by a G protein receptor kinase (GRK).[16] GRKs are represented by a family of seven related kinases. Phosphorylation of the GPCR at serine and threonine residues by GRKs allows the binding of an arrestin, which sterically uncouples the GPCR from the G protein, terminating the signal. Binding to the receptor also alters the conformation of the arrestin such that it interacts with components of the endocytosis system such as clathrin.[17] The GPCR is escorted to the sorting endosome where it either recycles back to the cell surface or is targeted to the lysosome for degradation. This system provides an efficient mechanism for homologous desensitization, in which there is receptor-specific downregulation of signaling pathways. This mechanism stands in contrast to negative regulation by second messenger–dependent protein kinases, which phosphorylate and inhibit all susceptible GPCRs regardless if occupied by ligand. In addition to its role in the modulation of G protein signaling, β-arrestin has a well-defined function as a signaling intermediate.



• **Fig. 2.5** Receptor tyrosine kinases. Three of the 16 families of receptor tyrosine kinases are represented. All receptor tyrosine kinases possess an extracellular domain containing the ligand-binding site, a single transmembrane domain, and an intracellular portion containing the tyrosine kinase domain. Several structural motifs (i.e., cysteine-rich domain, immunoglobulin-like domain, tyrosine kinase domain) in these receptor tyrosine kinases are indicated on the right side of the figure. *Dotted lines* indicate disulfide bonds. *Cys,* cysteine; *EGF,* epidermal growth factor; *Ig,* immunoglobulin; *PDGF,* platelet-derived growth factor.

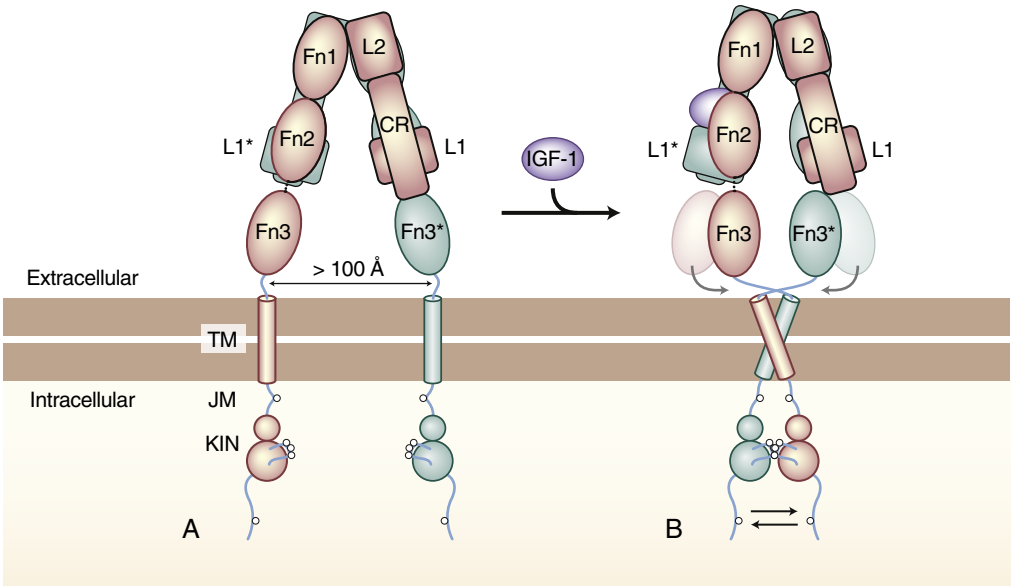
β-arrestin is now known to bind multiple members of the Src family of tyrosine kinases as well as other proteins, such as mitogen-activated protein kinases (MAPKs, also known as extracellular regulated kinases [ERKs]), phosphoinositide 3-kinase (PI3K), Akt, PDE4, and c-Jun N-terminal kinase-3.[18,19]

One of the most interesting aspects of GPCR signaling is the ability of GPCRs to undergo functional selectivity (also known as biased signaling), defined as the ability of ligands to stimulate distinct downstream signaling pathways, presumably due to stabilization of distinct conformational states of the receptor.[20] For GPCR receptors that can activate multiple G proteins, biased signaling refers to the ability to preferentially activate pathways downstream of a subset of the G proteins. For GPCR receptors that can activate arrestins, biased signaling usually refers to the ability of the receptor to favor the G protein response and minimize the arrestin response. Most of the research activity around biased signaling has taken place in the pharmaceutical industry, where the principle has been used in attempts to develop more specific therapeutics. For example, attempts have been made to develop opioid agonists that activate the analgesic effects mediated by G protein signaling but are devoid of arrestin-dependent desensitization and tolerance.[21,22] A similar strategy is being attempted to dissociate G protein–mediated opioid analgesia from arrestin-mediated constipation and respiratory depression.[21,22] Which downstream pathways are initiated by activation of a given GPCR is affected by the type and concentration of the ligand itself, and also the recruitment of specific GRKs, the subcellular location of the GPCR, and the time after ligand exposure.[23]

Receptor Tyrosine Protein Kinases as Cell Surface Receptors

The receptors that make up the receptor tyrosine protein kinase (RTK) family use a number of strategies to accomplish the same goal: to convert the binding of ligand to the exofacial portion of the receptor to a change in the activity of a tyrosine protein kinase domain residing in the interior of the cell. All of these receptors are type I transmembrane proteins with an N-terminal hormone-binding domain on the outside, a 25–amino acid hydrophobic segment that spans the membrane (the transmembrane domain), and a carboxy portion of the protein containing a kinase domain extending into the cytoplasm[24] (Fig. 2.5). The intracellular catalytic domain transfers phosphate from ATP to tyrosine residues in proteins, including the receptor itself. The 58 RTKs expressed in humans can be divided into about 20 subfamilies based on structural features. One of these groups is exemplified by the insulin receptor, which, unlike other RTKs, exists as a disulfide-linked tetramer in the basal state. Receptors in all other subgroups of RTKs, including receptors for fibroblast growth factor, platelet-derived growth factors (PDGFs), and epidermal growth factor (EGF), exist as monomers, though there is evidence that many associate noncovalently into larger structures in the basal state.

Biochemical experiments involving affinity cross-linking and biosynthetic labeling identified the structure of the insulin receptor and that of the highly related IGF1 receptor as a heterotetramer, composed of two 125-kDa α-subunits and two 90-kDa β-subunits linked by disulfide bonds[25,26] (Figs. 2.5 and 2.6). The receptor is synthesized as a single peptide with a cleavable signal sequence directing insertion cotranslationally into the membrane, and it is glycosylated and cleaved into the α and β chains in the Golgi complex.[27] Even though they exist as two separate peptides in the mature protein, each pair of α and β chains behaves much like a receptor monomer found in other growth factor receptors. Affinity labeling by insulin shows cross-linking to both the α-subunit and β-subunit, indicating that both are accessible to substances at the surface of the cell. Insulin binding has been long recognized to exhibit *negative cooperativity,* which means that the affinity for additional hormone decreases as the population of receptors binds more ligands.[28] In structural terms, this is explained by the presence of four binding sites on



• **Fig. 2.6** How insulin-like growth factor 1 (IGF1) activates its receptor. Each IGF1 receptor is made up of two half-receptors, which are linked by disulfide bonds (not shown). The six domains in the extracellular region of the first half-receptor (orange) are L1, CR, L2, Fn1, Fn2, and Fn3; the domains in the second half-receptor (green) are the same and labeled with an asterisk. The L1, CR, L2, and Fn1 are in the α-chain, and the Fn3 and the transmembrane and intracellular domains make up the β-chain of each half-receptor; the Fn2 domain is made up of contributions from both chains. The intracellular region comprises the juxtamembrane region (JM) and the tyrosine kinase domain (KIN). Sites of transphosphorylation are shown as circles. (A) When IGF-1 is not bound to the receptor, an interaction between L1* of the second half-receptor and Fn2 and Fn3 of the first half-receptor (and vice versa) is thought to maintain a large separation between the transmembrane (TM) helices *(double arrow)*. (B) When IGF1 binds to L1* (or to L1), it disrupts the L1*-Fn2 (or L1-Fn2*) interaction. This allows Fn2 and Fn3 of each half-receptor to pivot *(curved arrows)* toward each other (the previous positions of Fn2 and Fn3 are shown semitransparently). This in turn facilitates the dimerization of the TM helices in the membrane, which juxtaposes the kinase domains for efficient transphosphorylation *(black arrows)*. Binding of a single IGF1 molecule (shown as binding to the left side) is sufficient to activate the receptor, but exactly how this asymmetry affects the conformational changes in the receptor is unclear. It is believed that the same mechanism also applies to activation of the insulin receptor. (Modified from Hubbard SR, Miller WT. Closing in on a mechanism for activation. *eLife.* 2014;3:e04909.)

each holoreceptor—two of low affinity and two of high affinity. Insulin initially binds to a low-affinity site before binding to a high-affinity site on the contralateral α/β-dimer, thus effectively cross-linking the two halves of the receptor such that the stoichiometry of this high-affinity complex is one insulin molecule per insulin receptor. This stable structure prevents binding of hormone to the second high-affinity site. This structural organization for binding is largely conserved in the association of IGF1 with its receptor.[29] Other classes of RTKs use alternative strategies for ligand binding. For example, activation of the EGF receptor appears to require binding of one EGF molecule to the outer surface of one of two noncovalently associated EGF receptors,[30,31] while PDGF binds as a dimer to two noncovalently associated PDGF receptors.[32]

In general, activation of RTKs requires the formation of a receptor dimer. In some cases, such as the insulin, IGF1, FGF, and EGF receptors, the unbound receptors appear to consist of preformed dimers. In other cases, bivalent ligand binding to two receptor monomers is thought to promote dimer formation. Examples of receptors thought to act this way include the receptors for PDGF, vascular endothelial growth factor, and nerve growth factor.

Because some of the RTK receptors exist as a dimer in the basal state, it is clear that dimerization alone is not sufficient to activate RTKs; there must also be some fundamental change in the interaction between the two halves of the receptor. In the case of the insulin and IGF1 receptors, the extracellular portions of the unbound receptor exist in an inverted V conformation formed by the α-subunits and part of the β-subunits.[33] The base is continuous with and anchored by the transmembrane domains of the β-subunits. Insulin or IGF1 binding to its low-affinity site removes a brake on a molecular hinge, allowing the V to close and bring the transmembrane domains closer to each other[34,35] (see Fig. 2.6). This conformational change is transmitted to the cytoplasmic domains, where it has the effect of bringing the two kinase domains into closer proximity. In the unbound state, each kinase domain is inactive due to an intramolecular peptide, the *activation loop,* which is buried in the catalytic cleft and sterically hinders entry of substrates.[36] When the two cytoplasmic portions of the receptor domains are brought sufficiently close together, the kinase domain of one β-subunit phosphorylates the other on a cluster of tyrosine residues in the activation loop, forcing the loop out of the catalytic cleft, thus activating the kinase domain.[37] This is possible because of the kinetic nature of the receptor's inactive state, in which the catalytic site is always alternating between open and closed conformations, though in the basal state the activation loop is inaccessible most of the time. However, when the contralateral kinase domain is brought sufficiently close, it can phosphorylate the activation loop during the brief period it is in the extended

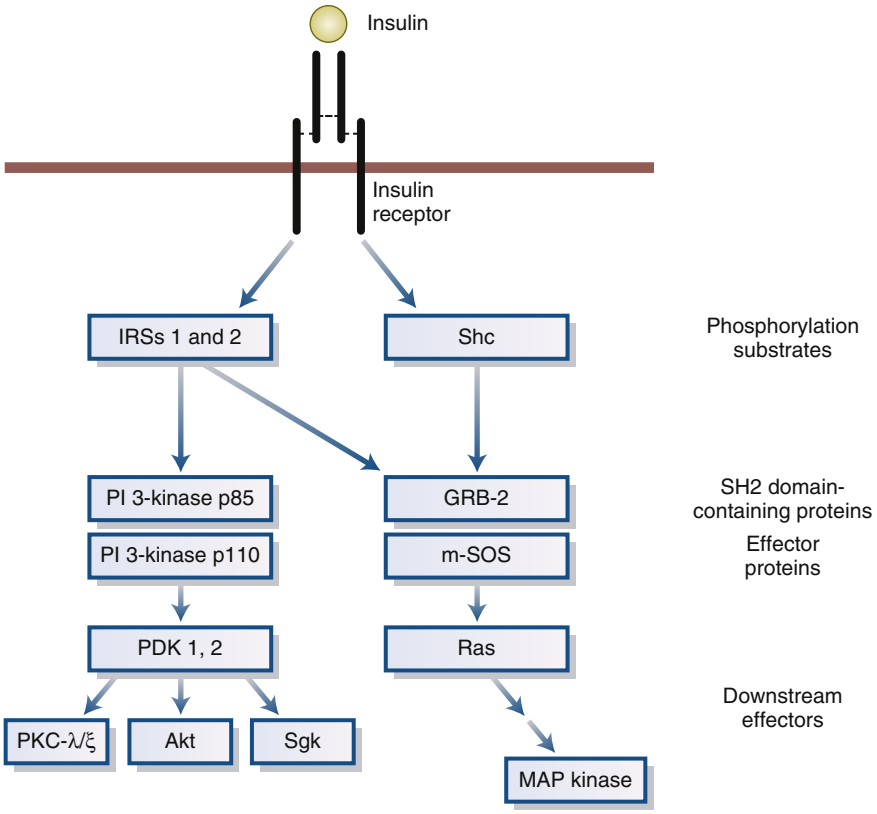
position, converting this to the more stable conformation. In this way, phosphorylation of one-half of the receptor increases its kinase activity, allowing the kinase in that half of the receptor to phosphorylate the activation loop in the other half and, ultimately, exogenous substrates.[38] Proximity-driven phosphorylation and activation of one monomer by the other are common features of RTK activation, but the precise strategies utilized to achieve this vary. Thus, although the active conformations of all tyrosine protein kinases are similar, the configurations of the inactive states differ enormously. An exception to the rule of activation by transphosphorylation is provided by the EGF receptor, in which activation depends on allosteric regulation of the kinase domain of one monomer by the other monomer, once again brought about by a conformational change bringing the two domains into adjacency. The critical interaction is between the C lobe of the activator kinase and the N lobe of the receiver kinase, which disrupts an autoinhibitory interaction present in the inactive monomer.[39]

Signaling by Receptor Tyrosine Protein Kinases

Because the insulin receptor is an enzyme with catalytic activity residing on the cytoplasmic surface of the plasma membrane, it stands to reason that it would transmit its signal by phosphorylating protein substrates within the cell. Nonetheless, though autophosphorylation sites within and outside the cytoplasmic kinase domain of the β-subunit have been long recognized, it proved difficult to identify robust, physiologically significant phosphorylation of tyrosine residues in other proteins. This seeming paradox is partially explained by the underlying mechanism of activation of signaling pathways by RTKs, which signal by recruiting a variety of signaling proteins to the different phosphorylated tyrosines in the receptor. These signaling proteins contain motifs such as the Src homology 2 (SH2) domain and the phosphotyrosine binding (PTB) domain that bind to phosphorylated tyrosines in specific contexts. In the case of the SH2 domain, a phosphorylated tyrosine residue in concert with some amino acids C-terminal to the phosphotyrosine serves as the binding interface for SH2 domains and therefore provides much of the specificity of the interaction.[40] For example, after PDGF binds to its receptor, autophosphorylation of tyrosines within the PDGF receptor in a context defined by the sequence tyrosine–methionine–any amino acid–methionine (YMXM) generates a binding site for the SH2 domains of the regulatory subunit of PI3K.[41] PI3K comprises a regulatory subunit that contains two SH2 domains in tandem and a catalytic subunit. Recruitment of PI3K to a phosphorylated receptor present in the plasma membrane both activates PI3K and brings the PI3K into proximity to its major physiologic substrate, the lipid phosphatidylinositol 4,5-bisphosphate ($PI4,5P_2$), which resides on the inner surface of the plasma membrane. PI3K phosphorylates $PI4,5P_2$ on the 3′-position of its inositol ring, generating phosphatidylinositol 3,4,5-trisphosphate (PIP_3), a potent signaling molecule by virtue of its ability to recruit protein kinases and other signaling molecules to the membrane. This illustrates an important principle governing RTK signaling: The initiation of intracellular events is often driven primarily by the spatial relationship of proteins and lipids rather than changes in the specific activity of assembled components. Although in some cases the hormone-bound receptor will modulate the activity of target protein by phosphorylation, the more important event is often the establishment of adjacency between two or more critical signaling molecules, such as PI3K and its substrate, $PI4,5P_2$. An additional example of this signaling mechanism is provided by activation of another proto-oncogene, c-Ras. In this case, signaling is initiated by recruitment of the adapter protein SH2 domain-containing protein (SHC) or growth factor receptor-bound protein 2 (GRB2) via their SH2 and/or PTB domain. When SHC is recruited, it is in turn tyrosyl phosphorylated by the RTK, enabling it to recruit GRB2 via its SH2 domain. GRB2 contains two Src homology 3 (SH3) domains that remain constitutively bound to a polyproline sequence in the son of sevenless (SOS) protein, which is thus, in turn, carried to the plasma membrane.[42,43] Association of SOS with the plasma membrane is necessary and sufficient for activation of the small G protein Ras.[44] SOS is a guanine nucleotide exchange factor (GEF) protein that activates Ras by catalyzing the removal of GDP from inactive Ras to allow binding of GTP. As noted earlier, the critical event that determines the activity of Ras is the positioning of SOS in proximity to Ras.[45,46]

The insulin and IGF1 receptors signal using a variation of the strategy described previously for the PDGF receptor (Fig. 2.7). Rather than assembling a signaling complex on the cytoplasmic domain of the receptor, they assemble the complex on members of a family of scaffolds called insulin receptor substrate (IRS) proteins.[47] There are at least three members of this family in humans, but IRS1 and IRS2 are thought to be the most important to physiologic signaling by insulin and IGF1. Like other members of the group, IRS1 and IRS2 lack intrinsic enzymatic activity; they serve solely as docking proteins to bring signaling molecules together into a multimeric complex. IRS1 and IRS2 are heavily tyrosine phosphorylated by activated insulin receptor, generating binding sites for the SH2 domains of PI3K, GRB2, and the phosphotyrosine phosphatase SHP2. A pleckstrin homology (PH) domain and PTB domain located at the N-terminus of IRS1/2 are instrumental in bringing the protein to the receptor.[48] Upon ligand engagement of the insulin or IGF1 receptor, IRS1/2 is rapidly phosphorylated on tyrosine residues and more slowly on serine/threonine residues, the latter by a number of cytoplasmic kinases, including protein kinase C (PKC), c-Jun N-terminal kinase (JNK), and pp70 S6 protein kinase. Serine/threonine phosphorylation of IRS proteins provides a strong negative feedback signal as it is thought to block further tyrosine phosphorylation and in some cases induces degradation of the protein.

There is some evidence that the insulin receptor is capable of signaling through scaffolds other than the IRS proteins, though the physiologic significance of these pathways remains unclear. The insulin receptor recruits SHC to a phosphotyrosine motif via SHC's PTB domain and phosphorylates SHC to generate a docking site for the SH2 domain of GRB2; this leads to activation of Ras as described earlier.[49] GRB10, and most likely its close relative GRB14, is an SH2 domain–containing protein that binds to the insulin receptor with high affinity.[50] However, unlike the IRS proteins, GRB10 binds to the three phosphorylated tyrosine residues in the activation loop and blocks the activity of the insulin receptor, inhibiting the insulin-dependent production of PIP_3.[51] GRB10 is stabilized via phosphorylation by mammalian (mechanistic) target of rapamycin complex 1 (mTORC1), itself activated downstream of insulin, providing another form of negative feedback.[52,53] Disruption of GRB10 in mice yields embryonic overgrowth, consistent with its role as a negative regulator of IGF1 signaling.[54] Both SH2B1 and SH2B2 (formerly known as APS) bind directly to the phosphorylated insulin receptor and both enhance the actions of insulin in vivo. However, while insulin sensitivity is decreased in SH2B1-deficient mice, consistent

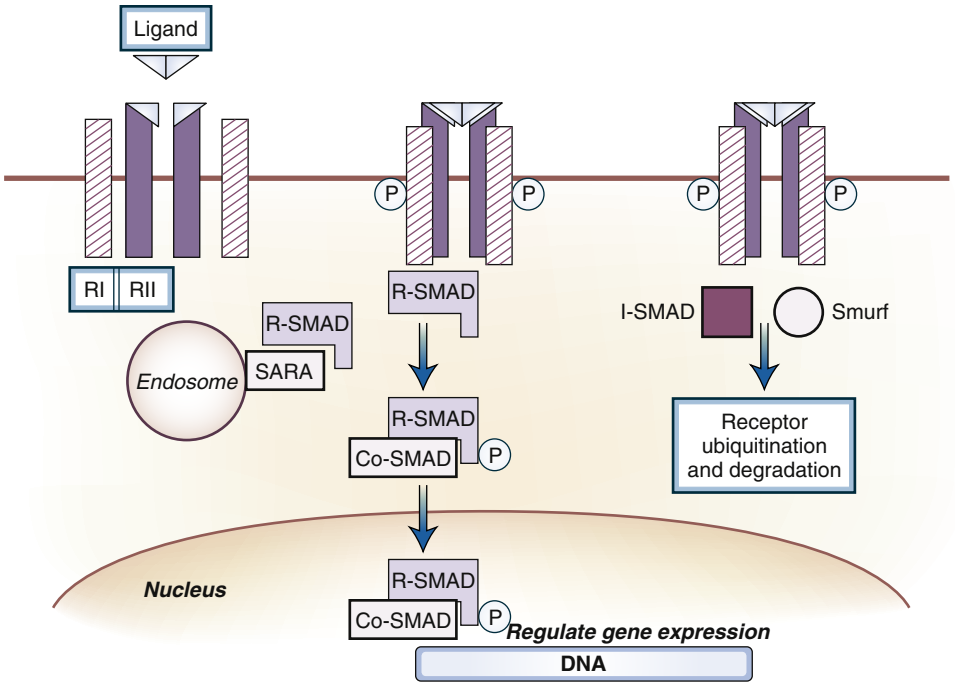


• **Fig. 2.7** Simplified model of signaling pathways downstream from the insulin receptor. Insulin binds to the insulin receptor, activating the receptor tyrosine kinase to phosphorylate tyrosine residues on insulin receptor substrates (IRSs), including IRS1 and IRS2. The phosphotyrosine residues in the IRS molecules bind to Src homology 2 (SH2) domains in molecules such as growth factor receptor–binding protein 2 (GRB2) and the p85 regulatory subunit of phosphoinositide (PI) 3-kinase (PI3K). These SH2 domain–containing proteins initiate two distinct branches of the signaling pathway. Activation of PI3K leads to activation of phosphoinositide-dependent kinases (PDKs) 1 and 2, which activate multiple protein kinases, including Akt/protein kinase B, atypical protein kinase C (PKC) isoforms, and serum-induced and glucocorticoid-induced protein kinases (Sgk). GRB2 interacts with m-SOS, a guanine nucleotide exchange factor that activates Ras. Activation of Ras triggers a cascade of protein kinases, leading to activation of mitogen-activated protein (MAP) kinase. *Shc,* Src, homology domain–containing protein.

with SH2B1 enhancing the actions of insulin, insulin sensitivity is modestly increased in SH2B2-deficient mice, suggesting that the SH2B2 gene product(s) may negatively regulate insulin sensitivity in animals.[51]

Receptor Serine/Threonine Protein Kinases

One of the more interesting variants on signaling by intracellular protein kinases is provided by a class of integral membrane receptors possessing intrinsic serine/threonine protein kinase activity. Ligands for these receptors are members of the transforming growth factor-β (TGFβ) family of first messengers. These 42 agonists encoded in the human genome can be classified into distinct groups typified by TGFβ itself, activin, inhibin, bone morphogenetic protein (BMP)/growth and differentiation factor (GDF), nodal, myostatin, and antimüllerian hormone. Each ligand is composed of a dimer of two peptides joined by hydrophobic interactions and often disulfide bonds. The hormone inhibin was isolated as an activity produced by gonadal tissue that blocks the secretion of follicle-stimulating hormone (FSH) from the pituitary.[55] Like other members of the TGFβ family, it is composed of two chains, an α-subunit and one of two related β-subunits. The hormone activin, which promotes the release of FSH, is formed by the assembly of homodimers of the β-subunit.[56] Like inhibin, activin was originally identified as a product of the gonads but is now known to be secreted by many tissues and to function in an autocrine or paracrine manner as well. The first indication that the TGFβ family of ligands exerts its actions via membrane protein kinases arose from the cloning of a complementary DNA encoding the activin receptor and recognition of a canonical kinase domain.[57] Like all receptors for ligands in the TGFβ superfamily, the activin receptor is composed of four transmembrane glycoproteins, two type 1 receptors and two type 2 receptors. Type 1 and 2 receptors have a similar primary structure, the major difference being an insertion of a conserved 30–amino acid sequence rich in glycine and serine (the GS domain) in the type 1 cytoplasmic domain preceding the kinase domain, which binds the immunophilin FKBP12. Activin interacts initially with type 2 receptors, which brings the type 1 and type 2 receptors into proximity so that the type 2 receptors can phosphorylate the GS domain of the partner type 1 receptors. This alleviates steric hindrance of the type 1 receptor kinase catalytic site and releases FKBP12, the two changes working in concert to activate the type 1 receptors, which allows the receptors to phosphorylate target substrates.[58] Inhibin exerts its inhibitory action by recruiting the transmembrane glycoprotein betaglycan (also called the type III receptor) to form a



• **Fig. 2.8** Mechanism of action for receptor serine kinases. Binding of dimeric ligand to the type II receptor (RII) subunit triggers assembly of the receptor into the heterotetrameric $[(RI)_2(RII)_2]$ state. RII transphosphorylates the type I receptor (RI), thereby activating phosphorylation of the receptor-regulated SMAD (R-SMAD) protein that is bound to the SMAD anchor for receptor activation (SARA) in endosomes. The phosphorylated R-SMAD associates with a comediator SMAD (Co-SMAD). Eventually, the R-SMAD is translocated into the nucleus, where it binds to DNA, enabling it to regulate gene transcription. The inhibitory SMAD (I-SMAD) can also bind to the activated receptor, promoting ubiquitination and degradation of the receptor. *P,* phosphorylation; *Smurf,* SMAD ubiquitination regulatory factor.

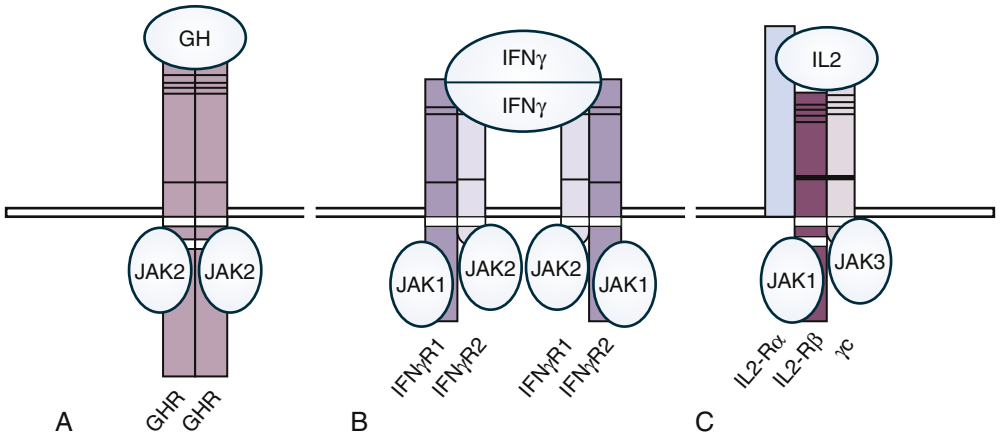
stable complex with type 2 receptors, thus sequestering them and preventing activation of the partner type 1 receptors.[59]

The major intracellular signaling mechanism utilized by all members of the TGFβ family involves SMAD proteins, which function as the major substrates for type I receptors (Fig. 2.8). There are eight human genes coding for SMAD proteins. Five of the human SMAD proteins, termed the receptor regulated SMADs or R-SMADs (SMADs 1, 2, 3, 5, and 8/9), contain a Ser-X-Ser phosphorylation site at their C-terminal tail and serve as substrates for the type I receptors. The activin receptor phosphorylates SMAD3 (and possibly SMAD2). Two R-SMADs then form a trimer with the common partner SMAD or co-SMAD (SMAD4) and are transported to the nucleus.[60] It is likely that other SMAD isoforms contribute to activin regulation of gene expression in vivo in a tissue-specific manner. Upon import into the nucleus, SMAD proteins are modified at their so-called linker domains by a complex set of phosphorylation events that serve both to enhance binding of SMAD proteins to transcriptional regulatory proteins and to target SMAD proteins for ubiquitin-dependent proteasomal degradation. SMAD proteins bind directly to DNA through a conserved N-terminal domain and interact with other transcription factors, which, in concert with the SMAD proteins, exert control over a transcriptional network defined by the cell type and activating ligand. A third class of SMADs, the inhibitory or I-SMADs (SMADs 6 and 7), can bind to the activated receptor and promote ubiquitination and degradation of the receptor.

One particularly interesting member of the TGFβ family is the hormone myostatin, formerly known as GDF8. Myostatin is secreted by skeletal muscle and negatively regulates muscle growth through binding to a type II (ActR-IIB) receptor and type 1 receptors (ALK4 and ALK5), which phosphorylate SMAD2 and SMAD3.[61] A deficiency of myostatin is responsible for the "double-muscled" phenotype of Belgian Blue and Piedmontese cattle, and deletion of its gene in mice and humans leads to massive muscle hypertrophy and hyperplasia.[62]

Signaling by Receptors That Associate With Enzymes

Another mode of signal transduction across the plasma membrane is provided by receptors that possess no intrinsic catalytic activity but that associate with a cytoplasmic, non–membrane-spanning tyrosine kinase. The best example of this is the family of class I and class II cytokine receptors, which are type 1 transmembrane proteins with the N-terminus on the outside of the cell and a cytoplasmic C-terminus (Fig. 2.9). As for RTKs, dimerization or higher order oligomerization appears important for activation of the receptor. In many cases, including the GH receptor, a single ligand molecule contains two distinct recognition sequences. The initial binding is to a high-affinity site, which is followed by a second lower affinity association with a site located on a second, associated monomer. The two monomers that compose the activated receptor make significant contact with each other, again in the exofacial domain close to where the receptor inserts in the membrane. For GH, prolactin, leptin, thrombopoietin, and erythropoietin (EPO), the receptor is a homodimer with two identical subunits. However, for some cytokines, the receptors consist of a ligand-specific monomer and one or more transmembrane chains shared with other cytokine receptors (see Fig. 2.9). For example, the interleukin 2 (IL2) receptor consists of an IL2 receptor–specific

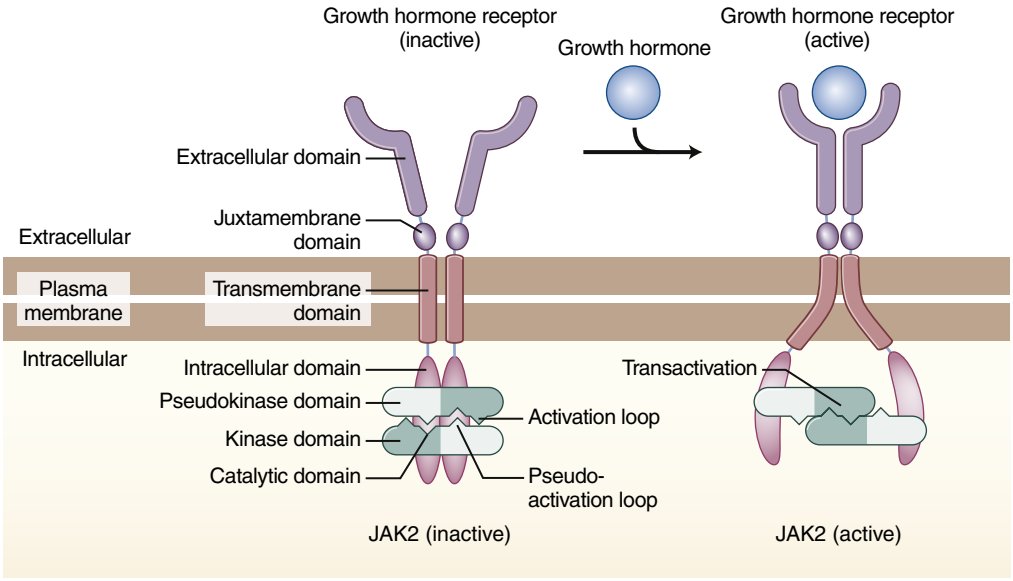


• **Fig. 2.9** Cytokine receptors are composed of multiple subunits and bind to one or more members of the Janus kinase (JAK) family of tyrosine kinases. (A) Growth hormone (GH), such as prolactin and leptin, binds to growth hormone receptor (GHR) homodimers and activates JAK2. (B) Interferon-γ (IFNγ) homodimers bind to their ligand-binding γR1 subunits. The γR2 subunits are then recruited, leading to activation of JAK1, which binds to the γR1 subunit, and JAK2, which binds to the γR2 subunit. Both subunits and both JAKs are necessary for responses to IFNγ. (C) Interleukin 2 (IL2) binds with high affinity to receptors composed of three subunits: a γc subunit shared with receptors for IL4, IL7, IL9, IL15, and IL21; an IL2Rβ subunit shared with the IL15 receptor; and a noncytokine receptor subunit, IL2Rα. IL2 activates JAK3, bound to the γc subunit, and JAK1, bound to IL2Rβ. Extracellular regions of homology are indicated by the *black lines* and *colored patterns*. Intracellular regions of homology are indicated by the *small white boxes*. Identical subunits are indicated by *identical colors*.

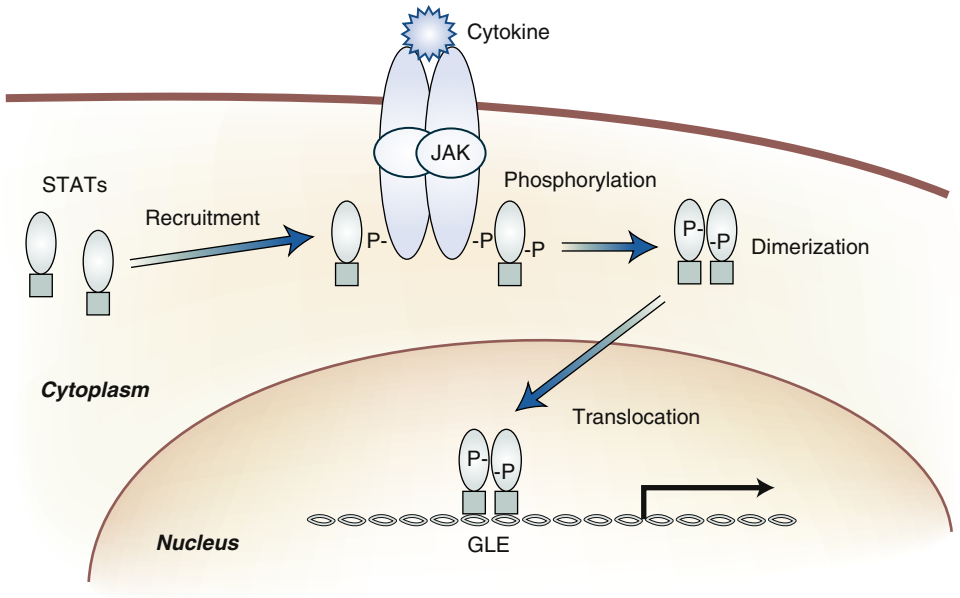
subunit (IR2Rα), a second subunit shared with the IL15 receptor (IL2Rβ), and a γc subunit that is shared with the receptors for IL4, IL7, IL9, and IL15. The IL6 receptor has a unique subunit but shares a glycoprotein 130 (GP130) subunit with at least five other receptors. As with RTKs, oligomerization appears important for activation of these receptors, as indicated by the observation that bivalent, but not monovalent, antibodies are capable of activating the receptors. However, also like RTKs, dimerization alone is insufficient to activate this class of receptors. This was recognized when the EPO receptor and subsequently the GH and prolactin receptors were examined in situ and found to exist as preformed dimers even in the unbound state.[63] The importance of dimerization of the GH receptor is illustrated by the effectiveness of the GH antagonist pegvisomant in treating acromegaly, a disease of excess GH secretion. Pegvisomant competes with native GH for its receptor and prevents functional dimerization.[64]

Proximal to the membrane on the inside of the cell, the class I and class II cytokine receptors have a conserved sequence that is critical to binding a protein tyrosine kinase of the JAK family. There are four members of this family: JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2), with JAK3 largely restricted to cells of the hematopoietic lineage.[65] For those receptors that function as homodimers, JAK2 is the predominant isoform involved in signaling. Cytokine receptors that function as heterodimers or higher order oligomers tend to bind more than one JAK family member. For example, the IFNγR1 subunit of the IFNγ receptor binds JAK1 and the IFNγR2 subunit binds JAK2, while the IL2 receptor recruits JAK1 to the IL2Rβ subunit and JAK3 to the γc subunit (see Fig. 2.9). The JAK proteins associate with a cytoplasmic, juxtamembrane portion of the cytokine receptors via a conserved, N-terminal domain structure called the FERM domain (named for its presence in Band 4.1 protein, ezrin, radixin, and moesin).[66] The carboxy half of JAK consists of two homologous regions in tandem, a pseudokinase domain followed by a kinase domain. The former has many of the conserved sequences that define a protein kinase, but it also has mutations of amino acids that are essential for catalytic activity. It is believed that in the non–ligand-bound receptor, the intracellular portions of two monomers are arranged in a way such that each pseudokinase domain binds to and suppresses the catalytic activity of the kinase on the other subunit, and vice versa. Binding of GH to its receptor results in a conformational change in the extracellular domain of the receptor, which induces a motion intracellularly like the opening of scissors, causing sliding of the two subunits of JAK in opposite directions. This relieves the allosteric inhibition of the kinases[67,68] (Fig. 2.10).

The major consequence of releasing the block to GH receptor-associated JAK2 activity is the JAK2-catalyzed transphosphorylation of the contralateral receptor subunit and its associated JAK2.[65] This allows binding of the SH2 and/or PTB domains of a number of signaling molecules, including IRS1/2 and PLCγ, thus recruiting them to the receptor and plasma membrane.[69] However, more important than these to the actions of GH on growth are members of the signal transducers and activators of the transcription (STAT) family (Fig. 2.11). The GH receptor binds a number of STAT family members, but STAT5b is most critical to its growth-promoting actions. There are seven STAT proteins with a shared domain structure. The N-termini are composed of four helical coils that function in binding to other proteins, followed by a DNA-binding domain (DBD).[70] The carboxy half of the proteins consists of a linker region, an SH2 domain, and a transcriptional transactivation domain. Several of the tyrosine residues in the GH receptor that undergo phosphorylation by JAK2 in response to ligand binding serve as docking sites for STAT5b. Once recruited to the receptor, STAT5b is itself phosphorylated, resulting in dimerization, with each STAT5b protein binding via its phosphorylated tyrosine to its partner's SH2 domain. At the same time, STAT5b dissociates from the receptor and translocates into the nucleus where it can regulate gene transcription. In addition to this basic pathway, there are numerous other layers of regulation. Serine/threonine protein kinases such as members of the MAPK and PKC families also phosphorylate STAT proteins; in some cases, this latter phosphorylation is required for maximal



• **Fig. 2.10** Scissor model for activation of the human growth hormone (hGH) receptor. In the basal state, the hGH receptor exists as an inactive dimer in which the two subunits are held together through weak interactions in the transmembrane membrane domain (TMD) and poised in the inactive state through electrostatic repulsion in the extracellular juxtamembrane domain (JMD) and pseudokinase inhibition in the associated JAK2 dimer *(left)*. Binding of hGH to the receptor *(right)* clamps the JMD such that it avoids the electrostatic repulsion and mechanically alters the TMD such that the intracellular domain is splayed outward. Splaying pulls on the JAK2 molecules to align their kinase domains. This triggers a wave of phosphorylation events, including the STAT proteins critical to receptor signaling. (Modified from Wells JA, Kossiakoff AA. New tricks for an old dimer. *Science*. 2014;344:703.)



• **Fig. 2.11** Cytokines activate signal transducers and activators of transcription (STATs). STAT proteins are latent cytoplasmic transcription factors. STATs bind through Src homology 2 (SH2) domains to one or more phosphorylated tyrosines (P) in activated receptor–Janus kinase (JAK) complexes. Once bound, STATs themselves are tyrosyl phosphorylated, presumably by the receptor-associated JAKs. STATs then dissociate from the receptor-JAK complex, homodimerize or heterodimerize with other STAT proteins, move to the nucleus, and bind to gamma-activated sequence-like elements (GLEs) in the promoters of cytokine-responsive genes. *P,* phosphorylation. (Modified from J. Herrington, used with permission.)

transcriptional activation. Using a different mechanism, SH2B1 binds to and enhances JAK2 activity.[69,71] STAT proteins can also heterodimerize with other STAT proteins or other transcription factors. For example, STAT5b has been shown to dimerize with the glucocorticoid receptor, with the latter acting as a coactivator for STAT5b to promote expression of GH-regulated genes (e.g., *IGF1*) implicated in body growth.[72]

Another important hormone that uses the JAK/STAT signaling pathway is leptin. Leptin is secreted by adipocytes; it acts on the arcuate nucleus of the hypothalamus as well as other regions in the brain to suppress appetite and, in rodents, increase metabolic rate. Humans deficient in leptin display massive obesity early in life.[73] Like GH, leptin binds to homodimers of a class I cytokine receptor and activates JAK2.[74] However, in contrast to the GH receptor, the leptin receptor recruits STAT3 as its primary signaling molecule, which binds to phosphotyrosines in a YXXQ motif. The phosphorylated leptin receptor also binds STAT5 and the SH2-containing protein tyrosine phosphatase 2 (SHP2; PTPN11). The latter is thought to act as a positive signaling module by mediating the first step in the activation of the ERK cascade.[75] On the other hand, the tyrosine phosphatase PTP1B dephosphorylates the leptin receptor and inhibits leptin action, and thus its deletion in mouse brain leads to obesity and insulin resistance.[76] JAK2 also phosphorylates IRS proteins, thereby engaging the PI3K pathway. The roles of the different signaling pathways activated downstream of leptin and JAK2 have been investigated using mice in which specific tyrosine residues in the receptor have been mutated. Replacement of tyrosine 1138 by serine completely blocks recruitment of STAT3, generating mice similar in their degree of obesity to those lacking leptin receptors, showing that STAT3 signaling is critical to the regulation of appetite and energy metabolism.[77]

Termination of class I cytokine signaling occurs in response to dephosphorylation of key phosphotyrosines; it is also promoted by the transcriptional induction of the suppressors of cytokine signaling, or SOCS proteins. The eight members of the SOCS family are direct targets of the STAT transcription factors and provide a potent negative feedback signal by binding to phosphorylated tyrosines in the receptors via the SOCS SH2 domain. Upon interacting with the receptors, SOCS proteins inhibit their action by reducing JAK activity, by competing for binding of other signaling molecules, and/or by inducing the degradation of receptor via the ubiquitin pathway due to the conserved SOCS box located at the C-terminus of the protein.[78] Mice deficient for SOCS2 appear normal when young but after weaning grow substantially larger than their wild-type littermates, consistent with enhanced GH signaling.[79] Cytokine signaling via STAT proteins can also be downregulated by members of the protein inhibitor of activated STAT (PIAS) family, which have been shown to regulate transcription through several mechanisms, including blocking the DNA-binding activity of transcription factors, recruiting transcriptional corepressors, and promoting protein sumoylation.

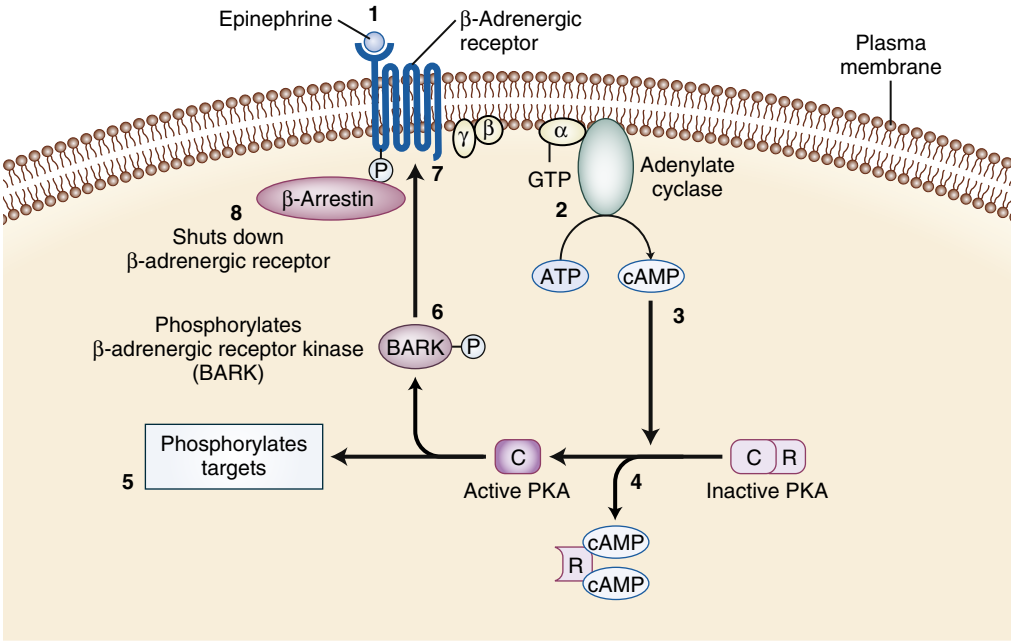
Coupling of Cell Surface Receptors to Intracellular Signaling

Downstream Signaling by Cyclic Adenosine Monophosphate

For the many hormones that bind exclusively to the outer surface of cells to carry out their actions, there must be some means of translating the extracellular signal into an intracellular response. The first example of a transduction system that was understood in some detail derived from investigating one of the key features of the fight-or-flight response, the mobilization of stored carbohydrate in the liver. The physiologic response to stress requires a supply of readily consumable energy, best provided in the form of blood glucose, which is stored as polysaccharide glycogen primarily in the liver. β-Adrenergic stimulation of hepatocytes by epinephrine leads rapidly to the hydrolysis of glycogen and the release of free sugar; glucagon also stimulates the breakdown of hepatic glycogen. The mechanism used to transmit this response is the prototypical example of a second messenger system, in which the agonist that interacts with the outside of the cell, in this case glucagon or epinephrine, is considered a *first messenger,* and a soluble, intracellular signaling molecule generated by hormone-receptor association is called a *second messenger*.[80] For hepatic glycogen breakdown in response to glucagon or β-adrenergic agents, the second messenger is cAMP, which is produced by a plasma membrane enzyme, adenylyl cyclase, from ATP (Fig. 2.12). Adenylyl cyclase is a direct target of $G\alpha_S$, which becomes GTP loaded and active in response to receptor occupancy.

The scope and diversity of hormones and other extracellular signals that activate adenylyl cyclase and increase the level of intracellular cAMP are remarkably extensive. Included in the long list of hormones that signal through this mechanism are β-adrenergic agents, glycoprotein hormones such as TSH, glucagon, adrenocorticotropic hormone (ACTH), hypothalamic hormones, and antidiuretic hormone. Moreover, the range of physiologic and biochemical events modulated by cAMP is equally vast. Thus, although the second messenger cAMP defines a commonly used mechanism for transducing signals from extracellular hormones, it also presents another problem in signaling: How do cells maintain selectivity in the way they respond to a given hormone? Much of this is accomplished by the subcellular compartmentalization of signaling complexes. A-kinase anchoring proteins (AKAPs), which are scaffolds localized to distinct intracellular sites, bind a number of proteins that modulate the actions of cAMP, including degrading enzymes and target kinases.[81] The regulated assembly of higher order structures confers a spatiotemporal resolution to cAMP signaling that can allow multiple biologic responses to exist within the same cell. For example, β-adrenergic agents and prostaglandin E_1 both act on the heart through elevations in cAMP, but each regulates a different cardiac function. This is accomplished through stimulation of distinct populations of cAMP target kinases, such that β-adrenergic agents are more potent than prostaglandins in their effects on the particulate fractions of the heart cell.[82] It is likely that AKAPs confer this specificity to the cardiomyocyte.

cAMP is degraded to AMP and phosphate by a specific PDE, and the balance between synthesis and degradation of cAMP determines the levels of the cyclic nucleotide. Although hormones generally use adenylyl cyclase as the means for modulating cAMP levels within the cell, the PDEs provide an additional site of regulation.[83] The cyclic nucleotide PDEs are a large and complex family of enzymes, whose diversity in both tissue and subcellular localization has made them favorite targets for the development of therapeutics. Caffeine and theophylline were two of the first drugs recognized to be inhibitors of PDE, but more recently, selective inhibitors of PDE5, an enzyme that degrades cyclic guanosine monophosphate (cGMP), have been widely used for the treatment of erectile dysfunction. In addition, PDE inhibitors are either currently being used or are in development for the treatment of a wide variety of diseases, including asthma, neurologic diseases, and pulmonary hypertension.



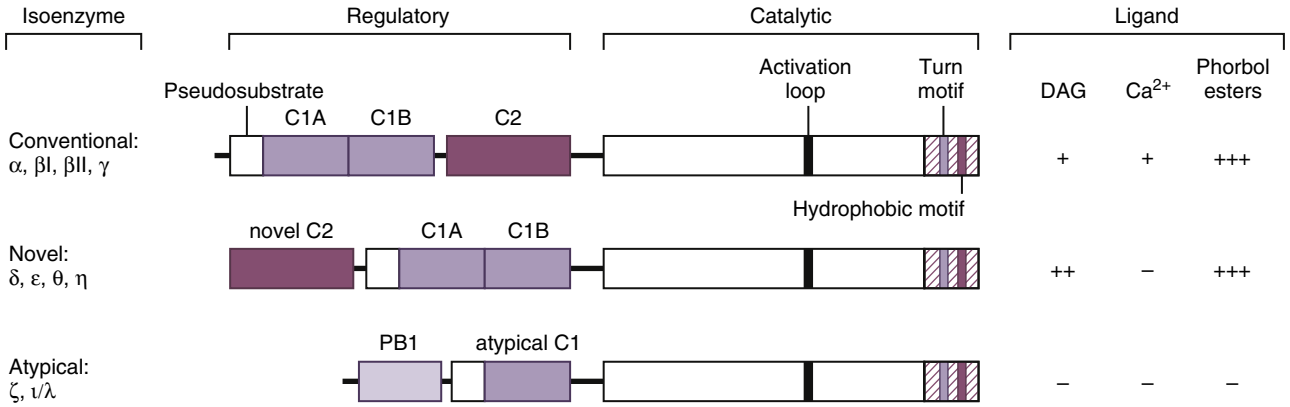
• **Fig. 2.12** Adenylate cyclase, protein kinase A (PKA), and β-adrenergic receptor kinase (BARK) activation by epinephrine. Step 1: Upon binding of epinephrine to the β-adrenergic receptor, G_s is activated. Step 2: $G_s\alpha$ binds to and stimulates adenylate cyclase. Step 3: Adenylate cyclase catalyzes the conversion of ATP to cAMP. Step 4: cAMP binds to the regulatory subunit (R) of PKA, releasing free catalytic subunit (C), which is active. Step 5: C phosphorylates a number of intracellular substrates in a manner determined by its location in the cell. Step 6: C phosphorylates serine and threonine residues on BARK. Step 7: BARK, itself a serine/threonine kinase, phosphorylates serine and threonine residues on the β-adrenergic receptor. Step 8: β-Arrestin binds to the phosphorylated receptor, which blocks further activation of G_s. β-Arrestin also initiates signaling cascades, which are not shown.

Glycogen metabolism in liver and muscle provided the initial example of a common mode of signaling initiated by second messengers—activation of a cascade of intracellular protein kinases. Signal transduction by protein phosphorylation stands as one of the most critical regulatory mechanisms in biology. The state of a phosphoprotein is regulated dynamically, determined by the relative rates of phosphorylation and dephosphorylation by protein kinases and phosphatases, respectively. In most cases, the turnover of the phosphate is rapid, allowing regulation by either the kinase or phosphatase, or in many instances both coordinately. Numerous endocrine signals exert control over intracellular metabolism, growth, and other functions via modulation of protein kinase activity. Originally, protein kinases were found to phosphorylate proteins on serine and threonine residues, but, as described earlier, tyrosine phosphorylation has emerged as another mode of signaling.

One advantage of such series of kinases is signal amplification. Amplification occurs because each individual kinase molecule can modify many downstream target proteins. When these downstream targets are also kinases that become activated upon phosphorylation, each one of them can in turn modify and activate many more proteins. In the case of cAMP-initiated signaling, one receptor:ligand pair creates multiple cAMP molecules. The multiple cAMP molecules activate the serine/threonine kinase protein kinase A (PKA), which in turn phosphorylates multiple downstream effector proteins. In the case of glycogen metabolism, PKA phosphorylates and activates glycogen phosphorylase kinase, which in turn phosphorylates and activates glycogen phosphorylase, which releases glucose-1-P from glycogen. In muscle, phosphorylase kinase is also stimulated by calcium, which is released from the sarcoplasmic reticulum during electrical stimulation and contraction. The mechanism by which cAMP activates PKA illustrates another theme in signal transduction: displacement or dissociation of intramolecular pseudosubstrates or substrates as a means to activate protein kinases, a mechanism also used by such protein kinases as PKC and myosin light chain kinase. cAMP binds to the two regulatory subunits of the heterotetrameric PKA, which causes them to dissociate from two catalytic subunits. A domain in the regulatory subunit resembles a PKA phosphorylation sequence but with the critical serine replaced by an alanine, which lacks the hydroxyl group required for transfer of the phosphate from ATP. When PKA is assembled into a heterotetramer of two regulatory subunits and two catalytic subunits, this pseudosubstrate interacts with the catalytic subunit, preventing it from phosphorylating target proteins.[84]

In addition to enhancing glycogen breakdown, PKA mediates the effects of a number of hormones in various tissues, including the positive inotropic and chronotropic effects of epinephrine on the heart, the trophic effects of the anterior pituitary hormones TSH and ACTH, and the effects of antidiuretic hormone on the permeability of the renal collecting duct to water. PKA also translocates into the nucleus to regulate gene transcription.[85] The best studied nuclear target of PKA is the cAMP-response element–binding protein (CREB), though it is still not clear how many of the physiologic actions of cAMP require this transcription factor to be phosphorylated. PKA also phosphorylates a number of coregulatory proteins, which also contribute to transcriptional outputs.

Importantly, cAMP also has actions that are independent of PKA. One of these is the direct regulation of ion channels;



• **Fig. 2.13** Domain structure and ligands of protein kinase C (PKC). The PKC family can be divided into three classes: the conventional, or classic, PKCs (cPKCs); the novel PKCs (nPKCs); and the atypical PKCs (aPKCs). The C1 domains bind diacylglycerol (DAG) or phorbol ester; the C2 domain binds calcium. A novel C2 domain in nPKCs does not bind calcium but mediates protein-protein interactions. Similarly, a PB1 domain in aPKCs is involved in protein-protein interactions. The aPKCs possess only one C1 domain and thus do not bind diacylglycerol. The conserved pseudosubstrate motif is represented by the *white boxes* in the regulatory domain. The activation loop and the turn and hydrophobic motifs are sites of regulatory phosphorylation.

another involves the exchange protein activated by cAMP (EPAC), which functions as a guanine nucleotide exchange factor (GEF) for the small GTP-binding protein Rap1.[86] Regulation of insulin secretion from pancreatic beta cells by glucagon-like peptide-1 (GLP1) and stabilization of the endothelial barrier by β-adrenergic agents are two processes thought to be mediated by EPAC.

Regulation by the Second Messengers Calcium and PKC

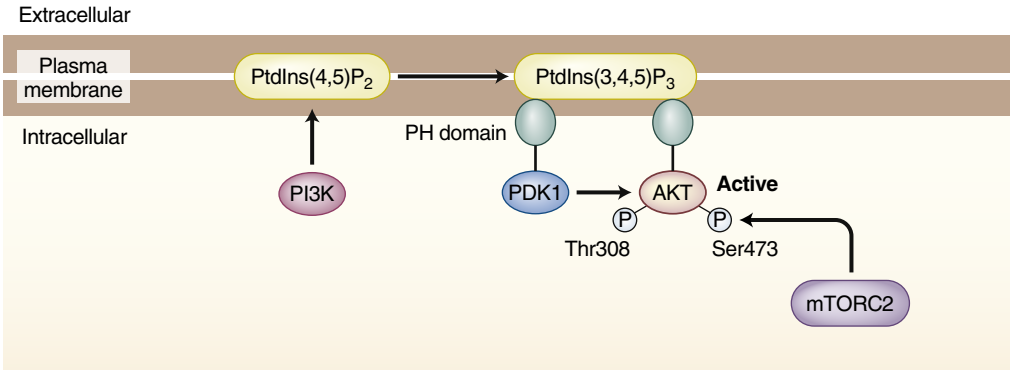
Many additional second messengers have been identified since the discovery of cAMP. These include calcium, cGMP, inositol polyphosphates, DAG, and nitric oxide. The calcium ion (Ca^{2+}) is one of the most common second messengers utilized by diverse cell types, and one that plays a particularly important role in the regulated secretion of hormones.[87] Ca^{2+} is maintained at low micromolar concentrations in the cytoplasm such that opening channels that lead to the outside of the cell or intracellular storage organelles results in a rapid increase in cytosolic Ca^{2+}. The heterotrimeric G proteins containing $G\alpha_q$ or $G\alpha_{11}$ cause increases in intracellular calcium by targeting the membrane-associated enzyme PLC. PLC catalyzes the hydrolysis of phosphatidylinositol 4',5'-bisphosphate into DAG and IP_3. Hormones that signal through G protein–dependent activation of PLC include angiotensin II, α-adrenergic catecholamines, growth hormone–releasing hormone (GHRH), and vasopressin. IP_3 binds to a receptor located on the cytoplasmic face of the endoplasmic reticulum, leading to the release of stored Ca^{2+} from that organelle. Ca^{2+} also interacts with the IP_3 receptor, further stimulating calcium discharge from the endoplasmic reticulum and providing a strong positive feedback loop. Another source of cytoplasmic Ca^{2+} is entry through receptor-operated channels in the plasma membrane, such as those activated by noradrenaline, endothelin, or histamine via heterotrimeric G proteins.

Ca^{2+} transmits its signal via a number of effectors, including protein kinases, in most cases through the intermediary binding protein, calmodulin, or its relative, troponin C. Calmodulin is a small, acidic protein that contains four copies of a canonical calcium-binding motif.[88] Calmodulin associates with and regulates in a Ca^{2+}-dependent manner glycogen phosphorylase kinase, myosin light chain kinase, and members of the family of calcium/calmodulin-dependent kinases. In addition to protein kinases, other calcium/calmodulin-dependent enzymes include the serine/threonine protein phosphatase, calcineurin, some adenylate cyclase and PDE isoforms, and nitric oxide synthase. Calcium interacts directly and independently of calmodulin with targets such as the protease calpain, synaptotagmin (a regulator of neurotransmitter and hormone exocytosis), and cytoskeletal proteins.

An important group of protein kinases directly activated by calcium is the PKC family. PKC, originally identified as the target of the tumor promoter phorbol ester, is a cyclic nucleotide-independent protein kinase regulated by the direct binding of DAG and calcium, two second messengers produced by the activation of PLC. The PKC family has been divided into three groups: classic (regulated by DAG, phosphatidylserine, and calcium), novel (regulated by DAG and phosphatidylserine), and atypical. All PKC proteins have a conserved kinase domain in their C-terminal portion and regulatory sequences in their N-terminal domain. For classic PKCs, the latter consist of a C1 domain, which binds DAG or phorbol ester, followed by a C2 domain, which associates with anionic lipids in a Ca^{2+}-dependent manner[89] (Fig. 2.13). Novel isoforms have a modified form of the C1 domain that confers a higher affinity for DAG than in the classic isoforms but lack the C2 domain, explaining the absence of Ca^{2+} regulation. Atypical PKCs have alterations in the C1 domain that eliminate DAG binding and also lack a site for Ca^{2+} binding. The regulation of PKC isoforms is complex, involving such covalent modifications as phosphorylation and proteolysis, as well as interaction with lipids and hydrophilic molecules other than those traditionally associated with activation of classic PKCs.[90]

Regulation of Protein Kinases by PI3K

Another important signaling pathway involves a family of related proteins that catalyzes phosphorylation of phosphoinositides on



• **Fig. 2.14** Mechanism of AKT activation. When phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) levels are low in the plasma membrane, AKT is in an inactive conformation in the cytoplasm and cannot be phosphorylated by the upstream activating 3-phosphoinositide-dependent protein kinase 1 (PDK1) (not shown). PtdIns(3,4,5)P_3 levels increase in the plasma membrane following the insulin-dependent recruitment to IRS1 and IRS2 of phosphoinositide 3-kinase (PI3K), which phosphorylates phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)P_2). AKT binds PIP_3 through its pleckstrin homology (PH) domain and induces a conformational change within the AKT kinase domain, allowing PDK1 to phosphorylate the critical residue in the activation loop required for AKT kinase activity, threonine 308 (Thr308). Mammalian target of rapamycin complex 2 (mTORC2) also phosphorylates AKT at the carboxy-terminal serine 473 (Ser473) site to fully activate its kinase activity. PDK1 has a PH domain that can bind PtdIns(3,4,5)P_3, but this interaction is not essential for PDK1 catalytic activity. (Redrawn from Finlay D, Cantrell DA. Metabolism, migration and memory in cytotoxic T cells. *Nat Rev Immunol.* 2011;11:109.)

the 3′ position of the inositol ring.[91] All class I PI3Ks are comprised of a catalytic protein associated with a regulatory subunit and use PI4,5P_2 as a preferred substrate; these isoforms are most important to signaling by RTK, GPCRs, and tyrosine kinase oncogenes. Class II PI3Ks phosphorylate PI and PI4P in vivo and lack stable regulatory subunits but probably associate with other proteins as modulating factors. They have been implicated in a diverse set of physiologic responses, but the downstream targets are largely unknown. Class III PI3K, which has one catalytic member also known as vacuolar protein sorting 34 (Vps34), binds tightly to the regulatory protein Vps15, uses exclusively PI as a substrate, and is involved primarily in membrane protein trafficking related to endocytosis, phagocytosis, and autophagy.

Class IA PI3Ks are defined by regulatory subunits containing SH2 domains, which target them to activated RTKs. The heterotrimeric G protein subunit pair Gβγ, when free, activates those class I PI3Ks containing regulatory subunits not bearing SH2 domains.

Class IA PI3Ks are thought to be the most important PI3Ks for the actions of hormones, particularly insulin and IGF1.[92] Activation of the receptors for either hormone leads to phosphorylation of IRS1 or IRS2 at sites specialized for docking with SH2 domains in the p85 regulatory subunit associated with the p110α catalytic subunit of PI3K. The bound PI3K catalyzes the production of PIP_3 and possibly PI3,4P_2, which serve to recruit additional proteins (including protein kinases) to the membrane by binding their PH domains.[93] PH domains are best known for their ability to bind phosphoinositides with high affinity and specificity, although only a small portion have been proven to do so. The serine/threonine protein kinase Akt, also named *protein kinase B* because of its structural similarities to PKA and PKC, contains an N-terminal PH domain that preferentially binds to PIP_3 and PI3,4P_2.[94] When insulin acts upon a target cell, the PH domain of Akt associates with the PIP_3 generated on the cytoplasmic face of the plasma membrane. The binding of the PH domain to PIP_3 serves two purposes: to recruit Akt to the membrane and to relieve steric hindrance of Akt's phosphorylation sites and catalytic domain by the PH domain. Also at the plasma membrane via its own PH domain is the enzyme 3-phosphoinositide-dependent protein kinase (PDK1), which phosphorylates Akt on a threonine in its activation loop (Fig. 2.14). mTORC2 also phosphorylates Akt on a serine in its C-terminus. Together, the PDK1 and mTORC2 phosphorylation events confer full activity to Akt. mTORC2 appears to be regulated by insulin, but the mechanism is unknown.

Akt is essential to many of the metabolic actions of insulin and growth effects of IGF1.[95,96] There are three Akt isoforms, each encoded by separate genes. Akt1 is the most widely expressed isoform and seems to be critical to the regulation of growth; Akt2 is enriched in insulin target tissues and is more important to the control of metabolism; and Akt3 is expressed primarily in the brain, where it controls growth of that tissue.[97] Indirect activation of mTORC1 by Akt and suppression of forkhead box (FOX)O–driven transcription are two of the critical targets for promoting organ growth, the Akt/mTORC1 pathway being particularly engaged in the regulation of cell size.[98] Members of the Rab GTPase-activating protein family, TBCD4 (also known as AS160) in fat cells and TBC1D1 in both muscle and fat, are phosphorylated and inhibited by Akt, contributing to the activation of glucose transport.[99]

Regulation of Protein Kinases by Ras

Routes to activation of Ras by GRB-SOS include both RTKs and GPCRs acting through β-arrestin.[100] GTP-bound Ras recruits several receptors to the plasma membrane, including the serine/threonine kinase Raf, which is activated by dimerization and a series of phosphorylation/dephosphorylation events.[101] Raf then phosphorylates MAPK/ERK kinase (MEK1), a tyrosine and serine/threonine–dual specificity protein kinase, initiating a protein kinase cascade centered on extracellular signal-regulated kinases 1 and 2 (ERK1/2). This represents one of four MAPK cascades,

the others involving c-Jun N-terminal kinase (JNK), the 38-kDa stress-activated kinases (p38), and ERK5. Specificity for MAPKs is conferred by scaffold proteins that bind most or all members of a given pathway, ensuring that each member phosphorylates only its appropriate target kinase.[102] Gonadotropin-releasing hormone, PTH, GH, angiotensin, and gastrin are just a few of the many hormones believed to signal at least in part through regulation of MAPKs.

Disease Caused by Defective Cell Surface Receptors

Numerous diseases develop as a result of dysfunctional binding to or signaling by hormone receptors. These *hormone resistance syndromes* invariably mimic the phenotype of the hormone-deficient state but present with high levels of biologically active hormones in the circulation.

Insulin Resistance Syndromes

The best studied inherited disease of hormone resistance is that caused by mutations in the insulin receptor. In addition to hyperinsulinism and the expected abnormalities in metabolism, patients with severe insulin resistance also display acanthosis nigricans (hyperpigmentation primarily in the skin folds) and often hyperandrogenism.[103] Beyond that, there is a range of syndromes that correlate with the degree of insulin signaling impairment. The strongest loss-of-function mutations result in leprechaunism, in which there are severe developmental defects presenting at birth. Some mutations of the insulin receptor gene cause a decrease in the number of receptors in the plasma membrane, in some cases accompanied by a decrease in the mRNA. Other mutations adversely affect hormone binding or the function of the kinase domain.[103] In contrast to insulin resistance caused by mutations in the receptor gene, sometimes referred to as type A insulin resistance, type B resistance presents at middle age, often with signs of autoimmunity such as vitiligo, alopecia, and arthritis. This syndrome is defined by the presence of antibodies directed against the insulin receptor; the levels of antibody correlate with the severity of the disease.

In many ways, the use of insulin resistance to describe the common syndrome associated with obesity or polycystic ovary syndrome (PCOS) is a misnomer. The term *resistance* was originally coined to describe the situation of hyperglycemia in the face of elevated concentrations of insulin in the blood.[105,106] However, the recognition that insulin has numerous physiologic actions in addition to those on carbohydrate metabolism has led to ambiguity in nomenclature. On the one hand, the term *insulin resistance* is often applied to abnormalities in insulin signaling to all outputs from the receptor; this typically occurs with mutations of the insulin receptor. However, in the insulin resistance of obesity or PCOS, some actions of insulin are preserved. This is demonstrated by a comparison of the phenotype of individuals with type 2 diabetes mellitus to those with genetically encoded partial defects in insulin receptor function.[107] Both groups share hyperglycemia, but only those with type A insulin resistance display defects in the regulation of hepatic lipid metabolism by insulin. Thus the metabolic phenotype associated with type A inherited insulin resistance is not faithfully phenocopied by the insulin resistance of obesity. Consistent with this, numerous pathologic mechanisms have been proposed to account for the insulin resistance associated with obesity, almost all of which involve a "post-receptor defect."

Defects in Cell Surface Receptors That Control Growth

One of the most clinically recognizable syndromes is resistance to the actions of GH, which results in shortness of stature. An inability to respond to GH results in Laron syndrome, characterized by high levels of circulating GH, very low levels of IGF1, and short stature.[108] Diverse molecular causes have been reported, including large deletions as well as missense, frameshift, and splicing mutations in the GH receptor. Similar syndromes of decreased growth can also result from mutations in STAT5b and deficiency in IGF-1 or defects in IGF-1 signaling. Recently, a syndrome has been described in which mutations in the *PIK3R1* gene, which encodes the p85α regulatory subunit of class I PI3K, lead to SHORT syndrome, which includes dysmorphic facial features and defects in growth (short stature, hyperextensibility, ocular depression, Rieger anomaly, and teething delay).[109] As might be expected by the similarities in IGF-1 and insulin signaling, individuals with SHORT syndrome also display lipodystrophy and insulin resistance.[110]

Diseases Caused by Mutations in GPCRs and G Proteins

A number of endocrine diseases can be attributed to mutations in the GPCR–G protein signaling system.[111,112] For GPCRs, many mutations are associated with some degree of loss of function and are inherited in a recessive manner (Table 2.3). Some examples include hypothyroidism from mutations in the thyrotropin-releasing hormone or TSH receptor, glucocorticoid deficiency from mutations in the melanocortin 2 receptor, extreme obesity from dysfunction of melanocortin 4 receptor, and infertility due to mutations in the receptor for luteinizing hormone or FSH. Gain-of-function mutations include those in the TSH receptor causing hyperthyroidism, in the α_2-adrenergic receptor, leading to diabetes mellitus, and in the calcium-sensing receptor resulting in hypoparathyroidism. Somatic activating mutations have been reported in the luteinizing hormone and TSH receptors.[111] A limited number of heterotrimeric G proteins are known to have mutations that cause human disease, and in all cases they affect the α-subunit. Mutation of the gene encoding the $G\alpha_t$ subunit of transducin is associated with night blindness. Dominant, activating mutations of $G\alpha_s$ cause pituitary adenomas, most often secreting GH, and more rarely, tumors of the thyroid, parathyroid, and adrenal glands.[112] Patients who inherit a loss of a functional allele in $G\alpha_s$ develop Albright hereditary osteodystrophy (AHO); those who inherit the mutant allele from their mothers also have pseudohypoparathyroidism type 1a in addition to AHO. This is due to imprinting of the $G\alpha_s$ gene, such that it is expressed preferentially from the maternal allele in a number of hormone target tissues, but biallelically in most other cell types.

Ligands That Act Through Nuclear Receptors

Many signaling molecules share with thyroid and steroid hormones the ability to function in the nucleus to convey intercellular and environmental signals. Lipophilic signaling molecules that use nuclear receptors include derivatives of vitamins A and D, endogenous metabolites such as oxysterols and bile acids, and

TABLE 2.3 Diseases Caused by G Protein–Coupled Receptor Loss-of-Function Mutations

Receptor	Disease	Inheritance
V_2 vasopressin	Nephrogenic diabetes insipidus	X-linked
ACTH	Familial ACTH resistance	AR
GHRH	Familial GH deficiency	AR
GnRH	Hypogonadotropic hypogonadism	AR
GPR54	Hypogonadotropic hypogonadism	AR
Prokineticin receptor 2	Hypogonadotropic hypogonadism	AD[a]
FSH	Hypergonadotropic ovarian dysgenesis	AR
LH	46 XY, intersex	AR
TSH	Familial hypothyroidism	AR
Ca^{2+} sensing receptor	Familial hypocalciuric hypercalcemia	AD
	Neonatal severe primary hyperparathyroidism	AR
Melanocortin 4	Obesity	AR
PTH/PTHrP	Blomstrand chondrodysplasia	AR

[a]With incomplete penetrance.

ACTH, Adrenocorticotropic hormone; *AD,* autosomal dominant; *AR,* autosomal recessive; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *GnRH,* gonadotropin-releasing hormone; *GPR54,* orphan G protein–coupled receptor 54; *LH,* luteinizing hormone; *PTH,* parathyroid hormone; *PTHrP,* parathyroid hormone–related protein; *TSH,* thyroid-stimulating hormone.

TABLE 2.4 Nuclear Receptor Ligands and Their Receptors

Ligand	Receptor
Classic Hormones	
Thyroid hormone	Thyroid hormone receptor (TR), subtypes α, β
Estrogen	Estrogen receptor (ER), subtypes α, β
Testosterone	Androgen receptor (AR)
Progesterone	Progesterone receptor (PR)
Aldosterone	Mineralocorticoid receptor (MR)
Cortisol	Glucocorticoid receptor (GR)
Vitamins	
$1,25\text{-}(OH)_2$-vitamin D_3	Vitamin D receptor (VDR)
All-*trans*-retinoic acid	Retinoic acid receptor, subtypes α, β, γ
9-*cis*-retinoic acid	Retinoid X receptor (RXR), subtypes α, β, γ
Metabolic Intermediates and Products	
Fatty acids	Peroxisome proliferator-activated receptor (PPAR), subtypes α, δ, γ
Oxysterols	Liver X receptor (LXR), subtypes α, β
Bile acids	Bile acid receptor (BAR, also called FXR)
Heme	Rev-Erb subtypes α, β
Phospholipids	Liver receptor homologue-1 (LRH1) Steroidogenic factor-1 (SF1)
Xenobiotics	Pregnane X receptor (PXR) Constitutive androstane receptor (CAR)

chemicals not naturally encountered in the environment (i.e., xenobiotics). These molecules are referred to as *nuclear receptor ligands*. The nuclear receptors for all of these signaling molecules are structurally related and are collectively referred to as the *nuclear receptor superfamily.* They are all transcription factors, serving to activate or repress specific gene sets that mediate their physiologic effects. The study of these receptors is a rapidly evolving field, and more detailed information can be obtained by visiting the Nuclear Receptor Signaling Atlas website.[113,114]

General Features of Nuclear Receptor Ligands

Unlike polypeptide hormones that function through cell surface receptors, no ligands for nuclear receptors are directly encoded in the genome. All nuclear receptor ligands are small (molecular mass <1000 Da) and lipophilic, enabling them to enter cells by passive diffusion, although in some cases a membrane transport protein is involved. For example, several active and specific thyroid hormone transporters have been identified, including monocarboxylate transporter 8 (MCT8), MCT10, and organic anion transporting polypeptide 1C1 (OATP1C1).[115]

All naturally occurring nuclear receptor ligands are derived from dietary, environmental, or metabolic precursors. In this sense, the function of these ligands and their receptors is to translate cues from the external and internal environments into changes in gene expression. Their critical role in maintaining homeostasis in multicellular organisms is highlighted by the fact that nuclear receptors are found in all vertebrates and insects but not in single-cell organisms such as yeast.[116]

Because nuclear receptor ligands are lipophilic, most are readily absorbed from the gastrointestinal tract. This makes nuclear receptors excellent targets for pharmaceutical interventions. In addition to natural ligands, many drugs in clinical use target nuclear receptors, ranging from drugs used to treat specific hormone deficiencies to those used to treat common multigenic conditions such as inflammation, cancer, and type 2 diabetes.

Subclasses of Nuclear Receptor Ligands

One classification of nuclear receptor ligands is outlined in Table 2.4 and is described in the following paragraphs.

Classic Hormones

The classic hormones that use nuclear receptors for signaling are thyroid hormone and steroids. Endogenous steroid hormones include cortisol, aldosterone, estradiol, progesterone, and testosterone. In some cases (e.g., thyroid hormone receptor α and β genes [*THRA* and *THRB*], estrogen receptor α and β genes [*ESR1* and *ESR2*]), more than one gene exists for a given type of hormone receptor. Each receptor gene may in turn encode additional receptors for the same hormone by alternative promoter usage or by alternative splicing (e.g., *THRB1* and *THRB2*).

Some receptors mediate the signals of multiple hormones. For example, the mineralocorticoid receptor, also known as the

aldosterone receptor, has equal affinity for aldosterone and cortisol and probably functions as a glucocorticoid receptor in some tissues, such as the brain.[117] Likewise, the androgen receptor binds and responds to both testosterone and dihydrotestosterone (DHT).[118]

Vitamins

Vitamins are essential constituents of a healthful diet. Two fat-soluble vitamins, A and D, are precursors of important signaling molecules that function as ligands for nuclear receptors.

Precursors of vitamin D are synthesized and stored in skin and activated by ultraviolet light; vitamin D can also be derived from dietary sources. Vitamin D is then converted in the liver to 25(OH)D (25-hydroxyvitamin D, calcidiol), which is subsequently converted by the kidney to $1,25(OH)_2D_3$ (1,25-dihydroxyvitamin D_3, calcitriol), the most potent natural ligand of the vitamin D receptor (VDR).[119] The 1-hydroxylation of calcidiol is tightly regulated, and calcitriol acts as a circulating hormone, arising in the kidney and circulating through the bloodstream to act on target tissues such as intestine and bone.

Vitamin A is stored in the liver and is activated by metabolism to all-*trans*-retinoic acid, which is a high-affinity ligand for retinoic acid receptors (RARs).[120] Retinoic acid functions as a signaling molecule in both a paracrine and an endocrine manner. Retinoic acid is also converted to its 9-*cis*-isomer, which is a ligand for another nuclear receptor, the retinoid X receptor (RXR).[121] These retinoids and their receptors are essential for normal development of multiple organs and tissues, and they have pharmaceutical utility for conditions ranging from skin diseases to leukemia.[122]

Metabolic Intermediates and Products

Certain nuclear receptors respond to naturally occurring endogenous metabolic products. The peroxisome proliferator-activated receptors (PPARs) constitute the best-defined subfamily of metabolite-sensing nuclear receptors.[123] All three PPAR subtypes are activated by polyunsaturated fatty acids, and although specific lipid species may act as selective PPAR ligands, the PPARs may also function as integrators of the concentration of a number of fatty acids.[124]

The natural ligand for PPARα has not been clearly identified, but may be a fatty acid derived from lipolysis.[125,126] The fibrate class of lipid-lowering pharmaceuticals are potent ligands for PPARα, and the very name of this receptor is derived from its ability to induce the proliferation of peroxisomes in the liver, organelles that break down very long-chain fatty acids through β-oxidation.[127] Indeed, stimulation of fatty acid oxidation is an important physiologic role of PPARα.

The other PPARs (δ and γ) are structurally related to PPARα but do not induce proliferation of peroxisomes when activated by their respective ligands. PPARδ, also known as PPARβ, is ubiquitous, and its ligands—other than fatty acids—are not well characterized. Activation of PPARδ increases oxidative metabolism in fat and skeletal muscle.[128] PPARγ is expressed primarily in adipocytes and is necessary for differentiation along the adipocyte lineage.[129] PPARγ is also expressed in other cell types, including colonic epithelial cells, macrophages, and vascular endothelial cells, where it may play physiologic and pathologic roles. The natural ligand for PPARγ is not known, but PPARγ is a major tissue target of thiazolidinedione (TZD) antidiabetic drugs that improve insulin sensitivity.[130,131] These pharmaceutical agents bind to PPARγ with nanomolar affinities. Non-TZD PPARγ ligands are also insulin sensitizers, further implicating PPARγ in this physiologic role.

Another metabolite-responsive nuclear receptor, the liver X receptor (LXR), is activated by oxysterol intermediates in cholesterol biosynthesis. Mice lacking LXRα have a dramatically impaired ability to metabolize cholesterol.[132] A related receptor known as farnesyl X receptor (FXR) binds and is activated by bile acids, and it plays a role in the regulation of bile synthesis and circulation in normal conditions and in disease states.[132]

Endobiotics and Xenobiotics

Other nuclear receptors appear to function as integrators of exogenous environmental signals, including natural endobiotics (medicinal agents and toxins found in plants) and xenobiotics (compounds that are not naturally occurring). In these cases, the role of the activated nuclear receptor is to induce cytochrome P450 enzymes that facilitate detoxification of potentially dangerous compounds in the liver. Receptors in this class include sterol and xenobiotic receptor (SXR), also known as *pregnane X receptor* (PXR), and constitutive androstane receptor (CAR).[133] Unlike other nuclear receptors that have high affinity for specific ligands, xenobiotic receptors have low affinity for a large number of ligands, reflecting their function in defense against a varied and challenging environment. Although these xenobiotic compounds are not hormones in the classic sense, the function of these nuclear receptors is consistent with the general theme of helping the organism to cope with environmental challenges.

Orphan Receptors

The nuclear receptor superfamily is one of the largest families of transcription factors, with 48 members in humans. The hormones and vitamins just described account for the functions of only a fraction of the nuclear receptors. The remaining receptors have been designated as *orphan receptors* because their putative ligands are not known.[134]

From analyses of mice and humans with mutations in various orphan receptors, it is clear that many of them are required for life or for the development of specific organs, ranging from brain nuclei to endocrine glands. Some orphan receptors appear to be active in the absence of any ligand (i.e., constitutively active) and may not respond to a natural ligand. Nevertheless, all of the receptors known to respond to metabolites and environmental compounds were originally discovered as orphans. Therefore future research will likely find that additional orphan receptors function as receptors for physiologic, pharmacologic, or environmental ligands. For example, the nuclear receptor NR1D1 (also known as Rev-Erbα), which is a regulator of circadian rhythms,[17] has been shown to be a receptor for heme,[135,136] although the physiologic significance of this remains to be determined.

Variant Receptors

The C-terminal domain of the nuclear receptors is responsible for hormone binding. In a few nuclear receptors, including THRA and the glucocorticoid receptor, alternative splicing produces variant receptors with unique C-termini that do not bind ligands.[137,138] These variant receptors are normally expressed, but their biologic relevance is uncertain. They may modulate the action of the classic receptor to which they are related by inhibiting its function.

Other normally occurring variant nuclear receptors lack a classic DBD (discussed later). These types include NR0B1 (also known as DAX1), which is mutated in human disease,[139] and PTPN6 (also known as SHP1).[140] Their ligands have not been identified, and it is likely that NR0B1 and PTPN6 bind to and repress the actions of other receptors.

TABLE 2.5 Mechanisms Regulating Ligand Levels

Precursor availability
Synthesis
Secretion
Activation (prohormone → active hormone)
Transport
Deactivation (active hormone → inactive hormone)
Elimination (hepatic, renal clearance)

Rare, naturally occurring mutations of hormone receptors can cause hormone resistance in affected patients. Inheritance of the hormone resistance phenotype is dominant if the mutant receptor inhibits the action of the normal receptor, as occurs with resistance to thyroid hormone or PPARγ ligands.[141] Inheritance is recessive if the mutation results in a complete loss of receptor function, as with the syndrome of hereditary calcitriol-resistant rickets, which is caused by mutations in the VDR.[142] Inheritance can also be X-linked, as with the mutated androgen receptor in androgen insensitivity syndromes.[143]

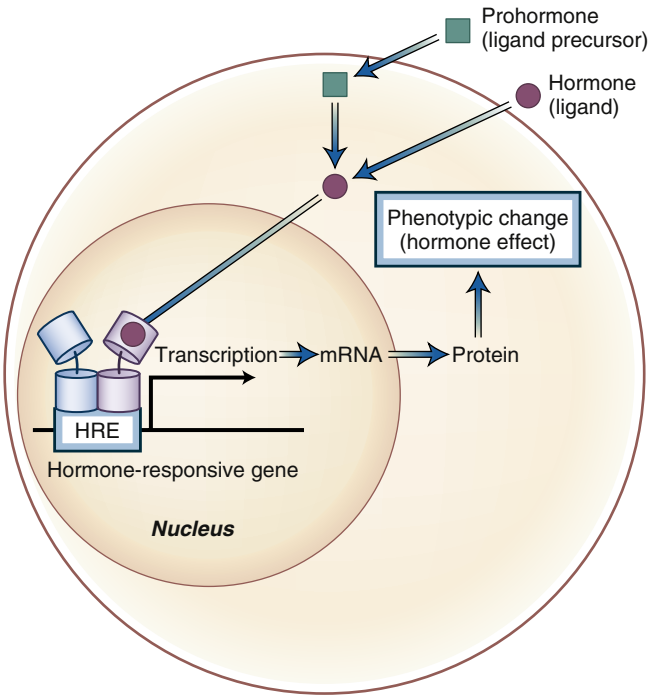
Regulation of Ligand Levels

Ligand levels can be regulated in several ways (Table 2.5). A dietary precursor may not be available in required amounts (e.g., hypothyroidism due to iodine deficiency). Pituitary hormones (e.g., TSH) regulate the synthesis and secretion of classic thyroid and steroid hormones. If the glands that synthesize these hormones fail, hormone deficiency can occur.

Many nuclear receptor ligands are enzymatically converted from inactive prohormones to biologically active hormones; examples include the 5′ deiodination of thyroxine (T_4) to triiodothyronine (T_3) (see Chapter 11). This can occur in the target cell itself or within other tissues that subsequently release T_3 to the circulation for action elsewhere in the body. In other cases, one hormone is a precursor for another, as illustrated by the aromatization of testosterone to estradiol. Biotransformation may occur in a specific tissue that is not the main target of the hormone, as with renal 1-hydroxylation of vitamin D (see Chapter 29), or it may occur primarily in target tissues (e.g., 5α-reduction of testosterone to DHT; see Chapter 19). Deficiency or pharmacologic inhibition of the enzymes responsible for these reactions can reduce hormone levels.

Transport into the target cell can also be a regulated process. T_3 and T_4, for example, do not penetrate the hydrophobic membrane by themselves; they require a transporter such as MCT8 or OATP1. Mutations in MCT8, for example, lead to neurologic issues, including severe intellectual disability and movement disorders with elevated serum T_3.[144] In this condition, it seems likely that the pathology is secondary to the inability of T_3 to enter neurons. Steroid hormones, by contrast, are believed to traverse the membrane by passive diffusion, although it remains possible that undiscovered binding proteins play a role.

Nuclear receptor ligands can be inactivated by hepatic or renal clearance or by more specific enzymatic processes. Mutations in genes encoding inactivating enzymes, or pharmacologic agents that inhibit these enzymes, can result in symptoms of hormone excess such as renal deactivation of cortisol by 11β-hydroxysteroid dehydrogenase (11βHSD). Because cortisol can activate the mineralocorticoid receptor, insufficient 11βHSD activity due to licorice ingestion, gene mutation, or extremely high cortisol levels causes syndromes of apparent mineralocorticoid excess.[145]



• **Fig. 2.15** Mechanism of signal transduction by hormones and other ligands that act through nuclear receptors. *HRE,* hormone response element; *mRNA,* messenger ribonucleic acid.

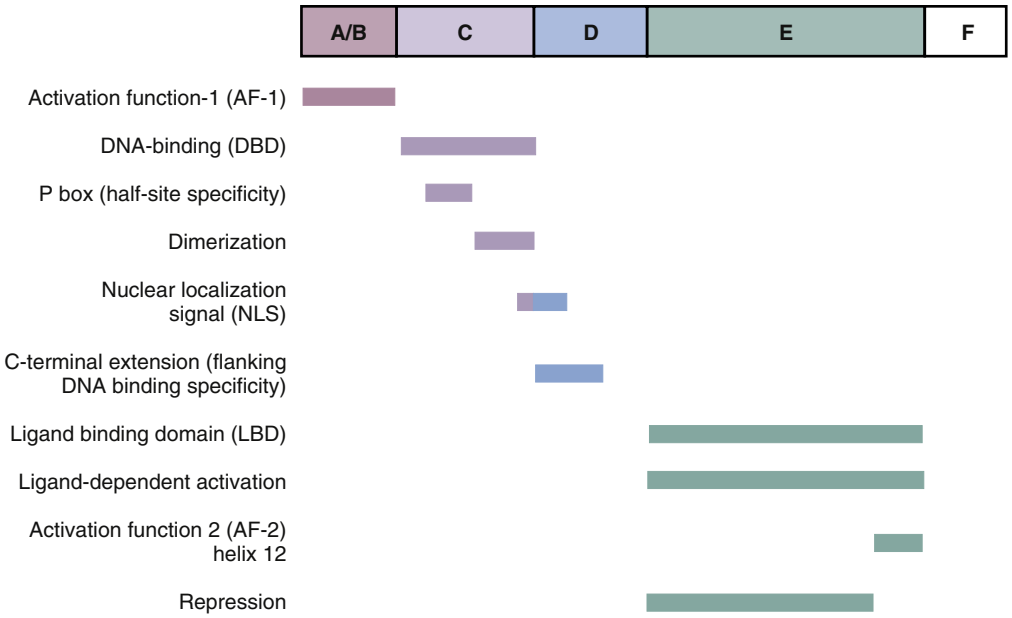
Nuclear Receptor Signaling Mechanisms

Nuclear receptors are multifunctional proteins that transduce the signals of their cognate ligands. General features of nuclear receptor signaling are illustrated in Fig. 2.15.

For hormone action, the ligand and the nuclear receptor must both get into the nucleus. The nuclear receptor also must bind its ligand with high affinity. Because a major function of the receptor is to selectively regulate target gene transcription, it must recognize and bind to promoter and enhancer elements in appropriate target genes. One discriminatory mechanism is dimerization of a receptor with a second copy of itself or with another nuclear receptor. The DNA-bound receptor must work in the context of chromatin to signal the basal transcription machinery to increase or decrease transcription of the target gene. In the regulation of signaling by nuclear receptors, some basic mechanisms are used by many or all members of the nuclear receptor superfamily, whereas other mechanisms impart the specificity that is crucial to the vastly different biologic effects of the many hormones and ligands that use these related receptors.

Domain Structure of Nuclear Receptors

Nuclear receptors are proteins with molecular masses between 50,000 and 100,000 Da. They share a common series of domains, referred to as domains A through F (Fig. 2.16). This linear depiction is useful for describing and comparing receptors, but it does not capture the roles of protein folding and tertiary structure in mediating various receptor functions. The structures of individual domains have now been solved for many receptors, as has the full-length structure of a more limited number of nuclear receptors.



• **Fig. 2.16** Domain structures of nuclear receptors.

Nuclear Localization

The nuclear receptors, like all cellular proteins, are synthesized on ribosomes that reside outside the nucleus. Import of the nuclear receptors into the nucleus requires the nuclear localization signal (NLS), which is located near the border of the C and D domains (see Fig. 2.16). As a result of their NLSs, most of the nuclear receptors reside in the nucleus, with or without their ligands. A major exception is the glucocorticoid receptor; in the absence of hormone, it is tethered in the cytoplasm to a complex of chaperone molecules, including heat shock proteins (HSPs). Hormone binding to the glucocorticoid receptor induces a conformational change that results in dissociation of the chaperone complex, allowing the hormone-activated glucocorticoid receptor to translocate to the nucleus by means of its NLS.

Hormone Binding

High-affinity binding of a lipophilic ligand is mediated by the C-terminal ligand-binding domain (LBD), domains D and E in Fig. 2.16. This region of the receptor has many other functions, including induction of dimerization and transcriptional regulation (see later discussions).

The structure of the LBD has been solved for a number of receptors. All share a similar overall structure consisting of 12 α-helical segments in a highly folded tertiary structure (Fig. 2.17A). The ligand binds within a hydrophobic pocket composed of amino acids in helices 3, 4, and 5 (H3, H4, and H5, respectively). The major structural change induced by ligand binding is an internal folding of the most C-terminal helix (H12), which forms a cap on the ligand-binding pocket (see Fig. 2.17B). Although the overall mechanism of ligand binding is similar for all receptors, the molecular details are essential for determining ligand specificity.[146,147] Ligand binding is the most critical determinant of receptor specificity.

Target Gene Recognition by Receptors

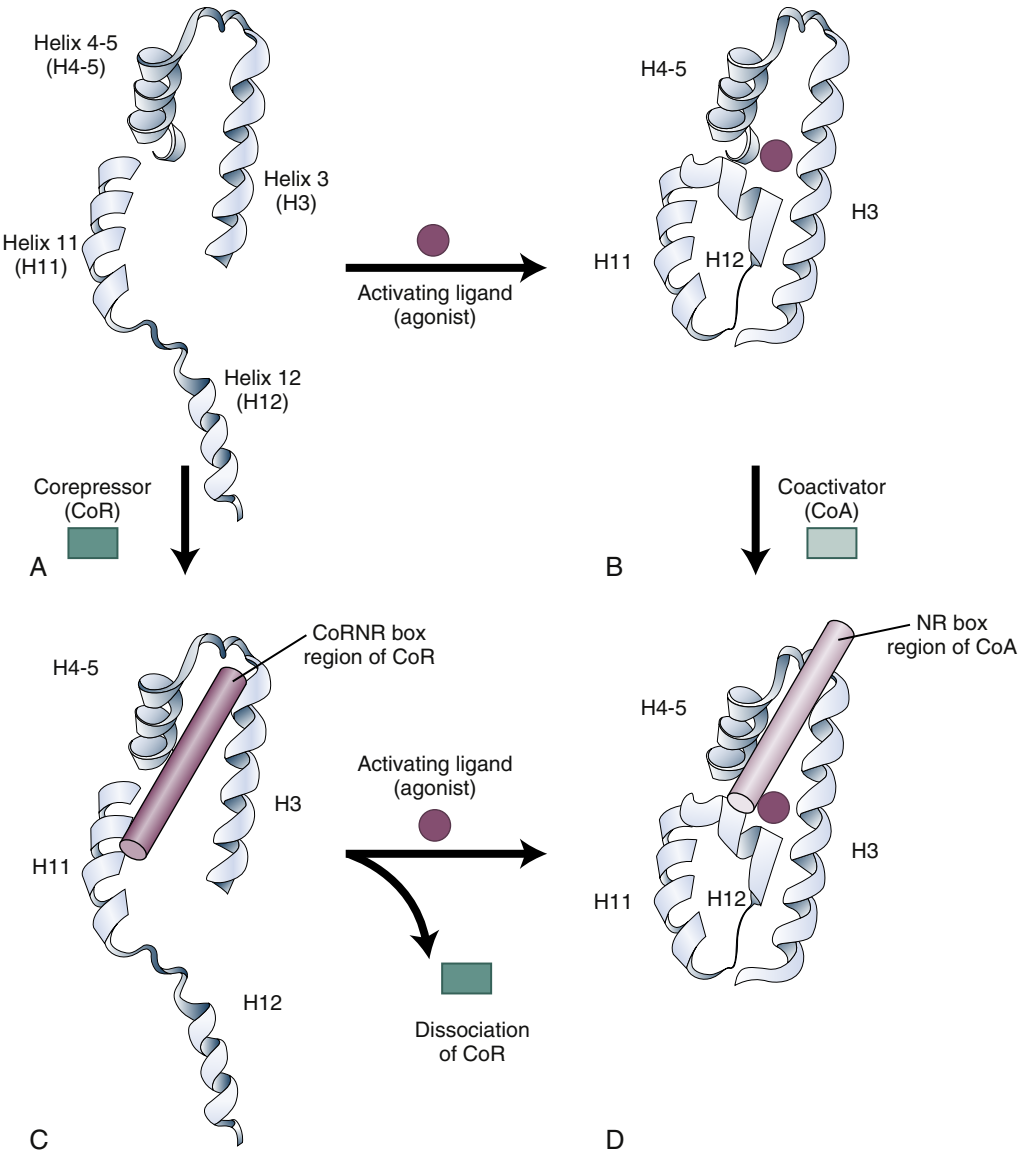
Another crucial specificity factor for nuclear receptors is their ability to recognize and bind to the subset of genes that is to be regulated by their cognate ligand. Target genes contain specific DNA sequences that are called *hormone response elements* (HREs). Binding to the HRE is mediated by the central C domain of the nuclear receptors (see Fig. 2.16). This region is typically composed of 66 to 68 amino acids, including two subdomains that are called *zinc fingers* because the structure of each subdomain is maintained by four cysteine residues coordinated with a zinc atom.

The first of these zinc-ordered modules contains basic amino acids that contact DNA; as with the LBD, the overall structure of the DBD is similar for all members of the nuclear receptor superfamily. The specificity of DNA binding is determined by multiple factors (Table 2.6). All steroid hormone receptors, except for the estrogen receptor, bind to the double-stranded DNA sequence AGAACA (Fig. 2.18).

By convention, the double-stranded sequence is described by the sequence of one of its complementary strands, with the bases ordered from the 5′ to the 3′ end. Other nuclear receptors recognize the sequence AGGTCA. The primary determinant of this specificity is a group of amino acid residues in the *P box* of the DBD (see Fig. 2.18). These hexamer DNA sequences are referred to as *half-sites*. The only difference between these hexameric half-sites is the central two base pairs (underlined in Fig. 2.18). For some nuclear receptors, the C-terminal extension of the DBD contributes specificity for extended half-sites containing additional, highly specific DNA sequences 5′ to the hexamer. Another source of specificity for target genes is the spacing and orientation of these half-sites, which in most cases are bound by receptor dimers.

Receptor Dimerization

The nuclear receptor DBD has affinity for the hexameric half-site or for extended half-sites; however, many HREs are composed of repeats of the half-site sequence, and most nuclear receptors bind these HREs as dimers.[148] Steroid receptors, including estrogen receptors, function primarily as homodimers, which preferentially bind to two half-sites oriented toward each other (i.e., inverted repeats [IRs]) with three base pairs in between (IR3) (see Fig. 2.18A). The major dimerization domain in steroid receptors is within the C domain, although the LBD contributes. Ligand



• **Fig. 2.17** Structural basis of nuclear receptor ligand binding and cofactor recruitment. (A and C) Apo-receptor (no ligand bound). (B and D) Ligand-bound receptor. (C and D) Structures showing the positional binding of a corepressor (CoR) (in C) or coactivator (CoA) (in D). *NR,* nuclear receptor.

binding facilitates dimerization and DNA binding of steroid hormone receptors. Most other receptors, including THR, RAR, PPAR, LXR, and VDR, bind to DNA as heterodimers with RXR (see Fig. 2.18B).

Heterodimerization with RXR is mediated by two distinct interactions, one involving LBDs and the other involving DBDs. The receptor LBD mediates the strongest interaction, which occurs even in the absence of DNA. These receptor heterodimers bind to two half-sites arranged as direct repeats (DRs) with a variable number of base pairs in between.

The spacing of the half-sites is a major determinant of target gene specificity; it results from the second receptor-receptor interaction, which involves the DBDs and is highly sensitive to the spacing of the half-sites. For example, VDR/RXR heterodimers bind preferentially to DRs separated by three bases (DR3 sites), TR/RXR binds DR4, and RAR/RXR binds DR5 with highest affinity.[149]

Studies of isolated DBD binding to DNA have shown that these spacing requirements are related to the fact that the RXR binds to the upstream half-site (i.e., farthest from the start of transcription). As a result of the periodicity of the DNA helix, each base pair separating the half-sites leads to a rotation of about 36 degrees of one half-site relative to the other. Subtle differences in the structure of the receptor DBDs make the interactions more or less favorable at different degrees of rotation.[150] Solution of the crystal structures of full-length nuclear receptor heterodimers bound to DNA has demonstrated remarkable diversity in the precise relationship between heterodimeric partners. For example, the PPARγ-RXR heterodimer forms a nonsymmetric complex, allowing the LBD of PPARγ to cooperate with both DBDs to enhance response element binding,[151] whereas the LXR-RXR heterodimer binds symmetrically to its target sequence.[152] Additional structures will be required to better understand the spectrum of RXR heterodimer binding to DNA.

The discovery of nuclear receptor binding sites has been largely empiric, based on the finding of binding sites in small numbers of known target genes. Unbiased analysis of thousands of

TABLE 2.6 Determinants of Target Gene Specificity of Nuclear Receptors

Specificity	Region of Receptor
1. Binding to DNA	DBD (C domain)
2. Binding to specific hexamer	P box in C domain (AGGTCA vs. AGAACA)
3. Binding to sequences 5′ to hexamer	Carboxy-terminal extension of DBD
4. Binding to hexamer repeats	Dimerization domain (C domain for steroid receptors; D, E, and F for others)
5. Recognition of hexamer spacing	RXR heterodimerization domain (nonsteroid receptors, D/E domains)
6. Cell-specific factors	Receptor-independent (cell-specific factors that open chromatin to permit receptor binding based on receptor-intrinsic properties above)

DBD, DNA-binding domain; *RXR*, retinoid X receptor.

nuclear receptor binding locations in living cells using chromatin immunoprecipitation followed by next generation sequencing has confirmed the canonical binding sequences for many nuclear receptors, including those of the estrogen receptor,[153] the androgen receptor,[154,155] the glucocorticoid receptor,[156] and PPARγ-RXR heterodimers.[157, 158] The complete set of cellular binding sites is referred to as the *cistrome*.[159] Although the sequence of the genome is the same in nearly all cells of the body, cistromes are context dependent, owing to cooperation with factors that open chromatin in a cell type or developmentally specific way, allowing the receptors to bind.

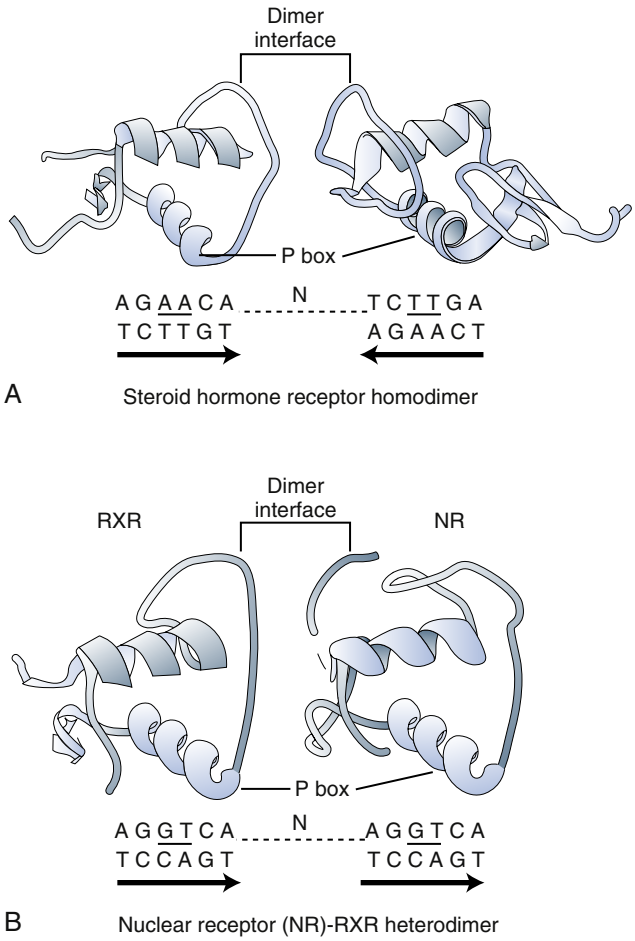
Receptor Regulation of Gene Transcription

Nuclear receptors mediate a variety of effects on gene transcription. The most common modes of regulation are ligand-dependent gene activation, ligand-independent repression of transcription, and ligand-dependent negative regulation of transcription (Table 2.7). Much of this regulation is mediated by interactions of nuclear receptors with proteins called *coregulators,* which include coactivators and corepressors.[160]

Ligand-Dependent Activation

Ligand-dependent activation is the best understood function of nuclear receptors and their ligands. The ligand-bound receptor increases transcription of a target gene to which it is bound. The DBD brings the receptor domains that mediate transcriptional activation to a specific gene. Transcriptional activation itself is mediated primarily by the LBD, which can function as an independent unit even when it is transferred to a DNA-binding protein that is not related to nuclear receptors. The activation function (AF) of the LBD is referred to as *AF2* (see Fig. 2.12).

Gene transcription is mediated by a large complex of factors that ultimately regulate the activity of ribonucleic acid (RNA) polymerase, the enzyme that uses the chromosomal DNA template to direct the synthesis of messenger RNA. Most mammalian genes are transcribed by RNA polymerase II using a large set of cofactor proteins that include basal transcription factors and



• **Fig. 2.18** Structural basis for nuclear receptor (NR) DNA-binding specificity is shown in the ribbon diagrams of receptor DNA-binding domains (DBDs). (A) Steroid hormone receptor binding as a homodimer to the inverted repeat *(arrows)* of the AGAACA half-site. The central base pairs are underlined. (B) RXR-NR heterodimer binding to the direct repeat of AGGTCA. The position of the P box, the region of the DBD that makes direct contact with DNA, is shown. *N,* number of base pairs between the two half-sites; *RXR,* retinoid X receptor.

TABLE 2.7 Regulation of Gene Transcription by Nuclear Receptors

Mode of Regulation	Examples
1. Ligand-dependent gene activation	DNA binding and recruitment of coactivators
2. Ligand-independent gene repression	DNA binding and recruitment of corepressors
3. Ligand-dependent negative regulation of gene expression	DNA binding and recruitment of corepressors, or coactivator redistribution

associated factors collectively referred to as *general transcription factors* (GTFs). Details about GTFs are of fundamental importance and are available elsewhere.

The ligand-bound nuclear receptor communicates stimulatory signals to GTFs on the gene to which it is bound. Ligands specifically recruit a subset of the coregulators to the nuclear receptor LBD. Positively acting coregulators, called *coactivators*,

specifically recognize the ligand-bound conformation of the LBD and bind to the nuclear receptor on DNA only when an activating (agonist) hormone or ligand is bound. A number of coactivator proteins that bind to liganded nuclear receptors have been described (Table 2.8).[161]

The most important determinant of coactivator binding is the position of H12, which changes dramatically when activating ligands bind receptors (see Fig. 2.17B). Along with H3, H4, and H5, H12 forms a hydrophobic cleft that is bound by short polypeptide regions of the coactivator molecules.[162–164] These polypeptides, called *NR boxes* (see Fig. 2.17D), have characteristic sequences of LxxLL, in which L is leucine and xx can be any two amino acids.[165]

Coactivators increase the rate of gene transcription. This is accomplished by enzymatic functions, including histone acetyltransferase (HAT) activity; some coactivators possess intrinsic HAT activity, while others act as scaffolds to recruit HAT proteins.[166] HAT activity is critically important for activation because chromosomal DNA is tightly wrapped in nucleosomal units composed of core histone proteins. Acetylation, as well as some other histone modifications, opens up this chromatin structure.

The best understood class of coactivator proteins is the p160 family, whose name is based on their protein size (approximately 160 kDa). The family contains at least three molecules, each with many names (see Table 2.8).[167] These factors possess HAT activity and recruit other coactivators, such as CREB-binding protein (CBP) and p300, which are also HATs. A third HAT, p300/CBP-associated factor (PCAF), is also recruited by liganded receptors. These HATs, along with a complex of SMARC molecules (SWI/SNF-related, matrix-associated, actin-dependent regulators of chromatin) that direct ATP-dependent DNA unwinding, create a chromatin structure that favors transcription (Fig. 2.19).[168]

Recruitment of multiple HATs may reflect different specificities for core histones and, potentially, for some nonhistone proteins. Some HATs also interact directly with GTFs and further enhance their activities. The mediator complex, which has also been called the thyroid hormone receptor–associated protein (TRAP) complex, and the vitamin D receptor–interacting protein (DRIP) complex link nuclear receptors to GTFs.[169] The HATs and TRAP factors are recruited to the liganded, target gene–bound receptor in an ordered manner that also involves cycling on and off the target receptor with a time scale of minutes.[170] Nuclear receptor interactions with the genome are even more complex, with on-off rates that have been measured to be on the order of milliseconds.[171]

TABLE 2.8 Nuclear Receptor Coregulators

Coactivators

Chromatin remodeling
SWI/SNF complex
Histone acetyltransferase p160 family (Srcs)
p300/CBP
PCAF (p300/CBP-associated factor)
Mediator

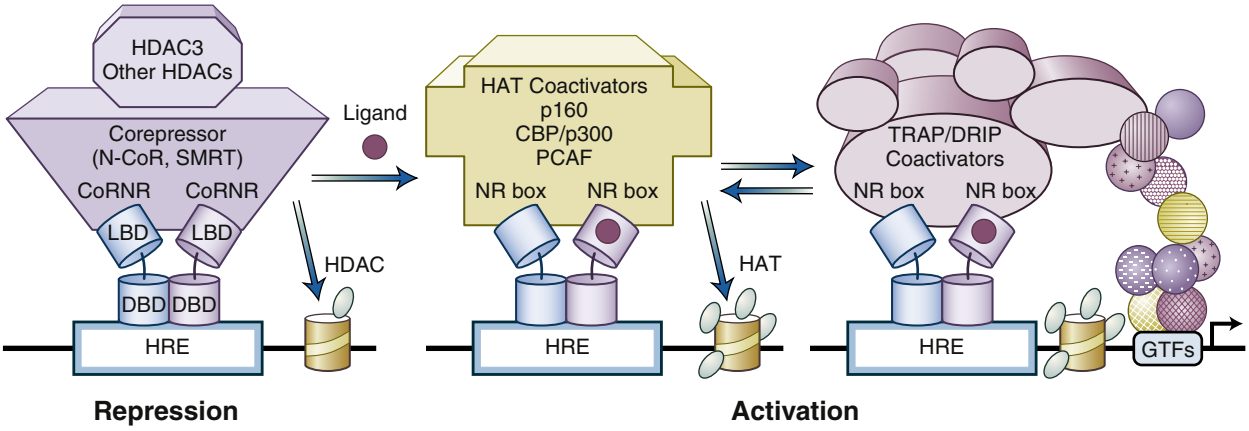
Corepressors

NCoR (nuclear receptor corepressor)
SMRT (silencing mediator for retinoid and thyroid hormone receptors)

CBP, CREB-binding protein (CREB = cAMP-response element–binding protein).

Repression of Gene Expression by Unliganded Receptor

Although DNA binding is ligand dependent for steroid hormone receptors, other nuclear receptors are bound to DNA even in the absence of their cognate ligand. In many cases, the unliganded, DNA-bound receptor actively represses transcription of the target gene. By reducing the expression of the target gene, this repressive function of the receptor amplifies the magnitude of the subsequent activation by hormone or ligand. For instance, if the level of gene transcription in the repressed state is 10% of the basal level in the absence of a receptor, hormone activation to 10-fold above that basal level represents a 100-fold difference of transcription rate between hormone-deficient (repressed) genes and hormone-activated genes (Fig. 2.20).[172] This phenomenon helps to explain why loss of hormone production can result in a much more profound phenotype than loss of the receptor. For example, hypothyroidism due to thyroid gland dysfunction or ablation or iodine



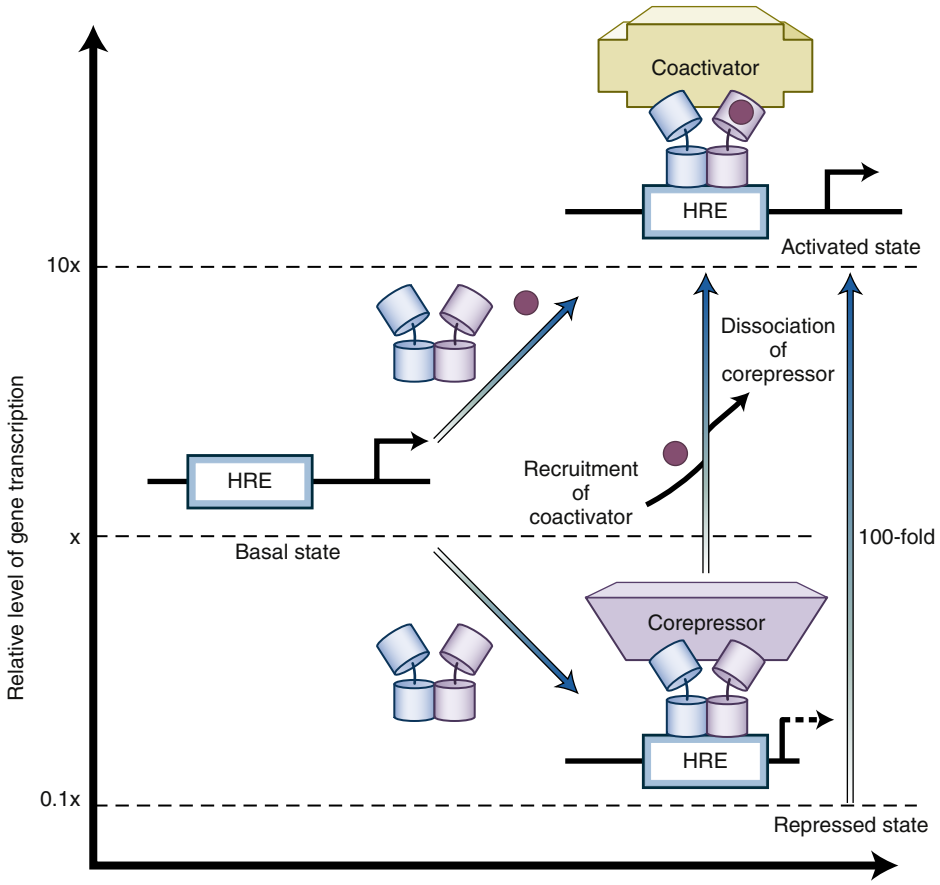
• **Fig. 2.19** Coactivators and corepressors in transcriptional regulation by nuclear receptors. *CBP,* CREB-binding protein; *CoRNR,* coreceptor nuclear receptor box; *DBD,* DNA-binding domain; *DRIP,* vitamin D receptor–interacting protein; *GTFs,* general transcription factors; *HAT,* histone acetyltransferase; *HDAC,* histone deacetylase; *HRE,* hormone response element; *LBD,* ligand-binding domain; *N-CoR,* nuclear receptor corepressor; *NR,* nuclear receptor; *PCAF,* CBP/p300-associated factor; *SMRT,* silencing mediator of retinoid and thyroid receptors; *TRAP,* thyroid hormone receptor–associated protein.

deficiency leads to severe consequences, up to and including cretinism, coma, and death. This is true in mice as well as people. On the other hand, mice lacking all thyroid hormone receptors are relatively mildly affected, with only moderate growth and fertility issues.[173,174] This discrepancy can be attributed to the fact that an unliganded receptor in the hormone deficient state exerts unchecked repressive activity, which has more severe consequences than loss of both repressive and activating functions of THR.

In many ways, the molecular mechanism of repression is the mirror image of ligand-dependent activation. The unliganded nuclear receptor recruits negatively acting coregulators, called *corepressors*, to the target gene. The two major corepressors are large (approximately 270 kDa) proteins: nuclear receptor corepressor (NCoR) and silencing mediator for retinoid and thyroid receptors (SMRT, also known as NCoR2).[175] NCoR and SMRT specifically recognize the unliganded conformation of nuclear receptors and use an amphipathic helical sequence similar to the NR box of coactivators to bind to a hydrophobic pocket in the receptor.

For corepressors, the peptide responsible for receptor binding is called the *CoRNR box* (see Fig. 2.17C), and it contains the sequence (I or L)xx(I or V)I, in which I is isoleucine, L is leucine, V is valine, and xx represents any two amino acids.[176] The receptor uses helices 3 to 5 to form the hydrophobic pocket, as in coactivator binding, but H12 does not promote and even hinders corepressor binding. This highlights the role of the ligand-dependent change in the position of H12 as the switch that determines repression versus activation by nuclear receptors (see Fig. 2.19).[177]

The transcriptional functions of NCoR and SMRT are the opposite of those of the coactivators. The corepressors themselves do not possess enzyme activity, but they recruit histone deacetylases (HDACs) to the target gene, thereby reversing the effects of histone acetylation described earlier and leading to a compact, repressed state of chromatin. Although the mammalian genome contains multiple HDACs, several of which may play a role in nuclear receptor function, the main one involved in repression is HDAC3, whose enzyme activity depends on interaction with NCoR or SMRT.[178] The ability of NCoR to bind and activate HDAC3 is required for normal metabolic and circadian physiology.[179] The corepressors interact directly with GTFs to inhibit their transcriptional activities, and they also exist in large, multiprotein complexes whose range of functions is not fully understood. Biology is complex, however, and recent studies suggest that in brown adipose tissue, HDAC3 foregoes its corepressor role and acts as a coactivator in concert with the nuclear receptor ERRα. In this case, the effect of HDAC3 is mediated at least in part by deacetylation of the key coactivator protein PGC-1, rather than a histone.[180]



• **Fig. 2.20** Repression and activation functions augment the dynamic range of transcriptional regulation by nuclear receptors. The magnitudes of activation and repression were arbitrarily set at 10-fold for this theoretical example. In cells, these magnitudes vary as a function of coactivator and corepressor concentration and in a target gene–specific manner. *HRE,* hormone response element.

Ligand-Dependent Negative Regulation of Gene Expression: Transrepression

The ligand-dependent switch between repressed and activated receptor conformations explains how nuclear receptor ligands activate gene expression. However, many important gene targets of such hormones are turned off in the presence of the ligand. This is referred to as *ligand-dependent negative regulation of transcription,* or *transrepression,* to distinguish it from the repression of basal transcription by unliganded receptors.

The mechanism of negative regulation is less well understood than ligand-dependent activation, and there may in fact be several mechanisms. One mechanism involves nuclear receptor binding to DNA-binding sites that reverse the paradigm of ligand-dependent activation (i.e., negative response elements). For example, when the unliganded thyroid receptor binds to the negative response element of the genes for the β-subunit of TSH or TSH-releasing hormone, transcription is activated,[181] although more recent studies suggest that the role and recruitment of coregulators in this process are complex.[182] In other cases, negative regulation may result from nuclear receptors that bind to, and inhibit, other transcription factors without binding DNA. This interaction leads to redistribution of coactivators from the other transcription factors that positively regulate the gene. Recent evidence supports this model, whereby inhibition of the activity of the positively acting factors results in the observed negative regulation.[183,184] Nuclear receptors can also lead to inhibition of gene expression indirectly by activating a gene that encodes a transcriptional repressor.

Roles of Other Nuclear Receptor Domains

The N-terminal A/B domain of the nuclear receptors is the most variable region among all members of the superfamily in terms of length and amino acid sequence. Subtypes of the same receptor often have completely different A/B domains, and the function of this domain is the least defined. It is not required for unliganded repression or for ligand-dependent activation. In many receptors, the A/B domain contains positive transcriptional activity, often referred to as activation function 1 (AF1) (see Fig. 2.16). Its activity is ligand independent, but it probably interacts with coactivators and may influence the magnitude of activation by agonists or partial agonists. This AF is tissue specific and tends to be more important for steroid hormone receptors, whose A/B domains are notably longer than those of other members of the superfamily.[185] The F domain of the nuclear receptors is hypervariable in length and sequence, and its function is not known.

Cross-Talk With Other Signaling Pathways

Hormones and cytokines that signal through cell surface receptors also regulate gene transcription, often by activating protein kinases that phosphorylate transcription factors such as CREB. Such signals can also lead to phosphorylation of nuclear receptors. Multiple signal-dependent kinases can phosphorylate nuclear receptors, leading to conformational changes that regulate function.[186] Phosphorylation can lead to changes in DNA binding, ligand binding, or coactivator binding, depending upon the specific kinase, receptor, and domain of the receptor that is phosphorylated. The properties of coactivators and corepressors are also regulated by phosphorylation.[187]

TABLE 2.9 Factors Modulating Receptor Activity in Different Tissues

- Concentration of receptor
- Cell specificity
- Variation within a given cell type
- Post-translational modification of receptor (e.g., phosphorylation)
- Regulation of intracellular ligand levels (see Table 2.5)
- Tissue-specific factors that open chromatin
- Function of ligand
  - Agonist
  - Partial agonist
  - Antagonist
- Concentration and types of coregulators
  - Coactivators
  - Corepressors

Receptor Antagonists

Certain ligands function as receptor antagonists by competing with agonists for the ligand-binding site. In the case of steroid hormone receptors, the position of H12 in the antagonist-bound receptor is not identical to that in the unliganded receptor or in the agonist-bound receptor. In antagonist-bound steroid receptors, H12, which has a sequence that resembles the NR box, binds to the coactivator-binding pocket of the receptor and thereby prevents coactivator binding.[188] This antagonist-bound conformation of the receptor also favors corepressor binding to steroid hormone receptors.

Tissue Selectivity of Ligands Interacting With Nuclear Receptors

Many endogenous hormones that act through nuclear receptors do so in a tissue-specific manner. The most obvious mechanism is differential expression of the receptors, both in space (e.g., cell type specificity)[189] and time (e.g., circadian variation).[190] Ligand levels may be regulated intracellularly (see earlier discussion and Table 2.5), and post-translational modification (e.g., phosphorylation) influences cell-specific receptor function. Although nuclear receptors bind at thousands of sites on genomic DNA, the specific binding sites are regulated in a cell type–specific manner. For example, the estrogen receptor binds to overlapping but clearly different sets of genomic sites in the uterus and in the breast, probably because of the differential actions of so-called pioneer transcription factors, which open tightly compacted chromatin in a tissue-specific manner and enable nuclear receptor and other transcription factors to bind.[191]

Some ligands function as antagonists in certain tissues but as full or partial agonists in others. These selective receptor modulators include compounds such as tamoxifen, a selective estrogen receptor modulator (SERM). SERMs are estrogen receptor antagonists with respect to the functions of AF2, including coregulator binding, and they require the AF1 for their agonist activity.[192] This agonism, like AF1 activity, tends to be tissue specific and therefore has great therapeutic utility.[193] Table 2.9 summarizes factors contributing to the tissue specificity of receptor activity.

Nongenomic Actions of Nuclear Receptor Ligands

Some actions of steroids and other nuclear receptor ligands occur within seconds or minutes, far quicker than should be possible

using the transcriptional mechanisms described in the chapter. This suggests that some traditional nuclear receptor ligands may have a discrete set of nongenomic actions. There is now reasonable evidence that thyroid hormone, estrogen, androgen, and possibly other ligands can bind and activate receptors outside of the nucleus. In most cases, these extranuclear receptors are splice variants of the same genes that encode the traditional nuclear receptor, often with loss of the DNA binding domain and NLS. Binding of ligands to these receptors causes activation of many classic signaling pathways, such as Src, ERK, and Akt pathways.[194] In some cases there may be binding of ligands to receptors that are completely unrelated to the nuclear receptors, though this is less clear. The nongenomic and genomic actions of ligands may act cooperatively (e.g., by causing phosphorylation of the nuclear form of the receptor).[195]

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3

Genetics of Endocrinology

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CHAPTER OUTLINE

KEY POINTS

- The genetic basis of each heritable endocrine disease/trait is quantified by its genetic architecture (1) the number of genetic variants/genes, (2) their frequency in the population, and (3) their respective contributions to disease risk/phenotypic variation.
- Mendelian endocrine disorders are caused by variants found rarely in the population, usually from a relatively small number of genes, and each variant has a large individual effect on disease risk so that in any one individual, most of the disease risk is explained by variants in a single gene. Mendelian variants can be highly penetrant, but this is not always the case.
- Common endocrine diseases/traits such as stature, type 2 diabetes, and serum lipids are polygenic—the result of combined, simultaneous effects of many variants in many genes, often found frequently in the general population, and with each variant contributing a small individual effect so that the phenotype in any one individual results from variants in many different genes.
- Genetic information enables endocrinologists to personalize therapy for patients.
- Comprehensive genetic testing (i.e., genome sequencing) can be standardized and automated, but drawing valid and clinically useful conclusions requires integration with patient history, physical examination, and other laboratory examinations.
- Genetic information is most likely to be of direct clinical use in patients with suspected mendelian syndromes.

The Role of Genetics in Endocrinology

The sequencing of the human genome has ushered in an era of genomic medicine. The catalog of protein-coding genes in humans is essentially complete, and the number of associations between genes and specific diseases is growing rapidly. Moreover, it is now feasible to identify nearly every genetic variant in an individual's protein-coding genes (whole-exome sequencing [WES]) or in his or her entire genome (whole-genome sequencing) due to revolutionary advances in sequencing technologies (collectively referred to as next generation sequencing [NGS]). The ability to interpret this variation is less advanced but is improving, as databases of variants and their clinical associations increase in both size and accuracy. Founded in 1982, the National Center for Biotechnology Information's GenBank now contains more than 200 million sequences and includes worldwide contributions from the DNA Data Bank of Japan and the European Nucleotide Archive.[1] Ongoing projects include the Genome Aggregation Database, Exome Aggregation Consortium (ExAC), United Kingdom 100,000 Genomes Project, and the National Center for Biotechnology Information's Single Nucleotide Polymorphism Database. Available sequencing data are growing exponentially, and the volume of information places increasing the demand for methods to distinguish pathologic variants from benign ones.

With the expanding reach of *precision medicine*—individualized diagnosis and therapy informed by genetics—we anticipate that increasing numbers of patients will have clinical indications for exome or genome sequencing, and others will come to clinical encounters with their sequences already in hand. Clinicians will be asked to interpret these genetic data to shed light on an individual's risk of developing disease, on diagnosis and prognosis for those already affected, on implications to family members, and on individualization of therapy. As such, it is critical that clinicians are able to draw valid and clinically useful connections between DNA sequence variation and human traits and diseases. Perhaps even more important, it is critical that clinicians understand the limits of such information.

In this chapter, we present a guide to help clinicians appreciate and critically interpret the relationship between a DNA sequence (genotype) and an individual's clinical presentation (phenotype). We first discuss principles of genetics to provide the framework for understanding and interpreting DNA variation in patients. We then focus on endocrine disorders, providing an overview of the genetics of endocrine diseases, with illustrative examples from both mendelian disorders (caused by mutations in single genes) and polygenic disorders (in which variation in many genes influences disease risk). Finally, we examine scenarios for clinical uses of genetic information in endocrinology and provide recommendations.

Most diseases, including endocrine disorders, are heritable, meaning that genetic variation contributes to disease risk in a population. These diseases range across the spectrum of rare, single-gene disorders, such as multiple endocrine neoplasia (see Chapter 42), Carney complex (see Chapter 15), and congenital adrenal hyperplasia (CAH) (see Chapter 24) to polygenic diseases, such as type 2 diabetes (see Chapter 34 and 35), Graves disease (see Chapter 12), and osteoporosis (see Chapter 29). The detailed discussions of the genetics of these and other disorders can be found throughout this textbook; this chapter provides illustrative examples that illuminate key concepts and refers the reader to the appropriate chapters for additional detail.

Principles of Genetics

A Brief Historical Perspective

In Western conception, the relationship between inheritance and physical characteristics (disease and nonpathologic) has been recognized since the time of Aristotle (323 BC). But it was not until 1865 that the Austrian abbot Gregor Mendel, after decades of careful experimentation in pea plants, posited and provided evidence for the modern genetic concept of *genes* (as coined by the botanist Wilhelm Johannsen in 1909).[2] Mendel deduced certain rules governing the passage of genotype (the collective versions of multiple genes in an individual) from parent to offspring, enabling the prediction of the resulting physical characteristics (phenotype) of the offspring. It was recognized in the early 20th century that certain human phenotypes, including diseases, were inherited according to the same rules that Mendel had described; these diseases are called *mendelian*.

Over the course of the next century, numerous breakthroughs established that genes were composed of DNA, physically connected on chromosomes, and encoded proteins. The first description of the molecular basis of a mendelian disease was made for sickle cell anemia, which involved a mutation in a single gene. In the 1970s, the ability to sequence DNA revealed natural and heritable sequence variation (genetic polymorphisms) in any given gene among different individuals. It was appreciated that the molecular basis of variation in the genotypes of individuals resulted from DNA sequence polymorphisms, which in turn effected alterations in phenotype. By tracing the transmission of these polymorphisms in families, it became possible to identify genes causing mendelian human disorders (those caused by altered function in a single gene and that consequently show distinctive patterns of inheritance in families).[3]

However, most human diseases and phenotypes are not mendelian. Biometricians had appreciated in the early 1900s that most continuous and commonly varying traits (e.g., height and blood pressure) did not follow mendelian patterns of inheritance. In 1919, R.A. Fisher[4] provided a general framework explaining continually varying traits as the consequence of polygenic inheritance—that is, polygenic phenotypes are a result of combined, small, and additive effects of variation in many genes simultaneously. In this framework, monogenic/mendelian traits were a special case. Despite this recognition, only a few genetic variants were convincingly connected with polygenic diseases/traits over the next 80 years. It would take a series of technological advances, including the sequencing of the human genome (Human Genome Project 1990–2003) and the systematic cataloging of DNA sequence polymorphisms across diverse human populations (International HapMap Project 2002–2005 Phase I), to systematically identify the genetic causes for common polygenic diseases.[5]

Heritability: An Estimate of the Importance of Genetic Factors to Disease Causation

Relatives resemble each other in many ways. Resemblance with respect to traits such as height or to diseases such as multiple endocrine neoplasia type 1 (MEN1) could be explained by shared genotypes passed down through generations, shared environments, and nonlinear interactions between genes and environment. Heritability quantifies, as a proportion, how much of this familial resemblance is due to genetic factors. A trait that has no genetic influence would have a heritability of 0%; a trait that is completely determined by inherited factors would have a heritability of 100%. Most clinically important traits have heritabilities ranging from 20% to 80% (Table 3.1). Appreciating the heritability of a trait is important when interpreting the contribution of genetic risk factors in disease: genetic factors are less influential for traits with low heritability and are likely to have more predictive or explanatory power for traits with high heritability.

In the past, the gold standard for heritability estimation was the comparison of monozygotic and dizygotic twin concordance rates for diseases/traits. Such studies relied on the rationale that an excess of disease correlation between genetically identical individuals (monozygotic twin pairs) compared with those who share only 50% of their genes (dizygotic twin pairs) pointed to the role of genetic factors. However, the validity of comparing twin concordance rates across different families relied on the assumption that the effect of environment was the same for the twin pairs, regardless of whether they were monozygotic or dizygotic twins. More recent methods for heritability estimation can overcome some of these limitations by leveraging subtle fluctuations in genetic similarities between sibling pairs.[6]

Regardless of the methodology employed, it is critical to appreciate that heritability is not a fixed property of a disease/trait. The heritability estimate from any study must be interpreted in the context of the population in which it is being measured, including the historical period, and variability in environmental factors such as socioeconomic status and nutrition. These factors likely explain the wide range of heritability estimates for type 2 diabetes, ranging from 40% in Finland[7] to 80% in Japan.[8] An illustrative example of the importance of history can be drawn by examining type 1 diabetes rates across the Scandinavian region of Karelia. In 1940, this region was divided between Finland and the former Soviet Union with little contact between the two sections over the next 60 years. Finnish Karelians have a sixfold increased rate of type 1 diabetes compared with Russian Karelians.[9] As a result, heritability for type 1 diabetes will be different when estimated in the combined Karelian populations than when estimated in Finnish or Russian Karelians alone. The difference in diabetes rate is likely due to environmental factors, because both Karelian populations recently originated from a common ancestry and therefore likely have similar genetic risk factors for type 1 diabetes.[10]

Human DNA Sequence Variation: Molecular Forms and Biologic Effects

Each human has two versions of his or her genome (one from each parent); each version consists of a sequence of approximately 3 billion DNA bases. When comparing two versions of a human genome, either within the same person or between two different people, about 1/1000 of these bases vary (i.e., 99.9% of them are the same) (Table 3.2). There are many possible ways in which DNA sequences can vary; several specific types of DNA sequence variants are frequently observed (Fig. 3.1).

TABLE 3.1 Heritable Endocrine Traits and Diseases

Common Form	Heritability	Reference[a]	Selected Mendelian Forms
Type 1 diabetes	80%	132	*KCNJ11, ABCC8* (permanent neonatal diabetes)
Type 2 diabetes	40–80%	23, 28, 133	*AGPAT2* (congenital generalized lipodystrophy), *LMNA* (familial partial lipodystrophy 1) *HNF4A, GCK, HNF1A* (MODY1-3)
Obesity	40–70%	134, 135	*MC4R, POMC*
Hypertension	30–70%	136	*MEN1, RET* (MEN2A/B), *VHL, SCNN1A* (Liddle syndrome), *CYP17A1* (17OHD), *HSD11B2* (AME)
Height	80%	24, 72	*GH1, FGFR3* (achondroplasia), *SHOX1* (Ullrich-Turner syndrome), *FBN1* (Marfan syndrome)
Pubertal timing	50–80%	137	*KAL1, KISS1R, FGFR1* (hypogonadotropic hypogonadism)
Hyperthyroidism	80%	138	*TSHR* (familial nonautoimmune hyperthyroidism)
Hypothyroidism	67%	139	*TSHR, SLC5A5, TG, TPO,* and *TSHB* (congenital hypothyroidism)
Osteoporosis	50–85%	140, 141	*COL1A1, COL1A2, IFITM5* (osteogenesis imperfecta)
Serum calcium	40%	142, 143	*CASR* (familial hypocalciuric hypercalcemia), *HRPT2* (hyperparathyroid jaw-tumor syndrome)
Lipids	40–60%	85, 86	*Low-density lipoprotein:* LDLR (familial hypercholesterolemia)
			High-density lipoprotein: CETP
			Triglycerides: APOE (familial dysbetalipoproteinemia)
Kidney stones	56%	144, 145	*CLCN5* (X-linked recessive nephrolithiasis), *NKCC2* (Bartter syndrome)

[a]Numbers in this column indicate references listed at the end of the chapter.

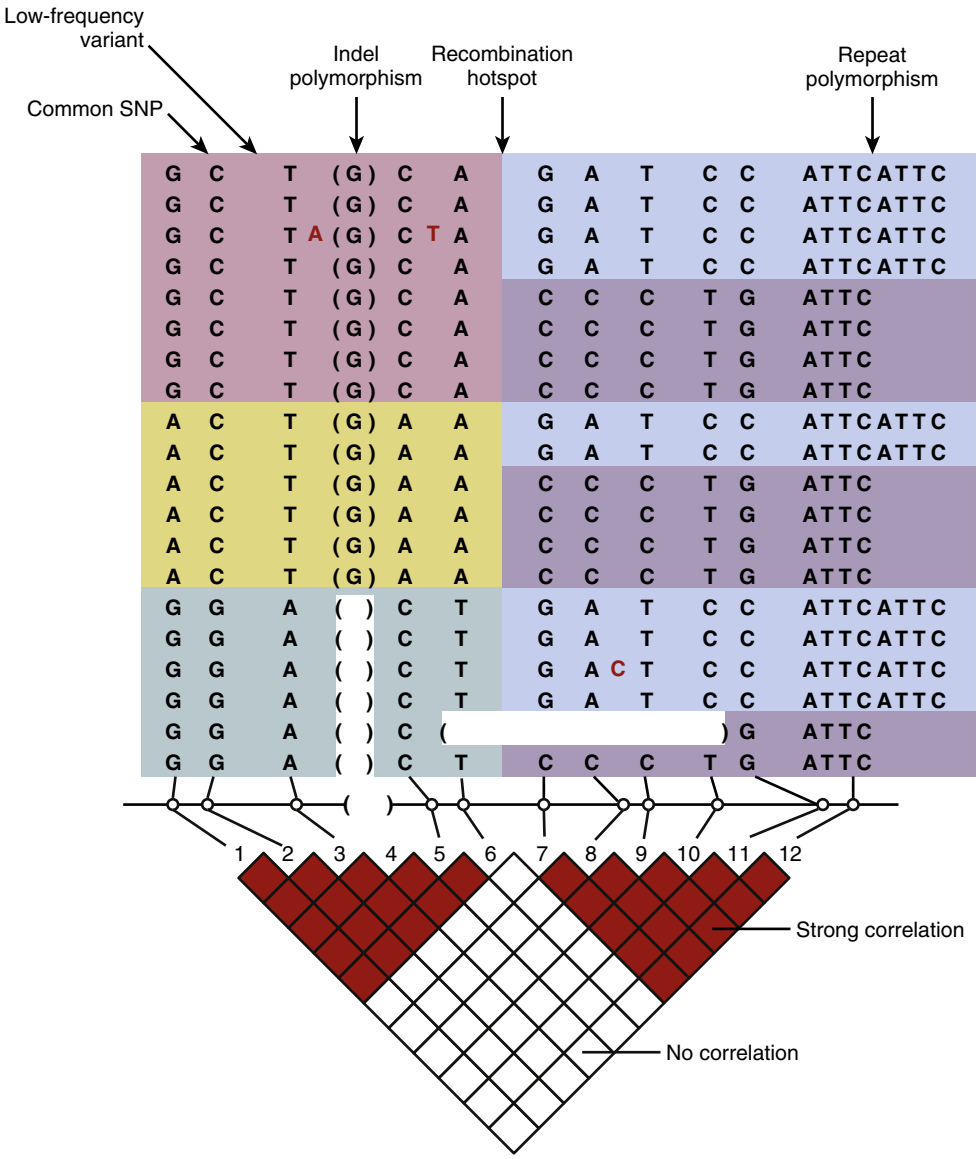
TABLE 3.2 Characteristics of Human Genome Sequence Variation

Characteristic	Frequency
Length of the human genome sequence (base pairs)	3 billion
Number of human genes (estimated)	20,000
Fraction of base pairs that differ between the genome sequence of a human and a chimpanzee	1.3% (1:80)
Fraction of base pairs that vary between the genome sequence of any two humans	0.1% (1:1000)
Fraction of coding region base pairs that vary in a manner that substantially alters the sequence of the encoded protein	0.02% (1:5000)
Number of sequence variants present in each individual as heterozygous sites	3 million
Number of amino acid–altering variants present in each individual as heterozygous sites	12,000
Number of sequence variants in any given human population with frequency >1%	10 million
Number of amino acid polymorphisms present in the human genome with a population frequency >1%	75,000
Fraction of all human heterozygosity attributable to variants with a frequency >1%	98%

Adapted from Altshuler D. The inherited basis of common diseases. In Goldman L, Schafer AI, eds. *Goldman's Cecil Medicine*, 24th ed. Philadelphia, PA: WB Saunders; 2012.

The most frequent form of variation, the single nucleotide polymorphism (SNP), refers to the situation in which a single base in the sequence of one individual is different from the base seen at the same position in the sequence of another individual. SNPs can exert a wide range of biologic effects, depending on where the variant occurs and whether it alters the function of the DNA sequence. Some SNPs occur within the portions of genes that are transcribed into RNA and then translated into proteins (protein-coding regions). Synonymous SNPs occur in the protein-coding portion of DNA but both versions (alleles) of the SNP encode the same amino acid, and so this sort of variation usually does not affect function. SNPs can be missense changes (alteration of a single amino acid in a protein-coding gene), as is the case of the C282Y mutation in the *HFE* gene responsible for autosomal recessive hereditary hemochromatosis (see Chapter 19). Some missense SNPs greatly alter function, whereas others appear to have no consequences. SNPs can also alter splice sites, disrupting the structure of the mRNA that is transcribed from the DNA during gene expression. For example, the most common cause of autosomal dominant isolated growth hormone (GH) deficiency is single-base mutations that inactivate a splice donor site of intron 3 in the *GH1* gene, causing skipping of exon 3 in *GH1* (see Chapter 25). SNPs can also introduce stop codons, leading to premature termination of translation and a truncated protein product. These nonsense variants typically dramatically impair or eliminate the function of the protein.

Changing the protein sequence is not the only way that SNPs (and other types of genetic variations) can alter gene function. Most of the human genome does not code for proteins (see Table 3.2), and most genetic variation occurs in this noncoding portion of the genome. For example, noncoding variants can alter the level, timing, or location of gene expression, without changing the sequence of the encoded protein. Noncoding variants often result in more



• **Fig. 3.1** DNA sequence variation in the human genome. Common and rare genetic variation in 10 individuals, carrying 20 distinct copies of the human genome. The amount of variation shown here is typical for a 5-kb stretch of genome and is centered on a strong recombination hotspot. The 12 common variations include 10 single-nucleotide polymorphisms (*SNP*s), an insertion-deletion polymorphism (indel), and a tetranucleotide repeat polymorphism. The six common polymorphisms on the left side are strongly correlated. Although these six polymorphisms could theoretically occur in 26 possible patterns, only 3 patterns are observed (indicated by pink, orange, and green). These patterns are called *haplotypes*. Similarly, the six common polymorphisms on the right side are strongly correlated and reside on only two haplotypes (indicated by blue and purple). The haplotypes occur because there has not been much genetic recombination between the sites. By contrast, there is little correlation between the two groups of polymorphisms because a hotspot of genetic recombination lies between them. In addition to the common polymorphisms, lower-frequency polymorphisms occur in the human genome. Five rare single-nucleotide polymorphisms are shown, with the variant nucleotide marked in red and the reference nucleotide not shown. In addition, on the second to last chromosome, a larger deletion variant is observed that removes several kilobases of DNA. Such larger deletion or duplication events (i.e., copy number variants) may be common and segregate as other DNA variants. (Redrawn from Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. *Science.* 2008;322[5903]:881-888.)

subtle biologic effects, and the mechanisms are still being uncovered. For example, some SNPs subtly influence type 1 diabetes risk and lie in enhancers (noncoding DNA segments that activate gene transcription at a distance) that appear to affect gene expression only in lymphoid cells.[11]

Insertions and deletions (collectively called *indels*) respectively refer to the addition or removal of one or more bases in the DNA sequence. Indels in protein-coding sequences are called *frameshift mutations*, as long as the number of bases inserted or deleted is not a multiple of three. Because the genetic code is composed of

triplets (every three bases encode one amino acid), a frameshift mutation alters how every subsequent base in the sequence is translated into a protein, resulting in profound molecular and clinical consequences. For example, classic salt-wasting CAH is often caused by frameshift deletions in the *CYP21A2* gene that ablate its function (see Chapter 15 and 24). Repeat polymorphisms (often referred to as copy number variants [CNVs] if the repeats are large) are a special case of indels in which DNA sequences are repeated in tandem, and the number of copies of the repeated sequence varies. For example, the *AR* gene (encoding the androgen receptor [*AR*]) contains a repeat polymorphism in which a CAG codon, encoding glutamine, is repeated 11 to 31 times (see Chapter 24). Structural variation can include both insertions and deletions, as well as rearrangement of large chunks of DNA sequence (translocations and other complex forms of genomic variation). Structural variation causes familial hyperaldosteronism type 1; the adrenocorticotropic hormone (ACTH, corticotropin)-responsive promoter of the *CYP11B1* gene is incorrectly located adjacent to the aldosterone synthase gene (*CYP11B2*), causing aldosterone to be produced by ACTH stimulation (see Chapter 16).

Factors Influencing the Biologic Impact of Genetic Variants in a Particular Gene

As discussed previously, the impact of a genetic variant on gene function will depend on the type of variant and its location with respect to the gene. For example, frameshift deletions in the *CYP21A2* gene eliminate 21-hydroxylase activity, whereas missense variants in *CYP21A2* often retain partial 21-hydroxylase activity (see Chapter 24). However, even a single, specific variant may have different effects in different individuals. The effect of any given genetic variant (genotype) on the phenotype can be modified by variants in other genes (gene-gene interactions) or by environmental factors (gene-environment interactions) or by random chance. It is usually not possible to measure or quantify these factors in any one person, but their combined effect can be quantified on a population level as *penetrance*, the proportion of individuals carrying a genetic variant who exhibit the phenotype. The penetrance of a genetic variant is highly context dependent with respect to phenotypic definition. For example, the hemochromatosis-associated C282Y allele in the *HFE* gene exhibits high penetrance for the biochemical phenotype of high ferritin (>60% of homozygous carriers manifest increased ferritin levels) but only 2% penetrance for the clinical phenotype of liver cirrhosis. Temporal context is also an important consideration, as disease incidence often increases with age. Carriers of mutations causing MEN1 have nearly 100% penetrance for parathyroid adenomas by age 40 but only 20% penetrance at age 20.

A common observation in members of a family carrying the same disease-causing genetic variant is that not all members of the family are equally affected. This range of phenotypic expression resulting from a particular genotype is referred to as variable *expressivity* and, as with penetrance, arises from the range of impacts of specific variants, as well as modifying influences of genetic background (gene-gene interactions), environment (gene-environment interactions), and random chance. For example, the same mutation in the *AR* (encoding an S703G substitution) resulted in a spectrum of clinical androgen insensitivity such that some individuals were raised as 46,XY females and others as males; other mutations in *AR* have different ranges of phenotypic effects (see Chapter 24).

TABLE 3.3 Origins of DNA Sequence Variation in Human Populations: Common Versus Rare Variants

The type of genetic variant (missense, frameshift, noncoding, etc.) provides clues to its possible consequences. In addition, the population frequency of a variant, whether it is common or rare, can also provide information about its likely impact on phenotype. The relative balance between common and rare genetic variation is strongly influenced by evolution and human demographic history. Modern humans likely originated from a small population residing in Africa that had been evolving over millions of years. Within the past 50,000 years, members of this ancestral population migrated "out of Africa," settled the globe, and only recently, over the past 5000 to 10,000 years, multiplied exponentially.[127] As a consequence of this demographic history, most of the 3 million genetic variants an individual inherits from his or her parents are common (typically >1% frequency in the population), can be traced back to the ancient African population, and are shared in many unrelated individuals in the population. Individuals also inherit thousands of genetic variants unique to themselves and their relatives. These rare genetic variants arose more recently from spontaneous mutation in the past 10 millennia, after the migration of many humans out of Africa, and are typically observed infrequently (<0.1% of all chromosomes) in the population.

Mosaicism, whereby cells within a single individual have different genotypes, is another mechanism that leads to variable expressivity. Most mutations known to influence disease are *germline* mutations—inherited from the sperm or egg and present in every cell—but some diseases can be caused by somatic mutations that occur after fertilization and are present in only some cells, leading to mosaicism. In these cases, which tissues or organs carry the mutation will influence the clinical outcome. The most familiar class of disease caused in large part by somatic mutations is neoplasia, including endocrine tumor syndromes such as Conn syndrome and Cushing disease. Another classic example from endocrinology is the McCune-Albright syndrome, in which the same activating mutation in *GNAS1* exhibits variable expressivity because of postzygotic mosaicism. The phenotype of patients with McCune-Albright syndrome depends on which tissues and what fraction of cells carry the *GNAS1* mutation. A minority of affected individuals (24%) display the classic triad of café au lait spots, polyostotic fibrous dysplasia, and gonadotropin-releasing hormone (GnRH)-independent precocious puberty; the majority express two or fewer features of the classic triad (see Chapter 26). The mechanism of variable expressivity likely maps to the zygotic stage in which the mutation arose: a mutation earlier in embryogenesis is present in more tissue lineages. Because mutations in a mosaic individual are not present in every cell, they can be hard to detect in DNA isolated from a blood sample if the cell in which the mutation occurred does not give rise to blood leukocytes. The *GNAS1* mutation responsible for the McCune-Albright syndrome is detected in only 8% to 46% of blood samples from affected individuals but is found in 90% of affected tissue sampled irrespective of clinical presentation (see Chapter 26). Conversely, blood cells can contain somatic variation that is absent in other tissues or the germline.[12]

It is important to remember that the base pair composition of a DNA sequence is not the only molecular determinant of phenotypic expression (Table 3.3). DNA is subject to other forms of modification besides sequence variation (termed *epigenetic variation*), such as cytosine methylation or packaging into nucleosomes

with various biochemically modified histones. Thus, the same molecular form of DNA sequence variation can vary in its cellular and phenotypic effect through epigenetic modifications. Indeed, epigenetic modification is a normal part of development and is the reason different cells have different properties even though they share the identical DNA sequence. A striking example of the effect of epigenetics is imprinting, the expression of a genetic variant in a parent-of-origin specific manner. For paternally imprinted genes, the copy that is inherited from the father is silenced, and only the mother's copy is expressed in the offspring. Imprinting can affect the impact of disease-causing mutations. Inactivating mutations in *SDHD* cause familial paraganglioma type 1. *SDHD* is maternally imprinted, so the mutation does not cause disease when inherited from the mother but is highly penetrant when inherited from the father. Imprinting can also be tissue specific. A paternally inherited inactivating mutation in *GNAS1* causes Albright hereditary osteodystrophy (pseudopseudohypoparathyroidism; see Chapter 29). The same mutation, when maternally inherited, manifests not only with Albright hereditary osteodystrophy but also with hypocalcemia secondary to parathyroid hormone resistance (pseudohypoparathyroidism type 1a [PHP1a]), because only the maternal copy of *GNAS1* is expressed in renal proximal tubules.

Evolution influences the frequency of variants that affect human phenotypes (e.g., endocrine diseases) through the process of natural selection. Variants that greatly increase the risk of a disease that is deleterious from a reproductive standpoint are less likely to be passed on to offspring and will be rare in the population (unless they have a compensatory benefit like malaria resistance in carriers of sickle cell disease). If a disease is at least mildly evolutionarily deleterious, then most common variants associated with that disease will only modestly increase disease risk. This is because those common variants, if they had strongly increased disease risk, would have then been subject to strong negative evolutionary selection and never would have risen in frequency to become common in the first place. By contrast, it is more plausible for rare/recent variants to exert strong effects on phenotype and greatly increase disease risk.

Finally, the number of genes that contribute to disease in a single individual (mendelian or polygenic disease) will be related to the strength of effect of any one variant on disease risk. By definition, variants that cause mendelian disorders have strong effects, whereas variants contributing to risk of polygenic diseases will typically have more modest effects. Thus, most variants with strong effects on disease will be rare, especially for those diseases that are clearly deleterious from an evolutionary standpoint (lethal before reproductive age). By contrast, common polygenic diseases and traits will have a much more substantial contribution from common genetic variants, although these considerations do not rule out an important role for rarer variants in polygenic phenotypes. As we will see later in this chapter, these patterns of genetic variation have important implications for identifying genetic variants that underlie disease and for interpreting the impact of genetic variation on disease.

Summary

To summarize this introductory section, we have briefly described several basic principles of genetics. Heritability describes the proportion of a disease/trait that can be explained by genetic factors; the heritability of most endocrine diseases ranges between 20% and 80% (see Table 3.1). Genetic variants can take many forms ranging from single-base changes (SNPs) to translocations of entire chromosomes (see Fig. 3.1). The biologic effect of these variants depends on the type of variant; where in the DNA they are located (e.g., within coding sequence, splice sites, enhancers); how severely the variant affects function; and for somatic mutations, the cells and tissues that carry the mutation. Biologic impact can also be modified by the presence of genetic variants in other genes (gene-gene interactions), the individual organism's environment (gene-environment interactions), and random chance. The demographic history of modern human populations explains the presence of common and rare genetic variants in the human genome (see Table 3.2). Common variants are mostly ancient and typically have relatively modest clinical effects, whereas rare variants tend to have arisen more recently and can exert larger clinical effects (Table 3.4).

Genetics of Endocrine Diseases

As described earlier, heritable diseases and traits, including endocrine phenotypes, span a range of genetic architectures ranging from single-gene mendelian disorders to common, polygenic diseases and traits. Mendelian and polygenic disorders represent two ends of a spectrum (Fig. 3.2) of genetic architectures. Although we distinguish between these two extremes of genetic architecture, it is important to appreciate that many disorders lie between these two extremes: rare variants of moderate effect can affect the common form of the disease, and genetic and nongenetic modifiers can strongly influence the outcome of mendelian disorders. Furthermore, many polygenic endocrine disorders also have rare mendelian forms (see Table 3.1).

The genes for a wide range of mendelian endocrine diseases have been mapped, revealing great mechanistic insight. Although mendelian diseases have offered valuable insights into pathophysiology, not all insights gained from mendelian forms of disease translate directly to the common forms of disease. For example, mendelian obesity caused by recessive inactivating mutations in the leptin receptor could be well treated by exogenous leptin, but this clinical insight did not apply to most obese individuals who actually demonstrate elevated leptin levels and do not respond to exogenous therapy with leptin (see Chapter 40). Obesity as a common trait is highly heritable (heritability 40–80%), and genome-wide association studies (GWASs) analysis has begun to identify risk variants for the common form.[13] Although some of the risk variants overlap with those causing mendelian syndromes (as is also true for other diseases), GWASs have pointed to additional genetic contributions outside the mendelian genes. And, of course, the variants that have strong effects on quite rare genetic syndromes do not explain much, if any, of the risk of the common forms of disease. Thus, genetics of both mendelian forms and common polygenic forms will have important and often complementary impacts on our understanding of disease and on patient care.

The sections that follow discuss representative examples of mendelian and polygenic endocrine disorders that illustrate important concepts in gene discovery, understanding of the impact of genetic variation on disease, and implications for clinical care and insights into new biology. We discuss several classes of mendelian diseases and highlight three polygenic endocrine diseases/traits: (1) type 2 diabetes, (2) stature, and (3) serum lipids. In each section, we discuss what is known about the underlying genetic contributors, the impact of genetics on our understanding of disease biology, and the translation into clinical care in the short and long term.

TABLE 3.4 Performing and Interpreting Genetic Studies

For any heritable disease, the success of genetic mapping efforts, the strategy employed, and the clinical utility of any resulting genotype-phenotype map depend on its genetic architecture: (1) the number of genetic variants/genes, (2) their frequency in the population, and (3) their respective contributions to risk (i.e., penetrance). On one end of the spectrum lie mendelian diseases, such as multiple endocrine neoplasia type 1, characterized by (1) few variants often in a single gene, (2) extremely rare frequency in the population (<1:1000), and (3) potentially high penetrance (>50-fold risk). On the other end of the spectrum lie the so-called common diseases, such as type 2 diabetes, characterized by (1) many variants in many genes (polygenic), (2) high frequency in the population (>1:20), and (3) often low penetrance (<1.5-fold risk) (see Fig. 3-2).

Because of their simple genetic architectures, mendelian endocrine disorders were ideally suited for genetic mapping using the techniques of familial linkage mapping developed in the 1980s.[3] Because they are rare and have strong effects on phenotype, mendelian variants were typically identified in families. As a result, the genotype-phenotype correlations for these variants could not be generalized to the population at large. For example, penetrance of mendelian variants could be accurately estimated only if these variants were ascertained in the general population rather than in selected families with a specific genetic background. Large-scale sequencing studies in the general population, which can identify all variants, rare and common, are now enabling such estimates. Such studies have found that, when ascertained in the general population, the so-called mendelian variants are less penetrant than was estimated from family-based studies.[146]

By contrast, the variants for common polygenic disorders have been identified through genetic association studies in the general population. Genetic association studies do not require the identification of rare families segregating disease, because they simply compare the frequency of a given genetic variant in disease cases and controls. Thus, they can be applied to identify genetic factors underlying diseases occurring in a population of unrelated individuals (i.e., common diseases). Unlike clinical risk factors/biomarkers association studies, correlation in genetic association studies implies causation, because genotype always precedes phenotype. Through the 1980s, genetic association studies were performed using single-nucleotide polymorphisms at candidate genes selected by educated guessing. Such studies yielded several common disease associations but were poorly reproducible and confounded by false-positive results arising from population stratification. The development of modern sequencing and genotyping technologies along with the cataloging of more than 10 million common variants (the International HapMap project[147]) enabled genome-wide association studies (GWASs), a systematic approach to simultaneously test all genes for associations that could account for population-based confounding.[5] GWASs have yielded a large number of reproducible genetic associations for diverse common/polygenic diseases/traits,[148] yielding insight into disease biology and genetic architecture.

When interpreting a result from any genetic study, it is important to bear in mind that the actual variant (usually a single-nucleotide polymorphism) tested in the study marks a haplotype (a combination of genetic variants inherited together) that can span millions of bases. The causal variant, in the sense that it is molecularly responsible for alteration in gene function leading to cellular and disease phenotype, may lie anywhere on this haplotype. As with the chromosomal linkage studies of the past, identifying the causal variants/genes on a haplotype necessitates a combination of further association analysis (fine-mapping)[149,150] and functional experimentation in model systems.[151]

Mendelian Endocrine Diseases

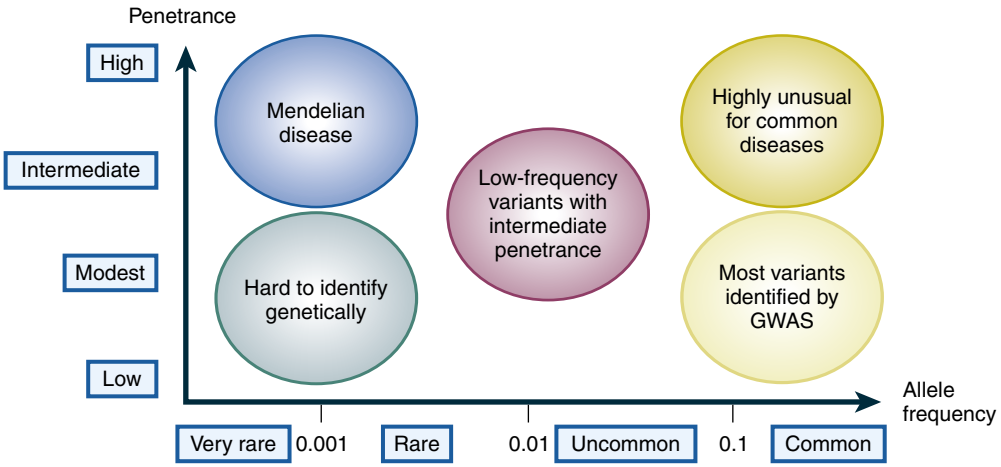
Genetic Architecture

Mendelian diseases represent one extreme of a spectrum of possible genetic architectures (see Fig. 3.2). The alleles causing mendelian diseases are found in a *small number of genes*, are typically *rare* (<1:1000), can be (although not always) *highly penetrant*, and follow simple patterns of dominant and recessive inheritance. They are considered monogenic in that a mutation in a single gene causes disease in an individual or family. But as different families segregating the same mendelian disease are identified and the causal genetic variants mapped, *genetic heterogeneity* is often observed: different alleles in different genes can cause the same disease. Some mendelian disorders (e.g., MEN2) demonstrate recurrent mutations in the same gene but of different molecular forms and locations, a phenomenon termed *allelic heterogeneity*. For other disorders (e.g., familial paraganglioma), multiple genes across different chromosomes are implicated, each causing the same/similar disease in different individuals. This phenomenon, variants in different genes causing the same disease, is termed *locus heterogeneity*. It is important to bear in mind that locus heterogeneity is intrinsically tied to the precision of disease definition. For example, CAH can be caused by defects in multiple genes encoding steroid biosynthetic enzymes (*CYP21A2*, *CYP11B1*, *CYP17A1*, *HSD3B2*, *POR*, *StAR*; see Chapter 15). However, if the CAH phenotype is refined to include biochemical measurements (mineralocorticoid, sex hormone, and electrolyte levels), individual subtypes emerge, each of which possesses a simpler genetic architecture (i.e., decreased locus heterogeneity).

When contrasted with common polygenic diseases, mendelian disorders exhibit relatively less locus heterogeneity. In other words, an appreciable fraction of mendelian disease cases can be largely explained by mutations in one or a few genes. For example, recurrent mutations in a single gene (the eponymous *MEN1*) account for 70% of families segregating the MEN1 clinical syndrome. Even in this classic mendelian case, however, the genetic architecture remains incompletely defined, as 30% of cases have no mutation in *MEN1*. Thus, much of the genetic architecture of mendelian diseases remains uncharted territory for genetic mapping. Modern sequencing technologies have facilitated a renaissance in mendelian disease gene mapping and will help improve our understanding of the genetic basis of mendelian disorders. By exome sequencing two individuals in a kindred with familial combined hypolipidemia (see Chapter 41), investigators identified two nonsense mutations in *ANGPTL3* that segregated with low serum lipoproteins when genotyped in other family members.[14] These mutations and the *ANGPTL3* gene were contained in the region identified by traditional linkage mapping[14] and could be quickly identified because the sequence of all exons in that region had been determined.

Disease Biology

Every endocrine organ ranging from the pituitary to adrenal is affected by well-described and less-described mendelian disorders. Mechanistic insight into disease biology has been gained from discovering the identities of the genes that lead to disease. When mutations in several different genes can all cause a disease (locus heterogeneity), additional mechanistic insight into molecular pathophysiology becomes possible. This makes intuitive sense in the context of a molecular understanding of genes as encoding proteins that act in concert to accomplish cellular functions. For example, Noonan syndrome (characterized endocrinologically by variable short stature, delayed puberty, and cryptorchidism in the



• **Fig. 3.2** Genetic architecture of common and mendelian diseases. On one end of the spectrum are mendelian diseases caused by few variants in few genes, each with a large individual effect on disease risk. On the other end of the spectrum are common diseases and traits caused by the combined effects of many variants, observed frequently in the population, each with a modest individual effect. *GWAS*, genome-wide association. (Redrawn from McCarthy MI, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet.* 2008;9[5]:356-369.)

setting of dysmorphic features and variable cardiac defects [see Chapter 24]) is typically caused by activating the RAS-MAPK (mitogen-activated protein kinase) signaling pathway. Dominant gain-of-function mutations in multiple pathway members (*PTPN11*, *SOS1*, *KRAS*, *RAF1*, *BRAF*, *NRAS*) have all been shown to cause Noonan syndrome. For other disorders, a more complex picture emerges in which multiple molecular pathways are implicated. For example, Kallmann syndrome (see Chapter 26), which arises from failure of migration of GnRH neurons during fetal development, demonstrates X-linked (*KAL1*), autosomal dominant (*FGFR1*), and autosomal recessive (*PROK2*) inheritance. The gene product of *KAL1*, a secreted protein called *anosmin*, is thought to interact with the fibroblast growth factor (FGF) receptor, whereas the gene product of *PROK2*, the secreted protein prokineticin 2, interacts with a different receptor. Both signaling pathways are required for GnRH neuronal migration.

At the level of a single gene/locus, genotype-phenotype correlations mapping allelic heterogeneity to phenotypic heterogeneity can provide detailed insight into how alterations in gene function affect disease severity. CAH caused by *CYP21A2* deficiency is a classic case. Several genetic variants including frameshifting deletions, splice site alterations, and missense mutations have been identified (see Chapter 24) in *CYP21A2*. This spectrum of alleles has been mapped to a biochemical spectrum of 21-hydroxylase enzyme activity, which in turn maps to a spectrum of clinical features along the axes of mineralocorticoid sufficiency, androgen excess, and ACTH elevation (see Chapter 24). In this disorder, it is possible to make predictions about clinical phenotype (categorized as salt wasting, simple virilizing, and nonclassic) based on genotype. Notably, the positive predictive value (PPV, the strength of the genotype-phenotype correlation) is strongest for variants that severely affect *CYP21A2* gene function and are predicted to cause severe disease (salt wasting, PPV ~100%). Predictive power is weaker for genetic variants that are expected to have more moderate effects on gene function and therefore result in milder disease (nonclassic, PPV ~60%). Some of this complexity is due to the potentially compensatory 21-hydroxylase enzyme activity of *CYP2C19* and *CYP3A4*, a form of gene-gene interaction. Genotype-phenotype correlations must be established empirically and are not possible in many cases. Even when mutations of varying molecular severity are identified, they may not predictably affect phenotype. For example, many individuals with genetic variants in the *SRD5A2* gene (encoding 5α-reductase type 2) of differing molecular severity and location have been identified (see Chapter 24), but no correlation between the genotype and the clinical degree of virilization is apparent.

A second example of synergy between NGS and classical genetic study design revealed gain-of-function mutations in the *KCNJ5* and *CACNA1D* genes as causes of hyperaldosteronism.[15,16] By sequencing exomes in a series of aldosterone-producing adenomas from individuals with primary hyperaldosteronism (Conn syndrome), investigators identified missense mutations in the *KCNJ5* gene in about one-third of tumors from unrelated individuals. They also identified a separate *KCNJ5* missense mutation in a mendelian family with hypertension, primary hyperaldosteronism, and massive adrenal hyperplasia (familial hyperaldosteronism type 3; see Chapter 16). Follow-up biochemical and electrophysiologic studies showed that this series of somatic and inherited missense mutations eliminated ion selectivity in the *KCNJ5* gene product, a potassium channel. The increased sodium conductance through these mutant channels caused membrane depolarization of adrenal cortical cells in the zona glomerulosa, stimulating aldosterone release and cell proliferation.

As demonstrated previously, modern genome sequencing technologies facilitates the identification of pathogenic mutations. Translation of mutations into a disease mechanism, however, requires relevant experimental model systems and close correlation with human phenotype. PHP1a (see Chapter 29) with gonadotropin-independent sexual precocity provides a classic example of the iterative relationship between mutations, human phenotypes, and laboratory experiments. Some individuals with PHP1a harbor the Ala366Ser missense mutation in a stimulatory G protein (G_s). This mutation destabilizes the protein, causing loss of function in most body tissues and thus hormone resistance. But these mutation carriers also exhibited paradoxical testotoxicosis consistent with gain of function in G_s. The paradox of the same mutation causing both loss and gain of function was

resolved with a series of experiments showing that the Ala366Ser mutation caused a temperature-sensitive effect physiologically relevant to the normal temperature for Leydig cells. In most body tissues maintained at 37° C, the mutant G_s protein was destabilized, whereas in Leydig cells (within the testes, maintained at a 3–5° C lower temperature), the mutant G_s demonstrated increased activity. Elucidating this mechanism required an appreciation of the discordant phenotype (testotoxicosis) and biochemical characterization in the relevant physiologic system (Leydig cells at a lower temperature).

Clinical Translation

Target discovery, risk prediction, and the tailoring of pharmacotherapy based on genotype are potential clinical applications of genotype-phenotype correlation. The existence of loss-of-function and gain-of-function variants in an allelic series and their concordance with opposing phenotypes can provide a rationale for therapeutically modulating gene function.[17] For example, inactivating mutations in the KISS1R receptor cause hypogonadotropic hypogonadism, whereas an Arg386Pro missense variant in *KISS1R* is associated with central precocious puberty. Kisspeptin, the agonist ligand of the KISS1R receptor, has shown promise as a fertility treatment.[18]

Genotype-phenotype correlation can be used to predict risk of disease in asymptomatic carriers. Prior to the identification and cloning of the *RET* proto-oncogene, MEN2 kindreds were monitored for evidence of medullary thyroid cancer (MTC) by calcitonin stimulation tests. Once mutations in *RET* were established as causing MEN2A/B and familial MTC, it became apparent that specific mutations could be mapped to the different syndromes. The *RET* gene product encodes a cell surface receptor tyrosine kinase. Mutations in the extracellular domain predispose to MEN2A (characterized by MTC, pheochromocytomas, and hyperparathyroidism), whereas mutations in the intracellular tyrosine kinase domain predispose to MEN2B (characterized by MTC, pheochromocytomas, and mucosal neuromas) (see Chapter 42). The clinical aggressiveness of MTC, the sine qua non of all three syndromes, is greatest in MEN2B, then less in MEN2A, with familial MTC demonstrating the least propensity to grow and metastasize. A well-defined genotype-phenotype correlation between specific *RET* mutations and clinical aggressiveness of MTC now dictates the timing of lifesaving prophylactic thyroidectomy in carriers of *RET* mutations.[19] The key to establishing clinically robust risk prediction based on genotype-phenotype correlations is a well-differentiated allelic series derived from multiple individuals/families. The consensus genotype-phenotype correlation for prophylactic thyroidectomy in *RET* mutation carriers was derived from analysis of more than 200 individuals from more than 100 families (see Chapter 42).

A genetic diagnosis in several mendelian disorders can also directly inform pharmacotherapies. A classic example includes obesity caused by leptin deficiency (see Chapter 40), which can be treated by exogenous leptin injections. Other examples include *HNF1A* MODY (maturity-onset diabetes of the young) and neonatal diabetes (discussed in detail later) caused by genes whose properties predict excellent response to sulfonylureas. In the case of congenital hyperinsulinism, autosomal recessive mutations in *ABCC8* or *KCNJ11* correlate with diffuse disease on spectroscopic imaging and lack of responsiveness to medical therapy (diazoxide); such individuals require near-total pancreatectomy for control of hypoglycemia.[20]

Type 2 Diabetes

Genetic Architecture

Type 2 diabetes (T2D) is a multifactorial, polygenic disorder for which manifestation depends on multiple interacting genetic and environmental risk factors. Heritability estimates show strong evidence of familial clustering, ranging from 40%[7] to 80%.[8] Approximately 5% of diabetes cases that may be classified as nonautoimmune arise from a single-gene disorder, follow mendelian patterns of inheritance, and cluster into clinically defined syndromes. These mendelian diabetes syndromes include neonatal diabetes, MODY, and congenital lipodystrophies.[21] To date, familial linkage studies have successfully implicated approximately 30 genes as monogenic causes of diabetes.[22]

The genetic factors underlying the majority of T2D cases (95% of cases) fit a polygenic model; genetic variants in multiple genes independently contribute to disease risk, each with a modest effect. The partial elucidation of these genetic risk factors required the advent of genetic association studies/GWASs and the assembly of cohorts of thousands of cases and control subjects.[23] As of 2014, about 70 loci have been identified from aggregated analysis on about 150,000 case controls. Taken together, these loci account for about 6% of heritability for T2D.[23] Of these loci, an SNP at *TCF7L2* (with the risk-increasing allele present at a frequency of ~30%) has the largest overall effect on risk, conferring a 1.4-fold increase in risk per allele.[23]

By contrast, type 1 diabetes shows a somewhat different genetic architecture, with common loci of large effect (a variant at the *HLA* locus found in 61% of the population confers a fivefold increase in risk,[24] and a common variant at the insulin gene confers a threefold increase in risk). This finding was consistent with prior studies from the 1980s estimating that 50% of the heritability of type 1 diabetes was explained by common haplotypes at the *HLA* locus.[25,26] Notably, the genes implicated in monogenic causes of diabetes also contribute to polygenic forms but through distinct genetic variants. Genes associated with mendelian diabetes syndromes, such as *KCNJ11* (neonatal diabetes), *HNF1A* (MODY2), and *PPARG* (familial partial lipodystrophy 3),[27] were found to harbor common variants that conferred risk for common T2D.[21,28] Conversely, genes first found associated with diabetes through GWASs have subsequently been identified as having rare, highly penetrant alleles. For example, noncoding common variants pointed to the *MTNR1B* gene (encoding the melatonin receptor) as a T2D-associated locus (1.15-fold risk).[29] Subsequently, large-scale resequencing studies identified multiple, rare coding variants of the same gene (present in <1:1000 individuals) that conferred a greater than fivefold increased risk of T2D.[30]

Mapping studies performed across various populations reveal both similarities and differences in the genetic risk factors contributing to diabetes among different ancestral groups. T2D GWASs performed in multiple populations/ancestries (European, South Asian, East Asian, Latino, African American)[31] reveal that many common variants are shared across populations with equivalent effects on disease risk, regardless of ancestry. This pattern is consistent with the origin of most common variants in an ancestral African population (see Table 3.3), but remarkable ancestry-specific effects have also been identified. A T2D GWAS performed in individuals of Latino and Mexican ancestry identified a common SNP at a locus containing the genes *SLC16A11/13* that confers a 1.25-fold increased risk of diabetes.[32] The same locus was identified by

another T2D GWAS performed in Japanese individuals.[33] Because the associated SNPs were rare in Europeans, the locus had not been detected in GWASs in European-ancestry populations. Similarly, a common variant in *TBC1D4* in individuals from Greenland (present in 17% of Greenland's population) strongly increases risk of T2D (10-fold increased risk).[34] This variant, which causes a premature truncation and is associated with elevated muscle insulin resistance, is extremely rare in continental Europe and likely became common in Greenland because it was present in the founding ancestors of Greenland's current population.

In summary, genetic mapping studies over the past three decades have revealed a genetic architecture for T2D with widespread locus and allelic heterogeneity. With regard to effect size and allele frequency, T2D genetic architecture so far consists of some very rare variants of large effect, some common variants with small to moderate effects (1.2- to 1.5-fold increased risk), and a larger number of common variants with even more modest effects on disease risk, with rare and common genetic variants spread out across multiple loci in the genome. This genetic architecture has proved to be typical for other common diseases[24] (see Fig. 3.2) and reflects both the underlying genetic architecture of the disease and the ability of large GWAS to more readily detect common variants of modest effect.

Disease Biology

The past three decades of genetic discoveries in T2D have nucleated a molecular understanding of disease mechanisms, highlighted differences between glycemia and T2D, and implicated previously unknown physiology in disease pathogenesis.

Supporting the current physiologic conception of T2D as a disorder of decreased insulin production, as well as decreased insulin sensitivity, genetic mapping has pointed to a molecular basis for both axes. Prediabetic individuals harboring T2D-associated variants in beta-cell genes (*SLC30A8*, *HNF1A*) and cell survival genes (*CDKAL1*) manifest with decreased insulin secretion (homeostatic model assessment B; see Chapter 34).[28] However, prediabetic individuals harboring T2D-associated variants in adipocyte genes (*PPARG*, *KLF14*) tend toward increases in homeostatic model assessment insulin resistance (see Chapter 34).[28] About 30% of T2D-associated SNPs point to insulin secretion/beta-cell function, and 15% point to insulin resistance.[22] Interestingly, the SNPs associated with insulin secretion in prediabetic individuals predict incident T2D, but the SNPs associated with insulin resistance do not.[35] These findings from genetic epidemiology are consistent with beta-cell failure being a final common pathway for manifestation of hyperglycemia and diagnosis of T2D. Importantly, more than half of the associated SNPs and the genes they point to cannot be connected with either insulin secretion or sensitivity. Their pathogenic mechanisms remain to be elucidated by physiologic and functional investigation.

Even without a full understanding of their molecular/cellular mechanism of causation, the large number of T2D-associated loci (~70 as of 2014) have been deployed to refine disease classification. By examining quantitative glycemic traits (insulin production, sensitivity, processing, and fasting glucose) in nondiabetic individuals genotyped for 37 T2D-associated common genetic variants, investigators were able cluster genes with glycemic traits to define unique diabetes subtypes.[36] For example, individuals harboring variants in *MNTR1B* and *GCK* manifested a combination of fasting hyperglycemia and decreased insulin secretion, whereas those harboring variants in *SLC30A8*, *CDKN2A/B*, *TCF7L2*, and other genes manifested primarily with decreased insulin secretion. Notably, many genes did not cluster with predefined glycemic traits, again suggesting that the current physiologic description of T2D remains incomplete.

Genetic mapping has also corroborated the epidemiologically identified intersection between T2D and obesity. An SNP in the second intron of the *FTO* gene was identified in parallel in GWASs for T2D [37] and obesity.[38-40] The association signal for T2D entirely disappeared with correction for body mass index (BMI), indicating that this SNP increased T2D risk by increasing BMI. Interestingly, this locus illustrates some of the difficulties in proceeding from GWAS signal to function. Although this SNP was initially thought to exert its effect on BMI by affecting *FTO* gene function, detailed mechanistic studies have revealed that it may function by altering expression levels of *IRX3*, a gene over a million bases away.[41] Although initial studies in mice showed that increasing *Fto* gene dosage increased food intake leading to increased fat mass,[42] no connection has been found between the disease-associated SNPs and *Fto* expression level or function.[43]

Even more recently, splice acceptor variants that generate premature stop codons and loss of function in *ADCY3* (a gene highly expressed in visceral adipose tissue) has been identified as causal for increased BMI and T2D risk. Concordant functional studies in mice with loss of function causing obesity, hyperphagia, and insulin resistance suggest that ADCY3 may be a new therapeutic target.[44]

Whereas T2D is diagnosed on the basis of hyperglycemia, genetic mapping has revealed that the genes that determine fasting glucose are partly distinct from those that are associated with T2D. Comparison of GWASs performed for blood glucose levels in nondiabetics versus T2D case-control studies revealed that glycemia and T2D have distinct genetic associations.[45] Some genes harbor variants that increase blood glucose levels and T2D risk, whereas others alter blood glucose levels but do not confer T2D risk. Thus, the two phenotypes have both common and distinct biology. Additionally, it is important to bear in mind that the genetic basis for surrogate measures of glycemia do not always point to genes specifically altering glycemic physiology. A particularly salient example is the association of hexokinase 1 (*HK1*) with hemoglobin A_{1c} levels but not with fasting or dynamic glycemia.[46] It is thought that the genetic variant in *HK1* alters hemoglobin A_{1c} levels as a result of the alteration of the red blood cell life span and anemia.[46]

Clinical Translation

The principle of genetics pointing to important therapeutic targets in T2D is well validated. Both rare and common genetic variants link *PPARG*, the drug target of thiazolidines, to syndromic and common T2D.[27] Similarly, rare variants in the sulfonylurea receptor (encoded by *ABCC8*) cause neonatal diabetes.[47] Although these oral hypoglycemics were identified in a pregenetic era, they point to an optimistic future of genetically guided drug discovery, one that will require detailed mechanistic understanding of the genes mapped by studies to date. A particularly attractive target nominated by genetics is *SLC30A8*, a gene that encodes a zinc transporter expressed almost exclusively in the endocrine pancreas (ZnT8). The common R325W missense variant in the protein encoded by the *SLC30A8* gene (present in ~1:3 individuals in most continental populations) was found to associate with protection from T2D (1.18-fold decreased risk).[48] Rare, protein-truncating variants in *SLC30A8* (present in ~2:1000 individuals) have also been associated with protection from T2D with a larger effect size (2.6-fold decreased risk).[49] The finding of human

heterozygous knockouts for *SLC30A8* who are protected from T2D and have no other deleterious phenotype offers a tantalizing therapeutic hypothesis that a chemical or antibody inhibitor of *SLC30A8* could treat diabetes and minimize side effects.

In the arena of risk prediction, genetics has not yet made a major impact on T2D because existing clinical risk factors already predict disease well. Common genetic variants, by virtue of their relatively high frequency, can explain a large part of heritability for a trait/disease but have small effects on the individual. For example, the common P12A variant in PPARG is associated with 1.25-fold risk of T2D.[50] Based on the high population frequency of the risk variant (85% P), if one were to theoretically substitute every P to A in the population, 20% of diabetes would be eliminated. Despite this incredible population-attributable risk, any given individual carrying the P variant only has a 25% increased risk of diabetes when compared with someone carrying the A variant. Given the high population frequency of common variants, many disease susceptibility variants are found in the same individual, each of which confers modest increases in risk. The progressive accessibility of genome sequencing makes it feasible to ascertain all known risk-conferring variants in an individual at once and combine these for potentially more clinically meaningful risk prediction. Investigators have attempted to combine common variants into a genetic risk score with modest success. For T2D, a risk score combining 18 common variants (including *PPARG* P12A) demonstrated 2.6-fold elevated risk in the high-risk versus low-risk group.[51] By contrast, simply reporting a family history of diabetes increases risk by threefold to sevenfold.[52]

Tailoring pharmacotherapy based on genotype has been successful in monogenic diabetes; the genome-sequencing era promises to bring this benefit to a wider group of individuals. The classic example of genotype guiding pharmacotherapy is that of individuals with MODY2 caused by autosomal dominant mutations in the *GCK* gene. Such individuals meet diagnostic criteria for diabetes but are able to regulate glycemia at a higher set-point, thus avoiding all secondary complications (see Chapter 35). Thus, a genetic diagnosis of *GCK*-related diabetes can allow such individuals to avoid pharmacotherapy. Individuals with permanent neonatal diabetes caused by *ABCC8* or *KCNJ11* mutations can be safely treated with high-dose sulfonylureas in place of insulin.[53,54] It is likely that individuals with functional mutations in *ABCC8* or *KCNJ11* will be found who do not present with the classic neonatal syndrome and are classified as common T2D but who may still respond preferentially to sulfonylureas. Proving this prediction will require identifying such individuals and performing prospective clinical trials. Prospective genotype-based intervention trials have been performed in individuals with MODY3 caused by *HNF1A* mutations and have shown the superiority of sulfonylureas over metformin.[55] Interestingly, exome sequencing of Latin American T2D case-control groups revealed the *HNF1A* E508K variant, previously annotated as MODY3, as conferring a fivefold risk of T2D in approximately 1:1000 individuals. These data demonstrate that the E508K is not fully penetrant; nevertheless, the individuals who carry it may still benefit preferentially from sulfonylurea treatment, as do their counterparts with clinically defined MODY3.

Large-scale sequencing of the known monogenic diabetes genes has uncovered many novel protein-coding variants; these present both an opportunity for pharmacogenetics and a challenge. As a case in point, sequencing of *PPARG* in approximately 20,000 T2D case controls identified 49 rare protein-coding variants. Three of the 49 had ever been identified before, were known to cause loss of function, and had been associated with mendelian lipodystrophy[56]; 46 variants were novel and of unknown function. Functional testing of each of the variants with respect to their ability to rescue adipocyte differentiation revealed nine of the novel variants causing loss of PPARG function, with the rest being indistinguishable from wild type.[56] The variants causing loss of function conferred a sevenfold increased risk of T2D, whereas the wild-type–like variants conferred no additional risk of T2D. To address the challenge of interpreting novel protein-coding variants at the scale they are being discovered (1:500 individuals in the general populations carry a rare protein-coding variant in *PPARG*) and which ones might be amenable to pharmacotherapy, investigators applied synthetic biology and high throughput sequencing to synthesize and functionally test all possible (~10,000) protein-coding variants in *PPARG*.[57] Using the resulting "lookup table" for PPARG function, clinical investigators identified two PPARG mutations (R308P and A261E) in patients affected by diabetes and partial lipodystrophy and demonstrated in vivo responsiveness to thiazolidinediones.[58] These studies demonstrate a proof of concept for the use of genetic information and high throughput functional characterization to guide pharmacotherapy in other monogenic disease genes.

Short Stature

Genetic Architecture

Adult height is a polygenic quantitative trait with a heritability of 80%.[6] Many mendelian syndromes manifest (see Chapter 25) large phenotypic variations in stature, and more than 150 genes are associated with monogenic short stature or overgrowth. The effect sizes for these rare, highly penetrant alleles are typically large, up to 300 mm (with 1 standard deviation [SD] for height equal to 55–60 mm). The genetic factors underlying most of human height variation are polygenic. GWASs aggregating more than 690,000 European samples have identified more than 3290 independent loci associated with height[59] through common variant association. Common variants account for about 60% of heritability.[59,60] The effect sizes for these common alleles are in the range of <1 to 15 mm (<0.02–0.20 SD). Taken together, these genetic mapping studies show that the genetics of height consists of the additive effects of common alleles with modest individual effects except in very short individuals (>2 SD below the mean).[61] In extremely short individuals, it is likely that rare alleles of large effect play a larger role.[61] Many of the genes (more than expected by chance) in height-associated loci identified by GWASs contain rare monogenic alleles. These height-associated genes frequently also harbor common alleles that contribute to polygenic stature variations.[59] A classic example is *GH1* (isolated GH deficiency 1a; see Chapter 25). This substantial overlap suggests that the genes in associated loci that have yet to demonstrate rare, large-effect alleles may be prime candidates for resequencing studies to discover new monogenic causes of alterations in stature.[62]

Genetic causes of short stature include Turner syndrome, mutations of the GH/insulin-like growth factor (IGF) pathway, and SHOX mutations. Previous GWASs have identified 700 common variants that explain approximately 20% of height variation in the population, with an additional 1.7% caused by rare coding variants. After excluding environmental factors such as nutrition, the cause of 60% to 80% of height variation remains unknown, but much of it likely is attributable to common variants that have not yet been identified as associated with height.[62] Short stature, but not tall stature, has been associated with deletions in the genome.

By systematically comparing CNVs across the genomes of more than 4000 individuals with developmental delay and congenital abnormalities with 7000 population-based control subjects, investigators observed that individuals with short stature harbored an excess number of low-frequency (found in <5% of the population) deletions.[63] Individuals in the cohort clinically diagnosed with short stature demonstrated an average loss of 900,000 bp from their genomes.[63]

In summary, the genetic architecture of height is consistent with classical theory and animal models[64] for a polygenic, quantitative trait: thousands of genetic variants in hundreds of genes contribute to the genetic variability in height. Most of the genetic variation in height (97%) occurs from the additive effects of common variants, each contributing a modest effect. Rare variants of large effect often causing mendelian syndromes cluster at the short extreme of height variation.

Disease Biology

Genetic mapping of genes influencing both normal variation and the extremes of stature have revealed diverse molecular pathways operating in multiple tissue types that work through both endocrine and cell-autonomous mechanisms.

Genetic mapping in individuals with low GH levels and short stature have delineated multiple components of the GH/IGF1 axis as a key endocrine pathway in human height regulation (see Chapter 25). These components include hormones, their receptors, binding proteins, and intracellular signaling proteins, such as GH1, GH receptor (GHR), GH-releasing hormone receptor (GHRHR), STAT5B, IGF acid-labile subunit (IGFALS), IGF1, and IGF1 receptor (IGF1R). IGF bioavailability varies according to its association with IGF binding proteins including IGFBP3. Recent WES in two families with short stature and microcephaly revealed the critical role of PAPP-A regulation in IGF signaling. PAPP-A is a metalloproteinase that cleaves IGF binding proteins to release the active hormone near IGF receptors. Inherited homozygous loss-of-function mutations in PAPP-A resulted in progressive growth failure in several family members despite elevated total circulating IGF.[65] A subsequent meta-analysis of exome array association data for height identified low-frequency coding variants in *STC2* (Stanniocalcin-2) with profound effects on height. STC2 covalently binds and inhibits PAPP-A.[66] Rare missense mutations in *STC2* decrease PAPP-A binding, resulting in increased PAPP-A activity, increased IGF availability, and an associated average 2 cm height increase. The same study identified rare *AR*, *CRISPLD2*, and *IHH* gene mutations, all of which decreased average height by approximately 2 cm. Remarkably, these rare IGF pathway gene variants have effect sizes that are often greater than 10 times the magnitude of common height variants.[67]

Genetics has also pointed to the importance of the pituitary gland as a key endocrine organ regulating height, as mutations in genes that encode transcriptional regulators of pituitary development, such as *HESX1*, *PITX1*, *PITX2*, *PROP1*, *POU1F1*, and *LHX3*, also lead to short stature when mutated in humans.[68] At the paracrine and cell-autonomous levels, genetic mapping has highlighted the importance of cellular proliferation, extracellular matrix deposition, and cartilage/bone development. These cellular processes were first implicated by monogenic short stature syndromes caused by mutations in genes such as *FGFR3* (achondroplasia) and *SHOX* (Langer mesomelic dysplasia) or tall stature syndromes, such as *FBN1* (Marfan syndrome) and *EZH2* (Weaver syndrome).

GWASs for height as a polygenic trait, combined with systematic clustering of genes in associated loci, have refined and expanded insights gained from mendelian genetics. Common variants associated with multiple members of the GH/IGF1 axis demonstrate the importance of this pathway in height regulation within the normal range.[69] The association of TGF-β itself and of its binding proteins, LTBP1 through LTBP3, complements the finding of *FBN1* mutations in Marfan syndrome in highlighting TGF-β signaling.[70] Similarly, the FGF signaling pathways are also highlighted, as common variants near *FGF4* are associated with height, complementing the finding of monogenic mutations in *FGFR3* causing achondroplasia. Clustering of GWAS-associated genes has also implicated hedgehog signaling (*GLI2*, *LAMA5*), Wnt signaling (*CTNNB1*, *FBXW11*, *WNT4*, *WNT5A*), and mammalian target of rapamycin (mTOR) signaling (*SMAD3*, *MTOR*) cascades in genetic control of height. Many associated loci do not cluster with the pathways above or other known tissue/cellular processes such as bone/cartilage formation, implicating previously unknown biology in height regulation. A notable example is the microRNA cluster *MIR17HG*, which was also identified as a syndromic cause of short stature.[71] By cross referencing GWAS-associated genes with gene-expression microarrays from thousands of human tissue samples, investigators have found that height-associated genes are predominantly expressed in the growth plate and to a lesser extent in cartilage, bone, and endocrine organs.[59,72]

Clinical Translation

Gene mapping and functional characterization in monogenic stature disorders have motivated therapeutic advances for diseases of overgrowth and short stature. A classic example is that of Marfan syndrome, in which excess TGF-β signaling resulting from the effects of disruptive mutations in *FBN1* has led to the development of TGF-β antagonist therapies.[73] Human trials of the angiotensin receptor blocker losartan, which exhibits TGF-β antagonist properties in in vivo preclinical models, were begun in cohorts of Marfan syndrome patients.[74] Results 3 years after treatment showed a decrease in aortic root diameter but not more so than conventional therapy with β-blockers.[75] The identification of the activating G380N mutation in *FGFR3* that causes 95% of achondroplasia has motivated the development of inhibitors of the tyrosine kinase activity of *FGFR3* through small molecules,[76] as well as through analogues of C-type natriuretic peptide.[77]

Due to the magnitude of its effect on height, *STC2* represents an intriguing novel therapeutic target for short stature and IGF-associated disease. Transgenic STC2 overexpression in mice causes severe postnatal growth retardation that is abolished when STC2 is replaced with a mutant protein lacking the ability to bind PAPP-A. STC2 overexpression may treat IGF excess in conditions including acromegaly and malignancies while knocking down STC2 with siRNA may help promote growth to treat short stature.[66,78] The diagnosis of short stature is an important application of genetic testing, particularly in pediatric populations. For example, the genotype-phenotype correlation of *SHOX* deficiency shows a clinical spectrum ranging from homozygous loss-of-function mutations causing the severe syndrome Langer mesomelic dysplasia to heterozygous defects found in patients with milder syndromic disease (Léri-Weill dyschondrosteosis, Ullrich-Turner syndrome) or idiopathic short stature.[79] Genetic diagnosis qualifies these patients for GH therapy. Genetic diagnosis also enables directed screening for comorbid conditions. For example, males with the likely underdiagnosed 3-M syndrome

(caused by mutations in *CUL7*, *OBSL1*, or *CCDC8*), clinically defined by severe postnatal growth retardation, characteristic facies, and radiographic findings of skeletal abnormalities, are at high risk of primary hypogonadism and need to be monitored.[79] Similarly, individuals with short stature secondary to Noonan syndrome are also often underdiagnosed and are at higher risk for cardiac disease.[80] Recent work added WES to targeted genetic testing to test whether sequencing would substantially improve genetic diagnosis. Of 565 patients with short stature referred for genetic evaluation, targeted genetic testing revealed the underlying genetic etiology in 13.6% of patients. Of the remaining 491 patients without a known diagnosis, 200 underwent WES, and a variant in a gene known to cause short stature was found in an additional 16.5% of patients in this population, suggesting that adding WES can increase diagnostic yield.[62] Genetic diagnosis could improve care for short stature by facilitating early identification and intervention prior to puberty and growth plate closure, screening for known comorbidities associated with the diagnosis, and potentially predicting the efficacy of interventions such as GH therapy.

Tailoring pharmacotherapy based on genotype can be a useful adjunct to biochemical testing in monogenic stature disorders. Physiologic stimulation and serum biochemistry are the gold standards for assessing GH sensitivity and resistance guiding its pharmacologic use. But genetic information plays an important role in solidifying diagnoses suggested by biochemical testing and suggesting alternative pharmacologic therapy. For example, children with defects in the GH receptor or post-GH receptor signaling (STAT5B) will be candidates for treatment with recombinant IGF1 (mecasermin).[81] Children with defects in IGFALS, a serum protein that stabilizes IGFs, respond poorly to both medications.[82] GH therapy is used to treat short stature arising from certain genetic defects outside the GH/IGF1 axis but is costly and not indicated in many others. Given the variable expressivity in many syndromic stature disorders, clinically distinguishing among syndromes presenting with short stature can be challenging and imprecise. Genetic diagnosis can resolve ambiguity, particularly in children before all the features of a syndrome are present. For example, GH is contraindicated in chromosomal breakage disorders. Bloom syndrome (caused by loss-of-function mutations in *BLM*, which encodes a DNA helicase) is one such disorder presenting with short stature. In the absence of genetic diagnosis with only clinical and biochemical testing, there are case examples of children being treated for years with GH until their clinical presentation evolved and Bloom syndrome was diagnosed.[83]

Lipids and Coronary Artery Disease

Genetic Architecture

Serum lipid levels are a complex polygenic trait significantly influenced by environmental factors such as diet. Heritability estimates suggest a large role for genetic factors: approximately 40% to 60% for high-density lipoprotein cholesterol (HDL-C), about 40% to 50% for low-density lipoprotein cholesterol (LDL-C), and about 35% to 48% for triglycerides (TG).[84] Monogenic disorders causing extremes of dyslipidemia have been associated with around 20 genes. Mendelian dyslipidemia syndromes can present with single or combined lipoprotein abnormalities (see Chapter 41). On the hyperlipidemic side, these abnormalities include syndromes of elevated LDL (familial hypercholesterolemia, sitosterolemia), elevated TG (lipoprotein lipase deficiency, APOCII deficiency), elevated HDL (CETP deficiency), and combined LDL/TG elevation (familial combined hyperlipidemia, dysbetalipoproteinemia). Monogenic disorders manifesting with extremely low lipid levels have also been identified and include low LDL (familial hypobetalipoproteinemia, *PCSK9* mutations), low HDL (familial hypoalphalipoproteinemia, lecithin cholesterol acyltransferase deficiency, Tangier disease), and combined low cholesterol/TG (abetalipoproteinemia, chylomicron retention syndrome).

The genetic factors underlying most serum lipid variations are polygenic. GWASs aggregating approximately 200,000 multiethnic samples have identified more than 150 independent loci associated with serum lipids through common variant association accounting for about 15% of heritability.[85] The effect sizes of common variants on lipid levels range from less than 1 mg/dL to about 15 mg/dL (SNP rs964184 at *APOA1* and TG).[86]

Among the loci identified, many alter one of the lipoproteins identified, a few (*CETP*, *TRIB1*, *FADS1-2-3*, *APOA1*) alter all lipoprotein levels, and a subset alters various combinations of lipoproteins.[85] These overlaps are consistent with observations in mendelian disorders and corroborate the shared metabolism and lipoprotein constituents of LDL, HDL, and TG.

Many of the genes identified as monogenic causes of dyslipidemia also alter lipid levels in the general population through both common and rare variants. *LDLR*, encoding the LDL receptor, provides a case in point of how the allelic spectrum of genetic variation ranges from rare mendelian alleles to common small-effect alleles with effect sizes inversely proportional to variant frequency. Disruptive mutations (those that frameshift or prematurely terminate the protein) in *LDLR* like the ones found in familial hypercholesterolemia are found in 2:1000 to 7:1000 individuals and increase LDL levels by 150 to 200 mg/dL.[87] Missense variants that decrease function of LDLR in cell models are found in about 1:100 individuals and increase LDL levels by about 100 mg/dL.[88] A common intronic SNP in *LDLR* found in 1:10 individuals decreases LDL levels by 7 mg/dL.[86]

In summary, the genetic architecture of serum lipids consists of a full spectrum of rare and common alleles in hundreds of genes throughout the genome acting in a polygenic fashion. Different variants at the same locus, based on varying impact on gene function, can alter lipid levels in a broad range. Variant frequency in the population is inversely correlated with the magnitude of effect on serum lipid levels. Many loci have an impact on multiple lipoprotein levels simultaneously.

Disease Biology

Genetic mapping for lipid traits has a rich history of synergy with biochemical and physiologic investigation to reveal the molecular mechanisms of lipoprotein metabolism and its relationship to cardiovascular disease in humans.

Mendelian hyperlipidemia syndromes were the first to yield pathophysiologic insight, starting with Brown and Goldstein's classic studies showing that LDL failed to suppress HMG-CoA (3-hydroxy-3-methyl-glutaryl coenzyme A) reductase activity in fibroblasts from subjects with familial hypercholesterolemia.[89] Subsequent studies of families with severe hypercholesterolemia (*LDLR*, *APOB*, *ABCG5*, *ABCG8*, *ARH*, *PCSK9*) yielded insights into basic mechanisms of cholesterol absorption and biliary excretion and contributed to basic biologic understanding of receptor-mediated endocytosis, recycling, and feedback regulation.[90,91] The association between high LDL-C and increased rates of myocardial infarction (MI) was also noticed in these families and complemented by observations of low rates of coronary disease in families segregating unusually low LDL-C levels (familial

hypobetalipoproteinemia: *APOB*, *PCSK9*, *ANGPTL3*).[91] Epidemiologic association and the success of statin therapy in preventing coronary heart disease (CHD) extended the relationship of LDL-C and CHD to the general population,[92] as have common LDL-C–associated SNPs identified by GWASs.[86]

The large number of associated loci for LDL-C, HDL-C, and TG combined with clinical outcome data on population-size cohorts of genotyped individuals has enabled causality testing for epidemiologic associations with important public health consequences. Elevated HDL-C levels have been associated with protection from MI, but the causality of this association is controversial. Should public health efforts be made to raise HDL levels in the population? Should drugs that increase HDL-C levels continue to be developed after early clinical failures?[93] By leveraging common variants associated with HDL-C levels, the causality of HDL-C in heart disease can be tested using an approach called *mendelian randomization*.[94] As described earlier, genetic associations imply causality because genotype precedes phenotype, mitigating epidemiologic issues of confounding, bias, and reverse causation. Mendelian randomization can be conceived of as a clinical trial performed by nature in which the subjects are randomized at conception to genetic variants associated with a risk factor (e.g., SNPs increasing levels of HDL-C). The subjects randomized to genotype are assessed for the outcome (e.g., MI), and their relative risk for the outcome is compared in patient groups with and without the genetic variants in question.

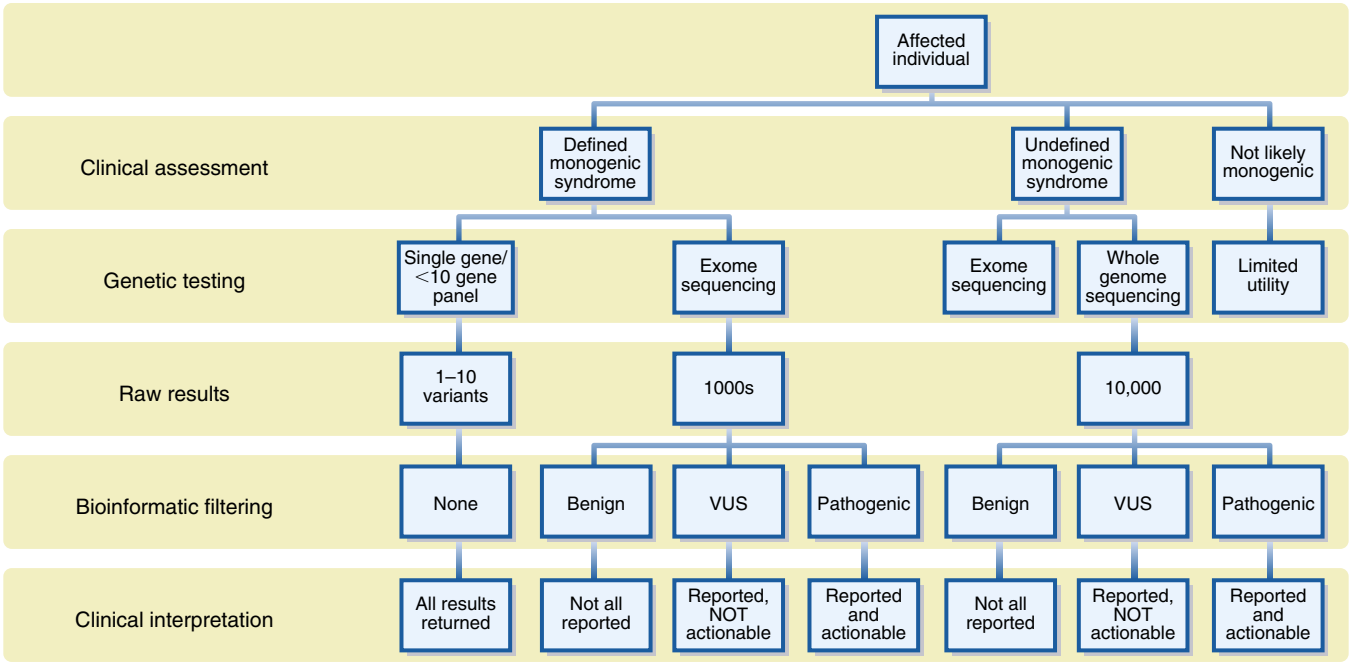
Although earlier observational studies showed a strong correlation between elevated HDL-C and CHD,[95] investigators tested the causality of this association using a mendelian randomization approach with SNPs quantitatively associated with lipid levels. They found that genetic variants increasing HDL-C were not protective for MI, whereas genetic variants decreasing LDL-C were protective of MI.[96,97] These findings are consistent with drug trials showing that LDL-C–lowering agents protect from MI, whereas multiple agents aimed at increasing HDL-C do not.[98] These results indicate that targets with support from human genetics are more likely to be clinically effective in humans.[99,100]

For TG, however, multiple lines of genetic evidence have supported a causal connection with CHD. First, rare, loss-of-function mutations in *APOC3* are associated with low levels of TG and are also protective for ischemic cardiovascular disease in European[101] and American cohorts.[102] Second, a mendelian randomization study showed that SNPs that raised serum TG levels also increased rates of CHD.[103] Finally, because of the interrelatedness of TG, LDL-C, and HDL-C, investigators have systematically examined all lipid-associated loci in cohorts phenotyped for CHD to dissect the contribution of TG to CHD risk apart from LDL-C and HDL-C.[85] By constructing a statistical framework to account for the pleiotropic effects of SNPs on all three lipoprotein levels, they demonstrated that (1) SNPs that alter LDL-C and TG in the same direction of effect are associated with CHD risk, (2) SNPs that exclusively alter TG levels are also associated with CHD, and (3) the strength of an SNP's effect on TG levels independently are correlated with the magnitude of effect on CHD risk.[85]

Clinical Translation

In the area of therapeutics, mapping of genes that affect lipid levels has exemplified a promising approach to drug target identification: genes that protect from disease when inactivated by nature might be useful pharmacologic targets. Statins, which inhibit HMG-CoA reductase (encoded by *HMGCR*), are among the most therapeutically successful drugs in lowering LDL-C levels and decreasing risk for CHD in both primary and secondary prevention settings. GWAS did identify *HMGCR* as a locus altering LDL-C levels (with an effect size of about 3 mg/dL),[86] but how would this particular locus be prioritized as a therapeutic candidate among more than 100 other associated loci? In some cases, experiments of nature (i.e., an allelic series) can be used to infer a dose-response curve of gene function that indicates how enhancement or suppression of the encoded protein's activity raises or lowers disease risk. In the well-known case of *PCSK9*, for instance, loss-of-function mutations decrease LDL-C and cardiovascular disease risk, whereas gain-of-function mutations increase LDL-C and cardiovascular disease risk.[104] Early clinical trials suggest that inhibiting the protein encoded by *PCSK9* is a promising strategy for lowering LDL-C and preventing cardiovascular disease.[105] Identifying genes that, when inactivated by nature, protect from disease offers several advantages for therapeutic targeting: (1) the target is already validated in humans, (2) designing inhibitors of gene/protein function is more tractable than increasing gene/protein function, and (3) nature has performed a lifelong clinical trial of inhibiting gene function, and the side effects of doing so are known. In the case of *PCSK9*, individuals with loss-of-function mutations demonstrated no phenotypic abnormalities other than low LDL-C levels and decreased risk of heart attack. In a similar example, naturally occurring mutations that disrupt *NPC1L1* function, the inhibitory target of ezetimibe, were found to be associated with reduced plasma LDL-C levels and a reduced risk of CHD.[106]

Genetic risk predictors of CHD based on lipid-associated loci alone have historically added little to the excellent risk prediction already provided by clinical risk factors, but recent work has shown significant promise in using combinations of SNPs to identify individuals at high risk. Working in the UK Biobank, investigators validated a polygenic risk score that contained SNP combinations conferring threefold risk in CHD, equivalent to the risk conferred by familial hypercholesterolemia. These high-risk polygenic combinations were identified in 8% of the study population, making them 20 times more prevalent than familial hypercholesterolemia. Given the broad indications for statins in primary and secondary CHD prevention,[107] genetics can help predict treatment efficacy and elucidate side effects. GWASs have identified a few reproducible loci (*APOE*, *LPA*, *SLCO1B1*, and *SORT1/CELSR2/PSRC1*) for the trait of LDL-C response following statin therapy.[108] *SLCO1B1* encodes the organic anion transporter, OATP1B1, which has been shown to regulate the hepatic uptake of statins.[109] When exposed to a single dose of simvastatin, individuals carrying a missense variant in *SLCO1B1* (V174A), which causes loss of function, have up to a 2.5-fold increase in plasma levels of statin.[109] A GWAS for statin-induced myopathy performed in a secondary prevention cohort receiving high-dose simvastatin identified the same genetic signal (via a noncoding SNP within the same haplotype) at *SLCO1B1*, which conferred a 4.5-fold risk of myopathy for one allele and a 16.9-fold risk of myopathy in homozygous individuals.[110] The investigators estimated that 60% of the myopathy cases in their cohort were due to this variant at *SLCO1B1*.[110] They performed a GWAS on only 200 case controls for myopathy, and it is likely that additional pharmacogenetically relevant loci like *SLCO1B1* will be identified in future studies with larger sample sizes. Several observational and intervention studies have also associated statin therapy with increased T2D risk. An important question is whether this risk is mediated by an off-target effect of statins or an on-target effect via HMG-CoA reductase. An off-target effect suggests that new,



• **Fig. 3.3** The suggested use of targeted and genome-wide genetic testing is shown in individuals suspected of harboring a monogenic/mendelian disease. *VUS*, variants of uncertain significance (see text).

more specific statins could be developed without this side effect, whereas an on-target effect implies that the development of more potent and specific statins would increase the risk of T2D. Genetics has begun to shed light on this question. Taking a mendelian randomization approach, investigators have found that SNPs at *HMGCR* that decrease LDL-C levels also increase BMI, insulin resistance, and risk for T2D, suggesting an on-target mechanism for statin-mediated T2D risk.

Considerations for Clinical Use of Genetic Information and Sequencing in Endocrinology

Generally speaking, medical application of laboratory testing requires three components. First, a validated test must be established with sufficient accuracy and precision for consistent measurement across testing centers and over time (clinical grade assay). Second, representative control populations of sufficient size must undergo testing to establish a normal clinical reference range. Third, mapping between test values and phenotypic outcome must be established using robust clinical studies. Ideally, these take the form of prospective randomized controlled trials. Expensive in their own right, these studies were prohibitive given early sequencing technology. NGS has provided the first requirement, furnishing high throughput, high-fidelity sequencing at an increasingly reasonable cost. The second requirement is currently under way. Genomics databases are growing around the world, providing a "reference range" for common gene sequences in healthy individuals.

As detailed previously, the clinical applications of genetic information include diagnosis, prognosis, risk prediction, and personalized therapy (e.g., pharmacogenetics). With the barriers of cost and feasibility diminishing every year, we believe that the use of genetic information will become commonplace in clinical practice. To maximize benefits to patients while minimizing the burden of false-positive and false-negative results, clinicians will have to select the right patients, deploy the appropriate genetic testing technology, and properly interpret the results. Specific clinical algorithms for patient/genetic test selection are being proposed for various endocrine diseases (e.g., short stature),[79] but they will take time to validate. With this in mind, we present a series of broad patient scenarios ranging from those with no clinically apparent disease to affected individuals with clinically identifiable genetic syndromes, summarizing benefits and caveats of genetic testing in each scenario. We subsequently review issues related to targeted and genome-wide testing and provide some guidance for patient and test selection. Finally, we provide an overview of disease-relevant classification of genetic findings, examine the interpretation of genetic information as presented in a clinical laboratory report, and make suggestions for clinical decision making (Fig. 3.3).

Genome Screening in the General Population

Population-based screening for mendelian mutations known to cause disease would seem to be an advantageous application of genome sequencing. As mentioned earlier, certain mutations in the *RET* gene predispose to aggressive MTC with such high penetrance that the American Thyroid Association recommends prophylactic thyroidectomy in infants under 1 year of age.[19] As genome sequencing becomes commonplace, it seems reasonable that genomes from the general population (e.g., as part of newborn screening) might be examined for *RET* mutations causing this rare but potentially fatal disease. Cases of cancer might be prevented and lives saved. However, one must consider that the clinical data on *RET* gene mutations have been obtained from kindreds affected by MEN2A/B and familial MTC (see Chapter 42). Are genotype-phenotype correlations between *RET* mutations and MTC, or between other gene mutations and the risk of other diseases, applicable to someone from the general population with no family history of disease?

An investigation of mendelian diabetes mutations ascertained in the general population suggests that genotype-phenotype correlations identified in mendelian disease families may not generally hold for the population at large. As a case in point, genome sequencing of a population-based US cohort phenotyped longitudinally for diabetes identified 25 individuals with mutations previously known to cause autosomal dominant diabetes (MODY). Despite harboring mutations from a curated catalogue of disease genes (Human Gene Mutation Database Professional[111]), only one of these individuals met the clinical criteria for MODY, and overall this group of MODY mutation carriers developed diabetes at a rate no different from that in the general population. As population-based sequencing becomes prevalent, it will become necessary to re-examine estimates of penetrance and genotype-phenotype correlations in the context of differing genetic backgrounds (e.g., ancestry) and environment. The current evidence and state of knowledge do not support genome sequencing for population-based screening.

Genetic Information and Sequencing in Individual Patients

Asymptomatic Individuals

Endocrinologists may be referred individuals with no apparent symptoms in whom risk of disease may be increased: (1) those with a family history of known genetic disease who have not yet been tested, and (2) those without family history who were tested and found with an apparently pathogenic mutation (i.e., a "genetic incidentaloma"). At present, this family history serves as a proxy for genetic predisposition or risk used to calculate pretest probability for screening. As with any medical test, the implication of genetic testing with regard to the likelihood of developing disease in the individual depends on a combination of inherent test characteristics (quantified by sensitivity/specificity) and the pretest likelihood of disease. For individuals with a family history of genetic disease, the pretest probability of disease can be as high as 1 in 2 for a highly penetrant autosomal dominant disorder or 1 in 4 for a sibling of an individual with a recessive disorder. Thus, for individuals with a family history of mendelian endocrine disease, genetic testing is often warranted (depending on the risks and benefits for the individual patient at hand, including psychosocial factors); this fits well with current clinical practice for asymptomatic individuals from families with mendelian disorders.

However, for individuals with no family history presenting with a mendelian-like "genetic incidentaloma," the pretest likelihood of disease is that of the population (1:10,000 to 1:100,000). Even with a mutation conferring 50-fold increased risk, the individual is far more likely to remain free of disease. So what reassurance can be offered to such individuals who carry mendelian mutations? Population-based sequencing surveys reveal that, on average, the genome of an apparently healthy individual contains approximately 100 mendelian-like disruptive mutations (i.e., frameshifting indels and SNPs resulting in premature stop codons), up to 20 of which are homozygously inactivated.[112] Thus, even the protein-coding portion of the human genome (comprising only 1–2% of the total genome) contains unexpected redundancy that protects from disease. Depending on the severity of clinical consequences and the estimate of penetrance in the general population, watchful waiting can be a prudent course of action.

Symptomatic Individuals

Individuals with symptomatic disease can present with clinically defined or unknown syndromes. In both cases, a genetic diagnosis can be of psychological benefit, inform family planning, and sometimes can direct therapeutic screening/intervention. NGS has the potential to improve diagnostic efficiency in these symptomatic individuals. Techniques including GWASs and WES shift the diagnostic workflow from a sequential search for candidate genes to parallel sequencing panels. By simultaneous testing a host of target genes, NGS avoids a lengthy process of diagnosis by exclusion. In addition, NGS panels can be designed to simultaneously ascertain second-order disease modifying mutations that are missed by classic sequential analysis.

The genotype-phenotype correlation of endocrine tumor syndromes (MEN1, MEN2; see previous discussion) are a classic example of the benefits of genetic diagnosis for directed screening and prophylactic interventions in both affected individuals and close relatives. For example, the genotype-phenotype correlation of *RET* oncogene mutations dictates the urgency of prophylactic thyroidectomy (ranging from <1 year old to >5 years old) in children.[19] Thus, even with a clinical diagnosis, genetic testing can have great prognostic value. Another example is the 3-M syndrome (clinically defined by short stature, facial dysmorphism, and skeletal abnormalities) caused by mutations in the *CUL7* gene (among others). Males with this syndrome are at high risk for hypogonadism and should be screened more vigilantly as a result of the genetic diagnosis to prepare for future infertility. Sometimes a genetic diagnosis can allow a patient to avoid lifelong treatment and testing. Individuals with diabetes from *GCK* mutations (MODY2) manifest hyperglycemia but are still able to regulate their blood sugar levels such that they neither require hypoglycemic therapy nor are at elevated risk for secondary complications of diabetes. More generally, and especially for heterogeneous endocrine diseases such as diabetes, genetic classification has the potential to guide pharmacotherapy. An existing example of genetically guided pharmacotherapy can be found in individuals with diabetes caused by *HNF1A* mutations. In clinical trials, these *HNF1A* mutation carriers were much more sensitive to sulfonylureas than noncarriers and maintained more durable glycemic control without additional agents.[55]

As in the cases described previously, genetic testing of affected patients is most likely to be informative when a high penetrance genetic variant is identified (i.e., for rare mendelian-type diseases). We offer the following criteria and mnemonic (SSSS), which should raise clinical suspicion for a mendelian disorder: (1) severe—more severe than usual for the disease; (2) segregating—multiple affected family members or family history of consanguinity; (3) syndromic—features such as dysmorphism, developmental abnormalities, and unusual biochemical phenotypes; and (4) too soon—early age of onset. The diagnostic yield of genetic testing is highly variable both due to technological reasons and underlying genetic (locus) heterogeneity. Even for well-described mendelian syndromes such as MEN1, 30% of kindreds have no identifiable mutations identified by *MEN1* gene sequencing. For affected individuals with unknown syndromes, or for whom targeted sequencing has failed to make a diagnosis, systematic gene sequencing using NGS methods would be required, and diagnostic yields are typically lower. A National Institutes of Health study of exome sequencing for rare disorders found a diagnosis in 20% of cases.[113]

Avoiding Invasive Diagnostics

As ultrasound, computed tomography, and magnetic resonance imaging continue to improve, the number of incidental endocrine findings also increases. NGS provides a tool to evaluate these

findings without resorting to invasive resections. Thyroid nodules present a classic diagnostic dilemma for the endocrinologist. Although most nodules are benign, fine-needle aspiration identifies 100,000 nodules with indeterminate cytology in the United States each year.[114] Several endocrine societies recommend surgical diagnosis via lobectomy or total thyroidectomy for indeterminate cases, although only 5% to 30% of these nodules prove to be malignant.[115] In these cases, genetic diagnosis may help reduce the need for surgery and lifelong thyroid replacement. A recent study applied genetic testing to 176 thyroid biopsies with indeterminate cytology. For these nodules, typical workup includes lobectomy, total thyroidectomy, or close observation. Instead, fine-needle aspiration biopsies were analyzed using NGS to check for mutations associated with thyroid malignancy. The resulting analysis demonstrated a specificity of 91% and prevented diagnostic hemithyroidectomy in 49 patients. Of 46 patients who presented for subsequent ultrasound follow-up, 45 had nodules that remained stable in size, and the exception was benign on repeat biopsy. Successful clinical application of genetic screening tools may thus save patients from the morbidity and cost of invasive surgical diagnosis.[116]

NGS can also help distinguish papillary thyroid carcinoma (PTC) from benign adenomatoid nodules. By analyzing patients with coincident benign nodules and PTC, Ye and colleagues were able to identify *BRAF* somatic mutations (22/32) that are only found in PTC, whereas mutations in *SPOP* (4/38), *ZNF148* (6/38), and *EZH1* (3/38) were enriched in adenomatoid nodules.[117] Several of the adenomatoid markers were found to be mutually exclusive, and phylogenetic tree analysis demonstrated that PTCs develop independently rather than progressing from adenomatoid nodules. This model reveals a distinct genetic origin for invasive thyroid neoplasms that differs from the progressive dysplasia evident in tissues such as colon. The data suggest that thyroid sequencing studies may rule out malignant potential in adenomatous nodules and reduce the need for further surveillance. In the future, specific genetic signatures may increase the specificity of aspiration biopsies and reduce the cost, care burden, and lifelong morbidity associated with thyroidectomy.[117]

Selection of Genetic Tests: Targeted Versus Genome-wide Approaches

If cost were no object, one might consider deploying whole-genome sequencing to maximize sensitivity and find the "smoking gun" mutation in every case. Using genome sequencing in this way is tantamount to performing thousands of genetic tests at once and clinically analogous to ordering every possible hormone level for every endocrine patient. Intuitively, this approach is not pursued because every test carries with it the possibility of false-positive results. The more tests one orders, the more likely at least one result will be false. The same logic applies to genetic tests and genome sequencing. Even if genetic variants are identified with 100% analytical sensitivity and specificity, their clinical sensitivity and specificity for disease risk are much less, due to incomplete penetrance, variable expressivity, and our incomplete knowledge of genotype-phenotype correlations.

Limitations of Genomic Testing

The molecular type of genetic variation (single-base changes or more complex variation, e.g., insertions/deletions/duplications) strongly affects the analytic sensitivity and specificity. The most commonly used gene-panel and genome-wide tests rely on NGS technology, which is currently well suited for detecting single or multiple base variations but poorly optimized for the detection of structural variants (chromosomal rearrangements), large indels, triplet repeat expansions, poly T and A sequences, novel breakpoints, and copy number variation.[118] For example, all false-positive genetic variants identified in a pilot clinical-grade genome sequencing study were indels or were near repetitive DNA stretches.[119] As NGS technology and genome reconstruction algorithms improve, so will the ability to accurately detect these more complex genetic variants, reducing both false-negative and false-positive results. Currently, alternative modes of testing, such as chromosomal microarrays (array CGH), are used to identify large (>50 kb) structural and CNVs.[120]

Even as technology addresses sequencing limitations, proper classification of gene variants limits deployment of sequencing in clinical practice. Recent data from the ExAC database focused on analysis of rare germline coding region, nonsynonymous, single-nucleotide variants (SNVs) in 38 endocrine disease genes. The study concluded that current variant prediction tools suffer from ascertainment bias and dependence on in silico pathogenicity prediction. Ascertainment bias results from dependence on studies that identified rare SNVs in disease cohorts. As genetic screening expands to the general population, the baseline prevalence of "pathogenic" SNVs must be validated in large healthy control populations. Again, a truly pathogenic variant should be enriched in affected individuals and absent in controls. At this stage, too many variants have been called *pathogenic* using disease cohorts without being appropriately excluded due to their presence in healthy controls. Meanwhile, in silico prediction for functional effects of mutations is much less robust for missense mutations than more dramatic frameshift and nonsense mutations. As a result of these two factors, genetic prediction models frequently misclassify benign variants as pathogenic, and the number of identified pathogenic gene variants far exceed reported disease prevalence. Several such examples emerge from the ExAC study in which individuals with mendelian disease are not included. For MEN1, apparently "pathogenic" missense mutations occur in 1:2000 individuals sequenced, whereas disease prevalence is 1:30,000.[121] Ultimately, healthy individuals carry a large number of misclassified "pathogenic alleles" initially identified as rare variants in individuals with disease. As genetic testing becomes more widespread, our definition of pathogenic variations and clinically actionable mutations will require increasing rigor.[121]

Targeted testing (either measuring variation in a requested set of genes or masking/only reporting back variation in the requested set of genes) is one solution to decrease false-positive results. In addition, clinical laboratories that focus on testing specific sets of genes may be particularly well versed in interpretation of variation in these genes, potentially improving sensitivity and specificity. Of course, when targeted testing is negative, then more comprehensive testing may be required to make a diagnosis. However, even beyond the issue of false-positive findings, targeted testing may offer superior analytic performance. Detection of variants can be highly variable in current versions of genome-wide sequencing, in which millions of genetic variants are identified simultaneously.[122] There is often a trade-off between the number of variants identified and the sensitivity/specificity for detecting and calling any individual variant. This analytic sensitivity/specificity relates to coverage, which is the depth of sequencing performed, or the number of independent times a particular nucleotide has been sequenced in a single test. Depth can vary greatly across the genome. For example, a clinical trial of whole-genome sequencing reported clinical-grade genome sequencing at "30× coverage on

average and at least 8× coverage for more than 95% of bases."[119] This means that of the 3 billion bases in the human genome, each base was sequenced independently 30 times on average, and more than 95% of bases were observed at least 8 times. However, 5% of the human genome was still poorly observed or missing. Thus, if clinical suspicion motivated examination of certain genes or genomic regions, a targeted approach might well have higher analytic sensitivity and specificity. The clinical-grade genome described previously is sufficient for detecting variants at a frequency found with germline heterozygosity (on average 50% of the molecules sequenced would contain the variant base), but for disorders requiring detection below germline heterozygosity, such as somatic mutation testing in tumors, higher coverage would be required.

Interpretation of Identified Genetic Variants

Once genetic variants are identified (from sequencing or otherwise), they must be interpreted for their impact on health and disease. This interpretation requires the integration of population data (to know whether a variant is seen at higher frequency than expected for a pathogenic variant), computational predictions, experimental evidence, and familial comparisons. Curated databases are being established to begin to accurately catalogue this information and assist in interpretation. The ClinVar archive aggregates information about genomic variation and its relationship to human health.[123]

Identified variants can be classified into three broad clinical categories: benign variants, pathogenic variants, and variants of uncertain significance (VUS), but intermediate categories have also been proposed.[124] In single-gene or disease-targeted testing, the number of identified variants is small enough to allow for the individual assessment of all variants in each patient, once common benign variations are curated. However, exome sequencing identifies tens of thousands of variants, and genome sequencing identifies several million in each individual.[125] Thus, automated filtering is necessary to point out a few pathogenic variants in a haystack of benign ones. Based on the assumption that the testing was done for a mendelian variant with high penetrance, most genetic variants can be filtered as benign based on having been observed at a frequency greater than 1% (or even lower thresholds for rare disorders) in suitable reference populations. Computational analysis can support a benign classification by showing a variant is *silent* (e.g., encodes the same amino acid as the reference base). This assumption, however, misses rare exceptions such as the *LMNA* Gly608Gly mutation in Hutchinson-Gilford progeria syndrome. Although mutating cytosine to thymine at position 1824 still codes for glycine, this variant introduces a new splice site in exon 11 that eliminates 50 amino acids from the final progerin protein.[126] Conversely, experimental data can provide evidence that a missense variant does not actually alter protein function in disease-relevant bioassays. In addition, family-based data can be extremely powerful for interpretation; for example, most variants for dominant diseases can be classified as benign if they are also observed in healthy relatives. Typically, the filtering of benign variants results in a 100- to 1000-fold reduction in the number of variants, requiring further analysis for 30 to 300 variants.[119,127]

Among these, a similar hierarchy of evidence can support a *pathogenic* designation, or the evidence can remain inconclusive, resulting in a VUS designation. Computational analysis showing predicted gene disruption (premature stop or frameshift variants) in a gene known to cause disease when inactivated provides strong evidence of pathogenicity. Experiments in disease-relevant bioassays can demonstrate deleteriousness of a variant, and family-based data can increase the conclusiveness of findings by showing that a proposed pathogenic variant segregates with disease or that it is absent from both parents in de novo cases of disease. Presence of the variant in databases such as the Human Gene Mutation Database can also provide supportive evidence, but the quality of evidence can vary widely among variants. Before final reporting, variants classified as pathogenic are typically validated by resequencing using traditional methods (i.e., Sanger sequencing),[124] although this practice may shift depending on the state of sequencing technology. In a recent pilot case-control study of 20 cardiomyopathy patients and controls subjected to genome sequencing, two to four variants per individual (in both cases and controls) were classified as pathogenic,[125] illustrating the challenges in interpreting genetic variation in isolation.

Using a Genetics Laboratory Report to Make Clinical Decisions

In the clinic, genetic testing is used to identify or confirm the cause of disease and to help the physicians make individualized treatment decisions (Table 3.5). Given the complexity of genetic testing, especially at the genomic (exome or genome) scale, physicians and clinical laboratories will have to work collaboratively to achieve useful results. For example, when a laboratory finds a rare or novel variant in the course of genomic sequencing, the director cannot assume it is relevant to a patient just because it is rare, or novel. The context of the patient's history, physical examinations, and previous laboratory examinations are key to distinguishing among causal variants for the patient's disorder, incidental findings, and benign variants.

How should identified variants be used in clinical decision making? Variant analysis contains uncertainties that are implicitly defined in the three-part classification described earlier: benign, pathogenic, and VUS. American College of Medical Genetics guidelines[124] recommend that variants classified as pathogenic can be used in clinical decision making. However, genetic evidence should not be the only evidence of disease and should be used in conjunction with complementary clinical information when possible, especially as some pathogenic variants may be misclassified. Examples of corroborative clinical data include enzyme assays, physical findings, and imaging studies. VUS should generally not be used in clinical decision making. Efforts to resolve the classification of the variant as pathogenic or benign should be undertaken, and interpretation in the context of the patient's clinical scenario is critical. Variants classified as benign can usually be assumed not to cause the patient's disorder. Detection of pathogenic variants incidental to the diagnostic motivation for sequencing, but of potential clinical relevance, will be an inevitable consequence of genomic testing. This scenario is analogous to the inevitable detection of adrenal masses in computed tomography scans or thyroid nodules on physical examination.

A clinician ordering genomic testing should be aware of laboratory policies and current ethical guidelines regarding such incidental or secondary findings. Current recommendations are to offer the patient the option not to receive such incidental findings, and laboratories may vary in their reporting of such incidental findings. From both the clinician and patient perspectives, incidental findings can also be specifically requested or declined. The laboratory should provide clear information about what constitutes a reportable incidental finding and how a finding may be requested

TABLE 3.5 Examining a Clinical Genetics Laboratory Report

A clinician considers several issues when examining a clinical genetics laboratory report.

Technical Summary of Genetic Testing

For targeted tests, the list of genes tested and coverage for each gene can be found at the Genetic Testing Registry (http://www.ncbi.nlm.nih.gov/sites/GeneTests). For genomic tests, average coverage (e.g., >30×) and minimum coverage (e.g., >8× for 95% of bases) should be considered in evaluating test quality. Genes with poor coverage or no coverage should be listed. For both targeted and genomic testing, the specific limitations of detection for different molecular classes of genetic variants given the technology utilized (e.g., copy number variants are not well captured by next generation sequencing) should be described.

Clinical Interpretation

With regard to the clinical indication for testing, was the test positive (an explanatory variant identified for the patient's condition), negative (no explanatory variants identified), or inconclusive (only variants of uncertain significance identified)? Does the potential explanatory variant fit with the clinical scenario or at least explain some of the patient's phenotypes? With regard to incidental findings, is there a carrier risk to future progeny or future risk of monogenic disease to the patient? A compendium of monogenic diseases and their patterns of inheritance can be found at the Online Mendelian Inheritance in Man website (https://www.omim.org/).

Variant Reporting and Classification

When applicable, the gene name, transcript, molecular form of variant (single-nucleotide polymorphism, indel, etc.), base changes, amino acid change, zygosity, population frequency, and classification (benign, pathogenic, variants of uncertain significance) should be provided. Naming conventions are determined by the HUGO Gene Nomenclature Committee (http://www.genenames.org). Variant frequencies in the population can be found at the 1000 Genomes Project (http://www.1000genomes.org) and for exomes at the Exome Aggregation Consortium (http://exac.broadinstitute.org/). The justification for variant classifications should be provided, taking into account family-based data if available and information from clinical databases. Previously reported relationships among variants and phenotypes can be found at ClinVar (http://www.ncbi.nlm.nih.gov/clinvar).[123] The analytic accuracy of each reported variant in terms of coverage and validation (e.g., Sanger resequencing) should be reported.

or declined. Guidelines have been set forth in the American College of Medical Genetics recommendations for reporting of incidental findings in clinical exome and genome sequencing.[128]

Future Perspectives and Summary

In the future, we anticipate that a genome sequence will become a standard accompaniment to the medical chart; thus, the question "Should we sequence?" will transmute to "What part of the sequence should we look at?" A rational clinical approach will require the discipline to not look at all of it, or at least to rigorously interpret sequence data in the clinical context. As detailed previously, every human genome is littered with thousands of VUS and multiple variants classified as pathogenic; clinical suspicion is essential to help direct where to look and how to interpret genetic variation. This approach recapitulates current clinical algorithms for genetic testing. For example, familial paraganglioma shows locus heterogeneity as a mendelian disorder with cases attributable to mutations in multiple genes encoding proteins for the succinate dehydrogenase complex. The current genetic testing algorithm is hierarchical, starting with the gene that best fits the clinical presentation of the index case (e.g., sequencing of the *SDHB* gene if the neoplasm is abdominal or malignant vs. *SDHD* if located in the head or neck). If no mutations are found in the most likely candidate gene, other complex members are tested (e.g., *SDHC*). In a genome-sequencing era, a clinical algorithm might hierarchically look up mutations in the succinate dehydrogenase complex members from a sequenced individual with familial paraganglioma syndrome. No additional sequencing costs would be incurred as each gene is subsequently tested, but honing in on the appropriate and interpretable areas of the genome will reduce the clinical burden of false-positive results.

As mentioned earlier, changes in DNA sequence are not the only means of passing on inherited information. Looking beyond primary sequencing data, epigenetic changes including DNA base methylation and histone modification are likely to become essential for accurate genetic diagnosis. Epigenetic changes can permanently and heritably alter the expression of genes and thus clinical phenotypes. Methylation of cytosine residues in CpG dinucleotides of DNA is so ubiquitous in the genome that it has been referred to as the "5th base."[129] Hypermethylation at promoters reduces gene expression by decreasing affinity for transcription factors and increasing recruitment of methylated DNA-binding gene repressors. DNA methyltransferases reproduce these methylation patterns in daughter cells and generate new methylation patterns according to environmental influences and extracellular signaling.

The clinical impact of inherited methylation on genetic disease is just beginning to be understood. Recent work assessed the genome-wide methylation state in more than 5000 individuals with respect to T2D and obesity in a process analytically similar to GWASs. Of the 187 methylation sites associated with increased adiposity and T2D, 38 were found in loci harboring genes such as *ABCG1*, a known regulator of lipid homeostasis and insulin resistance. These methylation loci predicted obesity and T2D independently of SNP variation and outperformed inflammatory markers such as C-reactive protein.[130]

In summary, genetic information is most likely to be of clinical use in individuals with suspected mendelian syndromes (see the SSSS criteria enumerated earlier). For individuals with a clinically defined syndrome, for which targeted panels exist and are well validated, a targeted approach (single-gene or gene panel testing) is currently recommended as an initial approach. For example, genome-wide sequencing is likely not needed when MEN2B is suspected on clinical grounds; sequencing *RET* will usually make a diagnosis. If results from targeted genetic testing are uninformative, and the suspicion of a genetic disorder is high, exome or genome sequencing will make additional diagnoses in some patients (see Fig. 3-3). We recommend primary genome-wide approaches that assess both structural variation and sequence variation for individuals with clinically unclassifiable genetic syndromes or when targeted panels are not available or well validated. Depending on technological progress, this may simply be an unmasking of data that had not been reported back in a targeted test or may require new sequencing. The exome comprises 1% to 2% of the genome yet contains nearly 85% of known disease-causing mutations.[131] Thus, given current technologies, exome sequencing supplemented with or following array CGH is a reasonable initial genome-wide approach.

Many other best practices will improve the outcome of genetic diagnosis through sequencing. Ideally, DNA from both parents should be obtained, if possible, and DNA from additional relatives may also aid in interpretation. If unaffected and affected tissues are identified, paired affected tissue-blood samples should be obtained when possible. Identified variants can be classified as benign, pathogenic, or VUS, based on cross referencing with databases of diseased and undiseased individuals, as well as family members; computational analysis; and experimental evidence. Indeed, to maximize interpretability of any genomic approach, it will be vital to interpret variation in the context of massive numbers of genome sequences obtained in healthy individuals and in patients with disease. Finally, accurate classification will require physicians and clinical laboratories to work collaboratively, and the resulting genetic information should always be used in conjunction with complementary data (chemistries, imaging, etc.) for clinical decision making.

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The complete list of references is available online at ExpertConsult.com.

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4

Laboratory Techniques for Recognition of Endocrine Disorders

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CHAPTER OUTLINE

KEY POINTS

- The practice of endocrinology relies heavily on accurate laboratory measurements. Small changes in hormone levels, biomarkers, or molecular markers are often more specific and earlier indicators of disease than the appearance of physical symptoms.
- Analytic methods for assessing endocrine problems are continually expanding. Traditional measurement of endocrine factors, protein, and steroid hormones and related factors has been supplemented by a wide array of disease biomarkers, particularly with respect to endocrine cancers.
- Newer systems are often manufactured outside the laboratory. Although the configurations are generally more user friendly, they also become more of a "black box," concealing most of the details of the system. Numeric values, especially when reported to several decimal places, can falsely suggest levels of accuracy and reproducibility beyond the technical limits of the technology used.
- Understanding the basic principles of method validation and quality control is essential if endocrinologists are to assess the reliability and robustness of numeric values reported and to work effectively with the laboratory to reconcile test values that do not match clinical presentations.
- Laboratory testing as practiced today contributes significantly, both directly and indirectly, to the cost of care, which over the past decade or so has increased faster than improvements in clinical outcomes. Clinicians and pathologists are increasingly required to understand the inner workings of laboratory medicine and work as a team in determining optimal management strategies to contain the costs of care without compromising quality.

Endocrinology is a practice of medicine that is highly dependent on accurate laboratory measurements. Small changes in hormone levels, biomarkers, or molecular markers often may be more specific and more sensitive for early disease detection (or risk) than the classic physical signs and symptoms. Most endocrinologists no longer have facilities to develop and validate laboratory assays. They must rely on centralized hospital or commercial reference laboratories. Understanding the nuances of laboratory testing can greatly aid the clinician in working with the laboratory, particularly when faced with disparate clinical observations and laboratory results.

Laboratory testing as practiced today contributes significantly, both directly and indirectly, to the cost of care, which, over the past decade or so, has increased faster than improvements in clinical outcomes.[1] Current treatment guidelines, especially in endocrine practice, rely heavily on early laboratory testing. Thus clinicians and pathologists are increasingly required to understand the inner workings of laboratory medicine and work as a team in determining optimal management strategies to contain the costs of care without compromising quality.

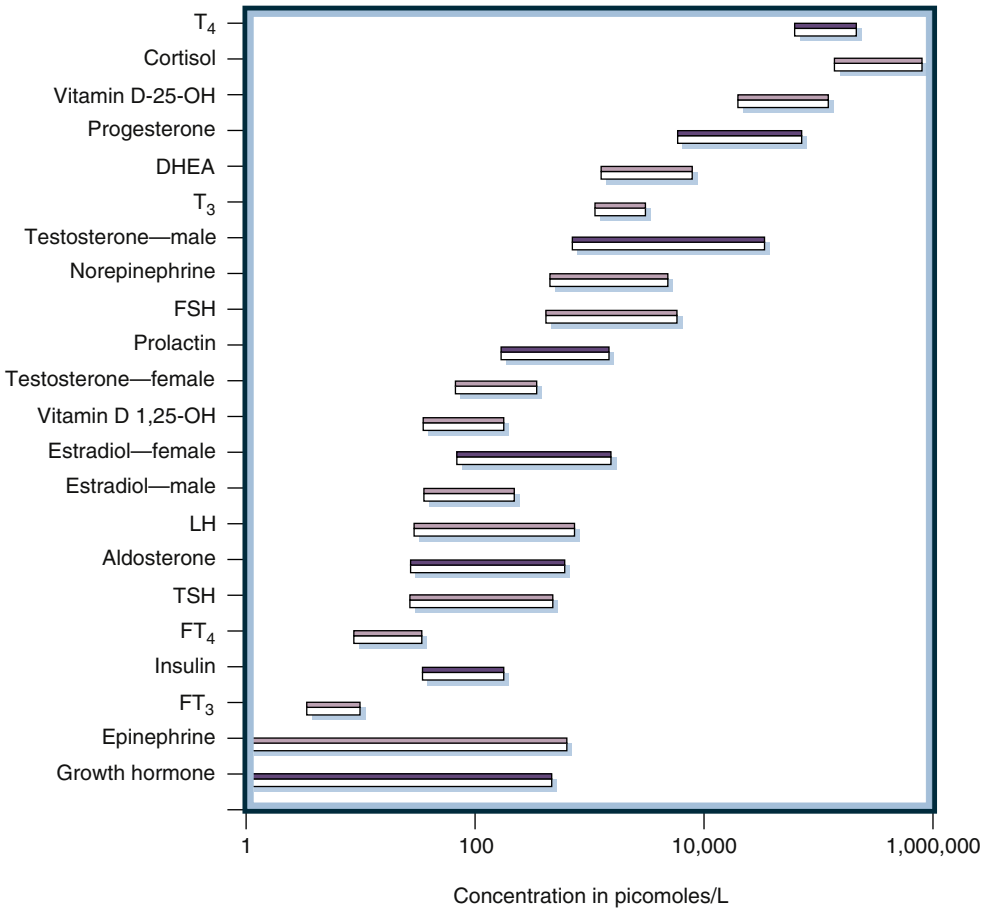
This chapter provides an overview of the analytic techniques typically used for diagnosing and monitoring the progress of endocrine disorders. Historically the quantitative measurement of endocrine factors, protein, and steroid hormones and related factors, such as steroid binding proteins, in blood and urine has been the primary goal. More recently, wide arrays of disease biomarkers, particularly with respect to endocrine cancers, have become valuable targets for measurement in the clinical laboratory. Analytic validation is then discussed. The parameters of analytic validation are not method specific. Principles are presented to help endocrinologists better assess the performance of the analytic systems that they are using. Techniques used by clinical laboratories to control

and assure quality testing results and services follow to provide guidance in appreciating the reliability and robustness of numeric values reported and in working with the laboratory to reconcile test values that do not match clinical presentations. Finally, especially for the academic practitioner, the classes of assays are discussed to provide some clarity on the regulatory requirements laboratories are required to meet in providing test results for patient care, federally supported human studies, and federally regulated clinical trials.

Laboratory Methods

Historically laboratory methods unique to the clinical practice of endocrinology were directed at the measurement of peripheral levels of hormones or hormone metabolites in serum, plasma, and/or urine. This measurement is analytically challenging because concentrations of most hormones are much lower than those of general chemistry analytes. Specialized techniques are necessary to measure these low concentrations that can be reported in molar units, mass units, or standardized units, such as the World Health Organization (WHO) international unit (IU). Fig. 4.1 illustrates the concentrations of representative hormones in plasma from healthy individuals. Expressed in molar units to allow direct comparisons, peripheral hormone levels range from 10^{-6} to 10^{-12} mol/L (i.e., micromolar to picomolar concentrations). Thus clinically useful analytic methods must have exquisite sensitivity. Furthermore, as also illustrated in Fig. 4.1, the range of concentrations is very broad (often several orders of magnitude), necessitating methods with a very wide dynamic range of measurement. Antibody-based methods are ideally suited to achieve sensitivity and wide dynamic ranges and were the first methods successfully used both to define endocrine systems and to be applied clinically in patient care. Because of their cost effectiveness, suitability for high throughput implementation, and potential for automation, antibody methods replaced earlier chromatographic/mass spectrometric methods that were used in the discovery and characterization of hormones, particularly steroid hormones. Initially, competitive binding assays using polyclonal antibodies were utilized; then with the development of monoclonal antibody technology in the 1980s immunometric, or double antibody, methods were utilized. Both of these analytic designs are automated and are in widespread use today: Competitive binding assays are used for measuring small molecules and immunometric assay is used for measuring antigens containing multiple antibody-binding epitopes (i.e., protein hormones and biomarkers).

As discussed in detail later, antibody-based assays are subject to interference and lack of specificity that can result in inaccurate measurements. Even when a given assay has been well validated and reference intervals are known (see discussion under "Analytic Validation"), this limitation is manifest as producing measurements that are method specific, vitiating the ability of clinicians to compare measurements reported using different assays (e.g., assays



• **Fig. 4.1** Six-logarithm range of normal plasma concentrations in endocrine tests. *DHEA,* dehydroepiandrosterone; *FSH,* follicle-stimulating hormone; *FT_4,* free thyroxine; *FT_3,* free triiodothyronine; *LH,* luteinizing hormone; *T_3,* triiodothyronine; *T_4,* thyroxine; *TSH,* thyrotropin.

from different laboratories) for the same hormone or biomarker. Although preanalytic methods such as extraction and chromatography have been tried to improve the accuracy of immunoassays used in research settings, these methods are seldom utilized in clinical laboratories today because of their high cost, complexity, and lack of commercial availability; all are by definition laboratory-developed tests (see "Classes of Assays").

Since the early 2000s, technologic advances in mass spectrometry–based assay systems have led to the rapid and ongoing replacement of antibody-based methods for the clinical measurement of hormones and biomarkers relevant to the endocrine practice. Currently these more complex and expensive methods are utilized primarily by commercial reference and large academic hospital laboratories, but as the technology becomes more cost effective and user friendly, its use will clearly increase. Thus it is important for clinicians to appreciate the principles of these assays as well as those of the older, albeit still widely used, antibody-based methods.

The final technologies considered in this section are molecular-based assays. These methods are not specifically designed for endocrine practice but are generic for identifying and in some cases quantifying genetic variance. Subsequent to the sequencing of the human genome and the continuing evolution of molecular methods and knowledge, these methods are rapidly penetrating endocrine practice. Although these methods are still in the early stages of clinical use and generally require specialized informatics and interpretative support, laboratories are increasingly providing molecular-based testing with respect to determining endocrine cancers, inherited disease, and individualized therapeutics.

Antibody-Based Methods

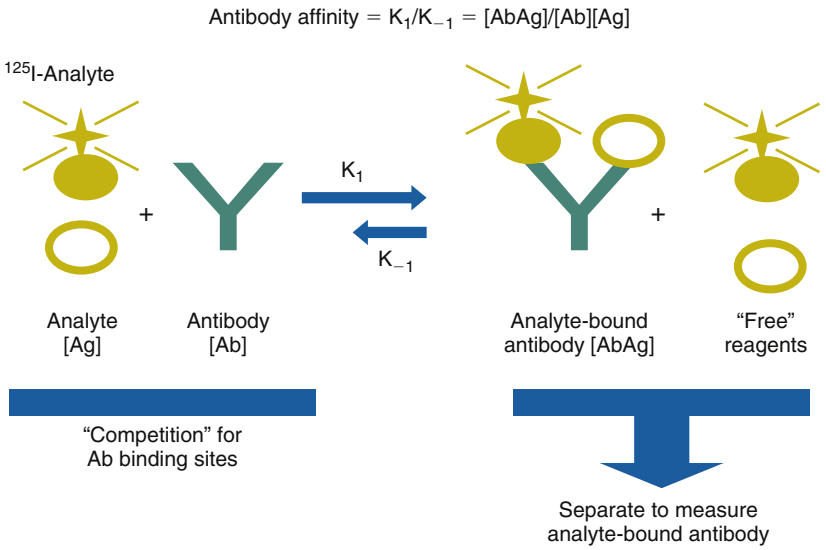
Classic Competitive Binding Immunoassays

The term *competitive binding assay* refers to a measurement method in which an analyte (e.g., a hormone or biomarker) in a specimen competes with a labeled reagent analyte for a limited number of binding sites on a binding protein. The earliest clinical assays used for the measurement of circulating concentrations of endocrine hormones utilized radioisotope-labeled analytes and antibodies in the classic radioimmunoassay format illustrated in Fig. 4.2. The three basic components of a competitive immunoassay are antibody, labeled analyte, and unlabeled analyte.[2,3] The basic principle of this methodology is to allow an equilibrium or steady-state condition (e.g., competition) to be established between a labeled analyte and the unlabeled analyte in calibrators or specimens binding to the antibody. The reaction obeys the law of mass action and is driven by the affinity of the antibody (Ab) for the target analyte, or antigen (Ag), as shown in Fig. 4.2. If the concentrations of antibody and labeled analyte are held constant, the amount of labeled analyte bound is inversely proportional to the concentration of the competing unlabeled analyte, as illustrated in Fig. 4.3. By comparing the percentage of bound antigen (% [bound/total]) generated by an unknown specimen to the dose-response curve generated by known concentrations of analyte (see Fig. 4.3B), the amount of analyte in a specimen can be quantified.

Competitive antibody-based assays are referred to generically as immunoassays. The analytic sensitivity of a competitive immunoassay is approximately inversely related to the affinity of the antiserum, such that an antiserum with an affinity constant of 10^9 L/M can be used to measure analytes in the nanomolar concentration range. This methodology has evolved significantly since the development of the prototypical radioimmunoassay in the late 1950s. Currently, although radioimmunoassays still have a role in the research laboratory, the immunoassays most widely utilized in clinical endocrine testing are fully automated, nonisotopic instrument systems whose manufacture and reagents are regulated by the Food and Drug Administration (FDA). Each of the component parts of the competitive immunoassay is discussed in detail next.

Antibody

Antibodies are ideal as the binding component in a competitive binding assay that is highly specific and can measure very low concentrations of analyte in complex mixtures such as serum or plasma. Antibodies are inherently specific, and both their specificity and affinity can be manipulated in developing immunoassays. Immunoassays developed prior to the mid-1980s relied upon polyclonal antiserum produced in animals. Limited quantities of high-affinity antisera that react primarily with the specific target antigen are obtained and can be used either as diluted antiserum or, most often, as purified immunoglobulins.



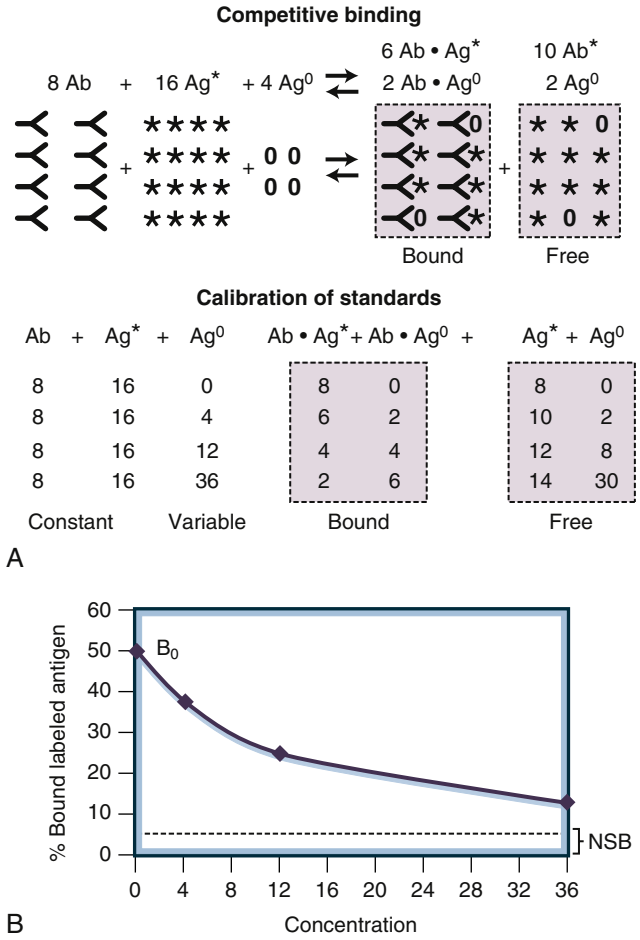
• **Fig. 4.2** Components of a radioimmunoassay; a prototype competitive binding methodology.

A polyclonal antiserum represents a composite of many immunologic clones, with each clone having a different affinity and different antigenic epitope specificity. Most clones have affinities in the range of 10^7 to 10^9 L/M. The affinity of the antiserum or purified immunoglobulins for the analyte (i.e., the immunogen) is the sum of the affinities of all the various clones. Antisera used in immunoassays typically have affinity constants above the 10^{12}-L/M range and can easily measure picomolar concentrations of analytes in biologic fluids. Various techniques are used to develop a specific antiserum. For example, the antigen may be altered chemically to block cross-reacting epitopes either before or after immunization. Historically immunoaffinity purification of antiserum to obtain epitope-specific immunoglobulins has been effectively used. This technology can also be applied to preanalytic assay steps to enhance the specificity of immunoassays as well as enrich specimens for chromatographic or mass spectrometric assays by selecting or eliminating cross-reacting factors.[4–6] The major disadvantage of a polyclonal antiserum is the limited quantity produced. Commercial manufacturers require large quantities of immunoassay reagents to support high-volume clinical testing performed by a large number of laboratories, and these reagents require rigorous validation. Thus the majority of commercial immunoassay systems available today are based on monoclonal antibodies that can be produced in virtually limitless quantities.

Monoclonal antibodies are used in most current immunoassays and are required for immunometric assays because they are epitope, as opposed to antigen, specific and can be produced virtually without limit. These antibodies are obtained by immunizing animals using techniques similar to those used for polyclonal antisera. Instead of harvesting the antisera from the blood, lymphocytes from the spleen are fused with myeloma cells to make cells (hybridomas) that will grow in culture continuously and produce monospecific antibodies.[7–10] These fused cells are separated into clones by means of dilution plating techniques similar to those used in subculturing bacteria. The supernatant of pure monoclonal cell lines (or ascites fluid if the cells are transplanted into carrier mice) contains monoclonal antibodies. The selection processes used to separate the initial clones can be targeted to identify specific clones, producing antibodies with high affinities and low cross-reactivity to related compounds.[11]

The epitope specificity of monoclonal antibodies allows assays to be designed for large analytes (containing multiple nonoverlapping epitopes), which do not depend on competition; these immunometric assays are often referred to as *two-site* or *sandwich assays* (see later). However, the high specificity of monoclonal-based assays can cause problems for some endocrine assays. Many hormones circulate in the blood as heterogeneous mixtures of multiple biologically active forms. Some of these forms are caused by genetic differences in patients; others are related to metabolic precursors and degradation products of the hormone. Genetic differences cause some patients to produce variant forms of a hormone, such as luteinizing hormone (LH). These genetic differences can cause marked variations in measurements made using assays with specific monoclonal antibodies, compared with more uniform measurements made using assays with polyclonal antisera that tend to cross-react with the multiple forms.[12] Well-characterized monoclonal antibodies can be mixed together to produce engineered polyclonal antibodies with improved sensitivity and specificity.[13]

Cross-reactivity with precursor forms of the analyte and with metabolic degradation products can cause major differences in assays. For example, the degree of cross-reactivity among six molecular forms of human chorionic gonadotropin (hCG) causes differences in hCG assays. Similarly, cross-reactivity among various circulating metabolic fragments causes method-specific differences in parathyroid hormone (PTH) assays.[14,15] Cortisol is an example of an analyte for which major cross-reactivity with other steroids, such as corticosterone, 11-deoxycortisol, cortisone, and numerous synthetic steroids, causes significant immunoassay interferences.[16] Matrix effects with albumin also can cause major differences in cortisol immunoassays (see "Mass Spectrometry" for a more robust method for measuring steroids).[17]



• **Fig. 4.3** Quantitation using competitive binding assays. (A) Principles of competitive binding assays. *Ab,* antibody; *Ag*,* labeled antigen; Ag^0, native antigen. See text for details. (B) Typical dose-response curve. The point on the curve labeled B_0 represents the percentage binding of the radiolabeled antigen when zero native antigen is present. The nonspecific binding (NSB) level is the minimal binding level of radiolabeled antigen at high concentrations of native antigen.

Labeled Antigen

In radioimmunoassays, radioactive iodine (^{125}I or ^{131}I) was originally used to label the antigen. Subsequently a large variety of methods have been developed to label the analytes.[18–23] Today most commercial kits and all automated immunoassays use nonisotopic signaling systems to measure hormone concentrations. These assays use colorimetric, fluorometric, or chemiluminescent signals rather than radioactivity to quantify the relative amount of antigen bound to the antibody used in the assay. The advantages of these nonisotopic labeling technologies include biosafety,

longer reagent shelf life, ease of automation, and reduced cost. On the other hand, they can be more subject to matrix interferences than radioactive detection systems. Radioactivity is not affected by changes in protein concentration, hemolysis, color, or drugs (except for other radioactive compounds), whereas many of the current signal systems can yield spurious results when such interferences are present. Later in this chapter, potential troubleshooting steps are outlined to help clinicians evaluate the integrity of test measurements when spurious results are suspected.

The specificity and accuracy of labeled antigen assays (e.g., competitive assays) depend on the purity of the labeled antigen. Especially with respect to labeling small molecules, such as steroid hormones, purification of the labeled antigen can be challenging and certainly contributes to lot-to-lot variance in assay performance. Additionally, sensitivity in this assay design is influenced by the specific activity of the labeled product (i.e., the amount of label incorporated into the antigen on a molar basis). An alternative design for competitive assays is to attach the antigen to a solid phase and label the antibody. A competitive binding assay is then achieved by allowing unlabeled antigen to compete with solid-phase antigen for labeled antibody binding. Although this addresses issues associated with antigen labeling, this format is subject to similar issues associated with modification of antibody binding characteristics as the antigen is chemically attached to a solid phase or due to restricting its conformation once the antigen is attached.

Unlabeled Antigen

Labeled and unlabeled antigens must compete for a limited number of binding sites. The competition is not always equal because the labeled antigen (tracer) and the native antigen may react differently with the antibody. This disparity in reactivity may be caused by alteration of the antigen due to labeling, as discussed earlier, or by differences in the endogenous antigen compared with the form of the antigen labeled for use as an assay reagent. The latter is a problem often encountered with protein hormones or biomarkers that often exhibit a wide range of isoforms and degradation products in peripheral circulation. For this reason, immunometric assay designs are preferable for proteins with multiple antibody-binding epitopes.

Because an assay can be calibrated with certified reference materials having known concentrations, differences in reactivity of labeled compared to unlabeled antigens do not prevent obtaining useful clinical measurements as long as the reactions are reproducible and appropriate reference intervals are established. Such differences do, however, result in method-specific measurements, and in this case assay results cannot be extrapolated across assays using different reagents.

Separation of Reactants/Automation

As illustrated in Figs. 4.2 and 4.3, immunoassays depend on detecting only the labeled antigen bound by the antibody. Thus the entire antibody-bound antigen component of the assay must be recovered and separated from any unbound reactants (i.e., labeled or unlabeled antigens not bound to antibody). Over the years since the introduction of radioimmunoassays, vast arrays of technologies have been developed to accomplish this separation. Approaches vary from methods to precipitate immunoglobulins and recover them by centrifugation or filtration to innovative ways to create solid-phase antibodies (i.e., antibodies attached to solid surfaces that can be washed to remove unreacted reagents after the binding process is completed).

Separation of immune complexes by precipitation and centrifugation is labor intensive and, like the use of radioactivity itself, not amenable to full automation. This approach is still widely used in research applications but seldom utilized in clinical testing. In contrast, solid-phase approaches are widely used and can be batch or fully automated. Three frequently used solid-phase materials are microtiter plates, polystyrene or latex beads, and paramagnetic particles. Most recently the use of immunoassay systems at the point of care and miniaturized assay systems are driving the development of novel solid-phase antibody systems.[21,23–25] Separation of solid-phase immune complexes from the unbound moieties is accomplished by plate washers, bead washers, magnetic wash stations, or microfluidics.

Antibodies can be attached to solid-phase materials directly or indirectly. Antibodies can be passively attached directly to plastic surfaces by hydrophobic interactions, and this method is often used in the manufacture of enzyme-linked immunosorbent assays (ELISA). Automated assays, requiring inline capture and washing systems, typically involve chemical procedures in which amino acid groups or carbohydrate groups on the Fc portion of immunoglobulins are covalently coupled to solid phases such as beads or magnetic particles. This can be achieved directly by coupling the antibody used in the assay to the solid phase or indirectly by covalently coupling a universal capture to the solid phase. Examples of universal capture systems are solid-phase particles with covalently attached streptavidin to capture biotinylated assay antibodies or solid-phase particles covalently coated with goat antimouse IgG as attachment moiety for mouse monoclonal-based assays. Another novel way of accomplishing this separation is to attach high-affinity linkers to antiserum, which then can be coupled to a complementary linker on the solid phase.

Quantitation

Fig. 4.3 illustrates the principles of quantitative measurement using competitive immunoassay techniques. In the schematic diagram, 8 units of antibody react with 16 units of labeled antigen and 4 units of native antigen. At equilibrium (assuming equal reactivity), 6 units of labeled antigen and 2 units of native antigen are bound to the limited supply of antibody. The antigen bound to the antibody is separated from the liquid antigen by any of several methods, and the amount of labeled antigen in the bound portion is quantified (see Fig. 4.3A). The assay is calibrated by measuring standards with known concentrations and cross-plotting the signal (i.e., counts of the gamma rays emitted from the radioactive label) versus the concentration of the standard to generate a dose-response curve. As the concentration increases, the signal decreases exponentially (see Fig. 4.3B).

Statistical data-processing techniques are needed to translate the assay signals into concentrations. These dose-response curves typically are not linear, and numerous curve-fitting algorithms have been developed. Before the introduction of microprocessors, tedious, error-prone manual calculations were required to mathematically transform the data into linear models. Today, curve fitting usually is accomplished electronically with the use of programs that test the robustness-of-fit of multiparameter curves after statistically eliminating discordant data points.[26,27] However, users of these systems must understand their limitations and should pay attention to any warnings presented by the programs during processing of the data. Commercial immunoanalyzers, used by the majority of clinical laboratories currently, are closed systems. The manufacturer validates not only the method (see "Analytic Validation") but

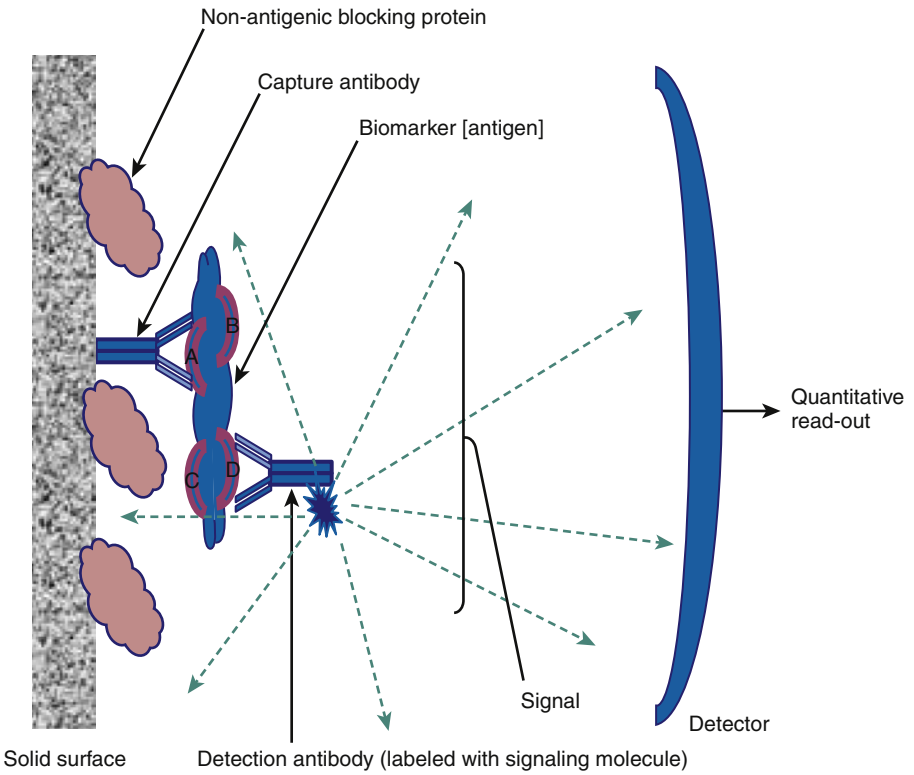
also the curve-fitting software, which cannot be altered by the user. Thus clinical laboratories and clinicians see only the final values for the signal generated and calculated analyte concentration for a given specimen.

In clinical practice today, competitive assays are used primarily for the measurement of small molecules, such as steroid hormones or bioactive peptides, which present only one antigenic epitope. For molecules in which multiple epitopes are present, allowing more than one antibody to bind each molecule, two-site or immunometric assays are used. Immunometric assays, discussed in detail in the following section, are advantageous because they do not require the time-consuming establishment of a binding steady-state condition and thus can be performed much faster. Speed of test performance is an important factor in the clinical laboratory supporting acute care. Speed is also directly related to high testing throughput, which is an important cost factor to optimize in modern clinical laboratories.

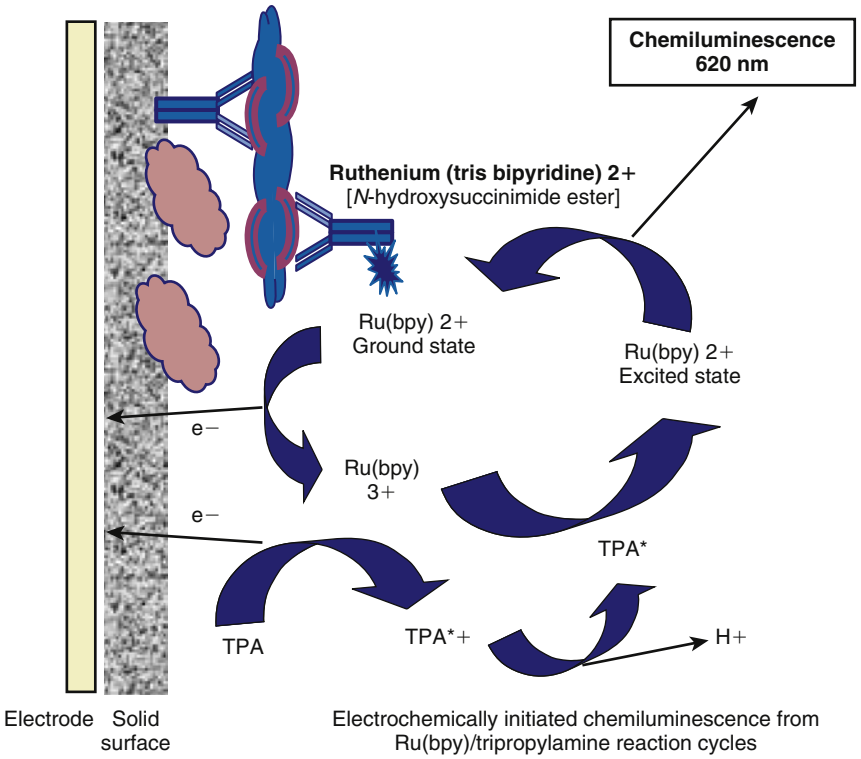
Immunoassays measure antigen concentrations rather than biologic activity. The reactive site for most antibodies is relatively small, about 5 to 10 amino acids for linear peptides. Some antibody reactions are specific for the tertiary structure that corresponds to unique molecular configurations. In either case, linear or conformational antigenic epitopes, the structural elements of the hormone involved in receptor activation and biologic signaling, are not necessarily identical to antigenic epitopes recognized in the assay. Clinicians must keep this in mind when interpreting the results of antibody-based assays. When measurements are ordered to identify abnormal secretion of hormones, the possible disparity between antigenic and biologic epitopes is not as relevant as when measurements are ordered to assess the endocrine stimulus received by the target glands.

Epitope-Specific Immunometric Assays

As briefly mentioned earlier, for larger analytes that contain more than one nonoverlapping antigenic epitope, the development of methods to produce monoclonal antibodies facilitates a unique assay design in which two antibodies are used. This format is illustrated in Fig. 4.4. The analyte in this example has four nonoverlapping epitopes: A, B, C, and D. A solid-phase monoclonal antibody (referred to as the capture antibody) that is specific to one site (in this example, A) can be used to bind the antigen in calibrators or specimens. Using a second, labeled monoclonal antibody (referred to as the detection antibody) that is specific to one of the other epitopes (in the example, D), the captured antigen can be quantified after washing away the unreacted reagents. Because there are four distinct antibody-binding sites on the analyte, 12 different assays can be configured using nonoverlapping epitope-specific monoclonal antibodies to each of these epitopes. It is important to realize that each of these 12 formats is a distinct assay with unique performance characteristics, each requiring validation. The detection systems used include all the options discussed earlier for labeling protein antigens in immunoassay formats. Fig. 4.5 illustrates one of the most common signaling systems used today in either fully automated clinical immunoanalyzers or as multiwell plate assays. The detection antibody is covalently labeled with ruthenium (tris bipyridine), which can be excited by an electric circuit that draws an electron from the molecule, leading ultimately to a high-energy state that will emit light when it decays; this is an electrochemiluminescent signaling system.[28] The assay buffer contains an excess of the electron donor tripropylamine (TPA). The Ru^{2+} (tris bipyridine ruthenium metal cation) complex is used as the chemical luminescent label. Ru^{2+} undergoes an electrochemical



• **Fig. 4.4** Components and design of an immunometric assay. See discussion in text. (From Sluss PM. Methodologies for measurement of cardiac markers. *Clin Lab Med.* 2014;34:167–185.)



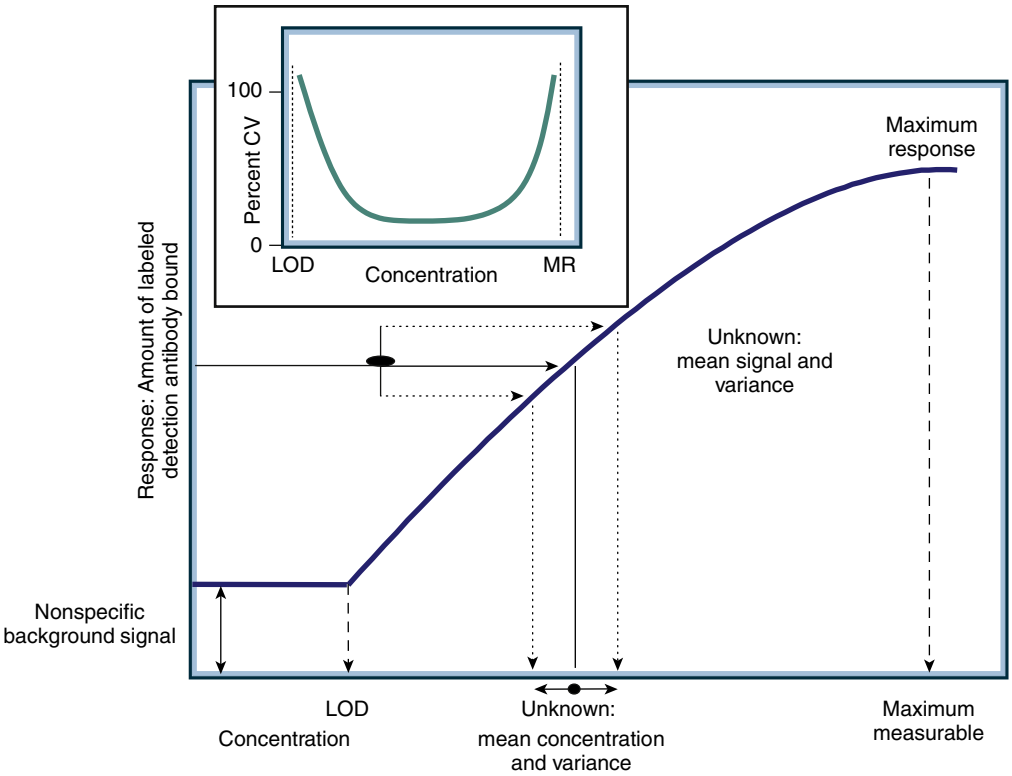
• **Fig. 4.5** Electrochemiluminescence detection system as employed in an immunometric assay. See text for details. *byp,* bipyridine; *TPA,* tripropylamine. (From Sluss PM. Methodologies for measurement of cardiac markers. *Clin Lab Med.* 2014;34:167–185.)

oxidation reaction on the electrode surface and transitions to an excited state to become Ru^{3+}. When the excited state returns to the ground state, light is emitted. The magnetic particles that are captured on the electrode are immunocomplexes that consist of sample and Ru metal complex (Ru^{2+}) and emit light at a specified voltage. The amount of light emitted is proportional to the amount of the immunocomplex and thus the analyte concentration in the sample. It can therefore be used for quantitative measurement. This design is typical of modern detection systems in that the signal generated is controlled in the analyzer (in this case, light production initiated by activating the electrode), and a regenerating system (in this case, TPA in the assay buffer) is used to enhance the signal generated, hence achieving high sensitivity detection.

In contrast to competitive immunoassays, immunometric (e.g., two-site, double antibody) assays use a large excess of antibody-binding sites relative to the concentration of antigen. The capture antibody immunoextracts the antigen from the sample, and the signal antibody binds to the capture antibody-antigen complex to form a tertiary complex. These assays are referred to as *immunometric* because the binding reaction is very fast (first-order kinetics due to excess antibody) and because it is not necessary to establish a binding steady state (a requirement for competitive assays) in the assay before quantifying the amount of label associated with the immune complex. Immunometric assays can be performed very quickly (5–20 minutes compared to 30 minutes to days for competitive assays) and typically have very broad measuring ranges (several log orders).

In contrast to competitive assays, the dose-response curve generated in an immunometric assay is directly proportional to the analyte concentration (Fig. 4.6). The signal increases progressively with the analyte concentration because the amount of labeled antibody bound increases proportionally to the amount of analyte present. Quantitative measurements are achieved in the same manner as those used in competitive assays. The signal generated by the specimen (the "unknown" in Fig. 4.6) is compared to a calibration curve generated by known concentrations of the analyte (plotted on the x-axis in Fig. 4.6).

As with any assay, there is a minimum detection limit (referred to as the limit of detection [LOD]) at which the signal generated by the analyte is not statistically different from that generated in the absence of analyte (referred to as nonspecific signal). Note that for an immunometric assay the LOD is associated with a small signal, but in a competitive assay the LOD is associated with a large signal (compare the dose-response curve in Fig. 4.6 to that illustrated in Fig. 4.3B). All antibody-based assays also have an upper limit of measurement associated with the maximum signal that can be generated by the assay. The working or dynamic range of the assay encompasses only analyte concentrations between the LOD and the maximum response. Analyte concentrations above the maximum response or below the LOD level do not generate signal changes (e.g., there is no dose-response relationship). Thus the measurement variance across the dynamic range of an antibody-based assay is heteroscedastic. The insert in Fig. 4.6 shows the measurement variance expressed as the percent coefficient of variance (CV) of repeated measurements of the same specimen. The CV is calculated as the standard deviation (SD) of the repeated measures divided by the mean value of the measurements. The highest percent CV (e.g., CV × 100) will occur at the extremes of the measurement where the analyte dose response is lost. This point



• **Fig. 4.6** Characteristics of the signal generated by an immunometric assay. Signal generated by the amount of detection antibody bound to the capture-analyte complex is directly proportional to the concentration of analyte in an immunometric assay. The concentration can be extrapolated from response (signal measured) by measuring known concentrations of "calibrators." As shown in the *inset,* the variance associated with measurement is heteroscedastic and increases significantly as the upper or lower limits of the assay are approached. *CV,* coefficient of variance; *LOD,* limit of detection; *MR,* maximum response. (From Sluss PM. Methodologies for measurement of cardiac markers. *Clin Lab Med.* 2014;34:167–185.)

is critical when interpreting assay results or monitoring quality control performance. Variance determined in the middle of the dynamic range of an assay will always underestimate the variance at the extremes.

Although the laboratory can control variance associated with high analyte concentrations by determining at what level of analyte to dilute and retest the specimen, variance associated with relatively low concentrations cannot be altered for a given assay without changing the kinetics of the system (i.e., the concentrations of reagents and/or incubation conditions). Changing the kinetics of a commercial clinical assay is not possible by the performing laboratory because the systems are "locked" to comply with FDA Good Manufacturing Practice regulations.

The combined specificity of two antibodies can produce exquisitely sensitive and specific immunoassays. In the past, a common problem with early competitive immunoassays was cross-reactivity among the structurally similar glycoprotein hormones: LH, follicle-stimulating hormone (FSH), thyrotropin (thyroid-stimulating hormone [TSH]), and human chorionic gonadotropin. The α-subunits of each of these hormones are identical, and the β-subunits, though unique, share considerable structural homology. The polyclonal antisera used for measuring one of these hormones in many of the earlier immunoassays had significant cross-reactivity for the other gonadotropins. The cross-reactivity of a pair of antibodies is less than the cross-reactivity of each of the individual antibodies because any cross-reacting substance must contain both of the binding epitopes to simultaneously bind to both antibodies. For example, consider two antibodies for LH, each having 1% cross-reactivity with hCG. The cross-reactivity of the pair is less than the product of the two cross-reactivities or, in this case, less than 0.01%. Most current immunoassays for LH have a cross-reactivity of less than 0.01%. This low cross-reactivity is important because pregnant patients or patients with choriocarcinoma can have very high hCG concentrations that could interfere with measurements of the other gonadotropin hormones. Most hormones circulate in the blood in multiple forms. Some hormones (e.g., prolactin, growth hormone) circulate with macro forms, which can cause difficulty in their analysis if specimens are not pretreated.[29,30] For hormones composed of subunits (e.g., the gonadotropins), both the intact and the free subunits circulate in blood. Immunometric assays can be made specific for intact molecules by pairing an antibody specific for the α-β bridge site of the subunits with a second antibody specific for the β-subunit. Assays using these antibody pairs retain the two-antibody low cross-reactivity needed for measuring gonadotropins and do not react with the free subunit forms of the hormones.

The heterogeneous forms of circulating hormones, and differences in specificity characteristics of immunoassays for these forms, make calibration and harmonization difficult. Two immunoassays calibrated with the same reference preparation still can give widely varying measurements on patient specimens. Consider the example of hCG in Table 4.1. The three assays are calibrated with a pure preparation of intact hCG,

such as the WHO Third International Reference Preparation. The three assays differ in their cross-reactivity with free β-hCG (0%, 100%, and 200%, respectively). These assays give identical measurements for a specimen containing only intact hCG but progressively disparate values as the percentage of free β-hCG in the specimen increases. In reality, the standardization issue is much more complex because multiple forms of hormones (i.e., intact hormone, free subunits, nicked forms, glycosylated forms, degradation products) circulate in patients, and each assay has different cross-reactivity characteristics with respect to these forms.[31–34]

Because of their speed, specificity, and sensitivity, immunometric assay designs have also been applied successfully to point-of-care testing devices. A typical design is shown in Fig. 4.7. In this example, a lateral flow assay system is shown with two solid-phase monoclonal antibodies affixed to the flow device. One antibody is specific for the analyte, and the other, located on a different section of the analytic strip, is directed at the capture antibody itself. This strip contains a reservoir of detection antibody covalently coupled to gold microparticles. A drop of specimen (e.g., blood, serum, plasma, urine) is placed on one end of the strip and carried across the analytic strip by lateral flow, passing first through the detection antibody reservoir and then over the capture antibodies in sequence. The final state, as illustrated in Fig. 4.7, results in a band of gold particles over the capture antibody region that is proportional to the amount of analyte in the specimen and a positive control band of gold particles over the antidetection antibody region. Such tests are generally qualitative but with the use of a standardized meter for measuring the gold bands and calibrator can be quantitative. Increasingly, these systems are being miniaturized and the detection methods optimized to enable accurate quantitative measurement at the point of care and in other nonlaboratory settings (e.g., field testing and low-resource settings). Although still primarily research tools, similar technologies are utilized in developing multiarray assays (e.g., "lab on a chip" assays) that will likely become part of the clinical laboratory's repertoire of tools in the future.[19,24,25,35,36]

TABLE 4.1 Effect of Immunoassay Specificity on Calibration of Human Chorionic Gonadotropin Assays

hCG Sample	Assay 1	Assay 2	Assay 3
Specificity for intact hCG standard (%)	100	100	100
Cross-reactivity with free β-hCG (%)	0	100	200
Measured values (IU/L)			
Specimen with 0% free β-hCG	10.0	10.0	10.0
Specimen with 10% free β-hCG	9.0	10.0	11.0
Specimen with 50% free β-hCG	5.0	10.0	15.0

hCG, Human chorionic gonadotropin.

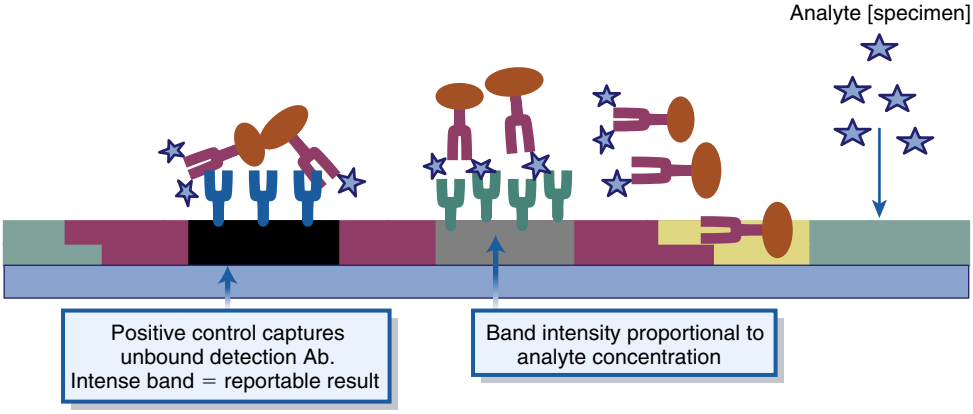
Molecular Structure–Based Methods

Extraction Methods

Extraction of hormones from serum and urine specimens before measurement is a technique used to enhance both the sensitivity and the specificity of immunoassays or mass spectrometry–based assays. Mass spectrometry–based assays are generally of limited sensitivity compared with immunoassay. Their application in endocrine testing relies on relatively large specimen volumes from which analyte can be concentrated by extraction. Extraction, especially in combination with chromatography, also results in the removal of interfering substances, which can produce inaccurate measurements in either immunoassays or mass spectrometry–based assays.

Generally, extraction procedures required for the measurement of serum steroids are based on the polarity or water solubility of the analytes. Extraction methods for proteins/peptides can be based on molecular size as well as polarity. It is essential in any extraction method that recovery (i.e., the amount of analyte extracted) is consistent across all specimens or adjusted quantitatively for each specimen tested. If the extraction recovery is less than 100% but consistent, the method will produce biased yet usable, albeit method-specific, results. If the recovery is different among specimens and cannot be corrected by monitoring, the assay is not valid.

Numerous extraction systems have been developed, including organic-aqueous partitioning to remove water-soluble interferences seen with steroids, solid-phase extraction with absorption, and selective elution from resins such as silica gels and immunoaffinity chromatography. Early immunoassays for steroids relied heavily on extraction prior to assay and provided a basis for assessing interference in subsequent direct assays.[37,38] However, extraction before immunoassay is seldom used in clinical assays today. Extraction techniques are difficult to automate, require



• **Fig. 4.7** Laminar flow immunometric assay design. See discussion in text. *Ab,* antibody. (From Sluss PM. Methodologies for measurement of cardiac markers. *Clin Lab Med.* 2014;34:167–185.)

skills and equipment not available in many clinical laboratories, and generally require correction based on measuring recovery. Monitoring recovery in automated immunoassays is very difficult and creates issues with regulatory compliance (i.e., modification of manufacturer methods). In contrast, extraction methods are a key element in preanalytic processing for mass spectrometry–based assays in which it is possible to measure recovery using an internal standard added to every specimen being tested. Extraction can also be applied to the measurement of proteins/peptides. Most current mass spectrometry assays for steroids involve deproteinization of the specimen (extraction of the steroids) prior to further extraction/purification preanalytically. Similarly, mass spectrometry–based assay of proteins/peptides generally utilizes a batch extraction based on molecular size or polarity. A good deal of progress has been made in developing preanalytic extraction methods prior to assay.[39–50]

Chromatographic Systems

The second major method of measuring hormone concentrations involves chromatographic separation of the various biochemical forms and quantitation of specific characteristics of the molecules. High-performance liquid chromatography (HPLC) systems use multiple forms of detection, including light absorption, fluorescence, and electrochemical properties.[51–53] Chromatography also is frequently combined with mass spectrometry. There are two major advantages of these techniques: They can be used to simultaneously measure multiple forms of an analyte, and they are not dependent on unique immunologic reagents. Therefore harmonization of measurements made with different assays is more feasible. The major disadvantages of these methods are their complexity and their limited availability.

Many chemical separation techniques are based on chromatography, but the two most commonly used for liquid chromatography are normal-phase HPLC and reverse-phase HPLC. In both systems, a bonded solid-phase column is made that interacts with the analytes as they flow through in a liquid solvent. In normal-phase HPLC, the functional groups of the stationary phase are polar (e.g., amino or nitrile ions) relative to the nonpolar mobile phase (e.g., hexane); in reverse-phase HPLC, a nonpolar stationary phase (e.g., C18 octadecylsilane molecules bonded to silica) is used. Polymeric packings made of mixed copolymers have been made with C4, C8, and C18 functional groups directly incorporated so that they are more stable over a wide pH range. The mobile and stationary phases are selected to optimize adherence of the analytes to the stationary phase. The adhered molecules can be eluted differentially from the solid phase, after washing to separate specific forms of the analyte from interfering substances. The elution is called an isocratic elution if the composition of the mobile phase remains constant throughout the run. If the mobile-phase composition is abruptly changed, then it is a step elution. If the composition is gradually changed throughout the run, then a gradient elution occurs.

The efficiency of separation in a chromatography system is a function of the flow rates of the different substances. The resolution of the system is a measure of the separation of the two solute bands in terms of their relative retention volumes (V_f) and their bandwidths (ω). Resolution (R_s) of solutes A and B is calculated as follows:

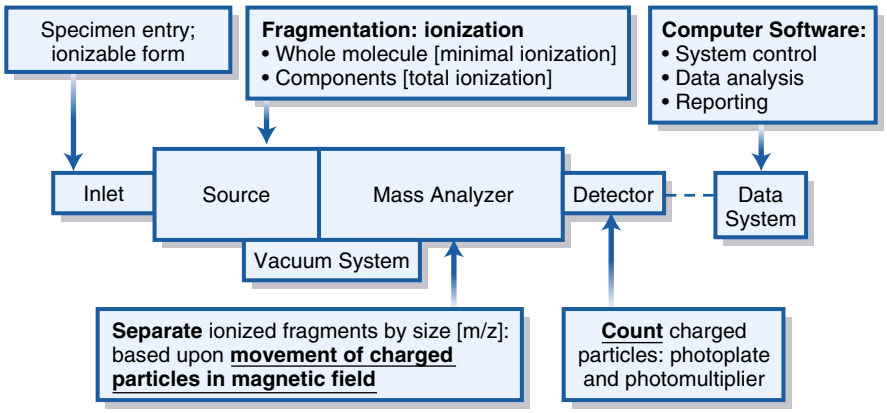
$$R_S = \frac{2\left[V_f(B) - V_f(A)\right]}{\omega(A) + \omega(B)}$$

Values of Rs less than 0.8 result in inadequate separation, and values greater than 1.25 correspond to baseline separation. The resolution of a chromatography column is a function of flow rates and thermodynamic factors.

HPLC remains the method of choice for clinical measurements of catecholamines in biologic fluids.[54,55] Simultaneous measurement of the three catecholamines (epinephrine, norepinephrine, and dopamine) can be obtained. Prior extraction by absorption on activated alumina and acid elution helps to improve specificity. Dihydroxybenzylamine, a molecule similar to endogenous catecholamines, can be used as an internal standard.

Mass Spectrometry

Mass spectrometry depends on the movement of charged particles through a magnetic field to separate and quantify them on the basis of their mass, or more rigorously their mass-to-charge ratio (m/z).[56] A mass spectrometer is an instrument designed to ionize analytes, accelerate them into a device (mass analyzer) that separates them based on their m/z, and quantifies their relative abundance. Fig. 4.8 illustrates the components and principles of a generic mass spectrometer. The heart of the system is the mass analyzer, which utilizes adjustable magnetic fields to accelerate or deflect volatile (e.g., in gaseous form) ions, typically in a vacuum so that the ion's flight path is determined only by the magnetic field. A source is used to ionize and, if necessary, volatilize and fragment analytes to introduce them into the mass analyzer. Analytes are introduced into the source via an inlet that can be as



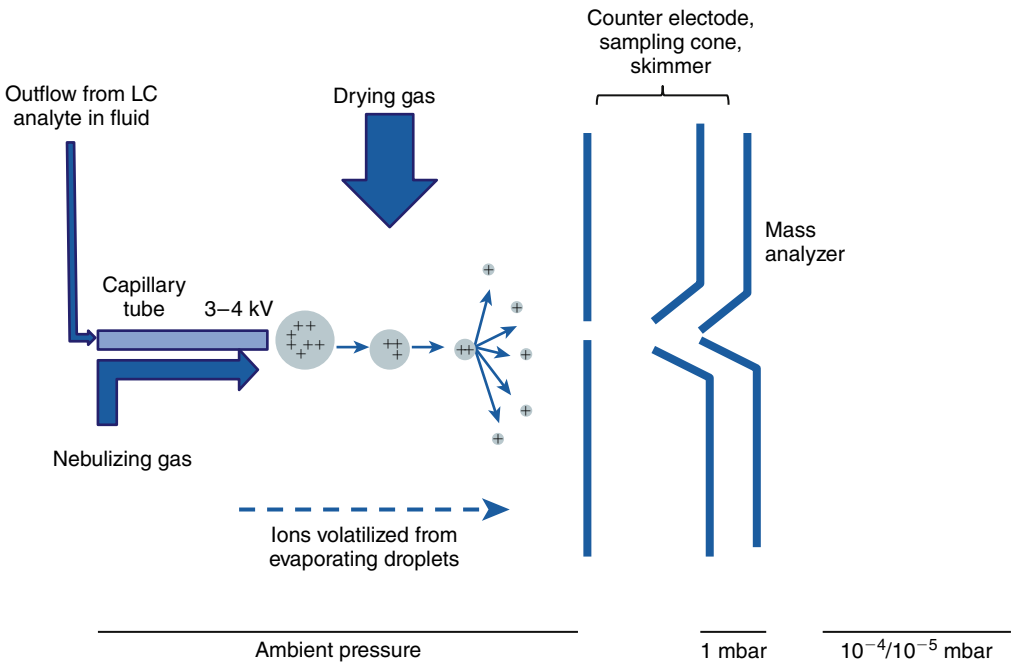
• **Fig. 4.8** Components and principles of a generic mass spectrometer. See discussion in text. *m/z*, mass-to-charge ratio.

simple as an injection port or as sophisticated as a laser-driven matrix desorption system or photoionization chamber. Charged particles passing through the mass analyzer are counted by a simple Faraday plate detector, which generates an electric current proportional in intensity to the frequency (abundance) of ions striking the detector. Mass spectrometers used in endocrine clinical testing are quite complex, with analytes being delivered to the inlet via a chromatography system, subtle selection of ions with *m*/*z* characteristics unique to the analyte, and measurement based on system calibration and recovery of internal standards. All of these aspects are controlled by the data system (computer software), which also generates data outputs that comply with clinical reporting requirements and increasingly can be integrated into fully electronic laboratory and medical record systems. These components are discussed in more detail later.

Analytic mass spectrometry was developed in tandem with the discovery and characterization of endocrine steroids during the 1930s, 1940s, and 1950s.[57] The source for these instruments ionized the analyte by electron impact (i.e., by bombarding gas molecules from the sample with electrons emitted from a heated filament), creating a full fragmentation of the analyte and multiple charged particles of each of the composite atoms. By determining the relative abundance and mass of each ionized particle, the molecular structure of the steroid could be constructed. However, the methodology requires that the steroids, indeed any analyte, be purified and volatilized prior to fragmentation and ionization in the source. Most steroid hormones are easily heat damaged and must be derivatized with molecules that can be volatilized and ionized before mass analysis. This methodology was used in strictly research applications in which it was invaluable in delineating the physiology of reproductive steroids. The development of gas chromatography, in conjunction with electron impact mass analyzers (GC/MS), led to the clinical use of mass spectrometry, which was applied first to endocrine steroids and subsequently to other small biologically important molecules. GC/MS, using quadrupole analyzers in scanning mode (see later), remains a key technology in the research laboratory today and arguably is the method of choice for the study of steroid hormone metabolites.[58] GC/MS was replaced for clinical endocrine steroid testing by the cheaper, higher throughput antibody-based assays, which remain the primary method in all but a few very large academic hospital or reference laboratories. However, dramatic advances in mass spectrometry design have led to the availability of instruments that are rapidly replacing many antibody-based assays, especially competitive immunoassays, in clinical laboratories.

The technologic advances leading up to modern mass spectrometers involve primarily the source and the mass analyzer components. The most dramatic advance in sources, with respect to clinical applications of mass spectrometry in endocrine testing, was the development of electrospray ionization (ESI).[59,60] This technology underlies the direct connection of liquid chromatography systems to mass spectrometry and is currently the method of choice for measuring analytes relevant to endocrinology, such as steroid hormones, in biologic fluids. The principle of ESI is illustrated in Fig. 4.9. The specimen to be measured in the effluent from a chromatography system is pushed at low speed through a capillary column into the source region of the spectrometer to create an aerosol when high voltage (positive or negative) is applied to the tube in the presence of a nebulizing gas. As the droplets in the aerosol dry, often with the aid of a drying gas, the molecules in the specimen become charged and volatilized. In gaseous form, these molecules then enter the mass analyzer.

ESI has directly resulted in the development of systems that are rapidly replacing the antibody-based assays in the clinical laboratory. The most obvious advantage is that liquid chromatography systems can be coupled directly to the mass spectrometer. This system allows the extensive knowledge of steroid and peptide purification by liquid chromatography to be directly applied in mass spectrometry systems that for the first time can be automated and support high throughput testing. Because the



• **Fig. 4.9** Principle of electrospray ionization for introducing analytes isolated by liquid chromatography (LC) directly into the mass spectrometer.

analysis time (seconds) in a mass analyzer is much shorter than the time required for chromatographic separations (minutes), several independent liquid chromatography systems can be supported by one mass spectrometer. Thus the technical advantages of the mass spectrometer (measurements based directly on the molecular composition of the analyte rather than indirect competition of the analyte for antibody binding) can be realized in the practical setting of clinical testing services.

The ionization achieved by ESI is also an important technologic advance. Although the exact mechanisms are still unclear, ESI is characterized by ionization at low temperatures and pressures and results in relatively little fragmentation of the analyte so that a molecular ion is always generated. This procedure has allowed the development of exquisitely specific methods. Most significant with respect to current clinical testing is tandem mass spectrometry, especially triple quadrupole mass analyzers linked together.

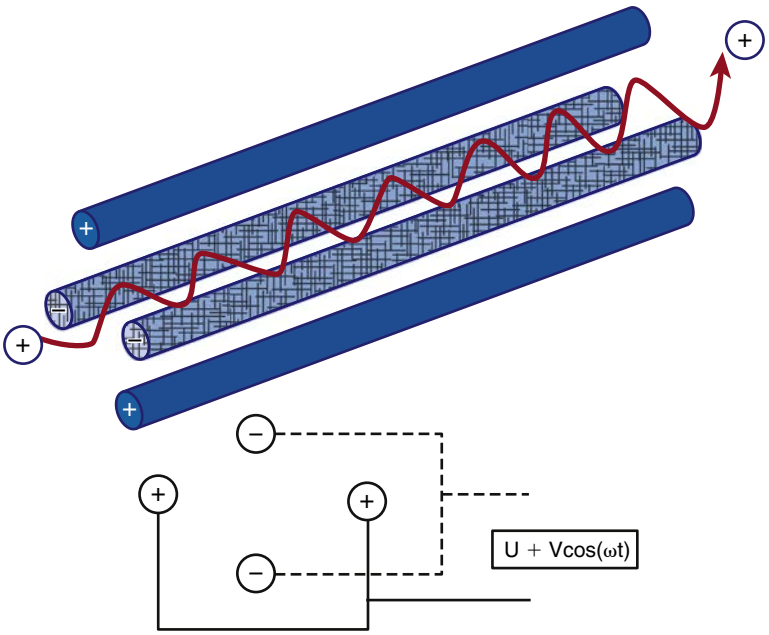
The design of a quadrupole mass analyzer is illustrated in Fig. 4.10. The analyzer is composed of four round electrodes. Voltage of the same polarity is applied to directly opposite electrodes, and opposite voltage polarity is applied to adjacent ones. An oscillating electric field is generated within the quadrupole when an alternating current (voltage V, frequency ω, and time t) is applied with a superimposed direct current (voltage U). Thus charged particles (ions) moving through the quadrupole follow oscillating paths and only ions with a specific *m*/*z* can pass through to the downstream detector. Ions with greater or lesser *m*/*z* collide with the electrodes and are not detected. By controlling the applied voltages, the analyzer can be operated to select ions of specific *m*/*z* for detection (or transit). Because ions are moving rapidly, and voltage can be controlled rapidly, the analysis time is very short. The analyzer thus can be operated in three distinct modes to (1) filter ions for the quantitation of only one *m*/*z*, (2) scan to sequentially quantify all ions by *m*/*z*, or (3) trap ions within the quadrupole.

Combining three quadrupole analyzers results in a very powerful system (Fig. 4.11), often referred to as the *triple quad mass spectrometer* and often in the clinical literature as simply LC/MS-MS or LC/tandem MS. The molecular ions generated by ESI can be filtered by the first mass analyzer (quadrupole) to capture, in a second mass analyzer, a molecular ion whose *m*/*z* is consistent with that of the target analyte. The captured molecular ion is fragmented and ionized in the second analyzer, which becomes the source for the third analyzer that either analyzes all the fragments or selects one that is unique to the parent ion. By operating the first and second quadrupoles in various modes, different analytic goals can be achieved. The primary approaches used for endocrine testing are multiple reaction monitoring and product ion scanning.

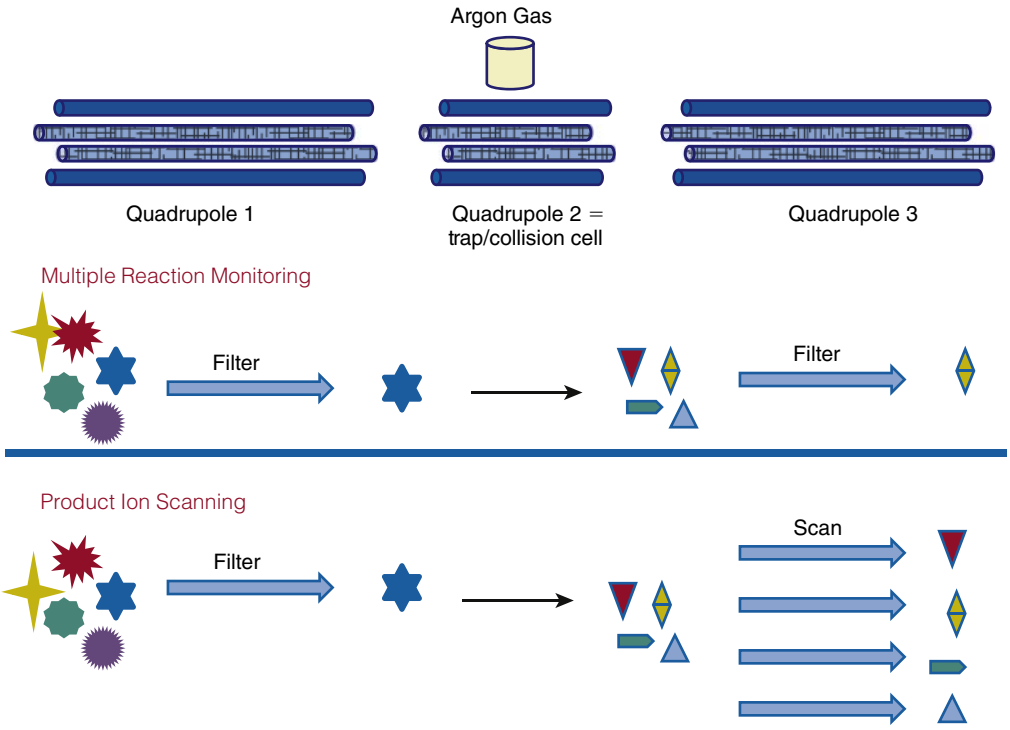
Multiple reaction monitoring mode allows both analytic analyzers (quadrupoles 1 and 3) to be fixed, selecting for a specific *m*/*z*. This adjustment increases specificity and sensitivity. This mode is used to monitor specific analytes and to confirm unambiguously the presence of a compound in a matrix. For example, two unique ions (first/second analyzer) for testosterone are 289.221/97.140 and 289.222/109.130. Because steroid hormones have well-known and unique elution times from LC systems, this mode is also widely used for steroid profiling, as illustrated in Fig. 4.12.

Product ion scanning allows a parent or precursor ion to be selected in quadrupole 1, and the scan in quadrupole 3 measures all the product ions resulting from fragmentation of that ion. This is a particularly useful method of operation for providing structural information concerning small organic molecules or for generating peptide sequence information.

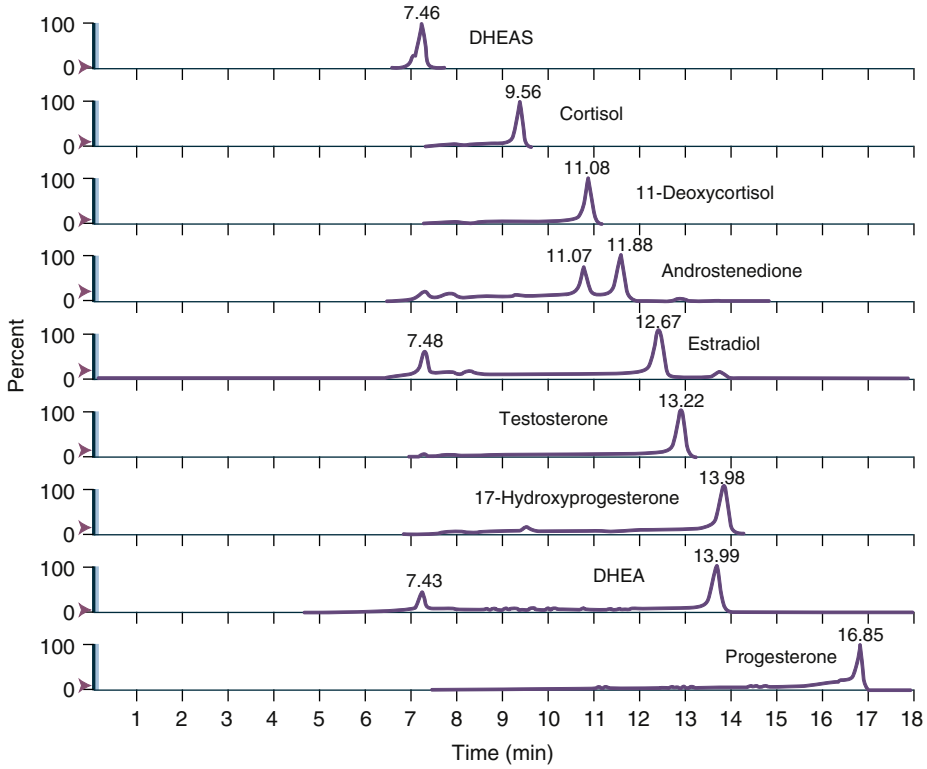
Mass analyzer design continues to evolve rapidly. Another system that deserves consideration with respect to endocrine clinical testing is time-of-flight mass spectrometers (TOFMSs). As illustrated in Fig. 4.13, TOFMSs are simple, albeit more highly engineered, instruments designed to determine *m*/*z* based on the time required to traverse a vacuum tube. The source is designed to align and accelerate ions after ionization so that they all enter the vacuum tube at the same time. The time required to traverse the vacuum tube is proportional to *m*/*z* (more precisely, the square root of the *m*/*z*); smaller or more highly charged ions will move faster to the detector. Most modern TOFMSs have electronic reflectors to effectively increase path length



• **Fig. 4.10** Design of the quadrupole mass analyzer. See discussion in text.



• **Fig. 4.11** Design of a triple quadrupole mass analyzer and modes of operation useful in endocrine testing. See text discussion.



• **Fig. 4.12** Liquid chromatography–tandem mass spectroscopy profiles of nine steroids. *DHEA,* dehydroepiandrosterone; *DHEAS,* dehydroepiandrosterone 3-sulfate.

and thus resolution; this design is often referred as a TOF-TOFMS. Time-of-flight spectrometers are especially well suited for the measurement of large molecules, including proteins larger than the range of quadrupole analyzers. The range of TOFMS includes DNA fragments and even whole microorganisms. Another particular advantage of TOFMSs is their compatibility with pulse ionization sources such as matric-assisted laser desorption/ionization (MALDI) or surface-enhanced laser desorption/ionization (SELDI) systems. Since the introduction of TOF spectrometry in the mid-1980s, a wide range of combination analyzers such as MALDI-TOF or SELDI-TOF

instruments have been developed and used in genomic, proteomic, and metabolomics studies as well as applied to biomarker discovery and microorganism identification.[61–66]

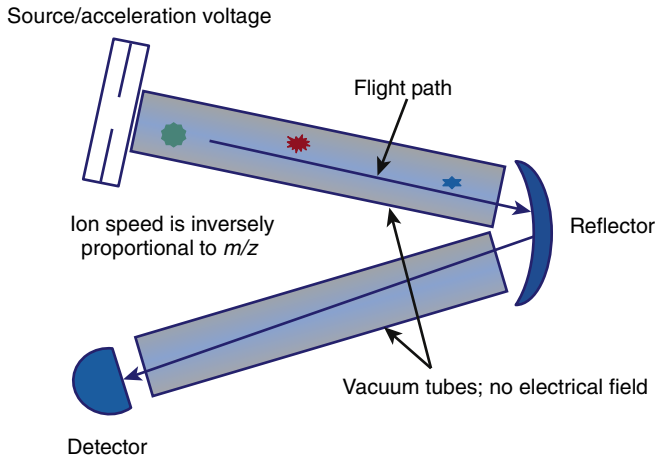
Translating the progress achieved by research applications of TOFMS into clinical diagnostics is still limited to the identification of microorganisms, mostly bacteria and fungi based on spectral libraries. Improved preanalytic, analytic, and postanalytic processes, including standardization, improved clinical sensitivity without loss of specificity for microorganism identification, and qualitative measurement of antibiotic sensitivity remain challenges to wider clinical applications.[67–71] In the near future, TOFMS is likely to find increasing applications in endocrine clinical laboratories. For example, current antibody-based methods for proteins such as TSH, prolactin, and thyroglobulin are often inaccurate in the presence of endogenous antibody. Mass spectrometry, particularly the MALDI-TOF given its ability to measure large proteins, is an attractive approach to addressing this issue.

Free Hormone Methods

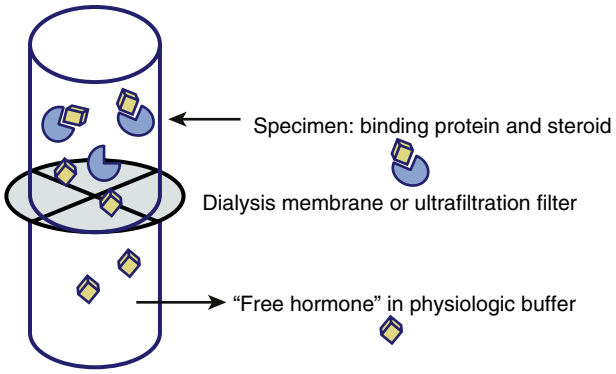
The design of assays, either antibody or mass spectrometry based, to measure steroid hormones and sterols (such as vitamin D) present special issues that warrant discussion. These analytes, which for the sake of simplicity are discussed as steroid hormones, are extremely hydrophobic. In aqueous environments, particularly blood and blood-derived specimens in which measurement is intended, steroid hormones are associated with hydrophobic regions of proteins or bound tightly to high-affinity, specific transport proteins. The former includes albumin, prealbumin, transthyretin, and apolipoproteins, among others, and the latter includes specific transport proteins listed in Table 4.2. Less than 5% to 10% of most steroid hormones circulate as free (unbound) analytes, and assay design requires that the protein-bound analyte be released or does not interfere in the assay in order to have an accurate measure of the total hormone present. Although not universally applicable, in many cases the physiologic effects of steroid hormones depend on the free hormone concentration rather than the total hormone concentration. Of course, under normal conditions the free and total hormone concentrations are directly related. This concept, known as the free hormone hypothesis, is the basis for the design of methods specifically intended to measure only the free hormone levels.[72–77] The free hormone hypothesis itself is controversial, and a critical discussion of it is beyond the scope of this chapter; however, the reader is directed to specific applications in the clinical chapters of this text. Here it is hoped that a technology-based discussion will give the reader an appreciation of the various methods that have been and are currently used to measure free hormones.

There are two basic types of assay designs for measuring free hormones: (1) assays based on the physical separation of bound and free hormones prior to measurement and (2) antibody-based binding assays designed to measure only the free hormones.

Fig. 4.14 illustrates the design of assays based on physically separating bound from free steroid hormones. Classically, a dialysis



• **Fig. 4.13** Design of a time-of-flight mass spectrometer. *m/z*, mass-to-charge ratio.



• **Fig. 4.14** Free hormone assay design: Physical separation of free hormone. Dialysis membranes or ultrafiltration allow the separation of free hormone from protein-bound hormone prior to measurement of free hormone directly or by determining the percent distribution of labeled hormone added to the specimen before processing.

TABLE 4.2 Circulating High-Affinity Protein Carriers of Steroid Hormones

Protein Carrier	Primary Ligand(s)	Note
Corticosteroid-binding globulin (CBG)	Glucocorticoids, mineralocorticoids	Also binds cell membranes
Sex hormone–binding globulin	Dihydrotestosterone, testosterone, estradiol	Also binds cell membranes
Thyroxine-binding globulin (TBG)	Thyroxine (T_4), triiodothyronine (T_3)	
Vitamin A–binding protein(s)	Vitamin A (retinol)	
Vitamin D–binding protein	25(OH) vitamin D_2, 25(OH) vitamin D_3, 1,25(OH) vitamin D_2, 1,25 vitamin D_3	Also binds cell membranes

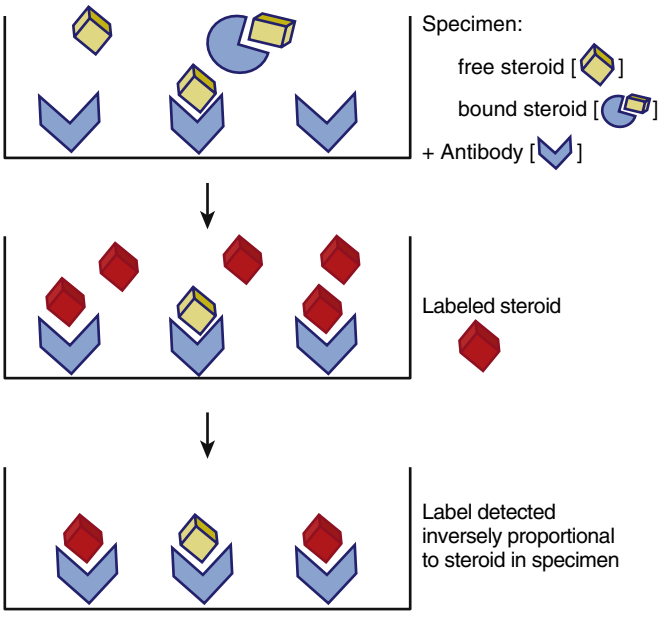
membrane was used to separate two fluid-filled chambers (e.g., tubes). The pore size of the dialysis membrane is specific to the analyte/binding proteins but in principle allows free movement of free steroid hormones while retaining the higher molecular-weight binding proteins and conjugated binding protein-steroid hormone complexes. Thus by placing the specimen in one chamber (top in Fig. 4.14) and matrix-appropriate buffer in the other and allowing the diffusion of free hormone to equilibrate, the free hormone can be measured directly by this equilibrium dialysis approach. Subsequent variations on the method include using an ultrafiltration membrane to allow faster (e.g., no need to wait for an equilibrium to be established) separation of bound from free steroid hormone (as illustrated in Fig. 4.14) or to chemically separate the high-molecular-weight bound hormone from the free hormone (e.g., precipitation of sex hormone–binding globulin–bound steroid using ammonium sulfate). The biggest challenge associated with this approach, regardless of how separating bound from free steroid hormone was achieved, is the measurement of the very low concentrations of free steroid hormone after separation (e.g., in the dialysate or lower chamber in Fig. 4.14). Thus a variation on the equilibrium dialysis design is to add labeled steroid hormone to the specimen prior to dialysis. High specific activity labels, such as radioisotopes, allow the detection of trace amounts of free hormone after dialysis. It is then possible to use the percentage of free hormone based on the distribution of labeled hormone to calculate the mass of free hormone from a direct measurement of total hormone by traditional methods.

One might easily get the misimpression, especially now that LC/MS-MS systems with sufficiently high sensitivity have been combined with it, that equilibrium dialysis is the method of choice or a gold standard method for measuring free hormones.[57,78,79] However, it must be emphasized that currently there is no established reference method for the measurement of free steroid hormones and that most separation methods, including equilibrium dialysis, have not been applied in a fashion that is necessarily valid or directly applicable to in vivo conditions.[73,77,80,81]

Antibody-based binding assays designed to measure only the free hormone can be divided into two classes: (1) two-step assays and (2) one-step assays.

The two-step immunoassay relies on labeled steroid and is illustrated in Fig. 4.15. Solid-phase antibody is used to capture the free hormone present in the specimen. The amount, if any, of bound hormone capture will depend on the relative affinity of the steroid for the antibody versus the binding protein. If the antibody affinity is much higher, the bound steroid will be stripped from the binding protein. If the antibody affinity is relatively low compared to the binding protein, only free hormone will be bound. In either case, after washing the solid-phase antibody, unoccupied antibody-binding sites are titrated using labeled steroid, which after a second wash step can be quantified. The signal generated by the captured labeled steroid is inversely proportional to the amount of free hormone in the specimens. It is important to note that free in this assay format is defined by the relative affinity of the antibody used and the endogenous steroid binding proteins.

One-step immunoassays are designed using either labeled steroid or labeled antibody. The basic formats are illustrated in Fig. 4.16. These assays are fast and easy to perform given their relatively simple format, which is also quite amenable to automation. The use of a labeled steroid analogue is summarized in Fig. 4.16A. The labeled analogue is not recognized by the binding protein but is able to compete with free hormone for antibody-binding sites on the solid phase. This type of assay depends on the validity of



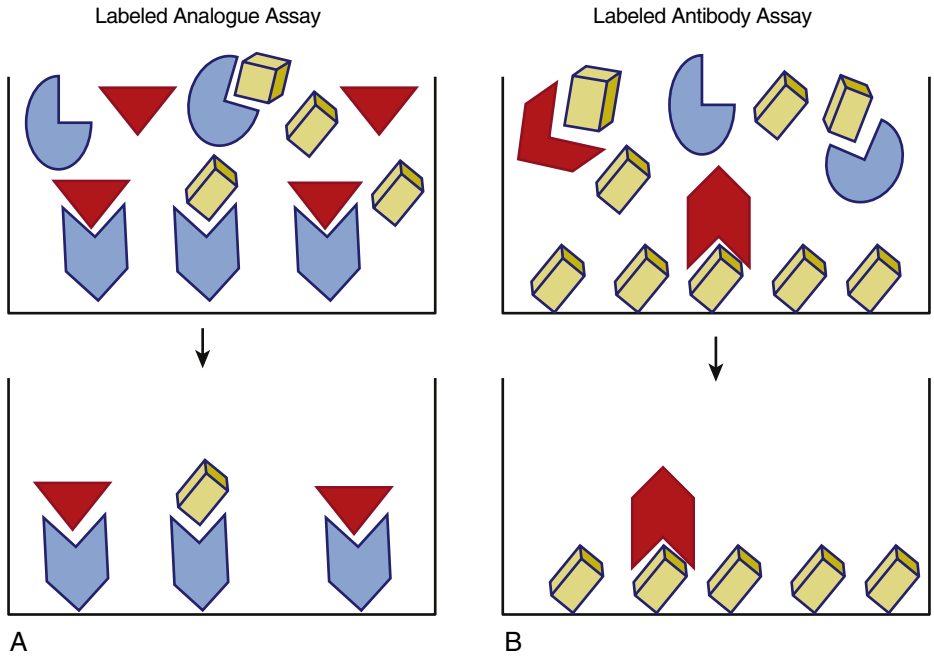
• **Fig. 4.15** Free hormone assay design: Solid-phase, indirect immunoassay. Excess solid-phase antibody binds the free steroid hormone during step 1 of this method. After washing, incubation with labeled steroid hormone (step 2) allows unbound antibody sites to be titrated. After a second wash, the amount of labeled steroid bound to the solid-phase antibody is inversely proportional to the amount of free hormone in the specimen.

the assumption that the signal generated, which is inversely proportional to the concentration of free steroid in the specimen, is solely due to the competition with free hormone. This has been shown not to be true for free testosterone assays and is likely valid only over a limited range for binding protein concentrations for free thyroxine assays.[77,82–87] An alternative approach is shown in Fig. 4.16B in which a labeled antibody is employed in an assay based upon its binding to solid-phase antigen (e.g., the analyte of interest). In this design, the signal generated reflects the amount of labeled antibody bound to the solid phase which, after reaching a steady state, is inversely proportional to the concentration of free steroid hormone in the specimen. The advantage of this newer approach is that a relatively higher signal is measured (e.g., improved sensitivity and precision), and it is not necessary to alter the structure of the steroid (other than that which may be associated with attachment to the solid phase).

It is important to recognize that for free steroid assay designs, the kinetics of competition and binding are very complex given the variety of proteins interacting with steroid hormones over a wide range of affinities. Specimens with low concentrations of binding proteins, including low-affinity but high-capacity binders such as albumin, are particularly challenging. As is true for any assay, all of these free hormone measurement methods require careful validation and method-specific reference intervals to be clinically useful.[73,74,80,81,88,89]

Nucleic Acid–Based Methods

Nucleic acid–based assays are designed to identify variations in an individual's DNA or RNA sequence that reflect molecular variance (e.g., mutations, rearrangements) that alters gene expression, regulatory pathways, and bioactive molecules in a fashion relevant to human disease (i.e., early diagnosis or increased disease



• **Fig. 4.16** Free hormone assay design: One-step immunoassays. (A) A labeled analogue steroid hormone that binds to antibody but not to binding proteins is used in a classic competitive binding format to measure only the free hormone in the specimen. (B) Labeled antibody is used in a single-step competitive assay in which free steroid hormone in the specimen competes with solid-phase steroid hormone for antibody binding. The amount of labeled antibody bound to the solid phase after washing is inversely proportional to the amount of free steroid hormone in the specimen. Red indicates labeled analogue steroid hormone in (A) and labeled antibody in (B).

susceptibility). Genetic variance results in a range of alterations from whole chromosome effects visible by karyotyping/cytogenetics to point mutations leading ultimately to changes in protein expression or functionality. As small molecules such as steroid hormones depend on protein enzymes, genetic alterations may affect all aspects of endocrine function and hence are important analytic targets. A plethora of analytic methods exist for the analysis of nucleic acids.

Discussion of the full range of methods is beyond the scope of this review, but these methods can be grouped into three major categories: (1) chromosome visualization methods, with or without application of sequence-selective enzymatic fragmentation; (2) assays based on binding of labeled nucleic acid probes, which obey Watson and Crick base-pairing rules and thus are sequence specific; and (3) direct sequencing of DNA or RNA. Methods in categories 2 and 3 are generally combined with methods for the amplification or selective enrichment of target sequences, but methods in category 1 generally depend on microscopy (whole chromosome analysis), fragmentation, gel electrophoresis, and blotting techniques (e.g., Southern blotting for DNA or Northern blotting for RNA). Thus the key elements to appreciate are hybridization, restriction enzyme fragmentation, electrophoretic separation, amplification, and nucleic acid sequencing. Methods composed of various combinations of these principles are available for scanning DNA sequences for new variants, scoring DNA sequences for known variants, and expression analysis of DNA or RNA target sequences. Direct sequencing effectively scores known variants as well as identifies new variants.

Currently, molecular methods being utilized by clinical laboratories are primarily for well-known inherited diseases, cancer diagnostics and management, and increasingly in infectious disease applications. Based on developments in research laboratories, particularly with respect to next generation sequencing (NGS), these methods are likely to affect clinical endocrine testing in the very near future.[90–92] The majority of nucleic acid–based assays are laboratory-developed methods applied in research settings or highly specialized clinical reference laboratories. However, this picture is rapidly changing as devices suitable for use in hospital clinical laboratories are increasingly becoming available.

Hybridization Assays (Mutation Assays, Genotyping)

Nucleic acid molecules have a unique ability to bind with high affinity to complementary base-pair sequences. When a fragment of a known sequence (probe) is mixed under specific conditions with a specimen containing a complementary sequence, hybridization occurs. This feature is analogous to the antibody-antigen binding used in immunoassays. Many of the formats used for immunoassay have been adapted to nucleic acid assays, including some of the same signal systems (e.g., radioactivity, fluorescence, chemiluminescence) and the same solid-phase capture systems (e.g., magnetic beads, biotin-streptavidin binding). In situ hybridization, which involves the binding of probes to intact tissue and cells, provides information about morphologic localization analogous to that provided by immunohistochemistry. Combining hybridization methods with enzymatic procedures to amplify, extend, and ligate DNA targets or probes greatly enhances the analytic sensitivity and specificity of hybridization-based methods. Hybridization methods, like other binding assays, are quite amenable to automation and incorporation into relatively simple devices suitable for clinical laboratory utilization.

Restriction Fragmentation

DNA restriction enzymes break DNA strands at specific sites based on the nucleic acid sequence. Thus digestion with a given restriction enzyme or combination of restriction enzymes will produce fragments of different lengths that are directly related to the DNA sequence. Mutations that alter the sequence of the enzyme cleavage site(s) will result in altered fragment size patterns, referred to as restriction fragment length polymorphisms (RFLPs), which can be visualized after fragment separation by gel electrophoresis or other separation methods. For known mutations the affected DNA sequence can be amplified (see later) prior to RFLP analysis (or by single-nucleotide extension if the mutation does not alter a restriction enzyme cleavage site). A large number of online tools are available to support researchers designing methods involving the use of restriction enzymes.[93,94] These tools can be useful in designing validation studies of commercially available assays for molecular variance.

Electrophoretic Separation

E.M. Southern invented an electrophoretic separation technique known as Southern blotting.[77] Restriction enzymes are used to digest a sample of DNA into fragments, and the product is subjected to electrophoresis. The separated bands of DNA are then transferred to a solid support and hybridized. Northern blotting is a similar technique in which RNA is used as the starting material. Western blotting refers to electrophoresis and transfer of proteins. Currently a wide range of methods for electrophoretic separation and blotting of DNA, RNA, and proteins is available and incorporated into clinically relevant methods. All are relatively complex laboratory-developed methods.

Amplification

Nucleic acid assays have an advantage in that low concentrations can be amplified in vitro before quantitation. The best-known amplification procedure is the polymerase chain reaction (PCR). The three steps in the process (denaturation, annealing, and elongation) occur rapidly at different temperatures. Each cycle of amplification can occur in less than 90 seconds by cycling the temperature. The target double-stranded DNA is denatured at high temperature to make two single-stranded DNA fragments. Oligonucleotide primers, which are specific for the target region, are annealed to the DNA when the temperature is lowered. Addition of DNA polymerase allows the primer DNA to extend across the amplification region, thus doubling the number of DNA copies.

At 85% to 90% efficiency, this process can amplify the DNA by about 250,000-fold in 20 cycles. This huge amplification is subject to major problems with contamination if special precautions are not taken.

Sequencing Methods

Traditionally, sequencing was performed using DNA polymerase to selectively incorporate dideoxynucleotides (causing chain termination) during in vitro DNA replication. This method, which was developed by Sanger and is now referred to as Sanger sequencing, remains the gold standard.[95,96] Although this method, which was used in the first sequencing of the human genome, is straightforward and reliable, it is primarily used in directed sequencing of relatively small lengths of DNA.

Next generation sequencing is a very different approach to sequencing and refers to a wide array of applications, including whole genome sequencing, exon sequencing, DNA-protein interaction assays, and RNA sequencing.[90,97–99] NGS methods, also referred to as high-throughput sequencing, all involve simultaneous identification (i.e., parallel sequencing) of nucleic acids released from DNA or RNA fragments rather than identification of nucleic acids released/identified in a serial fashion as is done in the earlier procedures (e.g., Sanger sequencing). NGS approaches hold tremendous clinical diagnostic potential because parallel sequencing is faster and orders of magnitude cheaper than serial sequencing.[100,101]

Methods encompassed in NGS are evolving very rapidly but currently include massively parallel signature sequencing, polony sequencing, pyrosequencing, dye sequencing (Illumina), and sequencing by ligation (Applied Biosystems) as well as a plethora of newer methods.[102–107] Currently the focus of new sequencing approaches is to develop methods capable of sequencing larger, more complex (e.g., high redundancy sequence) fragments of DNA. In particular, complex, repetitive, megabase DNA regions are subject to technical and computational limitations to accurately assessing sequence variance.[102,108,109]

NGS is still primarily a research tool providing unique information concerning the mechanisms of disease. NGS has already provided valuable insight into endocrine diseases by identifying sequence variants associated with (1) endocrine tumors, (2) disorders of bone and mineral metabolism, (3) adrenal disorders, (4) gonadal disorders, (5) pituitary and hypothalamic diseases, (6) thyroid disorders, (7) sex development disorders, and (8) multiple endocrinopathy syndromes.[109–119] The clinical value of NGS is clear, and its clinical use is a potential game-changer as it allows diagnosis of disease at a personal rather than a population level and can contribute to prognosis and/or suggest the most effective interventions for individual patients.[104,120,121] However, to truly achieve clinical value, NGS-based diagnostics must be fully validated, standardized, cost effective, provide for secure data storage, produce actionable results, and be compliant with ethical standards of patient care.[101,122–130]

Analytic Validation

In this section, the basic elements of method validation are outlined and are applicable to any quantitative assay method discussed in the methods sections earlier. It is only the degree to which the parameters are determined and the frequency with which they are verified that vary from method to method or as a function of assay class. Clearly, to be clinically valuable an analytic method must be valid; that is, the results or measurements generated are accurate and reproducible within the context of use (i.e., specified concentration limits, specimen types, clinical settings). This is often expressed as demonstrating that the method is "fit for use." In more straightforward terms, any given method is valid only within specifications of use. In practical terms, methods are validated, or more accurately, their validity is verified, by clinical laboratories to the extent required by appropriate regulatory guidelines (see "Classes of Assays").

The validation process begins with the design and development of the method, regardless of the technical processes involved. Clinical laboratories approach validation differently depending on the technologies and reagents used. Commercial systems (instruments and reagents) are validated by the manufacturer, who is also responsible for quality control of subsequent reagent lots and instrument change. Clinical laboratories conduct limited studies to verify the validation. When using instruments and reagents made or modified by the clinical laboratory, full validation is necessary. In both settings, the clinical laboratory relies on professional guidelines specific to the technology.

Failure to fully appreciate these subtleties can lead to very erroneous perspectives of the results reported by a given laboratory or obtained by a given method. For example, the majority of assays used to diagnose endocrine diseases are accurate only over specific ranges of analytes, only with reference to specific and generally nonstandardized calibration materials, and only when applied to specific specimen types. In many cases, results that are essential to patient care are method specific and cannot be extrapolated between methods and laboratories.

The basic elements of method validation are listed in Table 4.3 along with the typical studies conducted to characterize each parameter. The parameters that define an assay's analytic performance are dependent on the technology and reagents employed and are often referred to as intrinsic characteristics. These characteristics include sensitivity, specificity, precision, and accuracy. Validation must also include specification of the assay's utility and provide data to support the clinical interpretation of results generated by the assay; these are listed in Table 4.3 as utility and interpretation parameters. As illustrated in Fig. 4.17, intrinsic parameters are interrelated. For example, as illustrated in Fig. 4.18, accuracy and precision are related parameters and must be optimized and validated in conjunction with each other. In the context of method development, assay validation is typically an iterative process, as shown in Fig. 4.19. It is only after an assay is optimized analytically for specificity, sensitivity, and precision, and accuracy is applied to clinical testing, that these parameters can be fully evaluated and interpretive specifications can be established.

Intrinsic Performance Parameters

Analytic Specificity

Analytic specificity can be simply defined as the ability of the assay to measure only the intended analyte. In other words, the value obtained from a measurement reflects only the concentration of the target analyte. Clearly, then, specificity is closely related to accuracy; an assay cannot be accurate if it is not specific. On the other hand, an assay may be specific but not accurate if, for example, the assay measures only the target analyte but produces a value that overestimates or underestimates its concentration due to calibration, recovery, or other technical issues. From a more practical perspective, specificity is often defined based on the signal generated in the assay; that is, the signal produced in a specific assay is generated only by the target analyte. Few assays, regardless of the technology used, are truly specific in this sense; typically, the signal measured can be generated by components of the specimen or assay systems in addition to the target analyte. Thus practical validation of specificity encompasses not only specificity per se but also interferences, such as matrix effects or ion suppression that can alter the derived concentration resulting in an inaccurate measurement. It is important to emphasize that interference can be specimen specific and is a challenge to assay validation as well as quality control and quality assurance.

TABLE 4.3 Parameters and Studies for Method Validation

Parameter of Performance	Validation Study
Specificity	Cross-reactivity Interference
Sensitivity	Analytic sensitivity Limits
Precision	Intra-assay variance Inter-assay variance
Accuracy	Recovery Bias Linearity Carryover
Utility (robustness)	Specimen stability Reagent stability Assay stability
Interpretation	Reportable range Reference intervals Diagnostic power

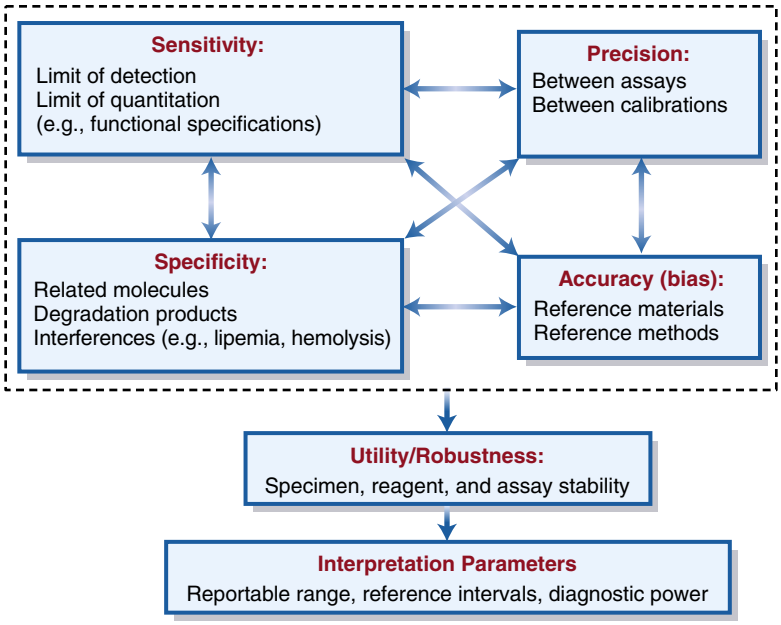
Cross-Reactivity

Assay cross-reactivity can be generically defined as signal generation by similar analytes. It is typically a definable and predictable assay characteristic (e.g., any specimen containing cross-reacting analytes will not be accurately measured).

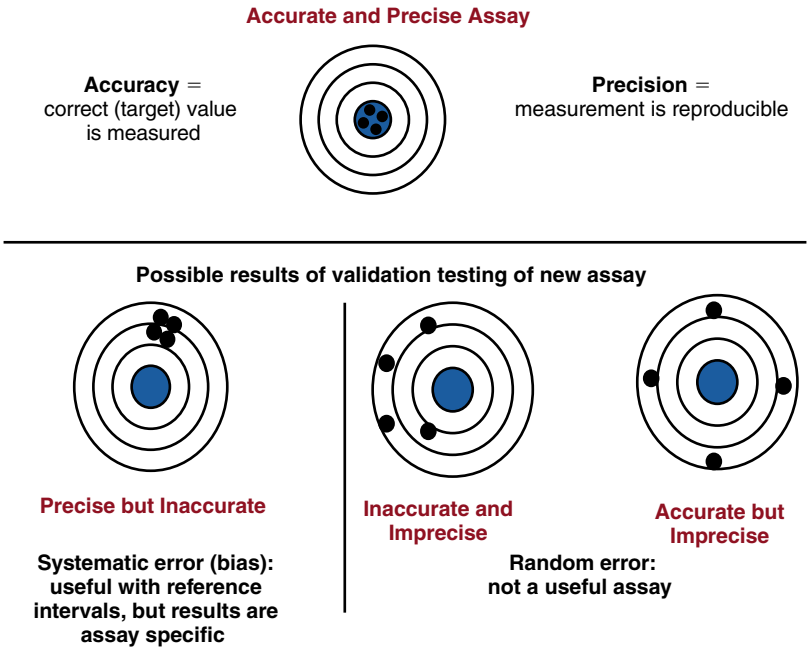
Cross-reactivity in antibody-based assays is due primarily to the specificity of the antibody-binding sites used in the assay. For example, steroids with a similar structure may bind to the antibody and compete with the labeled analyte to produce the same signal (decrease in labeled analyte binding) as the target analyte. Similarly, proteins containing a binding epitope similar to the ones targeted in an immunometric assay can generate signal (i.e., increased binding of the detection antibody). Cross-reactivity is not a term typically used with respect to structural or nucleic acid–based assays, but the concept is applicable. For example, if the fragment used for quantification can be generated by more than one analyte, the signal generated is not specific. Similarly, if the sequence target for a nucleic acid–binding assay is large, the detection probe may bind to more than one analyte. In all cases, the cross-reactivity is not necessarily complete in that the cross-reacting analyte may generate the same, more, or less signal than the target analyte. Thus the degree to which cross-reactivity vitiates assay measurements will be dependent on the concentration of cross-reacting analyte and the degree to which it cross-reacts.

Assays are validated with respect to cross-reactivity primarily by two approaches: (1) response curve comparison and (2) spiked specimen measurement.

Response curve comparisons are done by adding known amounts of analytes expected to cross-react (based on the design of the assay) to the appropriate matrix to generate a dose-response curve for each analyte to be tested. These response curves are compared to those used to quantify the target analyte (e.g., the calibration curve). When possible, the curves are compared at the half-maximal response point where precision and sensitivity (see later) are highest. The degree of cross-reactivity can then be expressed as a percentage. An example of the procedure is shown in Fig. 4.20. The half-maximal response (50% B_{max}/total labeled antibody bound) is generated by a concentration of 200 mass/mL of the target analyte. In contrast, 2000 mass/mL of the cross-reacting analyte is required to generate a half-maximal response. Thus the cross-reactivity of this cross-reacting analyte is 10% (i.e., % cross-reactivity = [200/2000] × 100). It is important to appreciate that this approach is valid only if the response curves are parallel.



• **Fig. 4.17** Relationships and sequencing of method validation parameters.

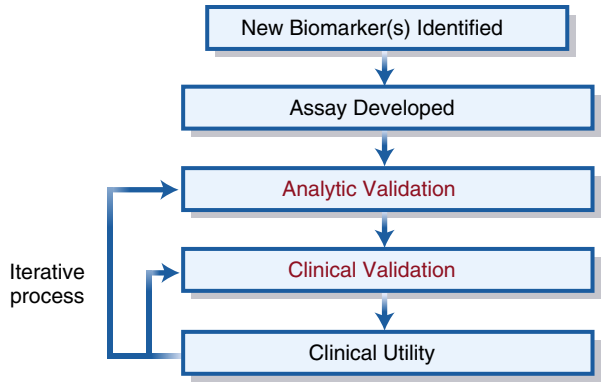


• **Fig. 4.18** Assay accuracy and precision are closely related parameters that must be optimized and validated together.

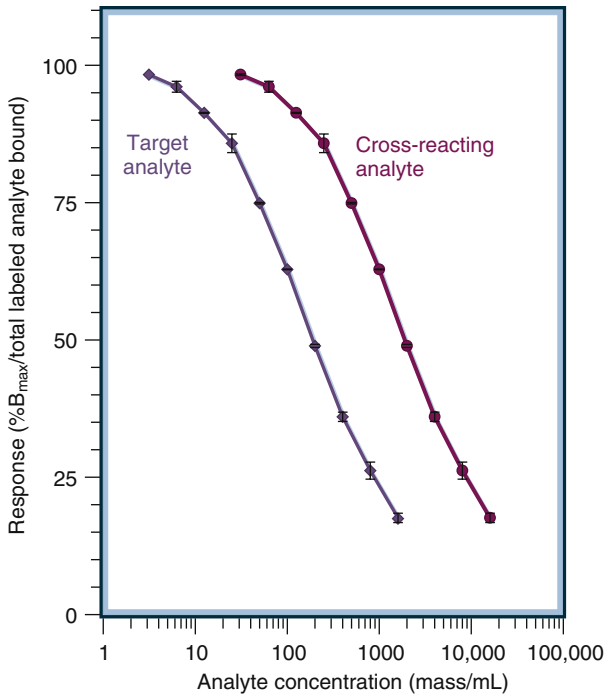
Spiked specimen measurement is often used to determine analyte cross-reactivity. This approach involves adding the cross-reacting analyte to a specimen that has been measured and then performing re-assay to determine if the added analyte cross-reacted. This approach is often seen in the package inserts of commercial assays. An example is shown in Table 4.4 for a commercial assay for the measurement of cortisol in human serum or plasma. The concentration achieved by spiking human serum is indicated for each potential cross-reactant listed. The unspiked human serum contained 12 µg/dL of endogenous cortisol as measured in the assay. Thus a cross-reactivity of 36.6% for fludrocortisone means that a cortisol value of 16.4 µg/dL was measured after the addition of 100 µg/dL of fludrocortisone to the specimen. Spiked specimen cross-reactivity data must be interpreted carefully as it assumes that the percent of cross-reactivity will be the same at all levels of cross-reactant and that the concentrations of cross-reactant tested are clinically relevant.

Interference

As alluded to previously, interference can be due to the influence of a specimen component on the signal generated by the target analyte or to the generation of signal by the interfering substance. In the latter case, what distinguishes interference



• **Fig. 4.19** Iterative nature of the assay development and validation process.



• **Fig. 4.20** Method for determining analyte cross-reactivity. See discussion of cross-reactivity in text.

from cross-reactivity is the lack of parallelism in the signal generation by interfering substances. Often the interfering substance or the mechanism of interference is something known; frequently encountered examples are given in the following paragraphs. In other cases, neither the mechanism nor interfering substance is known; in this case, interference is referred to as a matrix effect. Matrix effects are typically identified only during the validation of accuracy (see later). They can be specimen specific, in which case they are identified only during the investigation of results that are inconsistent with the clinical setting or other analytic results. It is critical that the clinical laboratory keep in mind that any analytic method can be subject to specimen-specific interferences unknown to the laboratory or indicated by routine quality control monitoring. Thus, despite a numeric value often to several decimal places and reported from a validated method, an analytic result from any single specimen must be interpreted in the overall clinical context.

TABLE 4.4 Example of Spiked Specimen Cross-Reactivity Data From a Commercial Immunoassay for the Measurement of Cortisol

Compound	Concentration (µg/dL)	Cross-Reactivity (%)
Aldosterone	1000	0
Beclomethasone	1000	0
Budesonide	1000	0
Canrenone	1000	0.1
Corticosterone	1000	0.9
Cortisol 21-glucuronide	1000	0.2
Cortisone	1000	2.7
β-Cortol	1000	0
β-Cortolone	1000	0
11-Deoxycorticosterone	100	0
11-Deoxycortisol	100	1.9
Dexamethasone	1000	0
DHEA	1000	0
DHEAS	1000	0
β-Estradiol	1000	0
Estriol	1000	0
Estrone	1000	0
Fludrocortisone	100	36.6
Fluticasone propionate	1000	0

DHEA, Dehydroepiandrosterone; *DHEAS*, dehydroepiandrosterone 3-sulfate.

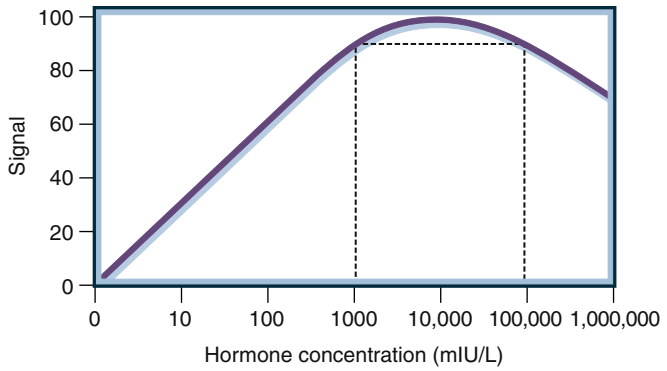
Well-known interference with assays that depend on light or fluorescence signaling can be due to hemolyzed, lipemic, and icteric specimens. Interference can also be a function of physically influencing the system. For example, a severe degree of lipemia can result in inaccurate measurement of water-soluble analytes. Interference can also be analyte specific. Some proteins are inaccurately measured in hemolyzed specimens due to digestion by proteases released during hemolysis (rather than due to a nonspecific color interference with light detection).

Two interferences that are well known to affect immunometric assays (but an issue to some extent for all antibody-based assays) are hook effects and heterophile antibody interferences.

The mechanism of the hook effect is illustrated in Fig. 4.21. As the antigen concentration approaches the effective binding capacity of the capture antibody system, the signal no longer increases. At this point, laboratory specifications for maximum reportable signal are exceeded and the specimen is diluted to obtain an accurate measurement. However, analyte concentrations vastly exceeding the binding capacity of the capture antibody result in also blocking the detection antibody, resulting in a decrease in signal back into the reportable range. Extending the principle to the extreme, it is theoretically possible to have so much analyte present that all the binding sites on both the capture and detection

antibodies are occupied; in this case, no detection antibody can be bound to the solid phase and the signal is baseline, which would be interpreted as no analyte present! Hook effects occur and are extremely important to recognize in the context of measuring hormones in patients with tumors that secrete large quantities of the hormone. Hook effects have been extensively reported for prolactin, hCG, thyroglobulin, calcitonin, and α-fetoprotein.[29,30,131–139]

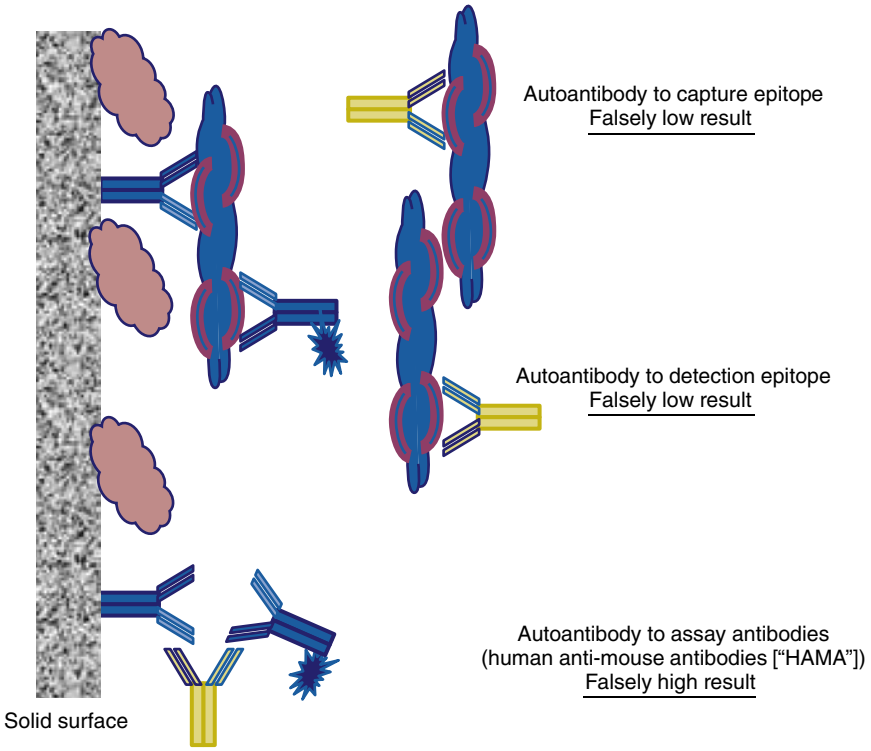
Heterophile antibody interference is, if not a misnomer, certainly a process that encompasses more than just the ability of endogenous antibodies to animal immunoglobulins to interfere in immunometric assays. The mechanism is simple and is illustrated in Fig. 4.22. Any analyte-independent process that alters the amount of detection antibody bound to the solid will result in inaccurate assay values. As shown in Fig. 4.22, endogenous antibodies (called heterophile antibodies) to animal immunoglobulins



• **Fig. 4.21** Immunometric high-dose hook effect. The response signal reaches a maximum and then decreases when the antigen concentration exceeds the limit of the assay.

can, because antibodies are bivalent, link the detection antibody to the solid-phase capture antibody in the absence of analyte, resulting in a falsely high value reported. Animal immunoglobulins are foreign proteins, and thus most humans are expected to have low titers of animal immunoglobulin antibodies. Immunometric assays are designed with blockers to eliminate heterophile antibody interference. However, some individuals have high titers of heterophile antibodies, which overcome the assay blockers and result in inaccurate measurements. In some cases, such as patients treated with drugs containing animal immunoglobulins (e.g., monoclonal-based therapeutics), it is clear why the patient has high titers; in others it is difficult to identify a priori individuals whose specimens might be subject to this artifact.[140–150] Heterophiles are not the only form of endogenous antibody that can interfere with immunometric assays by this mechanism (e.g., antibody-based analyte-independent interference). Fig. 4.22 shows that endogenous antibodies to epitopes on the target analyte can also result in inaccurate measurements—in this case, falsely low results—because the endogenous antibodies block the quantitative detection of analyte. Although the protein hormones and biomarkers relevant to endocrine practice do not elicit the formation of endogenous antibodies in healthy individuals, patients with various autoimmune conditions or other disease processes may have endogenous antibodies that interfere with specific immunometric assays. A classic example of nonheterophile endogenous antibody interference is the interference in thyroglobulin assays by thyroglobulin antibodies in cancer patients.[151–153]

Endogenous antibodies and binding proteins can also interfere with the interpretation of values obtained from antibody-based assays. For example, endogenous antibodies bound to prolactin create what is referred to as *macroprolactin*. Macroprolactin is not biologically active but is measured in many immunometric assays. This results in prolactin levels being reported that are discordant



• **Fig. 4.22** Heterophile antibody interference. See text for details. (From Sluss PM. Methodologies for measurement of cardiac markers. *Clin Lab Med.* 2014;34:167–185.)

with clinical manifestations of hyperprolactinemia.[135,154] Another example is the ability of competitive assays to measure small molecules, such as thyroid or sex hormones, that are inactive when bound to high-affinity carrier proteins, such as thyroxine-binding globulin or sex hormone–binding globulin. In this case, values reported by immunoassay can grossly overestimate the biologic signal represented by the hormone measurement.

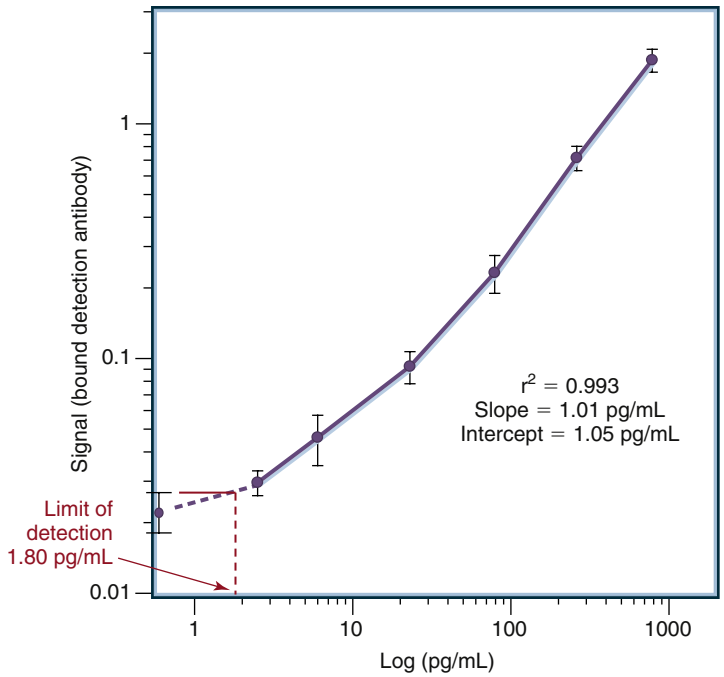
Antibody-based assays that utilize biotinylated antibodies or analytes are susceptible to interference from high levels of biotin in the assay sample, which can occur if the patient is taking very large doses of biotin as a health supplement. This can result in falsely elevated sample values in competitive binding immunoassays and falsely low sample values in immunometric assays. If, for example, free thyroxine and TSH assays both use biotin, the reported free thyroxine can be falsely high and the reported TSH can be falsely low, a situation that could be mistakenly interpreted as indicative of thyrotoxicosis. Biotin interference can be especially difficult to suspect where the artifact causes both a high free thyroxine (FT_4) and a low TSH.[155] There is an increasing awareness that oral biotin can interfere with the accuracy of results from not only thyroid assays but also a wide range of analytes measured by methods involving biotin-streptavidin binding.[156–158] As information regarding oral biotin use is typically not available to the laboratory, it is important for ordering physicians to be aware of this issue.

Analytic Sensitivity

Strictly, analytic sensitivity is the slope of the response curve. It is determined simply as the change in signal as a function of the change in analyte concentration and represents the smallest change in analyte concentration that can be measured. An example calibration curve is shown in Fig. 4.23. The slope of this calibration curve determined by least squares linear regression analysis was 1.01 pg/mL with a goodness of fit of $r^2 = 0.993$ and an intercept of 1.05. Thus the smallest difference that can be measured overall is 1.01 pg/mL with an LOD (intercept) of 1.08 pg/mL. This approach is useful only when the calibration curve is linear (or linearized by log transformation of the analyte concentrations) and the zero calibrator is accurately determined (i.e., there is no matrix effect on the blank measurement). Dose-response curves for clinical assays, regardless of technology employed, are seldom linear, and detector imprecision can be high. Thus limits of detection and direct estimates of variance at clinically meaningful analyte concentrations are typically more meaningful in describing assay performance.

The analytic LOD, often less rigorously referred to as sensitivity, is a statistical definition of the lowest concentration that can be measured (i.e., distinguished for zero analyte in the assay system). This concentration is mathematically determined as the upper 95% limit of replicate measurements of the zero standard, calculated from the average signal plus 2.0 SD. The LOD for the curve in Fig. 4.23 is 1.80 pg/mL. This minimal detection limit is valid only for the average of multiple replicate measurements. When individual determinations are performed on a specimen having a true concentration exactly at the minimal detection limit, the probability that the measurement is above the noise level of the assay is only about 50%.

A second parameter for the lowest level of reliable measurement for an assay is the functional detection limit, or the limit of quantitation. For this value to be determined, multiple pools with low concentrations are made and analyzed in replicate testing. A cross-plot of the coefficient of variation of the measurements versus concentration allows one to generate a precision profile. The analyte concentration corresponding to a coefficient of variation of 20% is the functional detection limit. This term typically applies to across-assay variation, but it also can be calculated for within-assay variation if one uses the tests to evaluate results measured within one run (e.g., provocative and suppression tests).



• **Fig. 4.23** Determination of analytic sensitivity and limit of detection. See text for details.

Precision

Precision is a measure of the replication of repeated measurements of the same specimen; it is a function of the time between repeats and the concentration of the analyte. Both short-term precision (within a run or within a day) and long-term precision (across calibrations and across batches of reagents) should be documented at clinically appropriate concentration levels.[159] In general, normal range, abnormally low range, and abnormally high range targets are chosen for precision studies; however, targets focused on critical medical decision limits may be more appropriate for some analytes.

Twenty measurements are generally considered minimal at each level for both short-term and long-term precision validations. Precision usually is expressed as the coefficient of variation, calculated as 100 times the SD divided by the average of the replicate measurements.[160] There is no universal agreement on the performance criteria for analytic precision, although numerous recommendations have been put forth. Two major approaches to defining these criteria have been (1) comparison with biologic variation and (2) expert opinion of clinicians based on their perceived impact of laboratory variation on clinical decisions.

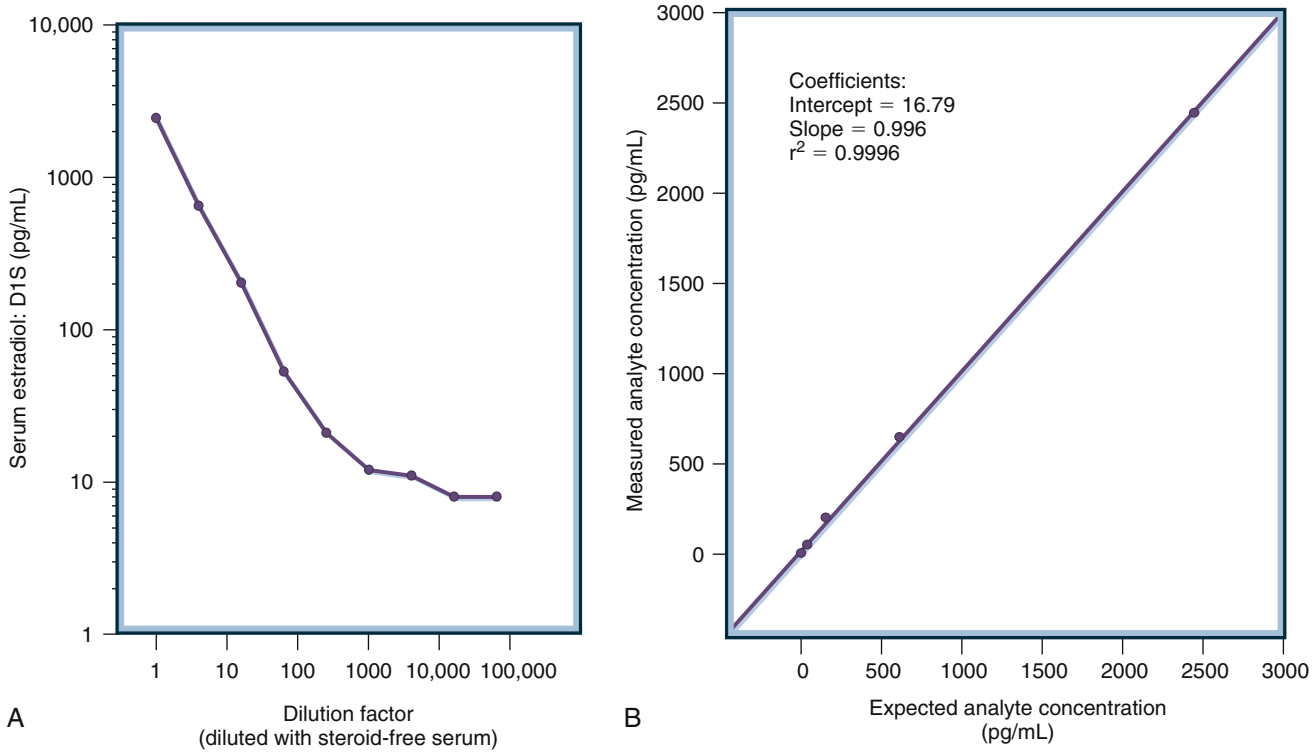
The total variation clinically observed in test measurements is a combination of the analytic and biologic variations. For instance, if the analytic SD is less than one-fourth of the biologic SD, the analytic component increases the SD of the total error by less than 3%. If the analytic precision is less than one-half of the biologic SD, the total error increases by only 12%. These observations have led to recommendations for maintaining precision of less than one-fourth or one-half of the biologic variation.

The expert opinion precision recommendations are based on estimates of the magnitude of change of a test value that would cause clinicians to alter their clinical decisions.

Accuracy

Two methods of assessing the recovery of assays are (1) measuring the proportional changes caused by mixing high-concentration and low-concentration specimens and (2) measuring the increase in test values after the reference analyte is added. Some analytes circulate in the blood in multiple forms, and some of these forms may be bound to carrier proteins. The recovery rate of pure substances added to a specimen may be low if the assay does not measure some of the bound forms. Mixtures of patient specimens may not be measured correctly if one of the specimens contains cross-reacting substances such as autoantibodies. A thorough understanding of the chemical forms of the analyte and their cross-reactivities in the assay is important during assessment of recovery data.

Measuring the proportional changes caused by mixing high-concentration and low-concentration specimens is referred to as a *linearity validation*. An example is shown in Fig. 4.24. A specimen containing a relatively high analyte concentration is diluted with a specimen containing "no" analyte. Practically, "no" analyte means analyte levels less than the detection limit of the assay as specimens with no analyte are typically not available. Fig. 4.24A shows the measurement of diluted specimens as a function of dilution. The point at which concentration no longer changes with increasing dilution is called the *limit of blank* (in this example, 10 pg/mL). The limit of blank can be significantly higher than the LOD or the limit of quantitation in some assays. Dilution linearity data (as in Fig. 4.24A) can be replotted (as in Fig. 4.24B) to evaluate the accuracy of an assay in terms of the analytic recovery of added analyte. Usually data are fitted by a linear regression. Assuming that the x-axes and y-axes have identical scaling, the slope*100 is the percent analytic recovery. Analytic recoveries less than or more than 100 reflect the bias of measurement for a given assay.



• **Fig. 4.24** Determination of assay accuracy. (A) Linear dilution recovery. (B) Spike analyte recovery.

Measuring the increase in test values after a reference analyte is added is referred to as a *spiked recovery validation*. The analytic approach is identical to that illustrated in Fig. 4.24B except that the expected analytic concentration is based on the addition of analyte to specimens rather than calculated based on the dilution of specimens. The most appropriate analytes for use in analytic recovery studies are certified reference materials, such as those from the WHO or the National Institute of Standards and Technology (NIST), although such well-characterized materials are not available for all analytes. Ideally, a rigorous method validation would also include comparison to a reference method (i.e., a method that has been carefully validated previously). These are generally performed by highly specialized laboratories, and reference methods do not yet exist for many analytes of interest in endocrine testing or for novel biomarkers.[161–169]

At a minimum, the evaluation of accuracy by any of the methods described previously should be conducted using specimens from healthy subjects and specimens from patients with the diseases being investigated.[170] When possible, the assay should be traceable to established reference standards or methods. Between 100 and 200 different specimens distributed over the assay range are recommended for method comparisons.[171–175] Although acceptable performance criteria for method comparisons are not well established, some important characteristics to examine are as follows:

- Any grossly discordant test values
- The degree of scatter about the regression curve
- The size of the regression offset on the vertical axis
- The number of points crossing between the low, normal, and high reference intervals for the two methods

The European Union has enacted the In Vitro Diagnostics Directive, which requires manufacturers marketing in the European Union to establish that their products are "traceable to reference standards and reference procedures of a higher order" when such references exist.[176] Hopefully, medically relevant performance characteristics that define the allowable ranges for differences between a specific assay's test values and the traceable standards will be linked with this traceability requirement. This combination of traceability and allowable error requirements could serve to harmonize many test methods worldwide because most diagnostic companies market internationally. Standardization and harmonization of hormone assays have become priorities for quality health care.[164,169,176,177]

Carryover

Many diagnostic systems use automated sample-handling devices. If a specimen to be tested is preceded by a specimen with a very high concentration, a trace amount remaining from the first specimen may significantly increase the reported concentration in the second specimen. The choice of the concentration that should be tested for carryover depends on the pathophysiology of the disease, but high values may need to be tested because some endocrine disorders can produce extremely high values. Validations also typically include assessing possible carryover from the sampling probe and for plate-based assays assessing detector carryover from nearby wells.

Utilization Parameters

Once an assay has been analytically validated, it is necessary to validate its utilization. The key aspects of utilization involve defining limits associated with specimen and reagent stability and ensuring that the assay is stable over time.

Specimen Stability

Validating specimen stability typically involves testing a series of aliquots exposed to different handling conditions to determine if the analyte measurement changes over time. This evaluation typically includes specimens representing the full range of specimen types to be tested (see later) and encompasses processing times and temperatures expected with respect to specimen collection and transport to the laboratory as well as stability during laboratory processing and on instrument time and during the assay itself. This is a critical aspect of method validation that can be very costly and labor intensive.

Reagent Stability

The stability of reagents used in the assay must also be defined. This includes both on-board stability and shelf-life stability for commercial, automated systems that are widely used in modern clinical laboratories. Although reagent expiration dates are determined and provided by commercial manufacturers, they must be verified under the actual working conditions of the laboratory and take into account workflow processes such as reconstituting lyophilized calibrators or refreezing calibrator/control aliquots. For laboratory-developed methods such as LC/MS-MS, the laboratory must also determine expiration dates for all reagent components and stock materials.

Robustness (Assay Stability)

Robustness is typically defined as the stability of measurement over time, which includes variance associated with reagent lot changes, equipment changes, and technologist performance.[178] Robustness validation provides the specifications for the reliability of the method during extended normal usage. These specifications become the basis for setting limits on variance, and it is critical that laboratories inform clinicians of changes that exceed these practice-relevant limits. For example, changes in antisera can cause significant changes in immunoassay performance, which in turn require revising reference interval and clinical decision points.

Interpretation Parameters

Reportable Range

The measuring range of an assay usually spans from the limit of quantitation to the concentration of the highest calibrator. Signal measured above the highest standard requires specimen dilution and retesting. This is typically done automatically in instrument systems but must be done manually in plate assay systems. In either case, an important aspect of method validation is to assess accuracy when a specimen must be diluted. Dilution is often associated with alternation of matrix effects and other interferences in an assay. Thus an assay that is accurate over the calibration range may not be accurate over the full reportable range when dilutions are utilized to obtain quantitative measurements. The validity of the analytic range is documented by the linearity and recovery studies. Most clinical laboratories confirm the reportable range of each assay at least twice a year.

Reference Intervals

Reference intervals, also commonly referred to as normal ranges, describe the analyte values expected from a given assay when healthy individuals are tested.[179–181] This is in contrast to clinical decision points, or cutoff values, determined for identifying patients with specific disease conditions (see "Diagnostic Power"). The development and validation of reference intervals

for endocrine tests can be very complex tasks as they require defining the healthy population by clinical evaluation[182,183] and testing large numbers of healthy individuals,[179,181] which often involves obtaining informed consent and related costly activities. Manufacturers of commercially available assays are required to provide reference intervals, but these do not necessarily represent the subpopulation served by a given laboratory.

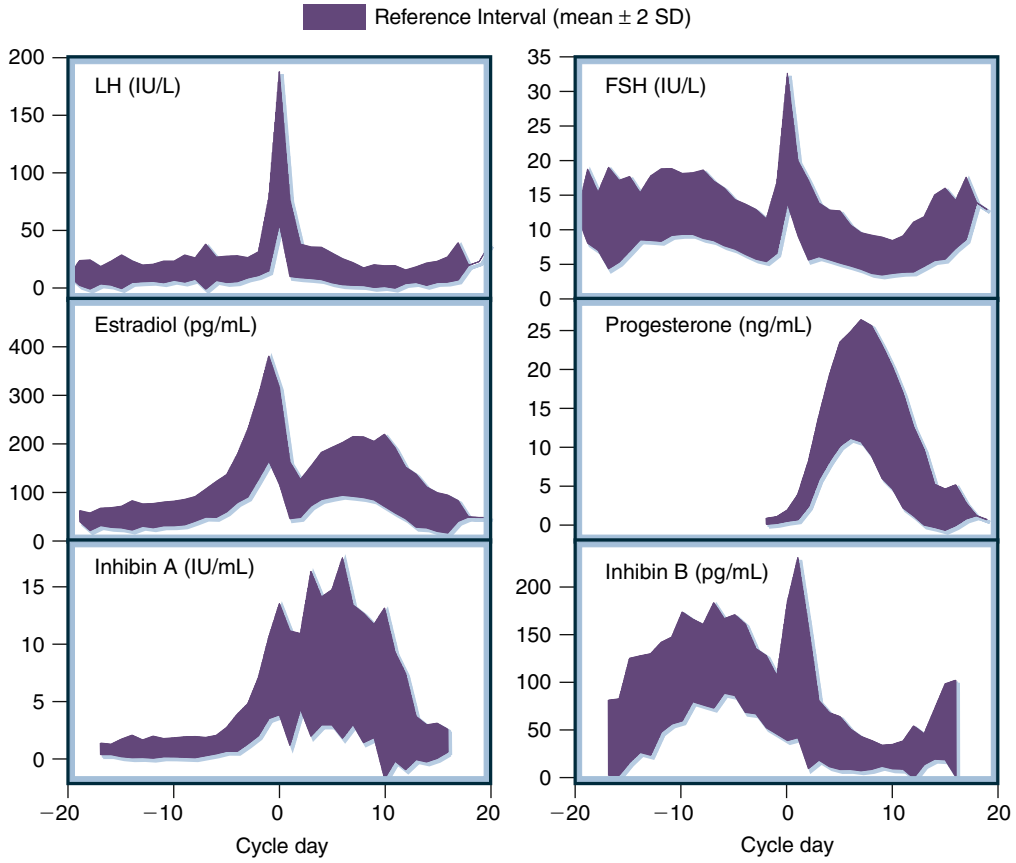
The normal reference interval for most laboratory tests is based on estimates of the central 95 percentile limits of measurements in healthy subjects. A minimum of 120 subjects is needed to reliably define the 2.5 and 97.5 percentiles. Formal statistic consultation is usually required to determine the appropriate number of subjects to test and to develop statistical models for defining multivariate reference ranges.[184–186]

The reference intervals for many endocrine tests depend on gender, age, developmental status, and other test values. Fig. 4.25 illustrates this complexity. Shown are the ranges of values (shaded areas) expected on a daily basis for hormone measurements in healthy young women across the menstrual cycle.

Diagnostic Power

Determining the clinical usefulness of the assay is typically the last step in validation. For commercially available assays the manufacturer is required to do this. The degree to which the assay is validated in this regard depends on specific FDA regulatory requirements (see "Classes of Assays"). Clinical laboratories using these assays are required to verify clinical utility claims depending on the specific requirements of the relevant accrediting organization, but generally the approach is to verify the clinical sensitivity and specificity of the assay using specimens from patients known to have or not have a specific clinical condition that the assay is designed to address. In contrast, for laboratory-developed methods, which include mass spectrometry and many molecular-based assays, the clinical laboratory is required to determine clinical decision points (cutoff values). In either case, good laboratory practice includes verification of clinical utility periodically as part of the laboratory's quality control (QC) and quality assurance (QA) programs. The details of clinical utility validation are beyond the scope of this chapter, but a high-level understanding of the processes is important because clinical sensitivity and specificity and cutoff parameters can help clinicians determine the relative weight to give assay results in the context of the entire clinical picture. Many excellent discussions have been published to provide in-depth details for interested readers.[186–191]

Clinical sensitivity and specificity are not to be confused with analytic sensitivity and specificity, which are assay characteristics. In contrast, clinical sensitivity and specificity quantify the ability of the assay, or any clinical diagnostic procedure, to correctly identify disease states, and these parameters are expressed as percentages. Clinical sensitivity is the percentage of patients with positive test results who actually have the target condition (i.e., they have the disease the assay is intended to identify); it is the true rate of accurate diagnosis. Subtraction of the clinical sensitivity from 100 provides the rate of false-positive results for an assay (i.e., the percentage of patients with positive test results who do not have the disease).



• **Fig. 4.25** High-resolution reference intervals required for the interpretation of reproductive hormone measurements across the menstrual cycle. *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone; *SD,* standard deviation.

Clinical specificity deals with patients with negative assay results. It is the percentage who actually do not have the disease (i.e., who are correctly identified as negative by the assay). Subtracting the clinical specificity from 100 gives the false-negative rate of the assay.

Clearly, to determine clinical sensitivity and specificity the laboratory must have two key things: (1) specimens from patients known to have or not have the disease and (2) either reference intervals or clinical cutoff points to provide a positive or negative interpretation to quantitative test results. Obtaining specimens from patients with known conditions for the purpose of assay validation/verification is challenging as the process requires accurate clinical information, Health Insurance Portability and Accountability Act (HIPAA) compliance, and specimens collected and handled in a fashion consistent with normal workflow. All these steps can be expensive and challenging and are best accomplished with the close collaboration of physicians who utilize the laboratory services.

Clinicians must appreciate that laboratory-derived clinical sensitivities and specificities are subject to many potential biases. First, in many cases laboratories rely on already established assays to provide the disease status information; in other words, they are comparing to predicate device results rather than clinical information. Even when using clinically characterized individuals to provide the disease-positive specimens, bias can be incorporated if the healthy group is not age or gender matched. Finally, using either clinical cutoffs or reference intervals to define a positive versus negative assay result is subject to statistical biases, such as normal versus nonnormal distribution of measurement.

Operational Parameters (Preanalytic Considerations)

Specimen Types

Many types of specimens are routinely used for the measurement of analytes in bodily fluids. The most common are whole blood, serum, plasma, urine, and saliva. Less frequently, fluids or cells derived from fine-needle aspirates are sent to the clinical laboratory for analysis. It is critical to understand that each type of specimen must be subjected to rigorous validation to ensure accurate measurements. Simply because an assay is valid for a given specimen type does not mean that it is valid for any specimen type. Even if an assay is capable of reproducible and specific measurement of an analyte in different specimen types, there may be clinically significant bias depending on the specimen type tested. Reference intervals and clinical cutoff values must be verified for each specimen type utilized.

Whole Blood

Whole blood specimens have both the limitation and the advantage of time dependency. The ability to detect rapid changes to a provocative stimulus is a strong advantage, whereas the unsuspected changes resulting from pulsatile secretions may be a major limitation. Whole blood is advantageous when the analyte is very labile as the specimen can be collected and tested quickly. The ability to test whole blood without processing at the point of care can be cost effective and enhance patient management as well as effectively address problems of specimen stability with respect to very labile analytes.

Blood drops collected on filter paper from punctures of a finger or heel are a convenient system for collecting, transporting, and measuring hormones.[192,193]

If standardized collection conditions and extraction techniques are used, these measurements correlate well with serum measurements. Integration of immunochemistry with computer chip technology has also led to immunochips that can measure multiple analytes using a single drop of blood.[194]

The use of whole blood is severely limited by several factors, most importantly: (1) Whole blood must be prevented from clotting during analysis, necessitating the use of anticoagulants, which often interfere in assays, and (2) whole blood is a very complex mixture of components that can directly interfere with analytic methods. Solutions to these problems include (1) dilution of the whole blood specimen in the assay, which in turn requires that the analyte being measured is in relatively high concentration or that the assay is extremely sensitive, and (2) preanalytic processing to remove the cellular fraction or reduce the complexity of the specimen. Traditionally specimen types most often used for endocrine testing are serum or plasma for these reasons.

Serum

Serum is obtained from whole blood specimens simply by allowing the blood to clot. Allowing whole blood to clot in glass tubes allows the serum to be completely and easily separated from the clot by centrifugation. The resultant serum specimen is free of cells, and many of the proteins involved in the clotting process are also removed. This has been the method of choice for large protein analytes such as immunoglobulins or for very stable analytes such as steroid hormones. In most laboratories glass phlebotomy tubes have been replaced with safer plastic ones that will not break during handling, especially in fully automated laboratories. Unfortunately, whole blood does not clot cleanly or quickly in plastic tubes, so clot activators or enhancers are added to the tubes. These factors can interfere with many analytic methods and must be carefully validated.

Plasma

Plasma is obtained by chemically preventing the clotting process and then centrifuging to remove the cellular components of whole blood. There are many approaches to preventing clotting; most commonly, ethylenediaminetetraacetic acid (EDTA), citrate, or heparin is added to prevent clotting. These chemicals, especially EDTA, have the additional advantage of inhibiting proteolysis and are thus advantageous when testing labile analytes such as adrenocorticotropic hormone (ACTH) or PTH. Other additives are added to stable plasma for specific tests; for example, sodium fluoride is added to EDTA tubes to inhibit glycolysis when glucose measurements are desired. Of course, all these additives have the potential to interfere with specific assay methods.

Separation of the cellular elements in anticoagulated whole blood can be enhanced by the use of gel separators. This type of tube is often preferred in automated aliquoting systems. Unfortunately, the gel used can interfere directly or indirectly (e.g., by trapping analytes) in some analytic methods.

Phlebotomy tubes with additives also create special considerations when collecting blood in multiple tube types. Tubes must be drawn in a specific order to avoid contamination with additives that are known to interfere in standard laboratory tests. Failure to collect additive tubes in the proper order is not identifiable once the tubes are received in the laboratory and can lead to serious inaccuracies in test results. The correct order for drawing the most commonly used phlebotomy tubes is shown in Fig. 4.26.[195] The stopper color is standardized to indicate what additives each tube contains. For example, the tube with a red stopper (3) contains

only clot activators; the yellow stopper tube (2) contains both clot activators and a separation gel. The lavender-topped tube (6) contains EDTA and must be drawn after a clot tube for serum (2 or 3) or tubes containing heparin (4 and 5).

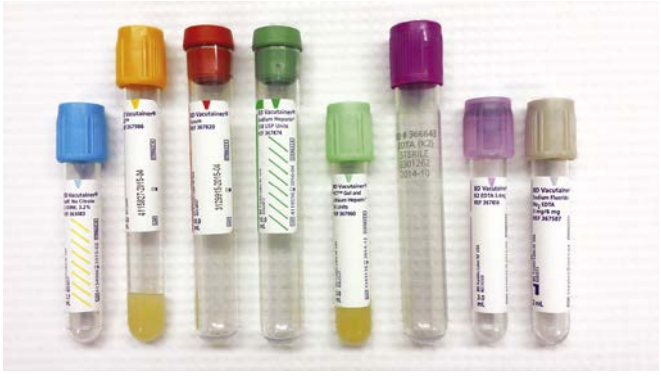
Urine

Urine often contains not only the original hormone but also key metabolites that may or may not have biologic activity. The 24-hour urine specimen is used for many endocrine tests. Such urine specimens represent a time average that integrates over the multiple pulsatile spikes of hormone secretion occurring throughout the day. The 24-hour urine specimen also has the advantage of better analytic sensitivity for some hormones and metabolites.[55,196–203]

Drawbacks include the inconvenience of collecting the 24-hour specimen and delays in collection. Another limitation of urine specimens is uncertainty regarding the completeness of the collection. Measurement of urinary creatinine concentrations helps in monitoring collection completeness, especially when this value is compared with the patient's muscle mass. Many urinary hormones are conjugated to carrier proteins before excretion. Therefore, both hepatic function and, to a lesser degree, renal function may alter urinary hormone values.

Saliva

Saliva is an attractive alternative specimen for measuring non–protein-bound hormones and small molecules.[204–207] Small analytes in blood pass into oral fluid by crossing capillary walls and basement membranes and by passing through lipophilic membranes of epithelial cells.[208] This transport involves passive diffusion, ultrafiltration, active transport, or some combination of these processes. The concentration in saliva depends on the concentration of the non–protein-bound analyte in blood, the salivary pH, the acid dissociation constant (pKa) of the analyte, and the size of the analyte. Analytes entering saliva by passive diffusion usually are less than 500 Da in size, non–protein bound, and nonionized. As with any specimen type, it is essential to fully validate the use of saliva in each analytic method and to establish the data needed for interpretation of test results, such as reference intervals.



• **Fig. 4.26** The order in which blood tubes containing no or various additives must be filled to avoid contamination and possible interference with accurate laboratory measurements. Tube types: 1, citrate; 2, clot tube (serum) with separator gel; 3, clot tube without separator gel; 4, heparin; 5, heparin with separator gel; 6 and 7, EDTA; 8, sodium fluoride/EDTA. (Courtesy Michael Purugganan.)

Saliva measurements reportedly correlate with blood measurements for some hormones such as cortisol, progesterone, estradiol, and testosterone, but they do not correlate well for others (e.g., thyroid and pituitary hormones).[209–217] Multiple preanalytic variables can affect the salivary measurement. Stimulation of oral fluid production by chewing or by sucking candy or drops that contain stimulants such as citric acid can increase oral fluid volume and stabilize pH but may alter some analyte concentrations. Several commercial devices are available for collection of oral fluid; however, these devices need to be validated for each analyte and each assay system to ensure they adequately recover each of the analytes.

Saliva is also an effective specimen type for obtaining genomic DNA and other nucleic acid assay applications. Its use in this regard is now well established and rapidly advancing.[218–220]

Fluids and Tissue From Fine-Needle Aspiration

Fine-needle aspiration (FNA) involves the insertion of a hollow needle into tissue, typically a suspicious lump or inflamed tissue, to withdraw fluid or cells for diagnostic evaluation. The procedure is usually performed manually by the surgeon or a cytopathologist when a palpable lump is present or the tissue target can be seen (e.g., during a surgical procedure). Deep sampling can be achieved using, for example, ultrasound guidance. Analysis of the cellular components of the FNA is done in the cytopathology laboratory and involves the examination of cells by histologic and immunohistologic procedures.

Thus FNA fluids represent a unique specimen type for analysis of biomarkers by traditional immunoassays or by LC/MS-MS methods. However, FNA fluids present special considerations with respect to handling, stability, validation, and interpretation. Aspirated fluids are typically obtained in volumes too small to directly assay and often will clot due to contamination with whole blood. Analyte stability as well as assay parameter validation must be determined using aspirated fluid and diluents required to provide the necessary volume and anticoagulation prior to testing. Interpretation is often difficult because reference intervals applicable to blood levels are seldom available and often analytes, such as thyroglobulin in neck mass aspirates, are high enough to cause artifacts in assays (such as hook effects in antibody-based assays).

Aspirated cells can provide sufficient DNA and RNA for genetic analysis. Currently this is an area of intensive investigation that has only just begun to be applied by clinical laboratories. Typical methods being applied include expression arrays, real-time PCR, and DNA methylation assays as well as the widespread and traditional application of immunohistochemical assays for specific biomarkers.[221] For example, expression arrays can provide clinically valuable information for the 15% to 30% of thyroid nodules subjected to FNA and characterized as indeterminate by cytologic tests.[222]

Other current clinical applications of FNA in conjunction with diagnostic assays include chorionic villus sampling[223,224] and measurement of biomarkers in aspirates from pancreatic tumors.[225–227]

Quality Control

Laboratory quality control procedures are intended to ensure that the tests are being performed within defined limits established during the validation of the assay.[228–232] The goal of these procedures is to identify circumstances when results obtained may not be accurate. They rely heavily on the testing of materials with known analyte concentrations. Quality control failures are meant

to detect instrument problems (hardware or instrument failures), reagent or calibration failures, and human mistakes (improper handling of reagents or specimens, training problems, or shift change communication failures).

Statistically there are two major forms of analytic errors: random and systematic. Random error relates to reproducibility; systematic error relates to the offset or bias of the test values from the target or reference value. Performance criteria can be defined for each of these parameters, and quality control systems can be programmed to monitor compliance with these criteria. Control systems must have low false-positive rates as well as high statistical power to detect assay deviations. The multirule algorithms developed by Westgard and colleagues use combinations of control rules—such as two consecutive controls outside warning limits, one control outside action limits, or moving average trend analyzers outside limits—to achieve good statistical error detection characteristics.[229–232] Traditionally, quality control programs have focused primarily on precision; however, analytic bias also can cause major clinical problems. If fixed decision levels are used to trigger clinical actions (e.g., therapy, additional investigations), changes in the analytic set-point of an assay can cause major changes in the number of follow-up cases. More modern quality control systems include using moving averages of patient test values to help monitor changes in analytic bias. Increasing numbers of web-based systems are available for laboratories to share quality performance data, allowing better statistical evaluations (larger numbers of values to identify shifts and drifts in quality control measurements).

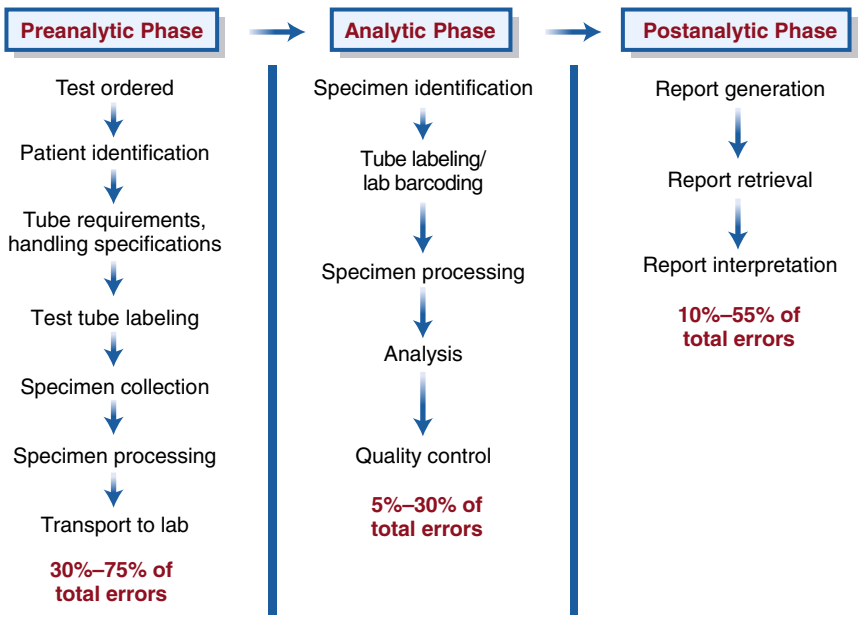
Quality Assurance

Quality assurance procedures go beyond monitoring test values for control materials. Testing of quality control materials only identifies errors that occur during testing per se (i.e., the analytic phase of the overall process from ordering a test until the results are reported back to the physician). A look at when errors typically occur provides important insight into the issues. As illustrated in Fig. 4.27, errors that occur during the analytic process represent less than a third of all errors associated with laboratory testing.

Quality assurance procedures are part of the regulatory requirements of a clinical laboratory. All laboratories have procedures to monitor things such as specimen transport times and report accuracy, which are processes that can be established solely within the laboratory. A key element of quality assurance, and one that should be emphasized for clinicians who are critical to its success, is the identification and investigation of test values that are discordant. Some of these discordant test values may be analytically correct, but others may be erroneous. Clinicians must help identify and investigate these suspicious test values by requesting laboratories to perform a few simple validation procedures.

Repeated testing of the same specimen is a valuable first step. The laboratory assumes that the specimen has been stored under stable conditions and allows for the propagation or error during repeated measurements. Typically, a result is considered to be confirmed if the absolute value of the difference between the initial and the repeated measurements is less than 3 SDs of the analytic error associated with the initial measurement.

Linearity and recovery are valuable techniques for evaluating test validity in individual specimens. If the initial test value is elevated, serial dilution of the specimen in the assay diluent and re-assay should be considered. If the initial value is low, one may consider adding known quantities of the analyte to part of the specimen. Analyzing these spiked or diluted specimens with the original specimen allows one to evaluate both reproducibility and recovery. It may be helpful to analyze the linearity or recovery of the assay standards at the same time, to provide internal controls of the dilution or spiking procedures and the appropriateness of the diluent and spiking material. If the replication, dilution, or recovery experiment appears successful, further analytic troubleshooting will vary according to the method used. For example, immunoassays may be affected by interference caused by heterophile antibodies or hook effects as described earlier. Addition of nonimmune mouse serum or heterophile antibody-blocking solutions may neutralize these effects. Chromatographic assays are usually more robust than immunoassays but often lack the specificity. Specimens with suspected interference on one type of assay can be reanalyzed by means of an alternative methodology. Interferences with cross-reacting drugs and metabolic products can be



• **Fig. 4.27** Distribution of errors during the entire process of clinical laboratory testing.

minimized with selective extraction or identified by adding a drug to nondiscordant specimens.

Classes of Assays

In the United States the regulations governing clinical laboratories fall into several categories based on the manufacture of assay components, the intended use of the assay, and how the assay service is billed. To be reimbursed by Medicare and other health insurance organizations, laboratories must comply with federal legislation known as the Clinical Laboratory Improvement Amendments (CLIA). This legislation requires that laboratories be certified by specified organizations (e.g., the College of American Pathologists or the Joint Commission for the Accreditation of Hospital Organizations) and is administered by the Centers for Medicare & Medicaid Services (CMS) within the US Department of Health and Human Services (DHHS). CMS also administers the HIPAA of 1996 and other quality standards that laboratories must comply with under federal law. CMS/CLIA certification requires quality inspections every 2 years to ensure that laboratories are meeting the standards outlined in the federal CLIA guidelines and the performance standards specified by the laboratory's inspecting agency. Inspecting agency standards are based on the CLIA guidelines.

In contrast, the manufacture and sale of assay reagents and instruments are regulated by guidelines called Current Good Manufacturing Practices (cGMPs), which cover very specific topics and are periodically amended.[233–238] Compliance with cGMPs is enforced by the FDA under federal law.[239] Besides the manufacture and sale of instruments and reagents, laboratory-developed methods used in patient care are encompassed within the jurisdiction of the FDA. The FDA does not certify compliance and conducts its own quality inspections. Failure to comply with cGMPs is a violation of federal law (as opposed to reimbursement requirements under CLIA) and can result in laboratory closure as well as potential fines and legal actions. Sale of assay instruments and reagents requires premarket approval by the FDA or FDA clearance under Section 510(k), depending on the clinical use of the product and its potential impact on patient care.

Common elements of clinical laboratory standards, CLIA guidelines, and cGMP regulations include laboratory evaluation and documentation of validation verification for commercially available reagents and instruments, which have been cleared or approved by the FDA and whose continued manufacture is overseen by the FDA. Requirements for laboratory-developed methods, which include any modifications of FDA-approved/cleared commercial procedures, are more extensive, and validation per se is expected.

Conclusion

The analytic methods of assessing endocrine problems in patients are continually expanding. The newer systems are often based on analytic techniques similar to those outlined in this chapter, but the configurations are generally more user friendly. These advances make the systems more convenient, but they also become more of a "black box" that conceals most of the details of the system. The methods, their descriptions, and approach to their validation, as outlined in this chapter, are intended to provide the clinician with insights into the inner workings of these systems and to encourage a more detailed level of interaction with the clinical laboratory in its ever more challenging endeavors to provide cost-effective yet high-quality support for patient care.

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The complete list of references is available online at ExpertConsult.com.

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5

The Global Burden of Endocrine Diseases

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CHAPTER OUTLINE

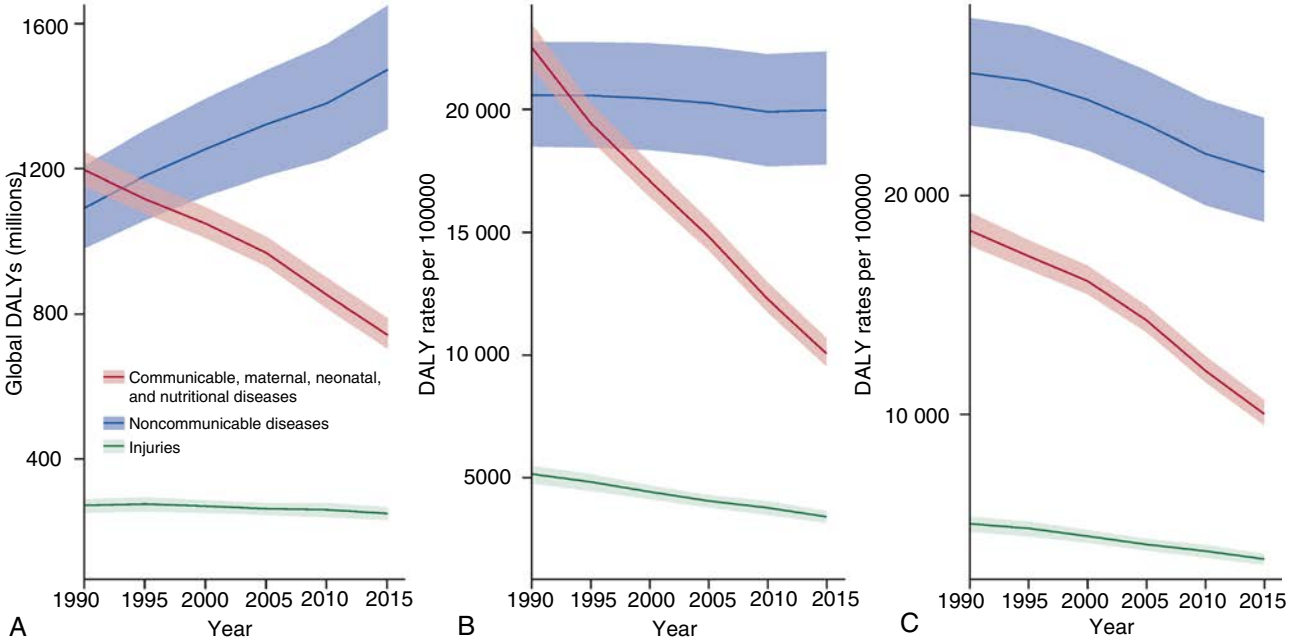
KEY POINTS

- High body mass index, including obesity, is now one of the world's top five risk factors causing disease burden.
- With the inexorable rise in the prevalence of excess weight, this burden is growing in all regions of the world.
- Diabetes incidence, prevalence, and burden have all increased in recent decades, with the aging of the population being a main driver of these trends. The scenario for burden, while stabilizing in countries with the highest degree of development, is most worrisome for those in the midrange of development.
- Given this scenario, diabetes, when joined with states of intermediate hyperglycemia, is now also one of the top five risk factors in the world.
- Thyroid diseases are among the most prevalent endocrinopathies across the world.
- Iodine deficiency is a highly prevalent public health problem in several countries.
- Incidence of thyroid cancer has increased globally over the last three decades, although a recent decline has been noted in countries with the highest level of development.
- The burden of thyroid cancer is higher in low-income and low-middle-income countries.

Introduction

The 20th and early 21st centuries have witnessed important social changes that have produced major demographic, nutritional, and epidemiologic transitions. As seen in Fig. 5.1A, from 1990 to 2015, the burden of disease (expressed as disability-adjusted life years [DALYs]) caused by communicable, maternal, neonatal, and nutritional disorders decreased remarkably, while that due to noncommunicable diseases (NCDs) increased.[1] The increase in NCD burden results, in part, from population growth and population aging. Fig. 5.1B shows that DALYs, when expressed as rates per 100,000, which account for population growth, are stable, and Fig. 5.1C shows that age-standardized DALYs, which also account for population aging over the period, are decreasing. Social progress and advances in scientific knowledge translated into clinical and population interventions were the main drivers of the declines seen in Fig. 5.1C. However, since the crude measures (see Fig. 5.1A and B) are the true indicators of population burden, the NCDs, many of which are caused by metabolic risk factors and include the principal endocrinopathies, have become, at the global level, the major cause of disease burden.

The 21st century is now faced with new and complex demands for action at both the population and clinical levels. Of major concern, progress in alleviating disease burden has not resolved longstanding inequities, leaving the less developed countries often with a double load of disease burden, the unresolved old ones and the new ones, thus posing difficult challenges to policymakers and to global health. We will address in this chapter the burden of endocrine diseases, namely obesity and excess weight, diabetes, iodine deficiency/thyroid goiter, and thyroid cancer, selected on the basis of their importance and the availability of reliable data. To facilitate the interpretation of burden presented, Table 5.1 summarizes the metrics utilized in its quantification throughout the chapter.



• **Fig. 5.1** Trends from 1990 to 2015, by GBD Level 1 disease groups, in (A) global DALYs, (B) crude DALY rates, and (C) age-standardized DALY rates. The difference in trends between (A) and (B) is caused by population growth, and the difference between (B) and (C) is caused by changes in the age distribution of the population. Shaded areas show 95% uncertainty intervals. *DALYs,* Disability-adjusted life years. (From GBD 2015 DALYs and HALE Collaborators. Global, regional, and national disability-adjusted life-years [DALYs] for 315 diseases and injuries and healthy life expectancy [HALE], 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet.* 2016;388:1603–1658.)

TABLE 5.1 Epidemiologic Indicators of Disease Presence and Burden

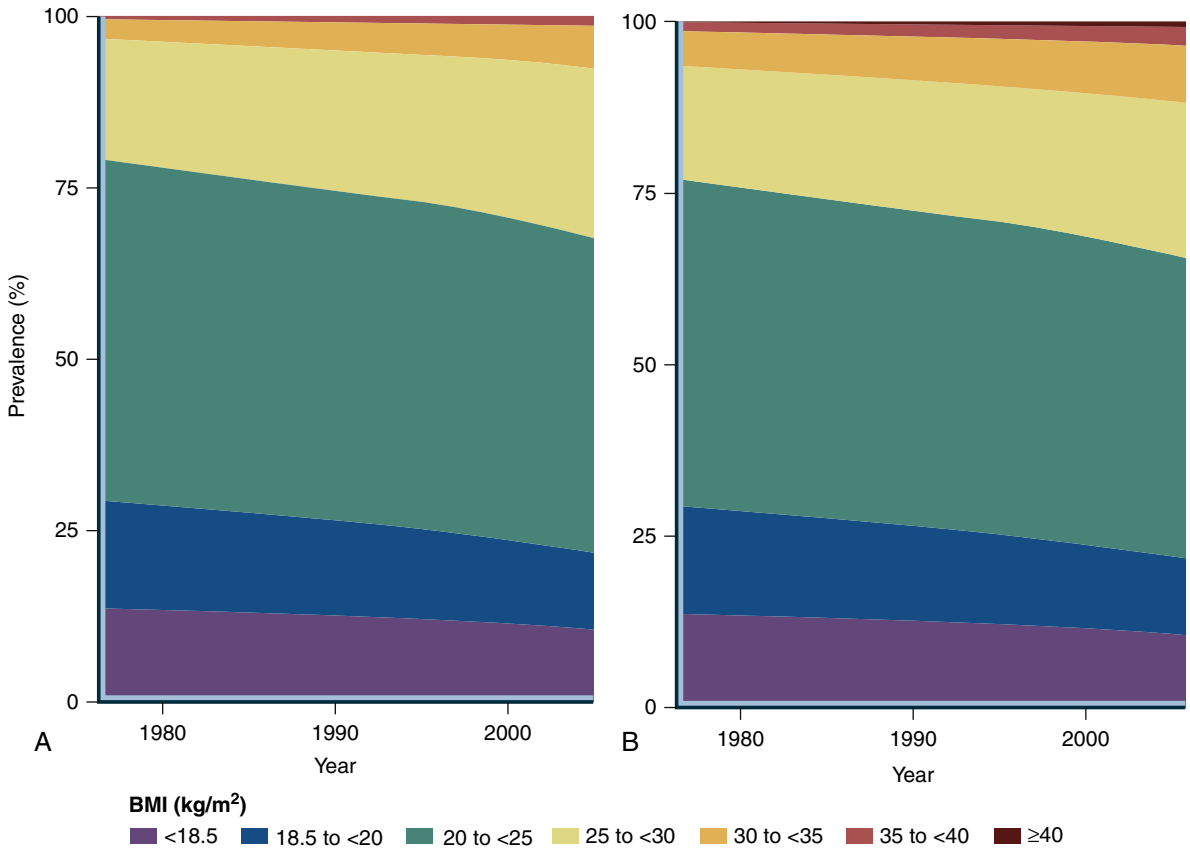
Indicator (Metric)	Abbreviation	Definition
Incidence	—	The number of new cases of a given disease during a given period in a specified population.
Prevalence	—	The total number of cases of a given disease in a specified population at a designated time.
Years lived with disability	YLDs	Morbidity burden: YLDs consider any short-term or long-term health loss. It is calculated by multiplying the prevalence of the condition causing health loss (e.g., blindness due to diabetes) by the disability weight (0.19) estimated for that condition (i.e., a year lived with blindness is worth 81% of a year lived in full health).
Years of life lost due to premature mortality	YLLs	Mortality burden: YLLs are calculated by subtracting the age at death from the longest possible life expectancy for a person at that age.
Disability-adjusted life years	DALYs	Overall disease burden: The sum of years lost due to premature death (YLLs) and years lived with disability (YLDs).

Data from Institute for Health Metrics and Evaluation.[11,12] More detailed information on calculations can be found in the methodology supplements to GBD Capstone papers published in the *Lancet* in 2017[13]; and the Institute for Health Metrics and Evaluation (IHME). Terms defined. http://www.healthdata.org/terms-defined; Institute for Health Metrics and Evaluation (IHME). Frequently asked questions. http://www.healthdata.org/gbd/faq; Institute for Health Metrics and Evaluation (IHME). GBD publications. http://www.healthdata.org/gbd/publications.

Obesity and Excess Weight

Prevalence and Trends

As extensively documented, the prevalence of obesity (body mass index [BMI] ≥30 kg/m^2) has increased dramatically over the past several decades throughout the world. From 1975 to 2014, as estimated by the NCD Risk Factor Collaboration,[2] global age-standardized mean BMI has increased 3.5 kg/m^2 for men, from 21.7 kg/m^2 (95% CI 21.3–22.1 kg/m^2) to 24.2 kg/m^2 (24–24.4 kg/m^2), and 2.3 kg/m^2 for women, from 22.1 kg/m^2 (21.7–22.5 kg/m^2) to 24.4 kg/m^2 (24.2–24.6 kg/m^2). These changes, expressed in the usual nutritional status categories, are visualized in Fig. 5.2. Similarly, from 1990 to 2017, as estimated by the Global Burden of Disease Study (GBD), the sum of risk across high levels of BMI increased 70%, and over the past decade increased 39%, the increase occurring at a constant rate of approximately 2% per year.[3]



• **Fig. 5.2** Worldwide trend in nutritional status categories. (A) Men and (B) women, 1975 to 2015. *BMI,* Body mass index. (From NCD Risk Factor Collaboration [NCD-RisC]. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. *Lancet.* 2016;387:1377–1396.)

Currently, GBD estimates that 15.2% of all adults—766 (758–774) million adults—are obese, prevalence being greater in women than in men. Additionally, 5.3% of the world's children and adolescents—130 (125–134) million—are obese. Over the lifespan, rates of obesity decrease slightly from age 2 to the beginning of adolescence, then increase steadily from age 15 and peak between the fourth and seventh decades. This peak comes earlier and at a lower level in men than in women and in countries of lesser versus greater sociodemographic development. At the end of life, prevalence declines,[4] in large part due to greater premature mortality among the obese.

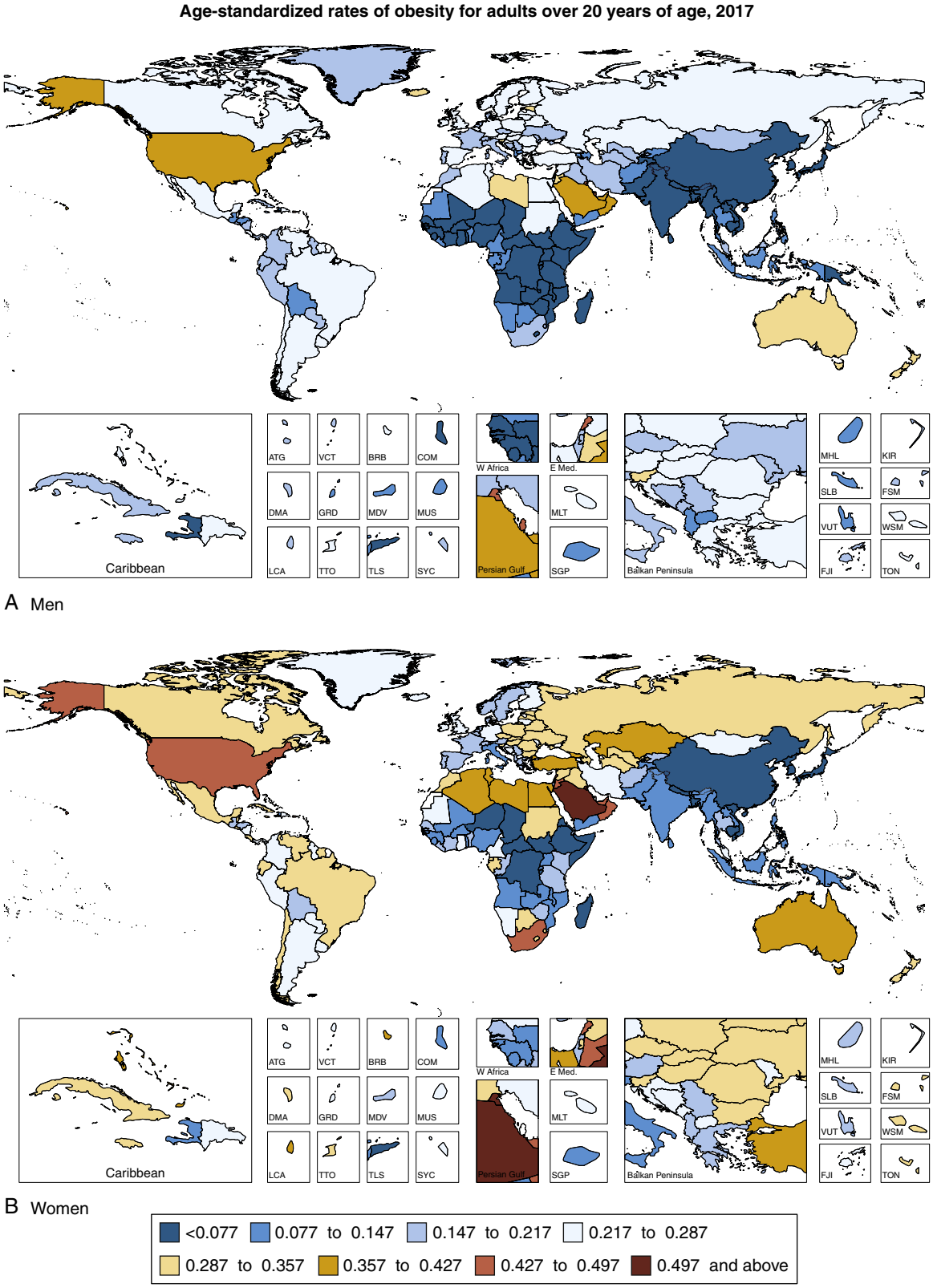
Although obesity rates are increasing in all geographic regions, both baseline values and rates of increase vary tremendously. As a result, obesity prevalence in countries extends across a broad range of values, as seen by the color coding in world maps (Fig. 5.3). In 2016, Pacific Islanders, followed by nations of the Middle East and then several higher income countries of Anglo-Saxon origin, had the greatest frequency of obesity. Lowest frequencies were seen in Asian countries. Examples of extreme country differences in obesity rates for women are seen comparing American Samoa (65.3%), Kuwait (47.1%), and the United States (36.5%) with Vietnam (2.7%) and Japan (3.9%).[5]

Part of this variability across nations can be explained by differences in socioeconomic development, as described by GBD sociodemographic index (SDI) categories, which are based on educational attainment, income, and fertility. As seen in Fig. 5.4, obesity rates among boys and men in low SDI locations have remained relatively stable over time, whereas rates in all other groups have risen steadily, for both children and adolescents, and for adults.

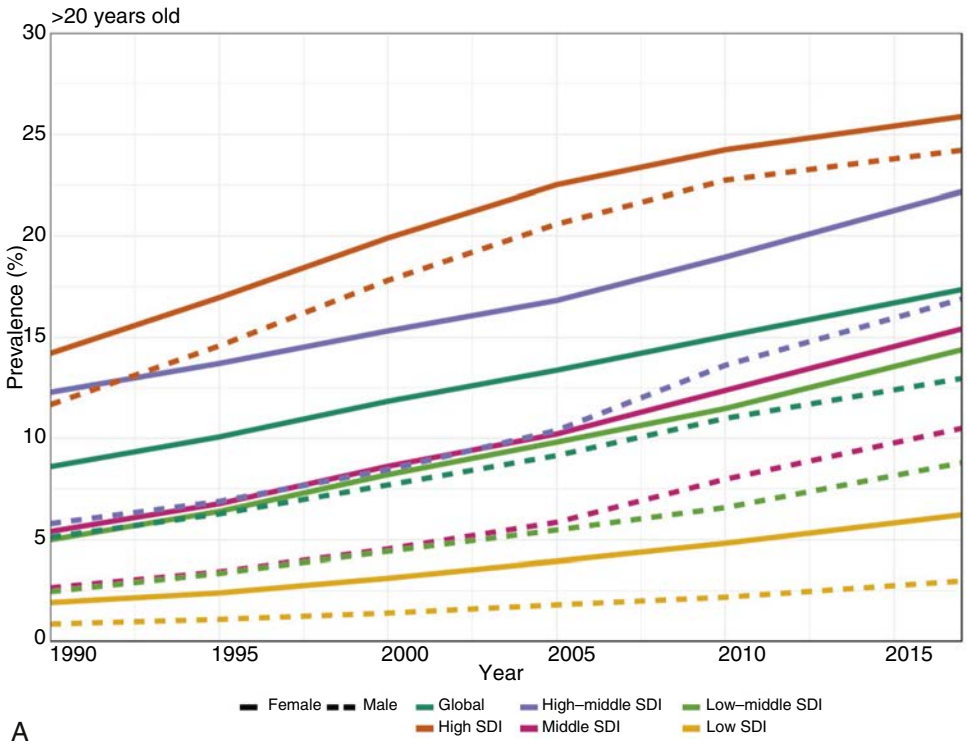
The Burden of Excess Weight

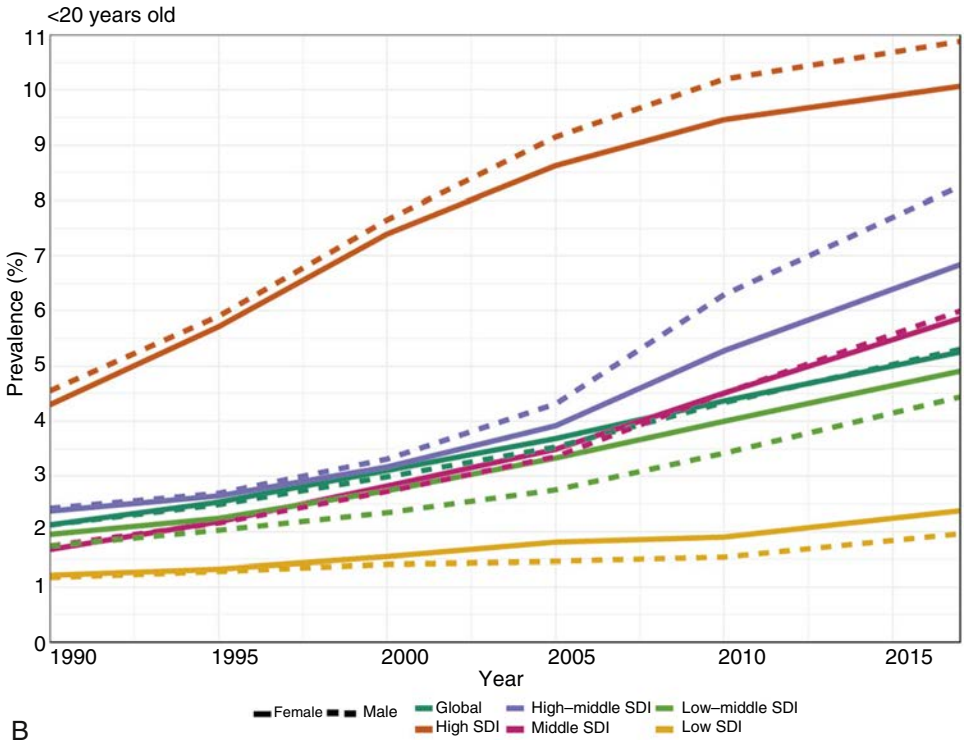
Fig. 5.5 shows the burden due to high BMI relative to that caused by other major cardiometabolic risk factors in 1990 and 2017. Within this comparison, the burden due to high BMI showed the greatest increase over this period. Currently, considering risk factors for all diseases, high BMI ranks 5th in terms of disease burden in the world, having risen from the 13th position in 1990.

High BMI causes both premature loss of life (72% of its burden) and disability among those living (the remaining 28%). The fraction due to disability has risen from 22% to 28% over recent decades and is anticipated to continue to rise in importance. In relative terms, considering unstandardized rates for the entire world population, high BMI is estimated to have caused 8.4% of deaths, 6.5% of premature mortality (years of life lost [YLLs]), 4.8% of morbidity (years lived with disability [YLDs]), and 5.9% of overall disease burden (DALYs). In relation to 1990, the burden it caused had increased 82% for deaths, 155% for YLLs, 90% for YLDs, and 133% for DALYs by 2017.



• **Fig. 5.3** Age-standardized fraction of the population presenting obesity by country, 2017. (A) Men and (B) women. (From Institute for Health Metrics and Evaluation; Global Burden of Disease [GBP] Group. www.healthdata.org/gbd.)

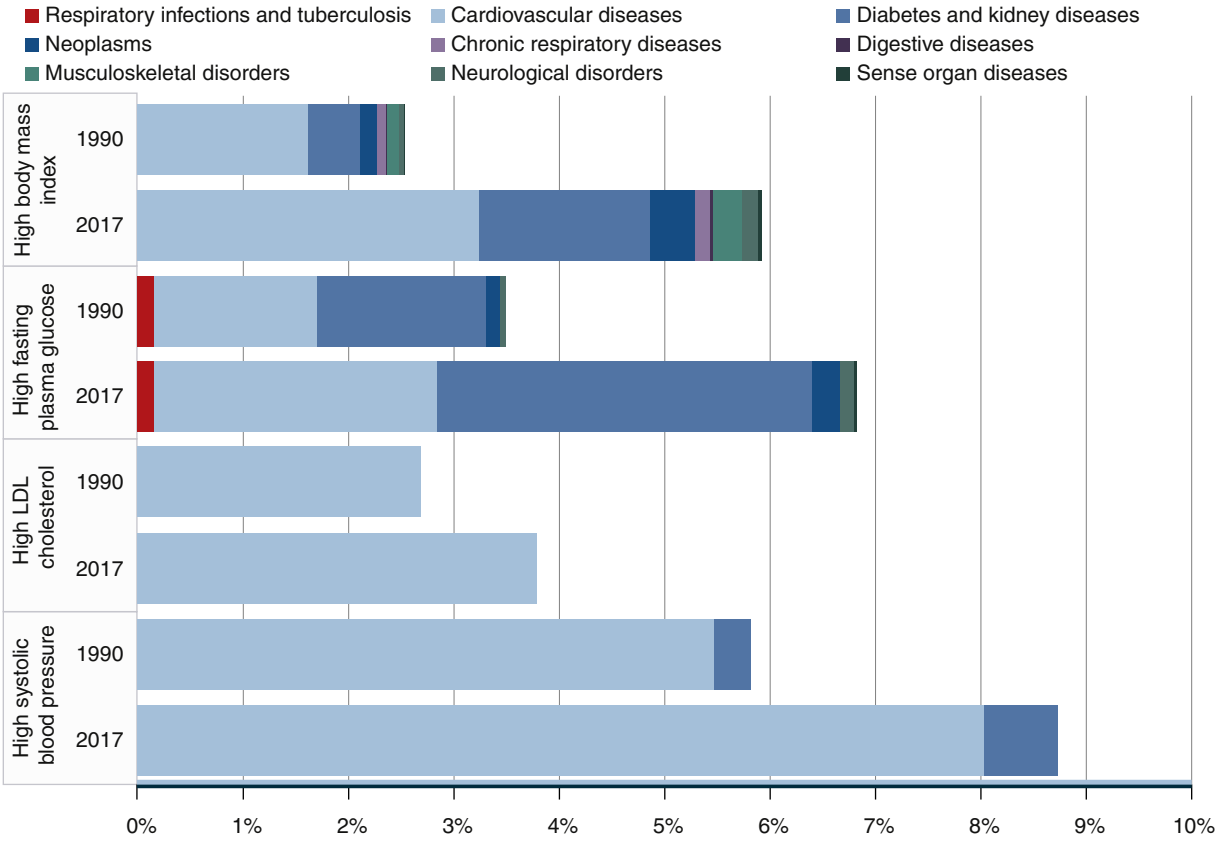




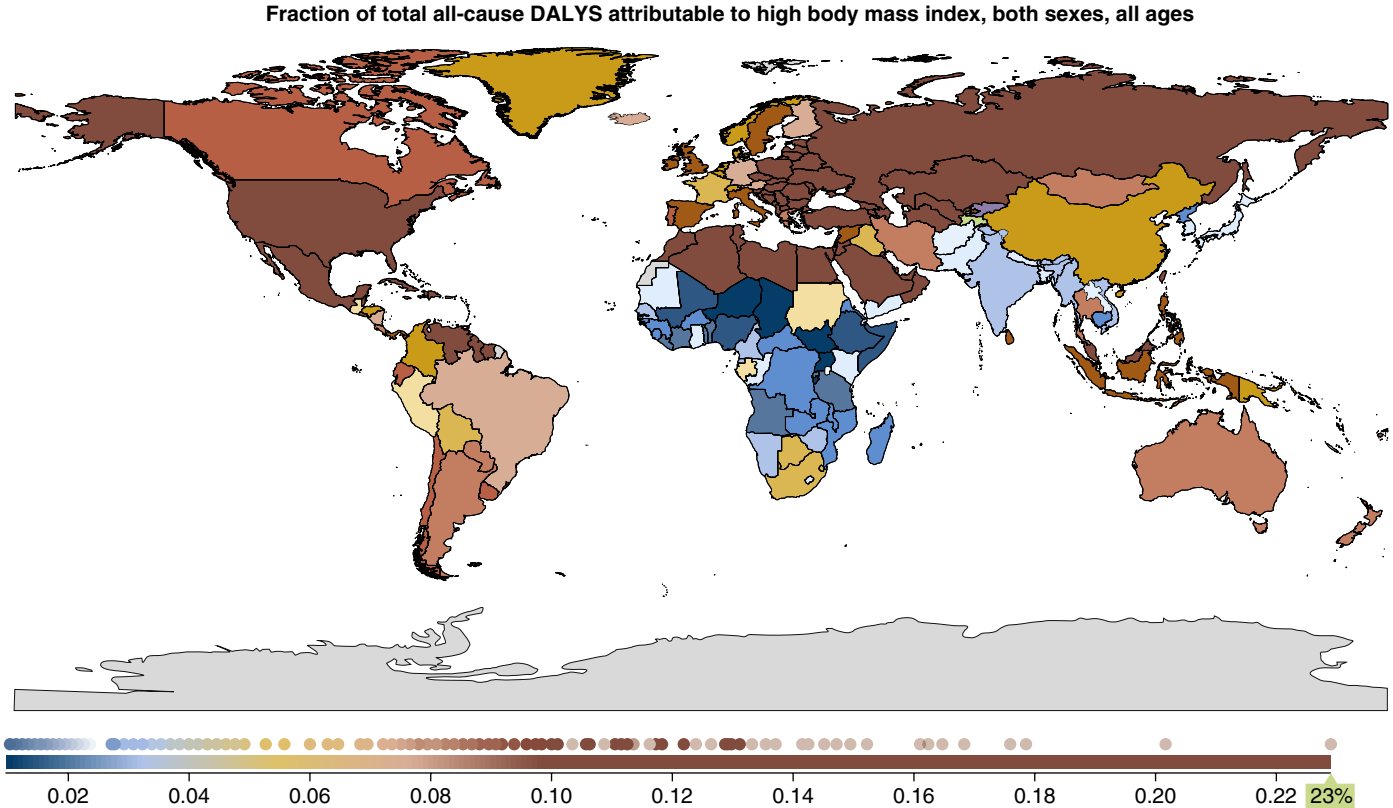
• **Fig. 5.4** Trends in the prevalence of obesity among children (A, aged >20) and adults (B, aged <20) from 1990 to 2017 overall and by level of sociodemographic development (SDI), separately for men and women. (From Institute for Health Metrics and Evaluation; Global Burden of Disease [GBP] Group. www.healthdata.org/gbd.)

As also seen in Fig. 5.5, the colors of the 2017 high BMI bar indicate that 55% of its burden resulted from cardiovascular diseases, 2% from chronic respiratory diseases, 28% from diabetes and renal diseases, 5% from musculoskeletal disorders, 7% from cancer, 3% from neurologic disorders (dementia), and 1% from remaining conditions.

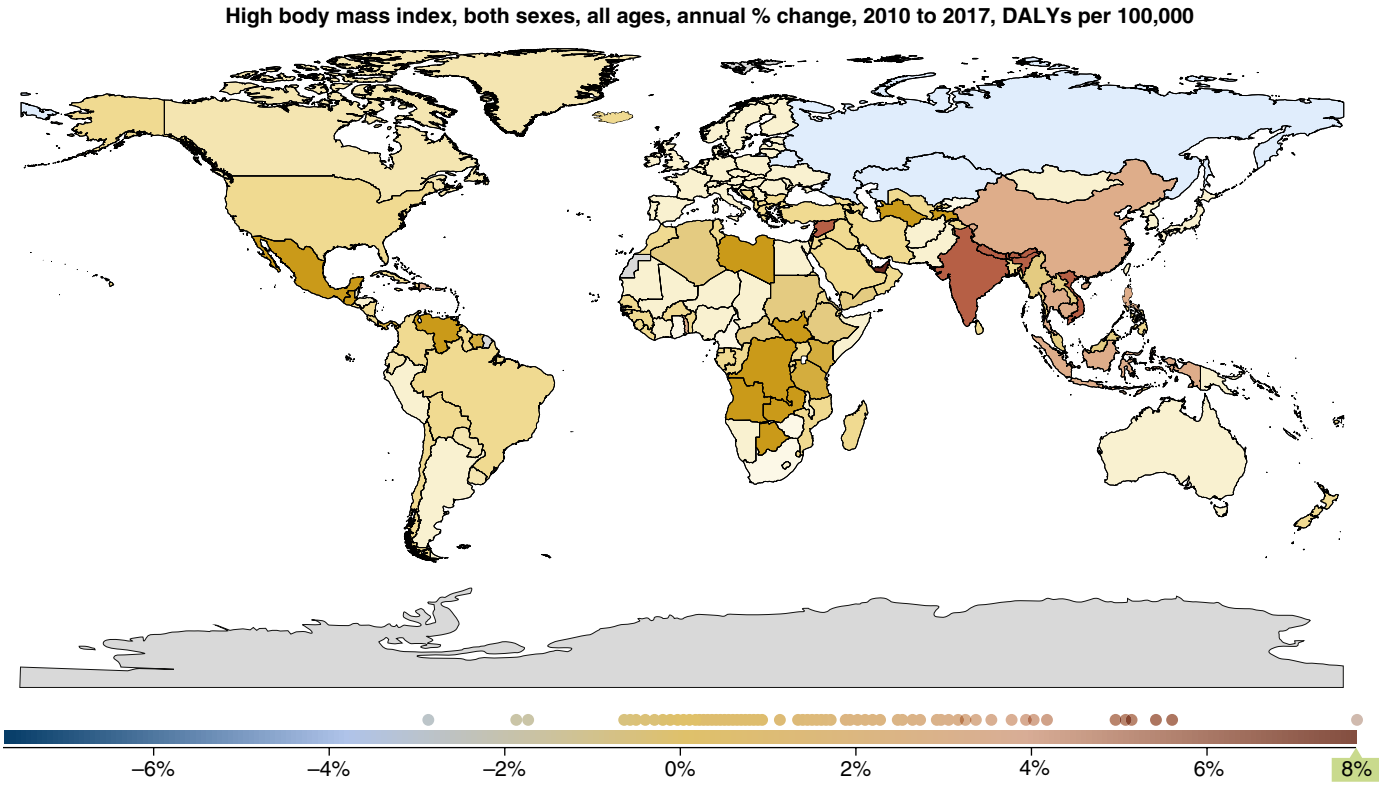
Like its prevalence, the burden of excess weight, though rising in all regions, is not evenly distributed among nations. Fig. 5.6



• **Fig. 5.5** Percent of total risk-factor–attributable disease burden due to the major risk factors for noncommunicable diseases, 1990 and 2017. The diseases causing this burden are shown by color codes. *LDL*, Low-density lipoprotein. (From Institute for Health Metrics and Evaluation [IHME]. GBD compare data visualization. 2017. https://vizhub.healthdata.org/gbd-compare/.)



• **Fig. 5.6** Crude fraction of all DALYs due to high BMI, men and women, 2017. (From Institute for Health Metrics and Evaluation [IHME]. GBD compare data visualization. 2017. https://vizhub.healthdata.org/gbd-compare/.)



• **Fig. 5.7** Relative annual percent change in the crude rate of DALYs attributable to high BMI, men and women, 2010 to 2017. (From Institute for Health Metrics and Evaluation [IHME]. GBD compare data visualization. 2017. https://vizhub.healthdata.org/gbd-compare/.)

shows the burden of high BMI, expressed as a fraction of disease burden, for the world's countries in 2017. For countries in dark brown greater than 10% of total disease burden was due to high BMI. For those in light brown, approximately 8% was due to high BMI; in dark yellow, approximately 6%; and in blue, less than 4%.

Fig. 5.7 shows the varying rates of growth of this burden, in terms of DALYs, from 2010 to 2017. The few countries shown in blue had a decrease in burden over this period and the small fraction of countries in yellow, a stable one. For those in tan, brown, light chocolate brown, and dark brown, burden has increased at annual rates of approximately 1%, 3%, 4% and 6%, respectively. The greatest increases are seen across the southern countries of Asia and much of Africa, both above and below the Sahara. Most of the Americas and much of Africa have experienced annual increases ranging from 1.5% to 3%. Europe and northern Asia, in general, have experienced the lowest increases.

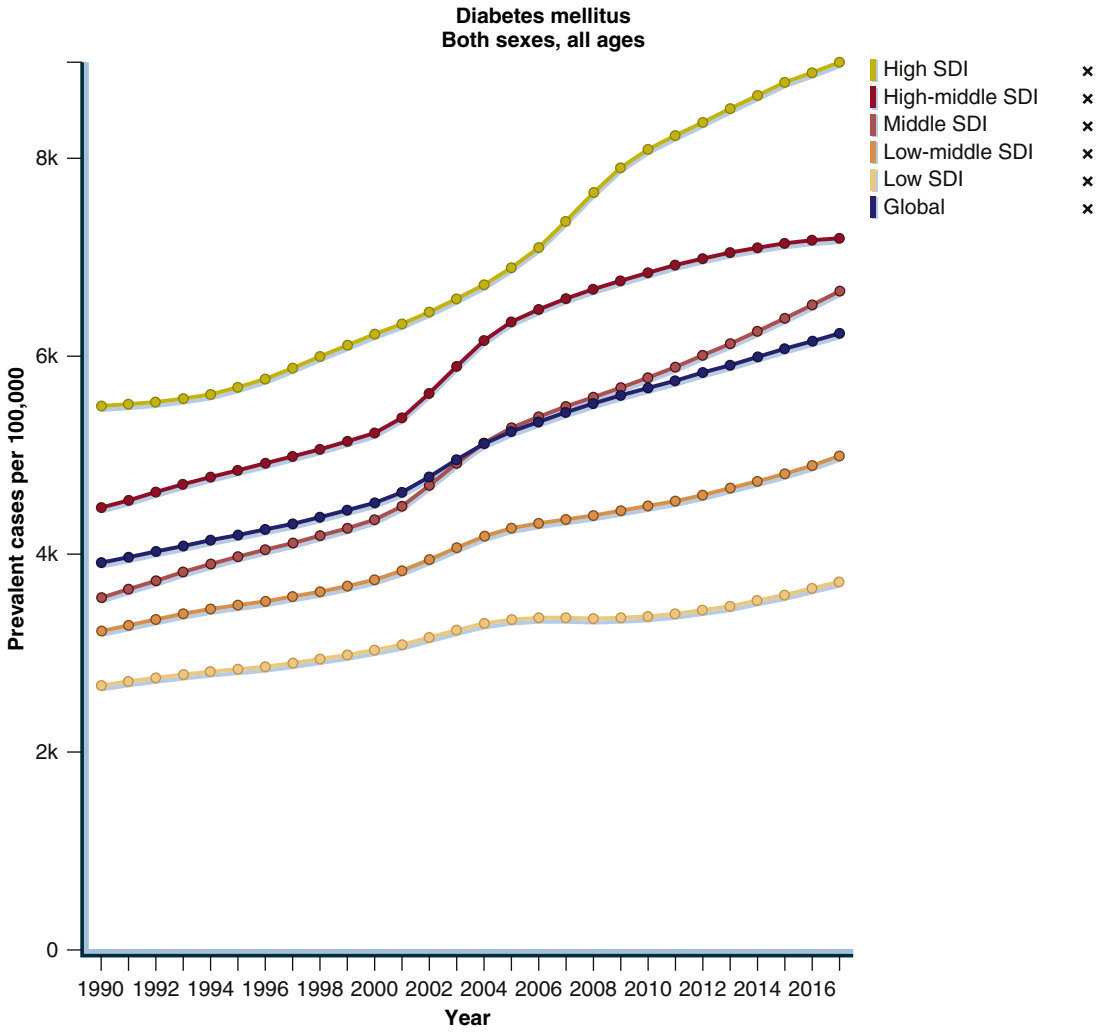
The Burden of Diabetes

Prevalence, Incidence, and Mortality Trends

From 1980 to 2014, as estimated by the NCD Risk Factor Collaboration,[6] the global age–standardized diabetes prevalence in adults aged 20 years and older more than doubled in men, from 4.3% (2.4–7%) to 9% (7.2–11.1%), and increased almost 60% in women, from 5% (2.9–7.9%) to 7.9% (6.4–9.7%). Given their large population size, China, India, the United States, Brazil, and Indonesia accounted for half of all adults with diabetes in 2014.

As seen in Fig. 5.8, from 1990 to 2017, the prevalence of diabetes has increased globally across all levels of development.[3] In large part this is due to the aging of the world's populations. As seen in Fig. 5.9, the crude incidence rates of diabetes generally increased over this period as well, driven by the aging of the population and the increase in certain risk factors, notably obesity. Also apparent from Fig. 5.9 is the stabilization of incidence in the high-SDI group and the decrease in incidence in high-middle SDI populations. It is noteworthy that the stabilization/decrease in incidence in higher SDI groups has not been sufficient to halt their rising prevalence of diabetes. The accentuated recent rise in prevalence in the high-SDI group is fueled in large part by the notable decline in diabetes mortality observed in this group since 2003, as seen in Fig. 5.10. Of note in this figure, all other SDI groups continue to show rising mortality due to diabetes.

An issue that has generated great concern in recent years is the rising prevalence/incidence of diabetes among the young populations. While incidence of type 1 diabetes has risen worldwide, the increase also results from the rising incidence of type 2 diabetes in the young.[7] Due to the early disease onset of type 2 diabetes, those affected will be exposed to metabolic and vascular disturbances for longer periods, resulting in an ominous prognosis, estimated to be worse than that predicted for type 1 diabetes manifesting in a similar period.[8] This new burden of young-onset diabetes coupled with the aging of the population heralds the emergence of growing populations of people with diabetes of extremely long duration manifesting premature complications, including renal failure, multiple morbidities, and frailty, and thus imposing new pressures on individuals, families, health care systems, and societies.



• **Fig. 5.8** Trends in the crude prevalence of diabetes globally and across sociodemographic index (SDI) quintiles, 1990 to 2017. (From Institute for Health Metrics and Evaluation [IHME]. GBD compare data visualization. 2017. https://vizhub.healthdata.org/gbd-compare/.)

Burden

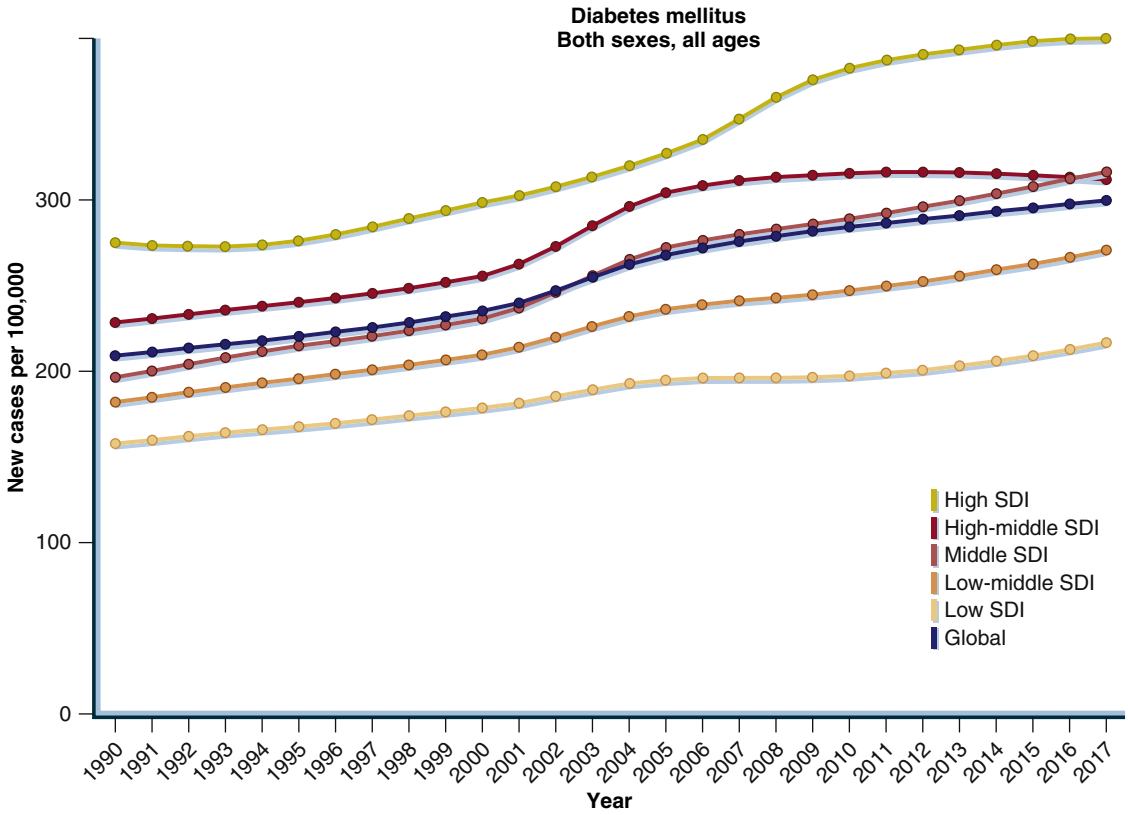
To characterize the burden of hyperglycemia along its continuous spectrum, GBD joins the burden of diabetes together with that of intermediate states of hyperglycemia, labeling it as burden due to "high fasting plasma glucose," defined as values above 4.5 to 5.4 mmol/L, their established range of minimal risk. High fasting plasma glucose in 2017 was the fourth leading risk factor for all-cause disease burden (crude DALYs) in the world, having risen from the ninth position in 1990.

Considering unstandardized rates for the world's population, high fasting plasma glucose is estimated to have caused 11.7% of deaths, 7.4% of YLLs, 5.8% of YLDs, and 6.8% of DALYs in 2017. In relation to 1990, this burden has increased 57% for deaths, 116% for YLL, 50% for YLDs, and 95% for DALYs. Fig. 5.5 shows that acute or microvascular complications (including renal disease) and living with diabetes in general produced 52% of the burden; cardiovascular diseases, 39%; cancer, 4%; tuberculosis, 2.5%; neurologic disorders (dementia), 2%; and cataract and glaucoma, <1%.

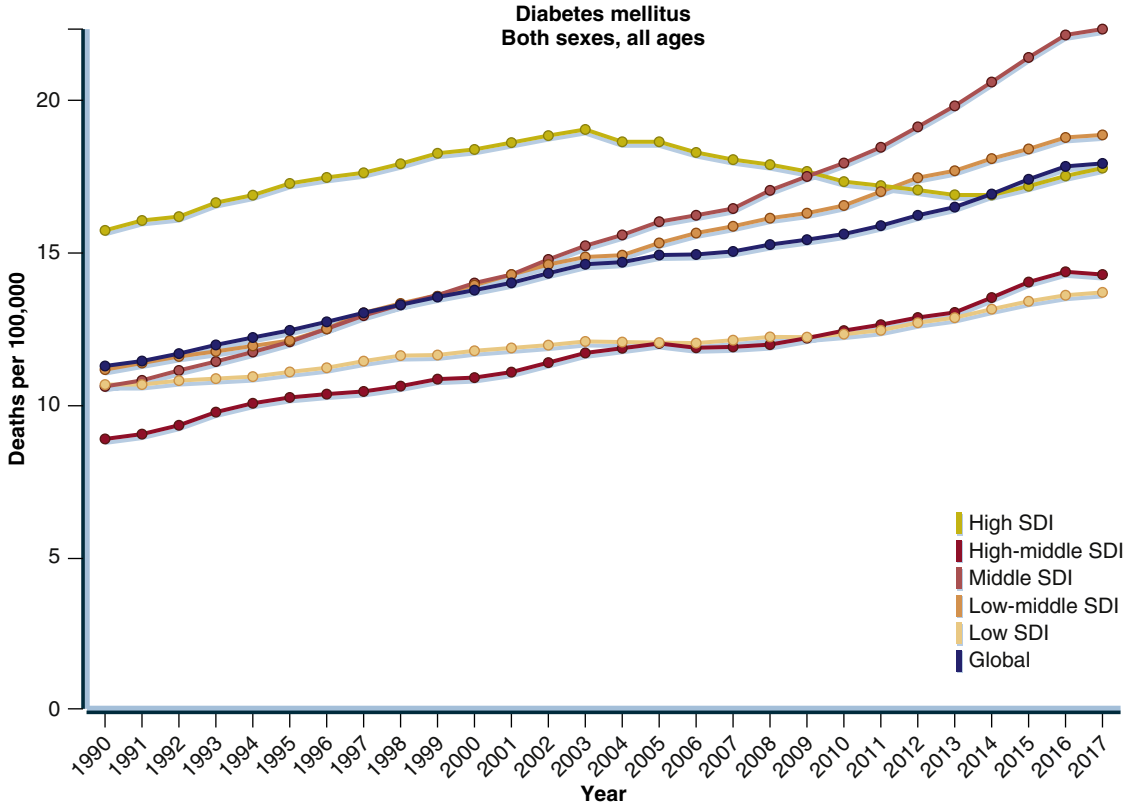
The Burden of Thyroid Diseases

GBD 2017 data provide information regarding the two clinically important subgroups of thyroid diseases: iodine deficiency/thyroid goiter and thyroid cancer.[3]

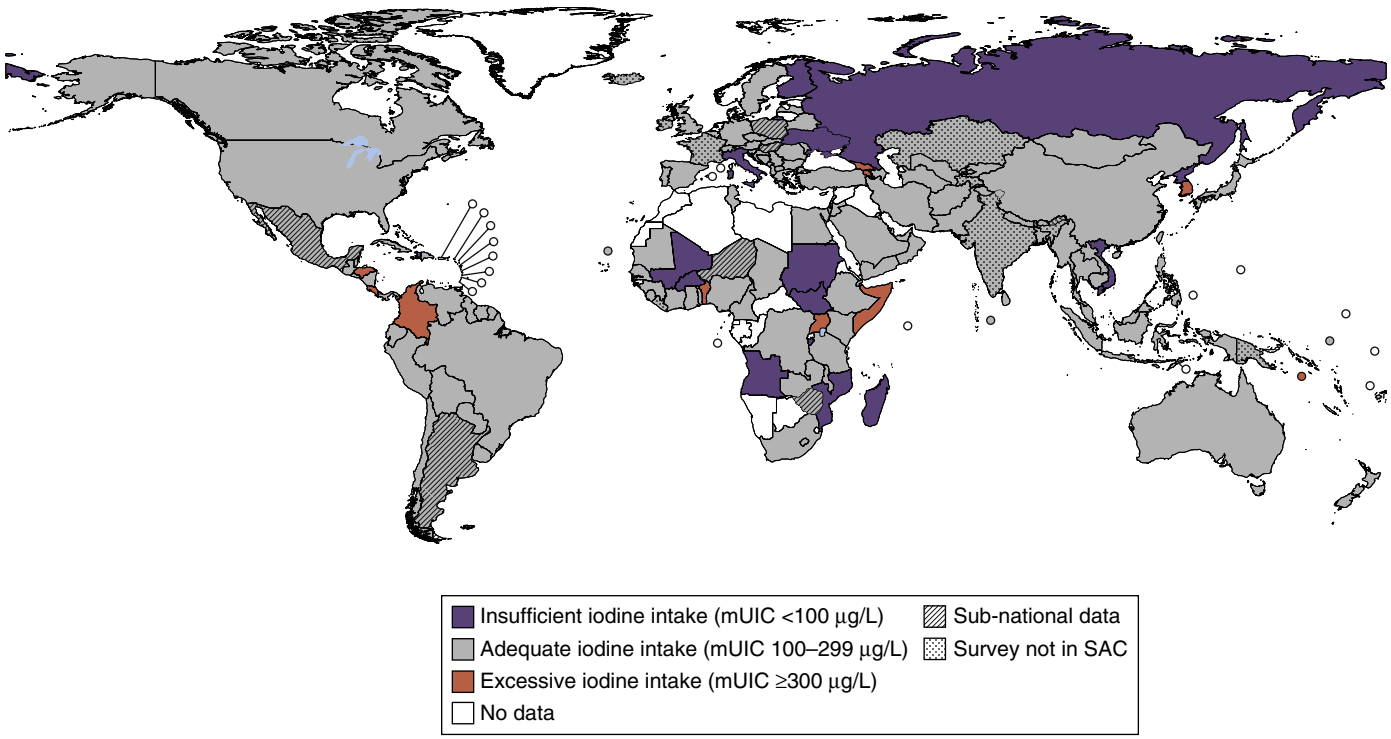
Deficiency of iodine, an essential component of the thyroid hormones, leads to inadequate thyroid hormone production. Thyroid hormones and thus iodine are necessary for many activities in the body. Iodine deficiency produces several consequences in humans, collectively called *iodine deficiency disorders*. It is one of the most prevalent preventable nutrient deficiencies in the world. Although the number of countries in which iodine intake is adequate has increased in recent decades, many nations still have a clinically relevant prevalence of deficiency. Most of them are low-income and middle-income countries, such as Angola, Burkina Faso, Burundi, Haiti, Mozambique, Sudan, and South Sudan. However, even countries in transition, such as Russia and the Ukraine, and some high-income countries (Finland, Israel, and Italy), present problematic levels.[9] Fig. 5.11 presents a global scorecard of iodine nutrition based on median urinary iodine concentration in school-age children and adults, in 2017.



• **Fig. 5.9** Trends in the crude incidence of diabetes globally and across sociodemographic index (SDI) quintiles, 1990 to 2017. (From Institute for Health Metrics and Evaluation [IHME]. GBD compare data visualization. 2017. https://vizhub.healthdata.org/gbd-compare/.)



• **Fig. 5.10** Trends in crude diabetes mortality globally and across sociodemographic index (SDI) quintiles, 1990 to 2017. (From Institute for Health Metrics and Evaluation [IHME]. GBD compare data visualization. 2017. https://vizhub.healthdata.org/gbd-compare/.)



• **Fig. 5.11** Global scorecard of iodine nutrition based on median urinary iodine concentration in school-age children (SAC) and adults, 2017. (From Iodine Global Network [IGN]. http://www.ign.org/index.cfm.)

The definition of iodine deficiency in GBD 2017 includes estimates of the subset of iodine deficiency associated with visible goiter (grade 2) and its associated sequelae because of the greater reliability and consistency of the clinical diagnosis of grade 2 goiter worldwide. It does not include estimates of subclinical iodine deficiency or nonvisible goiter (grade 1) induced by iodine deficiency. The global burden of iodine deficiency disorders, expressed in all-age DALYs, has shown improvement over the 1990–2017 period, declining 43.6%, from 61.7/100,000 (37.7–98.7/100,000) to 26.9/100,000 (16.3–42.6/100,000).[3]

Thyroid cancer incidence has increased in all continents, except in Africa. In fact, incidence rates are more than twofold higher in high-income countries compared with low-middle-income countries.[10] Fig. 5.12 illustrates the differences in age-standardized incidence rates for thyroid cancer in the 1990–2017 period, in countries categorized by the SDI. In spite of the overall increase in incidence in the last three decades, a recent decline has been noted in countries with high and high-middle SDI. Fig. 5.12 also highlights the discrepancy across the developmental continuum in incidence of thyroid cancer. This socioeconomic discrepancy is probably due to differences in access to diagnosis, which is more available in high-income countries.

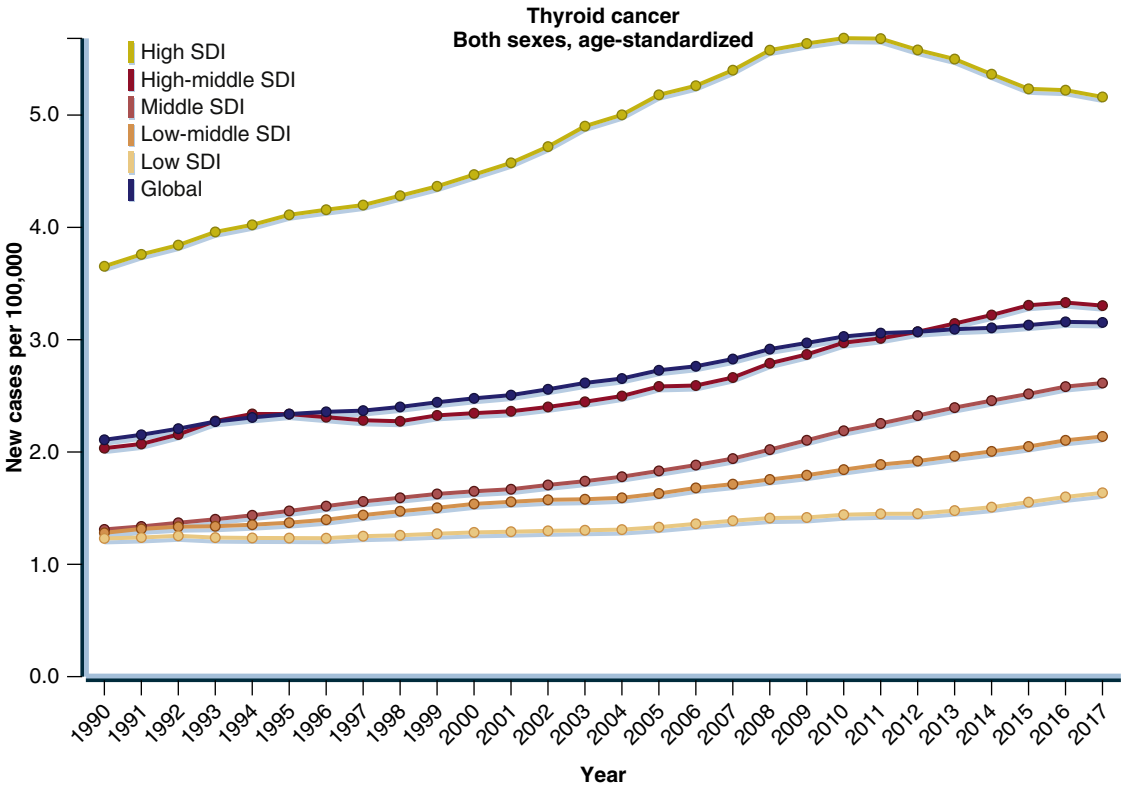
Despite this overall increase in incidence, age-standardized mortality rates of thyroid cancer have declined in countries with high, high-middle, and low SDI (by 18%, 11%, and 5%, respectively), while slightly increasing in countries with middle and low-middle SDI (by 6% and 8%, respectively), as shown in Fig. 5.13. As the decline is more evident in countries with high and high-middle SDI, it is likely due to earlier diagnosis with quick therapeutic intervention.

According to 2017 GDB data,[4] the crude global burden of thyroid cancer evaluated by DALYs in the 1990–2017 period increased from 12/100,000 (11–13.2) to 14.8/100,000 (14–16.1). Fig. 5.14 shows age-standardized DALYs due to thyroid cancer in countries categorized by SDI in this period. The global age–standardized DALY declined from 14.4/100,000 (13.4–15.8/100,00) to 14.1/100,00 (13.3–15.3/100,000). Despite the relatively accentuated decline in low-SDI countries, the burden of thyroid cancer (DALY) continues to be greater in countries with low or low-middle SDI. This socioeconomic discrepancy is reflecting differences in access to diagnosis and adequate treatment.

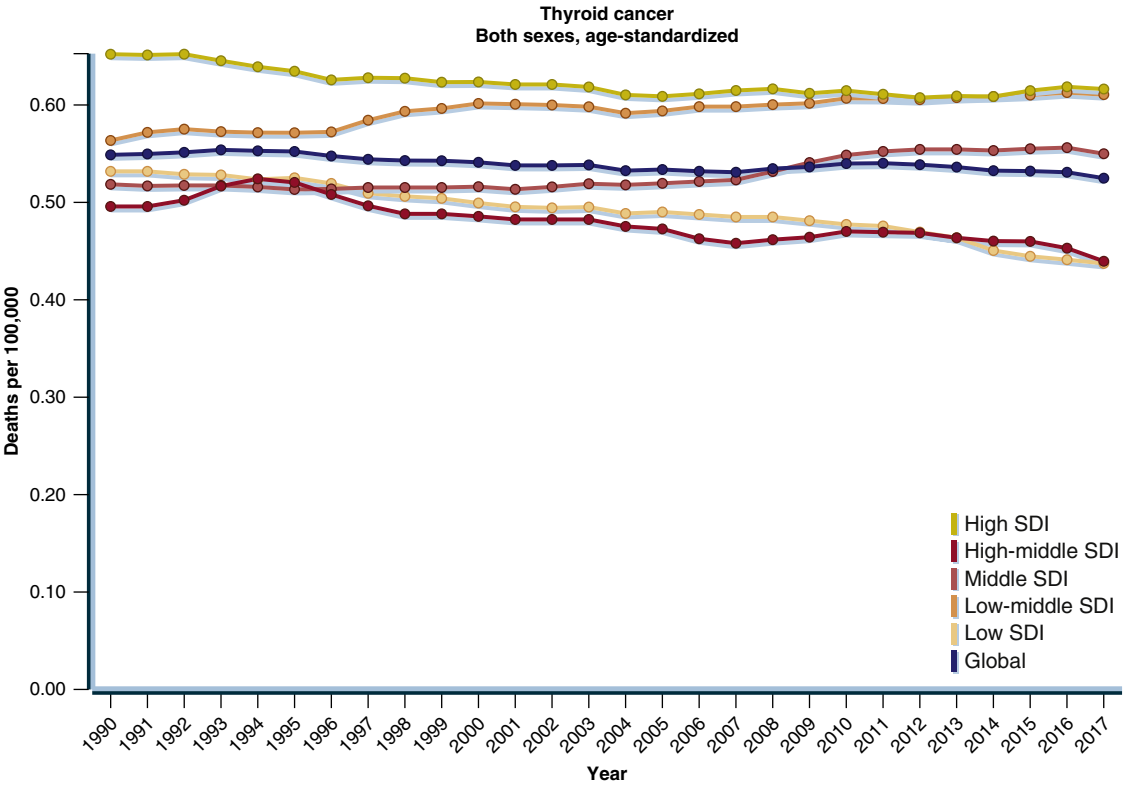
These data highlight the necessity to improve iodination programs and access to care for patients with thyroid diseases through the development of infrastructure and appropriate patient management in the developing world. These actions could save costs, improve quality of life, and produce a healthier workforce. These GDB results call attention to the need to include thyroid diseases in the public health agenda.

Perspectives for the Next Decade

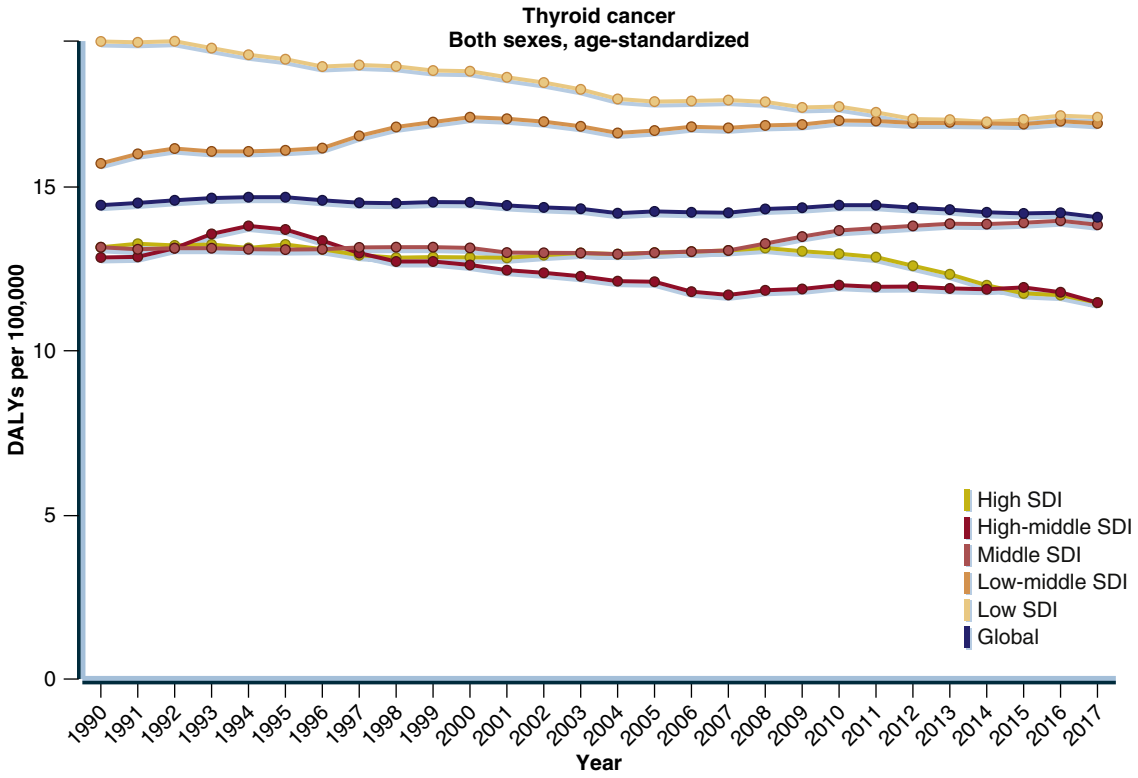
Recent trends in the prevalence of high BMI and hyperglycemia suggest that unless major public health or clinical breakthroughs occur, the burden of obesity and diabetes will continue to grow and demand commitment of ever-greater resources in the foreseeable future. The continuous aging of the world's population will be a major driver in these trends. On the other hand, extension of policies involving iodine supplementation will likely continue to decrease the burden of iodine-related thyroid disease and complications. Access to early diagnosis and treatment of thyroid cancer, especially in countries at low and middle levels of development, will hopefully lead to a continuing decrease in disease burden.



• **Fig. 5.12** Trends in age-standardized incidence rates (per 100,000) of thyroid cancer, for both sexes, in countries categorized in quintiles by the sociodemographic index (SDI), 1990 to 2017. (From Institute for Health Metrics and Evaluation [IHME]. GBD compare data visualization. 2017. https://vizhub.healthdata.org/gbd-compare/.)



• **Fig. 5.13** Trends in age-standardized mortality rates (per 100,000) by thyroid cancer in countries categorized in quintiles by the sociodemographic index (SDI), 1990 to 2017. (From Institute for Health Metrics and Evaluation [IHME]. GBD compare data visualization. 2017. https://vizhub.healthdata.org/gbd-compare/.)



• **Fig. 5.14** Trends in DALYs attributable to thyroid cancer in countries categorized in quintiles by the sociodemographic index (SDI), 1990 to 2017. (From Institute for Health Metrics and Evaluation [IHME]. GBD compare data visualization. 2017. https://vizhub.healthdata.org/gbd-compare/.)

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The complete list of references is available online at ExpertConsult.com.

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6

Navigating Through Clinical Practice Guidelines in Endocrinology

NAYKKY SINGH OSPINA, SPYRIDOULA MARAKA, RENE RODRIGUEZ-GUTIERREZ, JUAN P. BRITO, AND VICTOR MONTORI

CHAPTER OUTLINE

KEY POINTS

- Clinical practice guidelines provide actionable recommendations for clinicians to provide care.
- The GRADE system provides a systematic framework for an in-depth assessment of the quality of the evidence and considers important factors used in clinical decision making to determine the strength of clinical practice recommendations.
- A systematic evaluation of the available literature (body of evidence) and a multidisciplinary panel are important features of trustworthy guidelines.
- Recommendations have a direction (in favor of or against an intervention), a strength (strong or weak), and a judgment of the quality of the evidence supporting the recommendation (very low, low, moderate, high).
- To provide care supported by clinical guidelines, clinicians should engage in meaningful conversations with their patients to understand their situation because evidence alone is not sufficient to provide care.

Introduction

Clinical decision making is a complex process in which, ideally, clinicians and patients create plans of care that reflect the best available research evidence while also considering the patients' values, preferences, and context.[1–3] The increasing number of published trials and studies, unclear sources of evidence, increasing number of interventions, patients with more and complex comorbidities, and time constraints make clinical decision making and caring for patients even more challenging.[4–7] Clinical practice guidelines are statements that provide actionable recommendations intended to support clinicians in the decision-making process and improve patient care.[8,9] Because of their potential benefits, the production of clinical practice guidelines has increased during the last few years. In fact, the National Guideline Clearinghouse website had ~1500 available guidelines with more than 100 guidelines related to endocrine diseases.[10] The availability of this large number of clinical guidelines makes it imperative for clinicians to understand the process associated with their development, their applicability, and their downsides in clinical practice; at the same time, clinicians must recognize which guidelines can be trusted and are likely to result in improved patient care when implemented.

Development of Trustworthy Clinical Practice Guidelines

To be trustworthy, clinical practice guidelines should be based on one or usually multiple systematic reviews of the available evidence and include a multidisciplinary guideline panel (free or

transparent regarding financial relationships).[8] This panel should include members with methodologic and clinical expertise that can systematically evaluate the factors that may influence a clinical decision and provide recommendations that are clear and actionable. In addition, patient representatives can be included.[8,11–14] Although the best way to involve patients in the development of clinical practice guidelines has not been defined, their integration into these panels is feasible. Moreover, patient panels formulate similar recommendations when compared to physician panels when presented with evidence warranting moderate to high confidence.[14] The Institute of Medicine has suggested a series of criteria that can help different stakeholders evaluate the quality of the process used to develop clinical practice guidelines and their trustworthiness (Table 6.1).

Many systems have been developed to provide a framework to analyze clinical evidence and provide practice recommendations. Initially, many clinical practice guidelines were developed based on expert opinion without a systematic evaluation of the available literature, making them prone to bias and inclusion of untrustworthy recommendations.[9] Moreover, many of these approaches provided recommendations based on study design alone and did not include explicit judgments about the strength of recommendations (i.e., how trade-offs between the expected benefits, harms, and costs weigh in during development of the recommendation). Furthermore, most systems were found to have low reproducibility of judgments (suggesting evaluation of different factors when making recommendations) and did not fit the needs of stakeholders.[15] To overcome the shortcomings of these systems, the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system was developed.[11] The GRADE system provides a systematic framework for an in-depth assessment of the quality of the evidence that is based on more than study design (randomized control trial vs. observational studies).[11–13,16,17] This approach also includes additional factors affecting the quality of evidence (Table 6.2) as well as other factors to consider when making a recommendation, such as the balance between benefits and harms, integration of the patient's values, preference and context, resources, and effects on health equity (Table 6.3).[12,17] This framework was first adopted by the Endocrine Society (Table 6.4) and is now used by more than 100 other organizations for the development of trustworthy guidelines.[9,18,19]

The GRADE system suggests several steps to develop trustworthy clinical practice guideline recommendations.[11–13,16,17,20–27]

Identification of the Clinical Question

Guideline panelists should select important clinical questions and identify the population to which the recommendations will apply, the intervention and comparator to be studied, and the health outcomes to be evaluated. The selected outcomes should be classified according to their clinical significance. When possible, patient-important outcomes should receive more value in decision making than surrogate markers (e.g., fracture rate compared to bone mineral density changes).[11,20,21]

Systematic Review of the Literature

Once the clinical question is identified, a systematic search of the literature is performed to identify all the available clinical studies (i.e., the body of evidence). This systematic and reproducible

TABLE 6.1 Suggested Criteria for Developing Trustworthy Guidelines

Criteria	Comment
Include a systematic evaluation of the clinical evidence	Allows identification of all the available evidence and prevents recommendations based on a single study or solely expert opinion. Allows comparison of effect estimates across different studies (likely including different populations and settings).
Include a multidisciplinary panel with clinical and methodologic expertise and other important stakeholders	A collaborative and multidisciplinary panel creates a space for evaluation of the evidence and of recommendations from different perspectives. Inclusion of patients is recommended. This can help clarify expected values and preferences of patients.
Consider the importance of patients' values and preferences	Clinical evidence is not sufficient to provide patient-centered care. As such, consideration of how the values and preferences of patients can affect clinical decision making is required. This is extremely important when there is expected variability in the values and preferences of patients.
Follow a transparent process	Guidelines can have significant impact on patient care and policy. Following a transparent and systematic approach increases our confidence that the recommendations are made to improve patient care and are free of conflict of interest. Guidelines should clearly report their funding source and potential conflict of interest of panel members.
Explain the logical relationship between alternative care options and health outcomes	Clear explanations of the reasoning behind recommendations, including harms/benefits, rating of the quality of the evidence, the value of patients' preferences, and the strength of the recommendation, are required. Differences in opinion between panel members can also be included. This transparency allows understanding of how recommendations were derived.
Provide explicit ratings of the quality of the supporting evidence and the strength of the recommendations	A systematic approach should be followed when assessing the quality of the evidence supporting different strengths of recommendations and clearly presented with each recommendation.
Be revised as new evidence warrants modification of the recommendations	Clinical practice guidelines should be updated to reflect the best available evidence.

TABLE 6.2 Domains Used to Assess the Quality of the Evidence

Domain	Comment
Rate Down the Quality of the Evidence	
1. Risk of bias	Randomized clinical trials and observational studies might be at higher risk of bias depending on features of their design or conduct that can lead to a systematic deviation of their results from the truth (bias). The presence of multiple sources of bias decreases the quality of the evidence.
2. Inconsistency	Refers to the difference in the magnitude of the effect across studies. Inconsistency is present when point estimates vary widely across studies or if there is no or only minimal overlap between confidence intervals. In addition, statistical tests such as the test for heterogeneity and the I^2 can help identify inconsistency.
3. Indirectness	This is present when there are significant differences between the populations, interventions, and outcomes evaluated in a study compared to the ones we want to study. In addition, the lack of head-to-head comparison between two alternative management strategies is a cause for indirectness.
4. Imprecision	The confidence intervals of an estimate are used to judge for imprecision. If the 95% confidence interval is wide, this suggests that the range in which the truth possibly lies is also wide. In addition, if the number of events and the sample size are small or if the confidence interval crosses the line of no effect, the quality of the evidence can also be rated down for imprecision.
5. Publication bias	Studies with statistically significant results are more likely to be published than those with negative results; as such, evaluation of the published literature can lead to an inaccurate estimate of the effect of an intervention. Publication bias can be considered when only a small number of studies is available and if most are sponsored by industry. The funnel plot is a commonly used method to assess for publication bias.
Rate Up the Quality of the Evidence	
1. Large effect	A magnitude of effect is considered large when the relative risk is between 2 and 5 or 0.5 and 0.2 without possible confounders or very large if the relative risk is above 5 or below 0.2 without problems of serious risk of bias or precision.
2. Dose-response gradient	This gradient is considered when evaluating cause-effect relationships. This is present when the effect increases with the dose/exposure.
3. Evaluation of plausible residual confounding	Rigorous observational studies will perform an adjusted analysis based on important prognostic factors. If a positive or negative effect is found after this adjustment, careful clinical analysis might help identify large effects, despite the presence of uneven groups that are expected to result in no effect.

evaluation of the literature will allow panelists to inform their recommendations based on the complete spectrum of the available literature and not only on the most recent clinical study. In addition, systematic reviews help generate a best estimate of the effect of the clinical intervention based on the body of evidence (and not on a single study) and allow the comparison of this estimate across different studies, which together often encompass a broad range of populations and settings. Moreover, having a summary of the body of evidence allows assessment for the possibility of publication bias.[11,20,21,23]

Evaluation of the Quality of the Evidence

A pivotal step in the development of clinical practice guidelines is the evaluation of the quality of the evidence. The quality of the evidence reflects the extent of our confidence that the estimates of an effect are adequate to support a recommendation. When high-quality clinical evidence is available across outcomes of most importance, there is good certainty of the potential effects of an intervention, allowing panelists and clinicians to feel confident in the expected outcomes of recommendations. When the quality of evidence is poor, panelists have less confidence in effect estimates and expected outcomes of recommendations.[12,13,16,21–26]

This evaluation starts by identifying the study design. In general, randomized controlled trials (RCTs) provide a high quality of evidence. However, for this to be true, these studies should be conducted following the accepted standards for RCTs that aim to protect them against bias such as adequate allocation concealment, blinding, follow-up, analysis, and complete outcome reporting. On the other hand, observational studies are considered to provide low-quality evidence because the groups are not balanced in terms of known and unknown prognostic factors at the start of the study (i.e., confounders). However, similar to the RCTs, specific details about how these studies were conducted can improve this initial rating (e.g., adequate selection of patients, adjustment of unbalanced factors and confounders, and follow-up).[12,13,16,21–26]

After this initial evaluation of the studies based on their design, the body of evidence is systematically evaluated in eight domains (see Table 6.2) that will allow panel members to categorize the quality of evidence associated with a specific recommendation as high quality, moderate quality, low quality, or very low quality across each outcome of interest. It is possible that the quality of the evidence varies among outcomes. In such cases, the quality of the evidence associated with the most important outcome should guide the quality of the evidence for a particular recommendation.[12,13,16,21–26]

TABLE 6.3 Factors Contributing to the Grading of Recommendations

Factors	Effect
Balance between desirable and undesirable outcomes	When the gradient between desirable and undesirable effects is large, panels are more likely to make a strong recommendation. When the gradient between desirable and undesirable effects is narrow, panels are more likely to make a weak recommendation.
Quality of the evidence (on patient-important outcomes)	When high-quality evidence for patient-important outcomes is available, panels are more likely to make a strong recommendation. When only very-low-quality evidence for surrogate markers or less important outcomes is available, panels are more likely to make a weak recommendation.
Confidence in the expected values and preferences of patients	Values and preferences refer to the processes that patients use when considering between alternative treatment options (benefits, harms, cost, and impact on daily life). It can be argued that there is always a degree of uncertainty in terms of the values and preferences of each individual patient. When the patients' values and preferences are unknown or great variability is expected, panels are more likely to make a weak recommendation. When the variability in patients' values and preferences is expected to be low, panels are more likely to make a strong recommendation.
Resources	Reliable evidence regarding resource use should be identified and important differences in resource use between alternative management strategies should be considered when grading recommendations.
Acceptability and feasibility of implementation	Panelists should consider if the proposed recommendations will be accepted and implemented in clinical practice. For example, they might consider the burden of treatment of a particular treatment agent compared to the alternative (e.g., multiple injections per day, need to be taken at a specific time of the day).
Health inequity (differences in health care that are avoidable and unfair or unjust)	Panelists should consider the impact of recommendations on health inequity and groups that are considered disadvantaged.

These levels of the quality of evidence refer to the panel's level of confidence that the true effect of an intervention lies within a particular range or on one side of an important clinical threshold.[28]

From Quality of the Evidence to Strength of Recommendation

In contrast to the construct of quality of the evidence, the strength of a recommendation refers to the panel's confidence that the desirable effects of an intervention outweigh the undesirable effects. In making this assessment, panel members take into consideration the quality of the evidence regarding expected benefits and harms of an intervention in addition to other factors that are important in medical decision making such as the values and preferences of patients, the setting where the intervention will take place, and available resources[12,13,17] (Fig. 6.1; also see Table 6.3).

Clinical Practice Guidelines Recommendations

Recommendations have a direction (in favor of or against an intervention), a strength (strong or weak), and a judgment of the quality of the evidence supporting the recommendation (very low, low, moderate, high)[12,13,21] (see case study later in the chapter).

Strong Recommendations

Strong recommendations are those in which the panel is confident that the desirable effects of following a recommendation clearly outweigh the undesirable effects. In general, these recommendations are based on high-quality or moderate-quality evidence. Panel members usually frame these recommendations as "We recommend…"[12,13,21] (see case study later in the chapter).

Strong recommendations assume that most patients faced with this health care decision will choose the proposed intervention given the certainty of potential benefits in important outcomes.[12,13,21] Clinicians are encouraged to follow these recommendations, policy makers can consider them as part of the standard of care, and they usually serve as the basis for quality or performance measures.

In some circumstances, strong recommendation can be based on low-quality evidence.[12,13,21] For example[29]:

- When low-quality evidence suggests benefit in a life-threatening situation (e.g., increasing the glucocorticoid dose in patients with congenital adrenal hyperplasia during severe illness)
- When low-quality evidence suggests benefit or equivalence of two alternatives and high-quality evidence suggests harm for one of the alternatives (e.g., recommendation for unilateral laparoscopic adrenalectomy for patients with documented primary hyperaldosteronism, instead of open adrenalectomy)
- When high-quality evidence suggests equivalence and low-quality evidence suggests harm in one treatment alternative (e.g., using propylthiouracil instead of methimazole during the first trimester of pregnancy)

Weak Recommendations (Also Called Conditional or Discretionary)

Weak recommendations are those in which the panel concludes that the desirable effect of following these recommendations probably outweighs the undesirable effects, but they are not confident about this conclusion. Weak recommendations are usually based on low-quality or very-low-quality evidence. Panel members usually frame these recommendations as "We suggest…"[12,13,21] (see case study later in the chapter).

In addition, in cases when the systematic evaluation of the evidence provides high-quality evidence that the benefits and risks of

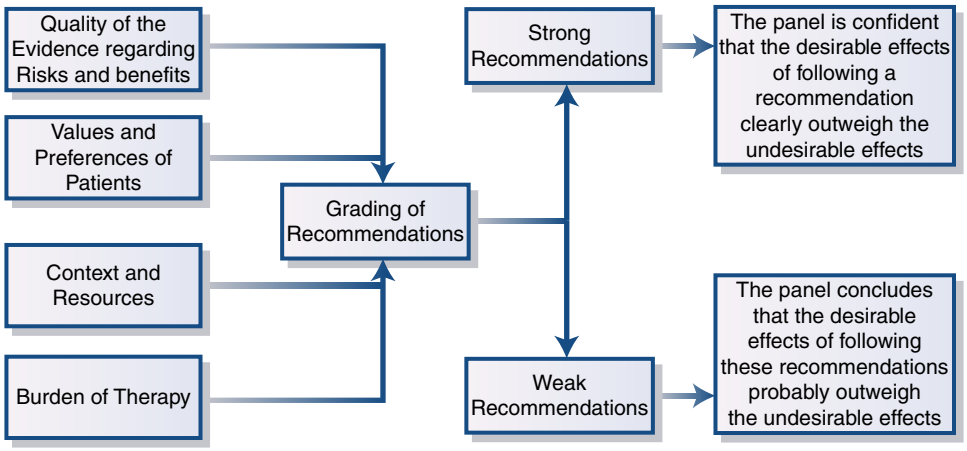
TABLE 6.4 Endocrine Society Clinical Practice Guidelines

Topic	Year of Publication
Adrenal Disease	
The management of primary aldosteronism: case detection, diagnosis, and treatment	2016
Diagnosis and treatment of primary adrenal insufficiency	2016
Treatment of Cushing syndrome	2015
Diagnosis and treatment of pheochromocytoma and paraganglioma	2014
Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency	2018
Diagnosis of Cushing syndrome	2008
Bone Health and Osteoporosis	
Pharmacologic management of osteoporosis in postmenopausal women	2019
Paget disease of bone	2014
Osteoporosis in men	2012
Evaluation, treatment, and prevention of vitamin D deficiency	2011
Cardiovascular Endocrinology	
Evaluation and treatment of hypertriglyceridemia	2012
Primary prevention of cardiovascular disease and type 2 diabetes in patients at metabolic risk	2008
Diabetes and Glucose Metabolism	
Treatment of diabetes in older adults	2019
Diabetes technology—continuous subcutaneous insulin infusion therapy and continuous glucose monitoring in adults	2016
Diabetes and pregnancy	2013
Management of hyperglycemia in hospitalized patients in a noncritical care setting	2012
Continuous glucose monitoring: an Endocrine Society clinical practice guideline	2011
Evaluation and management of adult hypoglycemic disorders	2009
Endocrine Neoplasia and Cancer	
Hypothalamic–pituitary and growth disorders in survivors of childhood cancer	2018
Diagnosis and treatment of pheochromocytoma and paraganglioma	2014
Female Reproductive Endocrinology	
Evaluation and treatment of hirsutism in premenopausal women	2018
Functional hypothalamic amenorrhea	2017
Treatment of symptoms of menopause	2015
Androgen therapy in women	2014
Diagnosis and treatment of polycystic ovary syndrome	2013
Male Reproductive Endocrinology	
Testosterone therapy in men with hypogonadism	2018
Neuroendocrinology	
Hormonal replacement in hypopituitarism in adults	2016
Acromegaly	2014
Diagnosis and treatment of hyperprolactinemia	2011
Evaluation and treatment of adult growth hormone deficiency	2011
Pituitary incidentaloma	2011
Obesity	
Pharmacologic management of obesity	2015
Endocrine and nutritional management of the post–bariatric surgery patient	2010

Continued

TABLE 6.4 Endocrine Society Clinical Practice Guidelines—cont'd

Topic	Year of Publication
Pediatric Endocrinology	
Pediatric obesity—assessment, treatment, and prevention	2017
Thyroid	
Management of thyroid dysfunction during pregnancy and postpartum	2012
Transgender Medicine	
Endocrine treatment of gender-dysphoric/gender-incongruent persons	2017



• **Fig. 6.1** Grading of recommendations.

alternative treatment are balanced, a weak recommendation can be made (see upcoming case study, question 2).[12,13,21] In general, the values and preferences of patients will significantly affect the medical decision when weak recommendations are made. Clinicians are encouraged to explore these values and preferences by engaging in shared decision making to reach the optimal medical decision based on the best available evidence and the values of patients.[3,12,13,30,31] In shared decision making, clinicians and patients work together to determine the best way to address the patient's situation.[3,30,31] Decision aids are tools that have shown to increase patients' knowledge and level of satisfaction with their medical decision and to enable patient-centered care; they can also help clinicians explore the values and preferences of patients.[30,32] These tools usually present a summary of the best available evidence about medical options for a particular clinical scenario and should help support the conversation between patients and clinicians.[30,32]

Good Practice Statements

This is a special category of clinical practice statements in which a formal evaluation of the literature is not necessary, as there is a large body of indirect evidence to support the benefit of the recommendation and where the evaluation of alternatives in clinical trials would be unproductive and unnecessary. These recommendations should be clear, necessary, and justified by panel members. In addition, guideline panelists should seldom use this type of recommendation, as there is the risk of using it to support strong recommendations in the absence of clinical evidence.[33] The following is an example of a Good Practice Statement from the Endocrine Society, titled "Endocrine Treatment of Gender-Dysphoric/Gender-Incongruent Persons"[34]:

> We advise that the clinician responsible for endocrine treatment and the primary care provider ensure appropriate medical clearance of transgender individuals for genital gender-affirming surgery and collaborate with the surgeon regarding hormone use during and after surgery.

In this case, it would be unreasonable to conduct a trial in which the endocrine team, the primary care provider, and the surgeon would not collaborate to achieve adequate surgical outcomes; in addition, there is indirect evidence that clinical collaboration is beneficial for patient care.

Clinical Practice Guidelines in Endocrinology

An analysis of the Endocrine Society clinical practice guidelines from 2008 to 2014, including 25 guidelines, found that up to 20% of the recommendations were based on very-low-quality evidence.[35] A similar study, including 17 Endocrine Society clinical practice guidelines published between 2005 and 2011, found that 58% of the recommendations were graded as strong (59% based on low-quality or very-low-quality evidence) and 42% were considered weak (92% based on low-quality or very-low-quality evidence).[29] These findings should highlight the importance of exploring patient values and preferences and engaging in shared decision making in endocrinology, given that a large number of recommendations are weak (conditional) and based on low-quality or very-low-quality evidence.[1,29,35] In addition, there is an urgent need of clinical research aimed at improving the quality of evidence supporting clinical practice guidelines in endocrinology. In fact, an assessment of current clinical trials in endocrinology found that only one in five recommendations that are based on

very-low-quality evidence has a clinical protocol in place trying to address this knowledge gap.[35]

Using Clinical Practice Guidelines in Patient Care

In clinical practice, guidelines can help physicians provide care that is based on the best available evidence and ideally supports patient-centered care.[9,12,13,36] Strong recommendations based on high-quality evidence can inform clinicians regarding the most effective and ineffective interventions, allowing them to adequately discuss the risks/benefits with their patients.[9] Weak recommendations based on low-quality evidence should lead to collaboration between patients and clinicians when deciding the next step in management.[3,30,31] The integration of decision aids and other tools to support shared decision making can help with the adequate implementation of clinical practice guidelines.[37,38]

Case Study: Management of Gestational Diabetes Using Clinical Practice Guidelines in Patient Care

An otherwise healthy 28-year-old gravida 1, para 0 at 25 weeks' gestation had an initial abnormal 50-g oral glucose challenge test (160 mg/dL, 8.8 mmol/L) followed by an abnormal 100-g 3-hour glucose tolerance test confirming the diagnosis of gestational diabetes. After 1 week of following medical nutrition therapy and daily moderate exercise for more than 30 minutes, she continues to have abnormal fasting glucose (95–109 mg/dL, 5.3–6.0 mmol/L) and postprandial hyperglycemia (140–160 mg/dL, 7.8–8.8 mmol/L).

Clinical Question 1: What should we do to improve pregnancy and offspring outcomes in this pregnant woman with gestational diabetes?

Answer: As per the Endocrine Society guidelines, "Diabetes and Pregnancy,"[39] using blood glucose-lowering pharmacologic therapy *is recommended* if lifestyle therapy is insufficient to maintain normoglycemia in women with gestational diabetes. (Strong recommendation, high-quality evidence)

The body of evidence is clear that maternal hyperglycemia has a deleterious effect on fetal, neonatal, and maternal outcomes.[40,41] Correction of maternal hyperglycemia, however, reduces or prevents adverse outcomes.[42] If this is not possible with nonpharmacologic management, well-conducted randomized clinical trials have shown that blood glucose–lowering pharmacologic therapy is effective at improving patient-important outcomes in this population (e.g., death, shoulder dystocia, large-for-gestational-age offspring).[42–45] Therefore, as the patient's clinician, you can strongly recommend initiation of a blood glucose–lowering medication, as it is expected that most otherwise healthy pregnant patients faced with this clinical recommendation would like to start a blood glucose–lowering medication.

Clinical Question 2: Of the available treatment options, which glucose-lowering medication would you recommend?

Answer: Providing evidence-based care for patients with gestational diabetes remains challenging because of the lack of well-conducted clinical trials in this population. Even among the major medical professional societies, including the Endocrine Society, the American Diabetes Association, and the American College of Obstetricians and Gynecologists (ACOG), consensus on management is often lacking.

As per the Endocrine Society guidelines, "Diabetes and Pregnancy,"[39] long-acting insulin analog detemir may be initiated during pregnancy for those women who require basal insulin and for whom neutral protamine Hagedorn (NPH) insulin, in appropriate doses, has previously resulted in, or for whom it is thought NPH insulin may result in, problematic hypoglycemia. (Weak recommendation, high-quality evidence)

It is also suggested that the rapid-acting insulin analogs lispro and aspart may be used over regular insulin. (Weak recommendation, moderate-quality evidence)

In addition, the Endocrine Society guidelines suggest that glyburide, an oral agent, is a suitable alternative to insulin therapy for glycemic control in women with gestational diabetes who fail to achieve sufficient glycemic control after a 1-week trial of medical nutrition therapy and exercise, except for women with a diagnosis of gestational diabetes before 25 weeks' gestation and for women with fasting plasma glucose levels above 110 mg/dL (6.1 mmol/L), in which case insulin therapy is preferred. (Weak recommendation, low-quality evidence.)

They also suggest that metformin therapy be used for glycemic control only for those women with gestational diabetes who do not have satisfactory glycemic control despite medical nutrition therapy and who prefer not to use insulin or glyburide and are not in the first trimester. (Weak recommendation, low-quality evidence.)

Therefore, in contrast to the confidence in recommending a blood glucose–lowering therapy for women with gestational diabetes based on the high-quality evidence supporting the medical guidelines, the decision about which agent to choose requires further discussion of the clinical evidence and the patient's situation. Health care professionals should fully and frankly discuss with women diagnosed with gestational diabetes the advantages and possible disadvantages of the available blood glucose–lowering pharmacologic therapy options and together reach a decision that makes intellectual, practical, and emotional sense to them.

Shortcomings of Clinical Practice Guidelines

Although clinical practice guidelines aim to help patients and clinicians collaborate in making better health decisions, there are still limitations in the development and implementation of clinical guidelines to improve care.

Guidelines as Tools That Support (Not Dictate) Care

There is high-quality evidence that statin therapy in patients with type 2 diabetes reduces the risk of adverse cardiovascular (CV) outcomes, including mortality.[46,47] Consequently, clinical practice diabetes guidelines unanimously advocate, as a strong recommendation, to use statins in this patient population.[48] However, not every patient will value the use of statins the same way as the panelists do. For instance, consider the case of Mr. Jones, a 54-year-old white man with a history of type 2 diabetes, obesity, controlled hypertension, depression, low back pain, sleep apnea, and new-onset dyslipidemia. His 10-year American College of Cardiology/American Heart Association CV risk is 10.0%.[49] If 100 patients

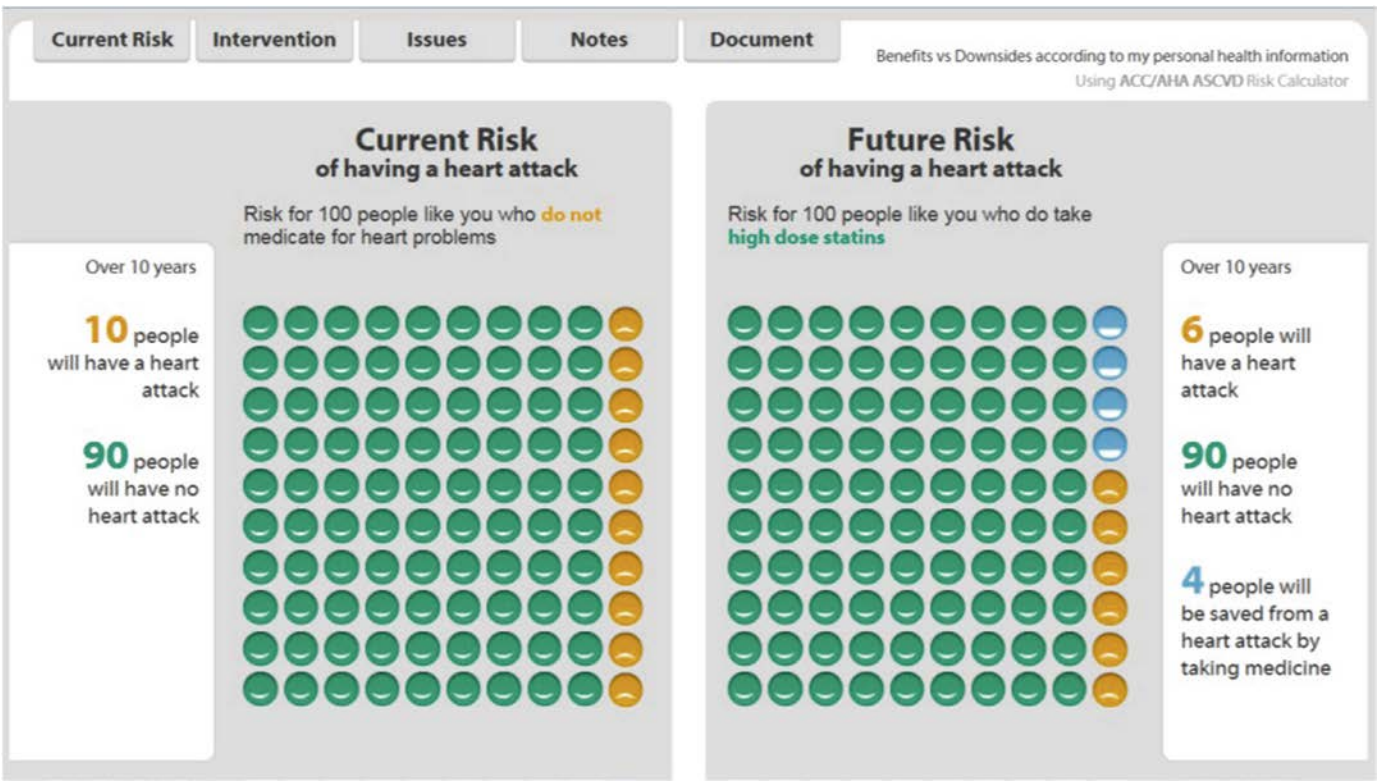
like Mr. Jones (Fig. 6.2) were actually *not to take* a statin for 10 years, 10 of them would have an adverse CV outcome while 90, despite not following the recommendation, would not have an adverse CV outcome. Alternatively, if all 100 patients *took* a statin for a decade, 6 patients would still have a fatal or nonfatal myocardial infarction and 4 patients would avoid a major CV event. This event would happen despite taking the statin every day for 10 straight years, with the risk of harm (i.e., muscle pain), out-of-pocket cost, and the required monitoring with lipid profile and consultation at least once or twice a year.[50] While for Mr. Jones there is little doubt that using a statin might have some CV benefit, at the moment taking statins does not fit his life. He has a high burden of illness (comorbidities), he takes more than 15 medications with many potential side effects, he has social and financial difficulties, and for Mr. Jones a 10-year risk of 10% versus 6% is simply not enough to start taking statins. Although guidelines recommend prescribing a statin for people *like* Mr. Jones, statin therapy may not be right *for* Mr. Jones.

Disease-Specific and Context-Blind Guidelines

Although almost a quarter of Americans have multiple chronic conditions, clinical guidelines are often disease specific and do not consider all aspects of care such as multimorbidity, socio-personal context, and patient preferences. A systematic search of clinical practice guidelines for patients with type 2 diabetes mellitus published between 2006 and 2012 demonstrated that there was a complete absence of incorporating the impact of comorbidities, socio-personal context, and patient preferences in 8 (29%), 11 (39%), and 16 (57%) of the 28 guidelines, respectively.[51] In other words, many guidelines recommendations are contextually blind. This limitation might be secondary to clinical evidence obtained from studies excluding patients with comorbidities. Guideline panelists should therefore be aware of this limitation and favor creating weak or conditional recommendations that promote a patient-centered approach such as shared decision making.[1] This approach allows clinicians to see how a particular recommendation fits into the life of the patient in front of them.[1]

Guideline Focus on Surrogate Outcomes (Not Patient-Important Outcomes)

Benefits ideally should be felt, improve quality of life, or reduce the risk of an undesired patient-important outcome (i.e., hard outcome); if not, they should at least provide the certainty that by treating a risk factor (i.e., surrogate outcome)—for instance, hemoglobin A1c or bone mineral density—the risk of the patient-important outcome (e.g., mortality and fracture, respectively) will be reduced.[8] Although there is no doubt that surrogate outcomes may help clinicians and researchers elucidate how and to what extent an intervention can affect health, there are a handful of RCTs that have demonstrated a nonlinear relationship between a surrogate marker (that physiologically sounds reasonable) and patient-important outcomes.[8,52,53] Patient-important outcomes have become more significant for patients with multiple chronic conditions in which the improvement of a surrogate marker may not always translate to the improvement of another surrogate marker from another disease and may be even harmful for the patient.[54] However, this problem has been systematically



• **Fig. 6.2** Decision aid for patients considering statin therapy. (From Mayo Foundation for Medical Education and Research. Statin choice decision aid [website]. Available at https://statindecisionaid.mayoclinic.org/index.php/statin/index.)

overlooked. While there is no data on guidelines, in ~200 diabetes trials in high-impact journals only 1 of 5 trials was focused on a patient-important outcome as the primary objective.[55]

Quality and Trustworthiness of Clinical Practice Guidelines

Ideally, clinical practice guidelines offer clinicians an easy and accessible way to be up to date with the best available evidence regarding a particular disease. However, it is important to acknowledge that delivering care drawn from "the best available evidence" is not an easy and straightforward task, as to do so means every recommendation should be linked to the best available evidence, preferably based on a systematic review. In addition, as mentioned in the previous discussion about how clinical guidelines are developed, there are strict steps that should be followed and guidelines should be systematically developed by authors with no conflicts of interest for them to be trustworthy.

The Appraisal of Guidelines and Research Evaluation II (AGREE II) instrument (by the AGREE Collaboration) can help clinicians assess the quality of clinical practice guidelines; it correlates with the principles described by the Institute of Medicine regarding trustworthy guidelines.[56] In addition, other tools that can help appraise clinical guidelines are available.[57]

When assessing the quality of clinical practice guidelines, the following domains are important to consider[56,58]:

1. Scope and purpose of the guideline: in which it is important to evaluate if the overall objectives, the clinical questions, and the patients to whom the guideline will apply are specifically described.
2. Stakeholder involvement: assess if the guideline development group or task force includes individuals from all pertinent professional groups and patients to provide the views and preferences of the targeted population.
3. Rigor in development: observe if systematic methods were used to search for the evidence, if methods for formulating recommendations are valid and adequately described, if there is an explicit link between recommendations and supporting evidence, and if a clear procedure for guideline updates has been provided.
4. Clarity of presentation: assess if the recommendations are specific or unambiguous, that when applicable, different options for management of the condition are clearly presented and that key recommendations are easily identifiable.
5. Applicability: assess if the guideline provides advice or tools that facilitate the clinical practice of the recommendation and the potential barriers and costs in applying the recommendations.
6. Editorial independence: it is critical to recognize the funding source and conflicts of interest of the guideline developers.

Conclusions

Clinical practice guidelines that are developed following rigorous and systematic methods by a multidisciplinary panel can provide recommendations that can support patient-centered care. In addition to being able to assess the trustworthiness of clinical practice guidelines, clinicians should understand how these recommendations can support care and collaborate with their patients to overcome their limitations.

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The complete list of references is available online at ExpertConsult.com.

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7

Neuroendocrinology

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CHAPTER OUTLINE

KEY POINTS

- An underlying principle of neuroendocrinology is that peptide and monoamine signaling molecules are secreted from specialized neurons directly into the circulation.
- Secretion of anterior pituitary hormones and expression of genes encoding these hormones are primarily regulated by releasing and inhibitory factors produced in hypophysiotropic hypothalamic neurons and secreted into the portal vessel system located in the median eminence.
- Homeostasis of each respective hypothalamic-pituitary axis is maintained by complex integration of positive and negative feedback loops involving the pituitary hormones themselves, downstream signals (including steroid hormones), and synaptic input from other brain areas onto the hypophysiotropic neurons.
- Hypothalamic neuropeptides are expressed in neurons throughout the brain to modulate activity of neural circuits and to coordinate a range of behavioral outputs that complement hormonal actions of the hypothalamic-pituitary axes.
- In addition to regulation of the pituitary gland, the hypothalamus subserves other important homeostatic functions, including thermoregulation and the sleep-wake cycle.
- Gene mutations, epigenetic alterations, tumors, inflammatory states, infections, vascular abnormalities, trauma, and psychogenic states can produce neuroendocrine disease involving the hypothalamus.
- Hypothalamic disease can present with nonendocrine manifestations in addition to alterations in hypothalamic-pituitary function.

Historic Perspective

The field of neuroendocrinology has expanded from its original focus on the control of pituitary hormone secretion by the hypothalamus to encompass multiple reciprocal interactions between the central nervous system (CNS) and endocrine systems in the control of homeostasis and physiologic responses to environmental stimuli. Although many of these concepts are relatively recent, the relationship between the hypothalamus and the pituitary gland was recognized as early as the 2nd century AD by Galen of Pergamon in *De Usu Partium*, the major treatise of Galenic physiology, and *Anatomicae Administrationes*, in which he described a physical connection between the infundibulum of the hypothalamic third ventricle and the pituitary gland and its association with a surrounding vascular network that he called the *rete mirabilis*. However, Galen thought the pituitary gland was a receptacle for brain "waste" in the form of mucus or phlegm (*pituita* in Latin) that is delivered to and "filtered" by the pituitary gland before exiting through the nasal cavities. These concepts dominated scientific thought about the hypothalamus and pituitary for more than a thousand years until the work of Andreas Vesalius in *De Humani Corporis Fabrica* (1543), in which he detailed the anatomic relationships between the hypothalamic infundibulum and the pituitary gland, and during the 17th century, by Thomas Willis in *Cerebri Anatome*, proposing that "humors" from blood perfusing the ventral surface of the brain are carried to the pituitary gland. The term *pituitary stalk* was not introduced into the literature until 1742 by Joseph Lieutaud, and the term *hypothalamus* was introduced in 1893 by the Swiss anatomist Wilhelm His. A series of major discoveries in the late 19th and early 20th centuries set the groundwork for rapid advancement in the understanding of the hypothalamus in the regulation of hormone secretion and energy homeostasis and continued to unravel throughout the 20th century and into the 21st century (see Anderson and Haymaker[1]

and Toni[2] for expanded historic perspective). Included were the discovery of the connection between the hypothalamus and the posterior pituitary (supraoptic-hypophysial tract) by Ramón Cajal in 1894 and subsequent work on neurosecretion in fish hypothalamus by Scharrer[3] in 1928, firmly establishing that neurons in the hypothalamus were the source of the axons that constitute the posterior pituitary; evidence by Popa and Fielding[4] in 1930 of an interconnection between the pituitary and hypothalamus through the "hypophyseo-portal vessels"; evidence by Wislocki and King[5] and Harris[6] that the blood flow in portal vessels is directed to the pituitary gland from the hypothalamic median eminence and electric stimulation of the hypothalamus is ineffective in eliciting a pituitary response if the pituitary stalk is severed; and the seminal studies by Hetherington and Ranson[7] in 1940 that destruction of the medial basal hypothalamus sparing the pituitary gland results in morbid obesity and neuroendocrine derangements recapitulating the syndrome of obesity, hypogonadotropic hypogonadism, and growth retardation described by Alfred Fröhlich[8] in 1901.

Subsequently, several important studies, especially those from Schally and colleagues and the Guillemin group, established that the anterior pituitary is tightly controlled by the hypothalamus.[9,10] Both groups identified several putative peptide hormone–releasing factors (see later sections). These fundamental studies resulted in the awarding of the Nobel Prize in Medicine in 1977 to Andrew Schally and Roger Guillemin. We now know that these releasing factors are the fundamental link between the CNS and the control of endocrine function. Furthermore, these neuropeptides are highly conserved across species and are essential for reproduction, growth, and metabolism. The anatomy, physiology, and genetics of these factors constitute a major portion of this chapter.

Over the past several decades, work in the field of neuroendocrinology has continued to advance across several fronts. Cloning and characterization of the specific G protein–coupled receptors (GPCRs) used by the hypothalamic-releasing factors have helped define signaling mechanisms utilized by the releasing factors. Characterization of the distribution of these receptors has universally demonstrated receptor expression in the brain and in peripheral tissues other than the pituitary, arguing for multiple physiologic roles for the neuropeptide-releasing factors. Finally, there have been tremendous advances in our understanding of both regulatory neuronal and humoral inputs to the hypophysiotropic neurons.

The adipostatic hormone, leptin, discovered in 1994,[11] is an example of a humoral factor that has profound effects on multiple neuroendocrine circuits.[12] Reduction in circulating leptin is responsible for suppression of the thyroid[13] and reproductive axes[14] during the starvation response. The subsequent discovery of ghrelin,[15] a stomach peptide that regulates appetite and also acts on multiple neuroendocrine axes, demonstrates that much remains to be learned regarding the regulation of the hypothalamic-releasing hormones. Traditionally, it has been extremely difficult to study releasing-factor gene expression or the specific regulation of the releasing-factor neurons because of their small quantities and, in some cases, diffuse distribution. Transformative technologies, including the development of transgenic mice in which expression of fluorescent marker proteins is specifically targeted to hormone-producing neurons such as gonadotropin-releasing hormone (GnRH) neurons[16] or arcuate pro-opiomelanocortin (POMC) neurons[17] (among many others), or the introduction of viral vectors into selective populations of hormone-producing neurons to modulate cellular activity and projection profiles, has provided new and powerful techniques to study properties of hypothalamic neurons both in vitro in the more native context of slice preparations or organotypic cultures and in vivo.

Although much of the field of neuroendocrinology has focused on hypothalamic-releasing factors and their control of reproduction, growth, development, fluid balance, and the stress response through their control of pituitary hormone production, the term *neuroendocrinology* has come to mean the study of interaction of the endocrine and nervous systems in the regulation of homeostasis. The field of neuroendocrinology has been further expanded by diverse areas of basic research, fundamental to understanding the neuroendocrine system including studies of neuropeptide structure, function, and mechanism of action; neural secretion; hypothalamic neuroanatomy; GPCR structure, function, and signaling; transport of substances into the brain; and the action of hormones on the brain. Moreover, homeostatic systems often involve integrated endocrine, autonomic, and behavioral responses. In many of these systems (e.g., energy homeostasis, immune function), the classic neuroendocrine axes are important but not autonomous pathways, and these subjects are also often studied in the context of neuroendocrinology.

In this chapter, the concepts of neural secretion, the neuroanatomy of the hypothalamic-pituitary unit, including CNS structures particularly relevant to the control of the neurohypophysis and adenohypophysis, and other homeostatic mechanisms regulated by the hypothalamus are presented. Each classic hypothalamic-pituitary axis is described, including a consideration of the immune system and its integration with neuroendocrine function. Then, modern concepts in the mechanisms involved in thermoregulation and the sleep-wake cycle are discussed. Finally, the pathophysiology of disorders of neural regulation of endocrine function are reviewed. The neuroendocrinology of energy homeostasis is fully considered in Chapter 39.

Neural Control of Endocrine Secretion

A fundamental principle of neuroendocrinology encompasses the regulated secretion of hormones, neurotransmitters, or neuromodulators by specialized cells.[3] Endocrine cells and neurons are prototypic secretory cells. Both have electrically excitable plasma membranes and specific ion conductances that regulate exocytosis of their signaling molecules from storage vesicles. Secretory cells are broadly classified by their topographic mechanisms of secretion. For example, *endocrine* cells secrete their contents directly into the bloodstream, allowing these substances to act globally as hormones. Cells classified as *paracrine* secrete their contents into the extracellular space and predominantly affect the function of closely neighboring cells. *Autocrine* secretory cells affect their own function by the local actions of their secretions. In contrast, secretory cells within *exocrine* glands secrete proteinaceous substances, including enzymes, and lipids into the lumen of ductal systems.

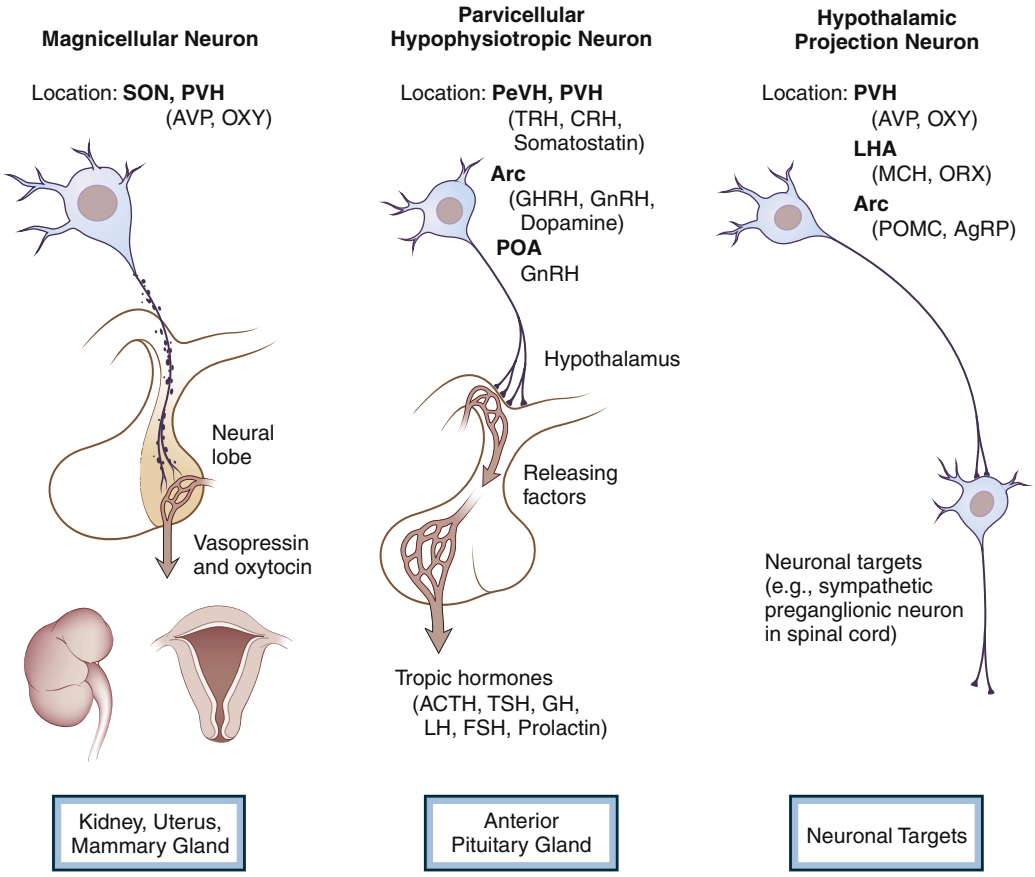
Neurosecretion

Neurons are excitable cells that send their axons throughout the nervous system to release their neurotransmitters and neuromodulators predominantly at specialized chemical synapses. Neurohumoral or neurosecretory cells constitute a unique subset of neurons whose axon terminals are not associated with classic synapses. Two examples of neurosecretory cells in the hypothalamus are neurohypophysial and hypophysiotropic cells. The prototypic

neurohypophysial cells are the magnicellular neurons of the paraventricular hypothalamic nucleus (PVH) and supraoptic nucleus (SON). *Hypophysiotropic cells* encompass all neurons that secrete their products into the pituitary portal vessels at the median eminence (Fig. 7.1).

In the most basic sense, neurosecretory cells are neurons that secrete substances directly into the bloodstream to act as hormones. The theory of neurosecretion evolved from the seminal work of Scharrer,[3] who used morphologic techniques to identify stained secretory granules in the SON and PVH neurons. They found that cutting the pituitary stalk led to an accumulation of these granules in the hypothalamus, which led them to hypothesize that hypothalamic neurons were the source of substances secreted by the neural lobe (posterior pituitary). It is now well established that the axon terminals in the neural lobe arise from the SON and PVH magnicellular neurons that contain oxytocin and the antidiuretic hormone, arginine vasopressin (AVP).

The modern definition of *neurosecretion* has evolved to include the release of any neuronal secretory product from a neuron. Indeed, a fundamental tenet of neuroscience is that all neurons in the CNS, including neurons that secrete AVP and oxytocin in the neural lobe, receive multiple synaptic inputs largely onto their dendrites and cell bodies. In addition, neurons have the basic ability to detect and integrate input from multiple neurons through specific receptors. They in turn fire action potentials that result in the release of neurotransmitters and neuromodulators into synapses formed with postsynaptic neurons. The vast majority of communications between neurons is accomplished by classic fast-acting neurotransmitters (e.g., glutamate, γ-aminobutyric acid [GABA], acetylcholine) and neuromodulators (e.g., dopamine, epinephrine, norepinephrine, neuropeptides) acting at chemical synapses.[18,19] Neurosecretion represents a fundamental concept in understanding the mechanisms used by the nervous system to control behavior and maintain homeostasis.



• **Fig. 7.1** Three types of hypothalamic neurosecretory cells. *Left*, A magnicellular neuron that secretes arginine vasopressin (AVP) or oxytocin (OXY). The cell body, which is located in the supraoptic nucleus (SON) or paraventricular hypothalamic nucleus (PVH), projects its neuronal process into the neural lobe, and neurohormone is released from nerve endings. *Center*, Parvicellular peptidergic neurons are located in the medial basal and dorsal hypothalamus in nuclear groups, including the periventricular hypothalamic nucleus (PeVH), the PVH, infundibular or arcuate nucleus of the hypothalamus (Arc), and preoptic area (POA). The neuropeptides in this case are released into the specialized blood supply to the pituitary to regulate its secretion. *Right*, A third category of hypothalamic peptidergic neurons terminates at chemical synapses on other neurons. These projection neurons are found in sites that include the PVH, Arc, and lateral hypothalamic area (LHA) that innervate multiple central nervous system nuclei, including autonomic preganglionic neurons in the brainstem and spinal cord. Such substances act as neurotransmitters or neuromodulators. *ACTH,* adrenocorticotropin hormone; *AgRP,* agouti-related peptide; *CRH,* corticotropin-releasing hormone; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone; *MCH,* melanin-concentrating hormone; *ORX,* orexin/hypocretin; *POMC,* pro-opiomelanocortin; *TRH,* thyrotropin-releasing hormone; *TSH,* thyrotropin.

In the era of optogenetics, multidimensional "omics," and personalized medicine, the importance of these early observations is often not fully appreciated. However, accounts of these early studies are illuminating, and it is not an overstatement that the confirmation of the neurosecretion hypothesis represented one of the major advances in the field of neuroscience and neuroendocrinology. Indeed, this and other early experiments, including the pioneering work of Geoffrey Harris,[6] led to the fundamental concept that the hypothalamus releases hormones directly into the bloodstream (neurohypophysial cells). These observations provided the principles on which the modern discipline of neuroendocrinology is built.

Contribution of the Autonomic Nervous System to Endocrine Control

Another major precept of neuroendocrinology is that the nervous system controls or modifies the function of both endocrine and exocrine glands. The exquisite control of the anterior pituitary gland is accomplished by the action of releasing-factor hormones (see "Hypophysiotropic Hormones and Neuroendocrine Axes"). Other endocrine and exocrine organs (e.g., pancreas and adrenal, pineal, and salivary glands) are also regulated through direct innervation from the cholinergic and noradrenergic inputs from the autonomic nervous system. An appreciation of the functional anatomy and pharmacology of the parasympathetic and sympathetic nervous systems is fundamental in understanding the neural control of endocrine function.[20]

The efferent arms of the autonomic nervous system comprise the sympathetic and parasympathetic systems. They have similar wiring diagrams characterized by a preganglionic neuron that innervates a postganglionic neuron that, in turn, targets an end organ.[21] Preganglionic and postganglionic parasympathetic neurons are cholinergic. In contrast, preganglionic sympathetic neurons are cholinergic, and postganglionic neurons are noradrenergic (except for those innervating sweat glands, which are cholinergic). Another basic concept is that autonomic neurons coexpress several neuropeptides. This coexpression is a common feature of neurons in the central and peripheral nervous systems.[18] For example, postganglionic noradrenergic neurons can coexpress somatostatin and neuropeptide Y (NPY), whereas postganglionic cholinergic neurons can coexpress vasoactive intestinal polypeptide (VIP) and calcitonin gene–related peptide (CGRP).

Most sympathetic preganglionic neurons lie in the intermediolateral cell column in the thoracolumbar regions of the spinal cord.[21] Most postganglionic neurons are located in sympathetic ganglia lying near the vertebral column (e.g., sympathetic chain and superior cervical ganglia). Postganglionic fibers innervate target organs. As a rule, sympathetic preganglionic fibers are relatively short and the postganglionic fibers are long. In contrast, the parasympathetic preganglionic neurons lie in the midbrain (perioculomotor area, long misidentified as the Edinger-Westphal nucleus[22]), the medulla oblongata (e.g., dorsal motor nucleus of the vagus and nucleus ambiguus), and the sacral spinal cord. Postganglionic neurons that innervate the eye and salivary glands arise from the ciliary, pterygopalatine, submandibular, and otic ganglia. Postganglionic parasympathetic neurons in the thorax and abdomen typically lie within the target organs, including the gut wall and pancreas.[21] Consequently, the parasympathetic preganglionic fibers are relatively long and the postganglionic fibers are short.

The dual autonomic innervation of the pancreas illustrates the importance of coordinated neural control of endocrine organs. The endocrine pancreas receives sympathetic (noradrenergic) and parasympathetic (cholinergic) innervation.[21,23] The latter activity is provided by the vagus nerve (dorsal motor nucleus of the vagus) and is an excellent example of neural modulation because the cholinergic tone of the beta cells affects their secretion of insulin. For example, vagal input is thought to modulate insulin secretion before (cephalic phase), during, and after ingestion of food.[24] In addition, noradrenergic stimulation of the endocrine pancreas can alter the secretion of glucagon and inhibit insulin release.[23] Of course, a major regulator of insulin secretion is the extracellular concentration of glucose,[25] and glucose can induce insulin secretion in the absence of neural input. However, the exquisite control by the nervous system is illustrated by the fact that populations of neurons in the hypothalamus, including ventromedial and perifornical hypothalamic neurons, and catecholamine neurons in the rostral ventral lateral medulla of the brainstem, like the pancreatic beta cell, have the ability to sense glucose levels in the bloodstream.[26] This information is particularly important to initiate counterregulatory responses to prevent hypoglycemia by altering the activity of the autonomic nervous system innervating the pancreas and adrenal glands while simultaneously facilitating arousal through projections to histaminergic neurons in the tuberomammillary nucleus (TMN) and inducing feeding responses.[27,28]

Autonomic regulation of glucose homeostasis is also modulated by gut-hypothalamic interactions and adipose tissue–derived substances such as leptin (see later). Included is hypothalamic sensing of bile acids, which after release into the small intestine, stimulates FGF-19 secretion that acts on mediobasal hypothalamic neurons to induce autonomic responses that lower blood glucose via a multisynaptic pathway.[29] Certainly, an increased understanding of the complex interplay between peripheral signals, the CNS, and endocrine function is necessary to diagnose and clinically manage endocrine disorders and may have particularly important implications for the treatment of diabetes mellitus.

Hypothalamic-Pituitary Unit

The hypothalamus is one of the most evolutionarily conserved and essential regions of the mammalian brain. Indeed, the hypothalamus is the ultimate brain structure that allows mammals to maintain homeostasis, and its destruction is not compatible with life. Hypothalamic control of homeostasis stems from the ability of this collection of neurons to orchestrate coordinated endocrine, autonomic, and behavioral responses. A key principle is that the hypothalamus receives sensory inputs from the external environment (e.g., light, nociception, temperature, odorants) and information regarding the internal environment (e.g., blood pressure, blood osmolality, blood glucose levels). Of particular relevance to neuroendocrine control, hormones (e.g., glucocorticoids, gonadal steroids, thyroid hormone, leptin) exert both negative and positive feedback directly on the hypothalamus.

The hypothalamus integrates diverse sensory and hormonal inputs and provides coordinated responses through motor outputs to key regulatory sites. These sites include the anterior pituitary gland, posterior pituitary gland, cerebral cortex, premotor and motor neurons in the brainstem and spinal cord, limbic system structures (including the amygdala, septum, hippocampus, and thalamic nuclei), and parasympathetic and sympathetic preganglionic neurons. The patterned hypothalamic outputs to these effector sites ultimately result in coordinated endocrine, behavioral, and autonomic responses that maintain homeostasis. The

hypothalamic control of the pituitary gland is an elegant system that underlies the ability of mammals to coordinate endocrine functions that are necessary for survival.

Development and Differentiation of Hypothalamic Nuclei

Tremendous advances in knowledge of the molecular and genetic bases for embryonic development of the hypothalamic-pituitary unit have occurred in the past few decades as a result of the genome sequencing projects and use of transgenic model systems. Pituitary development is detailed in Chapter 8; only a few key points most relevant to the physiology and pathophysiology of the neuroendocrine hypothalamus are presented here.

There has been considerable debate concerning the extent to which developmental studies in the rodent hypothalamic-pituitary system are applicable to the human. However, accumulating data suggest that the similarities outweigh the differences. Ontogenic analyses of the organization of the human hypothalamus utilizing a battery of neurochemical markers have reinforced its homologies to the better-studied rat brain.[30] The cytoarchitectonic boundaries of hypothalamic nuclei are much more easily discerned in the fetal human brain than in the adult brain, and for the most part correspond to homologous structures in the rodent hypothalamus. This finding has important implications for the validity of interspecies comparative analyses. Two examples further illustrate this point. First, the ventromedial nucleus of the hypothalamic core (ventromedial hypothalamus [VMH]), which plays a role in energy balance and female sexual behavior, differentiates from neuroblasts in both humans and rodents at a time-point intermediate to the earlier differentiation of lateral hypothalamic nuclei and later differentiation of the midline nuclei, including the suprachiasmatic nucleus (SCN), the arcuate nucleus, and the PVH.[30,31] Expression of the transcription factor SF1 (steroidogenic factor 1) has been shown to be restricted both temporally and spatially to cells in the VMH, and knockout of the *Sf1* gene in mice alters VMH development by influencing the migration of cells and hence their ultimate location.[31] A second example of interspecies homologies in hypothalamic development is the migration of GnRH-secreting neurons from their origins in rostral neuroepithelium to the anterior hypothalamus.[32] As discussed later, spontaneous and inherited mutations in genes that affect the migration of these neurons are an important cause of Kallmann syndrome or hypogonadotropic hypogonadism associated with anosmia.

In addition to SF1 and the genes associated with Kallmann syndrome, there is a growing list of genes primarily encoding transcription factors that have been implicated in human neuroendocrine disorders and characterized experimentally in rodent models (see Chapter 3).[33,34] This list includes the homeobox transcription factor OTP and the heterodimeric complex formed by the basic helix-loop-helix (bHLH) transcription factors SIM1 and ARNT2 mediating the expression of the POU III–related homeobox genes that include Brn2. These factors are required for the proper development of the PVH and SON and for expression of many key hypophysiotropic neuropeptide genes. The physiologic importance of SIM1 is illustrated by the development of an obesity phenotype in both mice and humans with a haploinsufficiency of SIM1 expression.[33] A major breakthrough in the understanding of factors controlling the development and terminal differentiation of human hypothalamic neurons is the ability to generate these neurons in vitro from induced pluripotential stem cells.[35,36]

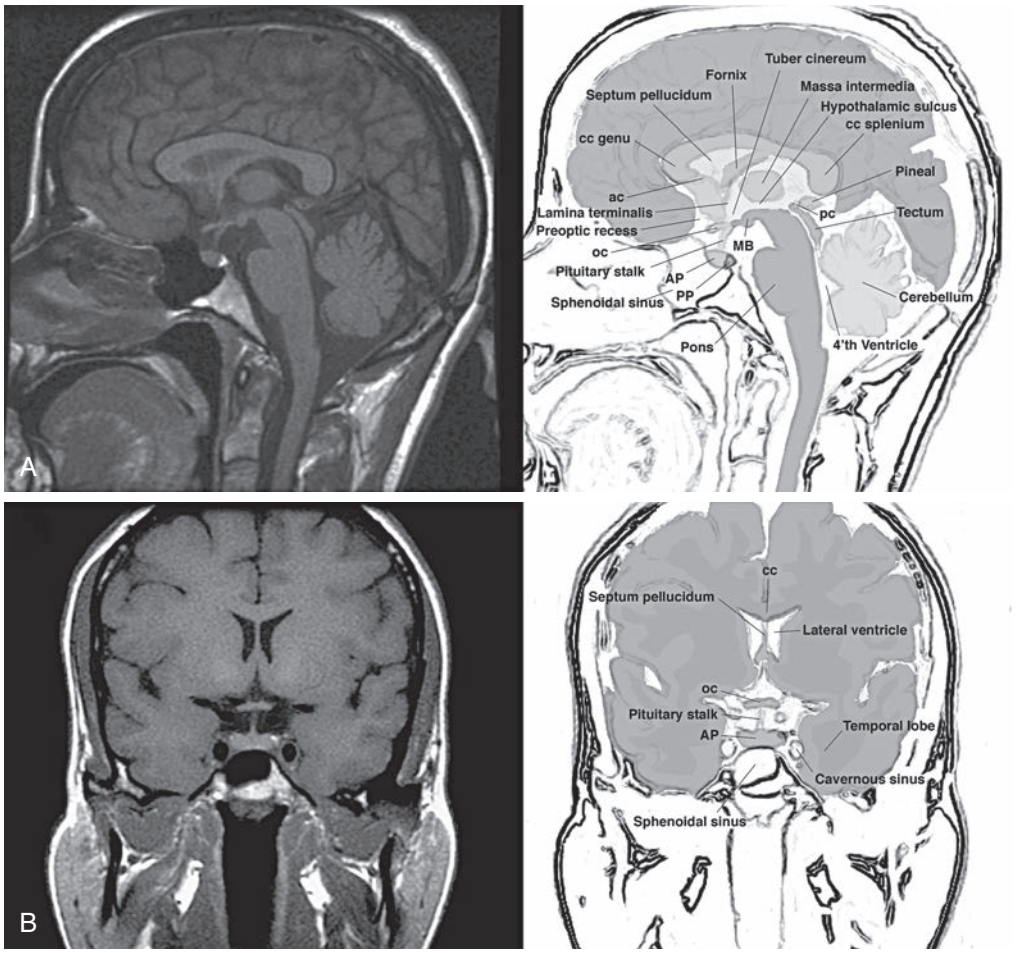
Two key concepts involved in CNS development, which also apply to the hypothalamus, are the balance between neurogenesis and cell death in the establishment of nuclei and the role of circulating hormones in providing organizational signals that regulate cell number and synaptic remodeling. The most thoroughly characterized examples are the effects of sex steroid hormones on the developing brain that result in key sexual dimorphisms of functional importance in later reproductive behaviors.[37] This principle has been extended recently to include organizational effects of other classes of hormones. For example, leptin plays an important role in the development of medial-basal hypothalamic circuits important for energy homeostasis.[38]

Anatomy of the Hypothalamic-Pituitary Unit

The pituitary gland is regulated by three interacting elements: hypothalamic inputs (releasing factors or hypophysiotropic hormones), feedback effects of circulating hormones, and paracrine and autocrine secretions of the pituitary itself. In humans, the pituitary gland (hypophysis) can be divided into two major parts, an anterior, epithelial lobe or adenohypophysis, and a posterior neural lobe or neurohypophysis, which are easily distinguishable from each other by T1-weighted magnetic resonance imaging (MRI) due to the hyperintense signal denoting the neurohypophysis (Fig. 7.2).[39] Women generally have a pituitary that is ~20% larger than men and increase the size and weight of the adenohypophysis during pregnancy due to lactotroph hyperplasia.[40,41] The adenohypophysis can be subdivided into three distinct lobes, the pars distalis (anterior lobe), pars intermedia (intermediate lobe), and pars tuberalis, with the pars distalis making up the bulk of the anterior pituitary and containing all of the hormone-secreting cells of the adenohypophysis. Whereas a well-developed intermediate lobe is found in most mammals, only rudimentary vestiges of the intermediate lobe are detectable in adult humans, with the bulk of intermediate lobe cells being dispersed in the anterior lobe.

The neurohypophysis is composed of the pars nervosa (also known as the neural or posterior lobe), the infundibular stalk, and the median eminence, the latter forming the central region of a mound, or tuber cinereum, at the base of the hypothalamus (see Fig 7.2). The infundibular stalk is surrounded by the pars tuberalis, and together they constitute the *hypophysial stalk*. The pituitary gland lies in the sella turcica (Turkish saddle) of the sphenoid bone and underlies the base of the hypothalamus. Pituitary tissue can also be present in the nasopharynx, commonly referred to as a pharyngeal pituitary, and is a residual of Rathke pouch during its migration from the oral cavity to the middle cranial fossa (see next).

The anterior and intermediate lobes of the pituitary derive from a dorsal invagination of the pharyngeal epithelium, called Rathke pouch. During development, Rathke pouch forms three diverticula, a single medial and two lateral. Precursor cells within the pouch undergo steps of organ determination, cell fate commitment to a pituitary phenotype, proliferation, and migration with cells in the medial diverticula differentiating into corticotrophs and thyrotrophs, lower portions of the lateral diverticulum into somatotrophs and gonadotrophs, and all three diverticula into lactotrophs. The upper part of the lateral diverticula gives rise to the pars tuberalis that differentiates into thyrotrophs and gonadotrophs. The intermediate lobe is in direct contact with the neural lobe and is the least prominent of the three lobes. With age, the human intermediate lobe decreases in size to leave a small, residual collection of POMC cells. In nonprimate species, these

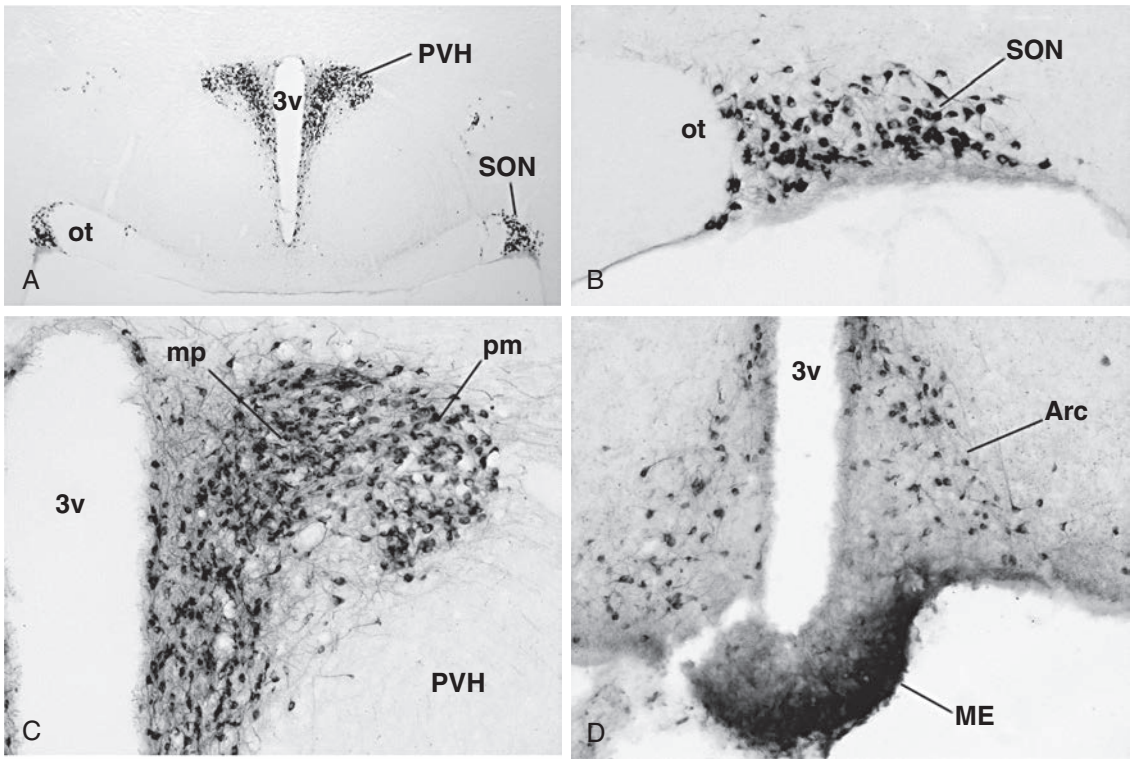


• **Fig. 7.2** Normal anatomy of the human hypothalamic-pituitary unit in (A) sagittal and (B) coronal planes. Structures that are visible in the T1-weighted magnetic resonance images *(left panels)* are identified in the corresponding diagrams *(right panels)*. The hypothalamus is bounded anteriorly by the optic chiasm, laterally by the sulci formed with the temporal lobes, and posteriorly by the mammillary bodies (in which the mammillary nuclei are located). Dorsally, the hypothalamus is delineated from the thalamus by the hypothalamic sulcus. The smooth, rounded base of the hypothalamus is the tuber cinereum; the pituitary stalk descends from its central region, which is termed the *median eminence*. The median eminence stands out from the rest of the tuber cinereum because of its dense vascularity, which is formed by the primary plexus of the hypothalamic-hypophysial portal system. The long portal veins run along the ventral surface of the pituitary stalk. Note the location of the pituitary stalk, the hyperintense signal *(white)* from the posterior pituitary (PP) (A, *left*), and the anatomic relationships of the pituitary gland to the optic chiasm (oc) and the sphenoidal and cavernous sinuses. *ac,* anterior commissure; *AP,* anterior pituitary; *cc,* corpus callosum; *MB,* mammillary body; *pc,* posterior commissure. (Magnetic resonance images courtesy Dr. D.M. Cook.)

cells are responsible for secreting the POMC-derived product, α-melanocyte-stimulating hormone (α-MSH).[42] Early contact of Rathke pouch with the primordial neurohypophysial tissue has a key role for histodifferentiation of anterior pituitary cell types as indicated by the lack of corticotrophs and gonadotrophs in anencephalic fetuses.[43] An expanding list of transcription factors involved in the development of the anterior pituitary gland and associated with genetic causes for human disorders of pituitary development and secretion has been recognized, with mutations of PROP1 being the most common genetic cause for combined pituitary hormone deficiency.[44]

The major component of the neural lobe is a collection of axon terminals arising from magnicellular secretory neurons located in the PVH and SON of the hypothalamus (Fig. 7.3; also see Fig. 7.1).[45] These axon terminals are in close association with a capillary plexus, and they secrete substances, including AVP and oxytocin, into the hypophysial veins and into the general circulation (Table 7.1). The blood supply to the neurohypophysis arises from the inferior hypophysial artery (a branch of the internal carotid artery). Glial-like cells called pituicytes comprise ~25% of the posterior pituitary and have an important role in regulating vasopressin and oxytocin secretion by at least two major mechanisms. These include structural modifications by engulfing or retracting from neurosecretory axon endings and the release of hormone modulators such as galanin-like peptide (GALP) and taurine that stimulate or inhibit the release of vasopressin, respectively.[46] As the source of AVP to the general circulation, the PVH and SON and their axon terminals in the neural lobe are the main effector arms for the central regulation of blood osmolality, fluid balance, and blood pressure (see Chapter 10).

The secretion of oxytocin by magnicellular neurons is critical at parturition, resulting in uterine myometrial contraction. In



• **Fig. 7.3** The tuberoinfundibular system is revealed by retrograde transport of cholera toxin subunit B (CtB). The location of hypothalamic cell bodies of neurons projecting to the median eminence (ME) and the posterior pituitary can be identified by microinjecting a small volume of the retrograde tracer CtB into the median eminence of the rat. (A) Retrogradely labeled cells can be seen in the paraventricular (PVH) and supraoptic nuclei of the hypothalamus (SON). (B) Magnicellular neurons are observed in the SON. (C) Labeled neurons are found in the posterior magnicellular group (pm) as well as the medial parvicellular subdivision (mp). The labeled cells in the PVH include those that contain corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH). (D) Retrogradely labeled cells are also found in the arcuate nucleus of the hypothalamus (Arc). These include neurons that release growth hormone–releasing hormone (GHRH) and dopamine. *ot,* optic tract; *3v,* third ventricle.

addition, the secretion of oxytocin is regulated by the classic milk let-down reflex. Mechanosensory information from the nipple reaches the magnicellular neurons, directly or indirectly, from the dorsal horn of the spinal cord, resulting in a synchronized burst of action potentials in the whole population of oxytocin neurons, followed by the release of oxytocin into the general circulation.[47] Oxytocin acts on receptors on myoepithelial cells in the mammary gland acini leading to the release of milk into the ductal system and, ultimately, the release of milk from the mammary gland. Oxytocin also acts on other regions of the brain, such as the medial preoptic area, ventral tegmental area, nucleus accumbens, and amygdala, to affect social behavior (particularly parental behavior) by reducing the stress response and increasing empathy, among others, whereas vasopressin may mediate various forms of aggression.[48] Vasopressin also synergizes with the corticotropin-releasing hormone (CRH) to release adrenocorticotropin hormone (ACTH) secretion (see upcoming discussion). A potential therapeutic role for oxytocin in the treatment of autism has been proposed.[49]

The Median Eminence and Hypophysiotropic Neuronal System

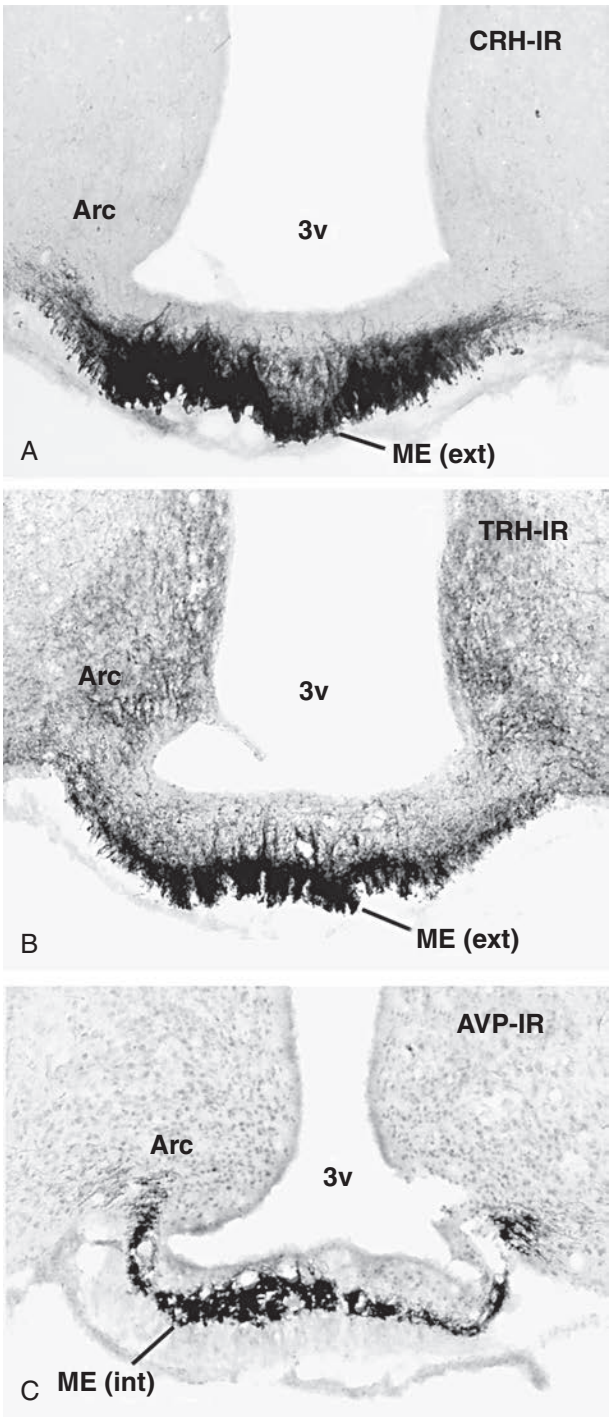
The median eminence is the functional link between the hypothalamus and the anterior pituitary gland. It lies in the center of the tuber cinereum and is composed of an extensive array of blood vessels and nerve endings (Fig. 7.4; also see Fig. 7.2).[50] Its extremely rich blood supply arises from the superior hypophysial artery (a branch of the internal carotid artery), which sends off many small branches that form capillary loops. The small capillary loops extend into the internal and external zones of the median eminence, form anastomoses, and drain into sinusoids that become the pituitary portal veins that enter the vascular pool of the pituitary gland. The flow of blood in these short loops is thought to be predominantly (if not exclusively) in a hypothalamic-to-pituitary direction. This well-developed plexus results in a tremendous increase in the vascular surface area. In addition, the vessels are fenestrated, allowing diffusion of the peptide-releasing factors to their site of action in the anterior pituitary gland. Because this vascular complex in the base of the hypothalamus and its "arteriolized" venous drainage to the pituitary compose a circulatory system analogous to the portal vein system of the liver, it has been termed the *hypothalamic-hypophysial–portal circulation*.

Three distinct compartments of the median eminence are recognized: the innermost ependymal layer, the internal zone, and the external zone (see Fig. 7.4).[50] Ependymal cells form the floor of the third ventricle and are unique in that they have microvilli rather than cilia. Tight junctions at the ventricular pole of the ependymal cells prevent the diffusion of high-molecular-weight substances between the cerebrospinal fluid (CSF) and the extracellular space within the median eminence. The ependymal layer also

TABLE 7.1 Neurotransmitters and Neuromodulators in the Paraventricular Nucleus and the Arcuate Nucleus of the Hypothalamus

Paraventricular Nucleus	Arcuate Nucleus
Magnicellular Division	Acetylcholine
Angiotensin II	γ-Aminobutyric acid (GABA)
Cholecystokinin (CCK)	Agouti-related peptide (AgRP)
Corticotropin-releasing hormone (CRH)	Cocaine-regulated and amphetamine-regulated transcript (CART)
Dynorphins	Dopamine
Glutamate	Dynorphin
Nitric oxide (NO)	Endocannabinoids
Oxytocin	Enkephalins
Phoenixin	Galanin
Spexin	Galanin-like peptide (GALP)
Urocortin 1	Ghrelin
Vasopressin (AVP)	Glutamate
Parvicellular Divisions	Gonadotropin-releasing hormone (GnRH)
γ-Aminobutyric acid (GABA)	Growth hormone–releasing hormone (GHRH)
Angiotensin II	Kisspeptins
Atrial natriuretic factor (ANF)	Melanocortins (ACTH, α-MSH, β-MSH, γ-MSH)
Bombesin-like peptides	Nesfatin-1
Cholecystokinin (CCK)	Neurokinin B (NKB)
Corticotropin-releasing hormone (CRH)	Neuromedin U
Dopamine	Neuropeptide Y (NPY)
Endocannabinoids	Neurotensin
Enkephalins	Nociceptin/orphanin FQ (OFQ)
Galanin	Opioids (β-endorphin) peptides
Glutamate	Pancreatic polypeptide
Interleukin 1 (IL-1)	Phoenixin
Nesfatin-1	Prolactin (PRL)
Neuropeptide Y (NPY)	Pro-opiomelanocortin (POMC)
Neurotensin	Pyro-glutamyl-RFamide peptide (QRFP)
Nitric oxide (NO)	Somatostatin (SST)
Peptide histidine isoleucine (PHI)	Substance P
Pituitary adenylate cyclase activating polypeptide (PACAP)	VGF
RFamide-related peptides (RFRP)	
Somatostatin (SST)	
Thyrotropin-releasing hormone (TRH)	
Vasopressin (AVP)	
Vasoactive intestinal peptide (VIP)	

contains specialized cells, called *tanycytes*,[51] that send processes into the other layers of the median eminence and into the mediobasal hypothalamus. Functionally, tanycytes comprise part of the blood-brain and blood-CSF barriers as they express tight junction proteins, including occludins, ZO1, and claudins,[52] regulating access of circulating substances and/or substances secreted into the median eminence from entering the hypothalamus or CSF.[53] However, it is becoming increasingly recognized that tanycytes have a number of important neuroendocrine functions. Included are hormone transport, regulation of hypophysiotropic hormone release, thyroid hormone homeostasis, regulation of reproduction, nutrient sensing, changes in energy homeostasis, and to function as stem/progenitor cells for neurogenesis and gliogenesis, even in adult animals.[54–57] Some of these functions are discussed in greater detail later in the chapter.



• **Fig. 7.4** The median eminence is the functional connection between the hypothalamus and the pituitary gland. (A and B) Distribution of corticotropin-releasing hormone and thyrotropin-releasing hormone immunoreactivity (CRH-IR and TRH-IR) in the external layer of the median eminence (ME ext) of the rat. CRH and TRH cell bodies reside in the medial division of the paraventricular hypothalamic nucleus. (C) Arginine vasopressin immunoreactivity (AVP-IR) in nerve endings in the internal layer of the median eminence (ME int). *Arc,* arcuate nucleus; *3v,* third ventricle.

The internal zone of the median eminence is composed of axons of the SON and PVH magnicellular neurons that contain characteristic, large neurosecretory granules or Herring bodies passing en route to the posterior pituitary (see Fig. 7.4C). In addition, axons of the hypophysiotropic neurons and processes of

tanycytes destined for the external layer of the median eminence are seen (see Fig. 7.4A and B).

Finally, the external zone of the median eminence represents the exchange point of the hypothalamic releasing factors and the pituitary portal vessels.[50] In addition to the fenestrated portal capillaries and cytoplasmic extensions and end-feet processes of tanycytes, it contains numerous fine-caliber, unmyelinated axons and axon terminals of hypophysiotropic neurons that contain all of the hypothalamic releasing and inhibiting substances. Two general types of neurons project to the external zone and comprise the so-called hypothalamic tuberoinfundibular system: (1) peptide-secreting (peptidergic) neurons, including thyrotropin-releasing hormone (TRH), CRH, growth hormone–releasing hormone (GHRH), somatostatin, and GnRH (see Fig. 7.1), and (2) neurons containing monoamines (e.g., dopamine, serotonin). Although the secretion of these substances into the portal circulation is an important control mechanism, some peptides and neurotransmitters in nerve endings are not released into the hypothalamic-hypophysial–portal circulation but instead function to regulate the secretion of other nerve terminals. In addition, some nerve terminals, such as GnRH nerve endings, are enveloped by tanycytes, which cover or uncover axonal endings in response to changes in functional status.[58,59] Thus supporting elements, with their own sets of receptors, can change the neuroregulatory milieu within the hypothalamus, median eminence, and pituitary (for further details, see "Physiologic Roles of Melatonin" and "Gonadotropin-Releasing Hormone and Control of the Reproductive Axis" [specifically "Regulatory Systems"]). As in the case of neurohormone secretion from the neurohypophysis, depolarization of hypothalamic neurons leads to the release of neuropeptides and monoamines at the median eminence.

The site of production, the genetics, and the regulation of synthesis and release of individual peptide-releasing factors are discussed in detail in later sections. Briefly, there are several cell groups in the medial hypothalamus that contain releasing factors that are secreted into the pituitary portal circulation (Table 7.2). These cell groups include the infundibular nucleus (called the *arcuate nucleus* in rodents) (see Fig. 7.3D), PVH (see Fig. 7.3A and C), periventricular nucleus, and a group of cells in the medial preoptic area near the organum vasculosum of the lamina terminalis (OVLT) (Fig. 7.5). As discussed earlier, magnicellular neurons in the SON and PVH send axons that predominantly traverse the internal zone of the median eminence to terminate in the neural lobe of the pituitary. However, a smaller number of magnicellular axons project directly to the external zone of the median eminence and may facilitate ACTH secretion.

In addition to axon terminals in the external zone of the median eminence, densely packed fibers derived from the sphenopalatine ganglion that contain vasoactive intestinal peptide (VIP) and the nitric oxide (NO)–synthesizing enzyme, nitric oxide synthase, are present on the ventral surface of the median eminence, surrounding portal vessels and innervating smooth muscle of precapillary arterioles that supply the portal capillary plexus.[60] Because both VIP and NO are potent vasodilators, these substances may have an important role in regulating the rate of blood flow to the median eminence and hence to the anterior pituitary.

The third structure often grouped as a component of the median eminence is a subdivision of the adenohypophysis called the *pars tuberalis*. It is a thin sheet of glandular tissue that lies around the infundibulum and pituitary stalk. In some animals, the epithelial component may make up as much as 10% of the total glandular tissue of the anterior pituitary. The pars tuberalis contains cells that produce luteinizing hormone (LH) and thyrotropin (thyroid-stimulating hormone [TSH]). As opposed to thyrotrophs in the pars distalis, however, TSH-producing cells in the pars tuberalis are smaller and lack receptors for TRH.[61] The physiologic role of the pars tuberalis in humans remains somewhat enigmatic, but it is clearly part of the photosensing pathway in the CNS as it expresses high concentrations of the melanocortin 1 receptor. Furthermore, in some mammals and birds, the pars tuberalis has an established role in the regulation of seasonal reproduction, energy balance, and body weight orchestrated through the release of melatonin.[62–65] The pars tuberalis also secretes a number of other substances, including neurokinin A, substance P, and endocannabinoids that are involved in the seasonal release of prolactin in some animal species through direct effects on the pars distalis.[66]

TABLE 7.2 Structural Formulas of Principal Human Hypothalamic Peptides Directly Related to Pituitary Hormone Secretion[a]

Vasopressin

Cys-Tyr-Phe-Gln-Asn-*Cys*-Pro-Arg-Gly-NH_2 (MW = 1084.38)

Oxytocin

Cys-Tyr-Ile-Gln-Asn-*Cys*-Pro-Leu-Gly-NH_2 (MW = 1007.35)

Thyrotropin-Releasing Hormone

pGlu-His-Pro-NH_2 (MW = 362.42)

Gonadotropin-Releasing Hormone

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 (MW = 1182.39)

Corticotropin-Releasing Hormone

Ser-Glu-Glu-Pro-Pro-Ile-Ser-Leu-Asp-Leu-Thr-Phe-His-Leu-Leu-Arg-Glu-Val-Leu-Glu-Met-Ala-Arg-Ala-Glu-Gln-Leu-Ala-Gln-Gln-Ala-His-Ser-Asn-Arg-Lys-Leu-Met-Glu-Ile-Ile-NH_2 (MW = 4758.14)

Growth Hormone–Releasing Hormone

Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-Gln-Gly-Glu-Ser-Asn-Gln-Glu-Arg-Gly-Ala-Arg-Ala-Arg-Leu-NH_2 (MW = 5040.4)

Somatostatin

Ala-Gly-*Cys*-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-*Cys* (MW = 1638.12)

Vasoactive Intestinal Peptide

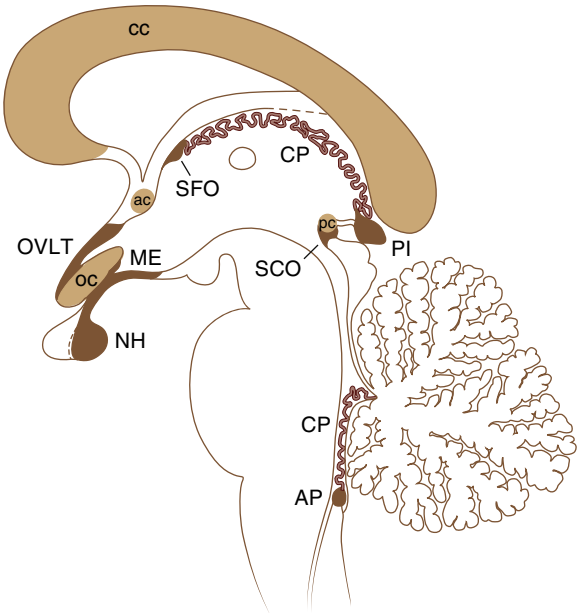
His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 (MW = 3326.26)

[a]Disulfide bonds between pairs of cystines that produce cyclization of the peptides are indicated by their italicized cognate *Cys* residues.

MW, Molecular weight; *pGlu,* pyroglutamyl.

Circumventricular Organs

A guiding principle of neurophysiology and neuropharmacology is that the brain, including the hypothalamus, resides in an environment that is protected from humoral signals.[58,67] The exclusion of macromolecules is due to the structural vascular specializations that make up the blood-brain barrier.[68] These specializations include tight and adherens junctions of brain vascular endothelial



• **Fig. 7.5** Median sagittal section through the human brain to show the circumventricular organs *(dark brown)*. Light brown areas are the optic chiasm (oc), corpus callosum (cc), anterior (ac) and posterior (pc) commissures. *AP,* area postrema; *CP,* choroid plexus; *ME,* median eminence; *NH,* neurohypophysis; *OVLT,* organum vasculosum of the lamina terminalis; *PI,* pineal gland; *SCO,* subcommissural organ; *SFO,* subfornical organ. (Adapted from Weindl A. Neuroendocrine aspects of circumventricular organs. In: Ganong WF, Martini L, eds. Frontiers in *Neuroendocrinology,* vol 3. New York: Oxford University Press; 1973:3–32.)

cells that preclude the free passage of polarized macromolecules, including peptides and hormones. In addition, astrocytic foot processes and perivascular microglial cells contribute to the integrity of the blood-brain barrier. However, to exert homeostatic control, the brain must assess key sensory information from the bloodstream, including hormone levels, metabolites, and potential toxins. For example, to monitor key signals the brain has "windows on the circulation," or circumventricular organs (CVOs), that serve as a conduit of peripheral cues into key neuronal cell groups that maintain homeostasis.[67]

As the name implies, CVOs are specialized structures that lie on the midline of the brain along the third and fourth ventricles. These structures include the OVLT, subfornical organ (SFO), median eminence, neurohypophysis (posterior pituitary), subcommissural organ (SCO), and area postrema (see Fig. 7.5). Unlike the vasculature in the rest of the brain, the blood vessels in CVOs have fenestrated capillaries that allow relatively free passage of molecules such as proteins and peptide hormones. Thus neurons and glial cells that reside within the CVOs have access to these macromolecules. In addition to the distinct nature of the vessels themselves, the CVOs have an unusually rich blood supply, allowing them to act as integrators at the interface of the blood-brain barrier. Several of the CVOs have major projections to hypothalamic nuclear groups that regulate homeostasis. Therefore the CVOs serve as a critical link between peripheral metabolic cues, hormones, and potential toxins and cell groups within the brain that regulate coordinated endocrine, autonomic, and behavioral responses. Detailed discussion of the physiologic roles of individual CVOs is beyond the scope of this chapter, but several in-depth reviews have assessed the function of each.[67,69–71]

Median Eminence

The median eminence (described earlier) has an essential role in the regulation of the pars distalis. The anatomic location of the median eminence places it in a position to serve as an afferent sensory organ as well as a functional link between the hypothalamus and the pituitary gland. Specifically, the median eminence is located adjacent to several neuroendocrine and autonomic regulatory nuclei at the tuberal level of the hypothalamus (see Fig. 7.3). These nuclear groups include the infundibular or arcuate, ventromedial, dorsomedial, and paraventricular nuclei.

The role of hypothalamic nuclei surrounding the median eminence as afferent sensory centers is supported by the observation that it is a portal of entry for hormones circulating in the bloodstream such as leptin and several gut-derived circulating hormones, including ghrelin. Both leptin and ghrelin are established mediators of body weight and neuroendocrine function that act on POMC and/or agouti-related protein (AgRP)/neuropeptide Y (NPY) neurons residing in the arcuate nucleus.[17,72–74] Thus it is likely that the median eminence is involved in conveying information from humoral factors to key hypothalamic regulatory neurons in the medial basal hypothalamus.[58]

Organum Vasculosum of the Lamina Terminalis and the Subfornical Organ

The OVLT and the SFO are located at the anterior wall of the third ventricle, the lamina terminalis. The OVLT and SFO lie, respectively, at the ventral and dorsal boundaries of the third ventricle (see Fig. 7.5). Because it lies at the rostral and ventral tip of the third ventricle, the OVLT is surrounded by cell groups of the preoptic region of the hypothalamus. Like other CVOs, the OVLT is composed of neurons, glial cells, and tanycytes. Axon terminals containing several neuropeptides and neurotransmitters, including somatostatin, angiotensin, dopamine, norepinephrine, serotonin, acetylcholine, oxytocin, AVP, and TRH, innervate the OVLT. Unlike the median eminence, however, blood from the OVLT does not drain into a portal plexus, but rather primarily to the medial preoptic region. However, in the rodent, neurons that contain GnRH and surround the OVLT possess unique projections with combined properties of dendrites and axons, termed *dendrons*, that bridge the distance between the OVLT and the median eminence.[75] In addition, the OVLT in the rat brain contains estrogen receptors, and the application of estrogen or electric stimulation at this site is capable of stimulating ovulation through GnRH-containing neurons that project to the median eminence.

Neurons in the OVLT project to many regions of the brain, including the preoptic nucleus, subfornical organ, arcuate nucleus, supraoptic nucleus, medial thalamus, and parts of the limbic system. Thus the OVLT is strategically placed to receive blood-borne information and then transmit this information to specific regions of the brain. Therefore, it is not surprising that the OVLT is involved in a diverse array of processes. For example, lesions of the OVLT and the surrounding preoptic area led to altered febrile responses after immunologic stimulation and disruptions in fluid and electrolyte balance, blood pressure, reproduction, and thermoregulation. Large lesions of the OVLT attenuate lipopolysaccharide-induced fever.[76] Consistent with this finding, it has been demonstrated that receptors for prostaglandin E_2 are located within and immediately surrounding the OVLT.[77] Because prostaglandin E_2 is thought to be an obligate endogenous pyrogen, the OVLT may be a critical regulator of febrile responses.

The OVLT is also likely to be involved in sensing serum osmolality through osmoreceptor cells that express the transient receptor potential vanilloid (TRPV) subfamilies 1 and 4 genes[78] and respond to circulating levels of angiotensin II and relaxin.[79,80] In addition, lesions of the OVLT attenuate AVP and oxytocin secretion in response to osmotic stimuli, whereas hypertonic saline administration induces activation in OVLT neurons.[81]

The SFO is located in the roof of the third ventricle below the fornix. This CVO critically regulates fluid homeostasis and contributes to blood pressure regulation.[67] Consistent with these functions, the SFO has receptors for angiotensin II and atrial natriuretic peptide.[82,83] Through direct projections to the paraventricular and supraoptic nuclei, SFO neurons induce the release of vasopressin from the posterior pituitary and activate paraventricular nucleus neurons that descend to sympathetic centers of the spinal cord that regulate vasoconstriction as well as other neuronal sites involved in fluid and blood pressure balance, including the median preoptic nucleus and OVLT.[84]

The critical importance of the SFO in maintaining fluid balance was demonstrated by Simpson and Routtenberg, showing that substances such as angiotensin II elicited drinking behavior when microinjected at low doses directly into the SFO.[85] Later studies demonstrated that SFO neurons have electrophysiologic responses to angiotensin II and that stimulation of the SFO elicits AVP secretion.[82] Like the OVLT, the SFO is activated by hypertonic saline administration.[81] Importantly, the use of Cre recombinase technology combined with optogenetics has enabled researchers to demonstrate that excitation of SFO neurons that express neuronal nitric oxide synthase (nNOS) and/or calmodulin kinase II (CaMKII) stimulates thirst, whereas excitation of vesicular GABA transporter–expressing neurons suppress thirst.[86] Subsequent studies have also established that chemogenetically activated CaMKII neurons in the SFO stimulate NaCl intake, indicating that the SFO is also the principal site for monitoring sodium levels.[87] Sodium sensing is mediated by NaX, the brain sodium-level sensor, which is expressed by glial cells in the SFO and by the release of lactate, activate GABAergic SFO neurons.[88] While most of the experimental work to establish the role of the SFO in salt and water balance has been done in rodents, clear evidence that the SFO mediates similar functions in humans is established by the demonstration in a pediatric patient with adipsic hypernatremia due to autoantibodies that target NaX.[89]

Area Postrema

The area postrema lies at the caudal end of the fourth ventricle adjacent to the nucleus of the tractus solitarius (NTS) (see Fig. 7.5). In rodents, it is a midline structure lying above the NTS.[67,90] However, in humans the area postrema is a bilateral structure. Because the area postrema overlies the NTS, it also receives direct visceral afferent input from the glossopharyngeal nerve and the vagus nerves. In addition, the area postrema receives direct input from several hypothalamic nuclei. The efferent projections of the area postrema include projections to the NTS, ventral lateral medulla, and parabrachial nucleus. Consistent with its role as a sensory organ, the area postrema is enriched with receptors for several neuropeptides, including glucagon-like peptide-1 and amylin.[91] It also contains chemosensory neurons that include osmoreceptors. The area postrema is thought to be critical in the detection of potential toxins and in the induction of vomiting in response to foreign substances. In fact, the area postrema is often referred to as the chemoreceptor trigger zone.[91]

The best described physiologic role of the area postrema is the coordinated control of blood pressure.[67] The area postrema contains binding sites for angiotensin II, AVP, and atrial natriuretic peptide. Lesions of the area postrema in rats blunt the rise in blood pressure induced by angiotensin II.[92] Finally, administration of angiotensin II induces the expression of c-Fos in neurons of the area postrema, an indication of its activation. The area postrema has also been hypothesized to play a role in responding to inflammatory cytokines during the acute febrile response, CNS glucose sensing, and the satiating effects of amylin.[92]

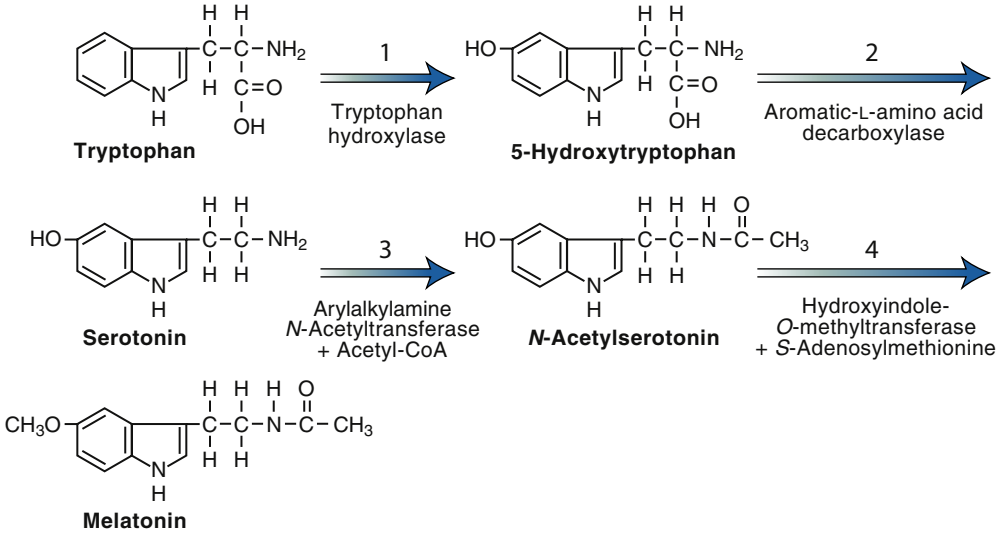
Subcommissural Organ

The SCO is located near the junction of the third ventricle and cerebral aqueduct below the posterior commissure and the pineal gland (see Fig. 7.5). It is composed of specialized ependymal cells that secrete a highly glycosylated protein of unknown function. The secretion of this protein leads to aggregation and formation of the so-called Reissner fibers.[93] The glycoproteins are extruded through the aqueduct, the fourth ventricle, and the spinal cord lumen to terminate in the caudal spinal canal. In humans, intracellular secretory granules are identifiable in the SCO, but Reissner fibers are absent. The SCO secretion in humans is therefore presumed to be more soluble and to be absorbed directly from the CSF. Compared with other CVOs, the physiologic role of the SCO is largely unknown. Hypothesized roles for the SCO include clearance of substances, including monoamines from the CSF.[93]

Pineal Gland

Descartes called the pineal gland the "seat of the soul." A more contemporary, although less colorful, viewpoint is that the pineal integrates information encoded by light into coordinated secretions that underlie biologic rhythmicity.[94] The pineal is both an endocrine gland and a CVO; it is derived from cells located in the roof of the third ventricle and lies above the posterior commissure near the level of the habenular complex and the sylvian aqueduct. The gland is composed of two cell types, pinealocytes and interstitial (glial-like) cells. Histologic studies suggest that the pineal gland cells are secretory in nature, and indeed the pineal is the principal source of melatonin in mammals.

The pineal gland is an epithalamic structure and consists of primordial photoreceptive cells. The gland retains its light sensitivity in lower vertebrates such as fish and amphibians but lacks direct photosensitivity in mammals and has evolved as a strictly secretory organ in higher vertebrates. However, neuroanatomic studies have established that light-encoded information is relayed to the pineal by a polysynaptic pathway.[95] This series of synapses ultimately results in innervation of the gland by noradrenergic sympathetic nerve terminals that are critical regulators of melatonin production and release. Specifically, retinal ganglion cells directly innervate the SCN of the hypothalamus through the retinohypothalamic tract. In turn, the SCN provides input to the dorsal parvicellular PVH, a key cell group in neuroendocrine and autonomic control. This pathway consists of direct and indirect intrahypothalamic projections. The PVH then provides direct innervation to sympathetic preganglionic neurons in the intermediolateral cell column of the thoracic regions of the spinal cord. Sympathetic preganglionic neurons innervate postganglionic neurons in the superior cervical ganglion and ultimately supply the noradrenergic innervation to the pineal gland (see "Hypothalamic-Pituitary Unit"). This circuitous pathway represents the anatomic substrate



• **Fig. 7.6** Biosynthesis of melatonin from tryptophan in the pineal gland. Step 1 is catalyzed by tryptophan hydroxylase, step 2 by aromatic-L-amino acid decarboxylase, step 3 by arylalkylamine *N*-acetyltransferase, and step 4 by hydroxyindole-*O*-methyltransferase. (From Wurtman RJ, Axelrod J, Kelly DE. Biochemistry of the pineal gland. In: Wurtman RJ, Axelrod J, Kelly DE, eds. *The Pineal*. New York: Academic Press; 1968:47–75.)

for light to regulate the secretion of melatonin through the release of norepinephrine. In the presence of light, whether morning light or a light impulse, norepinephrine release is inhibited, resulting in the shutdown of melatonin synthesis. In the absence of light input, the pineal gland rhythms persist but are not entrained to the external light-dark cycle.

The Pineal Is the Source of Melatonin

The predominant hormone secreted by the pineal gland is melatonin. However, the pineal contains other biogenic amines, peptides, and GABA. Pineal-derived melatonin is synthesized from tryptophan, which is first converted into 5-hydroxytrophan by tryptophan hydroxylase and then decarboxylated into serotonin (5-HT). Two additional steps transform 5-HT into melatonin, including the rate-limiting step catalyzed by the enzyme aralkylamine *N*-acetyltransferase (AANAT) to yield *N*-acetylserotonin, followed by the transfer of a methyl group from S-adenosyl methionine to the 5-hydroxy group of *N*-acetylserotonin by hydroxyindole-*O*-methyltransferase (HIOMT) (Fig. 7.6).[96] Melatonin plays a key role in regulating a myriad of circadian rhythms, and a fundamental principle of circadian biology is that the synthesis of melatonin is exquisitely controlled.[97] AANAT-mRNA levels, AANAT activity, and melatonin synthesis and release are regulated in a circadian fashion and are entrained by the light-dark cycle, with darkness thought to be the most important signal.[94,96] Melatonin and AANAT levels are highest during the dark and decrease sharply with the onset of light. Melatonin is not stored to any significant degree; it is released into the blood or CSF directly after its biosynthesis in proportion to AANAT activity but has a very rapid half-life (minutes) due to 6-hydroxylation in the liver.

Lack of light ultimately results in the release of norepinephrine from postganglionic sympathetic nerve terminals that act on β-adrenergic receptors in pinealocytes, resulting in an increase in adenylyl cyclase activity and synthesis of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate. Increased levels of intracellular cAMP activate downstream signal transduction cascades, including the catalytic subunits of protein kinase A and phosphorylation of cAMP response element (CRE) binding protein. CREs have been identified in the promoter of AANAT.[98] Therefore light (or lack of it) acting through the sympathetic nervous system induces an increase in cAMP, representing a fundamental regulator of AANAT transcription and melatonin synthesis that ultimately results in a dramatic change of melatonin levels across the day.[95] Interspecies comparative studies of melatonin's physiologic function must be tempered by knowledge of the key differences between rodent and human melatonin regulation, however. For example, significantly more light, as much as 4 log units, is required in humans to produce an equivalent nocturnal suppression of melatonin,[99] and the control of AANAT is largely post-transcriptional in humans rather than transcriptional.[96]

Physiologic Roles of Melatonin

One of the best-characterized roles of melatonin is the regulation of the reproductive axis, including gonadotropin secretion[100] and the timing and onset of puberty (see "Gonadotropin-Releasing Hormone and Control of the Reproductive Axis"). The potent regulation of the reproductive axis by melatonin is established in rodents and domestic animals such as sheep. It was observed experimentally with the demonstration that removal of the pineal leads to precocious puberty. In addition, male rats exposed to constant darkness or blinded by enucleation display testicular atrophy and decreased levels of testosterone. These profound effects of gonadal involution are normalized by removal of the pineal gland.[95] The physiologic significance of melatonin is probably most important in species referred to as seasonal breeders. Indeed, the role of melatonin in regulating reproductive capacity in species such as the sheep and the horse is now established. This type of reproductive strategy probably evolved to synchronize the length of day with the gestational period of the species to ensure that the offspring are born at favorable times of the year and maximize the viability of the young. Interestingly, although there is a strong and consistent correlation between altered melatonin secretion, day

length, and seasonal breeding in diverse species, the valence of the signal can be either positive or negative dependent on the ecologic niche for each species.

The signaling pathway by which melatonin regulates reproduction and puberty in seasonal species is becoming increasingly understood. Changes in the nocturnal duration of melatonin convey photoperiodic information to cells in the pars tuberalis via the MT1 receptor, which is expressed in high density on these cells to regulate clockwork proteins such as Cry 1 that are linked to the regulation of TSHβ synthesis.[101,102] TSHβ acting as a paracrine signal from the pars tuberalis binds to TSH receptors on tanycytes whose end-feet processes reside immediately dorsal to the pars tuberalis. TSHβ regulates the expression of type 2 (D2) and type 3 (D3) iodothyronine deiodinase in tanycytes, enzymes that either activate or inactivate the thyroid hormone, respectively, thus governing the bioavailabilty of the thyroid hormone in the mediobasal hypothalamus.[103–105] Under long day conditions (or short melatonin duration), D2 is upregulated, thereby increasing T3 availability, whereas under short day conditions (or long melatonin duration), D3 is upregulated, resulting in decreased T3 availability in the mediobasal hypothalamus. An increase in T3 bioavailabilty results in reproductive activation and can be replicated by implanting a T3 pellet into the hypothalamus of seasonal breeders maintained in short days that otherwise show reproductive inactivation.[106] The mechanism by which the thyroid hormone affects GnRH secretion involves regulation of the encasement of GnRH axon terminals in the external zone of the median eminence by tanycyte end-feet processes. When thyroid hormone is administered into the hypothalamus, it promotes retraction of these processes to allow direct access of secreted GnRH to the fenestrated portal vessels for conveyance to the pars distalis.[107,108] Thyroid hormone receptors are also plentiful throughout the hypothalamus and may be involved in the regulation of kisspeptin,[109] a hormone that has a critical role in the pulsatile release of GnRH (see "Gonadotropin-Releasing Hormone and Control of the Reproductive Axis").

Melatonin Receptors

Melatonin mediates some of its effects by acting on a family of GPCRs, which have been characterized by pharmacologic, neuroanatomic, and molecular approaches.[94,96,97] The first member of the family, MT1 (Mel_{1a}), is a high-affinity receptor that was isolated originally from *Xenopus* melanophores. The second, MT2 (Mel_{1b}), has approximately 60% homology with MT1. A third receptor in mammals, MT3, is not a GPCR but instead a high-affinity binding site on the cytosolic enzyme quinone reductase 2 that is involved in cellular detoxification and might explain some of melatonin's effects as an antioxidant.[94,96] Melatonin also acts directly as a free radical scavenger to detoxify reactive oxygen and nitrogen species.[95]

The mechanisms for melatonin's effects on regulating and entraining circadian rhythms are becoming increasingly understood. For example, melatonin inhibits the activity of neurons in the SCN of the hypothalamus, the master circadian pacemaker in the mammalian brain.[97,110,111] Melatonin can entrain several mammalian circadian rhythms, probably by the inhibition of neurons in the SCN. Neuroanatomic evidence suggests that many of the effects of melatonin on circadian rhythms involve actions on MT1 receptors in that the distribution of MT1 mRNA overlaps with radiolabeled melatonin binding sites in the relevant brain regions. These sites include the SCN, the retina, and the pars tuberalis of the adenohypophysis. The MT2 receptor is also expressed in the retina and brain, particularly the SCN, but evidently at much lower levels and more involved with phase-shifting effects of melatonin.[96,97,110]

Genetic studies in mice have also helped to illuminate the relative roles of each melatonin receptor in mediating the effects of this hormone. Targeted deletion (knockout) of the MT1, but not the MT2 receptor, abolished the ability of melatonin to inhibit the activity of SCN neurons.[111,112] Several studies have suggested that the inhibition of SCN neurons by melatonin is of great physiologic significance. Melatonin may underlie the mechanism by which light induces phase shifts. However, it should be noted that lack of the MT1 gene does not block the ability of melatonin to induce phase shifts. These unexpected and somewhat confusing results have resulted in the hypothesis that MT2 is involved in melatonin-induced phase shifts, as this receptor may be expressed in the SCN in the human brain.[96]

Melatonin Therapy in Humans

Melatonin is purported to exert multiple beneficial functions that include slowing or reversing the progression of aging, blood pressure and autonomic cardiovascular regulation, protecting against ischemic damage after vascular reperfusion, and enhancing immune function.[94–96,113,114] However, the most-studied and established role of melatonin in humans is that of phase shifting and resetting circadian rhythms. In this context, melatonin has been used to treat jet lag and may be effective in treating circadian-based sleep disorders.[115] In addition, melatonin administration has been shown to regulate sleep in humans, although two meta-analyses of the published reports on melatonin for the treatment of either primary or secondary sleep disorders concluded that there is limited evidence for significant clinical efficacy, but it is safe to use in the short term (≤3 months).[116,117] Melatonin therapy has also been suggested to have oncostatic effects on several tumors by acting as an antioxidant and to reduce blood pressure in patients with hypertension, improve major depressive disorder and anxiety, exert anti-inflammatory actions in rheumatoid arthritis and osteoarthritis, and because of its protective role against oxidative stress, potentially be efficacious for the treatment of Alzheimer disease, Parkinson disease, Huntington disease, and amyotrophic sclerosis.[118,119]

Hypophysiotropic Hormones and Neuroendocrine Axes

With the demonstration by the first half of the 1900s that pituitary secretion is controlled by hypothalamic hormones released into the portal circulation, the race was on to identify hypothalamic releasing factors. The search for hypothalamic neurohormones with anterior pituitary regulating properties focused on extracts of the stalk median eminence, neural lobe, and hypothalamus from sheep and pigs. To give some idea of the herculean nature of this effort, approximately 250,000 hypothalamic fragments were required to purify and characterize the first such factor, TRH.[10] Such hypophysiotropic substances were initially called *releasing factors* but are now more commonly called *releasing hormones*.

All of the principal hypothalamic-pituitary regulating hormones are peptides with the notable exception of dopamine, which is a biogenic amine and the major prolactin-inhibiting factor (PIF) (see later discussion and Table 7.2). All are available for clinical investigations or diagnostic tests, and therapeutic analogues for dopamine, GnRH, and somatostatin are widely prescribed.

In addition to regulating hormone release, some hypophysiotropic factors control pituitary cell differentiation, proliferation, and hormone synthesis. Some act on more than one pituitary hormone. For example, TRH is a potent releaser of prolactin (PRL) in addition to TSH, and under some circumstances, releases corticotropin (ACTH) and growth hormone (GH). GnRH releases both LH and follicle-stimulating hormone (FSH). Somatostatin inhibits the secretion of GH, TSH, and a wide variety of nonpituitary hormones. The principal inhibitor of PRL secretion, dopamine, also inhibits secretion of TSH, gonadotropin, and under certain conditions, GH. Dual control is exerted by the interaction of inhibitory and stimulatory hypothalamic hormones. For example, somatostatin interacts with growth hormone–releasing hormone and TRH to control secretion of GH and TSH, respectively, and dopamine interacts with prolactin-releasing factors (PRFs) to regulate PRL secretion. Some hypothalamic hormones act synergistically; for example, CRH and AVP cooperatively regulate the release of pituitary ACTH.

Secretion of the releasing hormones in turn is regulated by neurotransmitters and neuropeptides released by a complex array of neurons synapsing with hypophysiotropic neurons. Control of secretion is also exerted through feedback control by hormones such as glucocorticoids, gonadal steroids, thyroid hormone, anterior pituitary hormones (short-loop feedback control), and hypophysiotropic factors themselves (ultrashort-loop feedback control).

The distribution of the hypophysiotropic hormones is not limited to the hypothalamus and can be found in many different regions of the brain and its periphery, produced by nonhypophysiotropic cells where they mediate functions unrelated to pituitary regulation. Most of the peptides, hormones, and neurotransmitters involved in the regulation of hypothalamic-pituitary control transduce their signals through members of the extensive GPCR family.

Feedback Concepts in Neuroendocrinology

To understand the regulation of each hypothalamic-pituitary-target organ axis, it is important to understand some basic concepts of homeostatic systems. A simplified account of feedback control in relation to neuroendocrine regulation is presented here. Hormonal systems form part of a feedback loop in which the controlled variable (generally the blood hormone level or some biochemical surrogate of the hormone) determines the rate of secretion of the hormone. In negative feedback systems the controlled variable inhibits hormone output, and in positive feedback control systems it increases hormone secretion. Both negative and positive endocrine feedback control systems can be part of a closed loop, in which regulation is entirely restricted to the interacting regulatory glands, or an open loop, in which the nervous system influences the feedback loop. All pituitary feedback systems have nervous system inputs that either alter the set-point of the feedback control system or introduce open-loop elements that can influence or override the closed-loop control elements.

In engineering formulations of feedback, three controlled variables can be identified: a sensing element that detects the concentration of the controlled variable, a reference input that defines the proper control levels, and an error signal that determines the output of the system. The reference input is the set-point of the system.

Hormonal feedback control systems resemble engineering systems in that the concentration of the hormone in the blood (or some function of the hormone) regulates the output of the controlling gland. However, hormonal feedback differs from engineering systems in that the sensor element and the reference input element are not readily distinguishable. The set-point of the controlled variable is determined by a complex cascade beginning with the kinetics of binding to a receptor and the activities of successive intermediate messengers. Sophisticated models incorporating control elements, compartmental analysis, and hormone production and clearance rates exist for many systems. In fact, this sort of modeling applied to developmental programming, intracellular signaling cascades, and neural circuits in addition to endocrine feedback systems is commonly referred to as *systems biology*.[120]

Endocrine Rhythms

Virtually all functions of living animals (regardless of their position on the evolutionary scale) are subject to periodic or cyclic changes, many of which are influenced primarily by the nervous system (Table 7.3).[121,122] Most periodic changes are free running; that is, they are intrinsic to the organism, independent of the environment, and driven by a biologic clock.

Most free-running rhythms are coordinated (entrained) by external signals (cues), such as light-dark changes, meal patterns, cycles of the lunar periods, or the ratio of day length to night

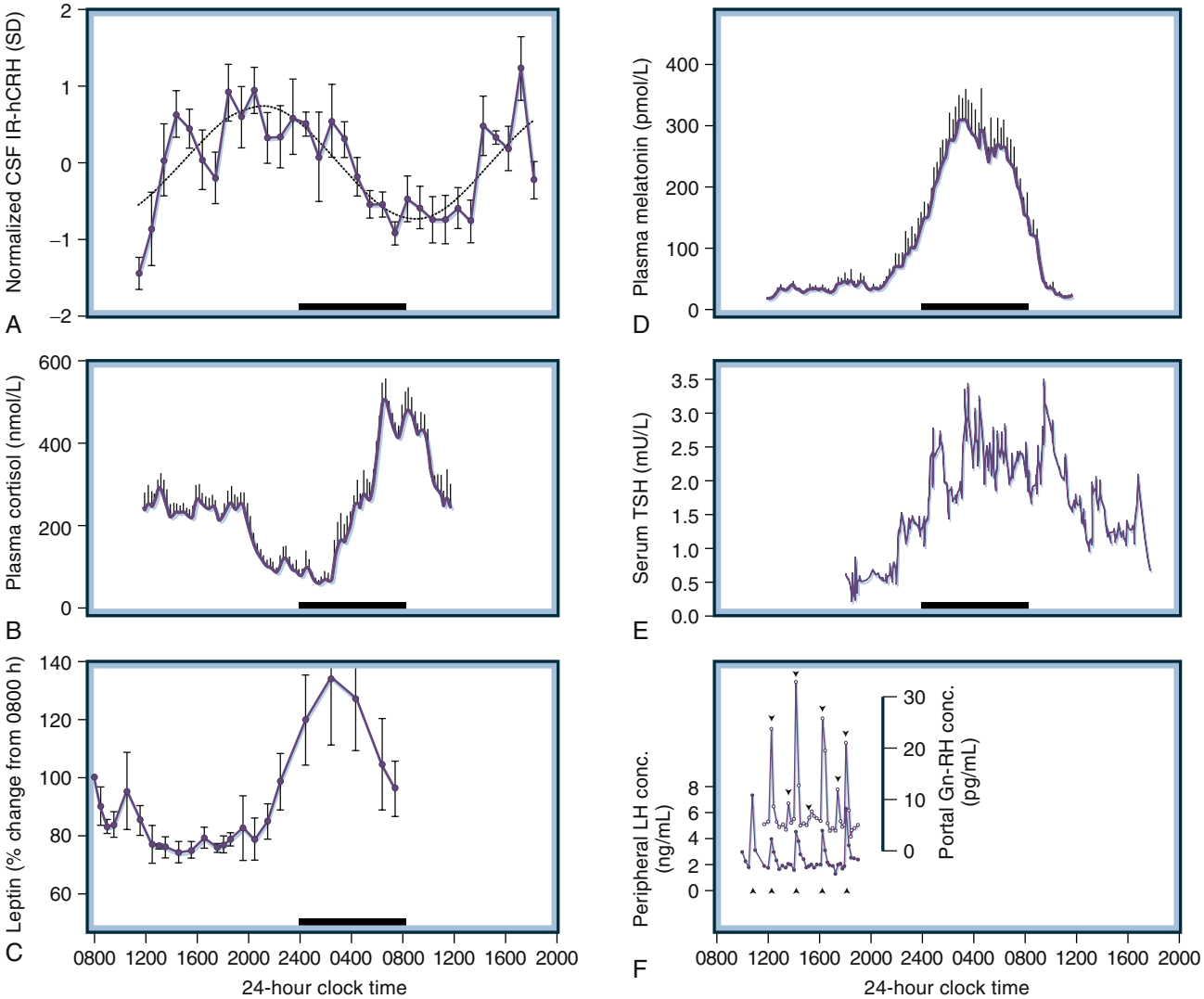
TABLE 7.3 Terms Used to Describe Cyclic Endocrine Phenomena

Term	Definition
Period	Length of the cycle
Circadian	About a day (24 hours)
Diurnal	Exactly a day
Ultradian	Less than a day (i.e., minutes or hours)
Infradian	Longer than a day (i.e., month or year)
Mean	Arithmetic mean of all values within a cycle
Range	Difference between the highest and lowest values
Nadir	Minimal level (inferred from mathematical curve-fitting calculations)
Acrophase	Time of maximal levels (inferred from curve fitting)
Zeitgeber	Time giver (German); the external cue, usually the light-dark cycle that synchronizes endogenous rhythms
Entrainment	The process by which an endogenous rhythm is regulated by a zeitgeber
Phase shift	Induced change in an endogenous rhythm
Intrinsic clock	Neural structures that possess intrinsic capacity for spontaneous rhythms; for circadian rhythms, these are located in the suprachiasmatic nucleus

Modified from Van Cauter E, Turek FW. Endocrine and other biological rhythms. In: DeGroot LJ, ed. *Endocrinology*. 3rd ed. Philadelphia: Saunders; 1995:2497–2548.

length. External signals of this type (*zeitgeber*, or time giver) do not bring about the rhythm but provide the synchronizing time cue. Many endogenous rhythms have a period of ~24 hours (circadian [around a day] or diurnal rhythms). Circadian changes follow an intrinsic program that is ~24 hours long, whereas diurnal rhythms can be either circadian or dependent on shifts in light and dark. Rhythms that occur more frequently than once a day are ultradian. Infradian rhythms have a period longer than 1 day, as in the ~27-day human menstrual cycle and the yearly breeding patterns of some animals.

Most endocrine rhythms are circadian (Fig. 7.7). The secretion of GH and PRL in humans is maximal shortly after the onset of sleep, and that of cortisol is maximal between 2 AM and 4 AM. TSH secretion is lowest between 4 PM and 7 PM and maximal between 9 PM and 5 AM. Gonadotropin secretion in adolescents is increased at night. Superimposed on the circadian cycle are ultradian bursts of hormone secretion. LH secretion during adolescence is characterized by rapid, high-amplitude pulsations at night, whereas in sexually mature individuals secretory episodes are lower in amplitude and occur throughout the 24 hours. GH, ACTH, and PRL are also secreted in brief, fairly regular pulses. The short-term fluctuations in hormonal secretion have important functional significance. In the case of LH, the normal endogenous rhythm of pituitary secretion reflects the pulsatile release



• **Fig. 7.7** Diurnal rhythms of (A) corticotropin-releasing hormone, (B) cortisol, (C) leptin, (D) melatonin, and (E) thyrotropin (TSH) in humans; (F) the relationship between gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) secretion in sheep. *CSF,* cerebrospinal fluid; *IR,* immunoreactive. (From Kling MA, DeBellis MD, O'Rourke DK, et al. Diurnal variation of cerebrospinal fluid immunoreactive corticotropin-releasing hormone levels in healthy volunteers. *J Clin Endocrinol Metab.* 1994;79:233–239, Fig. 3; van Coevorden A, Mockel J, Laurent E, et al. Neuroendocrine rhythms and sleep in aging men. *Am J Physiol.* 1991;260:E651–E661, Fig. 1A and C; Sinha MK, Ohannesian JP, Heiman ML, et al. Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects. *J Clin Invest.* 1996;97:1344–1347, Fig. 2; Brabant G, Prank K, Ranft U, et al. Physiological regulation of circadian and pulsatile thyrotropin secretion in normal man and woman. *J Clin Endocrinol Metab.* 1990;70:403–409, Fig. 2B; and Clarke IJ, Cummins JT. The temporal relationship between gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. *Endocrinology.* 1982;111:1737–1739, Fig. 2A.)

of GnRH. The period of ~90 minutes between LH peaks corresponds to the optimal timing of GnRH pulses to induce maximal pituitary stimulation. Episodic secretion of GH also enhances its biopotency, but for many rhythms the function is not clear. Most homeostatic activities are also rhythmic, including body temperature, water balance, blood volume, sleep, and activity.[123,124]

Assessment of endocrine function must take into account the variability of hormone levels in the blood. Thus appropriately obtained samples at different times of the day or night may provide useful dynamic indicators of hypothalamic-pituitary function. For example, the loss of diurnal rhythm of GH and ACTH secretion may be an early sign of hypothalamic dysfunction. Furthermore, the optimal timing for the administration of glucocorticoids to suppress ACTH secretion (as in therapy for congenital adrenal hyperplasia) must take into account the varying suppressibility of the axis at different times of the day.

The best-understood neural structures responsible for circadian rhythms are the SCNs, paired structures in the anterior hypothalamus above the optic chiasm.[121,124] Individual cells of the SCN have an intrinsic capacity to oscillate in a circadian pattern due to the existence of a cell-autonomous transcription-translation feedback loop involving the transcription factors CLOCK and BMAL1 that interact with the promoters of the *period* (*PER*) and *cryptochrome* (*CRY*) genes.[125] If lesioned bilaterally, free-running circadian rhythmicity is produced, characterized by disruption of the sleep-wake cycle and loss of predictable daily oscillations in feeding, drinking, melatonin secretion, and the secretion of some anterior pituitary hormones.[126,127] Normal rhythmicity can be restored if the SCN is transplanted back into the lesioned animals.[128]

In addition to the massive retinohypothalamic projection from the retina described earlier carrying pituitary adenyl cyclase-activating peptide (PACAP) and nitric oxide, the SCN receives neuronal input from many nuclei, including GABA-containing and NPY-containing axonal projections from the intergeniculate leaflet of the thalamus and serotonin neurons from the midbrain raphe, the limbic system (hippocampus, bed nucleus of the stria terminalis [BNST], septum), and the hypothalamus itself. These inputs have an important role in modulating the endogenous rhythms of the individual SCN pacemaker cells and contribute to phase-shifting effects on SCN pacemaker activity.[129] The SCN is also organized to permit many reciprocal neuron-neuron interactions mediated by GABA at direct synaptic contacts. It is especially rich in neuropeptides, including AVP, VIP, gastrin-releasing peptide (GRP), and calretinin. The SCN also responds to melatonin through melatonin receptors.[94,96] Studies have indicated that intrinsic pacemaker function is not unique to neurons of the SCN; circadian oscillators are also found in many peripheral tissues.[124]

Metabolic changes in the SCN, such as increased uptake of 2-deoxyglucose and an increased level of VIP, accompany circadian rhythms. This nucleus projects to the pineal gland indirectly via the PVH and the autonomic nervous system (see earlier discussion) and regulates its activity and to the medial preoptic hypothalamus indirectly via the dorsal subparaventricular zone to regulate body temperature set-point and food-dependent energy intake.[129,121] However, the bulk of SCN outflow occurs in a trunk coursing dorsolaterally through the ventral subparaventricular zone and terminating in the dorsal medial hypothalamic nucleus. Polysynaptic pathways involving these latter structures are responsible for the actions of the SCN to produce the circadian rhythms in thermoregulation, glucocorticoid secretion, sleep, arousal, and feeding.[121,125]

Circadian rhythms during fetal life are regulated by maternal circadian rhythms.[130] Circadian changes can be detected 2 to 3 days before birth, and SCN from fetuses of this age display spontaneous rhythmicity in vitro. Maternal regulation of fetal circadian rhythms may be mediated by circulating melatonin or by cyclic changes in the food intake of the mother. The timing of the circadian pacemaker can be shifted in humans by the administration of triazolam, a short-acting benzodiazepine, melatonin (described earlier), or by altered patterns of intense illumination.[99]

Thyrotropin-Releasing Hormone

Chemistry and Evolution

TRH, the short peptide hypophysiotropic hormone, is the tripeptide pyroGlu-His-Pro-NH_2. Six copies of the TRH peptide sequence are encoded within the human TRH pre-prohormone gene (Fig. 7.8).[131] The rat pro-TRH precursor contains five TRH peptide repeats flanked by dibasic residues (Lys-Arg or Arg-Arg), along with seven or more non-TRH peptides.[132] Two prohormone convertases, PC1 and PC2, cleave the carboxy-terminal (COOH-terminal) side of these dibasic residues as the prohormone molecule transits the regulated secretory pathway. Carboxypeptidase E then removes the dibasic residues, leaving the sequence Gln-His-Pro-Gly. This peptide is then amidated at the COOH-terminus by peptidylglycine α-amidating monooxygenase (PAM), with Gly acting as the amide donor. The amino-terminal (NH_2-terminal) pyroglutamate residue results from cyclization of the Gln.

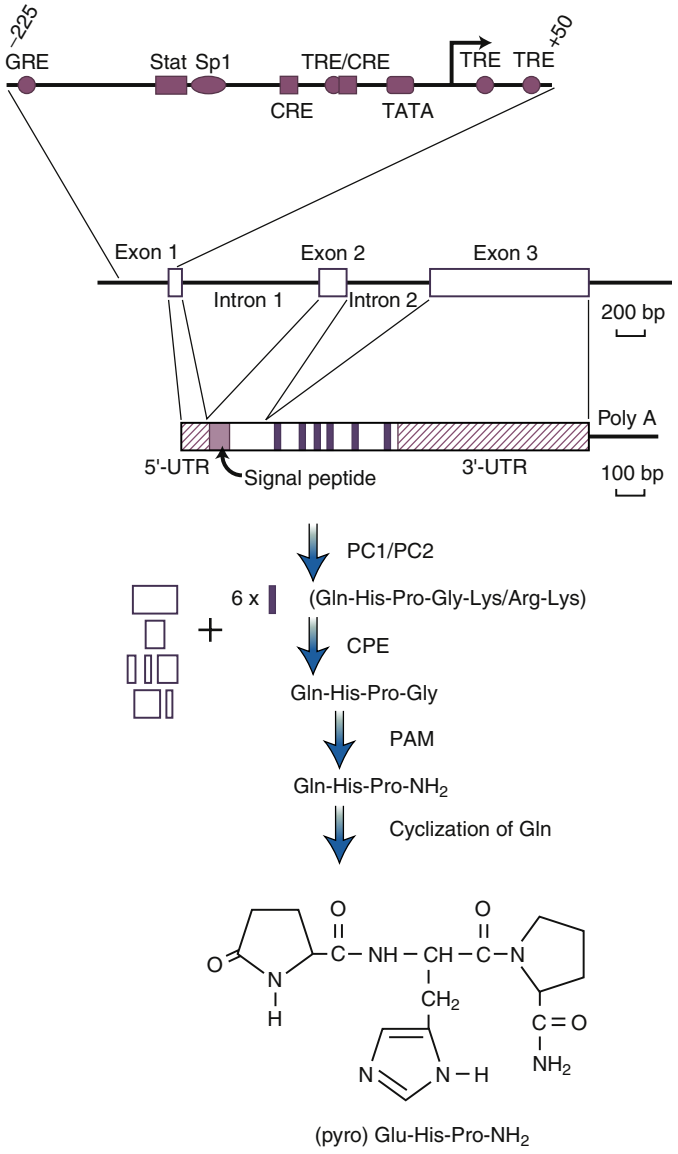
TRH is a phylogenetically ancient peptide; it has been isolated from primitive vertebrates such as the lamprey and even invertebrates such as the snail. TRH is widely expressed in both the CNS and its periphery in amphibians, reptiles, and fishes but does not stimulate TSH release in these poikilothermic vertebrates. Therefore TRH has multiple peripheral and central activities and was co-opted as a hypophysiotropic factor midway during the evolution of vertebrates, perhaps specifically as a factor needed for coordinated regulation of temperature homeostasis.

Although the TRH tripeptide is the only established hormone encoded within its large prohormone, rat pro-TRH yields seven additional peptides that have unique tissue distributions. Several biologic activities of these peptides have been observed: pro-TRH(160-169) may be a hypophysiotropic factor because it is released from hypothalamic slices and potentiates the TSH-releasing effects of TRH.[133] Pro-TRH(178-199) is also released from the median eminence and has been reported to stimulate PRL release or possibly function as a corticotropin release-inhibiting factor.[134]

Effects on the Pituitary Gland and Mechanism of Action

After intravenous injection of TRH in humans, serum TSH levels rise within a few minutes,[135] followed by a rise in serum triiodothyronine (T_3) levels; there is an increase in thyroxine (T_4) release as well, but a change in blood levels of T_4 is usually not demonstrable because the pool of circulating T_4 (most of which is bound to carrier proteins) is so large. TRH action on the pituitary is blocked by previous treatment with thyroid hormone, which is a crucial element in the negative feedback control of pituitary TSH secretion.

TRH is also a potent prolactin-releasing factor.[135] The time course of response of blood PRL levels to TRH, the dose-response characteristics, and the suppression by thyroid hormone pretreatment (all of which parallel changes in TSH secretion) suggest that



• **Fig. 7.8** Structure of the human thyrotropin-releasing hormone (TRH) gene, messenger RNA, and prohormone, showing six repeats of the TRH peptide sequence encoded within exon 3. *CPE,* carboxypeptidase E; *CRE,* cyclic AMP response element; *GRE,* glucocorticoid response element; *PAM,* peptidylglycine α-amidating monooxygenase; *PC1/PC2,* prohormone convertases 1 and 2; *Sp1,* specificity protein 1 binding sequence; *Stat,* signal transducer and activator of transcription binding sequence; *TATA,* Goldstein-Hogness box involved in binding RNA polymerase; *TRE,* thyroid hormone response element; *UTR,* untranslated. (Adapted from data in Yamada M, Radovick S, Wondisford FE, et al. Cloning and structure of human genomic DNA and hypothalamic cDNA encoding human preprothyrotropin-releasing hormone. *Mol Endocrinol.* 1990;4:551–556.)

TRH may be involved in the regulation of PRL secretion. Moreover, TRH is present in the hypothalamic-hypophysial–portal blood of lactating rats. However, it is unlikely to be a physiologic regulator of PRL secretion because the PRL response to nursing in humans is unaccompanied by changes in plasma TSH levels, and mice lacking TRH have normal lactotrophs and basal PRL secretion.[136] Nevertheless, elevated levels of TRH associated with hypothyroidism may occasionally cause hyperprolactinemia (with or without galactorrhea).

In normal individuals, TRH has no influence on the secretion of pituitary hormones other than TSH and PRL, but it enhances the release of GH in acromegaly and of ACTH in some patients with Cushing disease. Furthermore, prolonged stimulation of the normal pituitary with GHRH can sensitize it to the GH-releasing effects of TRH. TRH also causes the release of GH in some patients with uremia, hepatic disease, anorexia nervosa, and psychotic depression and in children with hypothyroidism.[135] TRH inhibits sleep-induced GH release through its actions in the CNS (see later discussion).

Stimulatory effects of TRH are initiated by binding of the peptide to its GPCR on the plasma membrane of the thyrotroph.[137] Thyroid hormone and somatostatin antagonize the effects of TRH but do not interfere with its binding. TRH action is mediated mainly through $G_{q/11}$ and hydrolysis of phosphatidylinositol, with phosphorylation of key protein kinases and an increase in intracellular free calcium (Ca^{2+}) as the crucial steps in postreceptor activation (see Chapter 2).[138] TRH effects can be mimicked by exposure to a Ca^{2+} ionophore and are partially abolished by a Ca^{2+}-free medium. TRH stimulates the formation of mRNAs coding for TSH and PRL in addition to regulating their secretion and stimulates the mitogenesis of thyrotrophs.

TRH is degraded to the dipeptide histidylprolineamide (His-Pro-NH_2) by pyroglutamyl aminopeptidase, following which the dipeptide is deaminated to yield His-Pro or cyclizes nonenzymatically to histidylproline diketopiperazine (cyclic His-Pro). Cyclic His-Pro is reported to act as a PRF and to have other neural effects, including reversal of ethanol-induced sleep (TRH is also effective in this system), elevation of brain cyclic guanosine monophosphate (cGMP) levels, an increase in stereotypical behavior, modification of body temperature, and inhibition of eating behavior. Some of the effects of TRH may be mediated through cyclic His-Pro, but the fact that cyclic His-Pro is abundant in some areas and is not proportional to the amount of TRH suggests that the peptide may not be derived solely from TRH. This latter assertion appears to be confirmed by the detection of substantial amounts of the dipeptide in brains of TRH knockout mice.[136]

Extrapituitary Function

In addition to the hypophysiotropic TRH neurons, TRH is also synthesized by nonhypophysiotropic neurons, including many neuronal groups in the hypothalamus and throughout the brain (amygdala, hippocampus, thalamus, brainstem, spinal cord).[139] The extensive distribution of TRH, its localization in nerve endings, and the presence of TRH receptors in brain tissue suggest that TRH serves as a neurotransmitter or neuromodulator in addition to its well-known function to regulate TSH secretion.[140,141] Central administration of TRH reduces appetite; increases locomotor activity; reduces anxiety; has an antiepileptic effect; induces hyperthermia indicative of a role in central thermoregulation[139]; has broad effects on autonomic regulation, resulting in increased blood pressure and heart rate, increased gastrointestinal motility, increased gastric acid secretion, and increased insulin secretion; and acts as an excitatory modulator of lower motoneuron function by directly synapsing on alpha-motoneurons in the ventral spinal cord.[142] TRH is also found outside the brain, including in anterior pituitary somatotrophs, where it may contribute to regulation of TSH secretion by a paracrine mechanism, retina, parafollicular cells of the thyroid, adrenal medulla, epididymis, prostate, Leydig cells of the testis, pancreatic islet cells, gastrointestinal tract, heart, spleen, lung, ovary, and hair follicles.

Clinical Applications

The use of TRH for the diagnosis of hyperthyroidism or to discriminate between hypothalamic and pituitary causes of TSH deficiency is uncommon since the development of ultrasensitive assays for TSH (see Chapter 11). TRH testing is also not of value in the differential diagnosis of causes of hyperprolactinemia but is useful for the demonstration of residual abnormal somatotropin-secreting cells in acromegalic patients who release GH in response to TRH before treatment.

Studies of the effect of TRH on depression have shown inconsistent results, possibly because of poor blood-brain barrier penetration and a short half-life.[139,141] Although a role for TRH in depression has not established, many depressed patients have a blunted TSH response to TRH, and changes in TRH responsiveness correlate with the clinical course. The mechanism by which blunting occurs is unknown.

TRH has been evaluated for the treatment of diverse neurobiologic disorders (for review see Gary and colleagues[139]), including spinal muscle atrophy and amyotrophic lateral sclerosis; transient improvement in strength was reported in both disorders, but the combined experience at many centers using a variety of treatment protocols (including long-term intrathecal administration) failed to confirm efficacy. TRH administration also reduces the severity of experimentally induced spinal and ischemic shock and may improve recovery after spinal cord injury and head trauma. TRH has been used to treat children with neurologic disorders, including West syndrome, Lennox-Gastaut syndrome, early infantile epileptic encephalopathy, and intractable epilepsy.[143] TRH has been proposed to be an analeptic agent. Sleeping or drug-sedated animals are awakened by the administration of TRH, and TRH reportedly reversed the sedative effects of ethanol in humans and awakened a patient with a profound sleep disorder caused by a hypothalamic and midbrain eosinophilic granuloma.[139]

Regulation of Thyrotropin Release

The secretion of TSH is regulated by two major, interacting elements: negative feedback by thyroid hormone and open-loop neural control by hypothalamic hypophysiotropic factors (Fig. 7.9). TSH secretion is also modified by tanycyte regulation of TRH secretion in the median eminence and other hormones, including estrogens, glucocorticoids, and possibly GH, and is inhibited by cytokines in the pituitary and hypothalamus.[133,144] Aspects of the pituitary-thyroid axis are considered further in Chapter 11.

Feedback Control: Hypothalamic-Pituitary-Thyroid Axis

In the context of a feedback system, the level of thyroid hormone in blood or of its unbound fraction is the controlled variable, and the set-point is the normal resting level of plasma thyroid hormone. The levels of thyroid hormone inversely regulate TSH secretion so that deviations from the set-point lead to appropriate changes in the rate of TSH secretion (Fig. 7.10). Factors that determine the rate of TSH secretion required to maintain a given level of thyroid hormone include the rate at which TSH and thyroid hormone disappear from the blood (turnover rate) and the rate at which T_4 is converted to its more active form, T_3.

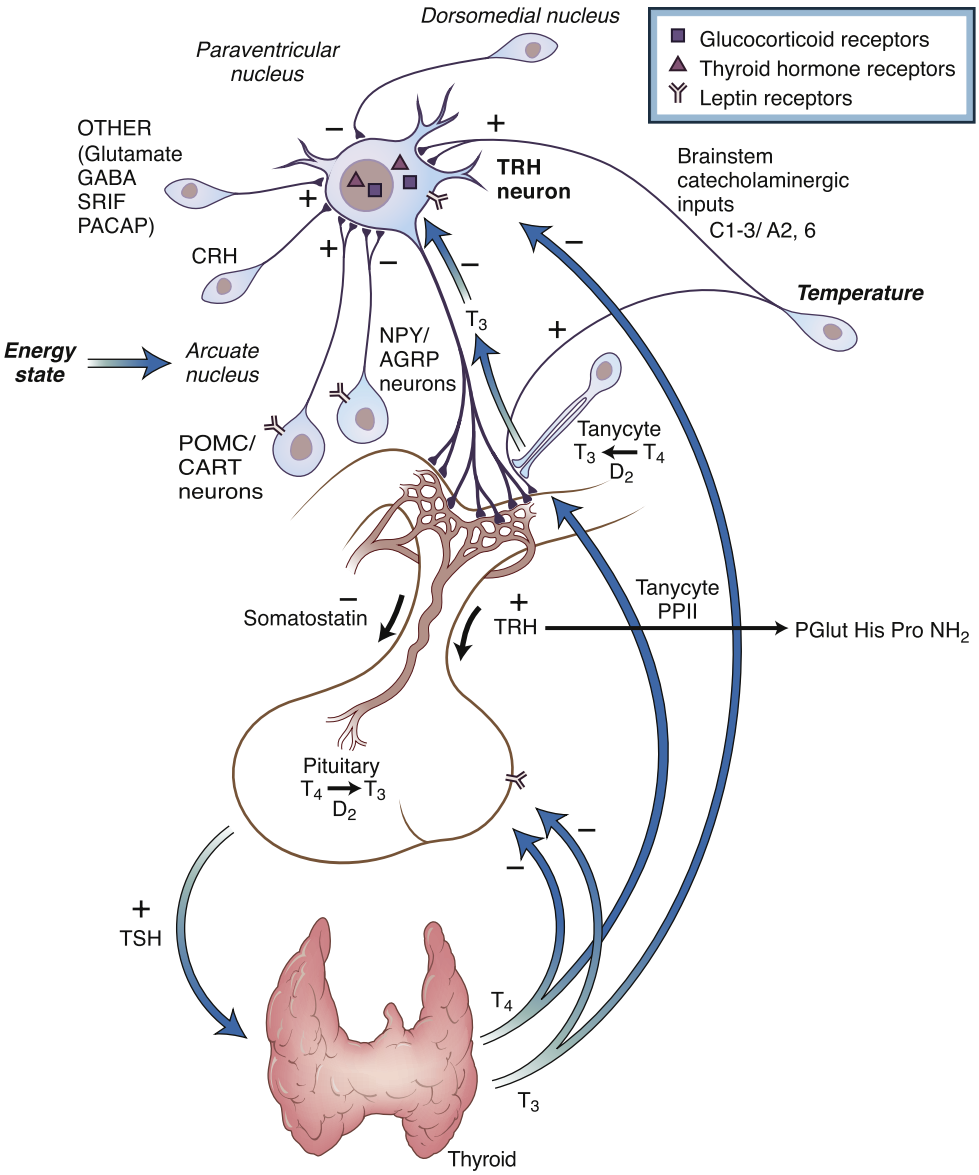
Thyroid hormones act on both the pituitary and the hypothalamus.[145] Feedback control of the pituitary by thyroid hormone is remarkably precise. Administration of small doses of T_3 and T_4 inhibit the TSH response to TRH, and barely detectable decreases in plasma thyroid hormone levels are sufficient to sensitize the pituitary to TRH. TRH stimulates TSH secretion within a few minutes through its action on a GPCR, whereas thyroid hormone actions, mediated by intranuclear receptors, require several hours to take effect.

The secretion of hypophysiotropic TRH is also regulated by thyroid hormone feedback. Systemic injections of T_3 or implantations of tiny T_3 pellets in the PVH of hypothyroid rats[146] (Fig. 7.11A and B) reduce the concentration of TRH mRNA and TRH prohormone in hypophysiotropic TRH-secreting cells that are located in the parvicellular subdivision of the PVH. The molecular mechanism governing negative regulation of TRH by thyroid hormone has not been firmly established but involves binding of the T_3-thyroid hormone receptor complex to a regulatory element in the TRH promoter called site 4 (see Fig. 7.8), the release of corepressors such as NCor and SMART, and recruitment of coactivators such as SRC-1.[145]

Thyroid hormone negative feedback regulation of hypophysiotropic TRH neurons is mediated by the beta 2 isoform of the thyroid hormone receptor ($TR\beta_2$),[147] which is expressed in hypophysiotropic, PVH TRH neurons. T_3 alone is not sufficient to normalize TRH expression in the PVH and requires the presence of T_4.[148] The mechanism is believed to involve the conversion of T_4 to T_3 by D_2 within tanycytes, following the uptake of T_4 circulating in the bloodstream or in the CSF by way of the thyroid hormone transporters OATP1 and MCT8 expressed on these cells.[133] T_3 released from tanycytes, can then be taken up by axon terminals of hypophysiotropic TRH neurons in the external zone of the median eminence that are in intimate contact with tanycyte end-foot processes and also express the thyroid hormone transporter MCT8, thereby inhibiting TRH following its retrograde axonal transport to cell bodies in the PVH.[133,148–150] Along these lines, it is of particular interest that D_3, the thyroid hormone degrading enzyme, is rarely expressed in hypophysiotropic TRH axons in the median eminence in contrast to axons originating from hypophysiotropic CRH, GnRH, and GHRH neurons.[151] Presumably this selectivity of D_3 expression allows hypophysiotropic TRH neurons to remain sensitive to changes in peripheral thyroid hormone levels while allowing other hypophysiotropic neuronal types to protect intracellular concentrations of T_3. Other central mechanisms contributing to feedback regulation of hypophysiotropic TRH by thyroid hormone include regulation of prohormone convertase enzymes in hypophysiotropic TRH neurons that are involved in the post-translational processing of proTRH, and the synthesis of the TRH degrading enzyme, pyroglutamyl peptidase II (PPII), by tanycytes that modulate the amount of TRH released into the portal circulation for conveyance to the anterior pituitary.[152,153]

Neural Control

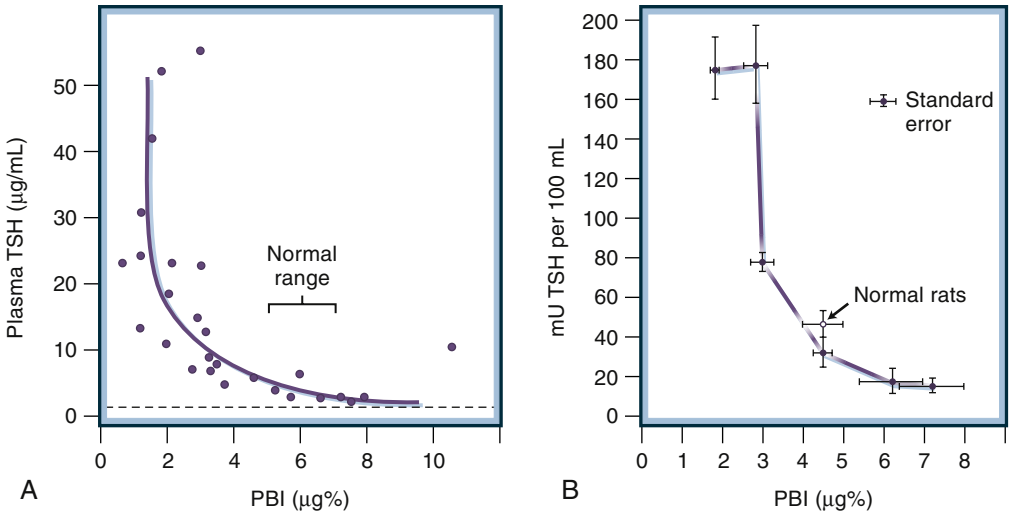
The hypothalamus determines the set-point of feedback control around which the usual feedback regulatory responses are elicited.[133] Lesions of the thyrotropic area lower basal thyroid hormone levels and make the pituitary more sensitive to inhibition by thyroid hormone, and high doses of TRH raise the levels of TSH and thyroid hormone. Synthesis of TRH in the paraventricular nuclei is regulated by feedback actions of thyroid hormone.[133,145] The hypothalamus can override normal feedback control through an open-loop mechanism involving neuronal inputs to the hypophysiotropic TRH neurons (see Fig. 7.9). Three main neuronal groups are known to project to hypophysiotropic TRH neurons, including two groups in the hypothalamus originating in the arcuate nucleus and dorsomedial nucleus and catecholamine-producing neurons in the brainstem.[133]



• **Fig. 7.9** Regulation of the hypothalamic-pituitary-thyroid axis. Central modulators of the hypothalamic tuberoinfundibular system include inputs from brainstem catecholamine neurons (C1–C3, A2, 6) that may also coproduce NPY and CART, NPY/AgRP, and α-MSH/CART neurons in the hypothalamic arcuate nucleus and the dorsomedial nucleus. Hypophysiotropic TRH neurons also receive a variety of other inputs, including CRH, glutamatergic, GABAergic, TRH, CRH, SRIF, and PACAP inputs. Feedback regulation by circulating thyroid hormone is mediated by the $TR\beta_2$ receptor expressed in hypophysiotropic TRH neurons. The conversion of T_4 to T_3 by type 2 iodothyronine 5′-monodeiodinase is required and proposed to take place in tanycytes lining the third ventricle. T_3 released from tanycytes can be taken up by TRH axon terminals in the median eminence that are in close contact with tanycyte end-feet processes and then transported retrogradely to the cell bodies in the hypothalamic PVH. *AGRP,* agouti-related peptide; *CART,* cocaine-regulated and amphetamine-regulated transcript; *CRH,* corticotropin-releasing hormone; *D_2,* type 2 iodothyronine deiodinase; *NPY,* neuropeptide Y; *POMC,* pro-opiomelanocortin; *PPII,* pyroglutamyl peptidase II; *T_3,* triiodothyronine; *T_4,* thyroxine; *TRH,* thyrotropin-releasing hormone; *TSH,* thyrotropin.

Neurons in the arcuate nucleus containing NPY, AgRP, α-MSH, and cocaine-regulated and amphetamine-regulated transcript (CART) have important roles in the regulation of energy homeostasis and respond to feeding-related signals in the bloodstream, including leptin, ghrelin, insulin, and glucose (see Chapter 39). These neurons establish numerous contacts with hypophysiotropic neurons and are involved in resetting the set-point for feedback regulation of thyroid hormone during nutritional insufficiency (see upcoming discussion about fasting and starvation). TRH neurons in the PVH of the human hypothalamus also receive inputs from NPY/AgRP neurons in the infundibular nucleus,[154] indicating evolutionary conservation of this important pathway.

The dorsomedial nucleus also has extensive projections to TRH neurons in the PVN[155] and, if ablated, results in an increase in circulating levels of T_3,[156] indicative of an inhibitory effect. Little is known about how the dorsomedial nucleus regulates hypophysiotropic TRH neurons, however, but it is hypothesized to be involved in circadian regulation of TSH (see "Circadian Rhythm").



• **Fig. 7.10** Relationship between plasma thyrotropin (TSH) levels and thyroid hormone as determined by plasma protein-bound iodine (PBI) measurements in humans and rats. These curves illustrate, in the human (A) and the rat (B), that plasma TSH levels are a curvilinear function of plasma thyroid hormone level. Human studies were carried out by giving myxedematous patients successive increments of thyroxine (T_4) at approximately 10-day intervals. Each point represents simultaneous measurements of plasma PBI and plasma TSH at various times in the six patients studied. The rat studies were performed by treating thyroidectomized animals with various doses of T_4 for 2 weeks before assay of plasma TSH and plasma PBI. These curves illustrate that the secretion of TSH is regulated over the entire range of thyroid hormone levels. At the normal set-point for T_4, the small changes above and below the control level are followed by appropriate increases or decreases in plasma TSH. (A, From Reichlin S, Utiger RD. Regulation of the pituitary thyroid axis in man: relationship of TSH concentration to concentration of free and total thyroxine in plasma. *J Clin Endocrinol Metab.* 1967;27:251–255, copyright by The Endocrine Society. B, From Reichlin S, Martin JB, Boshans RL, et al. Measurement of TSH in plasma and pituitary of the rat by a radioimmunoassay utilizing bovine TSH: effect of thyroidectomy or thyroxine administration on plasma TSH levels. *Endocrinology.* 1970;87:1022–1031, copyright by The Endocrine Society.)

Finally, hypophysiotropic TRH neurons receive a particularly dense innervation from catecholamine-producing neurons in the brainstem, including adrenergic neurons located in regions C1 to C3 in the rostroventrolateral medulla and within and adjacent to the nucleus tractus solitarius, and noradrenergic neurons located in A2 and A6 regions in the ventrolateral medulla and dorsal vagal complex.[157] Subpopulations of these catecholaminergic neurons coproduce peptides, contributing ~25% of the NPY innervation and ~60% of the CART innervation to TRH neurons in the PVN.[158,159] As will be described later, this projection pathway has a particularly important role in the regulation of TRH gene expression by cold.

Hypothalamic regulation of TSH secretion is also influenced by the inhibitory factor somatostatin. Antisomatostatin antibodies increase basal TSH levels and potentiate the response to stimuli that normally induce TSH release in the rat, such as cold exposure and TRH administration.[160] Thyroid hormone in turn inhibits the release of somatostatin, implying coordinated, reciprocal regulation of TRH and somatostatin by thyroid hormone. GH stimulates hypothalamic somatostatin synthesis and can inhibit TSH secretion. However, the physiologic role of somatostatin in the regulation of TSH secretion in humans is uncertain.

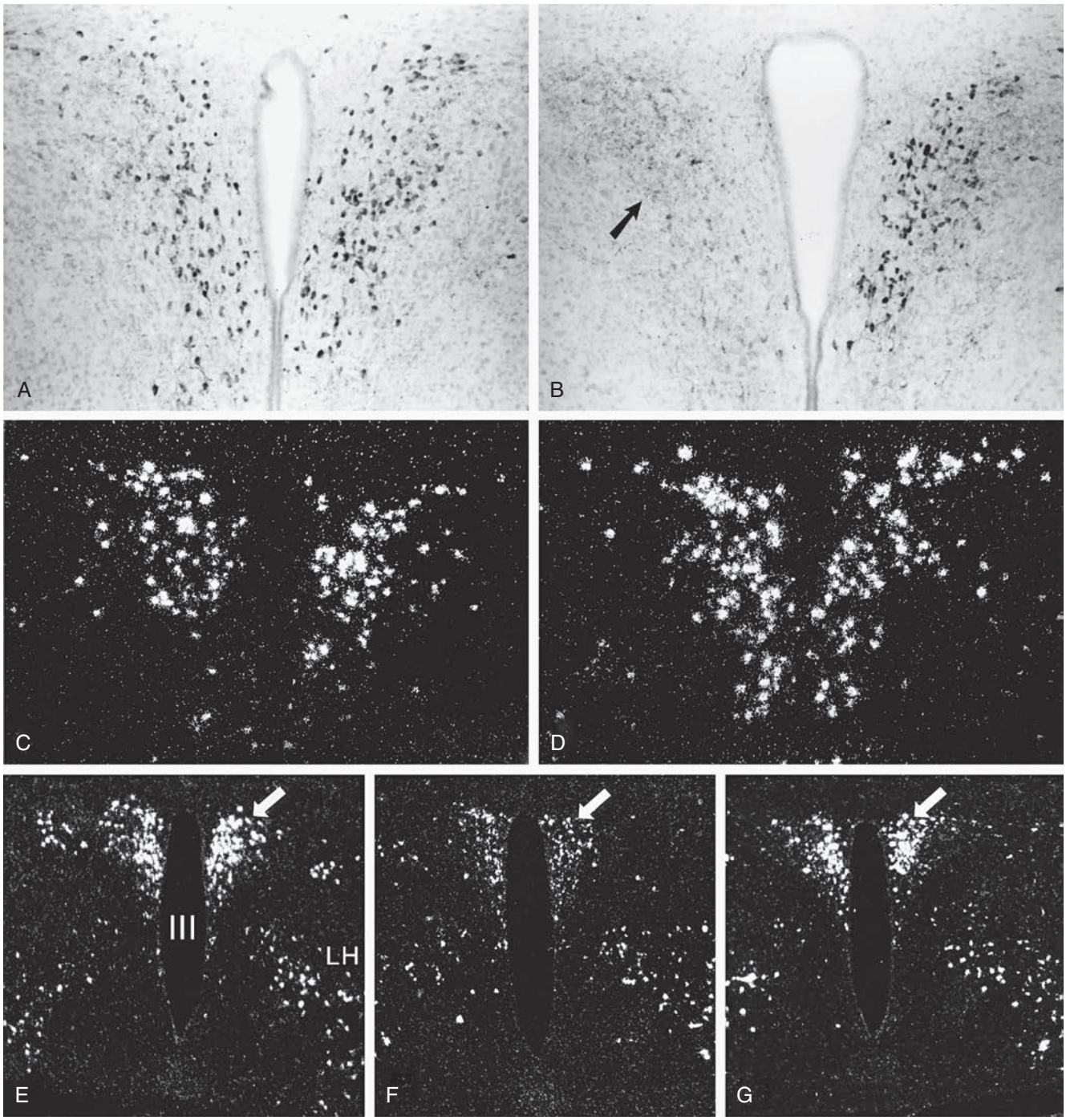
Circadian Rhythm

Plasma TSH in humans is characterized by a circadian periodicity, with a maximum between 9 PM and 5 AM and a minimum between 4 PM and 7 PM (see Fig. 7.7E).[161] Smaller ultradian TSH peaks occur every 90 to 180 minutes, probably because of bursts of TRH release from the hypothalamus, and are physiologically important in controlling the synthesis and glycosylation of TSH. Glycosylation is a determinant of TSH potency.[162]

Temperature

External cold exposure activates and high ambient temperature inhibits the pituitary-thyroid axis in animals, and analogous changes occur in humans under certain conditions.[163] Exposure of infants to cold at the time of delivery causes an increase in blood TSH levels, possibly because of alterations in the turnover and degradation of the thyroid hormones. Blood thyroid hormone levels are higher in the winter than in the summer among people living in cold climates but not in other climates. However, it is difficult to show that changes in environmental or body temperature in adults influence TSH secretion. For example, exposure to cold ambient temperature or central hypothalamic cooling does not modify TSH levels in young men. Behavioral changes, activation of the sympathetic nervous system, and shivering appear to be more important than the thyroid response for temperature regulation in adults.

The autonomic nervous system and the thyroid axis work together to maintain temperature homeostasis in mammals, and TRH plays a role in both pathways.[163] Hypothalamic TRH release is rapidly increased (30–45 minutes) in rats exposed to cold. Rapid inhibition of somatostatin release in the median eminence has also been documented, and both changes appear to play important roles in the rise in plasma TSH induced by cold exposure. TRH mRNA is elevated within an hour of cold exposure (see Fig. 7.11C and D). The regulation of hypophysiotropic



• **Fig. 7.11** (A and B) Direct effects of triiodothyronine (T_3) on thyrotropin-releasing hormone (TRH) synthesis in the rat hypothalamic paraventricular nucleus (parvicellular division) were shown in this experiment by immunohistochemical detection of pre-pro-TRH(25-50) after implantation of a pellet of either T_3 (B) or inactive diiodothyronine (T_2) as a control (A). The T_2 pellet had no effect on the concentration of pre-pro-TRH (A). In contrast, the TRH prohormone concentrations (B) were markedly reduced (*black arrow* indicates the unilateral pellet implantation). These studies indicate that thyroid hormone regulates the hypothalamic component of the pituitary-thyroid axis as well as the pituitary thyrotrope itself. (C and D) Effects of 1 hour at 4°C on TRH messenger ribonucleic acid (mRNA). (E to G) Effects on TRH mRNA levels of starvation (F) and leptin replacement during starvation (G). *White arrows* show the location of the paraventricular nucleus. *III,* 3rd ventricle; *LH,* lateral hypothalamus. (Photomicrographs in panels A and B, from Dyess EM, Segerson TP, Liposits Z, et al. Triiodothyronine exerts direct cell-specific regulation of thyrotropin-releasing hormone gene expression in the hypothalamic paraventricular nucleus. *Endocrinology*. 1988;123[5]:2291–2297; photomicrographs in panels E, F, and G, from Légrádi G, Emerson CH, Ahima RS, Flier JS, Lechan RM. Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. *Endocrinology*. 1997;138[6]:2569–2576; copyright by The Endocrine Society; photomicrographs in panels C and D, courtesy Dr. P. Joseph-Bravo.)

TRH release and expression by cold is largely mediated by catecholamines, and a rapid rise in TRH release is observed after norepinephrine treatment of hypothalamic fragments. As mentioned previously, catecholamines arise from neurons originating in the brainstem and can affect TRH secretion both through contacts on TRH perikarya in the PVH and their axon terminals in the median eminence (see Fig. 7.9).[164]

Stress

Stress is another determinant of TSH secretion.[165] In humans, physial stress inhibits TSH release, as indicated by the finding that low levels of T_3 and T_4 in patients with the nonthyroidal illness syndrome do not cause compensatory increases in TSH secretion as would occur in normal individuals.[166]

A number of observations demonstrate interactions between the thyroid and adrenal axes.[165] Physiologically, the bulk of evidence suggests that glucocorticoids in humans and rodents act to blunt the thyroid axis through actions in the CNS.[167] Some actions may be direct because the TRH gene (see Fig. 7.8) contains a glucocorticoid response element consensus half-site,[132] and hypophysiotropic TRH neurons appear to contain glucocorticoid receptors.[168] The diurnal rhythm of cortisol is opposite that of TSH (see Fig. 7.7), and acute administration of glucocorticoids can block the nocturnal rise in TSH, but disruption of cortisol synthesis with metyrapone only modestly affects the TSH circadian rhythm.

Nevertheless, several lines of evidence identify conditions in which elevated glucocorticoids are associated with stimulation of the thyroid axis. Human depression is often associated with hypercortisolism and hyperthyroxinemia, and TRH mRNA levels are elevated by glucocorticoids in a number of cell lines as well as in cultured fetal hypothalamic TRH neurons from the rat. Thus, although glucocorticoids can stimulate TRH production in TRH neurons by binding to glucocorticoid receptor binding sites in the TRH promoter, their overall inhibitory effect on the thyroid axis results from indirect glucocorticoid negative feedback on structures such as the hippocampus. Disruption of hippocampal suppression of the hypothalamic-pituitary-adrenal (HPA) axis is proposed to be involved in the hypercortisolemia commonly seen in affective illness, and disruption of hippocampal inputs to the hypothalamus has been shown to produce a rise in hypophysiotropic TRH in the rat.[169]

Suckling

Suckling also increases TRH gene expression in hypophysiotropic neurons but primarily to increase prolactin secretion from the anterior pituitary. TSH and thyroid hormone levels do not increase in response to suckling, perhaps due to the simultaneous release of CART that is coexpressed with TRH in hypophysiotropic neurons that attenuate TRH-induced TSH secretion.[170]

Starvation

The thyroid axis is suppressed during starvation, presumably to help conserve energy by depressing metabolism (see Fig. 7.11E–G). In humans, reduced T_3, T_4, and TSH are seen during starvation or fasting.[133] There are also changes in the thyroid axis in anorexia nervosa, such as low blood levels of T_3 and low normal levels of T_4. Inappropriately low levels of TSH are found, suggesting defective activation of TRH production by low thyroid hormone levels. During starvation in rodents, reduced TRH release into hypothalamic-hypophysial portal blood and reduced pro-TRH mRNA levels are seen, despite lowered thyroid hormone levels.[171] Reduced basal TSH levels are also usually present.

The hypothyroidism seen in fasting or in the leptin-deficient *ob/ob* mouse can be reversed by administration of leptin,[14] and evidence suggests that the mechanism involves leptin's ability to upregulate TRH gene expression in the PVH (see Fig. 7.11E–G).[13] Leptin appears to act both directly through leptin receptors on hypophysiotropic TRH neurons and indirectly through its actions on other hypothalamic cell groups, such as arcuate nucleus POMC and NPY/AgRP neurons.[172,173] TRH neurons in the PVH receive dense NPY/AgRP and POMC projections from the arcuate and express NPY and melanocortin-4 receptors (MC4R),[174] and α-MSH administration partially prevents the fasting-induced drop in thyroid hormone levels.[172,173] Indeed, the TRH promoter contains a signal transducer and activator of transcription (STAT) response element and a CRE that have been demonstrated to mediate induction of TRH gene expression by leptin and α-MSH, respectively, in a heterologous cell system (see Fig. 7.8).[174] The regulation of TRH by metabolic state is likely to be under redundant control, however, because leptin-deficient children, unlike rodents, are euthyroid,[175] whereas both humans and rodents with MC4R deficiency are euthyroid.[176]

Dehydration-Induced Anorexia

The addition of 2.5% NaCl to drinking water markedly reduces food intake by 80%.[177] Despite the very low food intake, TRH gene expression in the PVH increases rather than the anticipated decrease as described previously. The mechanism for this response is uncertain but may involve activating effects of CRH from neurons in the lateral hypothalamus that project to hypophysiotropic TRH neurons. Dehydration-induced anorexia (DIA) also increases CRH gene expression in lateral hypothalamic neurons, and as noted previously, CRH induces TRH release in cell culture.[178]

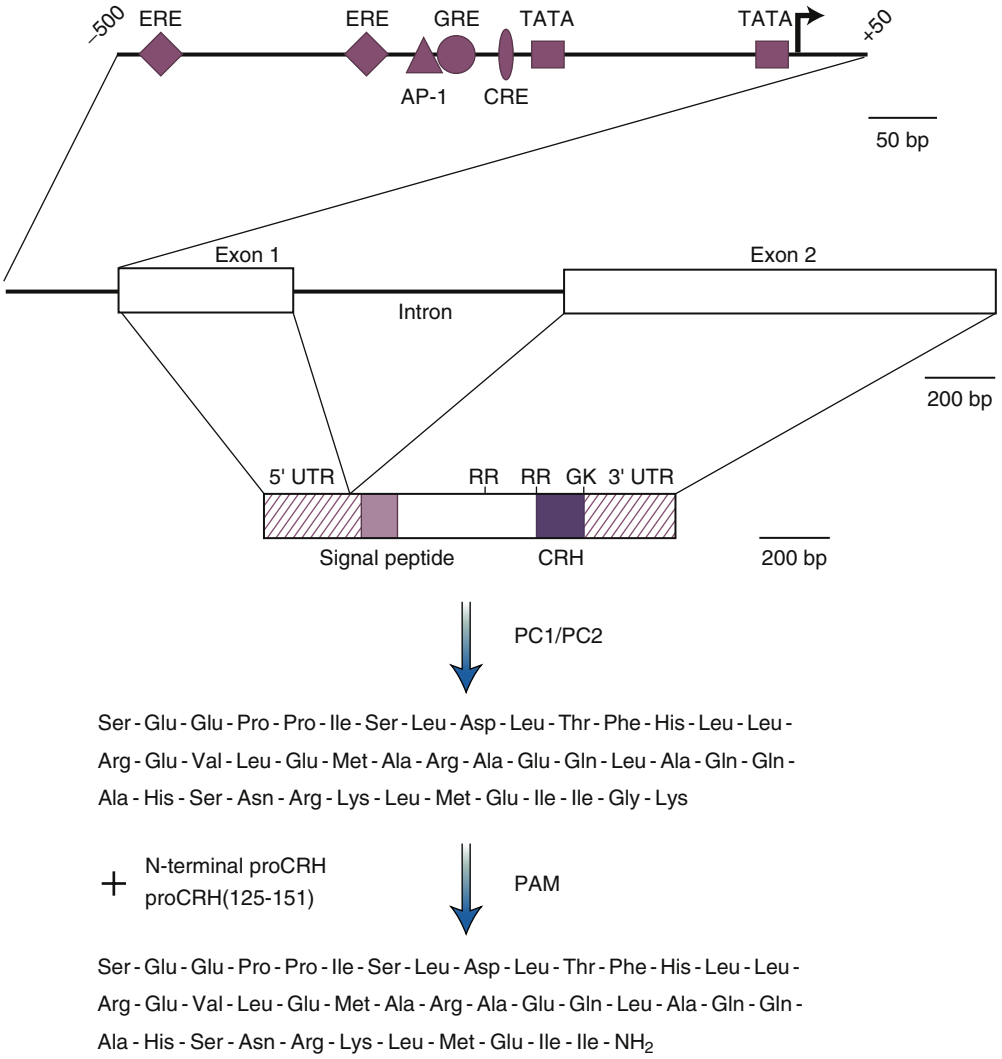
Infection and Inflammation

The molecular basis of infection-induced or inflammation-induced TSH suppression is partially established. TSH secretion is inhibited by sterile abscesses; by the injection of interleukin 1β (IL-1β), an endogenous pyrogen and secretory peptide of activated lymphocytes[179]; or by tumor necrosis factor-α (TNFα). IL-1β stimulates the secretion of somatostatin.[180] TNFα inhibits TSH secretion directly and induces functional changes in the rat characteristic of the sick euthyroid state.[181] It is likely that the TSH inhibition in animal models of the sick euthyroid syndrome is due to cytokine-induced changes in hypothalamic and pituitary function.[182] IL-6, IL-1, and TNFα contribute to the suppression of TSH in the nonthyroidal illness syndrome.[183] Other evidence suggests that bacterial lipopolysaccharide can directly stimulate tanycytes via their expression of toll-like receptor 4. The stimulated tanycytes express higher levels of type 2 deiodinase, perhaps directly induced by activation of nuclear factor-κB (NFκB), that in turn increases the levels of T_3 relative to T_4, causing feedback inhibition on TRH neurons by a mechanism described earlier.[133] Endotoxin also increases TSH production in the pars tuberalis, which, as described previously (see "Physiologic Roles of Melatonin"), can increase tanycyte D_2 expression.

Corticotropin-Releasing Hormone

Chemistry and Evolution

The HPA axis is the humoral component of an integrated neural and endocrine system that has an important role in the regulation of energy homeostasis through effects on hepatic glycogenolysis



• **Fig. 7.12** Structure of the human corticotropin-releasing hormone (CRH) gene, complementary DNA, and peptide. The sequence encoding CRH occurs at the carboxy-terminus of the prohormone. Dibasic amino acid cleavage sites (RR) and the penultimate Gly and terminal Lys (GK) are shown. *AP-1,* activator protein-1 binding sequence; *CRE,* cyclic adenosine monophosphate response element; *ERE,* estrogen response element; *GRE,* glucocorticoid response element; *PAM,* peptidylglycine α-amidating monooxygenase; *PC1/PC2,* prohormone convertases 1 and 2; *TATA,* Goldstein-Hogness box involved in binding RNA polymerase; *UTR,* untranslated. (Redrawn from data of Shibahara S, Morimoto Y, Furutani Y, et al. Isolation and sequence analysis of the human corticotropin-releasing factor precursor gene. *EMBO J.* 1983;2:775–779.)

and lipolysis and functions in response to acute and chronic internal and external challenges to homeostasis (stressors). The system is comprised of neuronal pathways linked to the hypothalamic-pituitary control of ACTH release and hence the release of glucocorticoids from the adrenal cortex and can coordinate with activation of the autonomic nervous system to induce the release of catecholamines from the adrenal medulla (fight-or-flight response). Pituitary ACTH secretion is stimulated primarily by CRH, derived from neurons located in the parvicellular division of the PVH, and to a lesser extent by AVP, originating from magnicellular neurons in the SON and PVN but also cosecreted from hypophysiotropic CRH neurons (see Figs. 7.3 and 7.4). In a broader context, the CRH system in the CNS is also vitally important for the behavioral responses to stress. This complex system also includes nonhypophysiotropic CRH neurons, three additional CRH-like peptides, urocortin 1 to 3, at least two cognate GPCRs, CRH receptors (including CRH-R1 and CRH-R2), and a high-affinity CRH-binding protein, each with distinct and complex distributions in the CNS.

The Schally and Guillemin laboratories demonstrated in 1955 that extracts from the hypothalamus stimulated ACTH release from the pituitary. The primary active principle, CRH, was purified and characterized from sheep in 1981. Human CRH is an amidated 41–amino acid peptide that is cleaved from the COOH-terminus of a 196–amino acid pre-prohormone precursor by PC1 and PC2 (Fig. 7.12).[184] CRH is highly conserved phylogenetically; the human peptide is identical in sequence to the mouse and rat peptides but differs at seven residues from the ovine sequence. Mammalian CRH, the three urocortin peptides, fish urotensin, anuran sauvagine, and the insect diuretic peptides are

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Human CRH                    SEEPPISLDLTFHLLREVLEMARAEQLAQQAHSNRKLMEII
Human urocortin               DNPSLSIDLTFHLLRTLLELARTQSQRERAEQNRIIFDSV
Human urocortin II(SRP) HPGSRIVLSLDVPIGLLQILLEQARARAAREQATTNARILARVGHC
Human urocortin III(SCP)    TKFTLSLDVPTNIMNLLFNIAKAKNLRAQAAANAHLMAQIGRRK
Frog  sauvagine               QGPPISIDLSLELLRKMIEIEKQEKEKQQAANNRLLLDTI
Carp  urotensin-I           NDDPPISIDLTFHLLRNMIEMARNENQREQAGLNRKYLDEV
```

• **Fig. 7.13** Sequence comparison of members of the corticotropin-releasing hormone (CRH) peptide family. Identical or highly conserved amino acids are indicated in *boldface letters*. *SCP,* stresscopin; *SRP,* stresscopin-related peptide.

members of an ancient family of peptides that evolved from an ancestral precursor early in the evolution of metazoans, ~500 million years ago.[185] Comparison of peptide sequences in vertebrates suggests a grouping of the peptides into two subfamilies, CRH-urotensin-urocortin 1-sauvagine and urocortin 2-urocortin 3 (Fig. 7.13).[186] Urocortin 1 and sauvagine appear to represent tetrapod orthologues of fish urotensin. Sauvagine, isolated originally from *Phyllomedusa sauvagii*, is an osmoregulatory peptide produced in the skin of certain frogs; urotensin is an osmoregulatory peptide produced in the caudal neurosecretory system of fish. Whereas isolation of CRH required 250,000 ovine hypothalami, the virtual cloning of urocortin 2 and 3 was accomplished by computer search of the human genome database.[186]

The CRH peptides signal by binding to CRH-R1[187] and CRH-R2,[188] receptors that couple to the stimulatory G protein (G_s) and adenylyl cyclase. Two splice variants of the latter receptor that differ in the extracellular NH_2-terminal domain, CRH-R2α and CRH-R2β, have been found in both rodents and humans,[189] and a third NH_2-terminal splice variant, CRH-R2γ, has been reported in humans.[190] In mammals, CHR-R1 is the predominant receptor expressed in the brain and anterior pituitary, thereby being the primary receptor for CRH-mediated ACTH release,[191] whereas CRH-R2 has a more limited and selective distribution in the brain.[192] CRH, urotensin, urocortin 1, and sauvagine are potent agonists of CRH-R1; urocortin 1 is also a potent agonist of CRH-R2; urocortins 2 and 3 are specific agonists of CRH-R2.[193] Thus CRH activation of the HPA axis is mediated exclusively through CRH-R1, which is expressed in anterior pituitary corticotrophs.

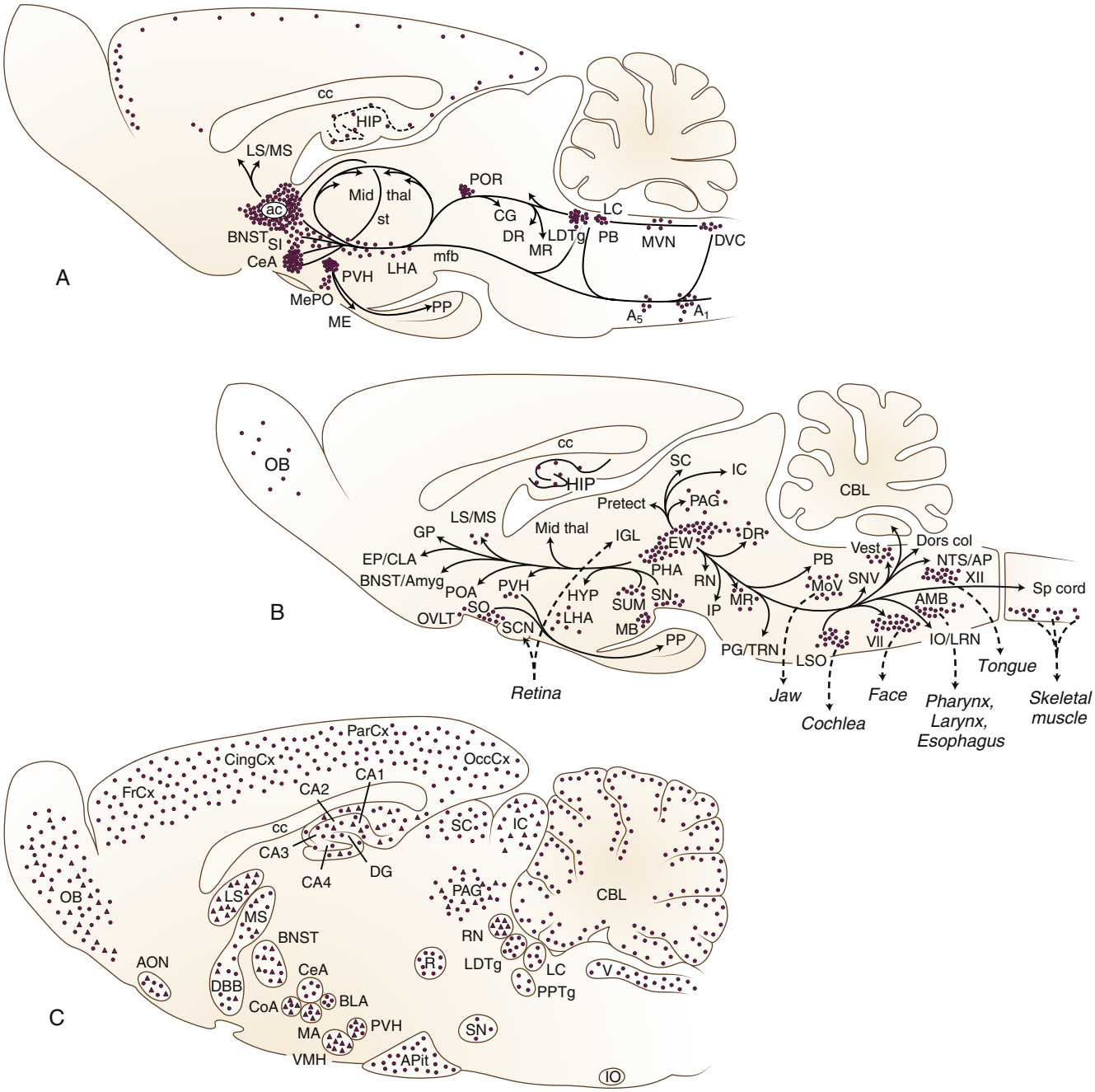
While CRH neurons projecting to the median eminence are found mostly in the PVH (Fig. 7.14A), some CRH fibers originating from the PVH also project to the brainstem. In addition, nonhypophysiotropic CRH neurons are abundant elsewhere but primarily in limbic structures involved in processing sensory information and in regulating the autonomic nervous system. Sites include the prefrontal, insular, and cingulate cortices; amygdala; substantia nigra; periaqueductal gray; locus coeruleus; NTS; and parabrachial nucleus. In the periphery, CRH is found in human placenta, lymphocytes, autonomic nerves, heart, lung, skin, kidney, testes, ovaries, and the gastrointestinal tract. Urocortin 1 is expressed at the highest levels in the nonpreganglionic Edinger-Westphal nucleus, the lateral superior olive, and the SON of the rodent brain, with additional sites such as the substantia nigra, ventral tegmental area, and dorsal raphe (see Fig. 7.14B). In the human, urocortin 1 is widely distributed, with highest levels in the frontal cortex, temporal cortex, and hypothalamus,[194] and has also been reported in the nonpreganglionic Edinger-Westphal nucleus.[22] In the periphery, urocortin 1 is seen in placenta, mucosal inflammatory cells of the gastrointestinal tract, lymphocytes, and cardiomyocytes. Urocortin 2 is expressed in hypothalamic neuroendocrine and stress-related cell groups in the mouse, including the locus coeruleus, whereas urocortin 3 is expressed in the hypothalamus and amygdala and particularly in pancreatic islet beta cells.[195,196]

In addition to its expression in pituitary corticotrophs, CRH-R1 is found in the neocortex and cerebellar cortex, subcortical limbic structures, and amygdala, with little to no expression in the hypothalamus (see Fig. 7.14C). CRH-R1 is also found in a variety of peripheral sites in humans, including ovary, endometrium, and skin. CRH-R2α is found mainly in the brain in rodents, with high levels of expression seen in the ventromedial hypothalamic nucleus and lateral septum (see Fig. 7.14C)[197]; CRH-R2β is found centrally in cerebral arterioles and peripherally in the gastrointestinal tract, heart, and muscle.[188,198] In humans, CRH-R2α is expressed in the brain and periphery, whereas the β and γ subtypes are primarily central.[189,190] Little CRH-R2 message is seen in the pituitary. CRH-R1 and CRH-R2 are expressed in the rodent adrenal cortex, suggesting a potential intra-adrenal CRH-ACTH system involved in the fine-tuning of adrenocortical glucocorticoid release.

Regulation of signaling at CRH-R1 and CRH-R2 in both the brain and periphery also occurs by a 37-kDa high-affinity CRH-binding protein (CRH-BP), which also binds urocortin 1 with equal or greater affinity than to the CRH receptors.[199,200] This factor was initially postulated from the observation that CRH levels rise dramatically during the second and third trimesters of pregnancy without activating the pituitary-adrenal axis. Among hypophysiotropic factors, CRH is the only one for which a specific binding protein (in addition to the receptor) exists in tissue or blood.

The functional significance of the CRH-binding protein is not fully understood. The CRH-binding protein does not bind to the CRH receptor but does inhibit CRH action. For this reason, the CRH-binding protein probably acts to modulate CRH actions at the cellular level. Corticotroph cells in the anterior pituitary have membrane CRH receptors and intracellular CRH-binding protein.[201] Conceivably, the binding protein acts to sequester or terminate the action of membrane-bound CRH and has been shown to attenuate ACTH-releasing activity of CRH in anterior pituitary cells.[202] The CRH-binding protein is also present in regions of the CNS that synthesize CRH or receive innervation from CRH-containing neurons such as the bed nucleus of the stria terminalis and central nucleus of the amygdala.[203] Most importantly, however, CRH-BP may have a regulatory role within the CNS to modulate the stress response (reviewed by Ketchesin and colleagues[200]). For example, CRH-BP increases in the amygdala following restraint stress, predator stress, and food deprivation,[204–206] suggesting that it may function to reduce the anxiety-promoting (anxiogenic) effects of CRH by decreasing CRH-R1 activation. Indeed, transgenic mice with gene deletion of CRH-BP demonstrate increased anxiety-like behavior when subjected to the elevated plus maze test.[207]

Structure-activity relationship studies have demonstrated that COOH-terminal amidation and an α-helical secondary structure



• **Fig. 7.14** Distribution of messenger RNA sequences for (A) corticotropin-releasing hormone (CRH), (B) urocortin, and (C) the CRH receptor 1 (CRH-R1, *circles*) and CRH-R2, *triangles*) in the rat brain. A_1, noradrenergic cell group 1; A_5, noradrenergic cell group 5; *ac,* anterior commissure; *AMB,* nucleus ambiguus; *APit,* anterior pituitary; *AP,* area postrema; *BLA,* basolateral amygdala; *BNST,* bed nucleus of the stria terminalis; *CBL,* cerebellum; *cc,* corpus callosum; *CeA,* central nucleus amygdala; *CG,* central gray; *DG,* dentate gyrus; *DR,* dorsal raphe; *DVC,* dorsal vagal complex; *EW,* Edinger-Westphal nucleus, noncholinergic; *HIP,* hippocampus; *IC,* inferior colliculus; *LC,* locus coeruleus; *LDTg,* laterodorsal tegmental nucleus; *LHA,* lateral hypothalamic area; *LS,* lateral septum; *MA,* medial amygdala; *ME,* median eminence; *mfb,* medial forebrain bundle; *Mid Thal,* midline thalamic nuclei; *MS,* medial septum; *MePO,* medial preoptic area; *MR,* medial raphe; *MVN,* medial vestibular nucleus; *OB,* olfactory bulb; *PAG,* periaqueductal gray; *PB,* parabrachial nucleus; *POR,* perioculomotor nucleus; *PP,* posterior pituitary; *PPTg,* pedunculopontine tegmental nucleus; *PVH,* paraventricular nucleus hypothalamus; *R,* raphe; *RN,* red nucleus; *SC,* superior colliculus; *SI,* substantia innominata; *st,* stria terminalis; *V/Vest,* vestibular nuclei; *VMH,* ventral medial nucleus hypothalamus. (From Swanson LW, Sawchenko PE, Rivier J, et al. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology.* 1983;36:165–186; Bittencourt JC, Vaughan J, Arias C, et al. Urocortin expression in rat brain: evidence against a pervasive relationship of urocortin-containing projections with targets bearing type 2 CRF receptors. *J Comp Neurol.* 1999;415:285–312, Fig. 17; and Steckler T, Holsboer F. Corticotropin-releasing hormone receptor subtypes and emotion. *Biol Psychol.* 1999;46:1480–1508, Fig. 1.)

are both important for biologic activity of CRH. The first CRH antagonist described was termed α-helical CRH(9-41).[208] A second, more potent antagonist, termed *astressin*, has the structure cyclo(30-33)(D-Phe^{12}, Nle^{21,38}, Glu^{30}, Lys^{33})hCRH(12-41).[209] Both peptides are somewhat nonspecific, antagonizing both CRH-R1 and CRH-R2. Because of the anxiogenic activity of CRH and urocortin 1, several pharmaceutic companies have developed small molecule CRH antagonists; many of these have been the subject of clinical trials for anxiety and depression (see later discussion). Thus far, this structurally diverse group of small molecule compounds, such as antalarmin, R121919, CP-154,526, NBI27914, NBI77860, BMS562086, and a novel series of thiazolol[4,5-*d*] pyrimidines-2-imine and 2-hydrazones, are potent antagonists of CRH-R1, with little activity at CRH-R2. The efficacy of these compounds across the entire behavioral, neuroendocrine, and autonomic repertoire of response to stress has been demonstrated in a number of laboratory animal studies. For example, oral administration of antalarmin in a social stress model in the primate (introduction of strange males) reduced behavioral measures of anxiety such as lack of exploratory behavior, decreased plasma ACTH and cortisol, and reduced plasma epinephrine and norepinephrine.[210] Activation of CRH-R2 by urocortin 2 or 3 in mice has also been shown to reduce anxiety and depression associated with the acute withdrawal of chronic nicotine treatment.[211,212] Thus selective CRH-2 agonists may also find clinical benefit.

Effects on the Pituitary and Mechanism of Action

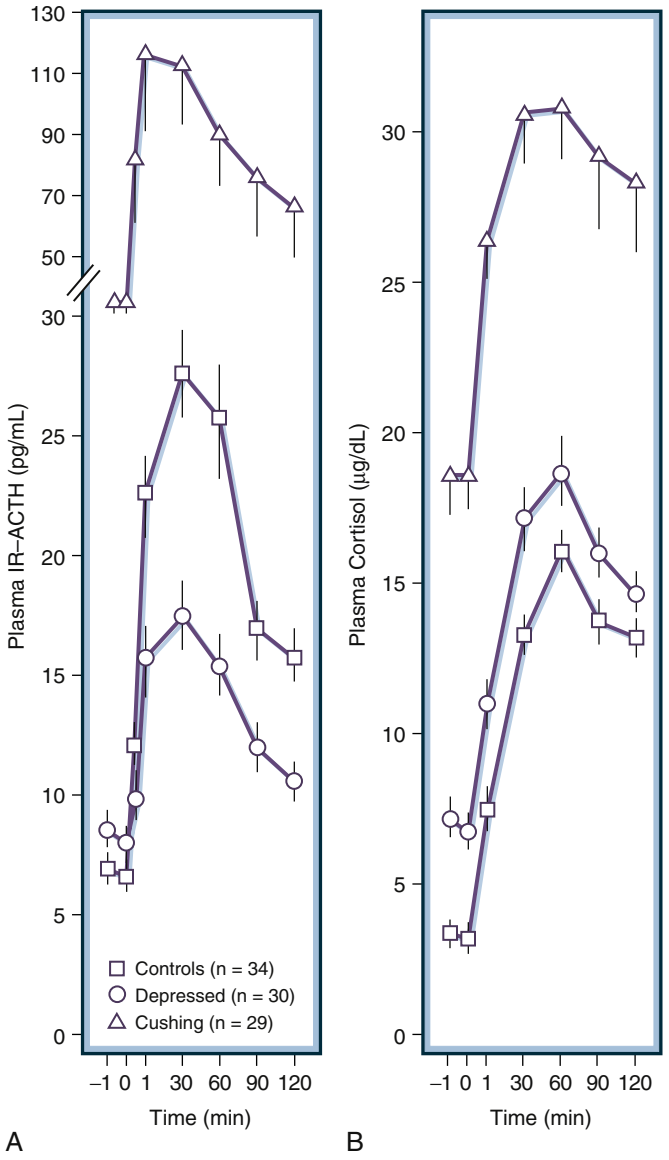
Administration of CRH to humans causes prompt release of ACTH into the blood, followed by secretion of cortisol (Fig. 7.15) and other adrenal steroids, including aldosterone. Most studies have used ovine CRH, which is more potent and longer acting than human CRH, but human and porcine CRHs appear to have equal diagnostic value. The effect of CRH is specific to ACTH release and is inhibited by glucocorticoids.

As mentioned, CRH acts on the pituitary corticotroph to regulate both basal-induced and stress-induced ACTH release, primarily by binding to CRH-R1 and activating adenylyl cyclase. The concentration of cAMP in the tissue is increased in parallel with the biologic effects and is reduced by glucocorticoids. The rate of transcription of the mRNA that encodes the ACTH prohormone POMC is also enhanced by CRH.

Extrapituitary Functions

CRH and the urocortin peptides have a wide range of biologic activities in addition to the hypophysiotropic role of CRH in regulating ACTH synthesis and release. Centrally, these peptides have behavioral activities in anxiety, mood, arousal, locomotion, reward, and feeding[213,214] and increase sympathetic activation independent of any effects on the HPA axis.[215] Many of the nonhypophysiotropic behavioral and autonomic functions of these peptides, however, can be viewed as complementary to activation of the HPA axis in the maintenance of homeostasis under exposure to stress. In the periphery, activities have been reported in immunity, cardiac function, gastrointestinal function, and reproduction.[216]

Hyperactivity of the HPA axis is a common neuroendocrine finding in affective disorders (see Fig. 7.15),[213,217] and normalization of HPA regulation is highly predictive of successful treatment. Defective dexamethasone suppression of CRH release, implying defective corticosteroid receptor signaling, is seen not only in depressed patients but also in healthy subjects with a family history of depression.[218] Depressed patients also show elevated levels of CRH in the CSF.[219] Extensive behavioral testing in a variety of mutant mouse models with genetically altered expression of either the CRH ligands or receptors generally supports the hypothesis that activation of central CRH pathways is a critical neurobiologic substrate of anxiety and depressive states.[214,220] Signaling through CRH-R1 also participates in addictive behavior, particularly in the central nucleus of the amygdala and bed nucleus of the stria terminalis. Acute withdrawal from drugs of abuse or withdrawal from ethanol or nicotine results in increases in CRH in these regions.[221,222] In addition, injections of CRH receptor antagonists into the central nucleus of the amygdala reduce self-administration of addictive substances (reviewed by Roberto and colleagues[223]). Activation of dopaminergic neurons in the ventral tegmental area by CRH may also be involved in mediating addictive behaviors by signaling through both CRH-R1 and CRH-R2.



• **Fig. 7.15** Comparison of plasma immunoreactive adrenocorticotropic hormone (IR-ACTH) (A) and plasma cortisol (B) responses to ovine corticotropin-releasing hormone in control subjects, patients with depression, and patients with Cushing disease. (From Gold PW, Loriaux DL, Roy A, et al. Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease: pathophysiologic and diagnostic implications. *N Engl J Med.* 1986;314:1329–1335.)

Central administration of CRH or urocortin activates the neuronal cell groups involved in cardiovascular control and increases blood pressure, heart rate, and cardiac output.[224] However, urocortin is expressed in cardiac myocytes, and intravenous administration of CRH or urocortin decreases blood pressure and increases heart rate in most species, including humans.[224] This hypotensive effect is probably mediated peripherally because ganglionic blockade did not disrupt the hypotensive effects of intravenous urocortin. Furthermore, high levels of CRH-R2β have been seen in the cardiac atria and ventricles,[188,198] and knockout of the *Crhr2* gene in the mouse eliminated the hypotensive effects of intravenous urocortin administration.[225]

Cytokines have an important role in extinguishing inflammatory responses through activation of CRH and AVP neurons in the PVH and subsequent elevation of anti-inflammatory glucocorticoids. Interestingly, CRH is generally proinflammatory in the periphery, where it is found in sympathetic efferents, in sensory afferent nerves, in leukocytes, and in macrophages in some species.[216,226] CRH also functions as a paracrine factor in the endometrium, where it may play a role in decidualization and implantation and act as a uterine vasodilator.[216]

The relative contributions of each of the CRH-urocortin peptides and receptors to the different biologic functions reported have been the topic of considerable analysis, given the receptor-specific antagonists already described as well as the CRH, CRH-R1, and CRH-R2 knockout mice available for study (reviewed by Bale and Vale[220] and Keck and colleagues[214]). Examination of three potent stressors—restraint, ether, and fasting—demonstrated that other ACTH secretagogues, such as AVP, oxytocin, and catecholamines, could not replace CRH in its role in mounting the stress response. In contrast, augmentation of glucocorticoid secretion by a stressor after prolonged stress was not defective in CRH knockout mice, implicating CRH-independent mechanisms.

CRH and urocortin peptides also have potent anorexigenic activity, implicating the CRH system in stress-induced inhibition of feeding. However, studies utilizing CRH, CRH-R1, and CRH-R2 knockout mice have not fully unraveled the complex interactions of these peptides and receptor signaling pathways in the acute effects of stress on feeding behavior.

Additional gene knockout studies have suggested that urocortin 2 plays a physiologic role in female mice to dampen basal daily rhythms of the HPA axis and reduce behavioral coping mechanisms in response to chronic stress.[195] Urocortin 3 may have a primary action to augment insulin secretion in response to the metabolic stress of excessive calorie intake.[196]

Clinical Applications

No approved therapeutic application of CRH or CRH-like peptides exists, although the peptide has been demonstrated to have a number of activities in human and primate studies. For example, intravenous administration of CRH was found to stimulate energy expenditure but is an unlikely pharmaceutic target for inducing weight loss. The development of small molecule, orally available, CRH-R1 antagonists such as R121919 produced considerable interest in their potential for treatment of anxiety and depression[219,227] and even reached a Phase IIa trial.[228] However, while still of major interest in the treatment of psychiatric conditions, subsequent trials have proved disappointing, perhaps indicating that the neurobiology of signaling through the CRH-R1 is more complex than currently appreciated.[229] Recent studies, however, have suggested promising results for the use of the CRH-R1 antagonist pexacerfont (BMS562086) to reduce craving and stress-induced eating, and verucerfont (NBI77860) in the treatment of hyperandrogenism associated with congenital adrenal hyperplasia.[229,230]

Feedback Control

The administration of glucocorticoids inhibits ACTH secretion; conversely, removal of the adrenals (or administration of drugs that impair secretion of glucocorticoids) leads to increased ACTH release. The set-point of pituitary feedback is determined by the hypothalamus acting through hypothalamic-releasing hormones CRH and AVP (see Chapter 8).[231–234] Glucocorticoids act on both the pituitary corticotrophs and the hypothalamic neurons that secrete CRH and AVP. These regulatory actions are analogous to the control of the pituitary-thyroid axis. However, whereas TSH becomes completely unresponsive to TRH when thyroid hormone levels are sufficiently high, severe neurogenic stress and large amounts of CRH can break through the feedback inhibition due to modulation of hypophysiotropic CRH neurons by afferent input from other regions of the brain, including brainstem catecholaminergic neurons (primarily the NTS), BNST, hippocampus, and prefrontal cortex, among others. Comprehensive reviews of regulation of the HPA axis have emphasized the complexity of this control beyond that of a simple closed-loop feedback.[235,236]

Glucocorticoids are lipid soluble and freely enter the brain through the blood-brain barrier.[233] In brain and pituitary they can bind to two receptors: Type I (encoded by *NR3C1*) is called the *mineralocorticoid receptor* because it binds aldosterone and glucocorticoids with high affinity. Type II (*NR3C2*), the glucocorticoid receptor, has low affinity for mineralocorticoids.[232–234] Classic glucocorticoid action involves binding of the steroid-receptor complex to regulator sequences in the genome. Type I receptors are saturated by basal levels of glucocorticoids, whereas type II receptors are not saturated under basal conditions but approach saturation during peak phases of the circadian rhythm and during stress. These differences and differences in regional distribution within the brain suggest that type I receptors determine basal activity of the hypothalamic-pituitary axis and that type II receptors mediate stress responses.[237]

In the pituitary, glucocorticoids inhibit secretion of ACTH by inhibiting CRH binding to pituitary cells and reducing cAMP and the synthesis of POMC mRNA; in the hypothalamus they inhibit secretion of CRH and AVP and the synthesis of their respective mRNAs, although with distinct temporal patterns.[233,234,238] The latency of the inhibitory effect is so short (<30 minutes) that it is likely that gene regulation is not the sole basis of the response.[239] However, long-term suppression (>1 hour) clearly acts through genomic mechanisms. Glucocorticoids can also exert additional rapid signaling on hypophysiotropic CRH neurons via an endocannabinoid-mediated suppression of synaptic excitation.[240] These events involve the action of glucocorticoids on a membrane-associated receptor to mobilize endocannabinoids (2-arachidonoylglycerol [2-AG] and N-arachidonoylethanolamine [AEA]) from CRH neurons that act through a cannabinoid receptor (CB1R) to inhibit the release of glutamate from neurons synapsing on hypophysiotropic CRH neurons.[241] In addition, glucocorticoid-mediated inhibition of hypophysiotropic CRH activity can occur at other loci within the brain, such as the hippocampus, prefrontal cortex, and NTS, to inhibit CRH neurons directly or through multisynaptic pathways (see "Neural Control"), perhaps of greatest importance in regulation of the HPA stress response (reviewed in Keller-Wood[235] and Herman and colleagues[236]). Lesioning or selective deletion of glucocorticoid receptors in these regions is

associated with increased activity of hypophysiotropic CRH neurons and the HPA axis[242,243] (see "Neural Control").

Neural Control

Significant physiologic or psychologic stressors evoke an adaptive response that commonly includes activation of both the HPA axis and the sympathoadrenal axis. The end products of these pathways then help to mobilize resources to cope with the physiologic demands in emergency situations, acutely through the fight-or-flight response and over the long term through systemic effects of glucocorticoids on functions such as gluconeogenesis and energy mobilization. The HPA axis also has unique stress-specific homeostatic roles, the best example being the role of glucocorticoids in downregulating immune responses after infection and other events that stimulate cytokine production by the immune system.

The PVH is the primary hypothalamic nucleus responsible for providing the integrated whole-animal response to stress.[238,244,245] This nucleus contains within it three major types of effector neurons that are spatially distinct from one another: magnicellular oxytocin and AVP neurons that project to the posterior pituitary and participate in the regulation of blood pressure, fluid homeostasis, lactation, and parturition; neurons projecting to the brainstem and spinal cord that regulate a variety of autonomic responses, including sympathoadrenal activation; and parvicellular CRH neurons that project to the median eminence and regulate ACTH synthesis and release. However, approximately 50% of CRH neurons coexpress AVP, which acts synergistically with CRH by activating the V1b receptor subtype on corticotrophs. AVP is regulated quite differently in parvicellular versus magnicellular neurons but is also regulated somewhat differently from CRH by stressors in parvicellular cells that express both peptides.[238] Different stressors result in different patterns of activation of the three major visceromotor cell groups within the PVH, as measured by the general neuronal activation marker c-Fos and nerve growth factor 1B (NGF1B) (Fig. 7.16). For example, salt loading downregulates CRH mRNA in parvicellular CRH cells and upregulates CRH in a small number of magnicellular CRH cells, but consistently activates magnicellular cells. Hemorrhage activates every division of the PVH, whereas cytokine administration primarily activates parvicellular CRH cells with some minor activation of magnicellular and autonomic divisions.

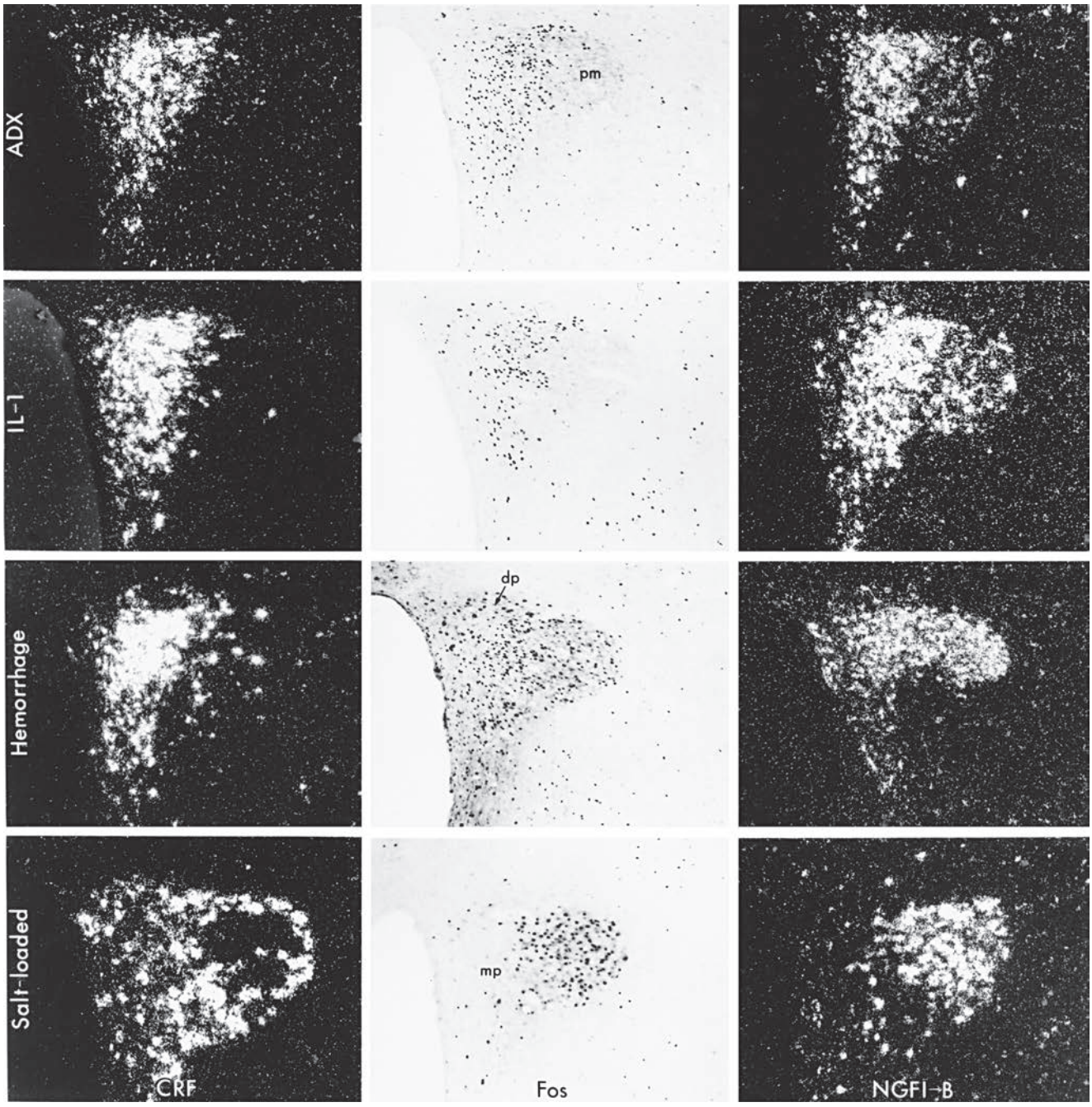
The synthesis and release of AVP, which regulates renal water absorption and vascular smooth muscle, are controlled mainly by the volume and tonicity of the blood. This information is relayed to the magnicellular AVP cell through the NTS and A1 noradrenergic cell group of the ventrolateral medulla and projections from a triad of CVOs lining the third ventricle, the SFO, the medial preoptic nucleus (MePO), and OVLT. Oxytocin is primarily involved in reproductive functions, such as parturition, lactation, and milk ejection, although it is cosecreted with AVP in response to osmotic and volume challenges, and oxytocin cells receive direct projections from the NTS as well as from the SFO, MePO, and OVLT. In contrast to the neurosecretory neurons functionally defined by the three peptides, CRH, oxytocin, and AVP, PVH neurons projecting to the brainstem and spinal cord include neurons expressing each of these peptides.

In the rodent, a wide variety of stressors has been determined to activate parvicellular CRH neurons, including cytokine injection, salt loading, hemorrhage, adrenalectomy, restraint, foot shock, hypoglycemia, fasting, and ether exposure. In contrast to the relative simplicity of inputs to magnicellular cells (Fig. 7.17A), as noted previously, parvicellular CRH neurons receive a diverse and complex assortment of inputs (Fig. 7.18; also see Fig. 7.17B). These inputs are divided into three major categories: brainstem, limbic forebrain, and hypothalamus. Because the PVH is not known to receive any direct projections from the cerebral cortex or thalamus, stressors involving emotional or cognitive processing must involve indirect relay to the PVH.

Visceral sensory input to the PVH involves primarily two pathways. The NTS, the primary recipient of sensory information from the thoracic and abdominal viscera via the vagus and glossopharyngeal nerves, sends dense catecholaminergic projections to the PVH from A2 noradrenergic and C2 adrenergic cell groups, both directly and through relays in the ventrolateral medulla. These brainstem projections also account for about half of the NPY fibers present in the PVH and contain other peptides such as glucagon-like peptide 1 and activin.[246,247] In a similar fashion, increased secretion of glucocorticoids in response to infection or inflammation is due to activation of catecholaminergic nerves in the NTS and ventrolateral medulla mediated by endotoxin and proinflammatory cytokines.[245] If the ascending catecholamine pathway to the PVN is transected, the ability of IL-1 to increase CRH mRNA in PVH neurons is reduced.[248] It is proposed that IL-1 exerts its effect on endothelial cells and/or perivascular microglia at the blood-brain interface, resulting in activation of cyclooxygenase-2, the release of prostaglandin E_2 into surrounding tissue, and ultimately activation of catecholaminergic neurons through prostaglandin receptors.[249] In addition, cytokines may act directly on vascular endothelial cells in the PVH to promote prostaglandin synthesis and activation of CRH neurons via EP3 receptors.[250] A second major input responsible for transducing signals from blood-borne substances derives from three CVOs adjacent to the third ventricle, the SFO, OVLT, and ME. Collectively, these pathways account for activation of CRH neurons by what are referred to as *systemic* or *physiologic stressors* such as systemic inflammation, blood loss, hypoxia, or disorders of fluid and electrolyte balance.[245]

By contrast, what are termed *neurogenic*, *emotional*, or *psychologic* stressors involve, in addition, nociceptive or somatosensory pathways as well as cognitive and affective brain centers. Using elevation of c-Fos as an indicator of neuronal activation, detailed studies have compared PVH-projecting neurons activated by IL-1 treatment (systemic stressor) versus foot shock (neurogenic stressor).[245] Only catecholaminergic solitary tract nucleus and ventrolateral medulla neurons were activated by moderate doses of IL-1. In contrast, foot shock activated not only neurons of the NTS and ventrolateral medulla but also cell groups in the limbic forebrain and hypothalamus. Notably, pharmacologic or mechanical disruption of the ascending catecholaminergic fibers blocked IL-1–mediated activation but not foot shock–mediated activation of the HPA axis.[244,245] Thus, while the end result to increase circulating levels of glucocorticoids is similar in all stress paradigms, depending upon the type of stress, different regions of the brain are recruited to allow activation of the hypophysiotropic CRH neurons.

Except for the catecholaminergic neurons of the NTS and ventrolateral medulla, parts of the BNST, and the dorsomedial nucleus of the hypothalamus, many inputs to the PVH, such as those deriving from the prefrontal cortex and lateral septum, are thought to act indirectly through local hypothalamic glutamatergic[251] and GABAergic neurons[252] with direct synapses to the CRH neurons. The BNST is the only limbic region with prominent direct projections to the PVH. With substantial projections

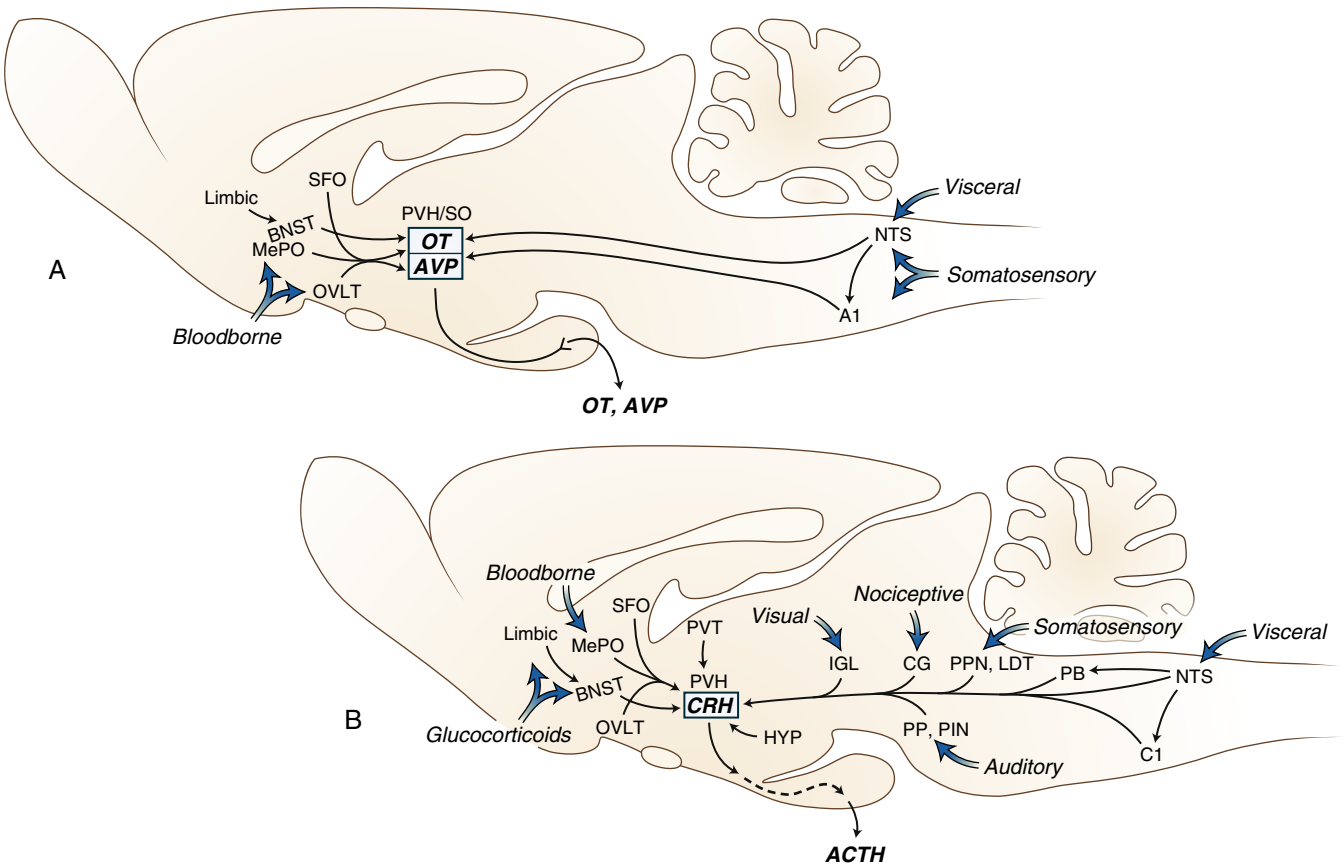


• **Fig. 7.16** Regulation of neurons of the paraventricular nucleus (PVH) by diverse stressors. *ADX,* adrenalectomy; *CRF,* corticotropin-releasing factor in situ hybridization *(dark-field)*; *dp,* dorsal parvicellular; *Fos,* c-Fos immunoreactivity *(bright-field)*; *IL-1,* interleukin 1; *mp,* medial parvicellular; *NGF1-B,* nerve growth factor 1-B in situ hybridization *(dark-field)*; *pm,* posterior magnicellular. (From Sawchenko PE, Brown ER, Chan RK, et al. The paraventricular nucleus of the hypothalamus and the functional neuroanatomy of visceromotor responses to stress. *Prog Brain Res.* 1996;107:201–222.)

from the amygdala, hippocampus, and septal nuclei, it may thus serve as a key integrative center for transmission of limbic information to the PVH.[244]

Inflammation and Cytokines

Stimulation of the immune system by foreign pathogens leads to a stereotyped set of responses orchestrated by the CNS. This constellation of stereotyped responses results from the complex interaction of the immune system and the CNS. They are mediated in large part by the hypothalamus and include coordinated autonomic, endocrine, and behavioral components with adaptive consequences to restore homeostasis. It is now clear that cytokines produced by peripheral circulating cells of the immune system and central glial cells mediate the CNS responses. Early evidence supporting this hypothesis was provided by the seminal observations that cytokines such as IL-1β can activate the HPA axis.[253–255] The resultant glucocorticoid secretion acts as a classic negative feedback to the immune system to dampen its response.



• **Fig. 7.17** (A) Neuronal inputs to magnicellular and (B) parvicellular neurons of the paraventricular nucleus (PVH). *AVP,* arginine vasopressin; *BNST,* bed nucleus of the stria terminalis; *CG,* central gray; *CRH,* corticotropin-releasing hormone; *HYP,* hypothalamus; *IGL,* intergeniculate leaflet; *LDT,* laterodorsal tegmental nucleus; *MePO,* medial preoptic nucleus; *NTS,* nucleus of the tractus solitarius; *OT,* oxytocin; *OVLT,* organum vasculosum of the lamina terminalis; *PB,* parabrachial nucleus; *PIN,* posterior intralaminar nucleus; *PP,* peripeduncular nucleus; *PPN,* pedunculopontine nucleus; *SFO,* subfornical organ; *SO,* supraoptic nucleus. (From Sawchenko PE, Brown ER, Chan RK, et al. The paraventricular nucleus of the hypothalamus and the functional neuroanatomy of visceromotor responses to stress. *Prog Brain Res.* 1996;107:201–222.)

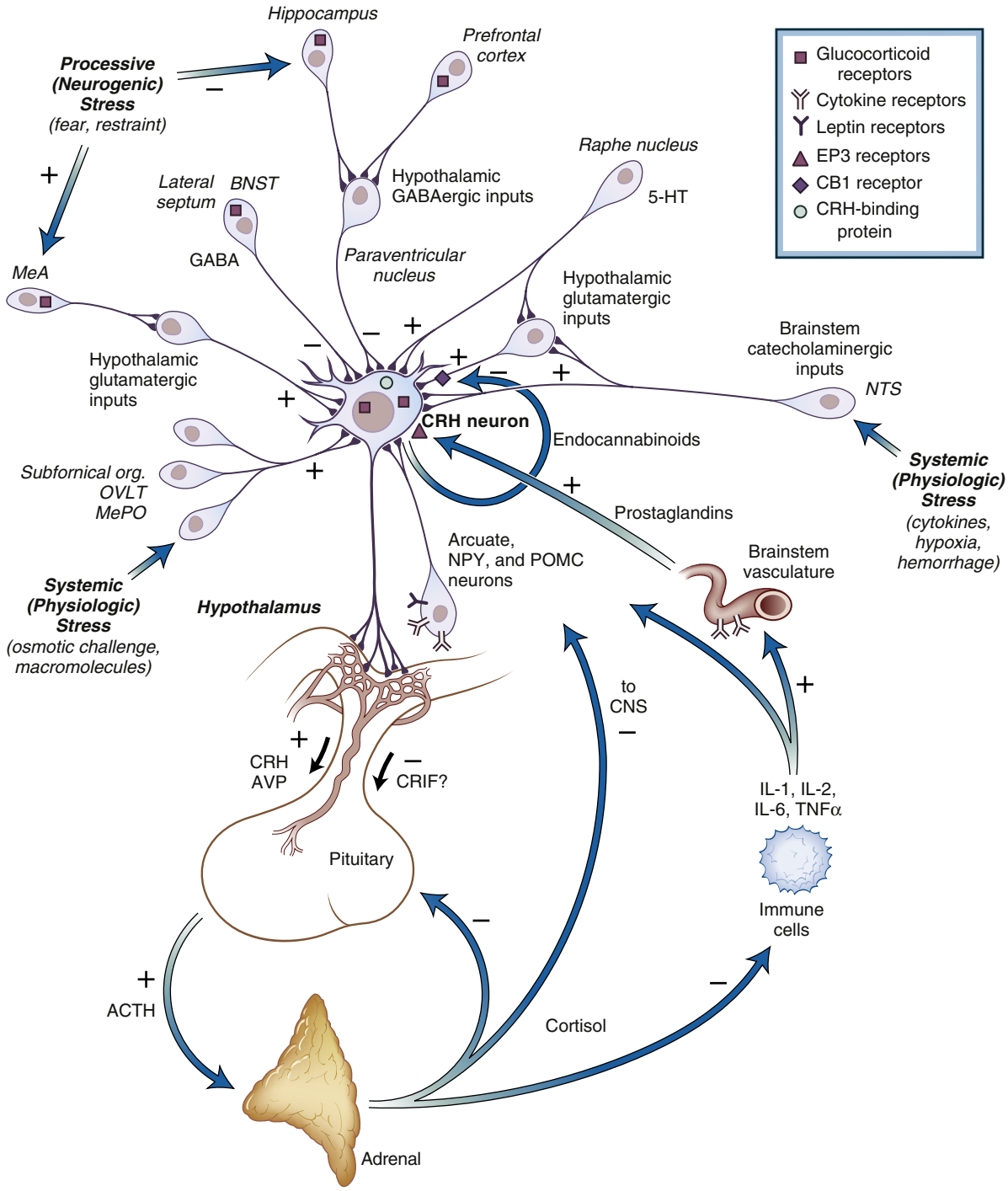
In general, glucocorticoids inhibit most limbs of the immune response, including lymphocyte proliferation, production of immunoglobulins, cytokines, and cytotoxicity. These inhibitory reactions form the basis of the anti-inflammatory actions of glucocorticoids.

Glucocorticoid feedback on immune responses is regulatory and beneficial because loss of this function makes animals with adrenal insufficiency vulnerable to inflammation. However, this feedback response can have pathophysiologic consequences, as chronic activation of the HPA axis can certainly be detrimental.[234,256] Indeed, prolonged exposure to elevated glucocorticoids is injurious to pyramidal neurons, and it is well established that chronic stress can lead to immunosuppression. The fact that products of inflammation such as IL-1β can activate the HPA axis suggests the operation of a negative feedback control loop to regulate the intensity of inflammation. The role of the hypothalamus in regulating pituitary-adrenal function is an excellent example of neuroimmunomodulation. Proposed models to explain how immune system signals might act upon the CNS to modulate homeostatic circuits by the integration of vagal input, peripheral cytokine interactions with receptors in the CVOs and cerebral blood vessels, and local production of cytokines within the CNS are explored in Chapter 39.

Other Factors Influencing Secretion of Corticotropin

Circadian Rhythms

In humans, levels of ACTH and cortisol peak in the early morning, drop during the day to reach a nadir at about midnight, and begin to rise between 1 AM and 4 AM (see Fig. 7.7). Within the circadian cycle approximately 15 to 18 pulses of ACTH can be discerned, their height varying with the time of day.[257] The set-point of feedback control by glucocorticoids also varies in a circadian pattern. Pituitary-adrenal rhythms are entrained to the light-dark cycle and can be changed over several days by exposure to an altered light schedule. It has long been assumed that the rhythm of ACTH secretion is driven by CRH rhythms, and CRH knockout mice were found to exhibit no circadian rhythm in corticosterone production. Remarkably, however, a diurnal rhythm in corticosterone was restored by a constant infusion of CRH to CRH knockout mice,[258] suggesting that CRH is necessary to permit pituitary or adrenal responsiveness to another diurnal rhythm generator.



• **Fig. 7.18** Regulation of the hypothalamic-pituitary-adrenal axis. *ACTH,* adrenocorticotropic hormone; *AVP,* arginine vasopressin; *BNST,* bed nucleus of the stria terminalis; *CNS,* central nervous system; *CRH,* corticotropin- releasing hormone; *CRIF,* corticotropin release–inhibiting factor; *GABA,* γ-aminobutyric acid; *5-HT,* 5-hydroxytryptamine; *IL-1,* interleukin 1; *MeA,* medial amygdala; *MePO,* medial preoptic nucleus; *NPY,* neuropeptide Y; *NTS,* nucleus of the tractus solitarius; *OVLT,* organum vasculosum of the lamina terminalis; *POMC,* pro-opiomelanocortin; *TNFα,* tumor necrosis factor-α.

Opiates

It has been long recognized that opiate analgesia can suppress the HPA axis, with approximately 10% of patients on chronic opiate therapy and 15% of patients receiving opiates intrathecally, developing abnormally low basal cortisol levels and flattened or reduced circadian rhythms of ACTH.[259,260] Profound adrenal insufficiency associated with hypotension and low ACTH and cortisol levels has also been reported.[261] The mechanisms for opioid-induced effects on the HPA axis are likely multifactorial but presumed to be mediated centrally through κ-receptors or δ-receptors, as there is relative insensitivity of the HPA axis to the potent μ-receptor agonist, naloxone.[262,263] Endomorphins injected directly into the hypothalamus acutely inhibit CRH gene expression,[264] but opiates may also act presynaptically on

noradrenergic terminals in the PVN to reduce CRH release.[265] Evidence that morphine inhibits the rise in ACTH to exogenous CRH further suggests an action of opiates on anterior pituitary corticotrophs.[266] It has been proposed that endogenous opiates may function to diminish the impact of the stress response, and during pregnancy, to protect the fetus from glucocorticoid excess.[267,268] Opiate-induced hyperprolactinemia may also contribute to suppression of the HPA axis given that chronic intracerebroventricular (ICV) infusion of antisense oligonucleotides against the prolactin receptor increase the stress-induced ACTH response, presumably by direct effects on CRH neurons or its noradrenergic input.[269]

Growth Hormone–Releasing Hormone

Chemistry and Evolution

Evidence for neural control of GH secretion originated from studies of its regulation in animals with lesions of the hypothalamus and from the demonstration that hypothalamic extracts stimulate the release of GH from the pituitary. When it was shown that GH is released episodically, follows a circadian rhythm, responds rapidly to stress, and is blocked by pituitary stalk section, the concept of neural control of GH secretion became a certainty. However, it was only with the discovery of the paraneoplastic syndrome of ectopic GHRH secretion by pancreatic adenomas in humans that sufficient starting material became available for peptide sequencing and subsequent cloning of a complementary deoxyribonucleic acid (cDNA).[270–272]

Two principal molecular forms of GHRH occur in the human hypothalamus: GHRH(1-44)-NH_2 and GHRH(1-40)-OH (Fig. 7.19).[273] As with other neuropeptides, the various forms of GHRH arise from post-translational modification of a larger prohormone.[270] The NH_2-terminal tyrosine of GHRH (or histidine in rodent GHRHs) is essential for bioactivity, but a COOH-terminal NH_2 group is not. A circulating type IV dipeptidyl peptidase potently inactivates GHRH to its principal and more stable metabolite, GHRH(3-44)-NH_2,[274] which accounts for most of the immunoreactive peptide detected in plasma. As in the case of GnRH, there are species differences among GHRHs; the peptides from seven species range in sequence homology with the human peptide from 93% in the pig to 67% in the rat.[273] The COOH-terminal end of GHRH exhibits the most sequence diversity among species, consistent with the exon arrangement of the gene and dispensability of these residues for GHRH receptor binding.

Despite its importance for the elucidation of GHRH structure, ectopic secretion of the peptide is a rare cause of acromegaly.[275] Fewer than 1% of acromegalic patients have elevated plasma levels of GHRH (see Chapters 8 and 9). Approximately 20% of pancreatic adenomas and 5% of carcinoid tumors contain immunoreactive GHRH, but most are clinically silent.[276]

In addition to expression in the hypothalamus, the *GHRH* gene is expressed eutopically in the human ovary, uterus, and placenta,[277] although its function in these tissues is not known. Studies in rat placenta indicate that an alternative transcriptional start site 10 kilobases upstream from the hypothalamic promoter is utilized together with an alternatively spliced exon 1a.[278]

Growth Hormone–Releasing Hormone Receptor

The GHRH receptor is a member of a subfamily of GPCRs that includes receptors for VIP, pituitary adenylyl cyclase–activating peptide, secretin, glucagon, glucagon-like peptide 1, calcitonin, parathyroid hormone or parathyroid hormone–related peptide, and gastric inhibitory polypeptide.[279] GHRH elevates intracellular cAMP by its receptor coupling to a G_s, which activates adenylyl cyclase, increases intracellular free Ca^{2+}, releases preformed GH, and stimulates GH mRNA transcription and new GH synthesis.[280] GHRH also increases pituitary phosphatidylinositol turnover. Nonsense mutations in the human GHRH receptor gene are the cause of rare familial forms of GH deficiency[281] and indicate that no other gene product can fully compensate for the specific receptor in the pituitary.

Effects on the Pituitary and Mechanism of Action

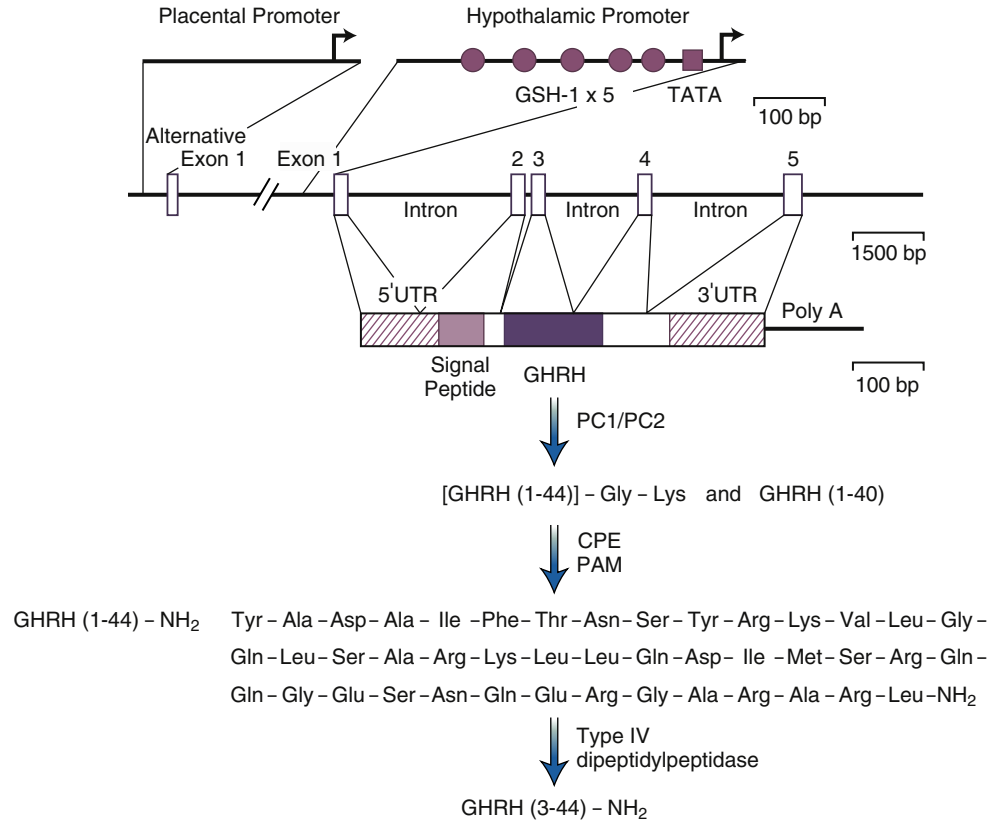
Intravenous administration of GHRH to individuals with normal pituitaries causes a prompt, dose-related increase in serum GH that peaks after 15 to 45 minutes, followed by a return to basal levels by 90 to 120 minutes (Fig. 7.20).[282] A maximally stimulating dose of GHRH is approximately 1 μg/kg, but the response differs considerably among individuals and within the same individual tested on different occasions, presumably because of endogenous cosecretagogue and somatostatin tone that exist at the time of GHRH injection. Repeated bolus administration or sustained infusions of GHRH over several hours cause a modest decrease in the subsequent GH secretory response to acute GHRH administration. However, unlike the marked desensitization of the GnRH receptor and decline in circulating gonadotropins that occur in response to continuous GnRH exposure, pulsatile GH secretion and insulin-like growth factor 1 (IGF-1) production are maintained by constant GHRH in the human.[282] This response suggests the involvement of additional factors that mediate the intrinsic diurnal rhythm of GH, and these factors are addressed in the following sections.

The pituitary effects of a single injection of GHRH are almost completely specific for GH secretion, and there is minimal evidence for any interaction between GHRH and the other classic hypophysiotropic-releasing hormones.[282] GHRH has no effect on gut peptide hormone secretion. The GH secretory response to GHRH is enhanced by estrogen administration, ghrelin, glucocorticoids, and starvation. Major factors known to blunt the response to GHRH are somatostatin, obesity, hyperinsulinemia, hyperglycemia, and advancing age. (For review see Steyn and colleagues[283].)

In addition to its role as a GH secretagogue, GHRH is a physiologically relevant growth factor for somatotrophs. Transgenic mice expressing a GHRH cDNA coupled to a suitable promoter developed diffuse somatotroph hyperplasia and eventually pituitary macroadenomas.[284] The intracellular signal transduction pathways mediating the mitogenic action of GHRH are not known with certainty but probably involve an elevation of adenylyl cyclase activity. Several lines of evidence support this conclusion, including the association of activating mutations of the $G_s\alpha$ polypeptide in many human somatotroph adenomas.[285]

Extrapituitary Functions

GHRH has few known extrapituitary functions. The most important may be its activity as a sleep regulator. The administration of nocturnal GHRH boluses to normal men significantly increases the density of slow-wave sleep and promotes nonrapid eye movement sleep,[286,287] mediated by GABAergic neurons in the preoptic nucleus and independent of GH.[286] Furthermore, there is a striking correlation between the age-related declines in slow-wave sleep and daily integrated GH secretion in healthy men.[288] These and other data suggest that central GHRH secretion is under circadian



• **Fig. 7.19** Diagram illustrating the genomic organization, messenger RNA structure, and post-translational processing of the human growth hormone–releasing hormone (GHRH) prohormone. Five GSH-1 homeodomain transcription factor–binding sites in the proximal promoter have been characterized in the rat gene. All of the amino acid residues required for bioactive GHRH peptides are encoded by exon 3. An amino-terminal exopeptidase that cleaves the Tyr-Ala dipeptide is primarily responsible for the inactivation of GHRH peptides in extracellular compartments. *CPE,* carboxypeptidase E; *PAM,* peptidylglycine α-amidating monooxygenase; *PC1/PC2,* prohormone convertases 1 and 2; *TATA,* Goldstein-Hogness box involved in binding RNA polymerase; *UTR,* untranslated region. (Compiled from data of Mayo KE, Cerelli GM, Lebo RV, et al. Gene encoding human growth hormone-releasing factor precursor: structure, sequence, and chromosomal assignment. *Proc Natl Acad Sci USA.* 1985;82:63–67; Frohman LA, Downs TR, Chomczynski P, et al. Growth hormone-releasing hormone: structure, gene expression and molecular heterogeneity. *Acta Paediatr Scand Suppl.* 1990;367:81–86; González-Crespo S, Boronat A. Expression of the rat growth hormone-releasing hormone gene in placenta is directed by an alternative promoter. *Proc Natl Acad Sci USA.* 1991;88:8749–8753; and Mutsuga N, Iwasaki Y, Morishita M, et al. Homeobox protein Gsh-1-dependent regulation of the rat GHRH gene promoter. *Mol Endocrinol.* 2001;15:2149–2156.)

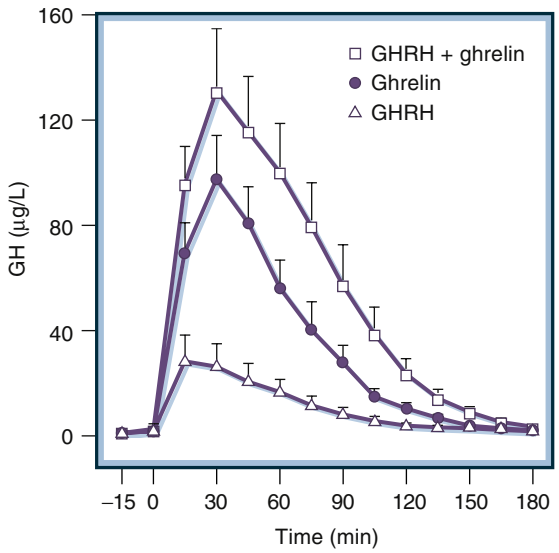
entrainment, and nocturnal elevations in GHRH pulse amplitude or frequency directly mediate sleep stage and sleep-induced increases in GH secretion.

GHRH has been reported to stimulate food intake in rats and sheep, but the effect is dependent on route of administration, time of administration, and macronutrient composition of the diet.[279] The neuropeptide's physiologic relevance to feeding in humans is unknown. Evidence suggests that nonpituitary GHRH modulates cell proliferation and promotes the healing of skin wounds.[289]

Growth Hormone–Releasing Peptides

In studies of the opioid control of GH secretion, several peptide analogues of met-enkephalin were found to be potent GH secretagogues (GHS). These include the GH-releasing peptide GHRP-6 (Fig. 7.21), hexarelin (His-D2MeTrp-Ala-Trp-DPhe-Lys-NH_2), and other more potent analogues, including cyclic peptides and modified pentapeptides.[279,290] Subsequently, a series of nonpeptidyl GHRP mimetics were synthesized with greater oral bioavailability, including the spiropiperidine MK-0677 and the shorter acting benzylpiperidine L-163,540 (see Fig. 7.21). Common to all these compounds, and the basis of their differentiation from GHRH analogues in pharmacologic activity screens, is their activation of phospholipase C and inositol 1,4,5-trisphosphate. This property was exploited in a cloning strategy that led to the identification of a novel GPCR GHS receptor that is highly selective for the GHS class of ligands.[291] The GHS-R is unrelated to the GHRH receptor and is highly expressed in the anterior pituitary gland and multiple brain areas, including GHRH neurons in the medial basal hypothalamus, suggesting both direct and indirect effects on GH secretion.[292,293]

Peptidyl and nonpeptidyl GHSs are active when administered by intranasal and oral routes, are more potent on a weight basis than GHRH itself, are more effective in vivo than in vitro, synergize with coadministered GHRH, and are almost ineffective in the absence of GHRH.[279,282] Prolonged infusions of GHRP amplify pulsatile GH secretion in normal men. GHRP administration,

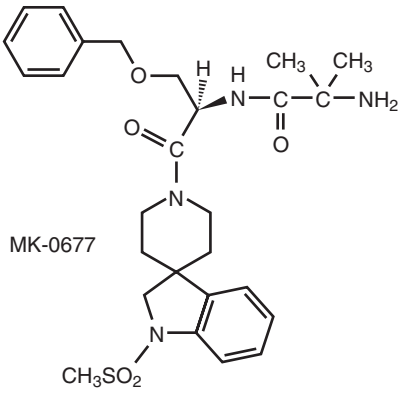


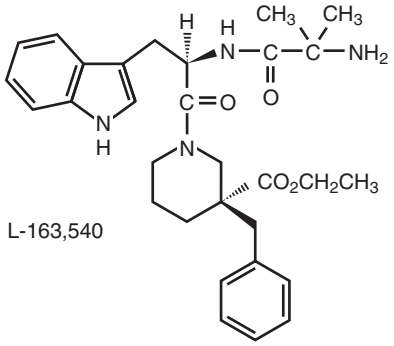
• **Fig. 7.20** Response of normal men to growth hormone–releasing hormone (GHRH)(1-29) (1 μg/kg), ghrelin (1 μg/kg), or the combination of GHRH(1-29) and ghrelin administered by intravenous injection. Note the prompt release of GH, followed by a rather prolonged fall in hormone level in response to both secretagogues. Ghrelin alone was more efficacious than GHRH(1-29), and there was an additive effect from the two peptides administered simultaneously. (From Arvat E, Macario M, Di Vito L, et al. Endocrine activities of ghrelin, a natural growth hormone secretagogue [GHS], in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. *J Clin Endocrinol Metab.* 2001;86:1169–1174.)

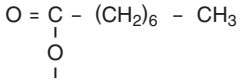
like that of GHRH, facilitates slow-wave sleep. Patients with hypothalamic disease leading to GHRH deficiency have low or no response to hexarelin; similarly, pediatric patients with complete absence of the pituitary stalk have no GH secretory response to hexarelin.[294]

The potent biologic effects of GHRPs and the identification of the GHS-R suggested the existence of a natural ligand for the receptor that is involved in the physiologic regulation of GH secretion. The 28–amino acid peptide, ghrelin, produced and secreted into the circulation from the stomach, is this ligand (Fig. 7.22).[15] The effects of ghrelin on GH secretion in humans are identical to or more potent than those of the nonnatural GHRPs (see Fig. 7.20).[295] In addition, ghrelin acutely increases circulating PRL, ACTH, cortisol, and aldosterone levels.[295] There is debate concerning the extent and localization of ghrelin expression in the brain that must be resolved before the implications of gastric-derived ghrelin in the regulation of pituitary hormone secretion are fully understood. Furthermore, post-translational processing of pro-ghrelin gives rise to a second neuropeptide, obestatin, which may also have functional roles in activity of the GH/IGF-1 axis and metabolism.[296] A proposed role for ghrelin and obestatin peptides in appetite and the regulation of food intake is discussed in Chapter 39. It is of particular interest that although GH secretory bursts are smaller in ghrelin knockout mice, they show little differences in weight and body length compared to normal controls, raising the possibility that ghrelin's main function on GH secretion is to maintain blood glucose levels during caloric restriction.[283]

GHRP-6: His–$_D$Trp–Ala–Trp–$_D$Phe–Lys–NH_2







Ghrelin: Gly – Ser – Ser – Phe – Leu – Ser – Pro – Glu – His – Gln – Arg – Val – Gln – Gln – Arg – Lys – Glu – Ser – Lys – Lys – Pro – Pro – Ala – Lys – Leu – Gln – Pro – Arg

• **Fig. 7.21** Structure of a synthetic peptidyl growth hormone (GH) secretagogue (GH-releasing peptide 6 [GHRP-6]) and nonpeptidyl growth hormone secretagogues (MK-0677 and L-163,540) and a natural ligand (ghrelin) that all bind and activate the growth hormone secretagogue (GHS) receptor. Ghrelin is an acylated 28–amino acid peptide. The *O-n*-octanoylation at Ser3 is essential for biologic activity and is a unique post-translational modification mediated enzymatically by ghrelin-*O*-acyltransferase (GOAT). (Adapted from Smith RG, Feighner S, Prendergast K, et al. A new orphan receptor involved in pulsatile growth hormone release. *Trends Endocrinol Metab.* 1999;10:128–135; Kojima M, Hosoda H, Date Y, et al. Ghrelin is a growth hormone-releasing acylated peptide from stomach. *Nature.* 1999;402:656–660.)

Clinical Applications

GHRH stimulates growth in children with intact pituitaries, but the optimal dosage, route, and frequency of administration, as well as possible usefulness by the nasal route, have not been determined. The availability of recombinant hGH (which requires less frequent injections than GHRH) and the development of the more potent GHSs with improved oral bioavailability have reduced enthusiasm for the clinical use of GHRH or its analogues. GHRH can be used to test for GH deficiency in adults, particularly when combined with arginine to improve diagnostic accuracy of the test; is not influenced by age, sex, or body mass index; and has a wider margin of safety than an insulin tolerance test.[297]

The potential clinical applications of GHSs are still being explored.[279,290] An area of intense interest is the normal, progressive decline in GH secretion with aging and its association with frailty, decreased muscle mass, central adiposity, cardiovascular morbidities, and increased fractures associated with somatopause. GH administration in healthy older individuals has been associated with increased lean body mass, increased muscle strength, and decreased fat mass, although there is a high incidence of adverse side effects, including edema, hypertension, carpal tunnel syndrome, and hyperglycemia. One must also take into consideration that animal models with growth hormone or IGF-1 deficiency tend to be associated with longevity and reduced cancer risk,[298–300] and this may also hold true for human longevity and slowing of the aging process.[301,302] Both GHRH and GHSs are also being investigated for the treatment of sleep disorders commonly associated with aging.

Neuroendocrine Regulation of Growth Hormone Secretion

GH secretion is regulated by two major hypothalamic peptides, GHRH that stimulates the release of GH and somatostatin that inhibits GH secretion, functioning in concert to give rise to the rhythmic 10 to 20 pulses of GH per 24 hours, depending upon the animal species. GHRH neurons are located in the arcuate nucleus or infundibular nucleus, whereas perikarya of the somatostatin neurons involved in GH regulation are located in the hypothalamic periventricular nucleus.[303] It is presumed that GH pulses are primarily driven by somatostatin, whereas the amplitude of the GH pulses is driven by GHRH.[304,305] However, other circulating hormones and additional modulatory peptides at the level of both the pituitary and the hypothalamus also contribute to the fine-tuning of GH secretion (see Fig. 7.22).[279,282,296,306–308] Additional background on somatostatin and its functions other than control of GH secretion are presented in "Somatostatin."

Feedback Control

Feedback control of GH secretion is regulated at both the pituitary and hypothalamus. GH release is mediated by negative feedback of GH, itself, and by IGF-1, the latter being synthesized in the liver and other tissues under the control of GH. Somatotrophs express GH receptors, and in cell culture their secretion is inhibited by the exogenous administration of GH.[309,310] IGF-1 also has a direct, major, inhibitory action on GH secretion as demonstrated by a rise in circulating GH levels when the IGF-1 receptor is selectively deleted from somatotrophs.[311] In addition, gene expression of both GH and the pituitary-specific transcription factor PIT1 is inhibited by IGF-1.[279]

GH also crosses the blood-brain barrier to feedback on the hypothalamus to regulate GH secretion, primarily by increasing somatostatin secretion into the portal capillary system. Indeed, the inhibitory effects of GH are abolished in knockout mice lacking somatostatin receptors (Fig. 7.23). As noted earlier, tuberoinfundibular somatostatin neurons are located in the hypothalamic periventricular nucleus and express GH receptors. GH receptors are also expressed by the majority of NPY neurons in the hypothalamic arcuate nucleus that show c-Fos expression in response to GH administration and are known to innervate somatostatin neurons in the periventricular nucleus.[312,313] In contrast to somatostatin neurons, however, only a small percentage of GHRH-producing neurons in the hypothalamus express GH receptors.[313] Thus, together with data from many other experiments, these results strongly support a model of GH negative feedback regulation that involves the activation of periventricular somatostatin neurons by GH using both direct and indirect (NPY) pathways. Similarly, in normal men, GH pretreatment blocks the subsequent GH secretory response to GHRH by a mechanism that is dependent on somatostatin.[314]

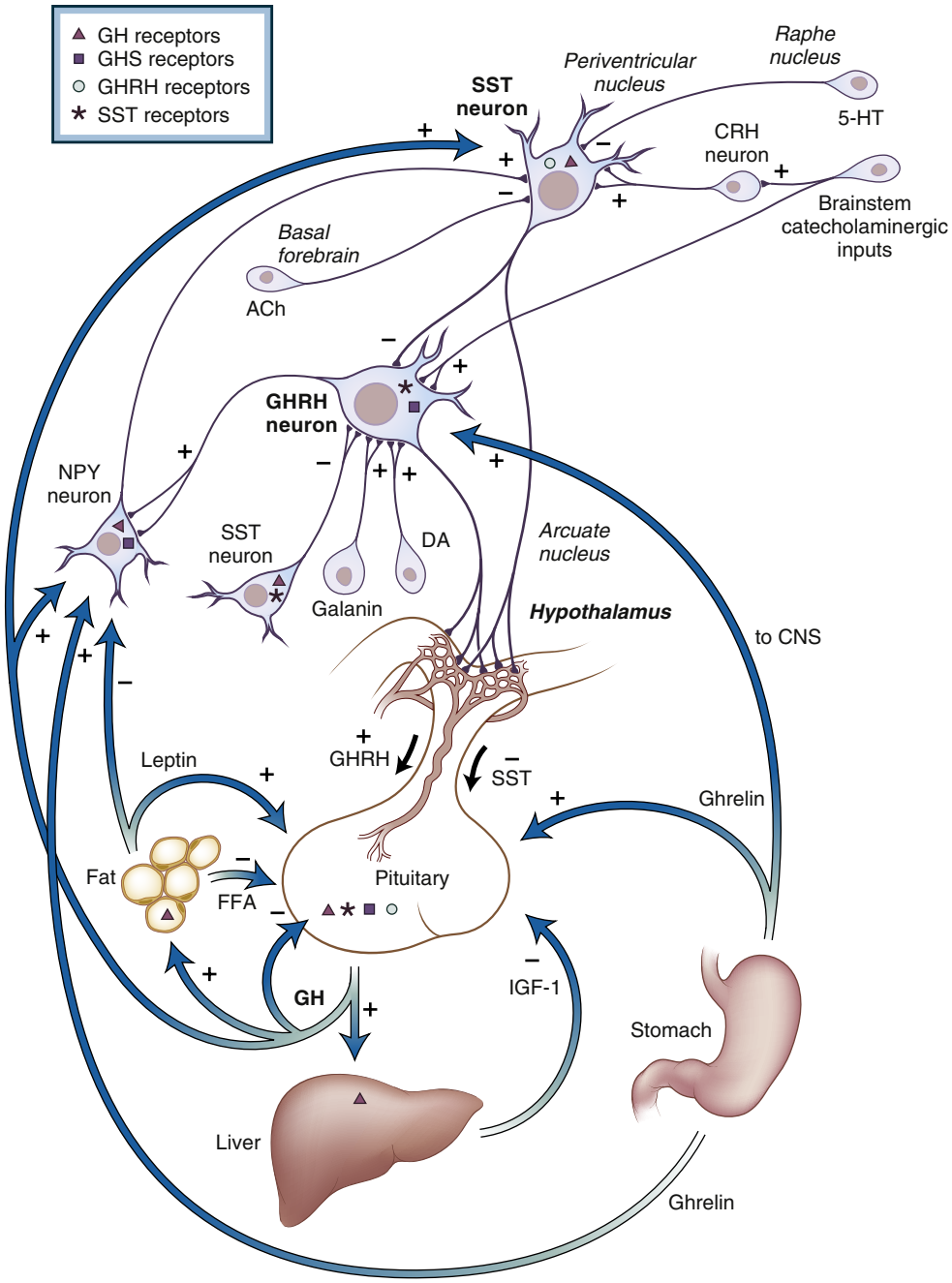
IGF-1 also has central effects to inhibit GH secretion that involves both an increase in somatostatin and a reduction in GHRH.[315] The feedback effects of IGF-1 account for the fact that serum GH levels are elevated in conditions in which circulating levels of IGF-1 are low, such as anorexia nervosa, protein-calorie starvation,[316] and Laron dwarfism (the result of a defect in the GH receptor).

GHRH and somatostatin also contribute to the pulsatile release of GH by exerting short-loop feedback effects on each other through bidirectional interactions.[317] Treatment of hypothalamic cultures with somatostatin inhibits GHRH, whereas treatment with GHRH induces somatostatin release.[318,319] In the human brain, however, there are very few GHRH contacts on somatostatin neurons, although the vast majority of GHRH neurons receive somatostatin contacts.[320] The feedback effects of GHRH on somatostatin neurons, however, may be indirect via arcuate nucleus NPY neurons (as described previously) that receive projections from GHRH neurons.[321] Evidence for extensive interactions among GHRH neurons in the human brain have also been observed and may be responsible for synchronizing their activity.[322]

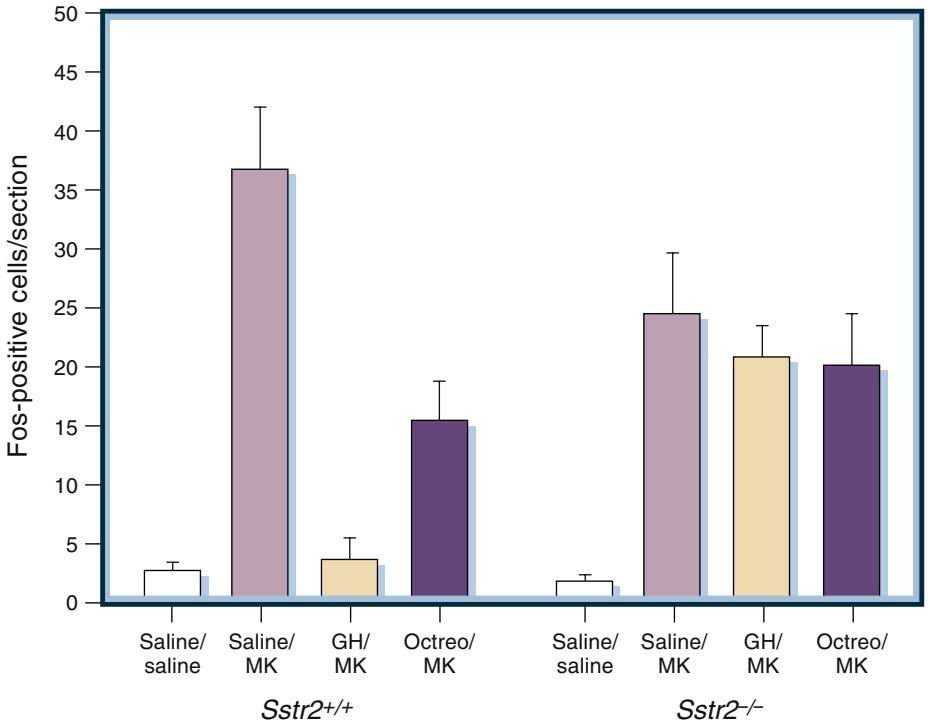
As mentioned previously, ghrelin receptors are highly expressed on somatotrophs in the anterior pituitary and in the hypothalamus, indicating both direct and indirect effects on GH secretion. When acylated with an eight-carbon fatty acid, ghrelin stimulates GHRH from hypothalamic explants, increases the firing rate of GHRH neurons, and when administered continuously, amplifies GH pulsatility.[323–325] The effect of ghrelin to increase GH secretion seems unaffected by the inhibitory effects of somatostatin, and in sheep, ghrelin also prevents the release of somatostatin into the portal capillary system, contributing to enhanced GH release.[326] Evidence for a gastro-hypophysial feedback loop has also been given as ghrelin levels fall in response to rising levels of GH.[327]

Neural Control

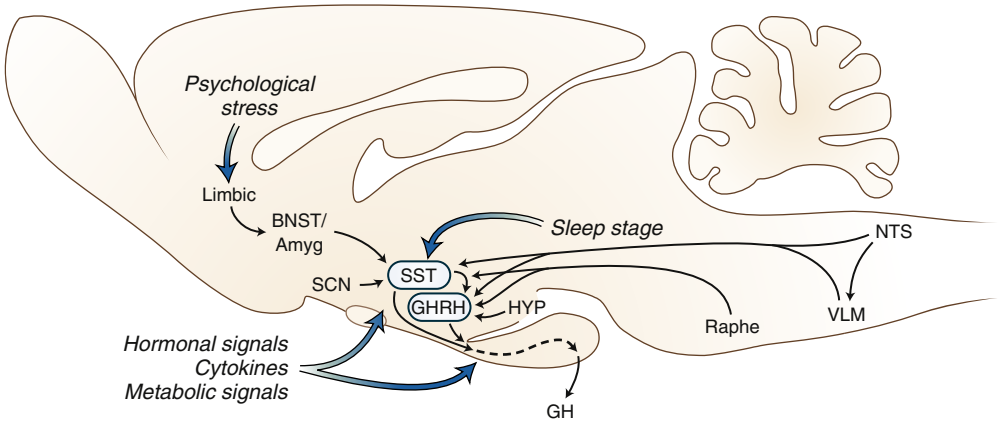
In addition to the regulatory mechanisms already described, multiple extrahypothalamic brain regions provide efferent connections to the hypothalamus and regulate GHRH and somatostatin neuronal activity (Fig. 7.24; also see Fig. 7.22). Somatosensory and affective information is integrated and filtered through the amygdaloid complex. The basolateral amygdala provides an excitatory input to the hypothalamus, and the central extended amygdala, which includes the central and medial nuclei of the amygdala together with the BNST, provides a GABAergic inhibitory input. Many intrinsic neurons of the hypothalamus also release GABA,



• **Fig. 7.22** Regulation of the hypothalamic-pituitary-growth hormone (GH) axis. GH secretion by the pituitary is stimulated by GH-releasing hormone (GHRH) and is inhibited by somatostatin (SST). Negative feedback control of GH secretion is exerted at the pituitary level by insulin-like growth factor 1 (IGF-1) and by free fatty acids (FFA). GH itself exerts a short-loop negative feedback through activation of SST neurons in the hypothalamic periventricular nucleus. These SST neurons directly synapse on arcuate GHRH neurons and project axon collaterals to the median eminence. Neuropeptide Y (NPY) neurons in the arcuate nucleus also indirectly modulate GH secretion by integrating peripheral GH, leptin, and ghrelin signals and projecting to periventricular SST neurons. Ghrelin is secreted from the stomach and is a natural ligand for the GH secretagogue (GHS) receptor that stimulates GH secretion at both the hypothalamic and pituitary levels. On the basis of indirect pharmacologic data, it appears that release of GHRH is stimulated by galanin, γ-aminobutyric acid (GABA), α_2-adrenergic, and dopaminergic inputs and inhibited by SST. Secretion of SST is inhibited by muscarinic acetylcholine (ACh) and 5-HT-1D receptor ligands, and increased by β_2-adrenergic stimuli and corticotropin-releasing hormone (CRH). *CNS,* central nervous system; *DA,* dopamine; *5-HT,* serotonin (5-hydroxytryptamine).



• **Fig. 7.23** Somatostatin and the somatostatin receptor 2 subtype are involved in the short-loop inhibitory feedback of growth hormone (GH) on arcuate neurons. Activation of neurons in the arcuate nucleus was determined by the quantification of immunoreactive Fos-positive cells after administration of the growth hormone secretagogue MK-0677 (MK). Preliminary treatment of wild-type mice *(Sstr2$^{+/+}$)* with either GH or the somatostatin analogue octreotide *(Octreo)* significantly attenuated the neuronal activation by MK-0677. In contrast, GH and octreotide had no effect on MK-0677 neuronal activation in somatostatin receptor 2–deficient mice *(Sstr2$^{-/-}$)*. (Adapted from Zheng H, Bailey A, Jian M-H, et al. Somatostatin receptor subtype 2 knockout mice are refractory to growth hormone-negative feedback on arcuate neurons. *Mol Endocrinol.* 1997;11:1709–1717.)



• **Fig. 7.24** Neural pathways involved in growth hormone (GH) regulation. This diagram illustrates the varied pathways by which impulses from the limbic system and brainstem ultimately impinge on the hypothalamic periventricular and arcuate nuclei to regulate GH release by opposing effects of somatostatin (SST) and growth hormone–releasing hormone (GHRH). Psychologic stress modulates hypothalamic function indirectly through the bed nucleus of the stria terminalis (BNST) and amygdalar complex (Amyg). Circadian rhythms are entrained in part by projections from the suprachiasmatic nucleus (SCN). Cortex and subcortical nuclei are involved in complex reciprocal interactions between sleep stage and GHRH release, but the detailed mechanisms are not known. Dopaminergic and histaminergic afferents originate from neurons located in the arcuate and mammillary nuclei, respectively, of the hypothalamus (HYP). Ascending catecholaminergic projections arise in both the nucleus of the tractus solitarius (NTS) and ventral lateral medulla (VLM). Serotoninergic (5-HT) afferents are from the raphe nuclei. In addition to these neural pathways, a variety of peripheral hormonal and metabolic signals and cytokines influence GH secretion by actions within the medial basal hypothalamus and pituitary gland.

often with a peptide cotransmitter. Excitatory cholinergic fibers arise to a small extent from forebrain projection nuclei but mostly from hypothalamic cholinergic interneurons, which densely innervate the external zone of the median eminence. Similarly, the origin of dopaminergic and histaminergic neurons is local with their cell bodies located in the hypothalamic arcuate and tuberomammillary bodies, respectively. Two important ascending pathways to the medial basal hypothalamus regulate GH secretion and originate from serotonergic neurons in the raphe nuclei and adrenergic neurons in the NTS and ventral lateral nucleus of the medulla.

Both GHRH and somatostatin neurons express presynaptic and postsynaptic receptors for multiple neurotransmitters and peptides (Table 7.4). The α_2-adrenoreceptor agonist clonidine reliably stimulates GH release; for this reason, a clonidine test was a standard diagnostic tool in pediatric endocrinology. The stimulatory effect is blocked by the specific α_2-antagonist yohimbine and appears to involve a dual mechanism of action: inhibition of somatostatin neurons and activation of GHRH neurons. In addition, partial attenuation of the effects of clonidine by mixed serotonin 5-HT1 and 5-HT2 antagonists suggests that some of the relevant α_2-receptors are located presynaptically on serotonergic nerve terminals and increase serotonin release. Both norepinephrine and epinephrine play physiologic roles in the adrenergic stimulation of GH secretion. The α_1-agonists have no effect on GH secretion in humans, but β_2-agonists such as salbutamol inhibit GH secretion by stimulating the release of somatostatin from nerve terminals in the median eminence. These effects are blocked by propranolol, a nonspecific β-receptor antagonist. Dopamine generally has a net effect to stimulate GH secretion and may be exerted directly on the pituitary gland[328] and/or GHRH neurons, as in the human brain. Dopamine-containing axonal varicosities heavily abut the majority of GHRH neurons.[329]

Serotonin's effect on GH release in humans is difficult to decipher because of the large number of receptor subtypes. However, clinical studies with the receptor-selective agonist sumatriptan clearly implicates the 5-HT1D receptor subtype in the stimulation of basal GH levels.[330] The drug also potentiates the effect of a maximal dose of GHRH, suggesting the recurring theme of GH disinhibition by inhibition of hypothalamic somatostatin neurons in its mechanism of action. Histaminergic pathways acting through H1 receptors play only a minor, conditional stimulatory role in GH secretion in humans.

Acetylcholine appears to be an important physiologic regulator of GH secretion and may contribute to the rise in GH during sleep.[331] Blockade of muscarinic acetylcholine receptors reduces or abolishes GH secretory responses to GHRH, glucagon and arginine, morphine, and exercise. In contrast, drugs that potentiate cholinergic transmission increase basal GH levels and enhance the GH response to GHRH. In vitro acetylcholine inhibits somatostatin release from hypothalamic fragments, and acetylcholine can act directly on the pituitary to inhibit GH release. There may even be a paracrine cholinergic control system within the pituitary. However, the sum of evidence suggests that the primary mechanism of action of M1 agonists is inhibition of somatostatin neuronal activity or the release of peptide from somatostatinergic terminals. Short-term cholinergic blockade with the M1 muscarinic receptor antagonist pirenzepine reduced the GH excess of patients with poorly controlled diabetes mellitus.[332]

Many neuropeptides in addition to GHRH and somatostatin are involved in the modulation of GH secretion in humans (see Table 7.4).[279,282] Among these, the evidence is most compelling for a stimulatory role of galanin acting in the human hypothalamus by a GHRH-dependent mechanism.[333] Many GHRH neurons are immunopositive for galanin as well as neurotensin and tyrosine hydroxylase. Galanin's actions may be explained, in part, by presynaptic facilitation of catecholamine release from nerve terminals and subsequent direct adrenergic

TABLE 7.4 Factors That Change Growth Hormone Secretion in Humans

Physiologic Factors	Hormones and Neurotransmitters	Pathologic Factors
Stimulatory Factors		
Episodic, spontaneous release	Insulin hypoglycemia	Acromegaly
Exercise	2-Deoxyglucose	TRH
Stress	Amino acid infusions	GnRH
Physical	Arginine, lysine	Glucose
Psychologic	Neuropeptides	Arginine
Slow-wave sleep	GHRH	Interleukins 1, 2, 6
Postprandial glucose decline	Ghrelin	Protein depletion
Fasting	Galanin	Starvation
	Opioids (μ-receptors)	Anorexia nervosa
	Melatonin	Renal failure
	Classic neurotransmitters	Liver cirrhosis
	α_2-Adrenergic agonists	Type 1 diabetes mellitus
	β-Adrenergic antagonists	
	M1 cholinergic agonists	
	5-HT1D receptor agonists	
	H1 histamine agonists	
	GABA (basal levels)	
	Dopamine (? D_2 receptor)	
	Estrogen	
	Testosterone	
	Glucocorticoids (acute)	
Inhibitory Factors[a]		
Postprandial hyperglycemia	Glucose infusion	Acromegaly L-Dopa
Elevated free fatty acids	Neuropeptides	D_2 receptor DA agonists
Elevated GH levels	Somatostatin	Phentolamine
Elevated IGF-1 (pituitary)	Calcitonin	Galanin
REM sleep	Neuropeptide Y (NPY[b])	Obesity
Senescence, aging	CRH[b]	Hypothyroidism
	Classic neurotransmitters	Hyperthyroidism
	$\alpha_{1/2}$-Adrenergic antagonists	
	β_2-Adrenergic agonists	
	H1 histamine antagonists	
	Serotonin antagonist	
	Nicotinic cholinergic agonists	
	Glucocorticoids (chronic)	

[a]In many instances, the inhibition can be demonstrated only as a suppression of GH release induced by a pharmacologic stimulus.

[b]The inhibitory actions of NPY and CRH on GH secretion are firmly established in the rodent and are secondary to increased somatostatin tone. Contradictory evidence exists in the human for both peptides, and further studies are required.

CRH, Corticotropin-releasing hormone; *DA,* dopamine; *GABA,* γ-aminobutyric acid; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *GnRH,* gonadotropin-releasing hormone; *IGF-1,* insulin-like growth factor type 1; *REM,* rapid eye movement; *TRH,* thyrotropin-releasing hormone.

stimulation of GHRH release.[334] Opioid peptides also stimulate GH release, probably by disinhibition of GHRH neurons, but under normal circumstances, endogenous opioid tone in the hypothalamus is presumed to be low because opioid antagonists have little acute effect on GH secretion. Studies on the human hypothalamus have indicated that enkephalin, but not endorphin or dynorphin, is the endogenous mediator of opiate action on GHRH neurons.[335]

Other Factors Influencing Secretion of Growth Hormone

Human Growth Hormone Rhythms

The deciphering of rhythmic GH secretion has relied on a combination of technical innovations in sampling and GH assay, and sophisticated mathematical modeling, including deconvolution analysis and the calculation of approximate entropy as a measure of orderliness or regularity in minute-to-minute secretory patterns.[282] At least three distinct categories of GH rhythms, which differ markedly in their time scales, can be considered here.

The daily GH secretion rate varies over two orders of magnitude from a maximum of nearly 2.0 mg/day in late puberty to a minimum of 20 μg/day in older or obese adults. The neonatal period is characterized by markedly amplified GH secretory bursts followed by a prepubertal decade of stable, moderate GH secretion of 200 to 600 μg/day. There is a marked increase in daily GH secretion during puberty that is accompanied by a commensurate rise in plasma IGF-1 to levels that constitute a state of physiologic hypersomatotropism. This pubertal increase in GH secretion is due to increased GH pulse amplitude rather than increased pulse frequency. Although the changes are clearly related to the increases in gonadal steroid hormones and can be mimicked by administration of estrogen or testosterone to hypogonadal children, the underlying neuroendocrine mechanisms are not fully understood. One hypothesis is that decreased sensitivity of the hypothalamic-pituitary axis to negative feedback of GH and IGF-1 leads to increased GHRH release and action, particularly given that IGF-1 levels also increase during puberty.[336] However, young adults have a return of daily GH secretion to prepubertal levels despite continued gonadal steroid elevation, perhaps associated with increased weight gain. The so-called somatopause is defined by an exponential decline in GH secretory rate with a half-life of 7 years, starting in the third decade of life, although more pronounced in men than women until women go through menopause.

GH secretion in young adults exhibits a true circadian rhythm over a 24-hour period, characterized by a greater nocturnal secretory mass that is independent of sleep onset.[337] However, as discussed earlier, GH release is further facilitated when slow-wave sleep coincides with the normal circadian peak. Under basal conditions, GH levels are low most of the time, with an ultradian rhythm of about 10 secretory pulses per 24 hours in men (20 in women) as calculated by deconvolution analysis.[338] Both sexes have an increased pulse frequency during the nighttime hours, but the fraction of total daily GH secretion associated with the nocturnal pulses is much greater in men. Overall, women have more continuous GH secretion and more frequent GH pulses that are of more uniform size than men.[307,338] A complementary study using approximate entropy analysis concluded that the nonpulsatile regularity of GH secretion is also significantly different in men and women.[339] These sexually dimorphic patterns in the human are actually quite similar to those in the rat, although the sex differences are not as extreme in humans.[282,339]

The neuroendocrine basis for sex differences in the ultradian rhythm of GH secretion is not fully understood. Gonadal sex steroids play both an organizational role during development of the hypothalamus and an activational role in the adult, regulating expression of the genes for many of the peptides and receptors central to GH regulation.[279,282] In the human, unlike the rat, the hypothalamic actions of testosterone appear to result predominantly from its aromatization to 17β-estradiol and interaction with estrogen receptors. Hypothalamic somatostatin appears to play a more prominent role in men than in women in the regulation of pulsatile GH secretion, and this difference is postulated to be a key factor in producing the sexual dimorphism.[338,340]

External and Metabolic Signals

The various peripheral signals that modulate GH secretion in humans are summarized in Table 7.4 (also see Figs. 7.22 and 7.24). Of particular importance are factors related to energy intake and metabolism because they provide a common signal between the peripheral tissues and hypothalamic centers regulating nonendocrine homeostatic pathways in addition to the classic hypophysiotropic neurons. It is also in this complex arena that species-specific regulatory responses are particularly prominent, making extrapolations between rodent experimental models and human GH regulation less reliable.[279,282]

Important triggers of GH release include the normal decrease in blood glucose level after intake of a carbohydrate-rich meal, absolute hypoglycemia, exercise, physical and emotional stress, and high intake of protein (mediated by amino acids). Some of the pathologic causes of elevated GH represent extremes of these physiologic signals and include protein-calorie starvation, anorexia nervosa, liver failure, and type 1 diabetes mellitus.[283] A critical concept is that many of these GH triggers work through the same final common mechanism of somatostatin withdrawal and consequent disinhibition of GH secretion. In contrast, postprandial hyperglycemia, glucose infusion, elevated plasma-free fatty acids, type 2 diabetes mellitus (with obesity and insulin resistance), and obesity result in inhibition of GH secretion.[283] The majority of these stimuli are associated with increased insulin secretion, which can exert direct, inhibitory action on somatotrophs through their expression of insulin receptors.[341] The specific role of leptin in modulating GH release is complicated by its multiple sites of action, coexistent secretory environment, and the animal species. For example, while leptin prevents the fasting-induced fall in GH in rodents, it does not affect GH levels in fasting human subjects.[342,343] Similarly, other members of the cytokine family, including IL-1, IL-2, IL-6, and endotoxin, have been inconsistently shown to stimulate GH in humans.

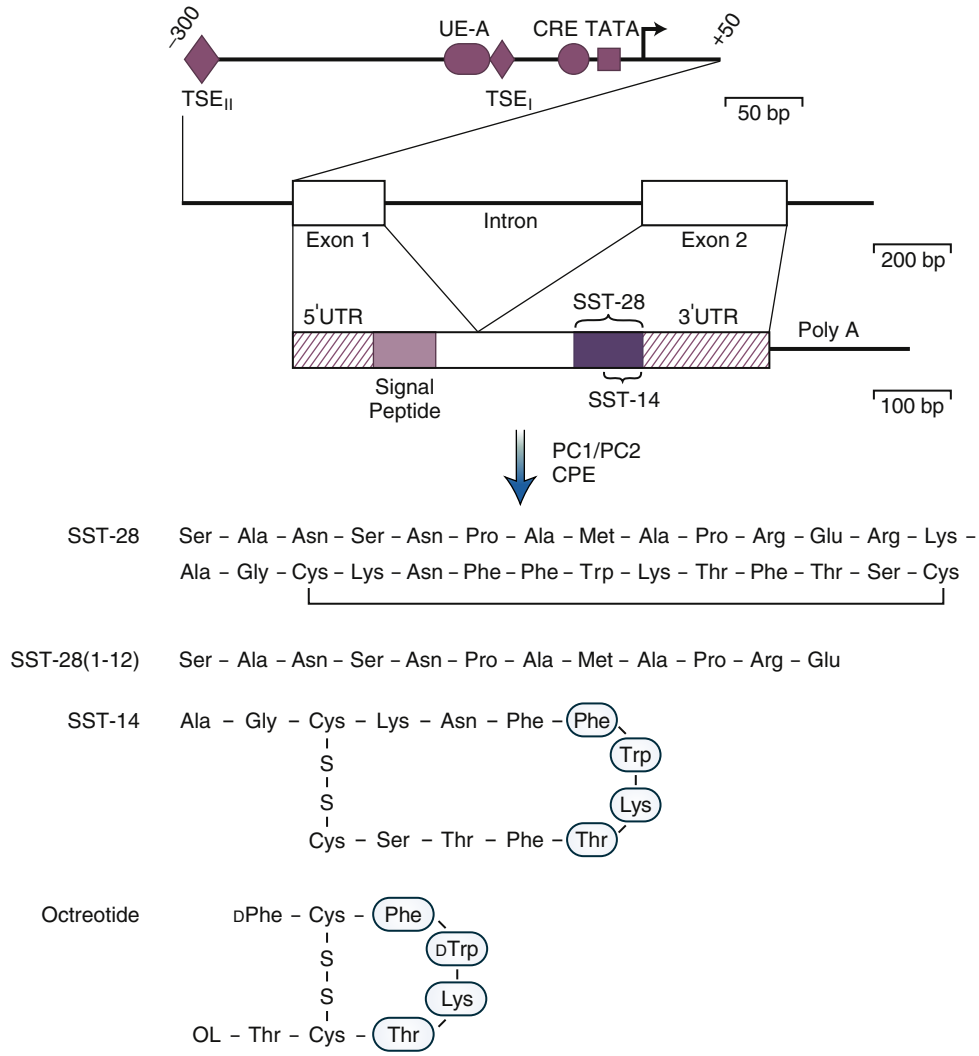
The actions of steroid hormones on GH secretion are complex because of their multiple loci of action within the proximal hypothalamic-pituitary components in addition to secondary effects on other neural and endocrine systems.[344] The acute administration of glucocorticoids results in an increase in GH through direct actions on somatotrophs and by increasing their sensitivity to GHRH.[345,346] High doses or long-term administration of glucocorticoids and individuals with Cushing disease have reduced GH secretion because of increased hypothalamic somatostatin tone and decreased GHRH.[347,348] CRH also has an inhibitory effect on GH secretion both by stimulating somatostatin release and through direct effects on GHRH neurons, contributing to impaired GH secretion associated with stress.[349,350] Similarly, physiologic levels of thyroid hormones are necessary to maintain GH secretion and promote GH gene expression. Excessive thyroid

hormone is also inhibitory to the GH axis, and the mechanism is speculated to be a combination of increased hypothalamic somatostatin tone, GHRH deficiency, and suppressed pituitary GH production.

Somatostatin

Chemistry and Evolution

A factor that potently inhibited GH release from pituitary in vitro was unexpectedly identified during early efforts to isolate GHRH from hypothalamic extracts. Somatostatin, the peptide responsible for this inhibition of GH secretion and the inhibition of insulin secretion by a pancreatic islet extract, was eventually isolated from the hypothalamus and sequenced by Brazeau and colleagues in 1973.[351] The term *somatostatin* was originally applied to a cyclic peptide containing 14 amino acids, also called *somatostatin-14* (SST-14) (Fig. 7.25). Subsequently, a second form, NH_2-terminal extended somatostatin-28 (SST-28), was identified as a secretory product. Both forms of somatostatin are derived by independent cleavage of a common prohormone by prohormone



• **Fig. 7.25** Diagram illustrating the genomic organization, messenger ribonucleic acid (mRNA) structure, and post-translational processing of the human somatostatin (SST) prohormone. Transcriptional regulation of the somatostatin gene, including the identification of tissue-specific elements (TSE), upstream elements (UE), and the cyclic adenosine monophosphate (cAMP) response element (CRE) that are binding sites for specific factors, has been studied extensively in pancreatic islet cell lines. It is not known whether all or some of these factors are also involved in the neural-specific expression of somatostatin. SST-28 and SST-14 are cyclic peptides containing a single covalent disulfide bond between a pair of cystine (Cys) residues. A β-turn containing the tetrapeptide Phe-Trp-Lys-Thr is stabilized by hydrogen bonds to produce the core receptor binding epitope. This minimal structure has been the model for conformationally restrained analogues of somatostatin, including octreotide. *CPE,* carboxypeptidase E; *PC1/PC2,* prohormone convertases 1 and 2; *TATA,* Goldstein-Hogness box involved in binding RNA polymerase; *UTR,* untranslated region. (Compiled from data by Shen LP, Rutter WJ. Sequence of the human somatostatin 1 gene. *Science.* 1984;224:168–171; Goudet G, Delhalle S, Biemar F, et al. Functional and cooperative interactions between the homeodomain PDX1, Pbx, and Prep1 factors on the somatostatin promoter. *J Biol Chem.* 1999;274:4067–4073; and Milner-White EJ. Predicting the biologically active conformations of short polypeptides. *Trends Pharmacol Sci.* 1989;10:70–74.)

convertases.[352] In addition, the isolation of SST-28(1-12) in some tissues suggests that SST-14 can be secondarily processed from SST-28. SST-14 is the predominant form in the brain, including the hypothalamus, whereas SST-28 is the major form in the gastrointestinal tract, especially the duodenum and jejunum.

The name *somatostatin* is descriptively inadequate because the molecule also inhibits TSH secretion from the pituitary and has nonpituitary roles, including activity as a neurotransmitter or neuromodulator in the central and peripheral nervous systems and as a regulatory peptide in gut and pancreas. As a pituitary regulator, somatostatin is a true neurohormone (i.e., a neuronal secretory product that enters the blood [hypothalamic-hypophysial–portal circulation] to affect cell function at remote sites). In the gut, somatostatin is present in the myenteric plexus, where it acts as a neurotransmitter, and in epithelial cells, where it influences the function of adjacent cells as a paracrine secretion. Somatostatin can influence its own secretion from delta cells (an autocrine function) in addition to acting as a paracrine factor in pancreatic islets. Gut exocrine secretion can be modulated by intraluminal action, so it is also a lumone. Because of its wide distribution, broad spectrum of regulatory effects, and evolutionary history, this peptide can be regarded as an archetypical pansystem modulator.

The genes that encode somatostatin in humans[353] (see Fig. 7.25) and a number of other species exhibit striking sequence homology, even in primitive fish such as the anglerfish. Furthermore, the amino acid sequence of SST-14 is identical in all vertebrates. Formerly, it was accepted that all tetrapods have a single gene encoding both SST-14 and SST-28, whereas teleost fish have two nonallelic preprosomatostatin genes (*PPSI* and *PPSII*), each of which encodes only one form of the mature somatostatin peptides. This situation implied that a common ancestral gene underwent a duplication event after the split of teleosts from the descendants of tetrapods.

However, both lampreys and amphibians, which predate and postdate the teleost evolutionary divergence, respectively, have now been shown to have at least two PPS genes.[354] A more distantly related gene has been identified in mammals that encodes cortistatin, a somatostatin-like peptide that is highly expressed in cortex and hippocampus.[355] Cortistatin-14 differs from SST-14 by three amino acid residues but has high affinity for all known subtypes of somatostatin receptors (see later discussion). The human gene sequence predicts a tripeptide-extended cortistatin-17 and a further NH_2-terminal extended cortistatin-29.[356] A revised evolutionary concept of the somatostatin gene family is that a primordial gene underwent duplication at or before the advent of chordates, and the two resulting genes underwent mutation at different rates to produce the distinct preprosomatostatin and preprocortistatin genes in mammals.[354] A second gene duplication probably occurred in teleosts to generate *PPSI* and *PPSII* from the ancestral somatostatin gene.

Apart from its expression in neurons of the periventricular and arcuate hypothalamic nuclei and involvement in GH secretion (discussed earlier), somatostatin is highly expressed in the cortex, lateral septum, extended amygdala, reticular nucleus of the thalamus, hippocampus, and many brainstem nuclei. Cortistatin is present in the brain at a small fraction of the levels of somatostatin and in a more limited distribution primarily confined to the cortex and hippocampus. The molecular mechanisms underlying the developmental and hormonal regulation of somatostatin gene transcription have been most extensively studied in pancreatic islet cells.[357,358] Less is known concerning the regulation of somatostatin gene expression in neurons except that activation is strongly controlled by binding of the phosphorylated transcription factor CRE-binding protein to its cognate CRE contained in the promoter sequence.[359,360] Enhancer elements in the somatostatin gene promoter that bind complexes of homeodomain-containing transcription factors (PAX6, PBX, PREP1) and upregulate gene expression in pancreatic islets may actually represent gene silencer elements in neurons (see Fig. 7.25, promoter elements TSE_{II} and UE-A). Conversely, another related *cis* element in the somatostatin gene (see Fig. 7.25, promoter element TSE_I) apparently binds a homeodomain transcription factor PDX1 (also called STF1/IDX1/IPF1) that is common to developing brain, pancreas, and foregut and regulates gene expression in both the CNS and gut.[361]

The function of somatostatin in GH and TSH regulation is considered earlier in this chapter. Its actions in the extrahypothalamic brain and diagnostic and therapeutic roles are considered in the remainder of this section and in Chapter 8. An additional function of somatostatin in pancreatic islet cell regulation is described in Chapter 33, and the manifestations of somatostatin excess as in somatostatinoma are described in Chapter 45.

Somatostatin Receptors

Five somatostatin receptor subtypes (SSTR1 to SSTR5) have been identified by gene cloning techniques, and one of these (SSTR2) is expressed in two alternatively spliced forms.[362] These subtypes are encoded by separate genes located on different chromosomes; they are expressed in unique or partially overlapping distributions in multiple target organs and differ in their coupling to second-messenger signaling molecules and in their range and mechanism of intracellular actions.[362] The subtypes also differ in their binding affinity to specific somatostatin analogues. Certain of these differences have important implications for the use of somatostatin analogues in therapy and in diagnostic imaging.

All SSTR subtypes are coupled to pertussis toxin–sensitive G proteins and bind SST-14 and SST-28 with high affinity in the low nanomolar range, although SST-28 has a uniquely high affinity for SSTR5. SSTR1 and SSTR2 are the two most abundant subtypes in the brain; they probably function as presynaptic autoreceptors in the hypothalamus and limbic forebrain, respectively, in addition to their postsynaptic actions. SSTR4 is most prominent in the hippocampus. All the subtypes are expressed in the pituitary, but SSTR2 and SSTR5 are the most abundant subtypes on somatotrophs. They are also the most physiologically important in pancreatic islets, with SSTR5 responsible for inhibition of insulin secretion from beta cells and SSTR2 responsible for inhibition of glucagon from alpha cells in mice.[363]

Binding of somatostatin to its receptor leads to activation of one or more plasma membrane–bound inhibitory G proteins ($G_{i/o}$), which in turn inhibit adenylyl cyclase activity and lower intracellular cAMP. Other G protein–mediated actions common to all SSTRs are activation of a vanadate-sensitive phosphotyrosine phosphatase and modulation of mitogen-activated protein kinase (MAPK). Different subsets of SSTRs are also coupled to inwardly rectifying K^+ channels, voltage-dependent Ca^{2+} channels, an Na^+/H^+ exchanger, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-kainate glutamate receptors, phospholipase C, and phospholipase A_2.[362] The lowering of intracellular cAMP and Ca^{2+} is the most important mechanism for inhibition of hormone secretion, and actions on phosphotyrosine phosphatase and MAPK are postulated to play a role in somatostatin's antiproliferative effect on tumor cells.

Effects on Target Tissues and Mechanism of Action

In the pituitary, somatostatin inhibits secretion of GH, TSH, and under certain conditions, PRL and ACTH. It exerts inhibitory effects on virtually all endocrine and exocrine secretions of the pancreas, gut, and gallbladder (Table 7.5). Somatostatin inhibits its secretion by the salivary glands and, under some conditions, the secretion of parathyroid hormone and calcitonin. Somatostatin blocks hormone release in many endocrine-secreting tumors, including insulinomas, glucagonomas, VIPomas, carcinoid tumors, and some gastrinomas.

The physiologic actions of somatostatin in the extrahypothalamic brain remain the subject of investigation.[364] In the striatum, somatostatin increases the release of dopamine from nerve terminals by a glutamate-dependent mechanism. It is widely expressed in GABAergic interneurons of the limbic cortex and hippocampus, where it modulates the excitability of pyramidal neurons. Temporal lobe epilepsy is associated with a marked reduction in somatostatin-expressing neurons in the hippocampus consistent with a putative inhibitory action on seizures.[365] In the amygdala, somatostatin has been associated with learning and expression of defensive responses.[366] A wealth of correlative data has linked reduced forebrain and CSF concentrations of somatostatin with Alzheimer disease, major depression, and other neuropsychiatric disorders, raising speculation about the role of somatostatin in modulating neural circuits underlying cognitive and affective behaviors.[367] A study using both genetic and pharmacologic methods to induce somatostatin deficiency in mice bolsters the hypothesis that the neuropeptide plays a physiologic role in the acquisition of contextual fear memory, possibly by altering long-term potentiation in hippocampal circuits.[368]

Clinical Applications of Somatostatin Analogues

An extensive pharmaceutic discovery program has produced somatostatin analogues with receptor subtype selectivity and improved pharmacokinetics and oral bioavailability compared with the native peptide. Initial efforts focused on the rational design of constrained cyclic peptides that incorporated D-amino acid residues and included the Trp^{9}-Lys^{10} dipeptide of somatostatin, which was shown by structure-function studies to be necessary for high-affinity binding to somatostatin receptors (see Fig. 7.25). Many such analogues have been studied in clinical trials, including octreotide, lanreotide, vapreotide, seglitide, and pasireotide.[362] These compounds are agonists with similarly high-affinity binding to SSTR2 and SSTR5, moderate binding to SSTR3, and no (or low) binding to SSTR1 (except for pasireotide) and SSTR4. A combinatorial chemistry approach has now led to a new generation of nonpeptidyl somatostatin agonists that bind selectively and with subnanomolar affinity to each of the five SSTR subtypes.[369] In contrast to the marked success in development of potent and selective somatostatin agonists, there is a relative paucity of useful antagonists.[370]

The somatostatin analogues have been effective in controlling excess secretion of GH in acromegaly in most patients and in shrinking the tumor size in about one-third of patients. They are also indicated for the treatment of recurrent TSH-secreting adenomas after surgery and a variety of functioning metastatic neuroendocrine tumors, including carcinoid, VIPoma, glucagonoma, and insulinoma, but they are seldom of use for the treatment of gastrinoma.[371] Somatostatin analogues are also useful in the management of many forms of diarrhea (acting on salt and water excretion mechanisms in the gut) and in reducing external secretions in pancreatic fistulas (thus permitting healing). A decrease in blood flow to the gastrointestinal tract is the basis for their use in bleeding esophageal varices, but they are not effective in the treatment of bleeding from a peptic ulcer.

TABLE 7.5 Biologic Actions of Somatostatin Outside the Central Nervous System

Hormone Secretion Inhibited (by Tissue)	Other Gastrointestinal Actions Inhibited
Pituitary gland	Gastric acid secretion
GH, thyrotropin, ACTH, prolactin	Gastric and jejunal fluid secretion
Gastrointestinal tract	Gastric emptying
Gastrin	Pancreatic bicarbonate secretion
Secretin	Pancreatic enzyme secretion
Motilin	Secretory diarrhea (stimulates intestinal absorption of water and electrolytes)
Glucagon-like peptide 1	Gastrointestinal blood flow
Glucose-dependent insulinotropic polypeptide	AVP-stimulated water transport
Vasoactive intestinal peptide	Bile flow
Pancreas	**Extragastrointestinal Actions Inhibited**
Insulin	Inhibits the function of activated immune cells
Glucagon	Inhibition of tumor growth
Somatostatin	
Genitourinary tract	
Renin	

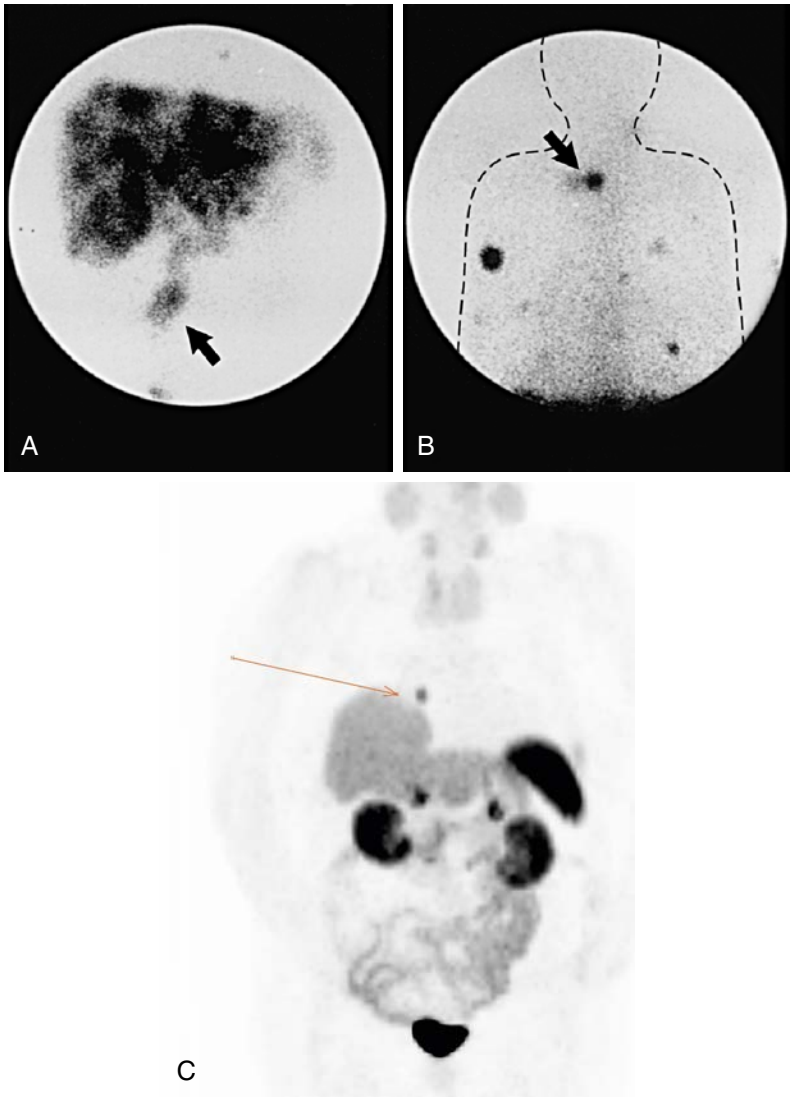
ACTH, Adrenocorticotropic hormone; *AVP,* arginine vasopressin; *GH,* growth hormone.

Somatostatin analogues labeled with a radioactive tracer have been used as external imaging agents for a wide range of disorders,[370,371] including indium-111 (^{111}In)-labeled and gallium-68 (^{68}Ga)-labeled somatostatin analogues, octreotate, DOTATATE, DOTATOC, and DOTANOC (Fig. 7.26). The majority of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) and bronchopulmonary NETs, pheochromocytomas/paragangliomas, medullary thyroid carcinoma, many pituitary tumors, and a variety of nonendocrine tumors that express somatostatin receptors such as breast cancer, renal cell carcinoma, meningioma, and astrocytoma can be visualized by external imaging techniques, using these agents by either scintigraphy or positron emission tomography (PET). Because activated T cells of the immune system display somatostatin receptors, inflammatory lesions such as sarcoidosis, Wegener granulomatosis, and tuberculosis, and many cases of Hodgkin disease and non-Hodgkin lymphoma also take up these radionucleotides.[372]

The ability of somatostatin to inhibit the growth of normal and some neoplastic cell lines and to reduce the growth of experimentally induced tumors in animal models has stimulated interest in somatostatin analogues for the treatment of cancer. Somatostatin's tumoristatic effects may be a combination of direct actions on tumor cells related to inhibition of growth factor receptor expression, inhibition of MAPK, and stimulation of phosphotyrosine phosphatase. Activation of SSTR1, SSTR2, SSTR4, and SSTR5 can all promote cell cycle arrest associated with induction of the tumor suppressor retinoblastoma (Rb) and p21 (CDKN1A), and SSTR3 activation can trigger apoptosis accompanied by induction of the tumor suppressor p53 and the proapoptotic protein Bax.[362] In addition, somatostatin has indirect effects on tumor growth through its inhibition of circulating, paracrine, and autocrine tumor growth–promoting factors, and it can modulate the

activity of immune cells and influence tumor blood supply. Two major studies, PROMID and CLARINET trials, demonstrated that treated patients with neuroendocrine tumors have significantly longer progression-free survival than patients treated with placebo.[373,374] Pasireotide, a novel multireceptor-targeted somatostatin analogue with 40-fold increased binding efficacy to SSTR5, has been effective in approximately one-third of patients with neuroendocrine tumors resistant to octreotide or lanreotide.

A treatment approach using radiolabeled somatostatin analogues has also been used to achieve the arrest of cancer cells.[370,371] The first is receptor-targeted radionuclide therapy using octreotide chelated to a variety of γ-emitting or β-emitting radioisotopes, including [^{90}Y-DOTA^{0},Ty^{3}]octreotide or DOTATATE chelated to lutetium 177. Theoretic calculations and empiric data suggest that radiolabeled somatostatin analogues can deliver a tumoricidal radiotherapeutic dose to tumors expressing somatostatin receptors either by simply binding to the surface receptors or following receptor-mediated endocytosis. Improved progression-free survival with these radionuclides in patients with metastatic GEP-NETs compared to the use of somatostatin analogues alone has been established in the NETTER-1 trial and in a recent, large Dutch cohort.[375,376] A variation on this theme is the chelation of a cytotoxic chemotherapeutic agent, such as doxorubicin, to a somatostatin analogue. An alternative approach involves somatic cell gene therapy to transfect SSTR-negative pancreatic cancer cells with an SSTR gene.[377]

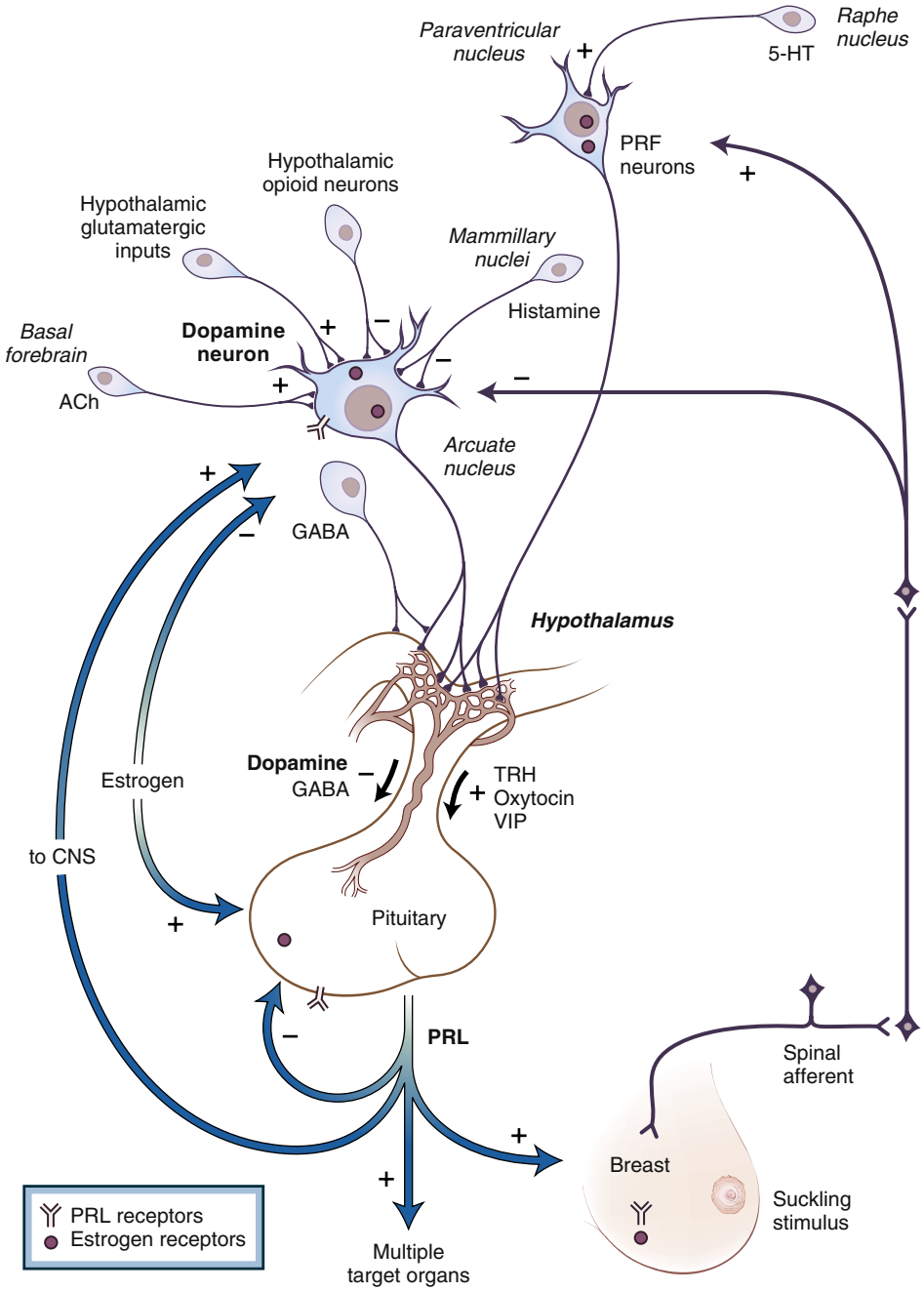


• **Fig. 7.26** The use of ^{111}In-labeled diethylenetriaminepenta-acetic acid (DTPA)-octreotide (A and B) and ^{68}Ga-DOTTATE (C) (radioactive somatostatin analogues) and external imaging techniques to localize a carcinoid tumor expressing somatostatin receptors. (A) Anterior view of the abdomen showing nodular metastases in an enlarged liver and the primary carcinoid tumor *(arrow)* in the wall of the jejunum of a patient with severe flushing and diarrhea. (B) Posterior view of the chest and neck showing a metastasis in a lymph node on the left side of the neck *(arrow)* and multiple metastases in the ribs and pleura. (C) Anterior view showing presence of a bronchopulmonary carcinoid tumor *(arrow)* causing ectopic ACTH syndrome and Cushing syndrome. (A and B, Modified from Lamberts SWJ, Krenning EP, Reubi J-C. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. *Endocrine Rev.* 1991;12:450–482. Copyright 1991, The Endocrine Society.)

Prolactin-Regulating Factors

Dopamine

It is well known that PRL secretion, unlike the secretion of other pituitary hormones, is primarily under tonic inhibitory control by the hypothalamus (Fig. 7.27).[378] Destruction of the stalk median eminence or transplantation of the pituitary gland to ectopic sites causes a marked constitutive increase in PRL secretion, in contrast to a decrease in the release of GH, TSH, ACTH, and the gonadotropins. Many lines of evidence indicate that dopamine is the principal physiologic PIF released from the hypothalamus.[379] Dopamine is present in hypothalamic-hypophysial–portal vessel



• **Fig. 7.27** Regulation of the hypothalamic-pituitary-prolactin (PRL) axis. The predominant effect of the hypothalamus is inhibitory, mediated principally by the tuberohypophyseal dopaminergic neuron system and dopamine D_2 receptors on lactotrophs. The dopamine neurons are stimulated by acetylcholine (ACh) and glutamate and inhibited by histamine and opioid peptides. One or more prolactin-releasing factors (PRFs) mediate acute release of PRL (e.g., in suckling, during stress). There are several candidate PRFs, including thyrotropin-releasing hormone (TRH), vasoactive intestinal polypeptide (VIP), and oxytocin. PRF neurons are activated by serotonin (5-HT). Estrogen sensitizes the pituitary to release PRL, which feeds back on the pituitary to regulate its own secretion (ultrashort-loop feedback) and also influences gonadotropin secretion by suppressing the release of gonadotropin-releasing hormone (GnRH). Short-loop feedback is also mediated indirectly by prolactin receptor regulation of hypothalamic dopamine synthesis, secretion, and turnover. *CNS,* central nervous system; *GABA,* γ-aminobutyric acid.

blood in sufficient concentration to inhibit PRL release, dopamine inhibits PRL secretion from lactotrophs both in vivo and in vitro, and dopamine D_2 receptors are expressed on the plasma membrane of lactotrophs. Mutant mice with a targeted disruption of the D_2 receptor gene uniformly develop lactotroph hyperplasia, hyperprolactinemia, and eventually lactotroph adenomas, further emphasizing the importance of dopamine in the physiologic regulation of lactotroph proliferation in addition to hormone secretion.[380]

The intrinsic dopamine neurons of the medial basal hypothalamus constitute a dopaminergic population with regulatory properties that are distinct from those in other areas of the brain. Notably, they lack D_2 autoreceptors but express PRL receptors, which are essential for positive feedback control (discussed in detail later). In rats, these neurons are subdivided by location into the A12 group of Dahlstrom and Fuxe within the arcuate nucleus and the A14 group in the hypothalamic periventricular nucleus. The dorsomedially located A12 dopamine neurons are further classified as tuberoinfundibular dopamine neurons (TIDA) because they project to the external zone of the median eminence and the main group of dopamine neurons responsible for prolactin regulation. The more rostral A12 group is classified as tuberohypophysial dopamine neurons (THDA) and project to the neural lobe and intermediate lobe. Finally, the A14 periventricular hypophysial dopaminergic neurons (PHDA) send their axons only to the intermediate lobe of the pituitary gland where they are primarily involved in the release of α-melanocyte–stimulating hormone.

Although the TIDA neurons are generally considered to be the major source of dopamine to the anterior lobe through the long portal vessels originating in the median eminence, dopamine can also reach the anterior lobe from the neural and intermediate lobes by the interconnecting short portal veins.[381] In addition to direct actions of dopamine on lactotrophs, central dopamine can indirectly affect PRL secretion by altering the activity of inhibitory interneurons that in turn synapse on the TIDA neurons. These effects are complicated by opposing intracellular signaling pathways linked to D_1 and D_2 receptors located on different populations of interneurons.[382]

The binding of dopamine or selective agonists such as bromocriptine to the D_2 receptor has multiple effects on lactotroph function. The D_2 receptor is a member of the cytokine superfamily and couples to pertussis toxin–sensitive G proteins to inhibit adenylyl cyclase and decrease intracellular cAMP levels. Other effects include activation of an inwardly rectifying K^+ channel, increase of voltage-activated K^+ currents, decrease of voltage-activated Ca^{2+} currents, and inhibition of inositol phosphate production. Together, this spectrum of intracellular signaling events decreases free Ca^{2+} concentrations and inhibits exocytosis of PRL secretory granules.[383]

There is continuing debate concerning the mechanism by which D_2 receptor activation inhibits transcription of the PRL gene. Likely pathways involve the inhibition of MAPK or protein kinase C, with a resultant reduction in the phosphorylation of Ets family transcription factors. Ets factors are important for the stimulatory responses of TRH, insulin, and epidermal growth factor on PRL expression, and they interact cooperatively with the pituitary-specific POU protein Pit1, which is essential for cAMP-mediated PRL gene expression.[384]

The second-messenger pathways used by the D_2 receptor to inhibit lactotroph cell division are also unsettled.[385] A study using primary pituitary cultures from rats demonstrated that forskolin treatment, which activates protein kinase A and elevates intracellular cAMP, and insulin treatment, which activates a potent receptor tyrosine kinase, were both effective mitogenic stimuli for lactotrophs. Bromocriptine competitively antagonized the proliferative response caused by elevated cAMP. Furthermore, inhibition of MAPK signaling by PD98059 markedly suppressed the mitogenic action of both insulin and forskolin, suggesting an interaction of MAPK and protein kinase A signaling.[386]

Another line of study has implicated the stimulation of phospholipase D activity by a Rho A–dependent, pertussis toxin–insensitive pathway in the antiproliferative effects of D_2 receptor activation in both GH4C1 pituitary cells and NCI-H69 small cell lung cancer cells.[387] Activation of the extracellular signal-regulated kinase 1/2 pathway and inhibition of the AKT/protein kinase B pathway have also been implicated in the action of the D_2 receptor to reduce lactotroph mitogenesis.[388] Therefore it is clear that dopamine actions on lactotrophs involve multiple different intracellular signaling pathways linked to activation of the D_2 receptor, but different combinations of these pathways are relevant for the inhibitory effects on PRL secretion, PRL gene transcription, and lactotroph proliferation.

The other major action of dopamine in the pituitary is the inhibition of hormone secretion from the POMC-expressing cells of the intermediate lobe[389]—although, as noted earlier, the adult human differs from most other mammals in the rudimentary nature of this lobe. THDA and PHDA axon terminals provide a dense plexus of synaptic-like contacts on melanotrophs. Dopamine release from these terminals is inversely correlated with serum MSH levels and also regulates POMC gene expression and melanotroph proliferation.

Other hypothalamic factors likely play a role secondary to that of dopamine as additional PIFs.[378] The primary reason to conjecture the existence of these PIFs is the frequent inconsistency between portal dopamine levels and circulating PRL in different rat models. GABA is the strongest candidate and most likely acts through $GABA_A$ inotropic receptors in the anterior pituitary. Melanotrophs, like lactotrophs, are inhibited by both dopamine and GABA but with the principal involvement of G protein–coupled, metabotropic $GABA_B$ receptors.[390] Because basal dopamine tone is high, the measurable inhibitory effects of GABA on PRL release are generally small under normal circumstances. Other putative PIFs include somatostatin and calcitonin.

Prolactin-Releasing Factors

Although tonic suppression of dopamine by prolactin is the dominant effect of the hypothalamus on PRL secretion, other mechanisms may also contribute by either contributing to dopamine suppression or directly promoting PRL release from the anterior pituitary (see Fig. 7.27). The most important of the putative PRFs are TRH, oxytocin, and VIP, but vasopressin, angiotensin II, NPY, galanin, substance P, bombesin-like peptides, and neurotensin can also trigger PRL release under different physiologic circumstances.[378] TRH has already been discussed. In humans there is an imperfect correlation between pulsatile PRL and TSH release, suggesting that TRH cannot be the sole physiologic PRF under basal conditions.[391]

Like TRH, oxytocin, vasopressin, and VIP fulfill all the basic criteria for a PRF. They are produced in PVH neurons that project to the median eminence. Concentrations of the hormones in portal blood are much higher than in the peripheral circulation and are sufficient to stimulate PRL secretion in vitro. Moreover, there are functional receptors for each of the neurohormones in the anterior pituitary gland, and either pharmacologic antagonism or passive immunization against each hormone can decrease PRL secretion, at least under certain circumstances.[392]

AVP is released during stress and hypovolemic shock, as is PRL, suggesting a specific role for vasopressin as a PRF in these contexts. Similarly, another candidate PRF, peptide histidine isoleucine, may be specifically involved in the secretion of PRL in response to stress. Peptide histidine isoleucine and the human homologue, peptide histidine methionine, are structurally related to VIP and are synthesized from the same prohormone precursor in their respective species.[393] Both peptides are coexpressed with CRH in parvicellular PVH neurons, and presumably they are released by the same stimuli that cause release of CRH into the hypothalamic-hypophysial portal vessels.[394]

Serotonin arising from the brainstem dorsal raphe also appears to have a major role in prolactin secretion, particularly during lactation.[395,396] These neurons innervate TIDA neurons to inhibit their secretion of dopamine and hence increase prolactin secretion but may also have an activating effect on PRF through direct innervation of TRH in the PVH. Endogenous opiates also may have a role during lactation by inhibiting TIDA secretion mediated by dynorphin-producing or beta endorphin–producing neurons in the arcuate nucleus.

Intrapituitary Regulation of Prolactin Secretion

Probably more than that of any other pituitary hormone, the secretion of PRL is regulated by autocrine-paracrine factors within the anterior lobe and by neurointermediate lobe factors that gain access to venous sinusoids of the anterior lobe by way of the short portal vessels. The wealth of local regulatory mechanisms within the anterior lobe has been reviewed extensively[378,397] and is also discussed in Chapter 8. In brief, that some gonadotrophs are closely apposed to lactotrophs and secrete substances in response to GnRH that stimulate prolactin secretion is well known.[398,399] Candidate substances derived from gonadotrophs abound and include pituitary glycoprotein hormone alpha subunit (αGSU), an N-terminal fragment of POMC (POMC 1-74), neurotensin, angiotensin II, PACAP, calcitonin, and CART, among a number of other substances.[400] Galanin, VIP, endothelin-like peptides, epidermal growth factor, basic fibroblast growth factor, and the cytokine IL-6 are also potent local stimulators of PRL secretion. Locally produced inhibitors include PRL itself, acetylcholine, transforming growth factor β, and calcitonin. Although none of these stimulatory or inhibitory factors have a dominant role in the regulation of lactotroph function and much of the research in this area has not been directly confirmed in human pituitary, it seems apparent that the local milieu of autocrine and paracrine factors plays an essential, modulatory role in determining the responsiveness of lactotrophs to hypothalamic factors in different physiologic states.

Cell clustering has also been observed in the anterior pituitary during lactation,[401] in which there is proliferation and reorganization of lactotrophs into a honeycomb-like structure with increased contacts between the cells. Presumably these changes are a way to integrate or amplify signals from the hypothalamus and coordinate increased output of prolactin. Recent advances in two-photon imaging of the pituitary and three-dimensional analyses of pituitary cell networks reinforce the importance of these local connections.[402]

Neuroendocrine Regulation of Prolactin Secretion

Secretion of PRL, like that of other anterior pituitary hormones, is regulated by hormonal feedback and neural influences from the hypothalamus.[378,379,403] Feedback is exerted by PRL itself at the level of the hypothalamus. PRL secretion is regulated by many physiologic states, including the estrous and menstrual cycles, pregnancy, and lactation. Furthermore, PRL is stimulated by several exteroceptive stimuli, including light, ultrasonic vocalization of pups (in rodents), olfactory cues, and various modalities of stress. Expression and secretion of PRL are also influenced strongly by estrogens at the level of both the lactotrophs and TIDA neurons[404] (see Fig. 7.27) and by paracrine regulators within the pituitary such as galanin and VIP.

Feedback Control

Negative feedback control of PRL secretion is mediated by a unique short-loop mechanism within the hypothalamus. PRL activates PRL receptors, which are expressed on all three subpopulations of A12 and A14 dopamine neurons, leading to increased tyrosine hydroxylase expression and increased dopamine synthesis and release.[404,405] Ames dwarf mice that secrete virtually no PRL, GH, or TSH have decreased numbers of arcuate dopamine neurons, and this hypoplasia can be reversed by neonatal administration of PRL, suggesting a trophic action on these neurons.[406] However, another mouse model of isolated PRL deficiency generated by gene targeting appears to have normal numbers of hypofunctioning dopamine neurons.[407] Ultrashort feedback control via autocrine or paracrine regulation of prolactin at the level of the pituitary gland also occurs as indicated by an increased sensitivity of lactotrophs to dopamine inhibition in transgenic mice with conditional deletion of the prolactin receptor, selectively in lactotrophs.[408]

Neural Control

Lactotrophs have spontaneously high secretory activity; therefore the predominant effect of the hypothalamus on PRL secretion is tonic suppression, which is mediated by regulatory hormones synthesized by THDA neurons. Secretory bursts of PRL are caused by the acute withdrawal of dopamine inhibition, stimulation by PRFs, or combinations of both events. At any given moment, locally produced autocrine and paracrine regulators further modulate the responsiveness of individual lactotrophs to neurohormonal PIFs and PRFs.

Multiple neurotransmitter systems impinge on the hypothalamic dopamine and PRF neurons to regulate their neurosecretion[378] (see Fig. 7.27). Nicotinic cholinergic and glutamatergic afferents activate TIDA neurons, whereas histamine, acting predominantly through H_2 receptors, inhibits these neurons. An inhibitory peptidergic input to TIDA neurons of major physiologic significance is that associated with the endogenous opioid peptides enkephalin and dynorphin and their cognate μ-receptor and κ-receptor subtypes.[409] Opioid inhibition of dopamine release has been associated with increased PRL secretion under virtually all physiologic conditions, including the basal state, different phases of the estrous cycle, lactation, and stress. Ascending serotonergic inputs from the dorsal raphe nucleus are the major activator of PRF neurons in the PVH. There is still debate concerning the identity of the specific 5-HT receptors involved in this activation.

The PRL regulatory system and its monoaminergic control have been scrutinized in detail because of the frequent occurrence of syndromes of PRL hypersecretion (see Chapter 8). Both the pituitary and the hypothalamus have dopamine receptors, and the response to dopamine receptor stimulation and blockade does not distinguish between central and peripheral actions of the drug. Many commonly used neuroleptic drugs influence PRL secretion. Reserpine (a catecholamine depleter) and phenothiazines such as chlorpromazine and haloperidol enhance PRL release by disinhibition of dopamine action on the pituitary, and the PRL response is an excellent predictor of the antipsychotic effects of phenothiazines because of its correlation with D_2 receptor binding

and activation.[410] The major antipsychotic neuroleptic agents act on brain dopamine receptors in the mesolimbic system and in the pituitary-regulating TIDA system. Consequently, treatment of such patients with dopamine agonists such as bromocriptine can reverse the psychiatric benefits of such drugs. A report of three patients with psychosis and concomitant prolactinomas recommended the combination of clozapine and quinagolide as the treatment of choice to manage both diseases simultaneously.[411]

Factors Influencing Secretion

Circadian Rhythm

PRL is detectable in plasma at all times during the day but is secreted in discrete pulses superimposed on basal secretion and exhibits a diurnal rhythm with peak values in the early morning hours.[412] In humans, this is a true circadian rhythm because it is maintained in a constant environment independently of the sleep rhythm.[413] The combined body of data examining TIDA neuronal activity, dopamine concentrations in the median eminence, and manipulations of the SCN suggests that endogenous diurnal alterations in dopamine tone that are entrained by light constitute the major neuroendocrine mechanism underlying the circadian rhythm of PRL secretion. The nocturnal rise in PRL secretion in nursing and nonnursing women may have evolved as a mechanism of milk maintenance during prolonged nonsuckling periods at night.

External Stimuli

The suckling stimulus is the most important physiologic regulator of PRL secretion. PRL levels rise within 1 to 3 minutes of nipple stimulation and remain elevated for 10 to 20 minutes.[414] This reflex is distinct from the milk let-down, which occurs nearly simultaneously with prolactin secretion during suckling and involves oxytocin release from the neurohypophysis and contraction of mammary alveolar myoepithelial cells. These reflexes provide a mechanism by which the infant regulates both the production and the delivery of milk. Of interest, in nonlactating rats, prolactin secretion inhibits oxytocin neurons and stimulates dopamine release from TIDA neurons, whereas during lactation, prolactin enhances oxytocin release and does not increase dopamine release. Mechanisms involved may include prolactin-induced sensitization of TRPV1 channels that are upregulated in oxytocin neurons during lactation, an indirect effect mediated by GABA release from TIDA neurons,[415] and inhibition of the prolactin feedback loop by the dissociation of electric activity and dopamine release to allow for sustained elevations in prolactin during suckling.[416] Signaling of prolactin to TIDA neurons may also be diminished as a result of upregulation of SOCS proteins that inhibit JAK-STAT signaling, induced by the binding of prolactin to the prolactin receptor.[417]

After stimulation of mechanoreceptors, pathways involved in the suckling reflex are carried to the spinal cord by way of spinal afferent neurons, ascend the spinal cord through spinothalamic tracts to the midbrain, and enter the hypothalamus by way of the median forebrain bundle (see Fig. 7.27). Neurons regulating the oxytocin-dependent milk let-down response accompany those involved in PRL regulation throughout most of this pathway and then separate at the level of the PVH nuclei, although the intrahypothalamic pathways involved have not been precisely elucidated. Using c-Fos as a marker for neuronal activation, several potential relay centers in the brainstem have been identified, including the ventrolateral medulla (A1) and dorsal vagal complex (A2) catecholamine cell groups, locus coeruleus, lateral parabrachial nucleus, caudal portion of the paralemniscal nucleus, and lateral and ventrolateral portions of the caudal part of the periaqueductal gray through direct or indirect synaptic connections to oxytocin neurons.[418] Noradrenergic neurons arising in the A1 and A2 groups directly innervate oxytocin neurons.[419] Synchronization of oxytocin neurons, which contributes to the coordinated pattern of pulsatile oxytocin release, is regulated by afferent inputs from glutamatergic neurons, in the lateral septum and bed nucleus of the stria terminalis.[47] Retraction of astroglial processes that normally separate oxytocin neurons, allowing increased interactions through somatic appositions, also contributes to the pulsatile pattern of oxytocin secretion and may be regulated by "intranuclear release" of oxytocin, in which oxytocin is released locally from dendrites and soma.[420] The suckling reflex brings about an inhibition of PIF activity and a release of PRFs, although an undisputed suckling-induced PRF has not been identified.

Suckling-induced prolactin secretion not only has an important role in lactation but coordinates a number of other physiologic responses that complement lactation, including the development of maternal behavior, increased appetite, and inhibition of reproductive function. These effects are facilitated by upregulation of prolactin receptors in the choroid plexus during lactation, allowing increased entry of prolactin from the circulation into the brain through a carrier-mediated transport system.[421] Binding of prolactin to prolactin receptors in the MePO may be important for maternal behavior as antagonizing prolactin receptors with a specific prolactin receptor antagonist injected into the preoptic area delays the onset of maternal behavior.[422] Tuberoinfundibular peptide of 39 residues (TIP39) may also be an important component of the mechanism involved in maternal motivation by lactation.[423] This peptide, produced by neurons in the thalamus, has extensive projections to galanin neurons in the preoptic area, which are known to be activated by suckling.[424] Suckling also results in a marked increase in NPY and AgRP gene expression in the arcuate nucleus mediated by neuronal afferents from the brainstem and the fall in circulating levels of leptin, brought about by a negative energy balance secondary to the milk production.[425] As NPY and AgRP are powerful orexigenic peptides (see Chapter 39 for overview of neuroendocrine regulation of appetite and satiety), their increase during suckling may explain the hyperphagia associated with lactation to meet the energy demands imposed by milk production. Indeed, total ablation of NPY/AgRP neurons by diphtheria toxin administered to transgenic mice expressing the diphtheria toxin receptor exclusively in NPY/AGRP neurons leads to a significant reduction in food intake in lactating animals.[426] Activation of arcuate nucleus NPY neurons may also contribute to the inhibition of reproductive function associated with suckling through direct actions on GnRH neurons mediated by NPY Y5 receptors.[427]

Although their significance for PRL regulation in humans is not certain, environmental stimuli from seasonal changes in light duration and auditory and olfactory cues are clearly of great importance to many mammalian species.[378] Seasonal breeders such as sheep exhibit a reduction in PRL secretion in response to shortened days. Vocalization of rodent pups are also potent stimuli for PRL secretion in lactating and virgin female rats by activation of the inferior colliculus and lateral geniculate of the posterior thalamus.[418] Olfactory stimuli from pheromones also have potent actions in rodents. A prime example is the Bruce effect, or spontaneous abortion, induced by exposure of a pregnant female rat to an unfamiliar male. It is mediated by a well-studied neural circuitry involving the vomeronasal nerves, the corticomedial amygdala, and the medial preoptic area of the hypothalamus, which results in activation of TIDA neurons and a reduction in circulating PRL

that is essential for maintenance of luteal function in the first half of pregnancy.

Stress in many forms dramatically affects PRL secretion, although the teleologic significance is uncertain. It may be related to actions of PRL on cells of the immune system or some other aspect of homeostasis. Different stressors are associated with either a reduction or an increase in PRL secretion, depending on the local regulatory environment at the time of the stress. However, whereas well-documented changes in PRL are associated with relatively severe forms of stress in laboratory animal models, the relevance to human physiology is not well established.

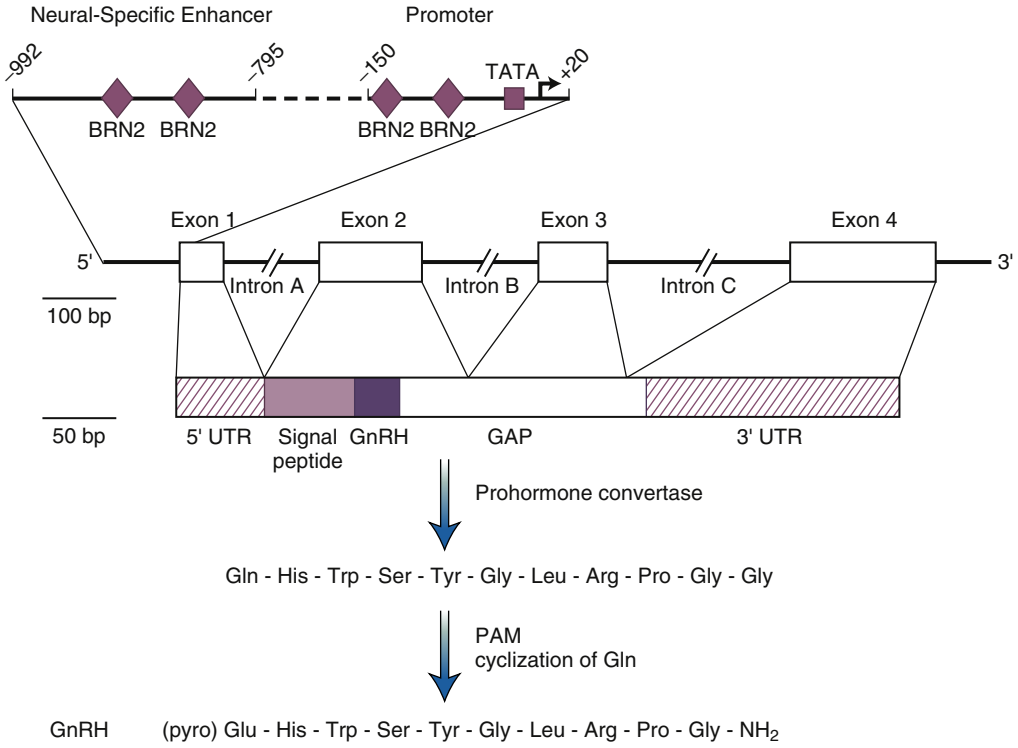
Gonadotropin-Releasing Hormone and Control of the Reproductive Axis

Chemistry and Evolution

GnRH is a 10–amino acid hypothalamic neuropeptide that controls the function of the reproductive axis. It is synthesized as part of a larger precursor molecule that is enzymatically cleaved to remove a signal peptide from the NH_2-terminus and GnRH-associated peptide (GAP) from the COOH-terminus (Fig. 7.28).[428] All forms of the decapeptide have a pyroGlu at the NH_2-terminus and Gly-amide at the COOH-terminus, indicating the functional importance of the terminal residues throughout evolution.

Two genes encoding GnRH have been identified within mammals.[429,430] The first, *GNRH1*, encodes a 92–amino acid precursor protein. This form of GnRH is found in hypothalamic neurons and serves as a releasing factor to regulate pituitary gonadotroph function.[431] The second GnRH gene, *GNRH2*, encodes a decapeptide that differs from the first by three amino acids.[432] This form of GnRH is found in the midbrain region and serves as a neurotransmitter rather than as a pituitary releasing factor. Both GnRH-I and GnRH-II are found in phylogenetically diverse species, from fish to mammals, suggesting that these multiple forms of GnRH diverged from one another early in vertebrate evolution.[431] A third form of GnRH, GnRH-III, has been identified in neurons of the telencephalon in teleost fish. GnRH is also found in cells outside the brain but is not well understood and is an area under current investigation.

All GnRH genes have the same basic structure, with the preprohormone mRNA encoded in four exons. Exon 1 contains the 5′ untranslated region of the gene; exon 2 contains the signal peptide, GnRH, and the NH_2-terminus of GAP; exon 3 contains



• **Fig. 7.28** Schematic diagram of the human gene for gonadotropin-releasing hormone-1 (*GNRH1*), the hypothalamic complementary deoxyribonucleic acid (cDNA), and post-translational processing of the GnRH prohormone. A cluster of binding sites for the homeodomain transcription factor BRN2 is present in both the proximal promoter and a distal enhancer region and is important for neuron-specific expression of the gene. Phylogenetically conserved homologous regions have been identified in the rat *Gnrh1* gene, but in that species the Oct1 transcription factor has been implicated in neuron-specific expression. The cDNA for GnRH-I isolated from human placenta has a longer 5′ untranslated region (UTR) because of differential splicing of the heterogeneous nuclear RNA (hnRNA) and inclusion of intron A sequences. *GAP,* GnRH-associated peptide; *PAM,* peptidylglycine α-amidating mono-oxygenase; *TATA,* Goldstein-Hogness box involved in binding RNA polymerase. (Compiled from data of Cheng CK, Leung PCK. Molecular biology of gonadotropin-releasing hormone [GnRH]-I, GnRH-II, and their receptors in humans. *Endocr Rev.* 2005;26:283–306; Wolfe A, Kim HH, Tobet S, et al. Identification of a discrete promoter region of the human GnRH gene that is sufficient for directing neuron-specific expression: a role for POU homeodomain transcription factors. *Mol Endocrinol.* 2002;16:435–449.)

the central portion of GAP; and exon 4 contains the COOH-terminus of GAP and the 3′ untranslated region (see Fig. 7.28).[431] Among species, the nucleotide sequences encoding the GnRH decapeptide are highly homologous. This section focuses on the hypothalamic GnRH that is derived from *GNRH1* mRNA and plays an important role in the regulation of the hypothalamic-pituitary-gonadal axis.

Two transcriptional start sites have been identified in the rat *GNRH1* gene, at the +1 and –579, with the +1 promoter being active in hypothalamic neurons and the other promoter active in placenta. The first 173 base pairs of the promoter are highly conserved among species. In the rat, this promoter region has been shown to contain two Oct1 binding sites, three regions that bind the POU domain family of transcription factors (SCIP, Oct-6, and Tst-1), and three regions that can bind the progesterone receptor.[433] In addition, a variety of hormones and second messengers have been shown to regulate GnRH gene expression, and a majority of the *cis*-acting elements, thus far characterized for hormonal control of GnRH transcription, are located in the proximal promoter region.[434,435] The 5′ flanking region of the rodent and human *GNRH1* genes also contains a distal 300–base pair enhancer region that is 1.8 or 0.9 kb, respectively, upstream of the transcription start site.[435,436] Studies have implicated the homeodomain transcription factors OCT1, MSX, and DLX in the specification of neuron expression and developmental activation.[436,437]

Anatomic Distribution

GnRH neurons are small, diffusely located cells that are not concentrated in a discrete nucleus. They are generally bipolar and fusiform in shape, with slender axons projecting predominantly to the median eminence and infundibular stalk. The location of hypothalamic GnRH neurons is species-dependent. In the rat, hypothalamic GnRH neurons are concentrated in rostral areas, including the medial preoptic area, the diagonal band of Broca, the septal areas, and the anterior hypothalamus. In humans and nonhuman primates, the majority of hypothalamic GnRH neurons are located more dorsally in the medial basal hypothalamus, the infundibulum, and the periventricular region. Throughout the hypothalamus, neurohypophysial GnRH neurons are interspersed with non-neuroendocrine GnRH neurons that extend their axons to other areas of the brain, including other hypothalamic regions and various regions of the cortex. GnRH secreted from non-neuroendocrine neurons has been implicated in the control of sexual behavior in rodents but not in higher primates.[438]

Embryonic Development

GnRH neuroendocrine neurons are an unusual neuronal population in that they originate outside the CNS, from the epithelial tissue of the nasal placode.[439] During embryonic development, GnRH neurons migrate across the surface of the brain and into the hypothalamus, with the final hypothalamic location differing somewhat among species. Migration is dependent on a scaffolding of neurons and glial cells along which the GnRH neurons move, with neural cell adhesion molecules playing a critical role in guiding the migration process.

Failure of GnRH neurons to migrate properly leads to the clinical condition Kallmann syndrome, in which GnRH neuroendocrine neurons do not reach their final destination and therefore do not stimulate pituitary gonadotropin secretion.[439,440] Patients with Kallmann syndrome do not enter puberty spontaneously. The X-linked form of Kallmann syndrome results from a deficiency of the *KAL1* gene, which encodes the extracellular glycoprotein termed *anosmin-1*. Mutations in the fibroblast growth factor receptor type 1 (*FGFR1*), *FGF8*, prokineticin 2 and its receptors (*PROK2* and *PROKR2*), and chromodomain helicase DNA-binding protein 7 (*CDH7*) genes produce an autosomal dominant form of Kallmann syndrome, and autosomal recessive forms of *PROK2* and *PROKR2* also occur. Other genes associated with Kallman syndrome include *CCDC141*, *DUSP6*, *FEZF1*, *FGF17*, *FLRT3*, *IL17RD*, *NELF*, *SEMA3A*, *SPRY4,* and *WDR11*.[441] These mutations, however, still account for only the minority of Kallmann cases, and other causes are yet to be characterized.[442] Administration of exogenous GnRH effectively treats this form of hypothalamic hypogonadism. Patients with Kallmann syndrome due to mutations in the *KAL1* gene often have other congenital midline defects, including anosmia, which results from hypoplasia of the olfactory bulb and tracts.

Action at the Pituitary

Receptors

GnRH binds to a membrane receptor on pituitary gonadotrophs and stimulates both LH and FSH synthesis and secretion. The GnRH receptor is a seven-transmembrane-domain GPCR, but it lacks a typical intracellular COOH-terminal cytoplasmic domain.[435] Under physiologic conditions, GnRH receptor number varies and is usually directly correlated with the gonadotropin secretory capacity of pituitary gonadotrophs. For example, across the rat estrous cycle, a rise in GnRH receptors is seen just before the surge of gonadotropins that occurs on the afternoon of proestrus. GnRH receptor message levels are regulated by a variety of hormones and second messengers, including steroid hormones (estradiol can both suppress and stimulate; progesterone suppresses), gonadotropins (which suppress), and calcium and protein kinase C (which stimulate).[433,435]

$G_{q/11}$ is the primary guanosine triphosphate–binding protein mediating GnRH responses; however, there is evidence that GnRH receptors can couple to other G proteins, including G_s and G_i.[435] With activation, the GnRH receptor couples to a phosphoinositide-specific phospholipase C, which leads to increases in calcium transport into gonadotrophs and calcium release from internal stores through a diacylglycerol-protein kinase C pathway. Increased calcium entry is a critical step in GnRH-stimulated release of gonadotropin secretion. However, GnRH also stimulates the MAPK cascade.

When there is a decline in GnRH stimulation to the pituitary, as occurs in a variety of physiologic conditions (including states of lactation, undernutrition, or seasonal periods of reproductive quiescence), the number of GnRH receptors on pituitary gonadotrophs declines dramatically. Subsequent exposure of the pituitary to pulses of GnRH restores receptor number by a Ca^{2+}-dependent mechanism that requires protein synthesis.[443] The effect of GnRH to induce its own receptor is termed *upregulation* or *self-priming*. Only certain physiologic frequencies of pulsatile GnRH can augment GnRH receptor production, and these frequencies appear to differ among species.[444] Upregulation of GnRH receptors after a period of low GnRH stimulation to the pituitary can take hours to days of exposure to pulsatile GnRH, depending on the duration and extent of the prior decrease in GnRH. The self-priming effect of GnRH to upregulate its own receptors also plays a crucial role in the production of the gonadotropin surge that occurs at midcycle in females of spontaneously ovulating species and triggers ovulation. Just before the gonadotropin surge, two factors—the

increased frequency of pulsatile GnRH release and a sensitization of the pituitary gonadotrophs by rising levels of estradiol—make the pituitary exquisitely sensitive to GnRH and allow an output of LH that is an order of magnitude greater than the release seen during the rest of the female reproductive cycle. This surge of LH triggers the ovulatory process at the ovary.

In contrast to upregulation of GnRH receptors by pulsatile regimens of GnRH, continuous exposure to GnRH leads to downregulation of GnRH receptors and an accompanying decrease in LH and FSH synthesis and secretion, termed *desensitization*.[445] Downregulation does not require calcium mobilization or gonadotropin secretion. It involves a rapid uncoupling of receptors from G proteins and sequestration of the receptors from the plasma membrane, followed by internalization and proteolytic degradation of the receptors.

The concept of downregulation has a number of clinical applications. For example, the most common current therapy for precocious puberty of hypothalamic origin (i.e., precocious GnRH secretion) is to treat it with a long-acting GnRH *superagonist* that downregulates pituitary GnRH receptors and effectively turns off the reproductive axis.[444,446] Children with precocious puberty can be maintained with long-acting GnRH agonists for years to suppress the premature activation of the reproductive axis, and at the normal age of puberty, agonist treatment can be withdrawn, allowing reactivation of pituitary gonadotrophs and a downstream increase in gonadal steroid hormone production (also see Chapter 17). Long-acting GnRH agonists are also used in the treatment of forms of breast cancer that are estrogen-dependent and of other gonadal steroid-dependent cancers.[444] Long-acting antagonists of GnRH have been developed that can also be used for these therapies.[447] Antagonists have the advantage of not having a flare effect (i.e., an acute stimulation of gonadotropin secretion that is seen during the initial treatment of individuals with superagonists).

Pulsatile Gonadotropin-Releasing Hormone Stimulation

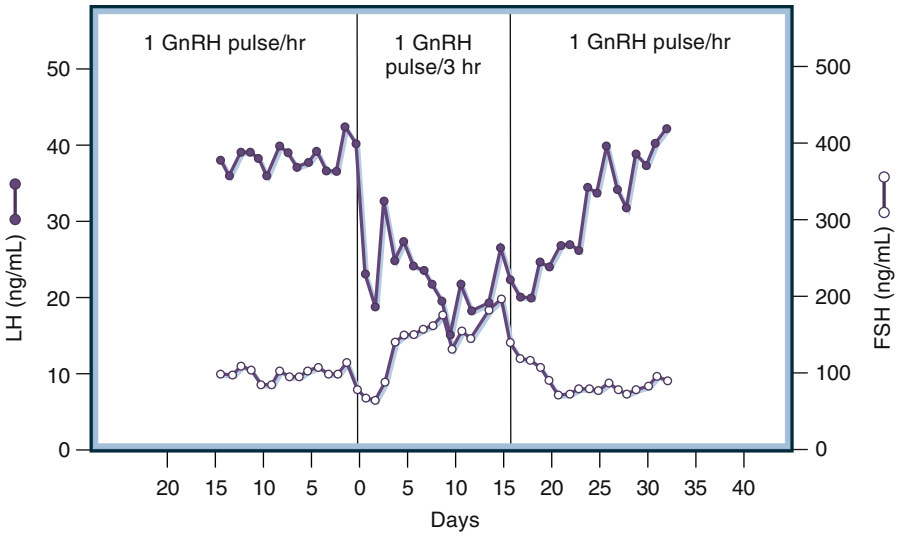
Because a single pulse of GnRH stimulates the release of both LH and FSH, and chronic exposure of the pituitary to pulsatile GnRH supports the synthesis of both LH and FSH, it is generally believed that there is only one releasing factor regulating the synthesis and secretion of LH and FSH. However, in a number of physiologic conditions, there are divergent patterns of LH and FSH secretion; thus a second FSH-releasing peptide has been proposed, but such a peptide has not been isolated to date. Other mechanisms, discussed in more detail later, are likely to account for the differential regulation of LH and FSH release.

The ensemble of GnRH neurons in the hypothalamus that send axons to the portal blood system in the median eminence fire in a coordinated, repetitive, episodic manner, producing distinct pulses of GnRH in the portal bloodstream. The pulsatile nature of GnRH stimulation to the pituitary leads to the release of distinct pulses of LH into the peripheral circulation. In experimental animals, in which it is possible to collect blood samples simultaneously from the portal and peripheral blood, GnRH and LH pulses have been found to correspond in about a 1:1 ratio at most physiologic rates of secretion.[448] Because the portal bloodstream is generally inaccessible in humans, the collection of frequent peripheral venous blood samples is used to define the pulsatile nature of LH secretion (i.e., frequency and amplitude of LH pulses), and pulsatile LH is used as an indirect measure of the activity of the GnRH secretory system. Indirect assessment of GnRH secretion by monitoring the rate of pulsatile LH secretion is also used in many animal studies examining the factors that govern the regulation of the pulsatile activity of the reproductive neuroendocrine axis. Unlike LH secretion, FSH secretion is not always pulsatile; even when it is pulsatile, there is only partial concordance between LH and FSH pulses.

It is possible to place multiple-unit recording electrodes in the medial basal hypothalamus of monkeys and other species and detect spikes of electrical activity that are concordant with the pulsatile secretion of LH secretion.[449] However, it is unknown whether these bursts of electrical activity reflect the activity of GnRH neurons themselves or the activity of neurons that impinge on GnRH neurons and govern their firing. With the development of mice in which the gene for green fluorescent protein has been put under the regulation of the GnRH promoter, it has been possible to identify GnRH neurons in hypothalamic tissue slices using fluorescence microscopy, to record from them intracellularly,[16] and to simultaneously measure GnRH release from the median eminence by fast-scan cyclic voltammetry.[450] These studies have shown that many, but not all, GnRH neurons show a bursting pattern of electrical activity. A central question in the field of reproductive neuroendocrinology is what causes GnRH neurons to pulse in a coordinated manner. Embryonic GnRH neurons from rhesus monkeys have shown intrinsic oscillatory changes in intracellular calcium concentration and synchronized calcium peaks among tens of neurons associated with GnRH release. A mathematical network model has been developed to further characterize this synchronization process.[451] The term *GnRH pulse generator* is often used to acknowledge the fact that GnRH secretion occurs in pulses and to refer to the central mechanisms responsible for pulsatile GnRH release.

A critical factor governing LH and FSH secretion is the rate of pulsatile GnRH stimulation of the gonadotrophs. Experimental studies in which the hypothalamus was lesioned and GnRH was replaced by pulsatile administration of exogenous GnRH showed that different frequencies of GnRH can lead to different ratios of LH to FSH secretion from the pituitary. Fig. 7.29 shows that in a monkey with a hypothalamic lesion, replacement of one pulse of GnRH per hour led to a relatively low ratio of FSH to LH secretion. Subsequent institution of a slower pulse frequency (one pulse of GnRH every 3 hours) led to a decrease in LH secretion but an increase in FSH secretion so that the ratio of FSH to LH secretion was greatly elevated. Thus, during the normal menstrual cycle, LH pulse frequency increases during the follicular phase as the midcycle ovulatory phase is approached, reflecting increased GnRH pulsatility, and then slows during the luteal phase. Abnormally low GnRH pulsatility is associated with delayed puberty, hypothalamic amenorrhea, opioid-induced hypogonadism, hyperprolactinemia, and obesity-related hypogonadism, whereas an inappropriately high GnRH pulsatility is associated with precocious puberty and polycystic ovarian syndrome (PCOS).[452] As discussed later, steroid hormones act at both the hypothalamus and pituitary to strongly influence the rate of pulsatile GnRH release and amount of LH and FSH secreted from the pituitary.

GnRH pulse frequency not only influences the rate of pulsatile gonadotropin release and the ratio of FSH to LH secretion, but also plays an important role in modulating the structural makeup of the gonadotropins. LH and FSH are structurally similar glycoprotein hormones. Each of these hormones is made up of an α-subunit and a β-subunit. LH, FSH, and TSH share a common α-subunit, and each has a unique β-subunit that conveys receptor specificity to the intact hormone. Before secretion of gonadotropins, terminal sugars are attached to each gonadotropin molecule.[162] The sugars include sialic acid, galactose, *N*-acetylglucosamine, and mannose,



• **Fig. 7.29** The influence of gonadotropin-releasing hormone (GnRH) pulse frequency on luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion in a female rhesus monkey with an arcuate nucleus lesion ablating endogenous GnRH support of the pituitary. Decreasing the GnRH pulse frequency from 1 pulse every hour to 1 pulse every 3 hours led to a decrease in plasma LH concentrations but an increase in plasma FSH concentrations. (Redrawn from Wildt L, Haulser A, Marshall G, et al. Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. *Endocrinology.* 1981;109:376–385.)

but the most important is sialic acid. The extent of glycosylation of LH and FSH is important for the physiologic function of these hormones.[162] Forms of gonadotropin with more sialic acid have a longer half-life because they are protected from degradation by the liver. Forms of gonadotropin with less sialic acid have more potent effects at their biologic receptors. Both the rate of GnRH stimulation and ovarian hormone feedback at the level of the pituitary regulate the degree of LH and FSH glycosylation. For example, slow frequencies of GnRH, seen during follicular development, are associated with greater degrees of FSH glycosylation, which would provide sustained FSH support to growing follicles. In contrast, faster frequencies of GnRH, seen just before the midcycle gonadotropin surge, are associated with lesser degrees of FSH glycosylation, providing a more potent but shorter lasting form of FSH at the time of ovulation.[453]

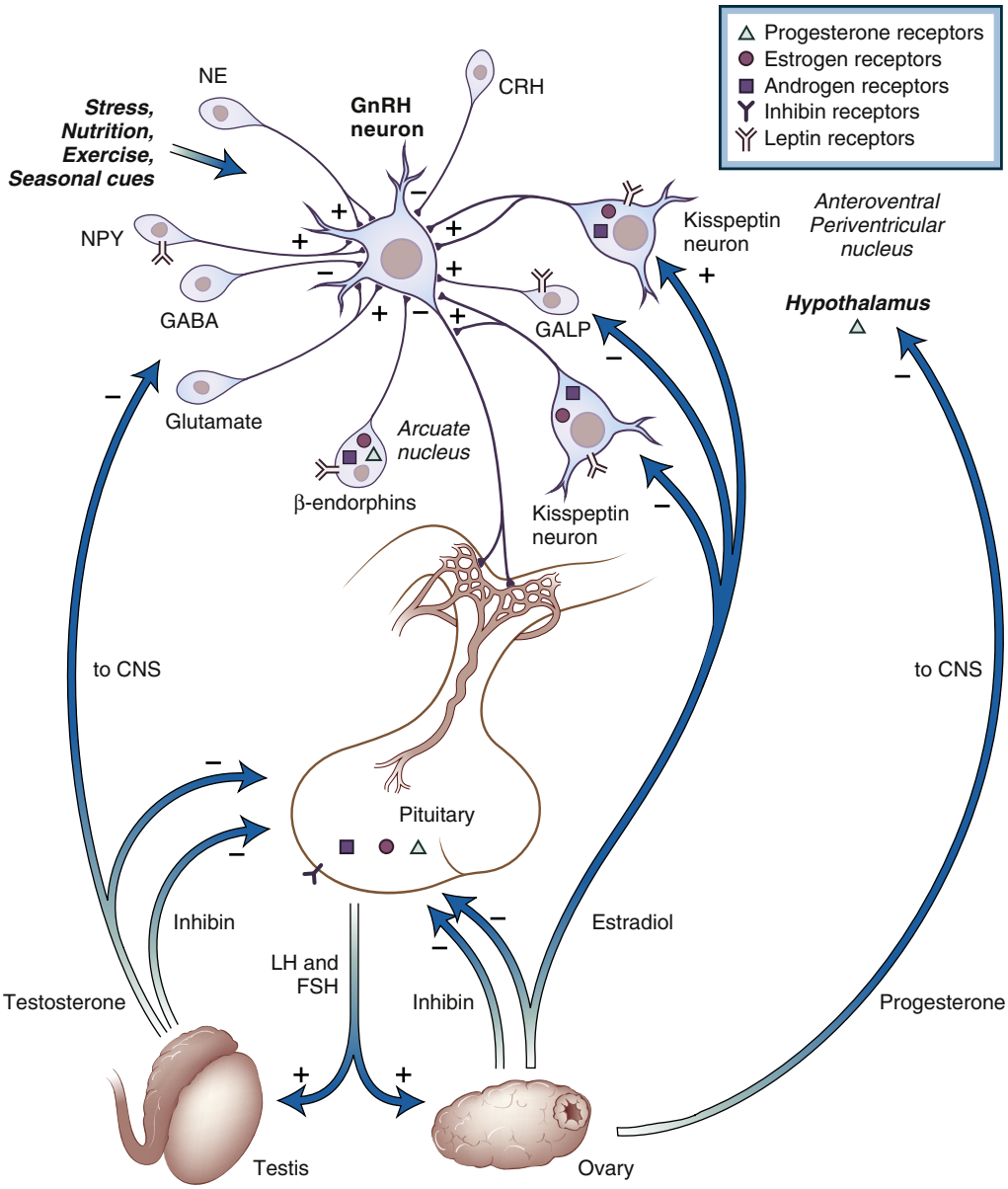
Regulatory Systems

Many neurotransmitter systems from the brainstem, limbic system, and other areas of the hypothalamus convey information to GnRH neurons (Fig. 7.30). These afferent systems include neurons that contain norepinephrine, dopamine, serotonin, GABA, glutamate, kisspeptin, endogenous opiate peptides, NPY, galanin, and a number of other peptide neurotransmitters. Glutamate and norepinephrine play important roles in providing stimulatory drive to the reproductive axis, whereas GABA and endogenous opioid peptides provide a substantial portion of the inhibitory drive to GnRH neurons. However, it is the kisspeptin neuronal network that is particularly essential for regulation of the GnRH neurons[454] and will be discussed in greater detail.

GnRH neuronal cell bodies are surrounded by astrocytes that may contribute to GnRH secretion through the release of prostaglandin E_2 (PGE_2) and/or transforming growth factor beta (TGFβ).[455] Changes in the steroid hormone milieu also influence the degree of glial sheathing of GnRH axon terminals in the external zone of the median eminence and have important roles in regulating delivery to the portal capillary plexus for conveyance to the anterior pituitary. Thus, during diestrus in the rat when GnRH output to the pituitary is very low, GnRH axon terminals in the median eminence are fully ensheathed by tanycyte end-feet processes, preventing GnRH from reaching the portal blood. The sprouting of tanycyte end-feet processes to engulf GnRH-containing axon terminals is mediated by transforming growth factor beta 1 (TGFβ1) and SEMA7A.[59] Conversely, during the preovulatory surge of proestrus when there is an increase in GnRH, estrogen binds to alpha-type estrogen receptors on tanycytes and induces retraction of tanycyte foot processes through PGE_2-dependent production of TGFβ1, allowing GnRH axon terminals to have direct contact with the fenestrated portal capillary system.[456] Estrogen may also affect the synthesis of adhesion molecules such as polysialylated neuronal cell adhesion molecule (PSA-N-CAM) and synaptic cell adhesion molecule (SynCAM) that facilitate glial-neuronal interactions and remodeling.[457] Endothelial cells of the portal capillary system may also contribute to tanycyte plasticity via estrogen-induced regulation of nitric oxide production that affects actin cytoskeleton remodeling.[458]

Feedback Regulation

Steroid hormone receptors are abundant in the hypothalamus and in many neural systems that impinge on GnRH neurons, including noradrenergic, serotonergic, kisspeptin, β-endorphin–containing, and NPY neurons. Early studies identifying regions of the brain that bound labeled estrogens showed that in rodents the preoptic area and VMH had the highest concentrations of estrogen receptors in the brain. Further localization studies, identifying estrogen receptors by immunocytochemistry or in situ hybridization, confirmed the strong presence of estrogen receptors in the hypothalamus and in brain areas with abundant connections to the hypothalamus, including the amygdala, septal nuclei, BNST, medial part of the NTS, and lateral portion of the parabrachial nucleus.[459] In 1986, a new member of the steroid hormone receptor superfamily with high-sequence homology to the classic estrogen receptor (now referred to as *estrogen receptor-α*) was isolated



• **Fig. 7.30** Regulation of the hypothalamic-pituitary-gonadal axis. Schematic diagram of the hypothalamic-pituitary-gonadal axis showing neural systems that regulate gonadotropin-releasing hormone (GnRH) secretion and feedback of gonadal steroid hormones at the level of the hypothalamus and pituitary. *CNS,* central nervous system; *CRH,* corticotropin-releasing hormone; *FSH,* follicle-stimulating hormone; *GABA,* γ-aminobutyric acid; *GALP,* galanin-like peptide; *LH,* luteinizing hormone; *NE,* norepinephrine; *NPY,* neuropeptide Y.

from rat prostate and named *estrogen receptor-β*. This novel estrogen receptor was shown to bind estradiol and to activate transcription by binding to estrogen response elements.[460]

In situ hybridization studies examining the localization of estrogen receptor-β mRNA have shown that these receptors are present throughout the rostral-caudal extent of the brain, with a high level of expression in the preoptic area, BNST, PVH and SON, amygdala, and laminae II to VI of the cerebral cortex.[461] Specific receptors for progesterone are induced by estrogen in hypothalamic regions of the brain, including the preoptic area, the ventromedial and ventrolateral nuclei, and the infundibular-arcuate nucleus, although there is also evidence for constitutive expression of progesterone receptors in some regions.[462] Androgen receptor mapping studies have shown considerable overlap in the distribution of androgen and estrogen receptors throughout the brain. The highest density of androgen receptors was found in hypothalamic nuclei known to participate in the control of reproduction and sexual behaviors, including the arcuate nucleus, PVH, MePO, ventromedial nucleus, and brain regions with strong connections to the hypothalamus (including the amygdala, nuclei of the septal region, BNST, NTS, and lateral division of the parabrachial nucleus).[459] The anterior pituitary also contains receptors for all of the gonadal steroid hormones.

Steroid hormones can dramatically alter the pattern of pulsatile release of GnRH and of the gonadotropins through actions at both the hypothalamus and the pituitary. At the hypothalamus, estradiol, progesterone, and testosterone can all act to slow the frequency of GnRH release into the portal bloodstream as part

of a closed negative feedback loop.[463] However, GnRH neurons lack estrogen receptors, indicating that the effects of estrogen on GnRH secretion is mediated by other neural systems that provide afferent input to GnRH neurons. Most important is the kisspeptin signaling pathway, which is now well established to be involved in the central regulation of puberty and reproduction by stimulating GnRH from the hypothalamus.

Kisspeptin, a RFamide-like peptide and the natural ligand for the former orphan GPCR, GPR54, is a 54–amino acid peptide that derives from the *KISS1* gene located on chromosome 1q33, although smaller forms that contain 10 to 14 amino acids have also been identified. Inactivating mutations of the *KISS1* gene result in hypogonadotropic hypogonadism or pubertal delay,[464] whereas activating mutations result in precocious puberty.[465] These neurons have been identified in two discrete areas in mammals: (1) the preoptic area and the infundibular nucleus in humans and primates and (2) the anteroventral periventricular nucleus (AVPV)/preoptic periventricular nucleus (defined together as the rostral periventricular area of the third ventricle [RP3V]) and arcuate nucleus in ruminants.[466–468]

Although GnRH neurons have an endogenous rhythmic activity in culture, they do not correlate well to the pulses of GnRH observed in vivo; therefore the pulse generator has largely been ascribed to the kisspeptin neurons. Kisspeptin neurons express estrogen (both α and β) and progesterone receptors and densely innervate GnRH neuronal cell bodies, particularly their axon terminals in the median eminence. In addition, GnRH neurons express the kisspeptin receptor (KISS1R).[469] Pulsatile secretion of GnRH is dependent upon pulsatile secretion of kisspeptin, as if kisspeptin is administered continuously, it results in suppression of LH secretion.[470] The mechanism by which pulsatile secretion of kisspeptin occurs is proposed to be mediated by at least two additional peptides coexpressed by kisspeptin neurons in the infundibular/arcuate nucleus with opposing actions, namely the tachykinin neurokinin B, which stimulates kisspeptin secretion, and dynorphin (or possibly enkephalin in humans[471]), which inhibits kisspeptin secretion.[454,472] For this reason, this population of kisspeptin neurons is commonly referred to as KNDy neurons.[472] This concept is supported by the presence of both tachykinin receptors and κ-opioid receptors on KNDy neurons and the extensive axosomatic and axodendritic interconnections among these neurons that coordinate their activity.[466] In addition, inactivating mutations of the genes encoding for neurokinin B or the neurokinin B receptor, NK3R, results in hypogonadotropic hypogonadism.[473,474] Substance P has also been shown to have an activating effect on KNDy neurons and is either synthesized within KNDy neurons themselves or by adjacent populations of neurons that innervate KNDy neurons, depending upon the animal species.[454,475] The action of kisspeptin on GnRH secretion, however, may occur primarily at axon terminals, as injection of a kisspeptin antagonist into the median eminence of monkeys can abolish GnRH pulses.[476]

Phoenixin-20 amide (PNX), a novel peptide that derives from the small, integral membrane protein 20 (SMIM20) and highly conserved among species, has also been found to have a role in regulation of the gonadal axis both by sensitizing gonadotrophs in the anterior pituitary to GnRH and through direct effects on GnRH neurons.[477] The observation that PNX is synthesized in kisspeptin neurons and increases KISS1 mRNA expression, and that kisspeptin neurons express GPR123 receptor for which PNX is a ligand, has raised the possibility that the peptide may activate kisspeptin neurons through an autocrine mechanism or through connections with other kisspeptin/PNS neurons.

Most of the time, the hypothalamic-pituitary axis is under the negative feedback influence of gonadal steroid hormones. If the gonads are removed surgically or their normal secretion of steroid hormones is suppressed pharmacologically, there is a dramatic increase (10-fold to 20-fold) in circulating levels of LH and FSH secretion in both males and females.[463] This type of *castration response* occurs normally at menopause in women, when ovarian follicular development and therefore ovarian production of large quantities of estradiol and progesterone decrease and eventually cease.

KNDy neurons in the infundibular nucleus are involved in mediating negative feedback regulation of GnRH release by steroid hormones, including estradiol (E_2), progesterone, and testosterone.[478–480] Estrogen suppresses kisspeptin and neurokinin B from infundibular KNDy neurons, and both kisspeptin and neurokinin B gene expression are upregulated in ovariectomized animals and postmenopausal women.[481] Negative feedback of steroid hormones can also occur directly at the level of the pituitary. For example, estradiol has been shown to be capable of binding to the pituitary, decreasing LH and FSH synthesis and release, and decreasing the sensitivity of pituitary gonadotrophs to the actions of GnRH so that less LH and FSH are released when a pulse of GnRH stimulates the pituitary. Evidence for such a direct pituitary action of estradiol came from studies with rhesus monkeys that had been rendered deficient in endogenous GnRH by a lesion in the arcuate nucleus and showed a decline in endogenous gonadotropin secretion. When these monkeys received a pulsatile regimen of GnRH treatments, subsequent estradiol infusions dramatically suppressed the responsiveness of the pituitary to GnRH and suppressed the gonadotropin secretion that was being driven by the pulsatile administration of GnRH.[482] Similarly, in a compound mutant mouse model on a GnRH-deficient (*Hpg*) genetic background, expression of a human FSH-β transgene was inhibited by testosterone directly at the pituitary level.[483] In primate species, including humans, there is considerable feedback of estradiol at the pituitary, but most of the progesterone and testosterone negative feedback occurs at the level of the hypothalamus.[463]

In addition to negative feedback, estradiol can have a positive feedback action at the level of the hypothalamus and pituitary to lead to a massive release of LH and FSH from the pituitary. This release of gonadotropins occurs once each menstrual cycle and is referred to as the *LH-FSH surge*. The positive feedback action of estradiol occurs as a response to the rising tide of estradiol that is produced during the process of dominant follicle development in the late follicular phase of the menstrual cycle. In women, elevated estradiol levels are generally maintained at about 300 to 500 pg/mL for about 36 hours before the stimulation of the gonadotropin surge.

Experiments have shown that both a critical concentration and duration of elevated estradiol are necessary to achieve positive feedback and a resulting gonadotropin surge. If supraphysiologic doses of estradiol are administered, the surge can occur as early as 18 hours after their administration. Because the ovary is responsible for the production of estradiol, and the time course and magnitude of estradiol release control the rate of positive feedback, the ovary has been referred to as the *zeitgeber* of the menstrual cycle. The dependence of the positive feedback system on the magnitude of estradiol production helps explain the fact that the portion of the menstrual cycle that varies most in length is the follicular phase. Production of higher levels of estradiol by

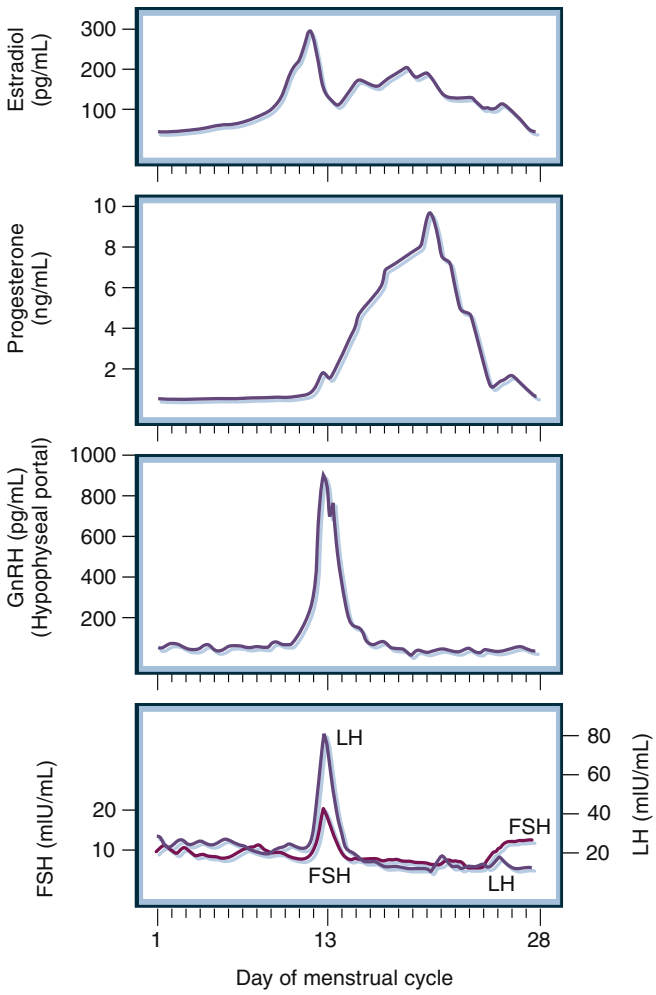
a dominant follicle in one cycle leads to a more rapid positive feedback action, with earlier ovulation and therefore a shorter follicular phase, compared to a cycle in which the dominant follicle produced lower levels of estradiol.

As with negative feedback in response to estradiol, the positive feedback actions of estradiol occur both at the hypothalamus to increase GnRH secretion and at the pituitary to enhance pituitary responsiveness to GnRH. Estradiol increases pituitary sensitivity to GnRH by increasing the synthesis of new GnRH receptors and by enhancing the responsiveness to GnRH at a postreceptor site of action. At the level of the hypothalamus, kisspeptin neurons in the preoptic and RPV3 nucleus are believed to function as the *surge center* to induce the ovulatory surge of GnRH. This particular subpopulation of kisspeptin neurons is activated in an estrogen-dependent manner immediately preceding the GnRH surge, as detected by Fos expression, and is postulated to play a key role in the positive feedback effects of estradiol on GnRH release.[484] In addition, lesions in areas adjacent to the medial preoptic area, near the anterior commissure and septal complex, block the ability of estrogen to induce a surge in these species without blocking its negative feedback effects.[485] It is also of note that kisspeptin expression in the RPV3 neurons is sexually diergic, with a much greater number of kisspeptin neurons in the female. The role of preoptic kisspeptin neurons in mediating the ovulatory surge in humans is controversial, however, as it is possible that KNDy neurons in the infundibular nucleus participate in the positive feedback effects of estrogen.[486] Cellular mechanisms that mediate the switch from negative to positive feedback of estrogen are not fully understood, but there is support for the concept that estrogen induction of various transcription factors and receptors may play an important role in mediating this switch.[487] Alternatively, KNDy neurons may not consist of a homogeneous population.

Regulation of the Ovarian Cycle

Cyclic activity in the ovary is controlled by an interplay between steroid hormones produced by the ovary and the hypothalamic-pituitary neuroendocrine components of the reproductive axis. The duration of each phase of the ovarian cycle is species dependent, but the general mechanisms controlling the cycle are similar in all species that have spontaneous ovarian cycles. In the human menstrual cycle, day 1 is designated as the first day of menstrual bleeding. At this time, small and medium-sized follicles are present in the ovaries, and only small amounts of estradiol are produced by the follicular cells. As a result, there is a low level of negative feedback to the hypothalamic-pituitary axis, LH pulse frequency is relatively fast (one pulse about every 60 minutes), and FSH concentrations are slightly elevated compared to much of the rest of the cycle (Fig. 7.31). FSH acts at the level of the ovarian follicles to stimulate development and causes an increase in follicular estradiol production, which in turn provides increased negative feedback to the hypothalamic-pituitary unit.

A result of the increased negative feedback is a slowing of pulsatile LH secretion over the course of the follicular phase to a rate of about one pulse every 90 minutes. However, as the growing follicle (or follicles, depending on the species) secretes more estradiol, the positive feedback action of estradiol is triggered, which leads to an increase in GnRH release and the LH-FSH surge. The surge of gonadotropins acts at the fully developed follicle to stimulate the dissolution of the follicular wall, leading to ovulation of the matured ovum into the nearby fallopian tube, where fertilization takes place if sperm are present. Ovulation results in a reorganization of the cells of the follicular wall; the cells undergo



• **Fig. 7.31** Diagrammatic representation of changes in plasma levels of estradiol, progesterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) and portal levels of gonadotropin-releasing hormone (GnRH) over the human menstrual cycle.

hypertrophy and hyperplasia and start to secrete large amounts of progesterone and some estradiol. Progesterone and estradiol have a negative feedback effect at the level of the hypothalamus and pituitary, so the LH pulse frequency becomes very slow during the luteal phase of the menstrual cycle. The corpus luteum has a fixed life span; without additional stimulation in the form of human chorionic gonadotropin (hCG) from a developing embryo, the corpus luteum regresses spontaneously after ~14 days and progesterone and estradiol secretions diminish. This reduces the negative feedback signals to the hypothalamus and pituitary and allows an increase in FSH and LH secretion. The fall in progesterone is also a withdrawal of steroid hormone support to the endometrial lining of the uterus; as a result, the endometrium is shed as menses, and a new cycle begins.

In other species, the interplay between the neuroendocrine and ovarian hormones is similar, but the timing of events is different, and other factors, such as circadian and seasonal regulatory factors, play a role in regulating the cycle. The rat has an ovarian cycle without menses (the endometrial lining is absorbed rather than shed) of 4 to 5 days. The rat also shows strong circadian rhythmicity in the timing of the LH-FSH surge, with the surge always occurring in the afternoon of the day of proestrus. Sheep are an example of a species that has a strongly seasonal pattern of

ovarian cyclicity. During the breeding season, ewes have 15-day cycles, with a very short follicular phase and an extended luteal phase; during the nonbreeding season, signals relaying information about day length through the visual system, pineal, and SCN cause a dramatic suppression of GnRH neuronal activity, and cyclic ovarian function is prevented by a decrease in trophic hormonal support from the pituitary (see "Physiologic Roles of Melatonin").

Early Development and Puberty

Neuroendocrine stimulation of the reproductive axis is initiated during fetal development, and in primates in midgestation, circulating levels of LH and FSH reach values similar to those in castrated adults.[488] Later in gestational development, gonadotropin levels decline, restrained by rising levels of circulating gonadal steroids. The steroids that have this effect are probably placental in origin because after parturition there is a rise in circulating gonadotropin levels that is apparent for variable periods of the first year of life, depending on the species. The decline in reproductive hormone secretion in the postnatal period appears to be due to a decrease in GnRH stimulation of the reproductive axis because it occurs even in the castrated state and because gonadotropin and gonadal steroid secretion can be supported by the administration of pulses of GnRH.[489]

Pubertal reawakening of the reproductive axis occurs in late childhood and is marked initially by nighttime elevations in gonadotropin and gonadal steroid hormone levels.[489,490] The mechanisms controlling the pubertal reawakening of the GnRH pulse generator have been an area of intense investigation.[489,491] Although the mechanisms are still not fully understood, significant progress has been made in identifying central changes in the hypothalamus that appear to play a role in this process. At puberty, there is both a decrease in transsynaptic inhibition to the GnRH neuronal system and an increase in stimulatory input to GnRH neurons.[489] One of the major inhibitory inputs to the GnRH system is provided by GABAergic neurons. Studies in rhesus monkeys have shown that hypothalamic levels of GABA decrease during early puberty and that blocking GABAergic input before puberty, by intrahypothalamic administration of antisense oligodeoxynucleotides against the enzymes responsible for GABA synthesis, results in premature activation of the GnRH neuronal system.

It has been suggested on the basis of findings that a subset of glutamate receptors (i.e., kainate receptors) increase in the hypothalamus at puberty and that the pubertal decrease in GABA tone may be caused by an increase in glutamatergic transmission. Further evidence for a role for glutamate comes from studies showing that administration of *N*-methyl-DL-aspartic acid (NMDA) to prepubertal rhesus monkeys can drive the reawakening of the reproductive axis.[492] Increased stimulatory drive to the GnRH neuronal system also appears to come from increases in norepinephrine and NPY at the time of puberty.[489] Furthermore, as discussed earlier, there is evidence that growth factors act through release of prostaglandin from glial cells at puberty to play a role in stimulating GnRH neurons.[493]

Despite an increased understanding of the neural changes occurring at puberty, the question of what signals trigger the pubertal awakening of the reproductive axis is unanswered at this time.[491,494] However, the kisspeptin neuronal system described earlier in the section on feedback regulation has become a prime integrative candidate for this function, perhaps mediated by the activating effects of neurokinin B and substance P or increased tachykinin sensitivity.[484,454] Also relevant to pubertal onset is galanin-like peptide (GALP), which is expressed specifically in the arcuate nucleus and binds with high affinity to galanin receptors. GALP is a potent central stimulator of gonadotropin release and sexual behavior in the rat and can reverse the decreased reproductive function associated with diabetes mellitus and hypoinsulinemia.[495] Whereas GnRH neurons do not express leptin receptors, both kisspeptin and GALP neurons are targets of leptin and are hypothesized to be involved in the well-known modulation of puberty and reproductive function by food availability and nutritional status (see the following section). Epigenetic mechanisms have been proposed to coordinate the changes in the expression of multiple genes that accompany the initiation of puberty.[491]

Reproductive Function and Stress

Many forms of physical stresses, such as fasting, exercise, temperature stress, infection, pain, and injury, as well as psychologic stresses, such as being subordinate in a dominance hierarchy, can suppress the activity of the reproductive axis.[496] If the stress exposure is brief, there may be acute suppression of circulating gonadotropins and gonadal steroid hormones; in females disruption of normal menstrual cyclicity may occur, but fertility is unlikely to be impaired.[496] In contrast, prolonged periods of significant stress exposure can lead to complete impairment of reproductive function, also characterized by low circulating levels of gonadotropins and gonadal steroids. Stress appears to decrease the activity of the reproductive axis by decreasing GnRH drive to the pituitary because in all cases in which it has been examined, administration of exogenous GnRH can reverse the effects of the stress-induced decline in reproductive hormone secretion.

In the case of foot shock stress in rats[497] or immune stress (i.e., injection of IL-1α) in primates,[498] the suppression of gonadotropin secretion that occurs was shown to be reversible by administration of a CRH antagonist, implying that endogenous CRH secretion mediates the effects of these stresses on GnRH neurons. In other studies, naloxone, a μ-opioid receptor antagonist, was shown to be capable of reversing restraint stress–induced suppression of gonadotropin secretion in monkeys; however, naloxone is ineffective in reversing the suppression of gonadotropin secretion that occurs during insulin-induced hypoglycemia.[499,500] In the case of metabolic stresses, multiple regulators appear to mediate changes in the neural drive to the reproductive axis.

Various metabolic fuels, including glucose and fatty acids, can regulate the function of the reproductive axis, and blocking cellular utilization of these fuels can lead to suppression of gonadotropin secretion and decreased gonadal activity. Leptin, a hormone produced by fat cells, can also modulate the activity of the reproductive axis. Mutant *ob/ob* mice that are deficient in leptin are infertile, but fertility can be restored by administration of leptin.[501] Moreover, leptin administration has been shown to reverse the suppressive effects of undernutrition on the reproductive axis in some situations.[502] Leptin receptors are found in several populations that are known to have a strong influence on the reproductive axis, particularly kisspeptin neurons.[484] Furthermore, treatment with a neurokinin B agonist and dynorphin antagonist can recover suppressed GnRH and LH secretion.[503]

In summary, it appears that a number of neural circuits can mediate the effects of stress on the GnRH neuronal system and that the neural systems involved are at least somewhat specific to the type of stress that is experienced.

Thermoregulation and Sleep-Wake Cycle

In addition to regulation of the anterior and posterior pituitary, the hypothalamus orchestrates a number of functions that are critical for normal homeostasis, including the maintenance of normal body temperature and its resetting under certain physiologic conditions and the regulation of sleep and wakefulness. Appetite regulation is discussed in Chapter 39.

Preoptic Area Is the Primary Hypothalamic Thermoregulatory Center

It has been long recognized that the brain plays an important role in the regulation of body temperature mediated by neurons that respond to heat, commonly referred to as warm-sensitive neurons, and neurons that respond to cold, referred to as cold-sensitive neurons,[504] with the preoptic area (POA) being the most important thermoregulatory locus (see Siemens and Kamm[505] for comprehensive review). Neurons in this region serve as a major integrating center that functions to maintain a constant body temperature by receiving information from surface thermoreceptors in the skin, spinal cord, and deep structures in the abdominal cavity that are relayed to the POA through the lateral parabrachial nucleus by multisynaptic pathways via the dorsal horn of the spinal cord or splanchnic and vagal afferents.[504] In addition, some neurons in the POA have the intrinsic ability to sense changes in local brain temperature. Preoptic warming reduces heat production and increases heat-loss responses by vasodilation, sweating, increased respiration, inhibition of UCP-1 in brown adipose tissue (BAT), and specific behavioral responses such as moving to a cooler area; conversely, preoptic cooling increases heat production and reduces heat losses by inducing vasoconstriction, shivering, activation of UCP-1 in brown adipose tissue, increasing intermediary metabolism in muscle and other parenchymal organs, and by specific behavioral responses such as increasing locomotor activity and food intake.[506] As the number of warm-sensitive neurons is significantly greater than the number of cold-sensitive neurons in the POA, lesioning this region of the hypothalamus typically results in hyperthermia.[507]

Mechanisms for Thermoreception

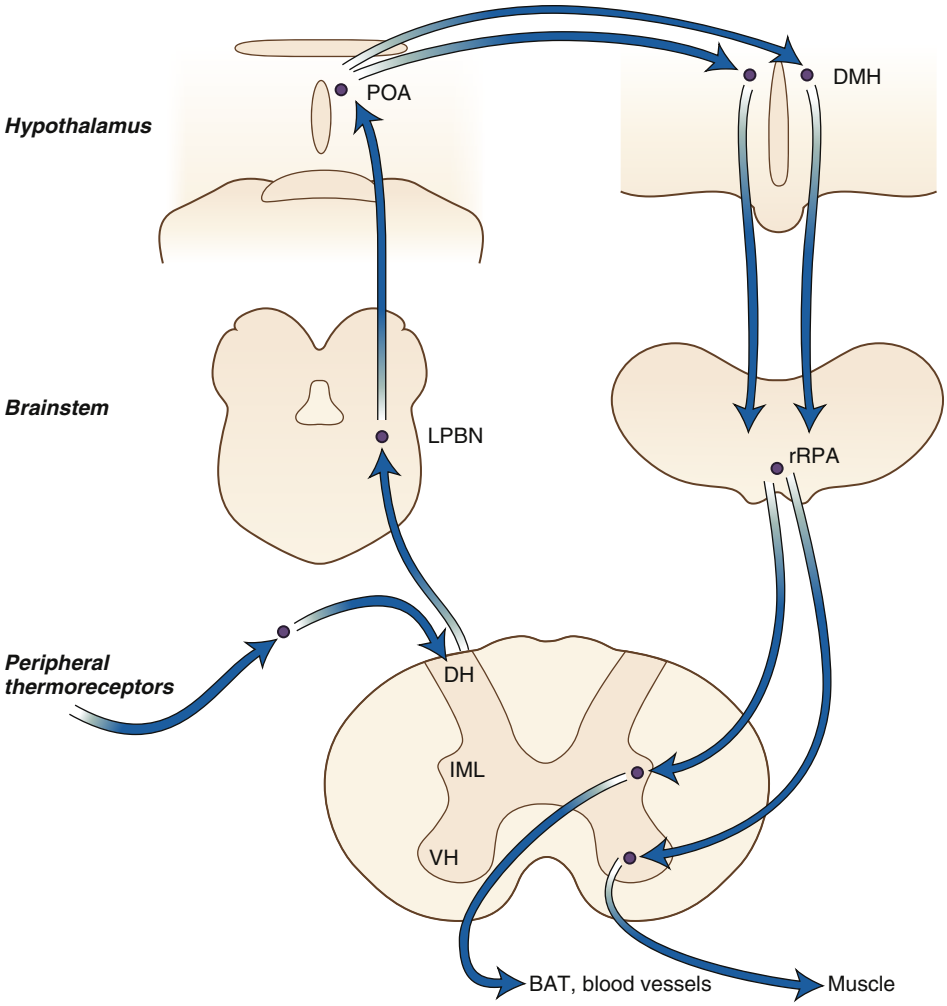
Within the periphery, primary sensory axon terminals express the TRP channel, transient receptor potential vanilloid 1 (TRPV1), which is activated by heat, and transient receptor potential cation channel subfamily M member 8 (TRPM8), which is activated by cold.[508] Animals with TRPM8 deficiency have impairment in maintaining normal body temperature when placed in a cold environment.[509] However, these animals can still thermoregulate when placed in extreme cold, indicating that other thermoreceptors and/or other mechanisms for temperature detection are also involved.

Within the POA, warm-sensitive neurons in the MePO and lateral preoptic nucleus (LPO) express transient receptor potential melastatin type 2 ion channels (TRPM2) that when selectively activated or inhibited results in profound hypothermia or hyperthermia, respectively.[510] In addition, transgenic animals deficient in TRPM2 have an exaggerated fever response. However, not all neurons in the POA involved in temperature regulation are intrinsically temperature sensitive. A subpopulation of neurons residing in the anterior ventromedial preoptic nucleus (VMPO), for example, respond to warming but do not express TRPM2 and therefore have been referred to as "warm-activated neurons."[511] These neurons receive inputs from skin receptors and are largely GABAergic but also coexpress the peptides BDNF and PACAP. When selectively activated using molecular techniques such as optogenetics, there is a decline in body temperature as a result of vasodilation and inhibition of heat generation from brown adipose tissue, as well as behavioral changes manifested by a preference for a colder environment.[511] Thus these warm-activated VMPO are capable of coupling three major heat-dissipating responses. Other subdivisions of the POA similarly trigger hypothermic responses when activated and receive thermal information from skin thermoreceptors, including the median preoptic nucleus (MnPO) and ventrolateral preoptic nucleus (VLPO).[512,513] Thus the POA is the primary locus for integrating a variety of thermoregulatory information, and through outputs to regions of the brain involved in regulation of the autonomic nervous system, behavior and other neuroendocrine responses orchestrate a series of responses to maintain normal core body temperature.

Central Circuitries Mediating Thermoregulation

Downstream targets of thermosensitive POA neurons are primarily components of the sympathetic nervous system, of which the dorsomedial hypothalamus (DMH) in the hypothalamus, sympathetic premotor neurons in the rostral ventromedial medulla that include the rostral raphe pallidus (rRPa), and preganglionic autonomic neurons of the intermediolateral cell column in the spinal cord, are particularly important (Fig. 7.32).[514]

On the basis of numerous anatomic and physiologic studies and more recent molecular approaches (see recent reviews by Siemens and Kamm[505] and Tan and Knight[515]), it is now believed that several regions in the POA are involved in relaying thermal information from peripheral thermoreceptors to the autonomic nervous system that may work in synchrony, and that no one discrete population of neurons in the POA mediates any single thermodefensive response (autonomic or behavioral). The downstream circuitry involved was initially recognized for the MnPO, which in response to skin cooling leads to a number of thermoregulatory responses that increase body temperature, such as shivering, heat generation from BAT, and vasoconstriction. It is proposed that the MnPO releases GABA to inhibit MePO neurons, as antagonizing $GABA_A$ receptors in the MePO block cooling-induced shivering.[516] These neurons have downstream projections to the DMH and rRPA, and the DMN also extensively innervates the rRPA. If either the DMN or rRPa is lesioned, the shivering response is blocked. In addition, selective activation of DMN thermosensitive neurons using DREADDS technology increases in temperature, along with an increase in energy expenditure and physical activity.[512] The rRPA then projects to α-motor neurons in the ventral horn of the spinal cord to mediate the shivering response but also to preganglionic neurons in the intermediolateral column of the spinal cord to regulate BAT thermogenesis, sweating, and vasomotor responses.[517] The MnPO also regulates cutaneous blood flow,[518] although it may do so by direct projections to the rRPa, bypassing the DMH, as cooling-induced cutaneous vasoconstriction is not affected by inhibiting DMH neurons.[519] The midbrain periaqueductal gray (PAG) may also be involved in the circuitry before proceeding to sympathetic preganglionic neurons in the spinal cord as PAG neurons show strong c-Fos induction following unilateral preoptic region heating[520] and induce cutaneous vasodilation when stimulated.[521]



• **Fig. 7.32** Simplified diagram of major loci and pathways involved in thermoregulatory responses. *BAT,* brown adipose tissue; *DH,* dorsal horn; *DMH,* dorsomedial hypothalamus; *IML,* intermediolateral cell column; *LPBN,* lateral parabrachial nucleus; *POA,* preoptic area; *rRPa,* rostral raphe pallidus; *VH,* ventral horn. (Redrawn and modified from Roth J, Blatteis CM. Mechanisms of fever production and lysis: lessons from experimental LPS fever. *Compr Physiol.* 2014;4:1563–1604.)

Using a technique of molecular profiling of neurons in the POA activated by heat, a population of GABAergic neurons in the VMPO that coproduce PACAP and BDNF was identified, and when activated by optogenetics leads to a rapid decline in core body temperature that persists as long as the stimulation continues.[511] Mechanisms for the heat loss included vasodilation and suppression of heat production from BAT, as well as behavioral responses because the animals move to colder temperatures. Mapping of these neurons not only demonstrated a direct projection to the DMH, but also the PVN, ventrolateral PAG, and structures associated with behavior (including BNST, paraventricular thalamus, and medial habenula), indicating that the VMPO mediates diverse autonomic and behavioral responses to heat.

Similarly, activation of GABAergic neurons in VLPO triggers a rapid reduction in temperature associated with a decrease in physical activity; conversely, inhibition raises core temperature.[512] These neurons also project to the DMH, and stimulating VLPO terminals locally in the DMN using optogenetics is similarly effective in reducing temperature by inhibiting DMH neuronal activity. Because the transsynaptic retrograde marker, pseudorabies virus, accumulates in the VLPO following injection into BAT,[522] the VLPO is also presumed to contribute to BAT thermogenesis.

Modulators of Thermoregulatory Responses

In addition to information relayed to the POA from thermosensory afferent pathways, body temperature can also be affected by peptide and hormonal mediators as well through circuitries within the CNS that serve to integrate thermoregulation with other homeostatic functions. Perhaps one of best-studied examples is the effect of thyroid hormone on temperature homeostasis.

Thyroid Hormone

Thyroid hormone is essential to sustain the effect of sympathetic activation of BAT, and in its absence, norepinephrine fails to increase BAT temperature, resulting in cold intolerance and hypothermia commonly observed in hypothyroid individuals.[523] Norepinephrine increases type 2 iodothyronine deiodinase (D_2) in BAT, which by converting T_4 to T_3 results in the upregulation of UCP-1 in mitochondria, allowing conversion of stored energy into heat. Thyroid hormone is similarly important for a maximal thermogenic response by skeletal muscle in which activation of the sympathetic nervous system increases D_2, leading to the activation of UCP-3.[524] T_3 also has a direct, central action on neurons in the hypothalamic ventromedial nucleus, which, by decreasing

the phosphorylation of AMP-activated kinase (AMPK), leads to increased sympathetic activity of nerves innervating BAT.[525] T_3 may also modulate feeding-related neurons in the hypothalamic arcuate nucleus that produce NPY and AgRP and contribute to the effect of these peptides on BAT activity.

Estrogen and Progesterone

The set-point for temperature regulation is sensitive to circulating levels of sex steroids, with core body temperature falling just prior to the midcycle surge in women and rising during the luteal phase.[526] Estrogen is responsible for the fall in temperature in the late follicular phase by activating warm-sensitive neurons in the POA,[527] whereas progesterone increases temperature in the luteal phase by decreasing the firing rate of these neurons and, perhaps, increasing the firing rate of cold-sensitive neurons.[528] Thermoregulatory effects of estrogen may also be mediated by KNDy neurons in the hypothalamic arcuate nucleus as further elaborated in "Neurokinin B." Estrogen and progesterone exert effects on peripheral blood vessels, with estrogen promoting vasodilation (and thereby heat dissipation) by activating nitric oxide synthetase (eNOS) in endothelial cells to produce nitric oxide,[529] and progesterone promoting vasoconstriction by enhancing adrenergic control through a prostaglandin-mediated mechanism.[530] It remains unclear as to the physiologic importance of these changes in body temperature over the menstrual cycle, although it has been proposed the increase in temperature during the luteal phase may facilitate implantation.[531]

Neurokinin B

Vasomotor symptoms, commonly referred to as hot flashes, are a frequent occurrence in menopausal women, can persist for many years, and disrupt sleep and daily activities. They are characterized by the sudden feeling of heat, leading to vasodilation and sweating as measures to increase heat loss. While the underlying etiology has long been known to be related to estrogen deficiency, the thermoregulatory mechanisms involved have only recently been elucidated with the discovery of kisspeptin and its role in regulation of GnRH (reviewed by Prague and Dhillo[532]). As mentioned previously (see "Gonadotropin-Releasing Hormone and Control of the Reproductive Axis"), kisspeptin neurons in the arcuate or infundibular nucleus coexpress neurokinin B and in some animal species, dynorphin, thereby coined KNDy neurons. During menopause, KNDy neurons hypertrophy and show increased neuronal excitability, presumably due to feedback effects of estrogen deficiency as these changes can be reversed by estrogen administration.[481,533] In addition, if KNDy neurons are selectively ablated, altered thermoregulatory responses are observed, suggesting that KNDY neurons are involved in the mechanism of heat dissipation.[534] Particularly noteworthy is that a population of thermosensitive neurons in the POA receive inputs from KNDy neurons[535] and also express the neurokinin 3 receptor (NK3R).[536] Thus, when a NK3R agonist is administered into the POA of experimental animals or neurokinin B is administered peripherally to human subjects, heat dissipation responses are observed.[537,538] Conversely, administration of an oral NK3R antagonist (MLE4901) to postmenopausal women reduces the frequency and severity of hot flashes.[539,540] As surmised by Prague and Dhillo,[532] the improvement in postmenopausal vasomotor symptoms observed with NK3R antagonists may provide an alternative to the use of estrogens, particularly in women with a history of or at risk of developing breast cancer, and for symptomatic men who are being treated for prostate cancer with androgen deprivation therapy.

Orexin-A

Orexin-A (sometimes referred to as hypocretin 1) derives exclusively from neurons in the lateral hypothalamic area, but it is contained in axons that are widely distributed throughout the central nervous system, including projections to thermoregulatory centers of the sympathetic nervous system such as the periaqueductal gray, rRPA, and intermediolateral cell column of the spinal cord.[541–543] While best known for its effects on arousal (see "Sleep-Wake Cycle") and its deficiency is etiologic for narcolepsy,[544] the peptide also regulates body temperature. Injection of orexin-A into the CSF increases sympathetic activity, including increased c-Fos in the rRPA,[545] and is associated with a rise in body temperature mediated by BAT thermogenesis.[546] The importance of this peptide (and/or associated co-neurotransmitters) in the maintenance of thermoregulatory homeostasis is indicated by a blunted hyperthermic response to cold exposure in mice with ablation of orexin neurons[547] and may explain dysregulation of thermogenesis in individuals with narcolepsy.[548]

Prostaglandins

Under certain circumstances, there is an adaptive advantage to elevate body temperature beyond the normal physiologic range that is highly conserved among animal species. Such is the situation during infection, when fever is a necessary response to facilitate recovery by improving the efficiency of immune cells and impairing replication of microorganisms.[549] A variety of cytokines, including IL-1, IL-6, and TNFα, are secreted in response to pathogens, resulting in the production of PGE_2 by the brain vasculature. GABAergic neurons in the MnPO and MePO express the prostaglandin EP_3 receptor, which when bound by PGE_2 alters the thermoregulatory set-point and induces fever.[550,551] EP_3 receptor POA neurons heavily project to DMN and rRPA,[552,553] disinhibiting these sympathoexcitatory neurons to provide excitatory drive for sympathetic outflow to BAT and to promote shivering through rRPa projections to ventral horn motor cells.[554]

In addition to inducing fever, endotoxin simultaneously activates an endogenous, counterregulatory, antipyretic response to prevent body temperature from rising too severely. This is largely achieved by stimulating the hypothalamic-pituitary-adrenal axis that exerts a dampening effect on the cytokine response, but more specifically by the direct antipyretic actions of α-MSH within the CNS.[555] As the VMPO is heavily innervated by axon terminals containing α-MSH, a direct effect on POA thermosensitive neurons is suggested.[556] Alpha-MSH is also contained in axons that heavily innervate autonomic regulatory neurons in the parvocellular PVN and the hypothalamic DMN, providing an alternative route for regulatory control over vasomotor responses and heat generation.

Opioids and Endocannabinoids

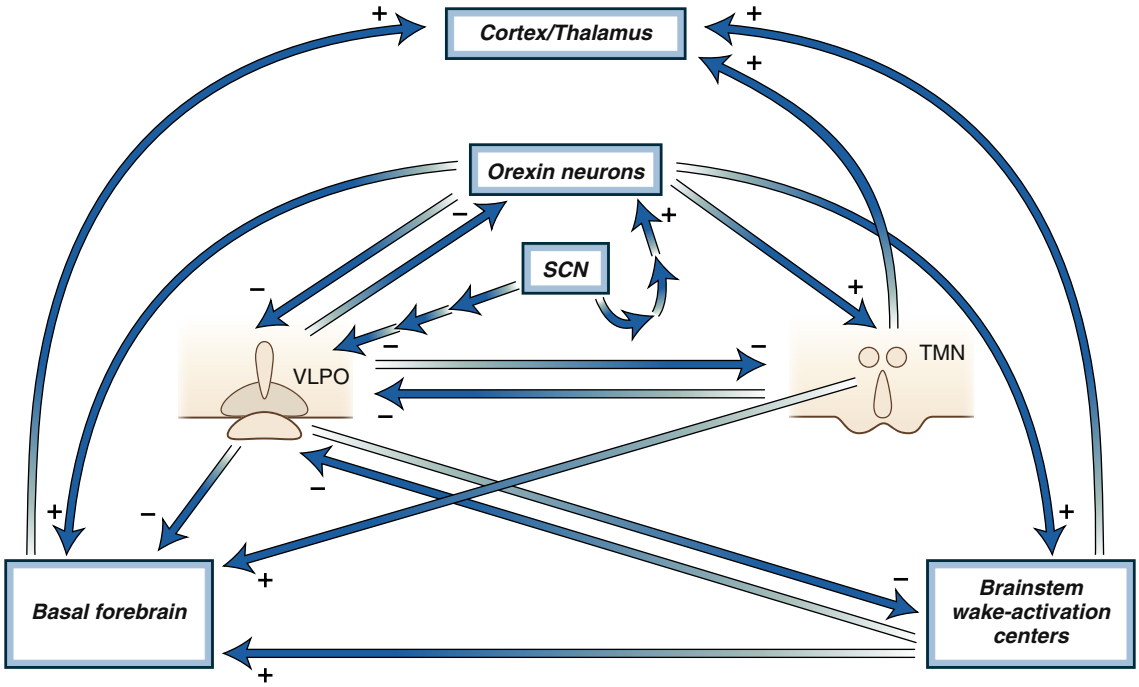
In addition to prostaglandins, there is evidence that opioids and endocannabinoids participate in febrile responses induced by pyrogens. Opioid receptors have been identified in the POA,[557] and activation of μ-opioid receptors causes hyperthermia.[558] In addition, fever induced by endotoxin administration of TNFα but not IL-1β can be reduced by a μ-opioid antagonist, indicating that the mechanism for the febrile response can differ among the various cytokines.[559] Similarly, endocannabinoids can also contribute to the febrile response as central administration of the CB1 receptor antagonist, AM251, reduces fever induced by TNFα, IL1β, and IL6.[560]

Sleep-Wake Cycle

Sleep is a natural state of altered consciousness that is divided into two distinct states: non–rapid eye movement (NREM) sleep characterized by the deepest sleep in stage 3 (slow-wave sleep) but with variable states of arousal, and rapid eye movement (REM) sleep, during which dreams are common and accompanied by autonomic activity. The neural organization of the sleep-wake cycle relies on multiple regions in the brain that produce either sleep or arousal and includes the hypothalamus. The primary sleep centers are located in the VLPO and MnPO of the hypothalamus and are composed of neurons that produce GABA and galanin.[561] Melanin-concentrating hormone (MCH) and GHRH neurons in the hypothalamus, somatostatin neurons in the basal forebrain, nNOS neurons in the cortex, and GABAergic neurons in the parafacial zone of the brainstem also contribute to sleep promotion.[562] Wakefulness centers are more numerous and include glutaminergic neurons in the parabrachial nucleus and pedunculopontine tegmental nucleus of the brainstem and GABAergic, cholinergic, and glutaminergic neurons in the basal forebrain.[562] Other nuclei involved in wakefulness include noradrenergic neurons in the locus coeruleus, serotonergic neurons in the dorsal raphe, dopaminergic neurons in the ventral periaqueductal gray, cholinergic cell groups in the pontine, and mesencephalic reticular formation, including the laterodorsal and pedunculopontine tegmentum, histaminergic neurons in the TMN, orexin (hypocretin)/glutamatergic and possibly GABAergic neurons in the lateral hypothalamic area, and neurons in the prefrontal cortex.[563] The reason for the need of such a large number of wake-promoting cell groups remains uncertain; perhaps they serve a redundant purpose, as with the exception of the parabrachial nucleus and pedunculopontine tegmentum, lesions of the other cell groups result in only partial loss of wake time (see Saper and colleagues[563] for review).

Central Circuitries Mediating the Sleep-Wake Cycle

The central pathways mediating the sleep-wake cycle are complex due to numerous interconnections among many regions of the brain, illustrated in Fig. 7.33 and reviewed in detail by Saper and colleagues,[563] Saper and Fuller,[562] Schwartz and Kilduff,[564] and Scammell and colleagues.[565] To briefly summarize salient points with particular reference to the hypothalamus, wake-promoting pathways ascend from the brainstem into two major divisions, a dorsal pathway that enters the thalamus and a ventral pathway that innervates the hypothalamus, basal forebrain, and cortex. Both pathways, however, are required for normal wakefulness.[566,567] Within the hypothalamus, TMN histaminergic neurons excite neurons in the cortex and thalamus and suppress VPLO activity. Orexin neurons excite the TMN but also project to the locus coeruleus and other arousal centers in the CNS.[568] NREM sleep is driven by the VLPO and MnPO, primarily by inhibiting wakefulness centers in the TMN, locus coeruleus, and dorsal raphe, and, if the VLPO is lesioned, there is profound reduction in NREM sleep.[569] Sleep-promoting neurons in the basal forebrain inhibit wake-promoting neurons in that same nucleus and directly inhibit cortical neurons.[565,570] The hypothalamic VLPO also contributes to REM sleep by innervating brainstem regions that promote REM sleep, such as the dorsal raphe and locus coeruleus. Hypothalamic neurons that synthesize MCH project to the subcoeruleus in the pons, producing muscle atonia that is characteristic of REM sleep,[571] and orexin neurons, although part of the wake-promoting system also appears to be involved in REM sleep as inhibiting these neurons increases REM sleep, presumably through brainstem projections.[572]



• **Fig. 7.33** Simplified diagram showing the connectivity of major regions of the brain involved in the sleep-wake cycle. *SCN,* suprachiasmatic nucleus; *TMN,* tuberomammillary nucleus; *VLPO,* ventrolateral preoptic nucleus.

Circadian Regulation of Sleep-Wake Cycle

Sleep is coupled to periods of wakefulness, giving rise to a circadian sleep-wake cycle whose timing is orchestrated to a 24-hour day-night cycle by photic stimuli transmitted to the hypothalamic SCN. As alluded to previously (see "Pineal Gland"), melatonin has an essential role in relaying this information to the SCN, ultimately attenuating the wake-promoting effects of the circadian clock. Thus sleep disorders are commonly observed in the blind due to the inability to synchronize with the day-night cycle (see Zisapel[573] for review), and ablation of the SCN or transgenic mice lacking various clock genes shows alterations in sleep time and sleep fragmentation.[574,575] The SCN is hardwired into the sleep-wake system, although indirectly. The main output of the SCN is to the subparaventricular zone of the hypothalamus that sends axons to the dorsomedial nucleus of the hypothalamus. The dorsomedial nucleus then projects to the VLPO to inhibit its activity and to orexin neurons in the lateral hypothalamus to activate its activity, hence promoting wakefulness.[576]

Somnogens

Somnogens are sleep-inducing substances and include adenosine, prostaglandin D_2, and cytokines, in particular IB-1β and TNF-α. Adenosine released from astrocytes is probably the most important somnogen and tends to increase during prolonged periods of wakefulness.[577,578] Prostaglandin D_2 is produced by the leptomeninges, and cytokines can either enter the CNS from the peripheral circulation or arise within the brain itself.[579] However, the somnogenic effect of cytokines may be mediated by prostaglandin D_2 as the effects of IL-1β on sleep can be suppressed by cyclooxygenase inhibitors, and the somnogenic effect of prostaglandin D_2 can be mediated by an increase in adenosine.[580] If adenosine A2A receptors are silenced, the somnogenic effect of prostaglandin D_2 is attenuated.[581] Adenosine A2A receptors promote NREM sleep by inhibiting histaminergic neurons in the TMN via activation of the VLPO.[582] This mechanism may also partly explain the arousing effects of caffeine, an adenosine antagonist that loses its wake-promoting effects in A2A knockout mice.[583]

As alluded to previously, GHRH has somnogenic effects (see "Growth Hormone–Releasing Hormone," then "Extrapituitary Functions"), and when administered ICV to experimental animals it stimulates NREM sleep that can be antagonized by somatostatin analogues.[584] The action of GHRH appears to be independent of GH and involves the actions of GHRH on the VLPO and MnPO as GABAergic neurons in these regions show increased c-Fos expression in response to GHRH. Evidence that GHRH also exerts somnogenic effects in humans is suggested by the well-recognized phenomenon of increased release of GH during sleep and individual reports demonstrating a reduction in sleep quality in individuals with GHRH resistance.[585]

Modifying Factors Influencing Sleep

The brain is also equipped to override the normal mechanisms involved in the sleep-wake cycle under situations when remaining awake is important for survival. Such a situation is food deprivation associated with increased wakefulness and locomotor activity. Orexin-producing neurons in the lateral hypothalamus have a major role in this response as transgenic animals in which orexin neurons have been ablated fail to respond to increased wakefulness with fasting.[586] Orexin neurons serve as an interface between wakefulness and energy homeostasis, maintaining arousal during periods of food unavailability. They are inhibited by glucose and leptin and activated by ghrelin, humeral signals that promote increased food intake and reduced energy expenditure (see Chapter 39). Orexin neurons project widely throughout the central nervous system and excite wakefulness centers (including histaminergic neurons in the TMN, monoaminergic neurons in the brainstem, and cholinergic neurons in the basal forebrain) and inhibit the VLPO.[587]

A variety of other factors can contribute to sleep disruption, including psychiatric disorders, psychosocial problems, alcoholism, pain, and chronic medical conditions such as diabetes mellitus, chronic renal failure, and Alzheimer disease, among many others (see Medic and colleagues[588] for review). However, the pathophysiology is likely multifactorial and not well established for most. Activation of the hypothalamic-pituitary-adrenal axis is likely involved for some conditions such as major depression[589] and due to elevations in cortisol, as similar sleep alterations are observed in individuals with Cushing syndrome.[590] However, CRH-induced activation of wakefulness centers, including orexin neurons in the lateral hypothalamus and norepinephrine neurons in the locus coeruleus, may also contribute to sleep disruption.[591] Stressed animals also show c-Fos activation in a variety of regions of the brain that may contribute to maintaining a waking state, including regions of the amygdala and infralimbic cortex.[592]

Neuroendocrine Disease

Disease of the hypothalamus can cause pituitary dysfunction, neuropsychiatric and behavioral disorders, and disturbances of autonomic and metabolic regulation. In the diagnosis and treatment of suspected hypothalamic or pituitary disease, four issues must be considered: the extent of the lesion, the physiologic impact, the specific cause, and the psychosocial setting. The cause of hypothalamic neuroendocrine disorders categorized by age and syndrome is summarized in Tables 7.6 and 7.7.

Manifestations of pituitary insufficiency secondary to hypothalamic or pituitary stalk damage are not identical to those of primary pituitary insufficiency. Hypothalamic injury causes decreased secretion of most pituitary hormones but can cause hypersecretion of hormones normally under inhibitory control by the hypothalamus, as in hypersecretion of PRL after damage to the pituitary stalk and precocious puberty caused by loss of the normal restraint over gonadotropin maturation. Impairment of inhibitory control of the neurohypophysis can lead to the syndrome of inappropriate vasopressin (antidiuretic hormone) secretion (SIADH) (see Chapter 10). More subtle abnormalities in secretion can result from impairment of the control system. For example, loss of the normal circadian rhythm of ACTH secretion may occur before loss of pituitary-adrenal secretory reserve, and responses to physiologic stimuli may be paradoxic. Because hypophysiotropic hormone levels cannot be measured directly and pituitary hormone secretion is regulated by complex, multilayered controls, assay of pituitary hormones in blood does not necessarily give a meaningful picture of events at hypothalamic and higher levels. Rarely, tumors secrete excessive amounts of releasing peptides and cause hypersecretion of hormones from the pituitary.

Disorders of the hypothalamic-pituitary unit can result from lesions at several levels. Defects can arise from destruction of the pituitary (as by tumor, infarct, inflammation, or autoimmune disease) or from a hereditary deficiency as with PROP1 mutations associated with GH, prolactin, and TSH deficiencies. Selective

TABLE 7.6 Etiology of Hypothalamic Disease by Age

Premature Infants and Neonates

Intraventricular hemorrhage
Meningitis: bacterial
Tumors: glioma, hemangioma
Trauma
Hydrocephalus, kernicterus

Age 1 Month to 2 Years

Tumors: optic glioma, histiocytosis X, hemangioma
Hydrocephalus
Meningitis
Congenital disorders: Kallmann syndrome, Laurence-Moon-Biedl syndrome, Prader-Willi syndrome

Age 2 to 10 Years

Tumors: craniopharyngioma, glioma, dysgerminoma, hamartoma, leukemia, histiocytosis X, ganglioneuroma, ependymoma, medulloblastoma
Meningitis: bacterial, tuberculous
Encephalitis: viral (exanthematous demyelinating)
Congenital diabetes insipidus
Radiation therapy
Diabetic ketoacidosis
Moyamoya disease, circle of Willis

Age 10 to 25 Years

Tumors: craniopharyngioma, glioma, hamartoma, dysgerminoma, histiocytosis X, leukemia, dermoid, lipoma, neuroblastoma
Trauma
Vascular: aneurysm, subarachnoid hemorrhage, arteriovenous malformation
Inflammatory disease: meningitis, encephalitis, sarcoidosis, tuberculosis
Structural brain defects: chronic hydrocephalus, increased intracranial pressure

Age 25 to 50 Years

Nutritional: Wernicke disease
Tumors: glioma, lymphoma, meningioma, craniopharyngioma, pituitary tumors, plasmacytoma, angioma, colloid cysts, sarcoma, ependymoma, histiocytosis X
Inflammatory diseases: sarcoidosis, tuberculosis, viral encephalitis
Vascular: aneurysm, subarachnoid hemorrhage, arteriovenous malformation
Damage from pituitary radiation therapy

Age 50 Years and Older

Nutritional: Wernicke disease
Tumors: pituitary tumors, sarcoma, glioblastoma, ependymoma, meningioma, colloid cysts, lymphoma
Vascular disease: infarct, subarachnoid hemorrhage, pituitary apoplexy
Inflammatory disease: encephalitis, sarcoidosis, meningitis
Damage from radiation therapy for ear-nose-throat carcinoma, pituitary tumors

Adapted from Plum F, Van Uitert R. Nonendocrine diseases and disorders of the hypothalamus. In: Reichlin S, Baldessarini RJ, Martin JB, eds. *The Hypothalamus*, vol 56. New York: Raven Press; 1978:415–473.

TABLE 7.7 Etiology of Endocrine Syndromes of Hypothalamic Origin

Hypophysiotropic Hormone Deficiency

Surgical pituitary stalk section
Inflammatory disease: basilar meningitis and granuloma, sarcoidosis, tuberculosis, sphenoid osteomyelitis, eosinophilic granuloma
Craniopharyngioma
Hypothalamic tumor: infundibuloma, teratoma (ectopic pinealoma), astrocytoma
Maternal deprivation syndrome, psychosocial dwarfism
Isolated GHRH deficiency
Hypothalamic hypothyroidism
Panhypophysiotropic failure

Disorders of Regulation of GnRH Secretion

Female

Precocious puberty: GnRH-secreting hamartoma, hCG-secreting germinoma
Delayed puberty
Neurogenic amenorrhea
Pseudocyesis
Anorexia nervosa
Functional amenorrhea and oligomenorrhea
Drug-induced amenorrhea
Kallman syndrome
GPR54 (KISS1R) mutations

Male

Precocious puberty
Anorexia nervosa
Fröhlich syndrome
Drug-induced hypogonadism
Kallmann syndrome
GPR54 (KISSR1) mutations

Disorders of Regulation of Prolactin-Regulating Factors

Tumor
Sarcoidosis
Drug induced
Reflex
Herpes zoster of chest wall
Post-thoracotomy
Nipple manipulation
Spinal cord tumor
Psychogenic
Hypothyroidism
Carbon dioxide narcosis

Disorders of Regulation of CRH

Paroxysmal corticotropin discharge (Wolff syndrome)
Loss of circadian variation
Depression
CRH-secreting gangliocytoma

CRH, Corticotropin-releasing hormone; *hCG,* human chorionic gonadotropin; *GHRH,* growth hormone–releasing hormone; *GnRH,* gonadotropin-releasing hormone.

loss of thyroid hormone receptors or GnRH receptors in the pituitary can give rise to increased TSH secretion and thyrotoxicosis or hypogonadotropic hypogonadism, respectively. Furthermore, disorders can arise through disruption of the contact zone between the stalk and the median eminence, the stalk itself, or the nerve terminals of the tuberoinfundibular system; such disruption occurs after surgical stalk section, with tumors involving the stalk, including metastatic disease, and in some inflammatory disorders such as sarcoidosis or neurohypophysitis. At a higher level, tonic inhibitory and excitatory inputs can be lost as manifested by absence of circadian rhythms or the development of precocious puberty. Physical stress, cytokine products of inflammatory cells, toxins, and reflex inputs from peripheral homeostatic monitors also impinge on the tuberoinfundibular system. At the highest

level of control, emotional stress and psychologic disorders can activate the pituitary-adrenal stress response and suppress gonadotropin secretion (e.g., psychogenic amenorrhea) or inhibit GH secretion (e.g., psychosocial dwarfism) (see Chapter 25). Intrinsic disease of the anterior pituitary is reviewed in Chapters 8 and 9, and disturbances in posterior pituitary function are discussed in Chapter 10. This chapter primarily considers diseases of the hypothalamic-pituitary unit.

Pituitary Isolation Syndrome

Destructive lesions of the pituitary stalk, as occur with head injury, surgical transection, tumor, or granuloma, produce a characteristic pattern of pituitary dysfunction.[593,594] Central diabetes insipidus (DI) develops in a large percentage of patients, depending on the level at which the stalk has been sectioned. If the cut is close to the hypothalamus, DI is almost always produced, but if the section is low on the stalk, the incidence is lower. The extent to which nerve terminals in the upper stalk are preserved determines the clinical course. The classic triphasic syndrome of initial polyuria followed by normal water control and then by AVP deficiency over a period of 1 week to 10 days occurs in fewer than half of the patients. The sequence is attributed to an initial loss of neurogenic control of the neural lobe, followed by autolysis of the neural lobe with release of AVP into the circulation, and finally by complete loss of AVP. However, full expression of polyuria requires adequate cortisol levels; if cortisol is deficient, AVP deficiency may be present with only minimal polyuria. DI can also develop after stalk injury without an overt transitional phase. When DI occurs after head injury or operative trauma, varying degrees of recovery can be seen even after months or years. Sprouting of nerve terminals in the stump of the pituitary stalk may give rise to sufficient functioning tissue to maintain water balance. In contrast to the effects of stalk section, nondestructive injury to the neurohypophysis or stalk, as during surgical resection of sellar tumors, can sometimes give rise to transient or delayed SIADH.[595]

Although head injury, granulomas, and tumors are the most common causes of acquired DI, other cases develop in the absence of a clear-cut cause.[596] Autoimmune disease of the hypothalamus may be the cause in some instances, as was suggested by the finding of autoantibodies to neurohypophyseal cells in a third of cases of idiopathic DI in one series.[597] However, autoantibodies were also frequently found in association with histiocytosis X. Later reports suggested the importance of continued vigilance in cases of idiopathic DI. A definite cause is frequently uncovered in time, including a high proportion of occult germinomas, whose detection by MRI may be preceded by elevated levels of hCG in CSF.[598] Congenital DI can be part of a hereditary disease. DI in the Brattleboro rat is due to an autosomal recessive genetic defect that impairs production of AVP but not of oxytocin. In contrast, inherited forms of DI in humans have been attributed to mutations in the vasopressin V_2 receptor gene or less frequently in the aquaporin or the AVP genes.[599–601]

Menstrual cycles cease after stalk section, although gonadotropins may still be detectable, unlike the situation after hypophysectomy. Plasma glucocorticoid levels and urinary excretion of cortisol decline after hypophysectomy and stalk section, but the change is slower after stalk section. A transient increase in cortisol secretion after stalk section is believed to be due to release of ACTH from preformed stores. The ACTH response to the lowering of blood cortisol is markedly reduced, but ACTH release after stress may be normal, possibly because of CRH-independent mechanisms. Reduction in thyroid function after stalk section is similar to that seen with hypophysectomy. The fall in GH secretion is said to be the most sensitive indication of damage to the stalk, but the insidious nature of this endocrinologic change in adults who have suffered traumatic brain injuries may cause it to be overlooked and therefore contribute to delayed rehabilitation.[602]

Humans with stalk sections or with tumors of the stalk region have widely varying levels of hyperprolactinemia and may have galactorrhea.[603] PRL responses to hypoglycemia and to TRH are blunted, in part because of loss of neural connections with the hypothalamus. PRL responses to dopamine agonists and antagonists in patients with pituitary isolation syndrome are similar to those in patients with prolactinomas. Interestingly, PRL secretion continues to show a diurnal variation in patients with either hypothalamic-pituitary disconnection or microprolactinoma.[412] Both forms of hyperprolactinemia are characterized by a similarly increased frequency of PRL pulses and a marked rise in nonpulsatile or basal PRL secretion, although the disruption is greater in the tumoral hyperprolactinemia.

An incomplete pituitary isolation syndrome may occur with the empty sella syndrome, intrasellar cysts, or pituitary adenomas.[604,605] Anterior pituitary failure after stalk section is in part due to loss of specific neural and vascular links to the hypothalamus and in part due to pituitary infarction.

Hypophysiotropic Hormone Deficiency

Selective pituitary failure can be due to a deficiency of specific pituitary cell types or a deficiency of one or more hypothalamic hormones. Isolated GnRH deficiency is the most common hypophysiotropic hormone deficiency. In X-linked Kallmann syndrome (gonadotropin deficiency commonly associated with hyposmia),[440] hereditary agenesis of the olfactory lobe may be demonstrable by MRI.[606] Abnormal development of the GnRH system is a result of defective migration of the GnRH-containing neurons from the olfactory nasal epithelium in early embryologic life (see earlier discussion). Other malformations of the cranial midline structures, such as absence of the septum pellucidum in septo-optic dysplasia (De Morsier syndrome), can cause hypogonadotropic hypogonadism or, less commonly, precocious puberty. A surprisingly large percentage of children with septo-optic dysplasia who otherwise have multiple hypothalamic-pituitary abnormalities actually retain normal gonadotropin function and enter puberty spontaneously.[607] The genetic basis of hypogonadotropic hypogonadism include X-linked mutations in *KAL1*, the Kallmann syndrome gene, and in *NROB1* (formerly *AHC* or *DAX1*), the gene that causes adrenal hypoplasia congenital with hypogonadotropic hypogonadism.[442,608] Autosomal recessive hypogonadotropic hypogonadism has been associated with mutations in the genes encoding the GnRH receptor, KISS1 receptor, leptin, leptin receptor, FSH, LH, PROP1 (combined pituitary deficiency), and HESX (septo-optic dysplasia) genes, and deficient FGFR1 function causes an autosomal dominant form of hypogonadotropic hypogonadism. Mutations in *PROK2* and *PROKR2*, which encode prokinectin-2 and its receptor, have been associated with heterozygous, homozygous, compound heterozygous, and oligogenic patterns of genetic penetrance.

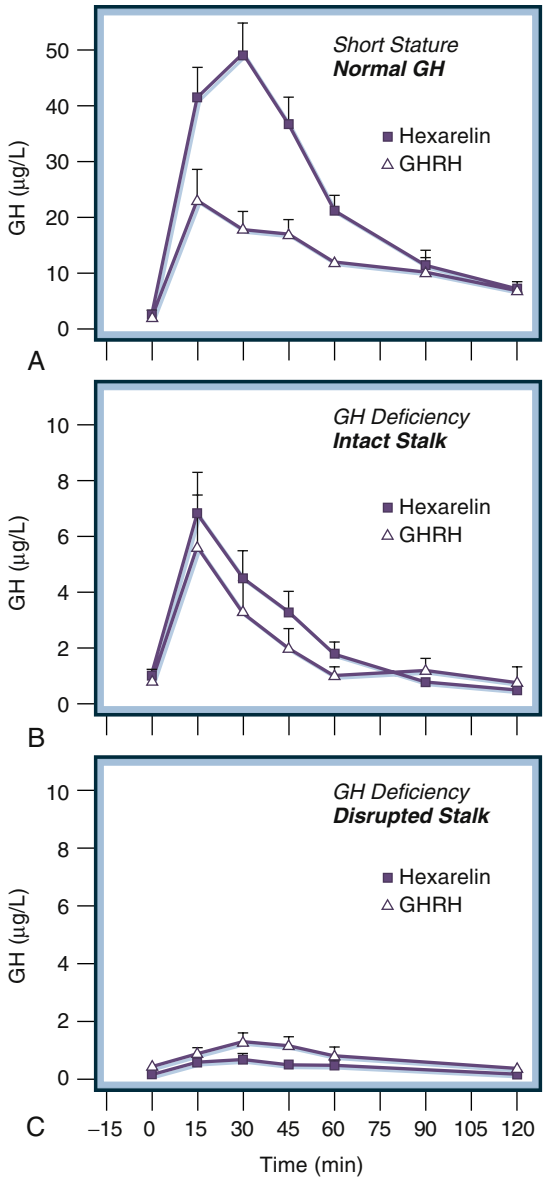
The GnRH stimulation test is of little value in the differential diagnosis of hypogonadotropic hypogonadism. Most patients with GnRH deficiency show little or no response to an initial test dose but normal responses after repeated injection. This slow response has been attributed to downregulation of GnRH

receptors in response to prolonged GnRH deficiency. In patients with intrinsic pituitary disease, the response to GnRH may be absent or normal. Consequently, it is not possible to distinguish between hypothalamic and pituitary disease with a single injection of GnRH. Prolonged infusions or repeated administration of GnRH agonists after hormone replacement therapy priming may aid in the diagnosis or provide therapeutic options for women with Kallmann syndrome who wish to become pregnant.[609,610]

Deficiency of TRH secretion gives rise to hypothalamic hypothyroidism, also called *tertiary hypothyroidism*, which can occur in hypothalamic disease or more rarely as an isolated defect.[611] Molecular genetic analyses have revealed infrequent autosomal recessive mutations in the TRH and TRH receptor genes in the cause of central hypothyroidism.[612] Hypothalamic and pituitary causes of TSH deficiency are most readily distinguished by imaging methods. Although it is theoretically reasonable to use the TRH stimulation test for the differentiation of hypothalamic disease from pituitary disease, the test is of limited value. The typical pituitary response to TRH administration in patients with TRH deficiency is an enhanced and somewhat delayed peak, whereas the response with pituitary failure is subnormal or absent. The hypothalamic type of response has been attributed to an associated GH deficiency that sensitizes the pituitary to TRH (possibly through suppression of somatostatin secretion), but GH also affects T_4 metabolism and may alter pituitary responses.[613] In practice, the responses to TRH in hypothalamic and pituitary disease overlap so much that they cannot be used reliably for a differential diagnosis. Persistent failure to demonstrate responses to TRH is good evidence for the presence of intrinsic pituitary disease, but the presence of a response does not mean that the pituitary is normal. Deficient TRH secretion leads to altered TSH biosynthesis by the pituitary, including impaired glycosylation. Poorly glycosylated TSH has low biologic activity, and dissociation of bioactive and immunoreactive TSH can lead to the paradox of normal or elevated levels of TSH in hypothalamic hypothyroidism.[611,614]

GHRH deficiency appears to be the principal cause of hGH deficiency in children with idiopathic dwarfism.[615] This condition is frequently associated with abnormal electroencephalograms (EEGs), a history of birth trauma, and breech delivery, although a cause-and-effect relationship has not been established. MRI scans show that most children with isolated, idiopathic hGH deficiency have a normal-sized or only slightly reduced anterior pituitary; less common findings are ectopic posterior pituitary, anterior pituitary hypoplasia, or empty sella.[616] In contrast, children with idiopathic combined pituitary hormone deficiency are significantly more likely to have evidence of moderate to severe anterior pituitary hypoplasia, ectopic posterior pituitary, complete agenesis of the pituitary stalk (both nervous and vascular components), and a variety of associated midline cerebral malformations.[616] Human GH is the most vulnerable of the anterior pituitary hormones when the pituitary stalk is damaged. It can be difficult to differentiate between primary pituitary disease and GHRH deficiency by standard tests of GH reserve. However, a substantial GH secretory response to a single administration of hexarelin occurs only in the presence of at least a partially intact vascular stalk (Fig. 7.34).[294]

In many children with dwarfism, the anatomic abnormalities of the intrasellar contents and pituitary stalk together with the frequent occurrence of other midline defects, such as those in septo-optic dysplasia, are consistent with the alternative hypothesis of a developmental defect occurring in embryogenesis.[616] There has been a remarkable advance in our understanding of the molecular ontogeny of the hypothalamic-pituitary unit, much of it based on mutant mouse models.[617] Parallel genetic analyses have been conducted in children with isolated GH deficiency or combined pituitary hormone deficiencies. These studies have identified autosomal recessive mutations in both structural and regulatory genes, including the genes encoding the GHRH receptor, PIT1, PROP1, HESX1, LHX3, and LHX4 that are responsible for a



• **Fig. 7.34** Effect of hypothalamic-pituitary disconnection on the growth hormone (GH) secretory responses to GH-releasing hormone (GHRH) (1 μg/kg) and the GH-secretagogue (GHS) receptor agonist, hexarelin (2 μg/kg), administered intravenously to children with GH deficiency. (A) Mean responses in a group of 24 prepubertal children with short stature secondary to familial short stature or constitutional growth delay are shown. (B) Children with GH deficiency and an intact vascular pituitary stalk as visualized by dynamic magnetic resonance imaging exhibited a clear, but blunted, GH response to both secretagogues. (C) In contrast, children with pituitary stalk agenesis (both vascular and neural components) had no response or a markedly attenuated response to both peptides. (From Maghnie M, Spica-Russotto V, Cappa M, et al. The growth hormone response to hexarelin in patients with different hypothalamic-pituitary abnormalities. *J Clin Endocrinol Metab.* 1998;83:3886–3889.)

sizable proportion of congenital hypothalamic-pituitary disorders once considered idiopathic.[281,615,616,618] Other mutations that are associated with variable GH deficiencies include ARNT2, GLI2, OTX2, PAX6, and SOX2.[44]

Adrenal insufficiency is another manifestation of hypothalamic disease and rarely is caused by CRH deficiency.[619] Isolated ACTH deficiency is uncommon, but there is suggestive evidence in at least one family of genetic linkage to the *CRH* gene locus.[620] More recent investigations have revealed mutations in *TBX19*, the gene encoding TPIT, a T-box transcription factor expressed only in pituitary corticotrophs and melanotrophs, which is associated with the majority of cases of isolated ACTH deficiency in neonates.[621] Mutations in ARNT2, a basic helix-loop-helix transcription factor and important in development of hypophysiotropic neurons in the PVH, can also result in ACTH and TSH deficiencies.[622] The CRH stimulation test does not reliably distinguish hypothalamic from pituitary failure as a cause of ACTH deficiency.[623]

Apart from intrinsic diseases of the hypothalamus such as tumors and granulomas, two environmental causes of central hypophysiotropic deficiencies are of increasing clinical importance: trauma to the brain,[594,602] particularly from motor vehicle accidents, and the sequelae of chemotherapy and radiation therapy for intracranial lesions in children and adults.[614,624,625] Improved short-term survival from head injuries associated with coma and CNS malignancies has greatly increased the prevalence of long-term neuroendocrine consequences.

Craniopharyngioma

Craniopharyngioma is the most common pediatric tumor occurring in the sellar and parasellar area (see Table 7.6) and accounts for 5% to 15% of all intracranial tumors in this age group.[626] Because of their location, these benign neoplasms frequently cause significant neuroendocrine dysfunction. Adamantinomatous tumors occur at all ages but are most common in children and usually contain both a cystic component filled with a turbid, cholesterol-rich fluid and a solid component characterized by organized epithelial cells.[627] Roughly 25% of craniopharyngiomas are diagnosed in patients over the age of 25 and include a papillary variety that is almost exclusively identified in adults. This subset of tumors tends to be solid and less likely to be calcified or cystic.[627] Both forms of craniopharyngiomas probably result from metaplastic changes in vestigial epithelial cell rests that originate in Rathke pouch and the craniopharyngeal duct during fetal development. The majority of adamantinomatous craniopharyngiomas have mutations of genes encoding β-catenin (*CTNNB1* and *APC*), whereas papillary craniopharyngiomas harbor BRAF V600E mutations.[628,629]

Common presenting symptoms are those due to a mass intracranial lesion and increased intracranial pressure. Visual field defects, papilledema, and optic atrophy can occur from compression of the optic chiasm or nerves. Between 80% and 90% of affected children have signs and symptoms of endocrine dysfunction, although these are not usually the chief complaint. The most frequent hormone deficiencies are GH and gonadotropins. The latter is almost universal in adolescents and adults and likely also present, but undetected, in prepubertal children with craniopharyngiomas. TSH and ACTH deficiency are also common, and approximately one-third of these patients develop diabetes insipidus. Even if not present at initial diagnosis, endocrine dysfunction often occurs subsequent to treatment that may also include obesity, imbalances in body temperature, autonomic dysfunction, sleep disturbances, and behavioral changes and necessitates long-term follow-up and retesting.[630,631]

MRI is the imaging modality of choice in cases of suspected craniopharyngioma.[632] A recommended examination includes T1-weighted thin sagittal and coronal sections through the sella and suprasellar regions, obtained before and after contrast administration. T2-weighted and fluid attenuation inversion recovery (FLAIR) images are useful to further delineate cysts and are hyperintense. Computed tomography scans can be useful to determine the presence of calcification.

Hypophysiotropic Hormone Hypersecretion

Pituitary hypersecretion is occasionally caused by tumors of the hypothalamus. GnRH-secreting hamartomas can cause precocious puberty.[633] CRH-secreting gangliocytomas can cause Cushing syndrome,[634] and GHRH-secreting gangliocytomas of the hypothalamus can cause acromegaly.[635] Although they do not arise from the hypothalamus, paraneoplastic syndromes can also cause pituitary hypersecretion, as with bronchial and pancreatic islet cell carcinoid tumors secreting CRH or GHRH.

Neuroendocrine Disorders of Gonadotropin Regulation

Precocious Puberty

The term *precocious puberty* is used when physiologically normal pituitary-gonadal function appears at an early age (see also Chapter 26).[636] By convention, it is defined as the onset of androgen secretion, and spermatogenesis must occur before the age of 9 or 10 years in boys and the onset of estrogen secretion and cyclic ovarian activity before age 7 or 8 in girls.[637,638] Central precocious puberty is due to disturbed CNS function, which may or may not have an identifiable structural basis. Pseudoprecocious puberty refers to premature sexual development resulting from excessive secretion of androgens, estrogens, or hCG; it is caused by tumors (both gonadal and extragonadal), administration of exogenous gonadal steroids, or genetically determined activation of gonadotropin receptors (see Chapter 25). Central precocious puberty with neurogenic causes and pineal gland disease are discussed in this chapter.

Idiopathic Sexual Precocity

Familial occurrence of idiopathic sexual precocity is uncommon, but there is a hereditary form of idiopathic sexual precocity that is largely confined to boys. Abnormal EEGs and behavioral disturbances, suggesting the presence of brain damage, have been reported occasionally in girls with idiopathic precocious puberty. The pathogenesis may be related to the rate of hypothalamic development or other as yet undetermined nutritional, environmental, or psychosocial factors. Many cases previously thought to be idiopathic are caused by small hypothalamic hamartomas (see later discussion). It has been argued that localized activation of discrete cellular subsets connected to GnRH neurons may be sufficient to initiate puberty.[639]

Neurogenic Precocious Puberty

Approximately two-thirds of hypothalamic lesions that influence the timing of human puberty are located in the posterior hypothalamus, but in the subset of patients who come to autopsy, damage is extensive. Specific lesions known to cause precocity include craniopharyngioma (although delayed puberty is far more common with these lesions), astrocytoma, pineal tumors, subarachnoid

cysts, encephalitis, miliary tuberculosis, tuberous sclerosis or neurofibromatosis type 1, the Sturge-Weber syndrome, porencephaly, craniosynostosis, microcephaly, hydrocephalus, empty sella syndrome, and Tay-Sachs disease.[640,641]

Hamartoma of the hypothalamus, a tumor-like collection of normal-appearing nerve tissue lodged in an abnormal location, can result in precocious puberty.[642,643] The parahypothalamic type consists of an encapsulated nodule of nerve tissue attached to the floor of the third ventricle or suspended from the floor by a peduncle, typically less than 1 cm in diameter. The intrahypothalamic or sessile type is enveloped by the posterior hypothalamus, which can distort the third ventricle. These hamartomas tend to be larger than the pedunculated variety, grow in the interpeduncular cistern, and are frequently accompanied by seizures, mental retardation, and developmental delays. Before the development of high-resolution scanning techniques, harmatoma was considered rare, but small ones can now be visualized. Miniature hamartomas of the tuber cinereum are common at autopsy. Precocious puberty occurs when the hamartoma makes connections with the median eminence and thus serves as an accessory hypothalamus. Peptidergic nerve terminals containing GnRH have been found in the tumors.[633] Early pubertal development is presumably due to unrestrained GnRH secretion, although the hamartomas almost certainly have an intrinsic pulse generator of GnRH secretion because pulsatility is required for stimulation of gonadotropin secretion (see earlier discussion).

Manifestations of premature puberty in patients with hamartomas are similar to those associated with other central causes of precocity. Hamartomas occur in both sexes and may be present as early as age 3 months. In the past, most cases were thought to be fatal by age 20 years, but many hamartomas cause no brain damage and need not be excised.[642] The interpeduncular fossa of the brain is difficult to approach, and surgical experience is somewhat limited. Early in the course of illness, epilepsy manifested as "brief, repetitive, stereotyped attacks of laughter"[644] may provide a clue to the disease. Late in the course, hypothalamic damage can cause severe neurologic defects and intractable seizures.

Hypothyroidism

Hypothyroidism can cause precocious puberty in girls that is reversible with thyroid therapy. Hyperprolactinemia and galactorrhea may be present. One possibility is that elevated TSH levels (in children with thyroid failure) cross-react with the FSH receptor.[645] Alternatively, low levels of thyroid hormone might simultaneously activate release of LH, FSH, and TSH. A third possibility is that hypothyroidism causes hypothalamic encephalopathy that impairs the normal tonic suppression of gonadotropin release by the hypothalamus. The high PRL levels that sometimes accompany this disorder may be due to a deficiency in PIF secretion, increased secretion of TRH, or increased sensitivity of the lactotrophs to TRH secretion.

Tumors of the Pineal Gland

Pineal gland tumors account for only a small percentage of intracranial neoplasms. They occur as a central midline mass with an enhancing lesion on MRI frequently accompanied by hydrocephalus. Pinealomas cause a variety of neurologic abnormalities. Parinaud syndrome, which consists of paralysis of upward gaze, pupillary areflexia (to light), paralysis of convergence, and a wide-based gait, occurs with about half of patients with pinealomas. Gait disturbances can also occur because of brainstem or cerebellar compression. Additional neurologic signs occurring with moderate frequency include spasticity, ataxia, nystagmus, syncope, vertigo, cranial nerve palsies other than VI and VIII, intention tremor, scotoma, and tinnitus.

Several discrete cytopathologic entities account for mass lesions in the pineal region (Table 7.8).[646] The most common nonneoplastic conditions are degenerative pineal cysts, arachnoid cysts, and cavernous hemangioma. Pinealocytes give rise to primitive neuroectodermal tumors, the so-called *small blue-cell tumors* that are immunopositive for the neuronal marker synaptophysin and negative for the lymphocyte marker CD45. True pinealomas can be relatively well-differentiated pineocytomas, intermediate mixed forms, or the less-differentiated pineoblastomas,[646,647] which are essentially identical to medulloblastomas, neuroblastomas, and oat cell carcinomas of the lung.

The most common tumors of the pineal gland are actually germinomas (a form of teratoma), so designated because of their presumed origin in germ cells. Germinomas may also occur in the anterior hypothalamus or the floor of the third ventricle, where they are often associated with the clinical triad of DI, pituitary insufficiency, and visual abnormalities.[641] Identical tumors can be found in the testis and anterior mediastinum. Intracranial

TABLE 7.8 Classification of Tumors of the Pineal Region

Germ Cell Tumors

- Germinoma
  - Posterior third ventricle and pineal lesions
  - Anterior third ventricle, suprasellar, or intrasellar lesions
  - Combined lesions in anterior and posterior third ventricle, apparently noncontiguous, with or without foci of cystic or solid teratoma
- Teratoma
  - Evidencing growth along two or three germ lines in varying degrees of differentiation
  - Dermoid and epidermoid cysts with or without solid foci of teratoma
  - Histologically malignant forms with or without differentiated foci of benign, solid, or cystic teratoma-teratocarcinoma, chorioepithelioma, embryonal carcinoma (endodermal–sinus tumor or yolk-sac carcinoma); combinations of these with or without foci of germinoma, chemodectoma

Pineal Parenchymal Tumors

- Pinealocytes
  - Pineocytoma
  - Pineoblastoma
  - Ganglioglioma and chemodectoma
  - Mixed forms exhibiting transitions among these types
- Glia
  - Astrocytoma
  - Ependymoma
  - Mixed forms and other less frequent gliomas (e.g., glioblastoma, oligodendroglioma)

Tumors of Supporting or Adjacent Structures

- Meningioma
- Hemangiopericytoma

Nonneoplastic Conditions of Neurosurgical Importance

- Degenerative cysts of pineal gland lined by fibrillary astrocytes
- Arachnoid cysts
- Cavernous hemangioma

From DeGirolami U. Pathology of tumors of the pineal region. In: Schmidek HH, ed. *Pineal Tumors*. New York: Masson; 1977:1–19.

germinomas have a tendency to spread locally, infiltrate the hypothalamus, and metastasize to the spinal cord and CSF. Extracranial metastases (to skin, lung, or liver) are rare. Teratomas derived from two or more germ cell layers also occur in the pineal region. Chorionic tissue in teratomas and germinomas may secrete hCG in sufficient amounts to cause gonadal maturation, and some of these tumors have the histologic and functional characteristics of choriocarcinomas. Diagnosis is confirmed by the combination of a mass lesion, cytologic analysis of CSF, and radioimmunoassay detection of hCG in the CSF.

Precocious puberty is a relatively unusual manifestation of pineal gland disease. When it occurs, neuroanatomic studies suggest that the cause is secondary to pressure or destructive effects of the pineal tumor on the function of the adjacent hypothalamus or to the secretion of hCG. Most patients have other evidence of hypothalamic involvement such as DI, polyphagia, somnolence, obesity, or behavioral disturbance. Choriocarcinoma of the pineal gland is associated with high plasma levels of hCG. The hCG can stimulate testosterone secretion from the testis but not estrogen secretion by the ovary; it therefore causes premature puberty almost exclusively in boys. The prevalence of elevated hCG levels in children with premature puberty related to tumors in the pineal region is unknown, but the fact that this phenomenon occurs further challenges the theory that nonparenchymal tumors cause precocious puberty by damaging the normal pineal gland. Rarely, pinealomas cause delayed puberty, raising speculation about the role of melatonin in inhibiting gonadotropin secretion in these cases.

Management of tumors in the pineal region is not straightforward.[646,648] Operative mortality rates can be high, but the rationale for an aggressive approach to the pineal region is based on the need to make a histologic diagnosis, the variety of lesions found in this region, the possibility of cure of an encapsulated lesion, and the effectiveness of chemotherapeutic agents for germinomas and choriocarcinoma. Stereotaxic biopsy of the pineal region provided diagnosis in 33 of 34 cases in one series, suggesting that this is a useful alternative to open surgical exploration for diagnostic purposes.[649] Long-term palliation or cure of many pineal region tumors is possible by combinations of surgery, radiation, gamma knife radiosurgery, or chemotherapy, depending on the nature of the lesion.[650]

Approach to the Patient with Precocious Puberty

Several groups have reviewed the diagnostic approach to suspected central precocious puberty (see Chapter 26).[651,652] Although guidelines differ, the index of suspicion is clearly inversely proportional to the age of the patient. A GnRH stimulation test to assess gonadotropin release and thereby differentiate between primed and inactive gonadotrophs is probably the single most important endocrinologic measure. If LH and FSH levels are not stimulated and there is no evidence of gonadal germ cell maturation, the cause of precocious puberty lies outside the hypothalamic-pituitary axis, and the diagnostic process should focus on the adrenal glands and gonads (see Chapters 15 and 17). MRI studies are central to the workup for exclusion or characterization of organic lesions in the areas of the sella, optic chiasm, suprasellar hypothalamus, and interpeduncular cistern.[653]

Management of Sexual Precocity

Structural lesions of the hypothalamus are treated by surgery, radiation, chemotherapy, or combinations of these as indicated by the pathologic diagnosis and extent of disease. Endocrinologic manifestations of precocious puberty are best treated by GnRH agonists with the therapeutic goals of delaying sexual maturation to a more appropriate age and achieving optimal linear growth and bone mass, possibly with the combined use of GH treatment.[654,655] Other approaches include the use of cyproterone acetate, testolactone, or spironolactone to antagonize or inhibit gonadal steroid biosynthesis.[656,657] Precocious puberty is stressful to both the child and the parents, and it is essential that psychologic support be provided.

Psychogenic Amenorrhea

Menstrual cycles can cease in young nonpregnant women with no demonstrable abnormalities of the brain, pituitary, or ovary in several situations,[658,659] including pseudocyesis (false pregnancy), anorexia nervosa, excessive exercise, psychogenic disorders, and hyperprolactinemic states (see Chapter 17). Psychogenic amenorrhea, the most common cause of secondary amenorrhea except for pregnancy, can occur with major psychopathology or minor psychic stress and is often temporary.

Exercise-induced amenorrhea may be a variant of psychogenic amenorrhea or may result from loss of body fat.[658,660] The syndrome is associated with intense and prolonged physical exertion such as running, swimming, or ballet dancing. Affected women are always below ideal body weight and have low stores of fat. If the activity is begun before puberty, normal sexual maturation can be delayed for many years. Fat mass may be a regulator of gonadotropin secretion with adipocyte-derived leptin as the principal mediator between peripheral energy stores and hypothalamic regulatory centers.[661] Studies in nonhuman primates showed a direct role of caloric intake in the pathogenesis of amenorrhea associated with long-distance running.[662] Exercise and psychogenic amenorrhea can have adverse effects because of the associated estrogen deficiency and accompanying infertility, osteopenia, and increased risk of stress fractures.[663,664] Nevertheless, because of a lack of clear benefit from oral estrogen administration on bone density, perhaps secondary to its suppression of IGF-1, short-term treatment with transdermal estrogen is recommended in women who have not had resumption in menses after a trial of nutritional, psychologic, and/or modified exercise interventions.[665]

Neurogenic Hypogonadism in Males

A discussion of neurogenic hypogonadism in males should begin with an account of Fröhlich syndrome (adiposogenital dystrophy), originally characterized as delayed puberty, hypogonadism, and obesity associated with a tumor that impinges on the hypothalamus.[8] It was subsequently recognized that either hypothalamic or pituitary dysfunction can induce hypogonadism and that the presence of obesity indicates that the appetite-regulating regions of the hypothalamus have been damaged. Several organic lesions of the hypothalamus can cause this syndrome, including tumors, encephalitis, microcephaly, Friedreich ataxia, and demyelinating diseases. Other important causes of hypogonadotropic hypogonadism are Kallmann syndrome and Prader-Willi syndrome.[666]

However, most males with delayed sexual development do not have serious neurologic conditions. Furthermore, most obese boys with delayed sexual development have no structural damage to the hypothalamus but have constitutional delayed puberty, which is commonly associated with obesity. It is not known whether there is a functional disorder of the hypothalamus in this condition. It is thought that psychosexual development of brain maturation depends on the presence of androgens within a critical developmental window corresponding to puberty; therefore hypogonadism in boys (regardless of cause) should be treated by the middle teen years (15 years of age at the latest).

In adult men, hypogonadism (including reduced spermatogenesis) can be induced by emotional stress or severe exercise,[667] but this abnormality is seldom diagnosed because the symptoms are more subtle than menstrual cycle changes in similarly stressed women. Prolonged physical stress and sleep and energy deficiency can also decrease testosterone and gonadotropin levels.[668] Chronic intrathecal administration of opiates for the control of intractable pain syndromes is strongly associated with hypogonadotropic hypogonadism and to a lesser extent with hypocorticism and GH deficiency in both men and women.[259] Finally, critical illness with multiple causes is well known to be associated with hypogonadism and ineffectual altered pulsation of GnRH.[669]

Neurogenic Disorders of Prolactin Regulation

Neurogenic causes of hyperprolactinemia include irritative lesions of the chest wall (e.g., herpes zoster, thoracotomy), excessive tactile stimulation of the nipple, and lesions within the spinal cord (e.g., ependymoma).[670] Prolonged mechanical stimulation of the nipples by suckling or the use of a breast pump can initiate lactation in some women who are not pregnant, and neurologic lesions that interrupt the hypothalamic-pituitary connection can cause hyperprolactinemia, as discussed earlier. Hyperprolactinemia also occurs after certain forms of epileptic seizures. In one series, six of eight patients with temporal lobe seizures had a marked increase in PRL, whereas only one of eight patients with frontal lobe seizures developed hyperprolactinemia.[671] Agents that block D_2-like dopamine receptors (e.g., phenothiazines, later generation atypical antipsychotics) or prevent dopamine release (e.g., reserpine, methyldopa, opiates) must be excluded in all cases.

Because the nervous system exerts such profound effects on PRL secretion, patients with hyperprolactinemia (including those with adenomas) may have a deficit of PIF or an excess of PRF activity. In studies of PRL secretion in patients apparently cured of hyperprolactinemia by removal of a pituitary microadenoma, regulatory abnormalities persisted in some but not all patients. Persistence of regulatory abnormalities may be due to incomplete removal of a tumor, abnormal function of the remaining part of the gland, or underlying hypothalamic abnormalities.[672]

Neurogenic Disorders of Growth Hormone Secretion

Hypothalamic Growth Failure

Loss of the normal nocturnal increase in GH secretion and loss of GH secretory responses to provocative stimuli occur early in the course of hypothalamic disease and may be the most sensitive endocrine indicator of hypothalamic dysfunction. As described earlier, anatomic malformations of midline cerebral structures are associated with abnormal GH secretion, presumably related to failure of the development of normal GH regulatory mechanisms. Such disorders include optic nerve dysplasia and midline prosencephalic malformations (absence of the septum pellucidum, abnormal third ventricle, and abnormal lamina terminalis). Certain complex genetic disorders, including Prader-Willi syndrome, also commonly involve reduced GH secretory capacity.[673] Idiopathic hypopituitarism with GH deficiency was considered earlier.

Maternal Deprivation Syndrome and Psychosocial Dwarfism

Infant neglect or abuse can impair growth and cause failure to thrive (the maternal deprivation syndrome). Malnutrition interacts with psychologic factors to cause growth failure in children with the maternal deprivation syndrome, and each case should be carefully evaluated from this point of view. Older children with growth failure in a setting of abuse or severe emotional disturbance (termed *psychosocial dwarfism*) may also have abnormal circadian rhythms and deficient hGH release after insulin-induced hypoglycemia or arginine infusion (see Chapter 25).[674] Deficient release of ACTH and gonadotropins may also be present. A variant termed *hyperphagic short stature* has been identified.[675] These disorders can be reversed by placing the child in a supportive milieu; growth and neuroendocrine hGH responses rapidly return to normal.[676] The pathogenesis of altered GH secretion in children in response to deprivation is unknown. Furthermore, in the adult human, physical or emotional stress usually causes an increase in hGH secretion (see earlier discussion).

Neuroregulatory Growth Hormone Deficiency

The availability of biosynthetic hGH for treatment of short stature has brought into focus a group of patients who grow at low rates (<3rd percentile) and have low levels of serum IGF-1 but a normal hGH secretory reserve. Studies of 24-hour hGH secretion profiles indicate that many of these children do not have normal spontaneous hGH secretion (i.e., abnormal ultradian and circadian rhythms or decreased number or amplitude of secretory bursts, or both). These children with idiopathic short stature may have a functional regulatory disturbance of the hypothalamus and appear to grow normally when given exogenous hGH.[677]

There is considerable uncertainty about the criteria for the diagnosis of neuroregulatory hGH deficiency. Many normally growing children have profiles of hGH secretion that are indistinguishable from those in children with the postulated syndrome.[678] Patterns of hGH secretion do not predict which child will benefit from therapy, and there is a poor correlation between hGH secretion and growth. Furthermore, the results of repeated tests in children show considerable variability. It has been suggested that specific genetic defects may underlie the pathogenesis of a subset of children with this heterogeneous syndrome of growth failure.[679] The prevalence of an hGH neuroregulatory deficiency syndrome is thus unclear, and the decision to treat short children with hGH should be made cautiously.[680,681]

Neurogenic Hypersecretion of Growth Hormone

Diencephalic Syndrome

Children and infants with tumors in and around the third ventricle frequently become cachectic, which is often associated with elevated hGH levels and paradoxical GH secretory responses to glucose and insulin.[682] GH hypersecretion may be due to a hypothalamic abnormality or to malnutrition. Deficits of pituitary-adrenal regulation are less common. A striking feature is an alert appearance and seeming euphoria despite the profound emaciation. A variety of associated neurologic abnormalities may be present, including nystagmus, irritability, hydrocephalus, optic atrophy, tremor, and excessive sweating. CSF abnormalities include increased protein and the presence of abnormal cells. Most cases are due to chiasmatic-hypothalamic gliomas, with the majority classified as astrocytomas.[682] Treatment options include surgical resection, radiation therapy, and chemotherapy.[683]

Growth Hormone Hypersecretion Associated With Metabolic Disturbances

Apparently inappropriate hGH hypersecretion occurs with uncontrolled diabetes mellitus, hepatic failure, uremia, anorexia nervosa, and protein-calorie malnutrition. Nutritional factors are probably

important in this response, because in normal persons obesity inhibits and fasting stimulates episodic GH hypersecretion.[684] In diabetes mellitus, cholinergic blockers reverse the abnormality,[332] possibly by inhibiting hypothalamic somatostatin secretion (see earlier discussion). Peripheral GH resistance leading to reduced IGF-1 synthesis and release may also contribute to increased levels of GH.

X-Linked Gigantism

Excess growth hormone secretion, resulting in acromegaly/gigantism, is most often associated with tumors of the anterior pituitary and, less commonly, ectopic secretion of GHRH from carcinoid tumors, pancreatic islet cell tumors, small cell lung carcinoma, adrenal adenomas, and pheochromocytomas (see Chapter 9). Genetic abnormalities resulting in gigantism have been associated with MEN1, MEN4, Carney complex, McCune-Albright syndrome, and *AIP* mutations. X-linked acrogigantism (XLAG) is a more recently identified disorder of early-onset GH excess associated with gigantism due to microduplications of the *GPR101* gene on the X chromosome. While the majority of affected individuals have pituitary adenomas, elevated circulating levels of GHRH, and the presence of diffuse hyperplasia of somatotrophs in some patients, expression of GPR101 in the arcuate nucleus has raised the possibility that increased hypothalamic secretion of GHRH underlies the pathogenesis for X-linked acrogigantism (see Trivellin and colleagues[685] for review). The presence of GPR101 in POMC neurons and increased food-seeking behavior in some affected individuals also raises the possibility of involvement of this gene in the regulation of energy homeostasis.

Neurogenic Disorders of Corticotropin Regulation

Hypothalamic CRH hypersecretion is the likely cause of sustained pituitary-adrenal hyperfunction in at least two situations: Cushing syndrome caused by the rare CRH-secreting gangliocytomas of the hypothalamus[686] and severe depression. Severe depression is associated with pituitary-adrenal abnormalities, including inappropriately elevated ACTH levels, abnormal cortisol circadian rhythms, and resistance to dexamethasone suppression.[213,218,219,687] The dexamethasone suppression test has in fact been used as an aid in the diagnosis of depressive illness. Another possible example of disordered neurogenic control of CRH associated with stress is the dysmetabolic syndrome.[688,689] This syndrome is characterized by mild hypercortisolism, blunted dexamethasone suppression of the HPA axis, visceral obesity, and hypertension and may be strongly associated with greater risks for cardiovascular disease and stroke.

A unique syndrome of ACTH hypersecretion termed *periodic hypothalamic discharge* (Wolff syndrome) has been described in one young man. The patient had a recurring cyclic disorder characterized by high fever, paroxysms of glucocorticoid hypersecretion, and electroencephalographic abnormalities.[690]

Nonendocrine Manifestations of Hypothalamic Disease

The hypothalamus is involved in the regulation of diverse functions and behaviors (Table 7.9). Psychologic abnormalities in hypothalamic disease include antisocial behavior; attacks of rage, laughing, and crying; disturbed sleep patterns; excessive sexuality; and hallucinations. Both somnolence (with posterior lesions) and pathologic wakefulness (with anterior lesions) occur, as do bulimia and profound anorexia. The abnormal eating patterns are analogous to the syndromes of hyperphagia produced in rats by destruction of the VMH or of connections to the PVH. Lateral hypothalamic damage causes profound anorexia. A more complete discussion of imbalance in energy homeostasis (both obesity and cachexia) associated with hypothalamic dysfunction and neuropeptides is presented in Chapter 39.

Patients with hypothalamic damage may experience hyperthermia, hypothermia, unexplained fluctuations in body temperature, and poikilothermy. Disturbances of sweating, acrocyanosis, loss of sphincter control, and diencephalic epilepsy are occasional manifestations. Hypothalamic damage also causes loss of recent memory, believed to be due to damage of the mammillothalamic pathways. Severe memory loss, obesity, and personality changes (e.g., apathy, loss of ability to concentrate, aggressive antisocial

TABLE 7.9 Neurologic Manifestations of Nonendocrine Hypothalamic Disease

Disorders of Temperature Regulation
Hyperthermia
Hypothermia
Poikilothermia

Disorders of Food Intake
Hyperphagia (bulimia)
Anorexia nervosa, aphagia
Cachexia

Disorders of Water Intake
Compulsive water drinking
Adipsia
Essential hypernatremia

Disorders of Sleep and Consciousness
Narcolepsy/cataplexy
Somnolence
Sleep rhythm reversal
Akinetic mutism
Coma
Delirium

Periodic Disease of Hypothalamic Origin
Paroxysmal sympathetic storms (diencephalic epilepsy)
Kleine-Levin syndrome
Periodic discharge syndrome of Wolff

Disorders of Psychic Function
Rage behavior
Hallucinations
Hypersexuality

Disorders of the Autonomic Nervous System
Pulmonary edema
Cardiac arrhythmias
Sphincter disturbance

Congenital Hypothalamic Disease
Prader-Willi syndrome
Laurence-Moon-Biedl syndrome

Miscellaneous
Diencephalic syndrome of infancy
Cerebral gigantism

behavior, severe food craving, inability to work or attend school) may occur with suprasellar extension of pituitary tumors, hypothalamic radiation, or damage incurred from surgical removal of parasellar tumors. Hypothalamic tumors grow slowly and may reach a large size while producing minimal disturbance of behavior or visceral homeostasis, whereas surgery of limited extent can produce striking functional abnormalities. Presumably this is because slowly growing lesions permit compensatory responses to develop. These potential consequences should be weighed carefully with the neurosurgeon, patient, and patient's family in planning the therapeutic approach. Adverse effects of treatment have led to more conservative surgical guidelines for the treatment of craniopharyngioma. A review from the University of Pittsburgh summarizes their individualized treatment program that includes microsurgical tumor resection, intracavitary ^{32}P radiotherapy, and gamma knife stereotactic radiosurgery to produce maximal benefit with minimal morbidity.[691]

Paroxysmal Sympathetic Storms (Diencephalic Epilepsy)

Diencephalic epilepsy is characterized by episodic, autonomic discharge that can include tachycardia, bradycardia, diaphoresis, pupillary dilation, flushing, elevations in blood pressure, tachypnea, hypothermia and hyperthermia, and abnormal extensor posturing. Although thought due to seizures in the earlier literature, EEGs do not generally show evidence for epileptiform activity, and affected individuals do not respond well to anticonvulsants. Therefore it has been proposed to better describe these episodes as paroxysmal sympathetic storms.[692] The underlying etiology for this disorder is unknown, but most often it has been associated with acute increases in intracranial pressure and traumatic brain injury, although individual case reports have also reported an association with intracranial tumors and congenital malformations.

Narcolepsy

A convergence of functional genomics from two animal species, the dog and mouse, has dramatically refocused attention on neuropeptide circuits of the hypothalamus in the control of sleep and wakefulness. Positional cloning was used to identify mutations in the hypocretin-orexin receptor 2 as a cause of canine narcolepsy.[693] Subsequently, knockout of the gene encoding the orexin-hypocretin peptide precursor produced an equivalent narcoleptic syndrome in mice,[694] further establishing this neuropeptide system as a major component of sleep-modulating neural circuits. Targeted ablation of orexin neurons in the lateral hypothalamus of rats by means of a hypocretin receptor 2–saporin conjugate produced narcoleptic-like sleep behavior,[695] closely paralleling the clinical findings and profound reduction in numbers of orexin-hypocretin neurons in the lateral hypothalamus of humans with narcolepsy.[544] The additional role of orexin-hypocretin in coordinating arousal states and feeding behavior is discussed earlier in the chapter (see "Sleep-Wake Cycle" and "Modifying Factors Influencing Sleep") and in Chapter 39.

Most cases of spontaneous narcolepsy with cataplexy result from a degenerative hypothalamic disorder, most likely autoimmune in pathogenesis, that produces a selective destruction of neuropeptidergic neurons. The absence of immunoreactive orexin-hypocretin in CSF is a sensitive diagnostic test for the disease. Future development of bioavailable, orexin-hypocretin receptor-selective compounds may provide a specific treatment alternative or adjunct to the stimulant and antidepressant drugs currently used for management of symptoms. More generally, these recent discoveries suggest the possibility that other cryptic hypothalamic disorders could be caused by selective disturbances in other neuropeptidergic circuits.

Kleine-Levin Syndrome

Kleine-Levin syndrome is a periodic sleep disorder, generally of male adolescents, characterized by relapsing episodes of hypersomnia that can last hours to days associated with cognitive and behavioral abnormalities, including hyperphagia and hypersexuality, but normal behavior between episodes.[696] In contrast to narcolepsy, CSF orexin concentrations are usually normal, and the disorder is associated with a favorable prognosis as it tends to be self-limiting and tends to spontaneously resolve. The etiology is unknown, although several hypotheses have been put forward and reviewed by Al Shareef and colleagues[697] Evidence for abnormal perfusion by functional imaging studies in several brain regions, including the hypothalamus, thalamus, and cortex, has raised the possibility that Kleine-Levin syndrome may be an inflammatory disorder either due to viral or autoimmune encephalitis.[698] The latter possibility is of particular interest because of the tendency for the disorder to run in families and its higher prevalence in Ashkenazi Jews. Along these lines, linkage analysis and exome sequencing have identified chromosome 3 and a heterozygous missense variant in *LMOD3* in a large family with seven affected members with Kleine-Levin syndrome and in three other sporadic cases.[699] Lmod is a member of the tropomodulin gene family (Tmod), which has structural proteins involved in the sequestration of actin monomers and stimulation of ATP hydrolysis to accelerate actin nucleation and polymerization. While initially thought to be present exclusively in heart and skeletal muscle, Lmod proteins have been identified in several regions of the brain, including nuclear groups involved in the regulation of the sleep-wake cycle such as orexin neurons in the lateral hypothalamus, TMN, locus coeruleus, dorsal raphe, and ventral tegmental area.[699]

Hypothalamus and Neuropsychiatric Disorders

The role of the hypothalamus in the pathophysiology of eating disorders such as anorexia nervosa, Prader-Willi syndrome, and lesions destroying the base of the hypothalamus is well recognized and discussed in detail in Chapter 39. However, there is evidence to suggest that the hypothalamus is also involved in the pathophysiology of a number of common neuropsychiatric disorders. Genome-wide pathway and functional analysis of susceptibility genes associated with schizophrenia, for example, identify the hypothalamus as one of five major regions in the brain where these genes are predominantly expressed.[700] Included are glutaminergic, serotonergic, GABAergic and dopamine receptors, calcium and potassium ion channels, solute transporters, and neurodevelopment genes. Enlargement of the third ventricle is also known to be associated with schizophrenia and major depressive illness and thought secondary to loss of periventricular gray matter (see Bernstein and colleagues[701] for review). Along these lines, a decreased number of neurophysin-containing and nNOS-containing cells in the PVN and reduction in its innervation by beta-endorphin have been observed in schizophrenics.[702–704] A 50% reduction in total neuron number but increased numbers of oxytocin and vasopressin neurons in the PVN occur, and a similar reduction in enkephalin innervation has been observed in major depressive disorder.[704–706] Alterations in the SCN have also been described, including a reduced number of vasopressin neurons and vasopressin mRNA

in schizophrenics[707,708] and increased VIP in major depressive illness.[709] Furthermore, analysis of panels of susceptibility genes common to several psychiatric disorders identified genes involved in serotonin and dopamine homeostasis, including the serotonin receptor 2A (HTR2A), tryptophan hydroxylase 2 (TPH2), and the dopamine receptor D2 (DRD2), supporting the potential involvement of the SCN.[710]

Aberrant HPA activity and/or excessive CRHR-1 receptor stimulation are perhaps the best-recognized abnormalities associated with neuropsychiatric disorders, including schizophrenia, depression, anxiety disorders, autism, and obsessive-compulsive disorder.[711,712] Mechanisms proposed include impaired feedback inhibition of cortisol due to reduction in glucocorticoid and mineralocorticoid receptors, polymorphisms in glucocorticoid receptors and 11β-hydroxysteroid dehydrogenase type 1, increased CRH and elevated CRH mRNA expression in the hypothalamic PVH, and polymorphisms in the genes for CRH and the CRH receptor, CRH-R1. Nevertheless, with the exception of very few studies, trials employing a variety of CRH-R1 receptor antagonists have not been particularly successful,[229,712] perhaps signifying the complexities involved in the pathophysiology for these disorders and the need for more refined methods to establish adequate receptor occupancy and/or identify individuals who are most likely to respond.

Dysregulation of the oxytocin and orexin systems has also been implicated in the pathophysiology of neuropsychiatric disorders, although the data have been somewhat inconsistent.[713,714] Oxytocin receptor gene polymorphisms have been identified in schizophrenia and major depressive disorder, and some patients have shown improvement following administration of nasal oxytocin (including cognition in schizophrenic patients) and a decrease in depressive and anxiety symptoms in patients with major depressive disorder.[715–717] Similarly, oxytocin has been proposed to be involved in the pathophysiology of autism, as indicated by a reduction in plasma oxytocin levels and an increase in a biologically inactive extended form of oxytocin, as well as improved social interactions and reduced repetitive behaviors following intranasal oxytocin administration.[713] Orexin deficiency and downregulation of orexin receptors have been observed in depression and may contribute to the disrupted sleep pattern associated with this disorder.[714,718] In addition, the orexin receptor-2 (OxR2) knockout mouse demonstrates increased depressive-like behaviors, and in other studies, ICV administration of orexin improved some of the manifestations of depression.[719,720] Antidepressant actions of kisspetin,[721] the potential antidepressant action of somatostatin mediated through heterodimerization of somatostatin with dopamine D2 receptors[722] and elevated levels of nesfatin-1 in major depressive disorder,[723] further indicate that there is much yet to be learned about the role of peptides and the hypothalamus in neuropsychiatric disease.

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8

Pituitary Physiology and Diagnostic Evaluation

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CHAPTER OUTLINE

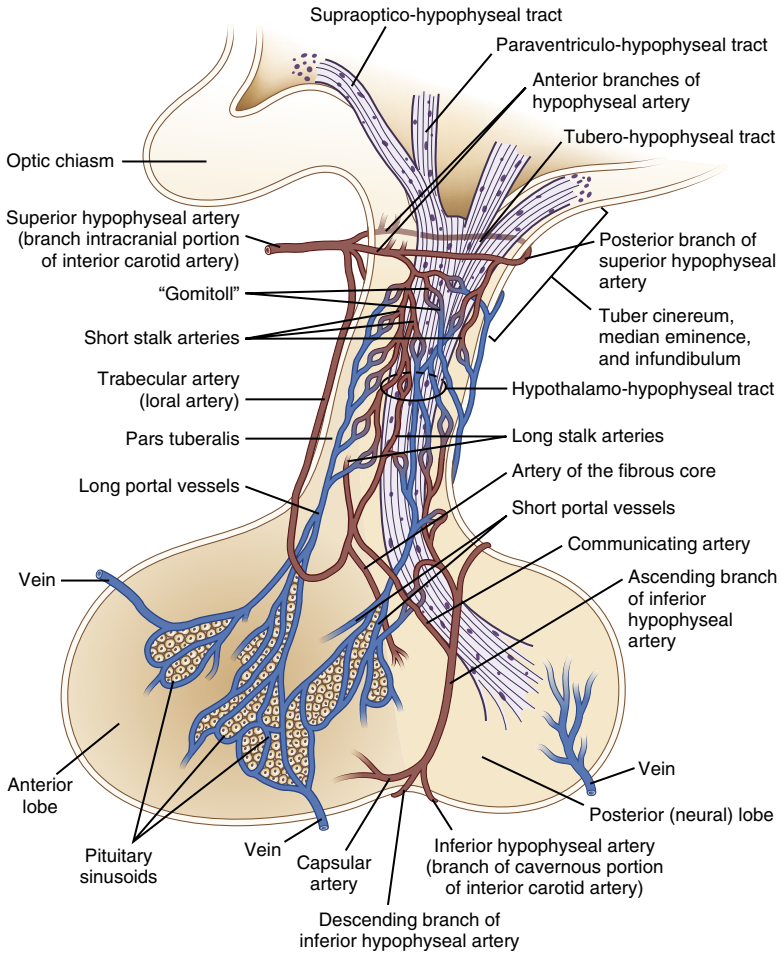
KEY POINTS

- The pituitary gland orchestrates endocrine system integrity through central hypothalamic and peripheral hormonal signals.
- Pituitary gland cells are organized into structural and functional networks formed during embryonic development but modified throughout life.
- Differentiated lactotrophs, somatotrophs, gonadotrophs, corticotrophs, and thyrotrophs are each regulated by system-specific factors.
- The growth hormone axis controls growth in childhood while regulating energy and substrate metabolism throughout life.
- Prolactin, responsible primarily for milk production during pregnancy and lactation, is uniquely under tonic inhibitory hypothalamic control by dopamine.
- The hypothalamic-pituitary-adrenal axis is the pivotal system subserving stress and survival, elaborated through effects on energy supply, fuel metabolism, immunity, and cardiovascular function.
- The hypothalamic-pituitary-gonadal axis plays a pivotal role in puberty, reproductive function, and fertility, controlling both gametogenesis and sex steroid hormone production.
- The hypothalamic-pituitary-thyroid axis plays a critical role in development, growth, and cellular metabolism, mediated by thyroid hormones.
- Pituitary gland failure can arise from developmental, heritable, and acquired disorders and is diagnosed by baseline and provocative pituitary and target gland hormone testing.

Anatomy, Development, and Overview of Control of Hormone Secretion

The pituitary gland, situated within the sella turcica, derives its name from the Greek *ptuo* and Latin *pituita*, meaning "phlegm," reflecting its nasopharyngeal origin. Galen hypothesized that nasal phlegm originated from the brain and drained via the pituitary gland. It is now clear that, together with the hypothalamus, the pituitary orchestrates the structural integrity and function of endocrine glands, including the thyroid, adrenal, and gonads, in addition to target tissues, including liver, cartilage, and breast. The pituitary stalk serves as an anatomic and functional connection to the hypothalamus. Preservation of the hypothalamo-pituitary unit is critical for integration of systemic and central nervous system (CNS) inputs for anterior pituitary control of sexual function and fertility, linear and organ growth, lactation, stress responses, energy, appetite, and temperature regulation, and secondarily for carbohydrate and mineral metabolism.

Integration of vital body functions by the brain was first proposed by Descartes in the 17th century. In 1733, Morgagni recorded the absence of adrenal glands in an anencephalic neonate, providing early evidence for a developmental and functional connection between the brain and the adrenal glands. In 1849, Claude Bernard set the stage for the subsequent advances in neuroendocrinology by demonstrating that central lesions to the area of the fourth ventricle resulted in polyuria.[1] Subsequent studies led to the identification and chemical isolation of pituitary hormones, and astute clinical observations led to the realization that pituitary tumors were associated with functional hypersecretory syndromes, including acromegaly and Cushing disease.[2–4] In 1948, Geoffrey Harris, the father of modern neuroendocrinology, reviewed the control of anterior pituitary gland hormones and proposed their hypothalamic regulation, predicting the subsequent discovery of specific hypothalamic regulating hormones.[5]



• **Fig. 8.1** Schematic representation of the blood supply of the hypothalamus and pituitary. (From Scheithauer BW. The hypothalamus and neurohypophysis. In: Kovacs K, Asa SL, eds. *Functional Endocrine Pathology.* Oxford, UK: Blackwell Scientific; 1991.)

Anatomy

The pituitary gland comprises the predominant anterior lobe, the posterior lobe, and a vestigial intermediate lobe (Fig. 8.1). The gland is situated within the bony sella turcica and is overlain by the dural diaphragma sellae through which the stalk connects to the median eminence of the hypothalamus. The adult pituitary weighs approximately 600 mg (range 400–900 mg) and measures approximately 13 mm in the longest transverse diameter, 6 to 9 mm in vertical height, and about 9 mm anteroposteriorly. Structural variation may occur in multiparous women, and gland volume also changes during the menstrual cycle. During pregnancy, these measurements may be increased in either dimension, with pituitary weight increasing up to 1 g. Pituitary hypertrophy without evidence for the presence of an adenoma was described in seven eugonadal women with pituitary height greater than 9 mm and a convex upper gland boundary observed on magnetic resonance imaging (MRI).[6]

The sella turcica, located at the base of the skull, forms the thin bony roof of the sphenoid sinus. The lateral walls comprise either bony or dural tissue about the cavernous sinuses, which are traversed by the third, fourth, and six cranial nerves and internal carotid arteries. Thus the cavernous sinus contents are vulnerable to intrasellar expansion. The dural roofing protects the gland from compression by fluctuant cerebrospinal fluid (CSF) pressure. The optic chiasm, located anterior to the pituitary stalk, is directly above the diaphragma sellae. The optic tracts and central structures are therefore vulnerable to pressure effects by an expanding pituitary mass, which typically follows the path of least tissue resistance. The posterior pituitary gland, in contrast to the anterior pituitary, is directly innervated by the supraopticohypophyseal and tuberohypophyseal nerve tracts of the posterior stalk. Hypothalamic neuronal lesions, stalk disruption, or systemically derived metastases to the hypothalamus are therefore often associated with attenuated vasopressin (diabetes insipidus) or oxytocin secretion.

The hypothalamus contains, among other neuronal populations, nerve cell bodies that synthesize hypophysiotropic releasing and inhibiting hormones, as well as the neurohypophyseal hormones of the posterior pituitary (arginine vasopressin [AVP] and oxytocin). Five distinct hormone-secreting cell types are present in the mature anterior pituitary gland:

1. Corticotroph cells express pro-opiomelanocortin (POMC) peptides, including adrenocorticotropic hormone (ACTH).
2. Somatotroph cells express growth hormone (GH).
3. Thyrotroph cells express the common glycoprotein α-subunit and the specific thyroid-stimulating hormone (TSH, thyrotropin) β-subunit.
4. Gonadotroph cells express the α-subunit and β-subunit for both follicle-stimulating hormone (FSH) and luteinizing hormone (LH).
5. Lactotroph cells express prolactin (PRL).

Each cell type is under highly specific signal controls that regulate their respective differentiated gene expression. The pituitary gland also contains support cells, known as pituicytes or folliculostellate cells.

Pituitary Blood Supply

The pituitary gland enjoys an abundant blood supply derived from several sources (see Fig. 8.1). The superior hypophyseal arteries branch from the internal carotid arteries to supply the hypothalamus, where they form a capillary network in the median eminence, which is external to the blood-brain barrier. Both long and short hypophyseal portal vessels originate from infundibular plexuses and the stalk, respectively. These vessels form the hypothalamic-portal circulation, the predominant blood supply to the anterior pituitary gland. They deliver hypothalamic releasing and inhibiting hormones to the trophic hormone-producing cells of the adenohypophysis, without significant systemic dilution, allowing the pituitary cells to be sensitively regulated by timed hypothalamic hormone secretion. Vascular transport of hypothalamic hormones is also locally regulated by a contractile internal capillary plexus (gomitoli), derived from stalk branches of the superior hypophyseal arteries.[7] Retrograde blood flow toward the median eminence also occurs, facilitating bidirectional functional hypothalamic-pituitary interactions.[8] Systemic arterial blood supply is maintained by inferior hypophyseal arterial branches, which predominantly supply the posterior pituitary. Disruption of stalk integrity may lead to compromised pituitary portal blood flow, depriving the anterior pituitary cells of hypothalamic hormone input.

Pituitary Development

The pituitary gland arises from within the rostral neural plate. Rathke pouch, a primitive ectodermal invagination anterior to the roof of the oral cavity, is formed by the fourth or fifth week of gestation[9] and gives rise to the anterior pituitary gland[10,11] (Fig. 8.2). The pouch is directly connected to the stalk and hypothalamic infundibulum and ultimately becomes distinct from the oral cavity and nasopharynx. Rathke pouch proliferates toward the third ventricle, where it fuses with the diverticulum and subsequently obliterates its lumen, which may persist as Rathke cleft. The anterior lobe is formed from Rathke pouch, and the diverticulum gives rise to the adjacent posterior lobe. Remnants of pituitary tissue may persist in the nasopharyngeal midline and rarely give rise to functional ectopic hormone-secreting tumors in the nasopharynx. The neurohypophysis arises from neural ectoderm associated with third ventricle development.[12]

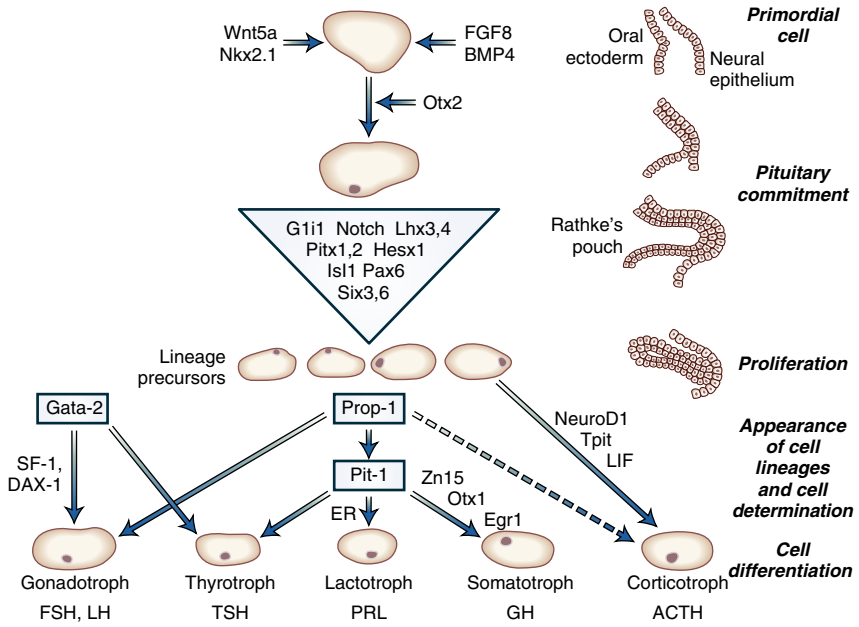
Functional development of the anterior pituitary cell types involves complex spatiotemporal regulation of cell lineage–specific transcription factors expressed in pluripotential pituitary stem cells, as well as dynamic gradients of locally acting soluble factors.[13–16] Critical neuroectodermal signals for organizing the dorsal gradient of pituitary morphogenesis include infundibular bone morphogenetic protein 4 (BMP4) required for the initial pouch invagination,[11] fibroblast growth factor 8 (FGF8), FGF10, Wnt5, and Wnt4.[17] Subsequent ventral developmental patterning and transcription factor expression are determined by spatial and graded expression of factors, including BMP2 and sonic hedgehog (SHH) protein, which appear critical for directing early patterns of cell proliferation.[18]

The human fetal Rathke pouch is evident at 3 weeks, and the pituitary grows rapidly in utero. By 7 weeks, the anterior pituitary vasculature begins to develop; by 20 weeks, the entire hypophyseal portal system is established. The anterior pituitary undergoes major cellular differentiation during the first 12 weeks, by which time all the major secretory cell compartments are structurally and functionally intact, except for lactotrophs. Totipotential pituitary stem cells give rise to acidophilic (mammosomatotroph, somatotroph, and lactotroph) and basophilic (corticotroph, thyrotroph, and gonadotroph) differentiated pituitary cell types, which appear at clearly demarcated developmental stages. At 6 weeks, corticotroph cells are morphologically identifiable, and immunoreactive ACTH is detectable by 7 weeks. At 8 weeks, somatotroph cells are evident with abundant immunoreactive cytoplasmic GH expression. Glycoprotein hormone–secreting cells express a common α-subunit, and at 12 weeks, differentiated thyrotrophs and gonadotrophs express immunoreactive β-subunits for TSH, LH, and FSH, respectively.[19] Fully differentiated PRL-expressing lactotrophs are only evident late in gestation (after 24 weeks). Prior to that time, immunoreactive PRL is only detectable in mixed mammosomatotrophs, also expressing GH, reflecting the common genetic origin of these two hormones.[20]

Pituitary Transcription Factors

Determination of anterior pituitary cell type lineages results from a temporally regulated cascade of homeodomain transcription factors.[13] Although most pituitary developmental information has been acquired from murine models,[21] histologic and pathogenetic observations in human subjects largely corroborate these developmental mechanisms (see Fig. 8.2; Tables 8.1 and 8.2). Early cell differentiation requires intracellular HESX1 and PITX expression. Rathke pouch expresses several transcription factors of the LIM homeodomain family, including LHX3, LHX4, and ISL1,[21] which are early determinants of functional pituitary development and are required for progenitor cell survival and proliferation. In contrast, activated Notch2 delays murine gonadotroph differentiation,[22] underscoring the role of NOTCH signaling pathways in the developmental cascade. Diversity of pituitary cell type determination is mediated by binary WNT/β-catenin signaling, leading to suppression of HESX1 and induction of Prophet of PIT 1 (PROP1).[23] These specific anterior pituitary transcription factors participate in a highly orchestrated cascade, ultimately leading to the commitment of the five differentiated cell types (see Fig. 8.2). The major proximal determinant of pituitary cell lineage is Prop1,[24] which determines subsequent development of POU1F1(PIT1)-dependent and gonadotroph cell lineages, while corticotroph cell commitment is directed by TBX19 protein.[25,26]

The bicoid homeodomain proteins, PITX1 and PITX2, behave as universal pituitary regulators and activate transcription of all the major anterior pituitary hormones.[27,28] PITX1 is expressed in the oral ectoderm and subsequently in all pituitary cell types, particularly those arising ventrally.[29] Rieger syndrome, characterized by defective eye, tooth, umbilical cord, and pituitary development, is caused by mutations in PITX2.[30] LHX3 determines GH, PRL, and TSH cell differentiation, and Prop1, a member of the paired-like family of homeodomain transcription factors, is expressed early in the development of Rathke pouch and behaves as a prerequisite for PIT1. PIT1 is a POU homeodomain transcription factor that determines development and appropriate temporal and spatial expression of GH, PRL, and TSH binding to specific DNA motifs to activate and regulate somatotroph, lactotroph,



Fetal appearance	12 weeks	12 weeks	12 weeks	8 weeks	8 weeks
Hormone	FSH LH	TSH	PRL	GH	POMC
Chromosomal gene locus	β-11p; β-19q	α-6q; β-1p	6	17q	2p
Protein	Glycoprotein α, β subunits	Glycoprotein α, β subunits	Polypeptide	Polypeptide	Polypeptide
Amino acids	210 204	211	199	191	266 (ACTH 1-39)
Stimulators	GnRH, estrogen	TRH	Estrogen, TRH	GHRH GHS	CRH, AVP gp-130 cytokines
Inhibitors	Sex steroids, inhibition	T3,T4, Dopamine, somatostatin glucocorticoids	Dopamine	Somatostatin, IGF activins	Glucocorticoids
Target gland	Ovary, testis	Thyroid	Breast, other tissues	Liver, bones, other tissues	Adrenal
Trophic effect	Sex steroid Follicle growth Germ cell maturation M, 5-20 IU/L F (basal) 5-20 IU/L	T4 Synthesis and secretion	Milk production	IGF-I production, growth induction, insulin antagonism	Steroid production
Normal range	M, 5-20 IU/L F (basal) 5-20 IU/L	0.1-5 mU/L	M <15; F <20 μg/L	<0.5 μg/L	ACTH, 4-22 pg/L

• **Fig. 8.2** Model for development of the human anterior pituitary gland and cell lineage determination by a cascade of transcription factors. Trophic cells are depicted with transcription factors known to determine cell-specific human or murine gene expression. *ACTH,* adrenocorticotropic hormone; *AVP,* arginine vasopressin; *CRH,* corticotropin-releasing hormone; *ER,* estrogen receptor; *F,* female; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *GHS,* growth hormone secretagogue; *GnRH,* gonadotropin-releasing hormone; *IGF,* insulin-like growth factor; *LH,* luteinizing hormone; *M,* male; *POMC,* pro-opiomelanocortin; *PRL,* prolactin; T_3, triiodothyronine; T_4, thyroxine; *TRH,* thyrotropin-releasing hormone; *TSH,* thyrotropin. (Adapted from Shimon I, Melmed S. Anterior pituitary hormones. In: Conn P, Melmed S, eds. *Scientific Basis of Endocrinology.* Totowa, NJ: Humana Press; 1996; Amselem S. Perspectives on the molecular basis of developmental defects in the human pituitary region. In: Rappaport R, Amselem S, eds. *Hypothalamic-Pituitary Development.* Basel, Switzerland: Karger; 2001; Dasen JS, O'Connell SM, Flynn SE, et al. Reciprocal interactions of PIT1 and GATA2 mediate signaling gradient-induced determination of pituitary cell types. *Cell.* 1999;97:587–598.)

and thyrotroph development and mature secretory function. Signal-dependent coactivating factors cooperate with PIT1 to determine specific hormone expression. Thus in POU1F1-containing cells, high estrogen receptor levels induce a commitment to express PRL, and thyrotroph embryonic factor (TEF) favors TSH expression. Selective pituitary cell type specificity is also perpetuated by binding of PIT1 to its own DNA regulatory elements as well as those contained within the GH, PRL, and TSH genes. Steroidogenic factor 1 (SF1) and DAX1 determine gonadotroph development.[31,32] TSH and gonadotropin-expressing cells share

TABLE 8.1 Etiology of Inherited Pituitary Deficiency

Mutation	Hormone Deficit
Receptor	
GHRH receptor	GH
CRH receptor	ACTH
GnRH receptor	FSH, LH
TRH receptor	TSH
Structural	
Pituitary aplasia	Any
Pituitary hypoplasia	Any
CNS masses; encephalocele	Any
Transcription Factor Defect	
HESX1	GH, PRL, TSH, LH, FSH, ACTH
SOX2/3	GH, PRL, TSH, LH, FSH, ACTH
LHX3/4	GH, PRL, TSH, LH, FSH
PITX2	GH
PROP1	GH, PRL, TSH, LH, FSH, ACTH
POU1F1	PRL, GH, TSH
IGSF1	PRL, GH, TSH
TBX19	ACTH
NR5A1	LH, FSH
NR0B1	LH, FSH
Hormone Mutation	
GH1	GH
Bioinactive GH	GH
FSHβ	FSH
LHβ	LH
TSHβ	TSH
POMC	ACTH
POMC processing defect	ACTH
PC1	ACTH, FSH, LH

ACTH, Adrenocorticotropic hormone; *CNS,* central nervous system; *CRH,* corticotropin-releasing hormone; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *GnRH,* gonadotropin-releasing hormone; *GPR,* orphan G protein–coupled receptor; *LH,* luteinizing hormone; *POMC,* pro-opiomelanocortin; *PRL,* prolactin; TSH, thyroid-stimulating hormone; *TRH,* thyrotropin-releasing hormone.

a common α-subunit (αGSU) expression under developmental control of GATA2.[33] FOXL2, a forkhead transcription factor, regulates differentiation of cell types expressing αGSU, including gonadotrophs and thyrotrophs, and transcription of αGSU and FSHβ.[17,34–37] Corticotroph cell commitment, occurring earliest during fetal development, is independent of PROP1-determined lineages, and TBX19 protein is a prerequisite for POMC expression.[38] Hereditary mutations arising within these transcription factors may result in isolated or combined pituitary hormone failure syndromes (see later).[39]

Pituitary Stem Cells

The adult pituitary gland exhibits plastic and regenerative trophic properties, which allow maintenance of homeostatic functions.[40] This characteristic is exemplified by pituitary lactotroph expansion during pregnancy and pituitary trophic hormone cell hyperplasia occurring after target organ ablation. Mechanisms underlying adult pituitary cell renewal and expansion are as yet unclear and may include intrinsic pituitary transdifferentiation, differentiation of previously uncommitted "null" cells, or expansion of already differentiated cells.

Several lines of evidence support the existence of cells with stem or progenitor characteristics in the adult pituitary gland.[41–45] Pituitary progenitor cells exhibit several characteristics of a stem cell phenotype, including an undifferentiated gene profile, clonality, the ability to form colonies, and expression of known stem cell markers, including Scal and CD133. Other markers, including Notch, Wnt, and SHH, are essential transcription factors for cell type determination and expansion of pituitary cell lineages.[46,47] Evidence has emerged that SOX2, SOX9, and OCT4 are markers of pituitary progenitors with properties for multipotent pituitary cell differentiation,[48] and a population of nestin-expressing murine pituitary cells fulfill criteria consistent with organ-specific multipotent stem cells.[45] These cells form differentiated pituitary-expressing progeny and contribute to an adult pituitary stem cell population distinct from embryonic precursor cells. Expression of potential stem cell markers such as nestin in the marginal zone around Rathke cleft has suggested that the stem cell population may exist in this marginal zone.[49] Cell-lineage tracing analysis has demonstrated that SOX2-expressing and SOX9-expressing progenitors can self-renew and give rise to pituitary endocrine cells in vivo, supporting the model of these as tissue stem cells.[43,50,51] Moreover, these cells can become mobilized and differentiate toward a specific cell fate in response to physiologic stress.[50,51] Advances have also been made toward recapitulation of pituitary differentiation in vitro: Three-dimensional (3D) cultures of embryonic stem cells have been stimulated to differentiate into Rathke pouch–like 3D structures, and various endocrine cells, including functional corticotrophs and somatotrophs, were subsequently produced, opening new avenues for the application of pluripotent stem cells to treat hypopituitarism.[52,53] Interestingly, adamantinomatous craniopharyngiomas share expression of stem cell markers with pituitary progenitor/stem cells, suggesting a common origin.[54]

Pituitary Control

The endocrine and nonendocrine cells of the pituitary gland are organized into structural and functional networks formed during embryonic development but modified throughout life.[55] The various endocrine cell types form distinct networks in spatial relationships with the vasculature, which can explain the marked secretory capacity not evident from those observed from dispersed cells in culture.[56] The functional characterization of the network activity of GH, PRL, and gonadotropin has provided firm evidence for cell organization in gene regulation, in magnitude, and in temporal facets of hormone secretion. As such, the existence of these endocrine cell networks confers the pituitary as more than a gland that simply responds to external regulation; rather, it acts as an oscillator that can imprint memory and adapt to coordinated network responses to hypothalamic inputs.[56]

Three levels of control subserve the regulation of anterior pituitary hormone secretion (Fig. 8.3). Hypothalamic control is mediated by adenohypophysiotropic hormones secreted into the hypothalamic portal system to impinge directly upon anterior

TABLE 8.2 Hereditary Pituitary Deficiency Caused by Transcription Factor Mutations

Gene	Chromosome	Pituitary Deficiency	MRI	Associated Malformations	Inheritance Mode
POU1F1	3p11	GH, PRL, ± TSH	Normal or hypoplastic anterior pituitary		Recessive, dominant
PROP1	5q35	GH, PRL, TSH, LH, FSH, ± ACTH	Normal, hypoplastic, hyperplastic, or cystic anterior pituitary		Recessive
HESX1	3p21	GH, PRL, TSH, LH, FSH, ACTH Posterior defects	Hypoplastic or hyperplastic anterior pituitary; normal or ectopic posterior pituitary	Septo-optic dysplasia	Recessive
PITX2	4q25	GH, PRL, TSH, FSH, LH		Rieger syndrome	Dominant
LHX3	9q34	GH, PRL, TSH, LH, FSH	Hypoplastic or hyperplastic anterior pituitary	Stubby neck with rigid cervical spine	Recessive
LHX4	1q25	GH, TSH, ACTH	Hypoplastic anterior pituitary Ectopic posterior pituitary		Dominant
TBX19	1q23	ACTH	Normal		Recessive
OTX2		GH, PRL, TSH, LH, FSH, ACTH	Hypoplastic anterior pituitary Ectopic posterior pituitary	Eye malformations	Dominant/negative
SIX6	14q22		Hypoplastic pituitary Absent chiasm	Brachio-otorenal and oculoauriculo-vertebral syndromes	Haplo-insufficiency
SOX2	3q26	GH, FSH, LH	Anterior pituitary hypoplasia Midbrain defects	Anophthalmia Esophageal atresia	
SOX3	Xq27	GH, TSH, ACTH, FSH, LH	Anterior pituitary hypoplasia Ectopic posterior pituitary		X-linked recessive
IGSF1	Xq25	GH, PRL, TSH		Testicular enlargement	X-linked recessive
NR5A1	9q33	FSH, LH		Adrenal insufficiency, gonadal defects, XY sex reversal	Dominant, recessive
NR0B1	Xp21.3	FSH, LH		Adrenal hypoplasia congenital, gonadal defects, XY sex reversal	X-linked dominant
GPR161	1q24.2	GH, TSH	Pituitary stalk interruption syndrome	Alopecia, ptosis, short fifth finger	Recessive
ARNT2	15q25.1	ACTH, TSH, GH, LH, FSH	Hypoplastic anterior pituitary and pituitary stalk interruption	Microcephaly, dysmorphic facies, visual and renal abnormalities	Recessive
NFKB2	10q24	ACTH, GH, TSH		Variable immune deficiency	Dominant

Genes involved in pituitary development or in maintaining integrity of the hypothalamic-pituitary axis. Functional defects include missense or frameshifts leading to truncated or deleted protein, DNA binding abnormality.

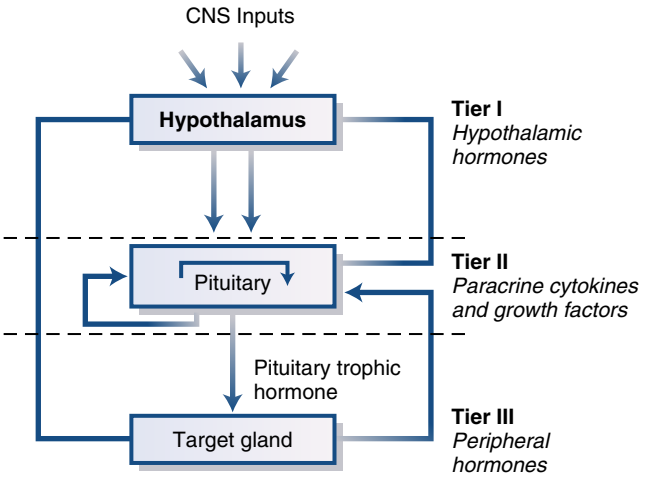
pituitary cell surface receptors. G protein–coupled cell surface membrane binding sites are highly selective and specific for each of the hypothalamic hormones and elicit positive or negative signals mediating pituitary hormone gene transcription and secretion. Peripheral hormones also participate in mediating pituitary cell function, predominantly by negative feedback regulation of trophic hormones by their respective target hormones. Intrapituitary paracrine and autocrine soluble growth factors and cytokines act to locally regulate neighboring cell development and function. The net result of these three tiers of complex intracellular signals is the controlled pulsatile secretion of the six pituitary trophic hormones—ACTH, GH, PRL, TSH, FSH, and LH (Fig. 8.4). Temporal and quantitative control of pituitary hormone secretion is critical for physiologic integration of peripheral hormonal systems, as exemplified by the menstrual cycle, which relies on complex and precisely regulated hormonal pulse control.

Physiology and Disorders of Pituitary Hormone Axes

Prolactin

Physiology

PRL is produced primarily by pituitary lactotrophs and is tonically inhibited by hypothalamic dopamine. The identification of PRL in humans was elusive until 1970 because human GH is highly lactogenic and active in bioassays used to isolate and measure PRL.[57]

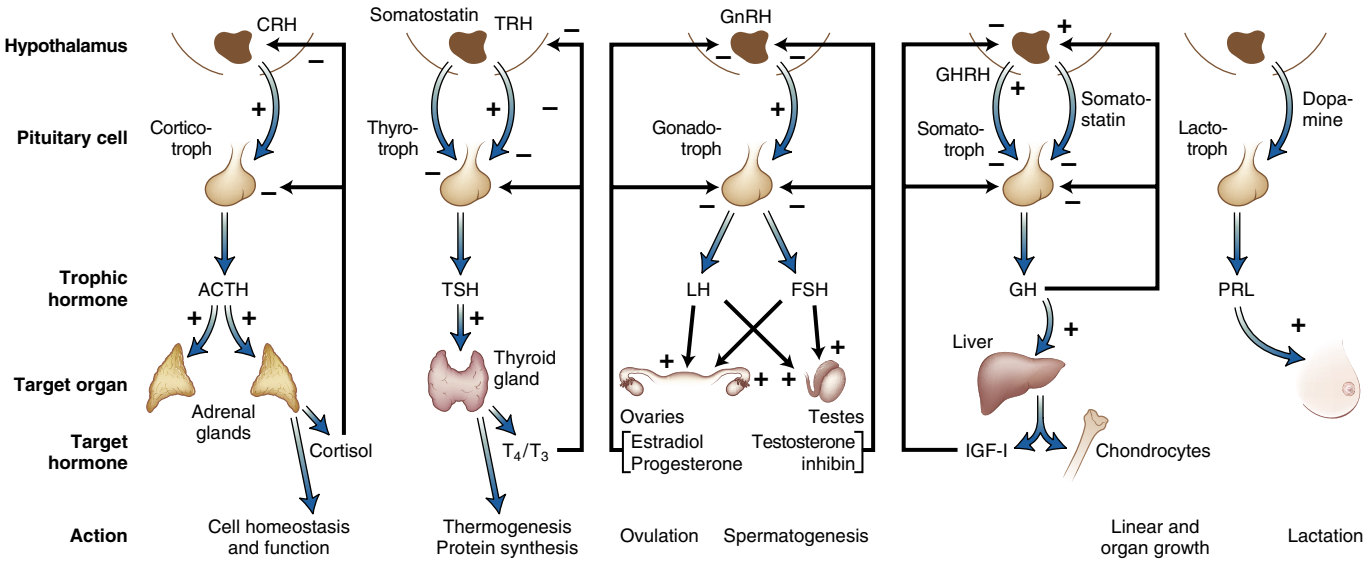


• **Fig. 8.3** Model for regulation of anterior pituitary hormone secretion by three tiers of control. Hypothalamic hormones traverse the portal system and impinge directly upon their respective target cells. Intrapituitary cytokines and growth factors regulate tropic cell function by paracrine (and autocrine) control. Peripheral hormones exert negative feedback inhibition of respective pituitary trophic hormone synthesis and secretion. *CNS*, central nervous system. (From Ray D, Melmed S. Pituitary cytokine and growth factor expression and action. *Endocr Rev.* 1997;18:206–228.)

Furthermore, GH is present in human pituitary glands in much higher concentrations (5–10 mg) than PRL (~100 μg).[58] To distinguish human PRL from GH, lactogenic activity derived from GH effects was neutralized with GH antiserum; using this assay, sera from postpartum women and patients with galactorrhea still had high lactogenic activity in the presence of GH antibodies.[59,60] Purification and isolation of PRL by Friesen and development of a specific radioimmunoassay underscored the utility of PRL measurements in understanding human disease.[61,62]

Lactotroph Cells

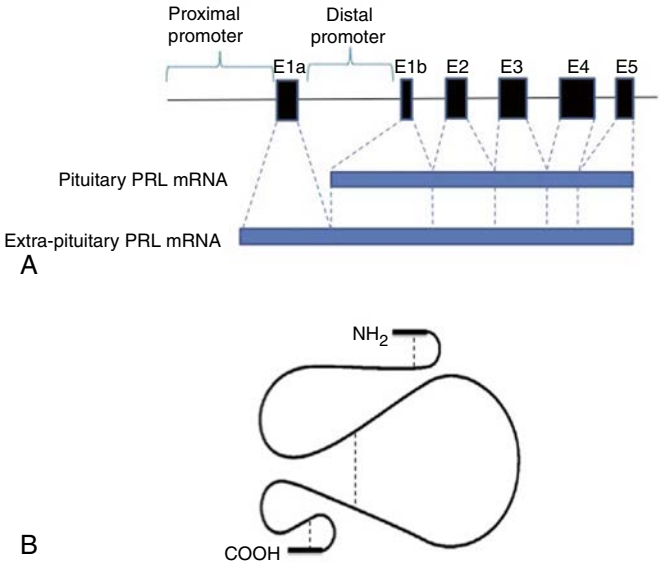
About 15% to 25% of functioning anterior pituitary cells are lactotroph cells. Most PRL-expressing cells appear to arise from GH-producing cells. Ablation of somatotrophs by expression of GH-diphtheria toxin and GH-thymidine kinase fusion genes inserted into the germline of transgenic mice eliminates most lactotrophs, suggesting that the majority of PRL-producing cells arise from postmitotic somatotrophs in mice.[63] Two cell forms expressing the PRL gene include large polyhedral cells found throughout the gland and smaller angulated or elongated cells clustered in the lateral wings and median wedge. Large PRL secretory granules (250–800 nm) are present in the evenly distributed cells, while the laterally localized cells are sparsely populated by smaller (200–350 nm) granules. Occasional mammosomatotroph cells cosecrete both PRL and GH, often stored within the same granule. In animal models, lactotroph cell function is heterogeneous. Thus dopamine or thyrotropin-releasing hormone (TRH) responsiveness, and shifting proportions of PRL versus GH secreting cells, may depend on cell localization within the pituitary, as well as the surrounding hormonal milieu, especially that of estrogen.[64] Although their absolute number is similar in men and women and does not change with age, lactotroph hyperplasia does occur during pregnancy and lactation[65] and resolves within several months of delivery.



• **Fig. 8.4** Control of hypothalamic-pituitary target organ axes. *ACTH,* adrenocorticotropic hormone; *CRH,* corticotropin-releasing hormone; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *GnRH,* gonadotropin-releasing hormone; *IGF,* insulin-like growth factor; *LH,* luteinizing hormone; *PRL,* prolactin; T_3, triiodothyronine; T_4, thyroxine; *TRH,* thyrotropin-releasing hormone; *TSH,* thyroid-stimulating hormone. (Adapted from Melmed S. Mechanisms for pituitary tumorigenesis: the plastic pituitary. *J Clin Invest.* 2003;112:1603–1618.)

Prolactin Structure

The human PRL gene, located on chromosome 6,[66] arose from a single common ancestral gene, which gave rise to the relatively homologous PRL, GH, and placental lactogen-related proteins.[67] Several factors influence PRL gene expression, including estrogen, dopamine, and TRH.[68] The PRL gene is transcribed from two alternative promoter regions, with the more proximal directing pituitary-specific expression and the more upstream promoter region directing extrapituitary expression (Fig. 8.5; see also Fig. 8.2).[69] PRL is a 199–amino acid polypeptide containing three intramolecular disulfide bonds (see Fig. 8.5). It circulates in blood in various sizes—monomeric PRL ("little prolactin"; 23 kDa), dimeric PRL ("big prolactin"; 48–56 kDa), and macroprolactin ("big, big prolactin," comprised of prolactin bound to immunoglobulins; >100 kDa).[69a–72] The monomeric form is the most bioactive PRL. In response to TRH, the proportion of the monomeric form increases. A glycosylated form of PRL, identified in pituitary extracts, is less biologically active than the nonglycosylated form.[73] Monomeric PRL is cleaved into 8-kDa and 16-kDa forms,[74] and the 16-kDa variant is antiangiogenic.[75,76] Indeed, this 16-kDa PRL cleavage product has been implicated in peripartum cardiomyopathy.[77] The mechanism underlying this association was recently shown to result from actions of the 16-kDa PRL, also referred to as vasoinhibin,[78] on capillary endothelial cells, stimulating the production of miR-146a, which in turn acts through a paracrine mechanism on cardiomyocytes to inhibit metabolism by blocking the activity of ERBB4, a tyrosine kinase receptor, to impair cardiomyocyte function. Cardiac dysfunction could be improved by treatment with the dopamine agonist bromocriptine, which results in a fall in miR-146a levels in parallel with an improvement in cardiac function.[79–81]



• **Fig. 8.5** Schematic representation of the human PRL gene and protein. (A) The *PRL* gene consists of five exons (E1–E5). PRL gene transcription is regulated by two independent promoter regions, upstream of two alternative first exons (1a and 1b). The proximal promoter regions directs pituitary-specific expression, whereas the distal promoter region directs extrapituitary expression. Exons 1a and 1b both encode 5′-untranslated sequences, so the protein-coding sequences of both transcripts are identical. (B) PRL is a 23-kDa protein comprised of 199 amino acids with three disulfide bonds *(hatched lines)*.

Regulation

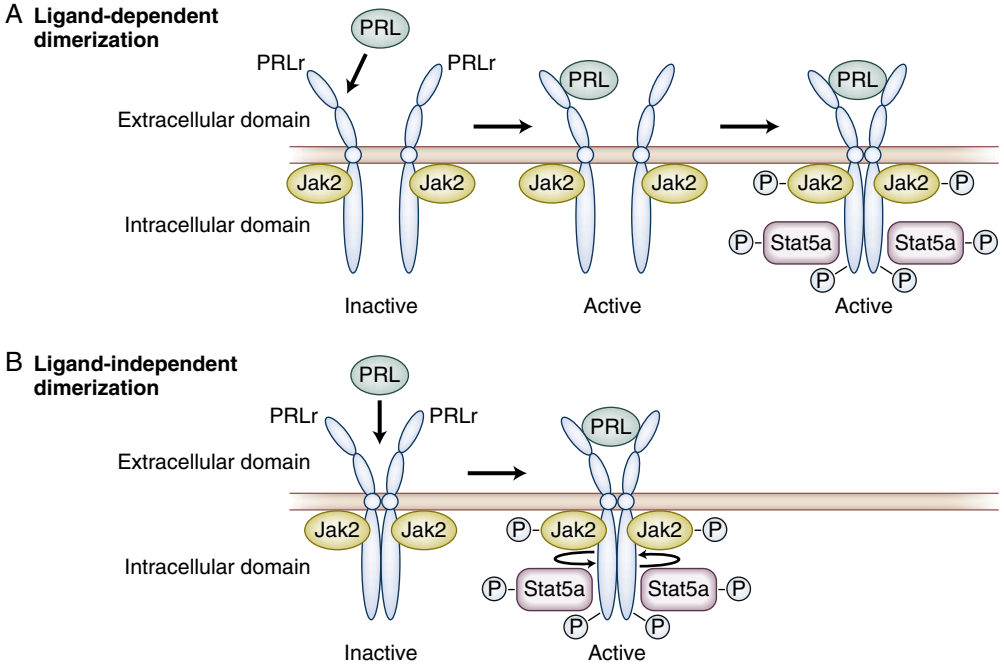
PRL secretion is under inhibitory control by dopamine, produced by the tuberoinfundibular (TIDA) cells and the hypothalamic tuberohypophyseal dopaminergic system.[82,83] Dopamine reaches the lactotrophs via the hypothalamic-pituitary portal system and binds to type 2 dopamine (D_2) receptors on pituitary lactotrophs to inhibit PRL secretion.[84] PRL, in turn, participates in negative feedback to control its release by increasing tyrosine hydroxylase activity, and thereby dopamine synthesis in the TIDA neurons.[83] In PRL-deficient mice, dopamine is decreased in the median eminence.[85] Mice lacking the D_2 receptor develop hyperprolactinemia and lactotroph proliferation.[84] Many other factors modulate PRL secretion, although their physiologic or clinical relevance remains unclear. Factors other than dopamine inhibit PRL secretion, including endothelin-1 and TGFβ1, which act as paracrine PRL inhibitors,[86,87] and calcitonin, which may be derived from the hypothalamus.[88] Several substances act as PRL-releasing factors. Growth factors such as basic FGF and epidermal growth factor (EGF) induce PRL synthesis and secretion. Neuropeptides acting through G protein-coupled receptors to stimulate PRL include vasoactive intestinal polypeptide (VIP), prolactin-releasing peptide (PrRP), opioid peptides, and TRH.[88a–88c,89] Estrogen stimulates PRL gene transcription and PRL secretion,[90] explaining why women have higher PRL levels, particularly during the periovulatory menstrual phase and in pregnancy.[91,92] The physiologic roles of other neuropeptides (e.g., neurotensin, substance P, bombesin, cholecystokinin, serotonin, and histamine) in regulating human PRL secretion are unresolved.[93] Evidence also suggests a short feedback loop with PRL negatively regulating its own secretion either via augmentation of hypothalamic TIDA turnover or through direct autocrine actions.[93–95]

Prolactin Secretion

The calculated production rate of PRL ranges from 200 to 536 μg/day/m^2, and the metabolic clearance rate ranges from 40 to 71 mL/minute per m^2.[96] PRL is cleared rapidly, with a calculated half-life in the circulation ranging from 26 to 47 minutes. PRL secretion occurs episodically, with 4 to 14 secretory pulses over 24 hours,[97,98] with the highest levels achieved during sleep and the lowest occurring between 10 AM and 12 noon.[99] The nocturnal elevation is sleep entrained, and a temporal relationship exists between rapid eye movement (REM) and non-REM sleep cycles.[100] PRL levels decline with age in both men and women. In older men, less PRL is produced with each secretory burst than in younger men.[101] Postmenopausal women have lower mean serum PRL levels and PRL pulse frequency than do premenopausal women, suggesting a stimulatory effect of estrogen on both of these parameters.[92]

Prolactin Action

The PRL receptor gene is a member of the cytokine receptor superfamily,[102] localized to chromosome 5p13, with an extracellular domain, a hydrophobic transmembrane domain, and an intracytoplasmic region homologous to the GH receptor[103] (Fig. 8.6). PRL receptor dimerization occurs in both ligand-dependent and ligand-independent manners, with a single PRL molecule binding to both components of the receptor dimer, with phosphorylation of intracellular Janus kinase/signal transducers and activators of transcription (JAK-STAT) molecules subsequent to PRL binding. Two ligand-receptor binding sites are critical for formation of the



• **Fig. 8.6** Ligand-dependent and ligand-independent dimerization of the prolactin (PRL) receptor (PRLr). (A) Ligand-dependent dimerization model. PRLr is in monomeric form at the cell membrane. One molecule of PRL first binds to one PRLr monomer via binding site 1, and this 1:1 complex then recruits the second PRLr via binding site 2. Dimerization of the two PRLrs leads to activating changes in the intracellular domain, leading to PRL signal transduction, such as phosphorylation (P) of Janus kinase 2 (Jak2), phosphorylation of the PRLr, and the recruitment and phosphorylation of the signal transducer and activator of transcription (Stat5). (B) Ligand-independent model. PRLr exists in dimeric form at the cell membrane in the absence of ligand. The receptors are held in an inactive form until binding of PRL to this preformed complex induces activating changes in the intracellular domain, leading to phosphorylation of Jak2, phosphorylation of the PRLr, and recruitment and phosphorylation of Stat5a. (From Clevenger C, Gadd SL, Zheng J. New mechanisms for PRLr action in breast cancer. *Trends Endocrinol Metab.* 2009;20:223–229.)

trimeric ligand-receptor complex and subsequent signaling.[104–106] PRL receptor induces protein tyrosine phosphorylation and activation of JAK2 and STAT1, STAT3, STAT5A, and STAT5B.[107,108] STAT5A phosphorylation is particularly important for mammary gland development and lactogenesis.[109]

PRL receptors are ubiquitously expressed in breast tissue as well as pituitary, liver, adrenal cortex, kidneys, prostate, ovary, testes, intestine, epidermis, pancreatic islets, lung, myocardium, brain, and lymphocytes. Regulation of milk production occurs via a cascade of intracellular events. Homozygous mice in which the PRL receptor has been inactivated are infertile.[104] A gain-of-function mutation conferring constitutive activity of the PRL receptor is present in a subset of patients with multiple breast fibroadenomas.[110] PRL receptor antagonists have been developed for targeting the receptor in PRL-sensitive disturbances, including resistant prolactinomas, breast tumors, and prostate tumors.[106,111]

PRL is essential for human species survival because it is responsible for milk production during pregnancy and lactation. Additional biologic functions ascribed to PRL include reproductive, metabolic, and immune effects.[69,93] Although PRL and its receptor are clearly crucial in lower animals,[112] the impact of PRL on maternal behavior in humans has not been fully delineated.

Mammary Gland Development

PRL is not essential for pubertal mammary development, which instead requires GH, the action of which is mediated by insulin-like growth factor 1 (IGF1).[113–115] At birth, the rodent mammary gland consists of a fat pad with small areas of ductal anlagen, which differentiate into pubertal mammary glandular elements under the influence of estrogen, GH, and IGF1. At puberty, a surge of estrogen begins the developmental process. Terminal end buds form and lead the process of mammary development by branching and extending into the substance of the mammary fat pad, leaving in its wake a network of ducts that virtually fill the mouse mammary fat pad.[116,117] GH acts on the mammary stoma compartment to produce IGF1, which in turn stimulates formation of terminal end buds and ducts in synergy with estrogen.[115,118] Parathyroid hormone–related protein is essential for fetal mammary development,[119] and epidermal growth is essential for pubertal mammary development.[120] Progesterone, possibly in association with GH and PRL, causes formation of lobular "decorations" along ducts, which are precursors to true glands.[121] In humans, pubertal mammary development begins in girls between the ages of 8 and 13 (see developmental scale of Tanner, Chapter 24). Once fully developed, the pubertal mammary gland remains quiescent until pregnancy, although cyclic changes occur during the menstrual cycle.

In human pregnancy, mammary gland alveolar elements proliferate and begin to produce milk proteins and colostrum. At 3 to 4 weeks of gestation, terminal ductal sprouting occurs, followed by lobular-alveolar formation, and true alveoli form at the end of the first trimester. Glandular elements proliferate further and secretory products appear in the alveolar lumina. During the third trimester, fat droplets are seen within alveolar cells, and the glands fill with colostrum.[122] A combination of estrogen, PRL, progesterone, and

possibly IGF1 and placental hormones are largely responsible for this phase of mammary development.[123] In mice with targeted disruption of the PRL gene, formation of alveolar structures is impaired.[84] Likewise, women with isolated PRL deficiency are unable to lactate.[124] Similarly, in mice lacking the progesterone receptor, lobular-alveolar formation does not occur.[125] Interestingly, only a minority of women have expressible milk during pregnancy, most likely due to the inhibitory effects of estradiol[126] and progesterone[127] on PRL-induced milk production.

Lactation

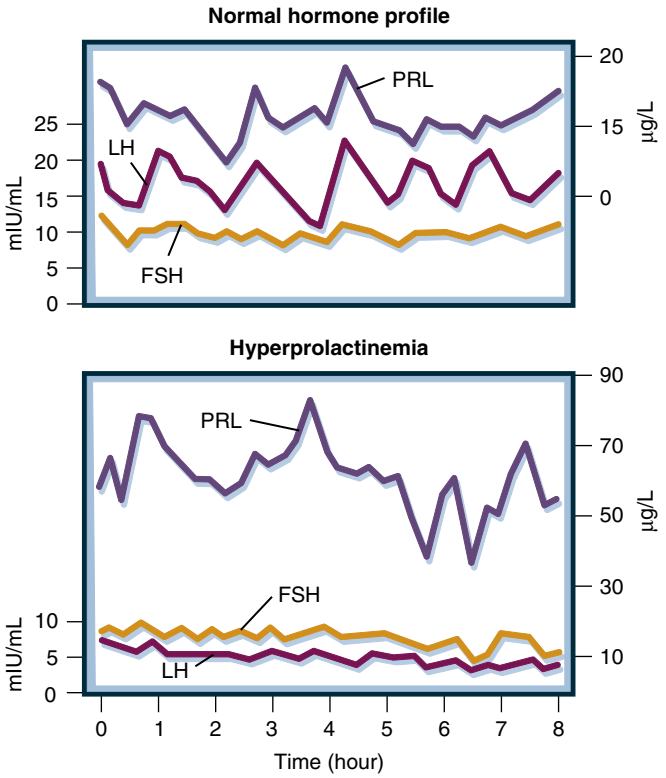
Active lactation is due in part to attenuation of estrogen and progesterone levels and elevation of PRL levels after delivery. Suckling also increases milk production after parturition and is essential for continued lactation because of its effect on pituitary hormone production and because it empties the mammary gland of milk.[128] Milk accumulation inhibits milk synthesis, explaining why a certain level of nursing activity is necessary for successful breastfeeding. In the absence of suckling, PRL concentrations, which rise throughout gestation, return to normal by 7 days postpartum.[129] Although PRL is essential for milk production, the milk yield does not correlate closely with serum PRL levels.[130] Suckling also stimulates posterior pituitary oxytocin release; unlike PRL, oxytocin responses to suckling do not decline for up to 6 months if nursing continues. Oxytocin induces myoepithelial cell contraction, thereby causing milk ejection.[131] Oxytocin also has important effects on alveolar proliferation.

Reproductive Function

Lactation results in amenorrhea and secondary infertility, and this natural form of contraception depends upon the frequency and duration of breastfeeding. The Kung hunter-gatherer women suckle their infants approximately four times an hour and at will during the night, and women bear a mean of 4.7 children during their reproductive years.[132] In contrast, the Hutterites of North America bear a mean of 10.6 children during their lifetimes, presumably because they nurse according to a rigid schedule, use supplemental feedings, and wean at 1 year. Amenorrhea and infertility result from PRL-mediated inhibitory effects on hypothalamic gonadotropin-releasing hormone (GnRH) and pituitary gonadotropin secretion of the gonadotropins, resulting in a reduction in both amplitude and frequency of LH pulses (Fig. 8.7).[133] In a hyperprolactinemic mouse model, hypothalamic kisspeptin immunoreactivity was reduced, and administration of kisspeptin restored estrous cyclicity, ovulation, and circulating LH and FSH levels. In addition, kisspeptin neurons have been shown to express PRL receptors.[134] Taken together, these data suggest that kisspeptin may be the missing link between hyperprolactinemia and the associated hypogonadotropic hypogonadism, anovulation, and infertility.[135,136] Indeed, kisspeptin administration to women with hyperprolactinemia-induced hypothalamic amenorrhea was shown to reactivate gonadotropin secretion.[136a] During lactation, additional metabolic factors induced by negative energy balance likely also contribute to disruption of pulsatile GnRH and LH secretion.

Other Actions

Early evidence indicated that PRL regulates immune function. However, some evidence indicates that PRL may not be important for immune function,[137] as innate immunity was not altered



• **Fig. 8.7** Effect of hyperprolactinemia on suppressing follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretory patterns leading to hypogonadotrophism in a female patient. *PRL,* prolactin. (Adapted from Tolis G. Prolactin: physiology and pathology. *Hosp Pract.* 1980;15:85–95.)

in transgenic mice devoid of either the PRL receptor ($PRLR^{-/-}$) or PRL ($PRL^{-/-}$).[84,138] Prolactin has been shown to have effects on maternal behavior in rodents, but its role in humans remains to be determined. Other roles that have been proposed for prolactin include effects on neurogenesis, metabolism and glucose homeostasis, appetite regulation, and bone and calcium homeostasis.[69,93]

Prolactin Measurements

Modern PRL measurements are based on immunoradiometric or chemiluminescent assays and are highly specific in distinguishing PRL from GH. The serum concentration of PRL can be given in mass concentration (μg/L or ng/mL), molar concentration (nmol/L or pmol/L), or international units (mIU/L). As these samples are usually assayed at a single dilution, extremely high PRL concentrations may saturate the capture and signal antibodies, preventing detection of very high PRL levels and resulting in a falsely low value being reported.[139] This "hook effect" may result in PRL-secreting macroadenomas diagnosed as clinically nonfunctioning adenomas. In patients harboring macroadenomas with clear-cut clinical features of hyperprolactinemia, serum samples should be subjected to at least 1:100 dilution prior to assay.

Conversely, macroprolactin, a physiologically inactive form of prolactin bound to IgG, can lead to a falsely elevated prolactin result, thus resulting in misdiagnosis of hyperprolactinemia. Misdiagnosis can be avoided by asking the laboratory to pretreat the serum with polyethylene glycol to precipitate macroprolactin before the immunoassay for prolactin in the supernatant.[140]

Prolactin Deficiency

Causes

Congenital PRL deficiency can occur in association with mutations in the transcription factors involved in lactotroph lineage development, including POU1F1, PROP1, LHX3, LHX4, and HESX1 (see Tables 8.1 and 8.2). In these cases, deficiencies in other anterior pituitary hormones occur in conjunction with PRL deficiency, with the spectrum of pituitary hormone deficiencies depending on the gene affected.[48] PRL deficiency has also been reported in conjunction with central hypothyroidism in a subset of patients with the immunoglobulin superfamily member 1 (IGSF1) deficiency syndrome.[167]

Manifestations

The only known clinical manifestation of PRL deficiency is the inability to lactate after delivery. Isolated PRL deficiency is rare but has been reported in association with autoantibodies directed specifically against PRL-secreting pituitary cells.[168] Most patients with acquired PRL deficiency have evidence of other pituitary hormone

TABLE 8.3 Etiology of Hyperprolactinemia

Physiologic

Pregnancy
Sucking
Stress
Sleep
Coitus
Exercise

Pathologic

Hypothalamic-Pituitary Stalk Damage

Tumors
Craniopharyngioma
Suprasellar pituitary mass extension
Meningioma
Dysgerminoma
Hypothalamic metastases
Granulomas
Infiltrations
Rathke cyst
Irradiation
Trauma: pituitary stalk section, sellar surgery, head trauma

Pituitary

Prolactinoma
Acromegaly
Macroadenoma (compressive)
Idiopathic
Plurihormonal adenoma
Lymphocytic hypophysitis
Parasellar mass
Macroprolactinemia

Systemic Disorders

Chronic renal failure
Polycystic ovary syndrome
Cirrhosis
Pseudocyesis
Epileptic seizures
Cranial irradiation
Chest: neurogenic, chest wall trauma, surgery, herpes zoster

Genetic

Inactivating prolactin receptor mutation

Pharmacologic

Neuropeptides

Thyrotropin-releasing hormone

Drug-Induced Hypersecretion

Dopamine Receptor Blockers

Phenothiazines: chlorpromazine, perphenazine
Butyrophenones: haloperidol
Thioxanthenes
Metoclopramide

Dopamine Synthesis Inhibitors

α-Methyldopa

Catecholamine Depleters

Reserpine

Cholinergic Agonists

Physostigmine

Antihypertensives

Labetalol
Reserpine
Verapamil

H_2 Antihistamines

Cimetidine
Ranitidine

Estrogens

Oral contraceptives
Oral contraceptive withdrawal

Anticonvulsants

Phenytoin

Neuroleptics

Chlorpromazine
Risperidone
Promazine
Promethazine
Trifluoperazine
Fluphenazine
Butaperazine
Perphenazine
Thiethylperazine
Thioridazine
Haloperidol
Pimozide
Thiothixene
Molindone

Opiates and Opiate Antagonists

Heroin
Methadone
Apomorphine
Morphine

Antidepressants

Tricyclic antidepressants: clomipramine, amitriptyline
Selective serotonin reuptake inhibitors: fluoxetine

deficiencies in association with pituitary injury.[169] Infarction of the pituitary gland after postpartum hemorrhage, known as Sheehan syndrome, has long been recognized as a cause of hypopituitarism.[170] In developed countries, postpartum hemorrhage now less often results in Sheehan syndrome than previously, largely due to improvements in obstetric care. No commercially available PRL preparation is available for these women, although studies with recombinant human PRL administration have been shown to increase milk volume in women with PRL deficiency or lactation insufficiency.[171]

Hyperprolactinemia

Causes

The causes of hyperprolactinemia may be physiologic, pathologic, or drug induced[141,142] (Table 8.3).

Physiologic Causes

Pregnancy. During pregnancy, the normal pituitary gland may double or more in size,[65] the result of a marked increase in the number of PRL-producing cells. Serum PRL concentrations rise to 10 times the normal concentration during pregnancy,[129] and amniotic fluid PRL concentrations are 100 times those of maternal or fetal blood.[129]

Suckling. Suckling increases serum PRL levels approximately 8.5-fold in actively nursing mothers.[143,144] As nursing continues, PRL concentrations fall, but each suckling episode continues to cause a subsequent episodic rise in serum PRL. Mean serum concentrations were 162 μg/L at 2 to 4 weeks postpartum, 130 μg/L at 5 to 14 weeks, and 77 μg/L at 15 to 24 weeks.[145] It is unclear why active milk production continues despite progressively lower PRL levels following parturition.

Idiopathic Hyperprolactinemia. An elevated circulating PRL level in patients in whom no cause is identified is considered idiopathic, and these patients are relatively resistant to dopamine agonist therapy. Mean serum PRL levels in patients with idiopathic hyperprolactinemia are usually less than 100 μg/L.[146]

Macroprolactinemia. PRL is a 23-kDa single-chain polypeptide but may also circulate in high-molecular-weight forms. High-molecular-weight PRL variants may, in some situations, represent 85% or more of total PRL, but under usual circumstances the 23-kDa variety predominates. Macroprolactinemia refers to these larger circulating PRL molecules, comprised of PRL bound to immunoglobulins, which exhibit markedly reduced bioactivity. Few of the expected clinical abnormalities usually associated with hyperprolactinemia (sexual dysfunction, hypogonadism, galactorrhea, osteoporosis) occur in patients with macroprolactinemia.[140,147] Screening for macroprolactinemia can be accomplished by polyethylene glycol precipitation of serum samples. In a 2005 survey, macroprolactinemia was detected in 22% of 2089 hyperprolactinemia samples.[148]

Pathologic Causes. Pathologic hyperprolactinemia may be caused by a prolactinoma, pituitary or sellar tumors that inhibit dopamine because of pressure on the pituitary stalk, or interruption of the vascular connections between the pituitary and hypothalamus. In a large series of histologically confirmed cases, a serum PRL level greater than 2000 mUL (~100 μg/L) was almost never encountered from stalk dysfunction.[149] However, prolactinomas can present with any level of PRL elevation.

Breast stimulation has only a minimal effect on serum PRL levels. In 18 normal women, serum PRL levels rose from a mean of 10 to 15 μg/L during breast pump stimulation.[143] Chest wall lesions (e.g., in association with herpes zoster [shingles]) can also be associated with mild hyperprolactinemia as a result of activation of neurogenic pathways that inhibit dopamine. Up to 20% of patients with hypothyroidism have elevated PRL levels.[150] Treatment of hypothyroidism with thyroid hormone normalizes serum PRL if the hyperprolactinemia is due to thyroid hormone deprivation.

PRL is moderately elevated (mean 28 μg/L) in patients with chronic renal failure and those on dialysis.[151] This increase is largely a result of an increase in PRL cleavage products, due in part to decreased glomerular filtration rate. Sexual dysfunction is common in men on dialysis, and reducing PRL with dopamine agonists improves sexual function[152] but does not normalize menses in women.[153] Side effects of dopamine agonists in patients with renal failure may be exacerbated because of fluid shifts and multiple medication interactions. PRL levels rise in response to stress, correlate with the degree of stress, and generally return to normal as stress abates. The mean peak serum PRL level in 19 women undergoing general anesthesia was 39 μg/L immediately prior to surgery, 173 μg/L at surgery, and still elevated at 47 μg/L 24 hours after surgery.[154] Traumatic brain injury can also result in hyperprolactinemia, often accompanied by diabetes insipidus or syndrome of inappropriate antidiuretic hormone (SIADH) secretion and other anterior pituitary hormone deficiencies. A meta-analysis found that 34% of patients develop hyperprolactinemia after cranial and hypothalamic irradiation, thought to be the result of decreased hypothalamic dopamine secretion.[154a]

Hyperprolactinemia was reported in association with an inactivating prolactin receptor mutation in three sisters with oligomenorrhea.[155] The mutation disrupted ligand binding and downstream signaling, demonstrated in vitro. The presence of hyperprolactinemia in these affected women suggested central negative feedback by prolactin on its secretion in humans, as previously demonstrated in animal studies.[84,85] Affected women were all heterozygous for the mutation; the presence of hyperprolactinemia suggests that the mutant receptor may interfere with signaling by the normal receptor, encoded by the wild-type allele, to result in PRL insensitivity. As the occurrence of oligomenorrhea and the ability to lactate is contrary to what might be predicted from a state of prolactin insensitivity, the function of PRL and its receptor in humans requires further study.[80]

Drug-Induced Causes. A variety of medications cause minimal or moderate prolactin elevations. Neuroleptic drugs elevate PRL because of their dopamine receptor antagonist properties, as do atypical antipsychotics that act by antagonizing both serotonin and dopamine receptors. Clozapine and olanzapine weakly induce PRL, and risperidone is a potent PRL stimulator.[156,157]

Unless patients exhibit hypogonadism, related osteoporosis, or troublesome galactorrhea, treatment of drug-induced hyperprolactinemia may not be necessary.[158] It should not always be assumed that hyperprolactinemia in patients on drugs known to elevate PRL is in fact due to those medications. Prolactinoma, other sellar lesions,[159] hypothyroidism, and renal failure should be considered as possible causes of hyperprolactinemia requiring active management. In patients taking neuroleptic medications, if the clinical situation permits, temporary drug withdrawal might be considered to determine if PRL levels normalize.[158] If PRL levels do not normalize or medication withdrawal is not possible, a pituitary MRI should be performed. When neuroleptics elevate PRL levels, switching to olanzapine may be attempted because the medication has minimal effects on PRL levels. In determining whether to discontinue a drug or whether to use alternative medication, the benefits should be weighed against the risks of drug replacement or cessation.[160] Although combined use of dopamine antagonists and dopamine agonists is not usually advised because of an increased risk of side effects, such as postural hypotension or

exacerbation of the underlying psychosis, some advocate the use of both formulations simultaneously.[161]

Clinical Features

Galactorrhea and reproductive dysfunction are the hallmarks of pathologic hyperprolactinemia. Women present with galactorrhea associated with a range of menstrual disturbances, including infertility, oligomenorrhea, and amenorrhea; men present with symptoms of hypogonadism and of tumor mass effects, with galactorrhea occurring infrequently.

Galactorrhea and amenorrhea were reported in the 19th century by Chiari and Frommel.[162] The Chiari-Frommel syndrome comprises postpartum galactorrhea, amenorrhea, and "uteroovarian atrophy" in patients not nursing. This disorder is usually self-limiting, and fertility eventually returns after normalization of PRL levels, sometimes without an intervening menstrual period. Patients with postpartum amenorrhea, hyperprolactinemia, and galactorrhea have sometimes subsequently been found to harbor prolactinomas. In the 1950s, Argonz and Del Castillo[163] and Forbes et al[164] associated galactorrhea and amenorrhea with pituitary tumors. In a report of 18 such patients, galactorrhea and amenorrhea were reported for up to 11 years after parturition with a mean PRL level of 45 µg/L.[150]

Inappropriate nipple secretion of milk-like substances may persist after childbirth or discontinuation of nursing for as long as 6 months. Thereafter, continued milk production is considered abnormal, and other causes for galactorrhea should be investigated. Galactorrhea can occur in either women or men and may be unilateral or bilateral, can be profuse or sparse, and can vary in color and thickness. If blood is present in the galactorrhea fluid, it could be the harbinger of an underlying pathologic process, such as a ductal papilloma or carcinoma, and mammography or sonography is indicated. Twenty-nine of 48 patients with pituitary tumors and galactorrhea had PRL concentrations of less than 200 µg/L, likely on the basis of stalk compression, suggesting that they harbored pituitary tumors other than prolactinomas.[150] It is likely that most patients with so-called idiopathic galactorrhea with amenorrhea harbor microprolactinomas. Fifty percent of patients with acromegaly also have galactorrhea, even in the absence of hyperprolactinemia, because human GH is a potent lactogen and can cause galactorrhea when elevated.[165] Normoprolactinemic galactorrhea with regular menses represents the most frequent cause of galactorrhea. In two-thirds of these patients, galactorrhea persists after parturition, despite the resumption of menses, and likely does not represent a pathologic entity. Normal PRL levels may still permit milk production because treatment of such patients with dopamine agonists alleviates galactorrhea. Galactorrhea may also develop transiently after surgical procedures to the chest wall, including mammoplasty, arising from neural reflexes due to intercostal nerve stimulation.[166] The optimal management of galactorrhea should be determined by identifying and treating the underlying cause. Regardless of the cause, galactorrhea associated with hyperprolactinemia responds to correction of hyperprolactinemia.

Growth Hormone

Physiology

Somatotroph Cells

Mammosomatotroph cells expressing both PRL and GH arise from the acidophilic stem cell and immunostain mainly for PRL. Somatotrophs are located predominantly in the lateral wings of the anterior pituitary gland and comprise 35% to 45% of pituitary cells (see Chapter 9, Fig. 9.1). These ovoid cells contain prominent secretory granules up to 700 µm in diameter. Juxtanuclear Golgi structures are particularly prominent with secretory granules in formation. The gland contains a total of 5 to 15 mg of GH.[172]

Structure

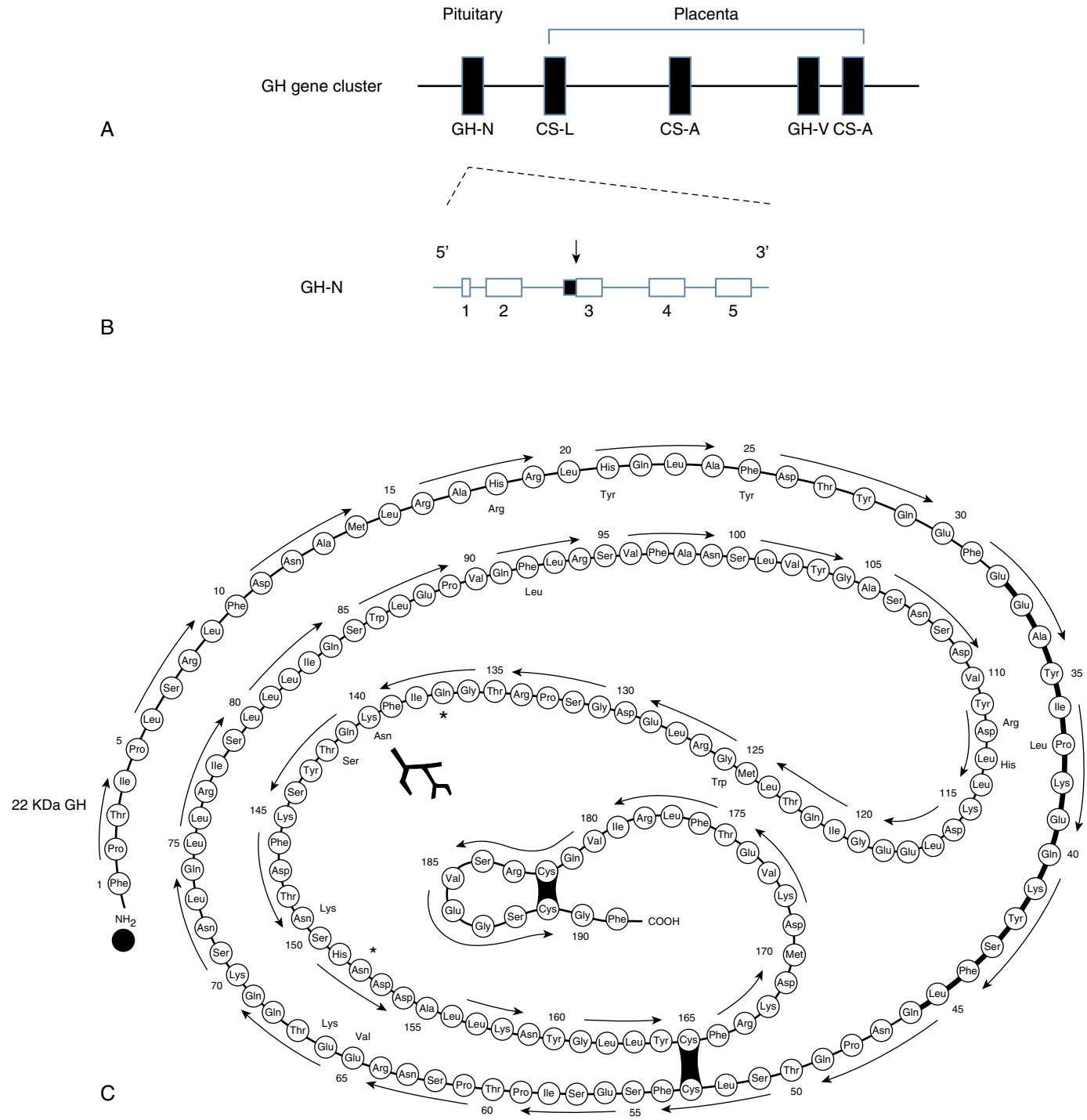
The human growth hormone (hGH) genome locus spans approximately 66 kb and contains a cluster of five highly conserved genes located on the long arm of human chromosome 17q22-24.[173] It encodes the following forms of hGH and human chorionic somatomammotropin (hCS): hGH-N, hCS-L, hCS-A, hGH-V, and hCS-B,[174] all of which consist of five exons separated by four introns (Fig. 8.8).

The GH-N gene is expressed in pituitary somatotrope cells and, to a minor extent, in lymphocytes, whereas GH-V and CS genes are expressed in the placenta. The level of GH gene expression in lymphocytes is sufficient to play a local paracrine/autocrine immunoregulatory role but is insufficient to fulfill a hormonal role at distant sites.

The hGH-N gene codes for a 22-kDa (191–amino acid) protein.[175] The hCS-A and hCS-B genes are expressed in placental trophoblasts.[176] hGH-V, expressed exclusively in placental syncytiotrophoblasts, encodes a 22-kDa protein that emerges from midgestation as hGH-V2. When hGH-V levels are elevated, hGH-N declines progressively, indicating feedback regulation of the maternal hypothalamic-pituitary axis. Postpartum circulating hGH-V levels drop rapidly and are undetectable 1 hour after delivery.[177] hCS-L is found in placental villi but has been considered a pseudogene.

The hGH promoter region contains *cis* elements that mediate both pituitary-specific and hormone-specific signaling. The POU1F1 transcription factor confers tissue-specific GH expression; a second, ubiquitous factor binds to a distal PIT1 site containing a consensus sequence for the Sp1 transcription factor. PIT1 and Sp1 both contribute to GH promoter activation, as mutation of the Sp1 binding site attenuates promoter activity.[178] DNase hypersensitive sites of a locus control region (LCR) located 14.5 kb upstream of the hGH-N promoter confers and restricts the expression of GH only to somatotrophs and mammosomatotrophs among the PIT1-positive cell population during development.[179] This distant locus also plays a major role in sustaining the level of hGH gene expression in somatotrophs. Growth hormone–releasing hormone (GHRH) stimulates GH synthesis and release mediated by cAMP. cAMP-response element binding (CREB) protein (CBP) is phosphorylated by protein kinase A and is a cofactor for PIT1-dependent human GH activation.

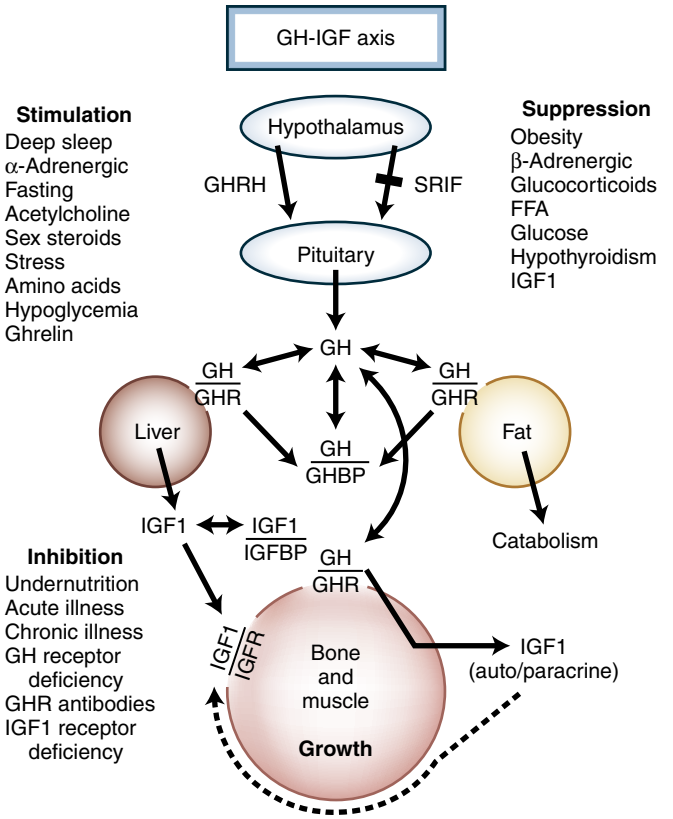
The GH molecule, a single-chain polypeptide hormone consisting of 191 amino acids, is synthesized, stored, and secreted by somatotroph cells. The crystal structure of hGH reveals four α-helixes.[180] Circulating GH molecules comprise several heterogeneous forms: 22-kDa and 20-kDa monomers, the latter lacking amino acids 32 through 46 deleted from alternative splicing of the GH gene (Fig. 8.8). The 22-kDa peptide is the major physiologic component, accounting for 75% of pituitary GH secretion, and the 20-kDa peptide accounting for about 10%. The 20-kDa GH has a slower metabolic clearance.[181] The pituitary gland also produces a number of other variants formed from post-translational modification, including two deamidated forms, acylated and glycosylated.[182]



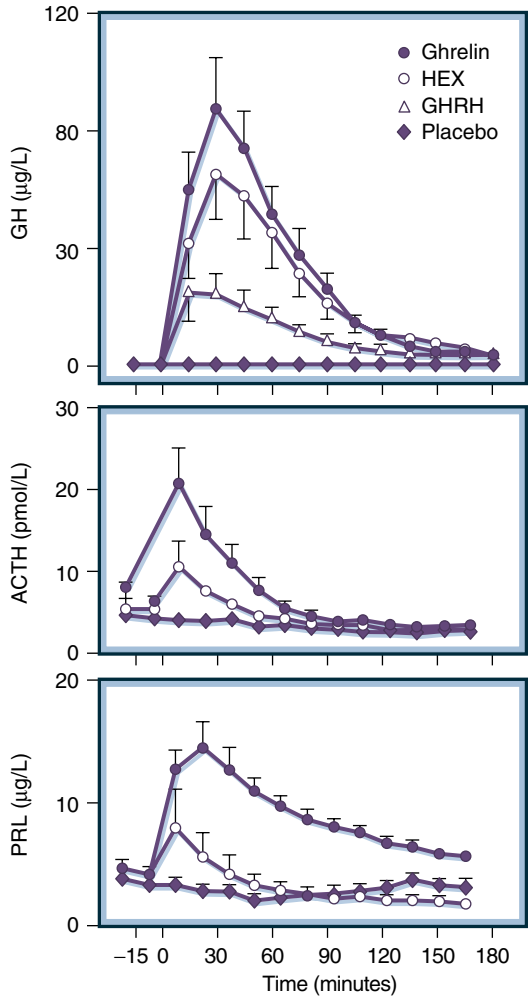
• **Fig. 8.8** Schematic presentation of the human growth hormone gene cluster (A) on chromosome 17 containing GH-N, CS-L, CS-A, GH-V and CS-A genes. GH-N is expressed in the pituitary gland while the rest are expressed in the placenta. CS-L and GH-V are the predominant forms expressed during pregnancy. The GH-N gene (B) encoding human growth hormone contains five exons and four introns, and generates a full-length 22-kDa protein (C). Exon 3 contains a splice acceptor site *(arrow)* that generates a 20-kDa isoform that lacks amino acids 32 to 46, shown with *thickened lines* in the lower panel within the 22-kDa hormone. (C, Courtesy Dr. G. Baumann.)

Regulation

Neuropeptides, neurotransmitters, and opiates impinge on the hypothalamus and modulate release of GHRH and somatostatin (somatotropin release–inhibiting factor [SRIF]). Integrated effects of these complex neurogenic influences determine the final secretory pattern of GH (Fig. 8.9). Apomorphine, a central dopamine receptor agonist, stimulates GH secretion,[183] as does levodopa (L-dopa). Oral L-dopa administration evokes a brisk serum GH response within an hour in healthy young subjects. Norepinephrine increases GH secretion via α-adrenergic pathways and inhibits GH



• **Fig. 8.9** The growth hormone/insulin-like growth factor (GH-IGF) axis. Simplified diagram of GH axis involving hypophysiotropic hormones controlling pituitary GH release, circulating GH-binding protein (GHBP) and its GH receptor (GHR) source, IGF1 and its largely GH-dependent binding proteins (IGFBP), and cellular responsiveness to GH and IGF1 interacting with their specific receptors. *GHRH,* growth hormone–releasing hormone; *IGFR,* IGF1 receptor; *FFA,* free fatty acids; *SRIF,* somatotropin release–inhibiting factor (somatostatin). (From Rosenbloom A. Growth hormone insensitivity: physiologic and genetic basis, phenotype and treatment. *J Pediatr.* 1999;135:280–289.)



• **Fig. 8.10** Effect of GH secretagogues on secretion of GH, adrenocorticotropic hormone (ACTH), and prolactin (PRL) in healthy subjects. Mean (± standard error of the mean [SEM]) curve responses after administration of ghrelin (1.0 μg/kg), hexarelin (HEX, 1.0 μg/kg), growth hormone–releasing hormone (GHRH) (1.0 μg/kg), or placebo. (Adapted from Arvat E, Maccario M, Di Vito L, et al. Endocrine activities of ghrelin, a natural growth hormone secretagogue [GHS], in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. *J Clin Endocrinol Metab.* 2001;86:1169–1174.)

release via β-adrenergic pathways. Insulin-induced hypoglycemia, clonidine, arginine administration, exercise, and L-dopa facilitate GH secretion by α-adrenergic effects.[184] β-Adrenergic blockade increases GHRH-induced GH release, possibly due to a direct pituitary action or by decreasing hypothalamic somatostatin release. Endorphins and enkephalins stimulate GH and may account for GH release during severe physical stress and extreme exercise[184] (see Fig. 8.9). Galanin, a 29–amino acid neuropeptide, induces GH release and enhances responses to GHRH. Cholinergic and serotonergic neurons and several neuropeptides stimulate GH, including neurotensin, VIP, motilin, cholecystokinin, and glucagon.

The somatotroph cell expresses specific receptors for GHRH,[185] GH secretagogues, and SRIF receptor subtypes 2 and 5, which mediate GH secretion.[186,187] GHRH selectively induces GH gene transcription and hormone release.[188] GHRH elicits a prompt rise in serum GH levels, with higher levels occurring in females.[189] SRIF suppresses secretion but not GH biosynthesis.

Ghrelin

The isolation of ghrelin from the stomach has revealed an additional control system for GH (see Chapter 39). Ghrelin is a 28–amino acid peptide that binds the growth hormone secretagogue (GHS) receptor to induce hypothalamic GHRH and pituitary GH release.[197] A unique *n*-octanoylated serine 3 residue confers GH-releasing activity to the molecule. Ghrelin is synthesized in peripheral tissues, especially gastric mucosal neuroendocrine cells, as well as in the hypothalamus. Control of GH secretion thus requires hypothalamic GHRH/SRIF as well as ghrelin.[198] Ghrelin dose-dependently evokes GH release. It requires the presence of GHRH to evoke GH release as evidence in patients with intact pituitary but disordered hypothalamic function, when GHS does not induce GH.[199] This is because GHRH acts as an allosteric coagonist for the ghrelin receptor.[200] GHS agonists also potentiate GH release in response to a maximal stimulating dose of exogenous GHRH. After a saturating dose of GHRH, subsequent GHRH administration is ineffective, although GHS agonists remain effective.[192] Hypothalamic ghrelin likely controls GH secretion interacting with GHRH and SRIF.[198,201,202] Ghrelin and synthetic hexapeptidergic analogues of the GHS receptor induce potent and reproducible GH release associated with modest increases in PRL and ACTH/cortisol that occur with some GHSs (Fig. 8.10). The GHS receptor uses intracellular

signaling pathways distinct from the GHRH receptor.[199,200] In addition to its GH-releasing action, there is strong evidence that ghrelin is a pleotropic hormone regulating appetite, energy balance, sleep-wake rhythm, gastric motility, glucose homeostasis, cell growth, and cardiac function.[203,204]

Extrapituitary GH

GH is expressed outside the pituitary gland in the brain, immune cells, reproductive tract (breast, ovary, testis, prostate), gastrointestinal system (liver, pancreas, gut), and lungs.[205] The regulation of GH in these tissues is poorly understood. Evidence suggests that GH subserves developmental and proliferative function, acting in an autocrine and paracrine manner. Autocrine GH binds GHRs that are translocated to the nucleus, inducing cell cycle proliferation.[206] Extrapituitary GH expression is strongly implicated in neoplastic transformation of breast, prostate, and colonic neoplasms.[207,208]

Secretion

GH secretion is episodic and exhibits a diurnal rhythm with approximately two-thirds of the total daily GH secretion produced at night triggered by the onset of slow-wave sleep. Major GH secretory pulses accounting for up to 70% of daily GH secretion occur with the first episode of slow-wave sleep.[209] Normal GH secretion is characterized by secretary episodes separated by troughs of minimal basal secretion, during which GH is undetectable for more than 50% of a 24-hour period. GH concentration is high in the fetal circulation, peaking at approximately 150 μg/L during midgestation. Neonatal levels are lower (~30 μg/L), possibly reflecting the negative feedback control by rising levels of circulating IGF1. GH output falls to a stable level during childhood, rising at the onset of puberty by a twofold to threefold level at late puberty. GH secretion starts to decline exponentially during the third decade of life, progressively declining with advancing age to about 50% of that observed in the third decade.[184] The decline in GH status occurs by a change in pulse amplitude rather than frequency. On average, the daily production of GH in the prepubertal state is 200 to 600 μg/day, rising to 1000 to 1800 μg/day at the peak of puberty.[184] In adulthood, production rates range from approximately 200 to 600 μg/day with rates higher in women than in men.[184,210] Adiposity accounts for a significant component of declining GH output with increasing age[211] (Table 8.4). Exercise and physical stress, including trauma with hypovolemic shock and sepsis, increase GH levels.[184] Emotional deprivation and endogenous depression suppress GH secretion.

The levels of IGF1 in childhood, adolescence, and adulthood reflect the status of GH output during the corresponding phases of life. IGF1 levels are stable postnatally and during childhood, rising at the start of puberty and peaking in late adolescence (Fig. 8.11).[583] This close correspondence throughout life renders age-stratified IGF1 level a valuable serum marker of GH status in both sexes.

Nutrition plays a major role in GH regulation, in part mediated by the inhibitory actions of insulin.[212] Malnutrition increases GH secretion, whereas obesity has the opposite effect. These nutritional effects occur acutely, as exemplified by fasting, which amplifies GH secretion within 12 hours[213] (Fig. 8.12), and glucose ingestion, which suppresses GH secretion. Central glucoreceptors appear to sense glucose fluctuations rather than absolute levels. Intravenous (IV) administration of single amino acids, such as arginine and leucine, stimulate GH secretion. Free fatty acids blunt the effects of arginine infusion, sleep, L-dopa, exercise, and GHRH on GH release.[214] Leptin plays a key role in regulation of food intake and energy expenditure[215] and may act as a metabolic signal in stimulating GH secretion[216] through interactions with somatostatin, GHRH, and the neuropeptide Y (NPY) system.

TABLE 8.4 Adult Growth Hormone Secretion[a]

Interval	Young Adult	Fasting	Obesity	Middle Age
24-h secretion (μg/24 h)	540 ± 44	2171 ± 333	77 ± 20	196 ± 65
Secretory bursts (number in 24 h)	12 ± 1	32 ± 2	3 ± 0.5	10 ± 1
GH burst (μg)	45 ± 4	64 ± 9	24 ± 5	10 ± 6

[a]Deconvolution analysis of growth hormone (GH) secretion in adult males.

From Thorner MO, Vance ML, Horvath E, et al. The anterior pituitary. In: Wilson JD, Foster D, eds. *Williams Textbook of Endocrinology*. 8th ed. Philadelphia: WB Saunders; 1992:221–310.

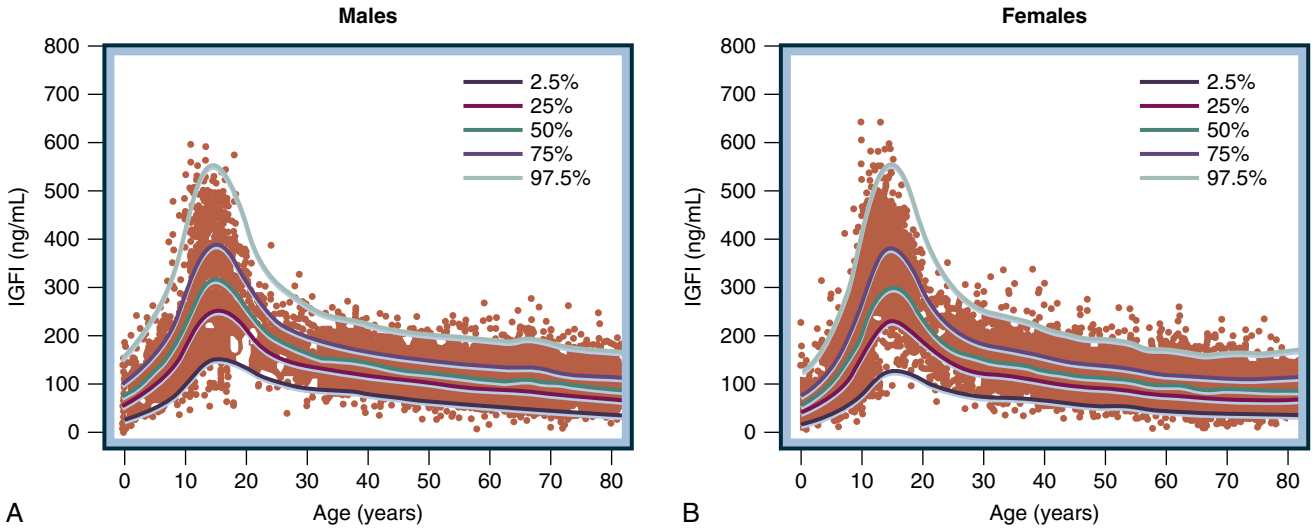
GHRH and SRIF Interaction

Hypothalamic SRIF and GHRH are secreted in independent waves and interact together to generate pulsatile GH release. The rat hypothalamus releases GHRH and SRIF 180 degrees out of phase every 3 to 4 hours, resulting in pulsatile GH levels. SRIF antibody administration elevates GH levels, with intact intervening GH pulses,[190] implying that hypothalamic SRIF secretion generates GH troughs. Similarly, GHRH antibodies eliminate spontaneous GH surges. In humans, GH pulsatility persists when GHRH is tonically elevated, as in ectopic tumor GHRH production or during GHRH infusion,[191] suggesting that hypothalamic SRIF is largely responsible for GH pulsatility. However, GHRH antagonists inhibit GH pulses, indicating an important role in the generation of pulsatile secretion in humans.[192] The periodic, pulsatile GH release is the result of coordinated SRIF and GHRH secretion and action.

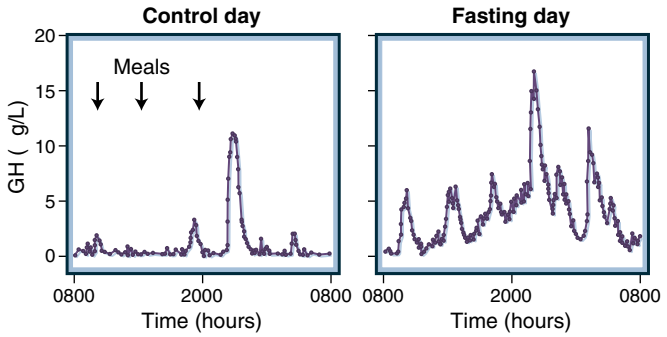
Chronic GHRH stimulation, either by continuous infusion or repeated bolus administration, eventually desensitizes GH release in vitro and in vivo, possibly due to depletion of a GHRH-sensitive pool of GH. GHRH pretreatment also decreases somatotroph GHRH binding sites.[193] GH stimulates hypothalamic SRIF, GHRH and SRIF autoregulate their own respective secretion, and GHRH stimulates SRIF release.[194] GH secretion is further regulated by its target growth factor, IGF1, which participates in a hypothalamic-pituitary peripheral regulatory feedback system.[195,196] IGF1 stimulates hypothalamic SRIF release and inhibits pituitary GH gene transcription and secretion.

Interaction With Other Hormone Axes

Glucocorticoids acutely stimulate GH secretion, but chronic steroid treatment inhibits GH. Three hours after acute glucocorticoid administration, GH levels rise and remain elevated for 2 hours.[217] Glucocorticoids administered to normal subjects dose-dependently inhibit GHRH-simulated GH secretion.[217] Thyroid hormones are required for the function of the GH system. Gonadal steroids regulate GH secretion and GH action in men and women. Testosterone stimulates GH secretion, an effect that is mediated centrally and dependent on prior aromatization to estrogen.[218] In women,



• **Fig. 8.11** Normal ranges for insulin-like growth factor 1 (IGF1). Individual points and fitted percentiles for IGF1 in males (A) and females (B). Displayed are the 2.5%, 25%, 50%, 75%, and 97.5% centiles. (Redrawn from Bidlingmaier M, Friedrich N, Emeny RT, et al. Reference intervals for insulin-like growth factor-1 (IGF-I) from birth to senescence: results from a multicenter study using a new automated chemiluminescence IGF-I immunoassay conforming to recent international recommendations. *J Clin Endocrinol Metab.* 2014;99:1712–1721.)



• **Fig. 8.12** Effect of fasting on growth hormone (GH) secretion patterns in a healthy male subject. (From Hartman ML, Veldhuis JD, Johnson ML, et al. Augmented growth hormone [GH] secretory burst frequency and amplitude mediate enhanced GH secretion during a two-day fast in normal men. *J Clin Endocrinol Metab.* 1992;74:757–765.)

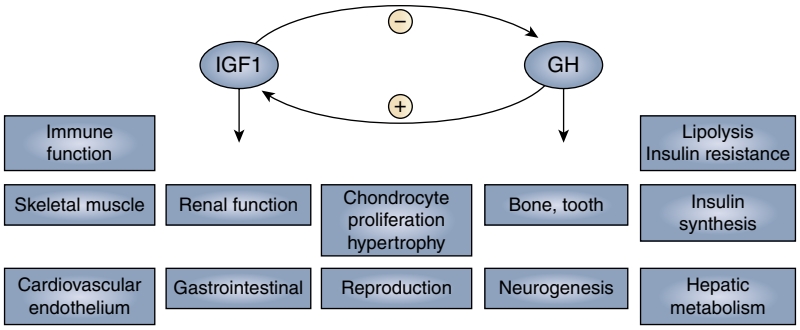
estrogen stimulates GH secretion, evident only with oral but not parenteral administration because of a first-pass effect hepatic, effect reducing IGF1 production[219] and enhancing GH secretion through reduced feedback inhibition.[219] In women, endogenous GH secretion is driven centrally by aromatization of androgens. Thus in women, estrogens act centrally in a paracrine manner in stimulating but act peripherally in an endocrine manner in enhancing GH secretion through reduced IGF1 feedback inhibition.[220]

Action

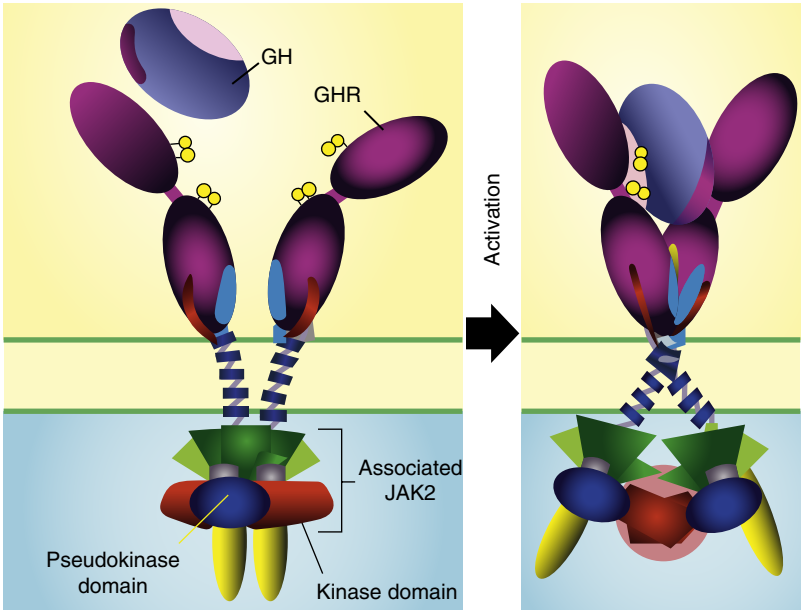
Growth hormone exerts its pleiotropic effects directly and indirectly through the actions of IGF1. These mediated actions stimulate growth and a host of functions in various tissues, organs, and systems (Fig. 8.13).

GHR Signaling

GH elicits intracellular signaling through a peripheral receptor, initiating a phosphorylation cascade involving the JAK-STAT pathway.[221] The GHR is a 620–amino acid 70-kDa protein of the class I cytokine/hematopoietin receptor superfamily that consists of an extracellular ligand-binding domain, a single membrane-spanning domain, and a cytoplasmic component.[222] The GHR superfamily is homologous with receptors for PRL, interleukin 2 (IL2) through IL7, erythropoietin, interferon, and colony-stimulating factor. These receptors exist as constitutive dimers. GHR activation is triggered by the binding of GH at two distinct sites to extracellular domains of each of the two receptor dimers (Fig. 8.14). The binding reorients and rotates the receptor separating the transmembrane domains, sliding the pseudokinase inhibitory domain of one JAK kinase away from the kinase domain of the other JAK within the receptor dimer-JAK complex (see Fig. 8.14).[223,224] This results in phosphorylation of intracellular signaling molecules,[223,224] including the signal transducing activators of transcription proteins (STAT1, STAT3, and STAT5).[225] Phosphorylated STAT proteins are directly translocated to the cell nucleus, where they elicit GH-specific target gene expression by binding to nuclear DNA. STAT1 and STAT5 may also interact directly with the GHR molecule.[225] GH also induces c-Fos induction, insulin receptor substrate 1 phosphorylation, and insulin synthesis. Additional intracellular signaling pathways induced by GH include mitogen-activated protein kinase (MAPK), protein kinase C, SH2β, SHP2, SIRPA, SHC, FAK, CrKll, C-SRC, paxillin, and tensin. Intracellular GH signaling is abrogated by suppression of cytokine signaling (SOCS) proteins, which disrupt the JAK-STAT pathway and thus disrupt GH action.[226] In transgenic mice with deletion of SOCS2, gigantism develops presumably due to unrestrained GH action. As SOCS proteins are also induced by proinflammatory cytokines, critically ill patients or those with renal failure may develop GH resistance due to cytokine-induced SOCS.[227] Unraveling STAT/SOCS regulation in syndromes associated with disordered GH signaling will likely yield mechanistic insights for dysregulated GH action. The GHR is expressed in virtually all tissues with liver, fat, muscle, and kidney showing highest levels of expression.[228] A polymorphism of the GHR, resulting in the deletion of exon 3, has attracted widespread interest since an observation in 2004 that signaling was 30% higher than that of the full-length receptor.[229] A recent meta-analysis found no



• **Fig. 8.13** Major physiologic actions of growth hormone and insulin-like growth factor 1 (IGF1) action. Given that growth hormone uses signal transducer and activator of transcription 5 (STAT5) to induce both IGF1 and for many of its direct actions, it is difficult to attribute specific roles to growth hormone or IGF1 for many biologic effects. However, those actions that involve more direct growth hormone action are placed to the right, whereas those involving induced IGF1 to the left, acknowledging that these assignments may change. Note that the growth hormone–IGF1 system is regulated by the tight negative feedback loop between hepatic IGF1 production and pituitary growth hormone secretion. *GH,* growth hormone; *IGF1,* insulin-like growth factor 1. (Redrawn from Brooks AJ, Waters MJ. The growth hormone receptor: mechanism of activation and clinical implications. *Nat Rev Endocrinol*. 2010;6:515–525.)



• **Fig. 8.14** Schematic representation of the GH receptor in the basal state *(left)* and the active state *(right)*. Binding of GH at two distinct sites to predimerized receptors leads to a crossover rotation and realignment, producing a separation of the lower transmembrane domains. This results in the separation of two associated JAK2s and the removal of the inhibitory pseudokinase domain from the kinase domain of the other JAK2, bringing the two kinase domains into position for transactivation, initiating tyrosine phosphorylation of the receptor cytoplasmic domain and signaling proteins such as STAT5. (Redrawn from Brooks AJ, Dai W, O'Mara ML, et al. Mechanism of activation of protein kinase JAK2 by the growth hormone receptor. *Science*. 2014;344:1249783.)

convincing influence of the exon 3–deleted GHR on stature, on GH-induced growth responsiveness in children, on metabolic benefits of GH replacement in adults with growth hormone deficiency (GHD), or on clinical manifestations and response to treatment in acromegaly.[230]

The pattern of GH secretion also determines tissue responses to GH in addition to the absolute amount secreted. Gender-specific patterns of GH secretion profiles determine sex-specific expression of cytochrome P450 enzymes. In turn, circulating steroids regulate neuroendocrine release of GH. SRIF, by suppressing interpulse GH levels, serves to masculinize the ultradian GH rhythm. In mice harboring a disrupted SRIF gene, plasma GH secretory patterns are elevated and liver enzyme induction loses its gender-specific dimorphism, but these animals retain sexually dimorphic growth patterns.[231] Linear growth patterns and liver enzyme induction are phenotypically gender specific, driven by higher GH pulse frequency rates involving STAT5B mediation. STAT5B is sensitive to repeated pulses of injected GH,[232] unlike other GH-induced responses, which are desensitized by repeated GH administration. Disruption of STAT5B in transgenic mice

impairs male pattern body growth[233] associated with female pattern IGF1 and testosterone levels. Appropriate GH pulsatility drives body growth mediated by STAT5B[234,235] but not metabolic GH effects. In humans, GH secretion is also sexually dimorphic and regulates the P450 liver enzymes.[235] A flatter pattern appears to evoke a higher IGF1 response and a lesser degree of lipolysis.[236]

IGF1 mediates growth-promoting activities of GH in an endocrine or paracrine manner.[237] In mice, paracrine IGF1 produced in extrahepatic tissues is critical for growth, which persists even when hepatic IGF1 is deleted.[238] GHR mutations are associated with partial or complete GH insensitivity and growth failure. These syndromes are associated with normal or high circulating GH levels, decreased circulating GHBP levels, and low levels of circulating IGF1. Multiple homozygous or heterozygous exonic and intronic GHR mutations have been described. These occur mostly in the extracellular ligand-binding receptor domain (see Chapter 25).

Growth Hormone–Binding Proteins

Two high-affinity and low-affinity circulating GH-binding proteins (GHBPs) include a 20-kDa low-affinity GHBP and a 60-kDa high-affinity GHBP, which corresponds to the extracellular domain of the GHR and binds half of the circulating 22-kDa GH form.[239,240] The high-affinity GHBP in humans is generated by proteolytic cleavage through the action of tumor necrosis factor-α–converting enzyme, a metalloprotease.[241] The 20-kDa GH binds preferentially to the low-affinity binding protein, which is unrelated to the GHR. It has been proposed that the circulating level of GHBP reflects global GHR expression in the body. The GHBPs dampen the acute oscillations in serum GH levels associated with pulsatile pituitary GH secretion, and plasma GH half-life is prolonged by decreased renal GH clearance of bound GH. The high-affinity binding protein also competes with GH for binding to surface GHRs and as such alters GH pharmacokinetics and distribution.

GHBP concentrations are unaffected by GH status such as in GHD or acromegaly.[242] Some patients with Laron dwarfism have absent or reduced levels of GHBP, reflecting mutations that result in absent translation of the GHR or of the extracellular domain.[243] Serum concentrations of GHBP are low in some children with idiopathic short stature[244] and in African pygmies, suggesting abnormalities in the gene for the GHR.[245] GHBP levels are increased in obesity, pregnancy, and in subjects undergoing refeeding[245] and by oral estrogen administration.[219] Levels are reduced in malnutrition, cirrhosis, and hypothyroidism and by glucocorticoids and androgens.[246]

Metabolic Action

GH functions as a major metabolic hormone in the adult, optimizing body composition and physical function as well as regulating energy and substrate metabolism. Metabolic actions of GH also closely interact with those of insulin in the control of fat, glucose, and protein metabolism during fasted and fed states.

GH promotes fat metabolism by enhancing lipolysis and fatty acid oxidation. This function is particularly important during the fasted state when GH secretion is enhanced, resulting in the partitioning of fuel utilization toward fat with the sparing of protein. Stimulation of lipolysis occurs indirectly through potentiating the activity of hormone-sensitive lipase by β-adrenergic stimulation. GH also regulates lipoprotein metabolism by enhancing low-density lipoprotein (LDL) clearance and by activating expression of hepatic LDL receptors.[247,248] The atherogenic profile of lipoproteins is increased in GHD and reduced by GH therapy.[249]

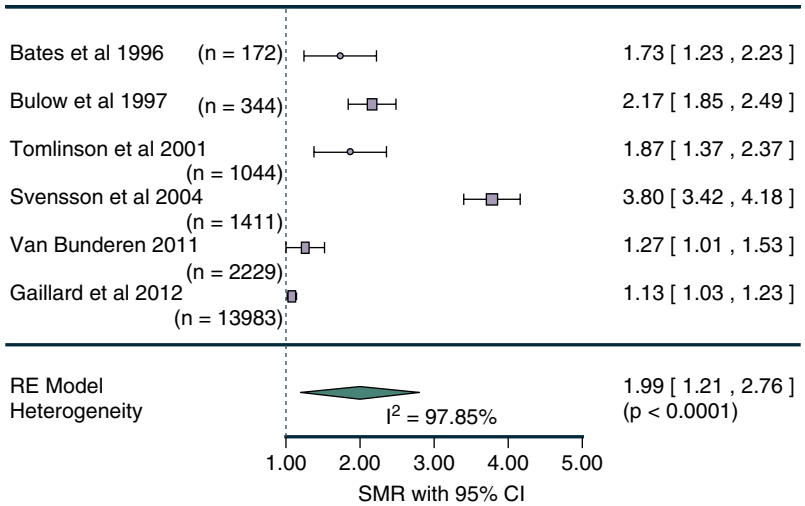
GH exerts profound effects on glucose metabolism either directly or by antagonizing insulin action. GH enhances glucose uptake and utilization in cells, referred to as its *insulin-like* effects.[250] At the whole-body level, GH suppresses glucose oxidation and utilization while enhancing hepatic glucose production subserving the nonoxidative[250] use of glucose. As GH is an important counterregulatory hormone, it is conceivable that this function protects against hypoglycemia.

Protein anabolism is a signature property of GH mediated by IGF1. However, GH directly stimulates amino acid uptake and incorporation into protein in vitro.[251] Arteriovenous measurements in the forearm report an acute increase in protein synthesis over a few hours of GH infusion, suggesting a direct non-IGF1 mediated effect.[252] Whole-body studies in humans using isotopes have consistently shown that GH reduces protein oxidation and stimulates protein synthesis.[148] The protein-sparing effect of GH is coupled to the availability and increased parallel utilization of free fatty acids, whereas pharmacologic reduction of free fatty acids during fasting augments the rate of protein breakdown.[250]

Growth Hormone Assays

Plasma GH is measured by radioimmunoassay (RIA; polyclonal or monoclonal) or by immunoradiometric assay (IRMA; dual monoclonal), but considerable differences exist in the results of GH measurements between assays by as much as threefold.[253,254] These differences are due mainly to heterogeneity in assay components and characteristics. An important contributor is the use of different calibrator materials[255] and international reference preparations, shifting from purified pituitary extracts to recombinant GH.[255] Not all assays are calibrated to the recombinant international GH reference preparation (98/574). Heterogeneity of secreted GH poses an additional problem as it consists of monomers, dimers, and other post-translational modified products. Their detection varies, with different antibodies having different specificity for the different molecular forms of GH. The reporting of assay results also varies. GH assay results are expressed in mass units but also in international units, which have been arbitrarily defined and do not have a clear relationship to mass.[255] Many factors interfere with GH measurements, including GHBP, which binds approximately 50% of circulating GH. Modern two-site monoclonal assays are engineered to detect solely 22-kDa GH and return lower values to polyclonal RIA, thus detecting various circulating forms. The inhomogeneity of GH immunoassay results poses a challenge in the definition of accepted standards for diagnosis of GHD where criteria are based on cutoffs. Clinicians should be aware of the nature of the GH assay and how values compare to those previously obtained by polyclonal RIA.

Similar issues apply to assays for IGF1. A significant issue arises from interference of IGF binding proteins, which bind over 95% of IGF1. This necessitates dissociating IGF1 from its binding proteins as a step before immunologic detection. Two-site assays have now overcome the need for prior dissociation. IGF1 measurements are particularly valuable in the management of GH-related disorders because GH status can only be estimated from repeated GH measurements over an extended period. Like GH, IGF1 status changes over the human lifespan, rising to a peak in late puberty and declining in early adulthood and gradually thereafter the fourth decade. A recent study employing a two-site chemiluminescent assay calibrated against a recombinant human



• **Fig. 8.15** Forest plot of standardized mortality ratios with 95% confidence intervals from six studies in patients with hypopituitarism. (Redrawn from Pappachan JM, Raskauskiene D, Kutty VR, et al. Excess mortality associated with hypopituitarism in adults: a meta-analysis of observational studies. *J Clin Endocrinol Metab*. 2015;100:1405–1411.)

IGF1 standard (02/254) in over 15,000 subjects has provided timely age-stratified and gender-stratified reference ranges from childhood to senescence (see Fig. 8.11).

Growth Hormone Deficiency

GH is the most abundant hormone in the adult pituitary gland, and it plays an important role in maintaining the metabolic process and the integrity and function of many tissues and systems after the cessation of growth. Life expectancy is reduced in hypopituitary patients with GHD. In a meta-analysis of six studies totaling over 19,000 patients, the mortality is twice that of the control population (Fig. 8.15) with the risk greater in women than men and greater in those who received radiotherapy.[256–260]

Pathophysiology

Adult GHD may be acquired or congenital[261] (Table 8.5). Surgical or radiation treatment of pituitary, parasellar, and brain tumors is the most common cause of GHD, accounting for nearly two-thirds of cases. The frequency of causes differs between patients with childhood-onset and adult-onset GHD (Fig. 8.16).[262] Idiopathic causes, representing the most commonly encountered group in childhood-onset GHD, likely represent a heterogeneous collection of congenital developmental abnormalities, including mutations of *PROP1* or *POU1F1* genes, causing GHD with other pituitary hormone deficiencies.[263] Isolated GHD may be complete or partial, and up to 67% of children initially diagnosed with idiopathic GHD had normal GH responses when subsequently retested as adults for GHD following cessation of GH treatment.[264] Children with GHD should be retested before GH treatment is continued into adulthood unless they have clearly documented panhypopituitarism or a defined genetic or developmental abnormality that causes complete and irreversible GHD. Mutations in the GH[265] and GHRH receptor genes,[266] and GH insensitivity as a result of primary GHR dysfunction,[267] result in a selective lack of GH action.

Presentation

The clinical consequences of GHD in adults are shown in Table 8.6. Symptoms of GHD are nonspecific and include fatigue, lack of energy, social isolation, low mood, poor concentration, and reduced physical function, contributing to a poor quality of life.[268] The signs are also nonspecific and include general and central adiposity and reduced lean tissue and bone mineral density (BMD), along with unfavorable biochemical changes such as hyperlipidemia[269,270] and glucose intolerance.[268] Some patients have established evidence of macrovascular disease such as increased carotid intimal thickness.[271,272] GH deficiency impairs cardiac function.[273,274] Cardiovascular risk abnormalities are less pronounced in adults with childhood-onset GHD than in those acquiring the deficiency during adulthood who have more pronounced disordered quality of life, lipids, and body composition.[270]

Subjects with isolated GHD or inactivating mutation of the GHR manifest some features that contrast with those of GHD as a component of multiple pituitary hormone deficits. Patients with selective absence of GH action display enhanced insulin sensitivity, lower prevalence of diabetes, absence of premature atherosclerosis, and normal life expectancy.[275,276] These findings suggest that the observed increased mortality rate in hypopituitary adults with GHD arises in part from suboptimal replacement regimens and coexisting comorbid conditions.

Evaluation

GHD is diagnosed biochemically within an appropriate clinical context. Biochemical testing for GHD occurs with a high probability in patients with a history of organic hypothalamic-pituitary dysfunction, cranial irradiation, known childhood-onset GHD, and traumatic brain injury (TBI).[277,278] The features of adult GHD are not particularly distinct and mimic body compositional and biochemical changes of the aging process. Thus clinical suspicion must be confirmed by accurate biochemical diagnosis to ensure that GH-deficient patients are accurately identified and treated.

Provocative Testing. The diagnosis of adult GHD is established by provocative testing of GH secretion (Table 8.7). Other hormone deficits should be corrected before testing. Several provocative tests include insulin tolerance test (ITT), arginine, glucagon, clonidine, growth hormone–releasing peptides (GHRPs), and

TABLE 8.5 Causes of Acquired Pituitary Insufficiency

Traumatic
- Surgical resection
- Radiation damage
- Traumatic brain injury

Infiltrative/Inflammatory
- Primary hypophysitis
  - Lymphocytic
  - Granulomatous
  - Xanthomatous
- Secondary hypophysitis
  - Sarcoidosis
  - Histiocytosis X
  - Infections
  - Wegener granulomatosis
  - Takayasu disease
  - Hemochromatosis

Infections
- Tuberculosis
- *Pneumocystis jirovecii* infection
- Fungal (histoplasmosis, aspergillosis)
- Parasites (toxoplasmosis)
- Viral (cytomegalovirus)

Vascular
- Pregnancy related
- Aneurysm
- Apoplexy
- Diabetes
- Hypotension
- Arteritis
- Sickle cell disease

Neoplastic
- Pituitary adenoma
- Parasellar mass
  - Rathke cyst
  - Dermoid cyst
  - Meningioma
  - Germinoma
  - Ependymoma
  - Glioma
- Craniopharyngioma
- Hypothalamic harmatoma, gangliocytoma
- Pituitary metastatic deposits
- Hematologic malignancy
  - Leukemia
  - Lymphoma

GHRH, alone or in combination with arginine or pyridostigmine. GHRPs are synthetic analogues of ghrelin. Because provocative tests vary in the ability to evoke GH release, a single value cannot be applied as a diagnostic threshold across different tests.[279] ITT is a more potent stimulator of GH release than arginine, clonidine, or L-dopa, whereas combinations such as arginine plus GHRH, or GHRP plus GHRH, are more potent than ITT alone.[280–282]

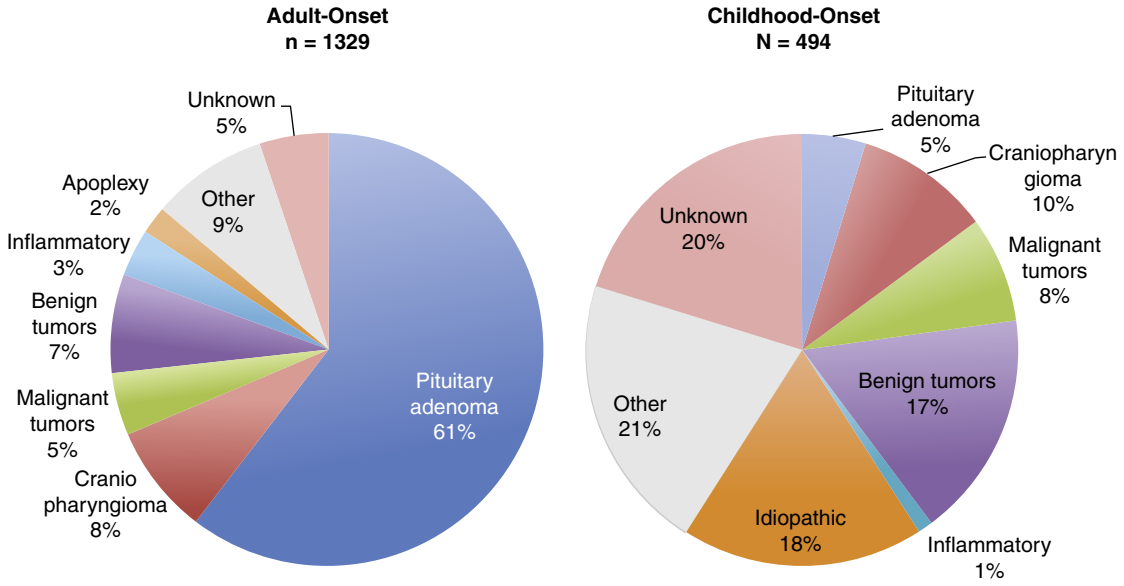
The ITT is the gold standard test for GHD. Normal subjects respond to insulin-induced hypoglycemia with peak GH concentrations of more than 5 μg/L[283] (Fig. 8.17). Severe GHD is defined by a peak GH response to hypoglycemia of less than 3 μg/L.[284] These cutoff values were defined using polyclonal GH RIAs.[284] The test is contraindicated in patients with electrocardiographic evidence or history of ischemic heart disease and in patients with seizure disorders.

Glucagon,[285] GHRH, and ghrelin mimetics (GHRP2, GHRP6, and macimorelin[286]), alone[287] or in combination,[282,288] are validated diagnostic tests.[281,282,288] The diagnostic thresholds for these tests are shown in Table 8.7. The unavailability of GHRH has brought glucagon greater attention as a simple diagnostic test for GHD when the ITT is not desirable.[289] The ITT evaluates the integrity of the hypothalamic-pituitary axis and has the added advantage of also stimulating ACTH secretion. Diagnostic tests employing GHRH or GHRPs, both of which directly stimulate GH release from the pituitary gland, may not identify GHD caused by hypothalamic disease.[290] This is exemplified by studies in patients treated with cranial irradiation, when the ITT shows the greatest sensitivity and specificity within the first 5 years after irradiation.[291] If peak GH levels are normal during a GHRH plus arginine test in patients who have received irradiation, then an ITT should also be performed. In irradiated patients as well as those with inflammatory and infiltrative parasellar lesions, GHD may develop many years after the initial insult. Therefore this group of patients should be followed in the long term with repeat testing. Obesity confounds the diagnostic testing of GHD as it blunts the peak GH response.[292] Except for the GHRH plus arginine test, body mass index (BMI)–stratified normal ranges have not yet been established for any of the provocative tests.

Growth Hormone–Responsive Markers. These markers include IGF1, IGF binding protein 3 (IGFBP3), and the acid-labile subunit of the IGFBP complex. IGF1 is useful for diagnosis only when age-adjusted normal ranges are used. Although IGF1 levels are reduced in adult GHD, a normal concentration does not exclude the diagnosis[283] (see Fig. 8.17). A subnormal IGF1 level in an adult patient with coexisting pituitary hormone deficits is strongly suggestive of GHD, particularly in the absence of conditions known to reduce IGF1 levels, such as malnutrition, liver disease, poorly controlled diabetes mellitus, and hypothyroidism. The separation of IGF1 values between GH-deficient and normal subjects is greatest in the young. As IGF1 levels decline with aging in normal subjects, IGF1 measurements become less reliable as a biochemical marker of GHD in patients older than 50 years when the values merge with those of normal age-matched subjects.[293] Measurement of IGFBP3 or the acid-labile subunit does not offer any diagnostic advantage over IGF1.[277,278]

In patients with organic hypothalamic-pituitary disease, the prevalence of GHD is strongly linked to the number of pituitary hormone deficits, ranging from approximately 25% to 40% in those with no other deficit to virtually 95% to 100% when more than three pituitary hormone deficiencies are present.[294] Patients with three or more pituitary hormone deficiencies and an IGF1 level below the reference range have a greater than 97% chance of being GH deficient (see Table 8.7)[295] and therefore do not require GH stimulation testing.[277,278]

Spontaneous GH Secretion. As pituitary GH secretion occurs episodically, accurate quantification of integrated GH secretion requires continuous measurement of secretion over 24 hours. This procedure requires insertion of a continuous withdrawal pump or patent indwelling catheter for frequent sampling. Continuous 24-hour GH measurement in the diagnosis of GHD is inferior to provocative testing[283] (see Fig. 8.17) and is cumbersome and expensive.



• **Fig. 8.16** Frequency of the causes of childhood-onset and adult-onset growth hormone deficiency in Denmark. "Other" includes cysts, hypophysitis, granulomas, trauma, and empty sella syndrome. (Data from Stochholm K, Gravholt CH, Laursen T, et al. Incidence of GH deficiency—a nationwide study. *Eur J Endocrinol*. 2006;155:61–71.)

Gene Expression Analysis

With the advent of high throughput and computational technology, there has been much interest in applying genomics and transcriptomics to identify dysfunction of the GH system and predict responsiveness to GH therapy. A recent study of well-characterized children with GH deficiency combining genotyping and gene expression analysis of peripheral blood mononuclear cells has identified a cluster of probe sets that strongly associate with the biochemical severity of GHD.[296] The results provide tantalizing evidence that gene expression analysis from a blood sample may aid in the diagnosis of GH deficiency. However, evidence that blood transcriptome changes are useful in predicting growth in children[297] or identifying GH abuse is poor.[298]

Growth Hormone Replacement Therapy

The effects of GH replacement were first reported in 1989. GH replacement induces profound effects on protein, fat, and energy metabolism, which result in increased lean body mass and decreased fat mass without a significant change in body weight within months[299] (Fig. 8.18). The greatest reduction of body fat occurs in abdominal and visceral adipose tissue[300] (Fig. 8.19). Significant increases in extracellular water occur as a consequence of the dose-dependent antinatriuretic properties of GH.[301] GH-induced reduction in abdominal and visceral fat is accompanied by a significant shift in lipoprotein metabolism to a less atherogenic profile with improvements in the cholesterol and in HDL-cholesterol ratio but little or no change in triglycerides.[249] Long-term experience of more than 10 years indicates that treatment provides sustained benefits in body composition and metabolic risk markers[302] (Fig. 8.20). GH treatment reduces intimal media thickness of the carotid arteries.[271] Proinflammatory factors such as C-reactive protein and IL6, strongly implicated in the pathogenesis of vascular disease, fall significantly with GH treatment.[269]

GH replacement activates bone formation and bone resorption, resulting in a transient decline before a net increase in bone mass by 12 months.[303–305] Markers of bone turnover increase over the first 12 months but return to baseline after 3 to 4 years.[306] The increase is greater in men and in younger subjects.[304] BMD may increase for up to 10 years in the lumbar spine, whereas a decline that occurs in the femoral neck may start sooner[307] (Fig. 8.21). In an observational study, the fracture incidence over a mean follow-up period of 4.9 years was 30% lower in patients replaced with GH compared to an unreplaced group providing evidence of long-term protective benefit.[308]

GH replacement improves physical function through gains in anaerobic[309] and aerobic capacity,[310,311] the latter mediated in part by an increase in red cell mass, plasma volume, and cardiac output.[312,313] Most studies assessing GH effects beyond 12 months have reported a significant improvement in muscle strength through gains in muscle mass,[314–316] without affecting contractile force or fiber composition.[317]

Most trials with GH replacement report improved quality of life.[318] Discrepant results likely arise from varying study tools, with some being generic health questionnaires and others being disease specific. Domains of energy and emotional reaction tend to show the greatest improvements. Disease-specific tools have reported unequivocal improvement in measures of life satisfaction after GH treatment.[319] A large survey of 304 patients showed improved quality of life and significant reduction in the numbers of sick leave days and doctor visits during 12 months of GH therapy.[320,321]

Growth Hormone Administration. GH secretion is greater in the young and greater in women than in men. It is recommended that the starting dose of GH in young men and women be 0.2 and 0.3 mg/day, respectively, and in older individuals 0.1 mg/day,[277,278] which is then titrated according to serum IGF1 concentrations and at a rate that minimizes side effects[322] (Fig. 8.22). If side effects occur, the dose should be reduced; if no side effects are reported, the therapeutic goal is to maintain IGF1 levels in the normal age-matched and gender-matched range while avoiding levels in the upper quintile or above. Dose determination based on body weight

TABLE 8.6 Adult Somatotropin Deficiency

Clinical Consequence	Effect of GH Replacement
Body Composition	
General and central adiposity	Decrease
Reduced lean mass	Increase
Reduced bone mass	Increase
Function	
Reduced exercise capacity	Improve
Impaired anaerobic capacity	Improve
Muscle weakness	Increase
Impaired cardiac function	Improve
Hypohydrosis	Increase
Quality of Life	
Low mood	Improve
Fatigue	Improve
Low motivation	Improve
Reduced satisfaction	Improve
Cardiovascular Risk Profile	
Abnormal lipid profile	Improve
Insulin resistance	No change
Increased inflammatory markers	Decrease
Intimal media thickening	Decrease
Laboratory	
Blunted peak GH to stimulation (see Table 8.7)	
Low IGF1 (in 50–60%)	Increase
Hyperinsulinemia	Improve
High LDL and low HDL cholesterol	Improve

GH, Growth hormone; *HDL*, high-density lipoprotein; *IGF1*, insulin-like growth factor 1; *LDL*, low-density lipoprotein.

TABLE 8.7 Validated Diagnostic Tests for the Diagnosis of Growth Hormone Deficiency in Adults

Test	Subject Numbers Normal/GHD	GH Threshold (μg/L)	Reference
Insulin-induced[a] Hypoglycemia	35/23	<5	Hoffman et al[283]
Arginine-GHRH[a]	74/49	<9	Aimaretti et al[281]
Glucagon[a]	46/73	<3	Gomez et al[285]
GHRP 6-GHRH[a]	125/125	<15	Popovic et al[282]
GHRP 2-GHRH[a]	30/36	<17	Mahajan et al[288]
GHRP 2	77/58	<15	Chihara et al[287]
Macimorelin	25/114	<5.1	Gomez et al[285]; Garcia et al[286]
Low IGF1 and ≥3 PHDs[a]	785	N/A	Hartman et al[295]

[a]Recommended by the Growth Hormone Research Society and the Endocrine Society.

GH, Growth hormone; *GHD*, growth hormone deficiency; *GHRH*, growth hormone–releasing hormone; *GHRP*, growth hormone–releasing peptide; *IGF1*, insulin-like growth factor 1; *N/A*, not applicable; *PHD*, pituitary hormone deficiency.

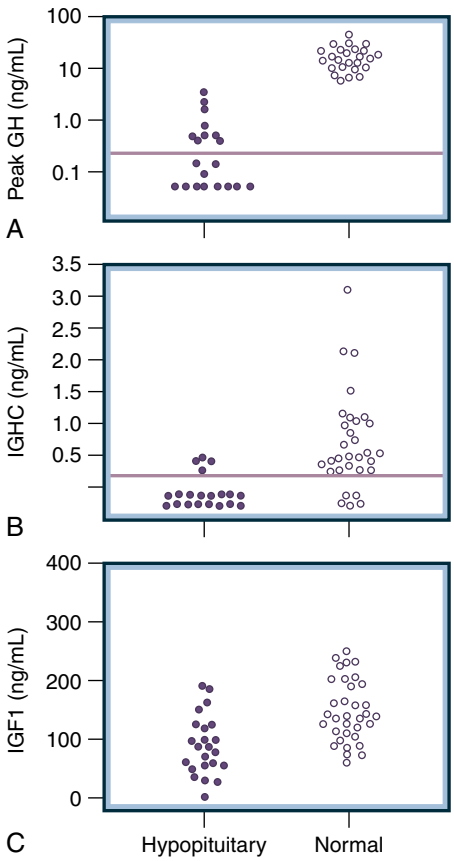
is not recommended because GH secretion is reduced in the obese.[277,278] GH is administered by nightly subcutaneous injection to mimic the greater secretion of GH at night. Side effects of GH in children are considerably fewer than those observed in adults.

Women with GHD require higher doses of hGH when also receiving oral rather than transdermal estrogen[323,324] because of a first-pass hepatic effect. Fifty percent more GH was required during oral estrogen treatment to maintain an IGF1 level equivalent to that achieved during transdermal administration; the waste is even greater when contraceptive instead of replacement estrogen doses are prescribed (Fig. 8.23).[325] In contrast, androgens enhance metabolic effects of GH.[148] The divergent effects of estrogens and androgens on GH action largely explain why women are less responsive than men to GH.[302]

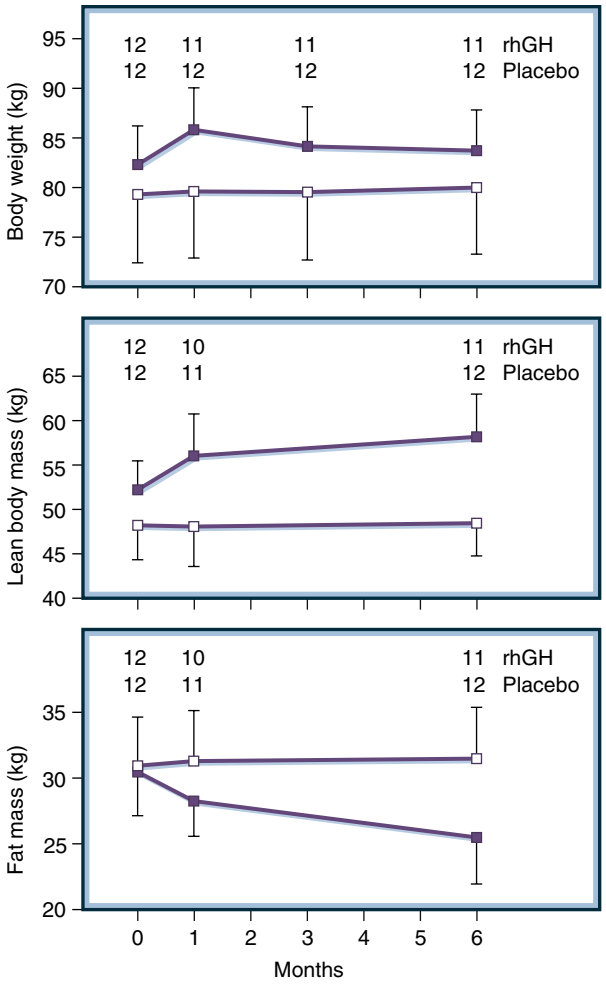
Transition-Age Patients. GH treatment of the GH-deficient child normally is terminated when final height and epiphysis closure are reached.[326] These GHD patients who transition from the time of cessation of linear growth do not attain the somatic and structural skeletal maturity that continues to occur in the early years of normal adulthood. GH-deficient children should continue GH treatment after puberty to complete somatic maturation of muscle and bone.[326] A significant proportion of patients with childhood-onset GHD exhibit normal GH response when retested at the end of GH treatment as young adults. Therefore patients considered for continuation of GH replacement in the transition years must be retested. GH retesting is not required for those patients with a transcription factor mutation (e.g., POU1F1, PROP1) or those with more than three demonstrated pituitary hormone deficits.[277,278]

Precautions and Caveats of Treating With Human Growth Hormone. The most common side effects of hGH replacement include edema, arthralgias, and myalgias (Table 8.8). However, these symptoms are mild, dose related, and resolve in the majority of patients either spontaneously or with dosage reduction.[322] Although GH antagonizes insulin action, the risk of developing hyperglycemia is very low (see Fig. 8.20). A meta-analysis of 13 placebo-controlled trials involving 511 patients found a mean elevation of fasting blood glucose of 0.22 mmol/L compared to placebo levels.[249] However, the propensity for developing diabetes is increased up to eightfold in GHD adults who are obese.[327]

Patients with active malignancies should not be treated with GH. The possibility that hGH might initiate new cancers or stimulate growth of preexisting benign tumors is an important theoretic issue. The incidence of cancer is markedly reduced among subjects with inactivating mutations of the GHR.[275] A number of epidemiologic studies have reported association between higher, albeit normal, IGF1 levels and later risk of developing prostate cancer,[328] breast cancer in premenopausal women,[329] and colon and lung cancer.[330]



• **Fig. 8.17** Comparison of peak growth hormone (GH) concentration obtained during an insulin-tolerance test (A), integrated GH concentration (IGHC) obtained from blood samples withdrawn every 20 minutes over 24 hours (B), and insulin-like growth factor 1 (IGF1) concentrations (C) in patients with organic hypopituitarism and sex-matched normal subjects. *Horizontal lines* represent the limit of reading. (Modified from Hoffman DM, O'Sullivan AJ, Baxter RC, et al. Diagnosis of growth hormone deficiency in adults. *Lancet.* 1994;343:1064–1068.)



• **Fig. 8.18** Effects of growth hormone (GH) replacement on lean body mass and fat mass in adults with GH deficiency. Effect of GH replacement on lean body mass and fat mass in 24 adults with GH deficiency. *rhGH,* recombinant human growth hormone. (From Salomon F, Cuneo RC, Hesp R, et al. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *N Engl J Med.* 1989;321:1797–1803.)

In contrast, patients with acromegaly do not have an increased incidence of either breast or prostate cancer or cancer in general. The overall risk for cancer in acromegaly is lower than expected. However, these patients have a significantly increased mortality risk from colon cancer.[331,332] Pediatric experience shows no convincing evidence for a causal link between GH treatment and tumor recurrence or the development of neoplasia, including leukemia.[333,334] When comparing the relative risk of brain tumor recurrence in 180 children treated with hGH versus 891 who did not receive hGH, the risk of recurrence after a mean of 6.4 years was lower in the treated group than in those not receiving hGH.[335] GH treatment does, however, increase the risk of radiation-induced second tumor formation, especially meningiomas.[336]

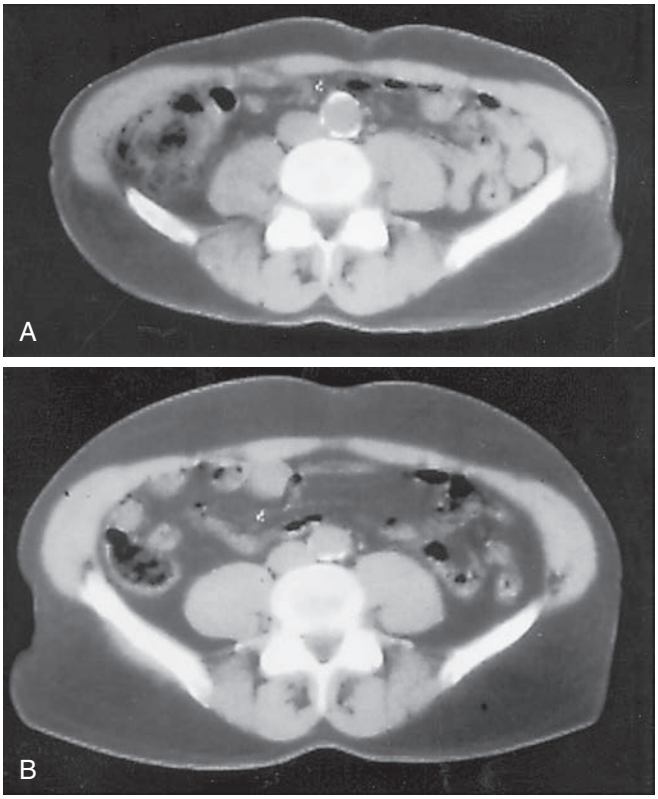
From observational studies, the incidence of malignancies in hypopituitary patients not receiving GH is higher than the general population but not in those replaced with GH.[337] The recurrence rate of craniopharyngiomas[338] and the recurrence rate and life expectancy in patients with nonfunctioning pituitary adenomas[339] were not different between patients replaced and unreplaced with GH over a follow-up period in excess of 10 years. Although observational studies are subject to selection bias, these findings provide some evidence supporting the long-term safety of GH replacement therapy. Nevertheless, long-term surveillance with adequate control groups in adults being treated for adult GHD is required to ensure that adult GH replacement does not increase the incidence of new cancers or growth of existing benign tumors.

Investigational Uses of Growth Hormone

Catabolic States. The anabolic actions of GH have prompted investigational use of GH in catabolic states, including surgery, trauma, burns, parenteral nutrition, and organ failure. The negative nitrogen balance in critically ill patients is partly attributable to GH resistance as well as to decreased IGF-1 production and action.[340] Beneficial effects of GH have been reported in patients with extensive burns, in those receiving high-dose glucocorticoid treatment, and in those with chronic obstructive pulmonary disease. There is some evidence that GH treatment in people with large burns results in more rapid healing of the burn wound and donor sites, and in reduced length of hospital stay, but there is an increased risk of hyperglycemia.[341] A study in which critically ill patients received very high doses of GH (up to 7 mg/day) approximating 15 to 20 times normal daily production rates was prematurely terminated due to increased fatality.[342] It has been suggested that GH may have had an adverse effect on acute-phase protein

in these patients.[343] Caution is advised for nonapproved uses of GH in adults.[344]

Osteoporosis. There is strong evidence that GH administered to otherwise healthy subjects with idiopathic osteoporosis improves bone mineral mass.[345] A double-blind, placebo-controlled treatment study for 18 months observed improvements in bone mineral content at the lumbar spine, in the femoral neck, and of the whole skeleton by up to 14%, which was sustained at 4 years.[346] Unclear long-term side effects, cost, and lack of comparative studies with other therapies for osteoporosis limit the potential use of GH in osteoporosis.

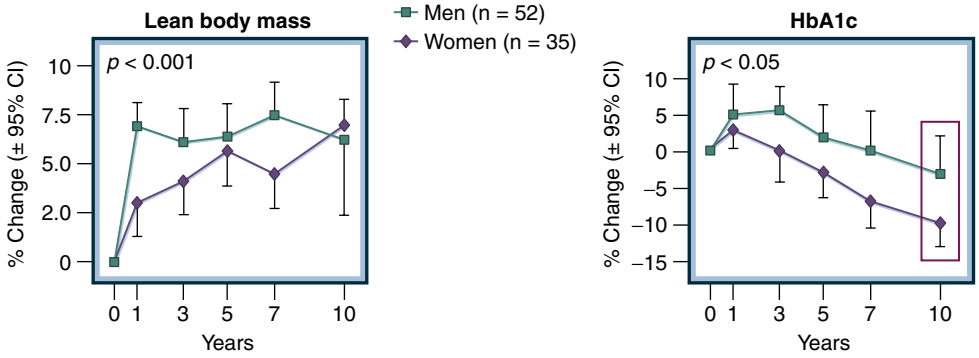


• **Fig. 8.19** Computed tomography scan through the abdomen before (A) and after treatment with human growth hormone (GH) (hGH) (B) in a GH-deficient patient. (Courtesy B.A. Bengtsson.)

Human Immunodeficiency Virus Infection. GH is Food and Drug Administration (FDA) approved for administration to adult patients with human immunodeficiency virus (HIV)–associated cachexia and results in positive nitrogen balance, increased lean body mass, decreased body fat, and improved work output.[347] One large study using supraphysiologic GH doses of 4 mg/day reported impairment in glucose tolerance.[348] A study using a physiologic dosing to maintain IGF1 in the high-normal range reported beneficial body compositional changes of lesser magnitude, reduced triglycerides, and lowered diastolic blood pressure but impaired glucose tolerance.[349] Treatment with a GH-releasing factor analogue for 26 weeks improved visceral fat and lipid profiles.[350] However, long-term beneficial effects of GH on survival and quality of life in those with HIV infection have not yet been reported.

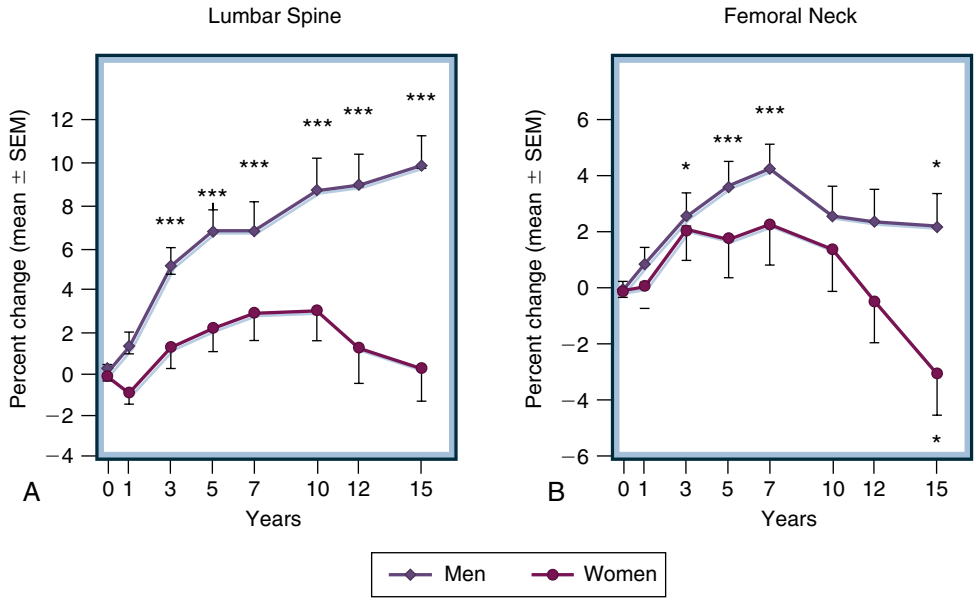
Sports. The public policy issues of GH abuse in competitive sports have received much attention. GH has been widely abused by athletes to enhance performance.[351] A systematic review concluded that claims that GH enhances physical performance are not supported by the scientific literature evaluating effects on aerobic capacity, strength, and power[352] and that more research is needed to conclusively determine the effects of GH on athletic performance. A double-blind, placebo-controlled study reported that GH enhances anaerobic sprint capacity but not aerobic capacity, strength, or power in recreational athletes.[353] The benefit to sprinting provides the first evidence justifying the prohibition of GH as a performance-enhancing drug.

Aging. Disease mongering of declining GH status is rampant and spawned by unsubstantiated claims that GH is an antiaging

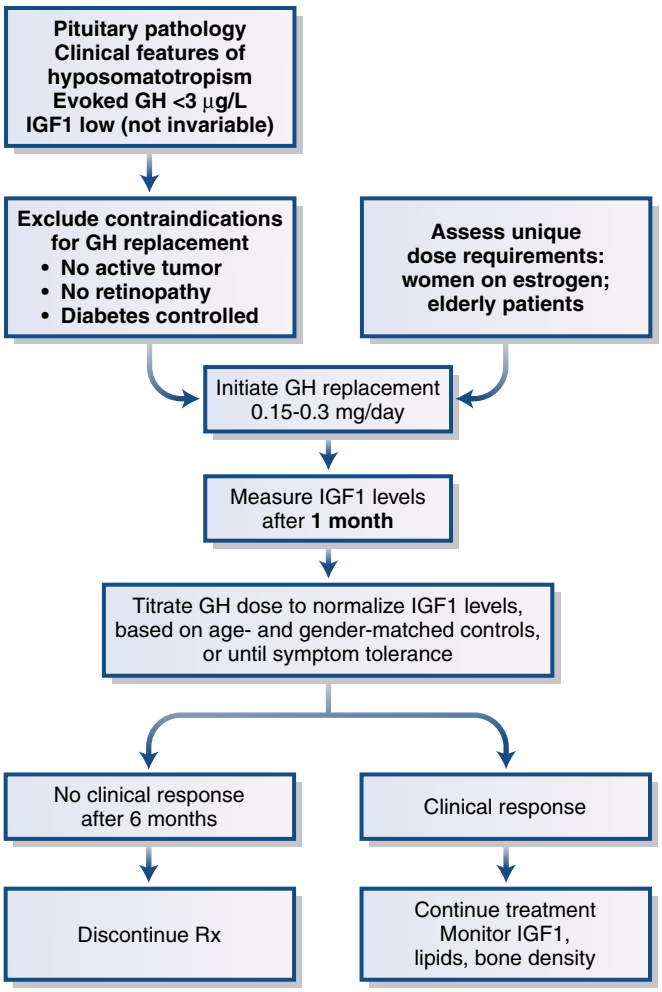


	Baseline	1 yr	3 yr	5 yr	7 yr	10 yr
GH Dose (mg/d)	0.98	0.65	0.53	0.50	0.48	0.47
Metabolic (mmol/L)						
TC	5.9					5.37
HDL-C	1.21					1.36
LDL-C	3.89					3.22
Glucose	4.00					4.96

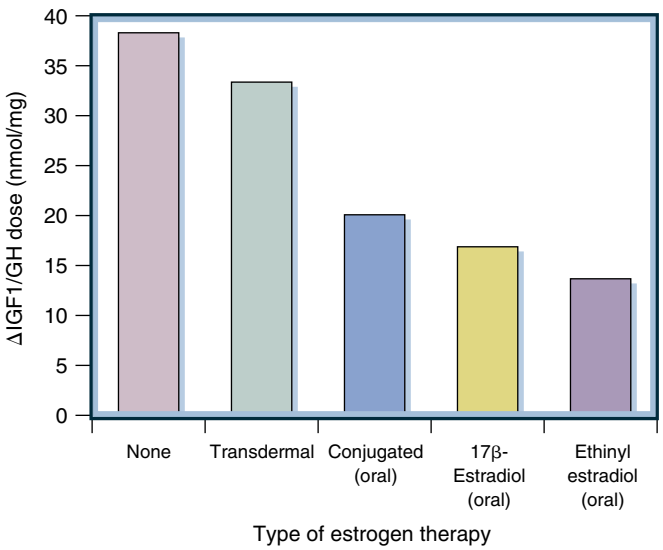
• **Fig. 8.20** Ten-year growth hormone (GH) therapy in 87 GH-deficient adults.[302] *CI,* confidence interval; *HbA1c,* hemoglobin A_{1c}; *HDL-C,* high-density lipoprotein-cholesterol; *LDL-C,* low-density lipoprotein-cholesterol; *TC,* total cholesterol. (Modified from Melmed S. Update in pituitary disease. *J Clin Endocrinol Metab.* 2008;93:331–338.)



• **Fig. 8.21** Fifteen years of growth hormone (GH) replacement on bone mineral densities at the lumbar (L2–L4) spine (A) and femur neck (B) in adult men and women with GH deficiency. The results are shown as percent change from baseline. The *vertical bars* indicate the SEM (standard error of the mean) values shown. $*p < 0.05$; $***p < 0.001$ versus baseline. $p < 0.01$ for men versus women. (From Elbornsson M, Gotherstrom G, Bosaeus I, et al. Fifteen years of GH replacement increases bone mineral density in hypopituitary patients with adult-onset GH deficiency. *Eur J Endocrinol.* 2012;166:787–795.)



• **Fig. 8.22** Management of somatotropin deficiency in adults. Patients older than 60 years require lower maintenance doses. Women receiving oral estrogen require higher doses than those receiving transdermal estrogen preparations. *GH,* growth hormone; *IGF1,* insulin-like growth factor 1; *Rx,* treatment.



• **Fig. 8.23** The effect of the route and type of estrogen therapy on the efficacy of growth hormone (GH) therapy in women with hypopituitarism. The GH sensitivity index was calculated as change in insulin-like growth factor 1 *(IGF1)* level *(nmol/L)* by dose of GH *(mg)*. Created using data from several studies. (From Birznieve V, Ho KKY. Growth and development: patching up a better pill for GH-deficient women. *Nat Rev Endo*. 2012;8:197–198.)

hormone.[354] The claims are based on observations that body composition changes that accompany the physical decline of the aging process superficially recapitulate the features of adults with organic GHD for which beneficial effects of GH replacement are established. A systematic review of literature published on randomized controlled trials in the healthy elderly indicates that GH supplementation is associated with small changes in body composition, no functional or cognitive benefit, but increased rates of

TABLE 8.8 Side Effects of Adult Growth Hormone Treatment

Edema
Arthralgias
Myalgias
Muscle stiffness
Paresthesias
Carpal tunnel syndrome
Atrial fibrillation
Headache
Benign intracranial hypertension
Increase in melanocytic nevi
Hyperglycemia
Sleep apnea
Iatrogenic acromegaly

adverse events.[355] GH cannot be recommended as an antiaging therapy.[354]

GH Excess

GH secretion is stimulated by fasting and by inadequate nutrition. This arises from the removal of substrates such as glucose and fatty acids, and of insulin, all of which suppress GH secretion.[213] The liver, being the major source of circulating IGF1, plays an important role in modulating feedback control of GH secretion. Thus liver disease and systemic illness affecting hepatic function and nutritional intake cause reversible hypersecretion of GH.[356,357] GH secretion is enhanced in type1 diabetes and falls when glycemic control improves.[358] Oral estrogen therapy enhances GH secretion by reducing blood IGF1 levels.[219] Under these circumstances episodic GH secretion is amplified, retaining a diurnal profile associated with subnormal IGF1 concentrations. This stands in contrast to the hypersecretion in acromegaly where secretion is autonomous and associated with elevated IGF1 concentration.

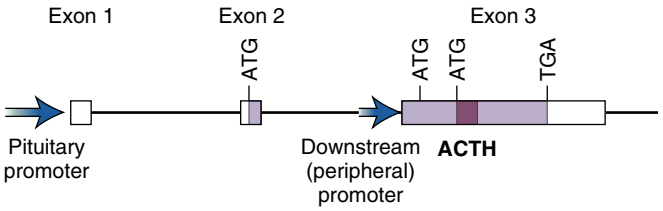
Adrenocorticotropic Hormone

Physiology

The hypothalamic-pituitary-adrenal (HPA) axis is the major system subserving stress and survival. Key components of the stress response are aimed at providing adequate amounts of glucocorticoids, which exert vital pleotrophic effects on energy supply, fuel metabolism, immunity, and cardiovascular function.

Corticotroph Cells

Corticotroph cells comprise about 20% of functional anterior pituitary cells and are the earliest detectable human fetal pituitary cell type, appearing by the eighth week of gestation. Corticotrophs are clustered mainly in the central median pituitary wedge. They are large, irregular cells, and their ultrastructural features include prominent neurosecretory granules (150–400 nm), endoplasmic reticulum, and Golgi bodies.[359] These cells produce the POMC gene products, including ACTH(1-39), β-lipotropin (βLPH), and endorphins. Because of the rich carbohydrate moiety of these molecules, the cells are strongly positive for periodic acid–Schiff (PAS). In the presence of excess glucocorticoid, characteristic hyaline deposits develop. In the normal human pituitary, POMC is expressed only in corticotroph cells. Most mammals possess an intermediate lobe comprising POMC-expressing melanotroph cells; however, this lobe is not developed in adult humans. Tpit, now referred to as Tbx19,[38] is a critical transcriptional factor for corticotroph cell differentiation during pituitary development and for transcription of the POMC gene.[38]



• **Fig. 8.24** Structure of POMC gene. Exon 1 encodes the RNA leader sequence, and exon 2 encodes the initiator methionine (ATG), the signal peptide, and several N-terminal residues of the precursor peptide, the remainder of which is encoded by exon 3. Corticotroph expression is determined by the upstream pituitary promoter *(longer arrowhead)*, whereas peripheral expression of the short POMC mRNA is determined by the downstream promoter *(shorter arrowhead)*. Translation of these shorter transcripts initiates from the initiator methionines *(ATG)* indicated in exon 3. The precursor peptide coding region is lightly shaded, and the ACTH coding region is darkly shaded. (From Adrian JL, Clark AJL, Swords FM. Molecular pathology of corticotroph function. In: Rappaport R, Amselem S, eds. *Hypothalamic-Pituitary Development*. Basel, Switzerland: Karger; 2001.)

Structure

The primary translation product of POMC is a 266–amino acid pre-prohormone molecule encoding corticotrophic, opioid, and melanotropic peptides. The peptide contains a leader sequence and multiple dibasic proteolytic cleavage sites for glycosylation, acetylation, and amidation. Products of this processing include ACTH(1-39) and βLPH, which in turn give rise to γLPH and β-endorphin, also containing metenkephalin. ACTH itself may also be cleaved to give rise to α-melanocyte–stimulating hormone (MSH)(1-13) and corticotropin-like intermediate lobe peptide (CLIP)(18-39).

The 8-kb human POMC gene, located on chromosome 2p23,[38,360] consists of three exons interspersed with two intervening introns (Fig. 8.24). The first exon encodes a leader sequence, the second encodes the signal initiation sequence and the amino-terminal (N-terminal) portion of the POMC peptide, and the third exon encodes most of the mature peptide sequences, including ACTH and βLPH.[361] The POMC gene is expressed in pituitary and nonpituitary tissues, including brain, skin, placenta, gonads, gastrointestinal tissues, liver, kidney, adrenal medulla, lung, and lymphocytes. The pattern of POMC processing differs between pituitary and extrapituitary issues and is determined by tissue-specific expression and activity of cleavage enzymes.

In corticotroph cells, a pituitary-selective promoter region for POMC generates a POMC messenger RNA (mRNA) transcript of approximately 1200 nucleotides. The 800 nucleotides of the coding region are translated into a pre-POMC molecule that includes a 26–amino acid signal peptide, which is rapidly cleaved. The parent POMC protein of 241 amino acids enters the secretory pathway for subsequent processing.

In extrapituitary tissues, POMC gene expression is regulated differently from that in the pituitary. An upstream promoter generates a longer transcript of approximately 1350 nucleotides. A downstream promoter generates short truncated transcripts of approximately 800 nucleotides arising from the 5′ end of exon 3. In the brain, however, neurons in the arcuate nucleus express

POMC mRNA that is identical to that of the pituitary.[362] In these neurons, POMC serves as a precursor to brain β-endorphin, αMSH, and other peptides, which have important functions on food intake and energy homeostasis.

Regulation

Pituitary POMC expression is primarily under the positive regulation of corticotropin-releasing factor (CRF) and negative regulation of glucocorticoids. However, multiple other signals regulate pituitary POMC gene expression: AVP, cytokines, catecholamines, and VIP activate while somatostatin and ANP inhibit.[363]

By contrast, POMC expression in the hypothalamus is stimulated by glucocorticoids, in turn enhancing the production of α-MSH, which mediates the suppression of appetite.[363] Leptin and insulin also enhance hypothalamic POMC gene expression.[364,365]

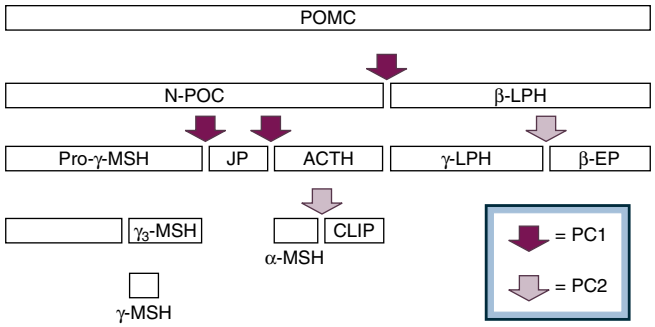
The corticotropin-releasing hormone (CRH) type 1 receptor is predominantly expressed on the corticotroph,[366] and receptor activation increases cAMP, protein kinase A, and CREB induction of CRH-binding protein (CRHBP) to the promoter, leading to POMC transcription.[367] CRH also activates an AP1 site within the first exon by a MAPK-mediated pathway. This receptor also appears critical for fear and anxiety responses, possibly by a related ligand, urocortin.[368] The type 2 CRH receptor predominantly regulates cardiovascular function.[369]

The corticotroph is regulated by inflammatory and nutritional signals that potentiate or inhibit CRF.[370] CRH action is potentiated by vasopressin and β-adrenergic catecholamines, enhancing POMC transcription and synthesis and increasing ACTH secretion.[363] Glucocorticoid inhibition of POMC transcription involves GR binding 5′-regulatory elements via two cooperative binding sites.[362] Normal pituitary corticotrophs express somatostatin receptors. Of the five subtypes, subtypes 2 and 5 are predominantly expressed. Somatostatin inhibits ACTH secretion, but the sensitivity is strongly regulated by glucocorticoids, which nullify the inhibition via downregulating somatostatin receptor expression.[371] Dopamine receptors have not been characterized in normal human corticotrophs, although they are highly expressed in the intermediate lobe of the rat pituitary.

POMC Processing

Several post-translational POMC modification steps are required for ultimate polypeptide hormone secretion (Fig. 8.25). First, the N-terminal signal sequence is removed, followed by glycosylation via an *O*-linkage to Thr45 and *N*-linkage to Asn65.[372] Serine-phosphorylation then occurs within the Golgi apparatus. After being transported to secretory vesicles, the constituent peptides are cleaved at dibasic amino acid residues, and ACTH-related peptides are stored in dense secretory granules for ultimate regulated release. Some POMC products also undergo carboxy-terminal (C-terminal) amidation mediated by peptidylglycine-amidating mono-oxygenase (PAM), peptidylhydroxyglycine-amidating lyase (PAL),[373] and N-terminal acetylation.

POMC proteolytic processing occurs at Lys-Arg or Arg-Arg residues by enzymes called prohormone convertases (PCs), mediated by either or two called PC1 or PC2. They are part of a superfamily of subtilisin/kexin proteinases. Tissue distribution of the two PCs are distinct and determine the function of the cleaved peptides at the tissue level. PC1 is most abundant in the anterior pituitary and in the hypothalamus. PC2 is present in the neurointermediate lobe, hypothalamus, various brain regions, skin, and pancreatic islets but is absent in the pituitary.



• **Fig. 8.25** Processing and cleavage of pro-opiomelanocortin (POMC). The mature POMC precursor peptide is sequentially cleaved by prohormone convertase 1 (PC1) in the anterior pituitary corticotroph. In the neurointermediate lobe and other cell types, cleavage by PC2 allows release of βMSH or β-endorphin, or both. Carboxypeptidase H *(not shown)* removes residual basic amino acids at cleavage sites. *ACTH,* adrenocorticotropic hormone; *CLIP,* corticotropin-like intermediate lobe peptide; *EP,* endorphin; *JP,* joining peptide; *LPH,* lipotropin; *MSH,* melanocyte-stimulating hormone; *N-POC,* N-terminal pro-POMC fragment. (From Clark AJL, Swords FM. Molecular pathology of corticotroph function. In: Rappaport R, Amselem S, eds. *Hypothalamic-Pituitary Development.* Basel, Switzerland: Karger; 2001.)

In corticotrophs, PC1 expression results in cleavage limited to four sites, with ACTH and ß-lipotrophin being major end products (see Fig. 8.25). In the hypothalamus and CNS, both PC1 and PC2 allow coordinated proteolysis, resulting in the generation of αMSH, CLIP, γLPH, and ß-endorphin. Heterozygous mutations of the PC1 gene are associated with childhood obesity, adrenal insufficiency, hyperproinsulinemia, and postprandial hypoglycemia[374] with elevated levels of plasma ACTH precursors.

Extrapituitary and CNS Expression of POMC

POMC is also expressed in the gonads, lung, skin, gastrointestinal and adrenal medullary neuroendocrine cells, and white blood cells. The biology of extrapituitary and extra-CNS POMC is poorly understood. There is little evidence that these tissues contain enzymes required for the production and secretion of the processed peptides.

Melanocortin Receptors

The major secreted POMC products from the pituitary are ACTH, ßLPH, and ß-endorphin. ACTH-derived and POMC-derived peptides bind to specific melanocortin receptors (MCR). MCRs are members of the rhodopsin-like receptors, a branch of the seven transmembrane-spanning domain G protein–coupled receptor (GPCR) superfamily. There are five MCRs, each showing different tissue distribution. All MCRs except for MC2R, the ACTH receptor, bind melanocortin peptides containing the conserved heptapeptide core MEHFRWG, found in αMSH, while the ACTH receptor further requires a peptide motif C-terminal to the 13 amino acids found in αMSH. Each MCR mediates diverse physiologic responses to αMSH, or ACTH, in the case of MC2R.[375] The distribution of MCR types is shown in Table 8.9.

The MC1Rs are found primarily in melanocytes that regulate pigmentation of skin and hair follicles. ACTH, βLPH, and γLPH produced from the corticotroph share a common heptapeptide sequence (Met-Glu-His-Phe-Arg-Trp-Gly) required to

TABLE 8.9 Tissue Distribution of Melanocortin Receptor (MCR) Types

MC Receptor Types	Tissue
MCR1	Skin, hair follicles
MCR2	Adrenal cortex
MCR3	Hypothalamus, limbic system
MCR4	Hypothalamus, various brain areas,[a] intestines
MCR5	Exocrine glands, skeletal muscle, adrenal cortex

[a]Cortex, thalamus, brainstem, and spinal cord.

activate MC1R. These peptides are responsible for inducing skin pigmentation in Addison disease because the other melanostimulating peptides, αMSH and βMSH, are not produced in the pituitary. Some MC1R variations show impaired signaling pathways strongly associated with red hair color, fair skin, and poor tanning ability.[376] POMC is also expressed in skin melanocytes, the expression of which is simulated by ultraviolet light, increasing local α-MSH and ß-endorphin production in parallel with PC1 and PC2 expression. ß-endorphin production alleviates pain and is thought to explain UV-seeking addictive behavior.[377]

MC3R and MCR4 are expressed almost exclusively in the CNS, especially the hypothalamus where they exert pivotal roles in satiety and energy homeostasis. Genetic and pharmacologic abrogation of the central melanocortin system causes profound obesity. POMC-deficient mice and humans are hyperphagic; MC4Rs are present in the L cells of the gut where they stimulate the production of anorexigenic peptides. The role of pituitary-secreted POMC peptides on gut MC4Rs is unknown.[378] MC5Rs play a major role in the regulation of exocrine gland function, especially of the sebaceous glands.[379] Recent findings provide evidence that MC5Rs in skeletal muscle enhance glucose uptake by αMSH independent of insulin.[380]

Adrenal Action

ACTH is a polypeptide of 39 amino acids with a molecular weight of 4.5 kDa. It is the only POMC-derived peptide with adrenocorticotrophic function, and it is the ligand of the MC2R. The function of MCR2 is dependent on a protein, melanocortin 2 receptor assessor protein (MRAP) required to traffic the receptor to the cell surface.[381] MRAP mutations cause familial glucocorticoid deficiency. MC2R activation results in production of adrenal glucocorticoids, androgenic steroids, and to a lesser extent, mineralocorticoids. The highly conserved 12 N-terminal amino acid residues are critical for adrenal gland steroid synthesis. N-terminal peptide POMC(1-28) exerts an independent mitogenic and growth-sustaining effect on the adrenal gland.[382]

ACTH signals via adenyl cyclase to regulate P450 enzyme transcription of cortisol, aldosterone, 17-hydroxyprogesterone, and to a lesser extent, adrenal androgens.[383] ACTH stimulates mitochondrial cholesterol transport and regulates the rate-limiting, side-chain cleavage of cholesterol to pregnenolone.[384] Adrenal cortisol response to ACTH is sensitive to the background ambient ACTH milieu. In states of chronic ACTH deficiency, adrenal reserve is compromised, although during ongoing ACTH hypersecretion the gland is primed such that a given ACTH bolus elicits a higher cortisol response. Both basal and stimulated (e.g., by CRH) ACTH secretion is blunted by glucocorticoids.

ACTH Secretion. The complex control of ACTH secretion reflects the integrated neuroendocrine control of the stress response. Similar to other anterior pituitary hormones, ACTH regulation is subserved by at least three tiers of control (see Fig. 8.3). First, the brain and hypothalamus release regulatory molecules (including CRH, vasopressin, and dopamine) that traverse the portal system and directly regulate corticotrophic function. Second, intrapituitary cytokines and growth factors act locally to regulate ACTH, either in concert with hypothalamic factors or independently. These paracrine controls often overlap and induce sensitive intracellular molecules that limit the ACTH response, preventing chronic ACTH hypersecretion. Third, glucocorticoids maintain regulatory feedback control of corticotroph secretion by rapidly inhibiting hypothalamic CRH and pituitary ACTH secretion. In a short feedback loop, pituitary ACTH inhibits hypothalamic CRH, and in an ultrashort loop, it may also suppress the corticotroph itself.

Stress Response

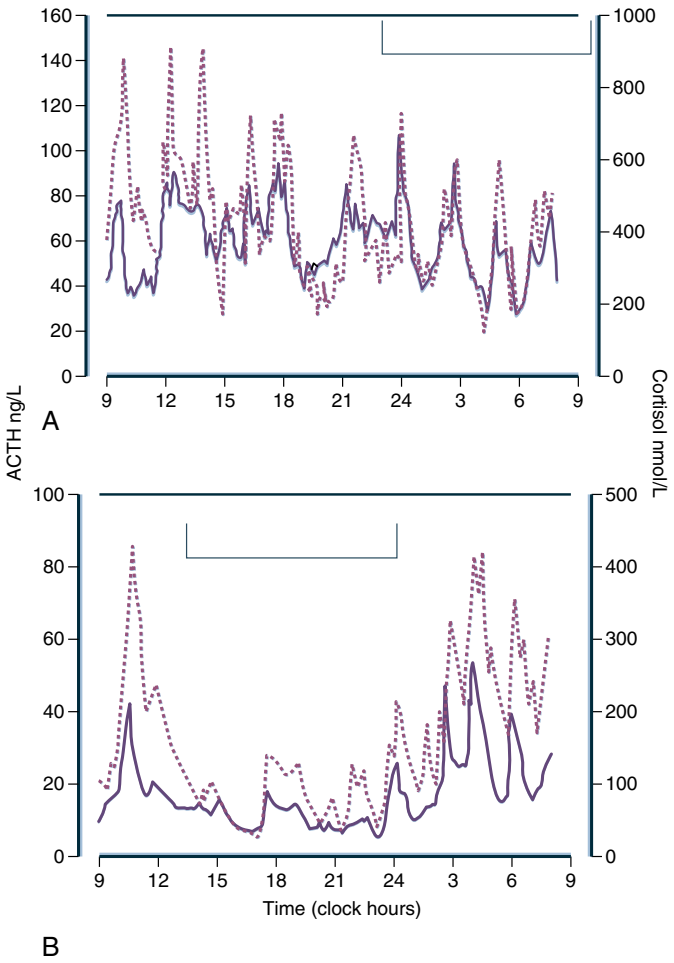
The HPA axes respond to a range of stressors, including pain, infection, inflammation, hemorrhage, hypovolemia, trauma, psychologic stress, hypoglycemia, and critical illness. The adaptation to stress involves vasovagal and catecholamine activation, cytokine secretion, and action. A tightly controlled immunoneuroendocrine interface regulates the ACTH response to peripheral stressors, which include pain, infection, inflammation, hemorrhage, hypovolemia, trauma, psychologic stress, and hypoglycemia. These signals vary in their ability to generate ACTH secretion and to sensitize the glucocorticoid response to ACTH. In addition to CRH, proinflammatory cytokines potently induce POMC transcription and ACTH secretion.[363] Sensitive intracellular signals within the corticotroph also serve to override the ACTH response to stress, thus preventing persistent and chronic hypercortisolemia.

Cytokines such as IL6 and leukemia inhibitory factor (LIF) activate the HPA axis and enhance glucocorticoid production, protecting the organism against lethality by constraining the inflammatory response.[370] Thus mice with inactivated CRH or LIF genes mount an inadequate neuroendocrine response to stress, inflammation, or endotoxins. During stress, glucocorticoid inhibition of ACTH is also prevented by nuclear factor κB activation, which interferes with pituitary glucocorticoid receptor function, thus further exaggerating enhanced ACTH secretion.[385]

Hypoglycemia evokes acute release of ACTH secretion. During the ITT, ACTH levels increase fivefold to sixfold, peaking by about 45 minutes, which is followed by peak cortisol at 60 to 90 minutes. Acute nutrient deprivation activates the secretion of ACTH. Exercise is a physiologic stimulus of ACTH release. Exercising to 90% of maximum oxygen capacity causes a significant elevation of ACTH, similar to levels observed during surgery or hypoglycemia.[386,387]

Circadian Periodicity

ACTH is secreted with both circadian periodicity and ultradian pulsatility under the control of the suprachiasmatic nucleus. The secretion of cortisol is tightly linked to and follows that of cortisol by 5 to 10 minutes (Fig. 8.26).[388] β-endorphin, coreleased with ACTH, also displays pulsatile, circadian, and ultradian rhythmicity with a close temporal coupling to cortisol.[389] The circadian pattern of ACTH secretion typically begins at about 4



• **Fig. 8.26** Plasma concentrations of ACTH *(dotted line)* and cortisol *(continuous line)* in a patient with Cushing disease (A) and a normal subject (B), showing the concordance between episodic ACTH and cortisol concentrations in blood. The ultradian pattern of ACTH and cortisol present in the normal subject is lost in the patient with Cushing disease. (Redrawn from Roelfsema F, Pincus SM, Veldhuis JD. Patients with Cushing's disease secrete adrenocorticotropin and cortisol jointly more asynchronously than healthy subjects. *J Clin Endocrinol Metab.* 1998;83:688–692.)

AM, peaking before 7 AM, with both ACTH and adrenal steroid levels reaching their nadir between 11 PM and 3 AM. Within this overall 24-hour diurnal cycle, periodic ACTH secretory bursts occur at a frequency of 40 pulses per 24 hours; amplitude rather than frequency modulation contributes to diurnal changes in ACTH profile.[390] ACTH circadian rhythm is entrained by visual cues, and the light-dark cycle is centrally controlled by CRH and other factors[391] and is lost in Cushing disease (see Fig. 8.26).[388]

The HPA axis is a critical component of a circadian system regulating substrate metabolism, immunity, cognition, and stress adaptation through the actions of glucocorticoids. This 24-hour rhythm partly reflects the activity of a master circadian pacemaker located in the suprachiasmatic nucleus, that is regulated by an oscillatory system governed by clock genes cued by light-dark and feeding cycles. The same molecular circuitry of core clock elements is present in multiple, if not all, peripheral tissues, including the heart, kidney, muscle, liver, pancreas, blood, fat, and adrenal gland.[392] The central clock also entrains peripheral clocks via neural and hormonal systems, the latter through the HPA axis. The physiologic effects of glucocorticoids are optimized when the central signal that controls the rhythm of glucocorticoid release and the peripheral rhythms in tissues expressing glucocorticoid receptors are aligned. There is strong evidence that a regular 24-hour temporal organization underpins good health. The early-morning peak subserves the metabolic and CNS preparation of physical activity and stressors at the start with declining levels favoring the optimization of insulin sensitivity for the rest of the day. The disruption of the circadian system brought by the lifestyles and social demands of modern civilization such as shift work and jet lag brings adverse health outcomes.[392] The 24-hour profile of cortisol concentrations does not adapt rapidly to acute shifts in light-dark, activity-rest, and/or feeding cycles. Under these circumstances the asynchrony between meal times and ultradian rhythm of cortisol secretion impairs substrate metabolism and worsens glucose tolerance (Fig. 8.27).[393] There is mounting evidence that the standard regimen of glucocorticoid replacement therapy does not restore physical, metabolic, and psychologic well-being among patients with adrenal insufficiency, from the failure to replicate the 24-hour rhythm of cortisol concentration.[394]

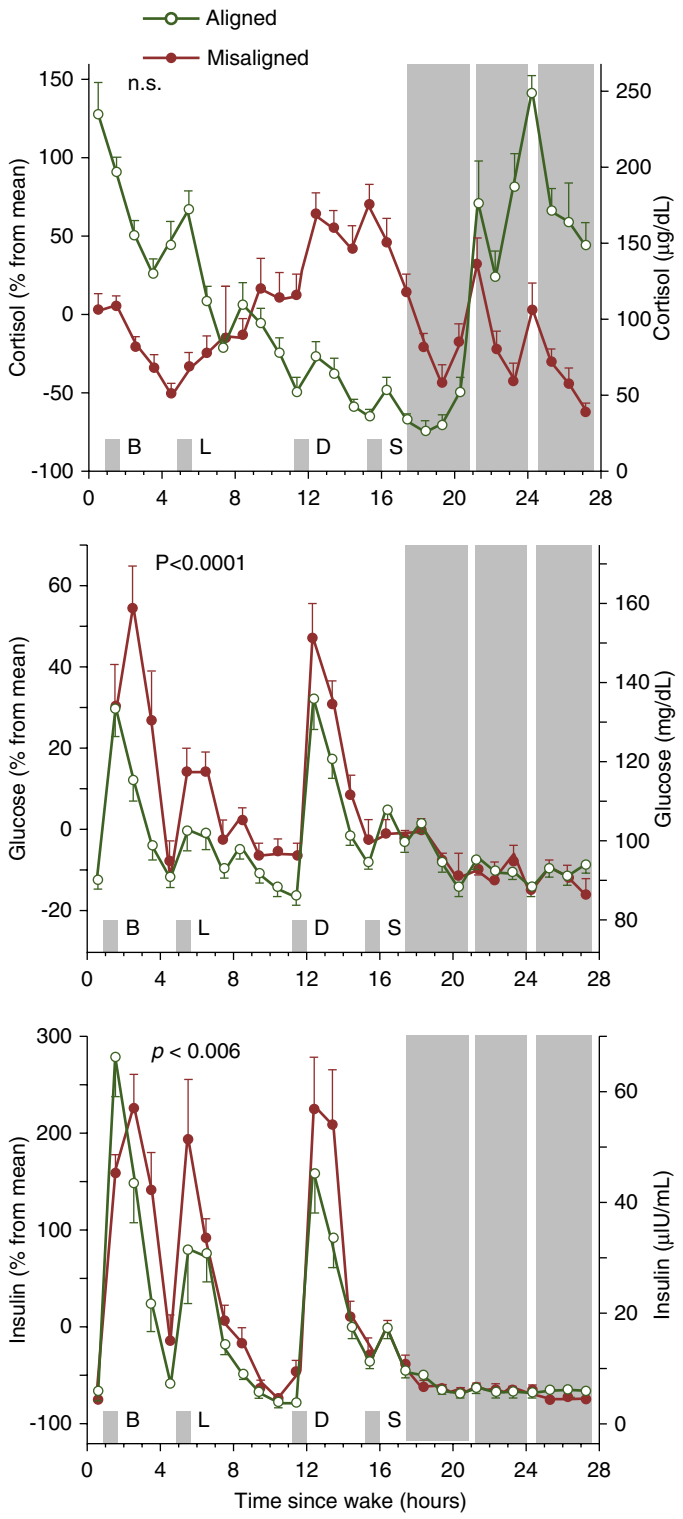
Measurement of ACTH

Measurements of plasma ACTH are extremely useful in the diagnosis of both Cushing syndrome and adrenal insufficiency. ACTH assays have evolved considerably since the first radioimmunoassay. Current commercially available two-site immunometric assays display high specificity and analytic sensitivity of less than 0.5 ng/L.[395] However, significant variability in precision and performance exists between commercial assays.[396] ACTH and other POMC-derived peptides such as αMSH, βLPH, or β-endorphin can also be measured with precision with current assays. Awareness of the assay peptide specificity may be especially critical when evaluating ectopic POMC products secreted by lung tumors. Ideally, nonstressed resting subjects should have venous blood withdrawn between 6 AM and 9 AM. As ACTH is relatively unstable at room temperature and has a propensity to adhere to glass, plasma samples should immediately be separated in iced siliconized glass tubes containing ethylenediaminetetra-acetic acid (EDTA) and stored below –20°C for transport. Morning (8 AM) plasma ACTH levels range from 8 to 25 ng/L. Episodic secretion and short plasma half-life result in wide and rapid fluctuation of plasma measurements. Cortisol values at 4 PM are about half those of morning levels, and 11 PM levels are usually less than 5 μg/dL. Plasma ACTH levels fluctuate broadly within the same individual and are highly sensitive to stress, time of collection, and gender. Pregnant females have higher ambient ACTH levels, possibly because of placental CRH secretion.[397]

ACTH Deficiency

Causes

Congenital ACTH deficiency may occur as an isolated pituitary defect or as a component of a wider spectrum of multiple pituitary hormone deficiencies. A mutation of *TBX19*, encoding Tpit, a transcription factor involved in corticotroph differentiation, is a cause of isolated ACTH deficiency (see Tables 8.1 and 8.2).[38] Mutations of transcription factors involved in early stages of pituitary cell differentiation or midline brain development may also give rise to ACTH deficiency as a component of multiple hormone deficiencies. These genes with mutations include *LHX4* and *HESX1* (see Table 8.2). Secondary causes include pituitary tumors, sellar mass lesions, trauma, irradiation, and lymphocytic



• **Fig. 8.27** Consequences of circadian misalignment on metabolic, autonomic, and endocrine function. Data are plotted according to "time since wake," during normal circadian alignment (*open green symbols*; scheduled awakening at habitual wake time) and during circadian misalignment (*filled red symbols*; scheduled awakening 12 hours out of phase from habitual wake time). P-values, statistical significance for effect of misalignment (based on 24-hour cycle for variable mainly driven by circadian cycle [cortisol] and 28-hour cycle for variables mainly driven by behavior cycle [others]). *Gray area*, scheduled sleep episode; *short vertical gray bars*, meal times: *B,* breakfast; *L,* lunch; *D,* dinner; *S,* snack. (Modified from Scheer FA, Hilton MF, Mantzoros CS, et al. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci USA.* 2009;106:4453–4458.)

hypophysitis, which may be associated with other autoimmune manifestations (see Table 8.5).

Clinical Features

The manifestations of ACTH deficiency are clinically indistinguishable from glucocorticoid deficiency of any cause. The clinical features are dependent on severity, the time of onset, and clinical context. In the newborn, ACTH deficiency may present as hypoglycemia and failure to thrive. In the adult, there is slowly progressive weight and appetite loss, anorexia, and generalized fatigue, mimicking a wasting syndrome. As adrenal mineralocorticoid secretion is largely unimpaired, salt wasting, volume contraction, and hyperkalemia, commonly encountered features in Addison disease, are not manifest. Furthermore, hyperpigmentation, usually associated with exuberant ACTH-related peptide secretion in the face of adrenal damage, does not occur.

Evaluation

Diagnostic evaluation of adrenal insufficiency requires concurrent measurement of cortisol and ACTH levels. Morning serum cortisol levels lower than 3 µg/dL suggest ACTH deficiency, but basal morning cortisol levels higher than 18 µg/dL usually indicate normal ACTH reserve. Provocative tests are required to diagnose ACTH deficiency. As cortisol is highly bound to cortisol-binding globulin (CBG), the level of CBG may confound interpretation of cortisol values. Cirrhosis and hyperthyroidism lower CBG concentrations, whereas estrogens elevate them.

Dynamic Testing for ACTH Deficiency

Hypothalamic Testing. Insulin hypoglycemia is a potent endogenous stressor that evokes ACTH secretion as well as GH release. Insulin (0.1–0.15 U/kg) is injected intravenously after an overnight fast to achieve symptomatic hypoglycemia and a blood glucose level of less than 40 mg/dL. This test must be performed under supervision. Normal HPA response to this stressor evokes cortisol levels higher than 20 µg/dL. As hypoglycemia acts centrally, a normal response implies integrity of all three tiers of HPA axis control.[398] Venous samples are collected at –15, 0, 15, 30, 45, 60, 90, and 120 minutes for measurement of glucose, ACTH, and cortisol levels. After the test, oral glucose should be administered *to ensure euglycemia.* Both intraindividual variations in blood glucose levels attained by a given dose of insulin and fluctuations in central sensitivity to glucose and activation of catecholamines may lead to difficulties in reproducibility. Contraindications are similar to those for the diagnosis of GH deficiency described earlier. If pronounced adrenal insufficiency is likely, insulin injection may provoke an adrenal crisis as a result of inadequate adrenal reserve, and hydrocortisone (100 mg) should be available for urgent IV use, if required, and oral glucocorticoid replacement therapy should be initiated pending confirmation of the diagnosis.

The metyrapone test is an alternative to the ITT for hypothalamic evaluation of the HPA axis. In blocking cortisol synthesis by inhibiting adrenal 11β-hydroxylase, it releases the HPA axis from negative feedback by cortisol, normally resulting in an ACTH surge and elevated levels of 11-deoxycortisol. A single oral dose (2–3 g) is given at midnight and serum levels of ACTH, 11-deoxycortisol, and cortisol are measured at 8 AM the following day. The test is only valid when cortisol levels fall to less than 10 µg/dL. In normal subjects, peak ACTH values higher than 200 ng/L are achieved. Side effects include nausea, gastrointestinal upset, and insomnia.[399] False-positive results

may be obtained with phenytoin, which prevents adequate enzymatic blockade. This test should be performed under observation in a hospital because acute adrenal insufficiency may ensue.

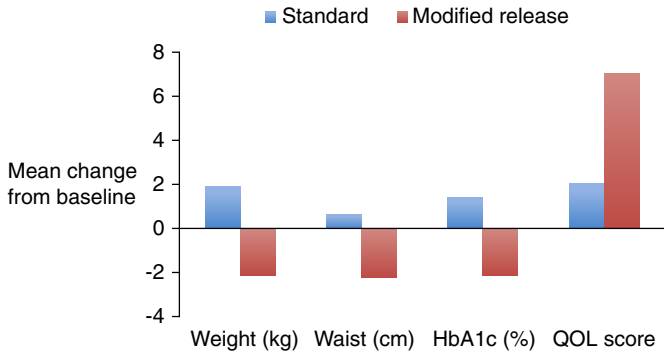
Pituitary Stimulation. Pituitary ACTH secretion is evoked by injecting either CRH or AVP. Ovine or human CRH (100 µg or 1 µg/kg) is administered intravenously, and cortisol and ACTH are measured at –5, –1, 0, 15, 30, 60, 90, and 120 minutes. Normally, maximal ACTH responses (twofold to fourfold above baseline) are evoked at 30 minutes,[400] and cortisol levels peak (>20 µg/dL) at 60 minutes or increase more than 10 µg/dL above baseline. Although CRH readily induces ACTH secretion and may demonstrate corticotrophic ACTH deficiency or ACTH excess, the wide variation of responses observed has limited the utility of this test. A useful application of the CRH test is in making the diagnosis of Cushing disease (see Chapter 15).

Adrenal Stimulation. The acute response of the adrenal gland to a bolus ACTH injection reflects ambient ACTH concentrations to which the gland has been exposed. Thus the cortisol response to an acute ACTH injection will be blunted if the subject has experienced chronic pituitary ACTH hyposecretion, with resultant adrenal atrophy and diminished cortisol reserve.[401] This time-honored test involves intramuscular or intravenous injection of 250 µg ACTH(1-24) (Cortrosyn or Synacthen) and cortisol levels measured before injection and then 30 minutes and 60 minutes after injection. Cortisol values greater than 20 µg/dL reflect a normal adrenal reserve response. The utility of this test in diagnosing diminished pituitary ACTH reserve has been challenged as a 250-µg dose may evoke a "normal" cortisol response in hypopituitary subjects. An unacceptably high false-negative rate (~65%) has been determined in a large series,[402] although peak cortisol levels at 30 minutes correlate well with peak responses to ITT.[403,404] Low-dose stimulation with 1 µg Synacthen evokes maximal serum cortisol levels at 30 minutes, and these correlate well with values observed after insulin or high-dose ACTH administration.[403] A cutoff value of more than 500 nmol/L provides almost 100% sensitivity and a specificity of 80% to 100%.[405] Failure to respond to low-dose ACTH should be corroborated by a standard dose of insulin or ACTH stimulation.

Adrenal Steroid Replacement

Hydrocortisone is widely used for glucocorticoid replacement. The normal secretory rate of cortisol is 15 to 20 mg/day, which is the recommended total daily dose. As plasma circulating half-life of cortisol is less than 2 hours, three times daily dosing of a total daily requirement of 10 to 20 mg (5–10 mg in the morning, 2.5–5 mg at noon, and 2.5–5 mg in the evening) is recommended.[406] Other synthetic glucocorticoids, including prednisolone (2.5–5 mg/day) and dexamethasone (0.25–0.5 mg/day), are suitable alternatives. Having longer half-lives, they can be administered once daily, but they are difficult to monitor biochemically. There is no consensus for monitoring treatment. Central diabetes insipidus may be unmasked after initial glucocorticoid replacement. Mineralocorticoid replacement is not required for treating secondary hypoadrenalism. Adrenal androgen replacement with dehydroepiandrosterone (DHEA) at doses of 25 mg/day may improve the sense of well-being, relieve fatigue, and improve sexual function in patients with primary and secondary adrenal insufficiency.[407,408]

However, conventional regimens do not recapitulate the physiologic pattern of cortisol release, and this may explain the high prevalence of poor quality of life and osteoporosis in these patients.[409] Two modified release formulations aimed at mimicking diurnal profiles have been evaluated in clinical trials.[410,411] One of these, a dual-release hydrocortisone tablet (DuoCort) containing an immediate-release coating and an extended-release core, was approved by the European Medicines Agency for once-daily therapy of adrenal insufficiency in 2012. In a 12-week randomized crossover study of 64 patients with adrenal insufficiency, DuoCort exhibited improved weight, glucose, and blood pressure control compared to conventional thrice-daily hydrocortisone dosing at the same total daily dose.[410] In a 26-week randomized controlled trial of 89 patients with adrenal insufficiency, significant benefits in weight, metabolic, immune, and quality-of-life measures were observed in the group randomized to once-daily, dual-release hydrocortisone (DuoCort) treatment compared to the group who continued with the conventional regimen of same daily dose administered thrice daily (Fig. 8.28).[394] Thus modified-release preparations mimicking cortisol diurnal profiles are a significant advance in the management of glucocorticoid insufficiency.



• **Fig. 8.28** Changes in body weight, waist circumference, HbA1c, and quality of life (QOL) in patients with hypoadrenalism randomized to standard treatment or modified-release hydrocortisone formulation for 26 weeks. Differences between treatments are all statistically significant ($p < 0.05$). (Data from Isidori AM, Venneri MA, Graziadio C, et al. Effect of once-daily, modified-release hydrocortisone versus standard glucocorticoid therapy on metabolism and innate immunity in patients with adrenal insufficiency [DREAM]: a single-blind, randomised controlled trial. *Lancet Diabetes Endocrinol.* 2018;6:173–185.)

ACTH Excess

Causes

Ectopic ACTH production occurs in tumors capable of generating high amounts of the pituitary-like 1072-nucleotide mRNA. However, the clinical manifestation depends on whether PC enzymes are appropriately expressed. Small cell lung cancers preferentially release intact POMC, but carcinoid tumors tend to process the precursor, releasing ACTH and smaller peptides.[412] Extrapituitary neuroendocrine tumors associated with ectopic ACTH secretion do not process the prohormone efficiently because of a general defect in PC expression. As ACTH is synthesized in nontumorous neuroendocrine cells, ectopic tumor hormone production may in fact reflect inappropriate ACTH processing. These patients also exhibit a higher ratio of circulating ACTH precursors, as well as smaller peptides, including CLIP. Where sufficient ACTH or ACTH precursors that are biologically active are secreted, these tumors can cause florid morbidity from excessive cortisol production.

Clinical Features

ACTH-induced adrenal hyperfunction causes a syndrome of hypercortisolism and of androgen excess in women. Manifestations arise from appetite stimulation (weight gain), altered fat distribution (moon facies, buffalo hump, central obesity), catabolism (skin thinning, muscle wasting), mood disturbance (depression, anxiety), sodium retention (hypertension), and androgen excess (menstrual irregularity, hirsutism, acne, oily skin). The evaluation and management of patients with Cushing disease is fully described in Chapter 15.

Gonadotropins

Physiology

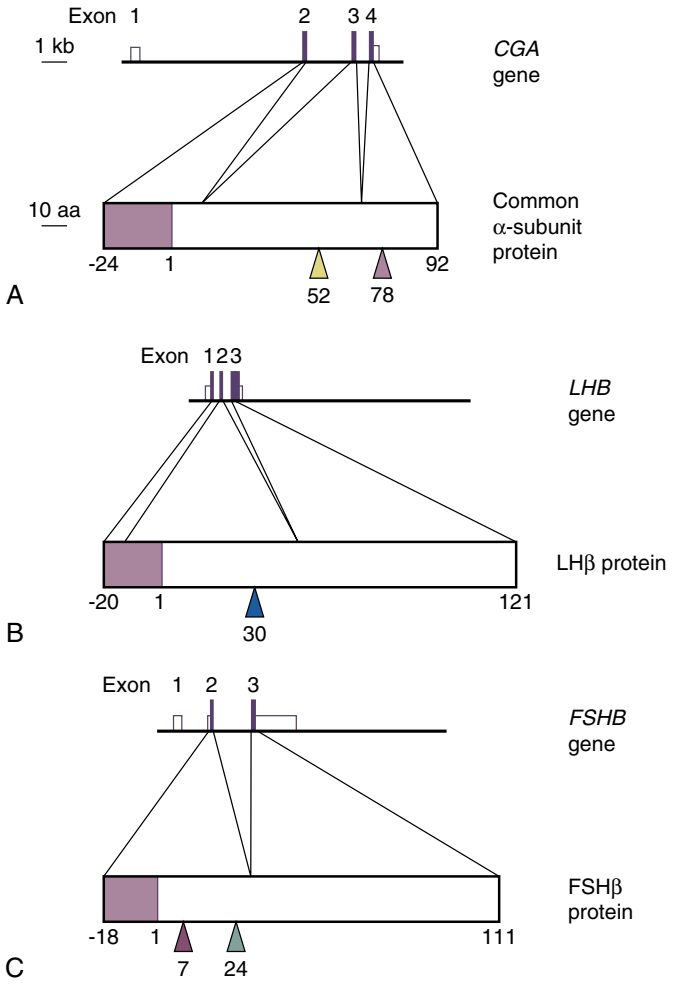
The gonadotropins, LH and FSH, are produced by pituitary gonadotrophs. These hormones play an essential role in the reproductive process. LH and FSH act on the ovaries and testes to direct gametogenesis and sex steroid hormone synthesis. Reproduction is a tightly regulated function, influenced by genetic, nutritional, environmental, and socioeconomic factors. Befitting their important roles, the synthesis and secretion of LH and FSH are under complex regulation by hypothalamic input (e.g., GnRH), by positive and negative feedback from gonadal sex steroid and peptide hormones, and by paracrine modulation from local factors produced within the pituitary gland itself (e.g., activins, inhibins, follistatin).[34]

Gonadotroph Cells

Gonadotroph cells comprise about 10% to 15% of the functional anterior pituitary cells. They are a heterogeneous cell population, with large round cell bodies with prominent rough endoplasmic reticulum and Golgi apparatus. Immunocytochemical studies have demonstrated the presence of both bihormonal and monohormonal groups of gonadotrophs. Cells with LH secretory granules often accumulate peripherally, and their Golgi structures may be less prominent. Gonadotrophs are characterized also by GnRH receptor expression and by SF1 and DAX1 nuclear receptors, which contribute to gonadotroph-specific gene expression.

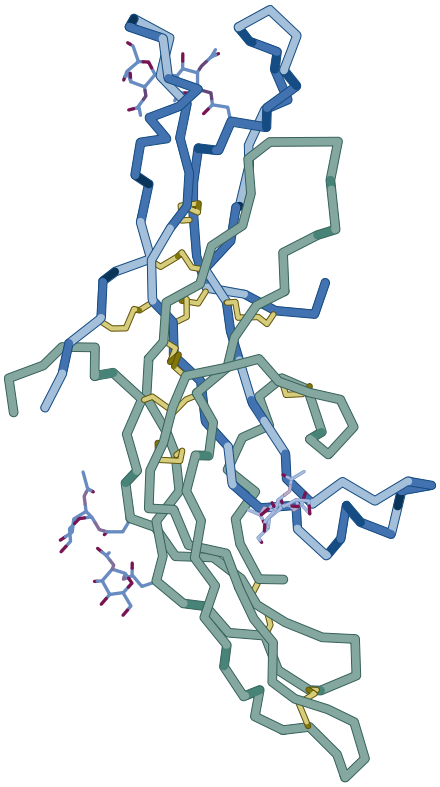
Gonadotropin Structure

FSH and LH function to regulate gonadal steroid hormone biosynthesis and initiate and maintain germ cell development in concert with peripheral hormones and paracrine soluble factors. The four heterodimeric glycoprotein hormones (LH, FSH, TSH, and human chorionic gonadotropin [hCG]) share structural homology, having evolved from a common ancestral gene. Although both the homologous LH and FSH molecules are cosecreted by gonadotrophs, their regulatory mechanisms are not uniformly concordant. The αGSU, LHβ, and FSHβ subunits are encoded by different genes, located on chromosomes 6, 11, and 19, respectively (Figs. 8.29 and 8.30; see also Fig. 8.2).[413] The heterodimeric structure of the common—and unique—subunits of LH and FSH is essential for biologic activity. Disulfide linkages within each subunit result in a tertiary structure that enables and maintains noncovalent heterodimerization, which also determines the ultrastructure of the mature folded molecule to facilitate specific ligand-receptor interaction.[414] Glycosylation of the subunits occurs by the transfer of oligosaccharide complexes to asparagine residues.[415] Post-translational processing of carbohydrate side chains is critical for hormone signaling, distinct for LH and FSH, and may even vary physiologically to influence

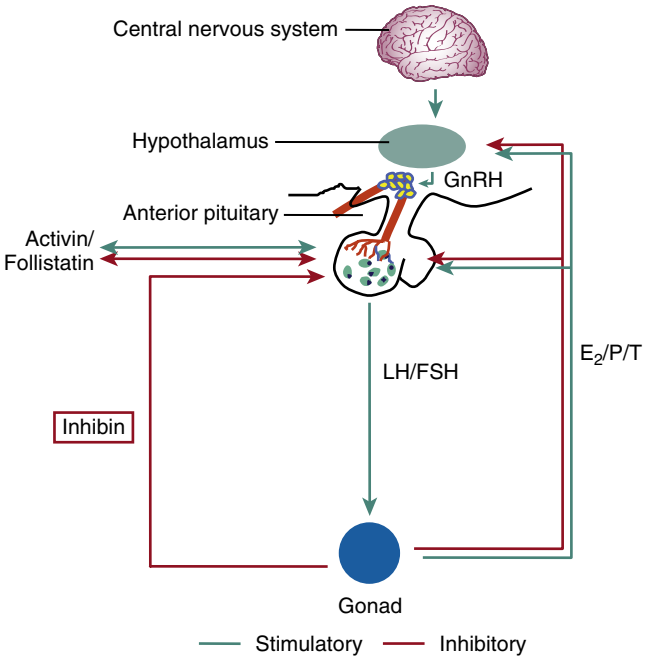


• **Fig. 8.29** Schematic representation of the human gonadotropin subunit genes. (A) Common α-subunit. (B) LHβ. (C) FSHβ. The *top part* of each scheme depicts the gene structure. *Open bars* indicate noncoding sequences; *solid bars* indicate coding sequences. The *bottom bar* of each scheme shows the protein structure. Signal peptides are *shaded;* the mature peptide is depicted by an *open bar*. The positions of N-linked glycosylation sites are depicted by *triangles*. Amino acid positions are depicted by *numbers*. (Redrawn and modified from Themmen APN, Huhtaniemi IT. Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary-gonadal function. *Endocr Rev*. 2000;21:551–583.)

biologic activity and metabolic clearance rates.[415,416] The human LHβ/CGβ gene cluster comprises seven genes, arising from gene duplication, of which one gene encodes LHβ, one encodes CGβ, and the remainder are pseudogenes. Unlike LH, hCG is present only in primate and equine species and is expressed primarily in the placenta. The LHβ and CGβ genes have different promoters and transcriptional start sites, accounting for their different tissue distribution patterns of expression.[417] LHβ includes a 24–amino acid signal peptide followed by a 121–amino acid mature protein. In contrast, the mature hCGβ protein is 145 amino acids in length and does not include a leader peptide, but it contains a 24–amino acid C-terminal extension important for the longer biologic half-life of hCG.[413,418] The FSHβ gene, on chromosome 11, is organized similarly to the other glycoprotein hormone β genes, encoding a mature peptide of 111 amino acids, with two glycosylation sites, and like LHβ, it is expressed only in gonadotrophs.



• **Fig. 8.30** The subunit structure and glycosylation sites of the four glycoprotein hormone heterodimers (α-subunit, *blue*; β-subunit, *green*). (From the University of Glasgow protein crystallography website. Available at http://www.chem.gla.ac.uk/protein/glyco/GPH.html.)



• **Fig. 8.31** The hypothalamic-pituitary-gonadal axis. See text for discussion. *E_2/P/T,* estrogen/progesterone/testosterone; *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone. (From Kaiser UB. Gonadotrophin hormones. In: Melmed S, ed. *The Pituitary.* 3rd ed. San Diego, CA: Elsevier; 2011:205–260.)

Regulation

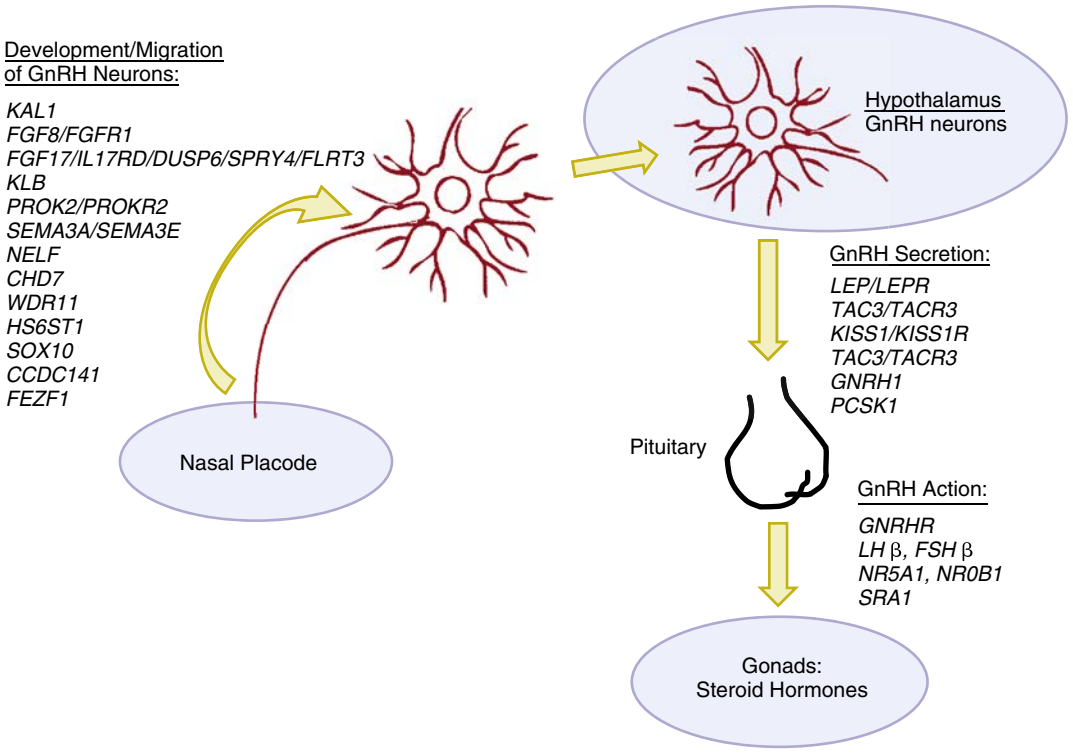
LH and FSH secretion patterns reflect the integration of sensitive complex hypothalamic signals (mediated primarily via GnRH), paracrine intrapituitary factors (primarily activin and follistatin), and peripheral feedback (both gonadal sex steroids and gonadal peptide hormones) (Fig. 8.31).

Gonadotropin-Releasing Hormone

Hypothalamic control of gonadotropin secretion occurs primarily through actions of GnRH. Hypothalamic GnRH neurons represent the pivotal integrators of peripheral signals in regulation of the pituitary-gonadal axis. Insights into the mechanisms that regulate GnRH secretion have been provided by the identification and study of genetic abnormalities in patients with pubertal disorders or infertility and by the use of animal models. Molecular defects that manifest as GnRH deficiency, or hypogonadotropic hypogonadism (HH), can be classified as defects in GnRH neuronal development, defects in control of GnRH secretion, and defects in GnRH action (Fig. 8.32; see Table 8.10). Conversely, genetic defects in *MKRN3* and *DLK1* were found to cause premature reactivation of GnRH secretion and central precocious puberty. *MKRN3* encodes a putative ubiquitin ligase and appears to act as a repressor or inhibitor of GnRH secretion during childhood.[419] *DLK1,* also known as preadipocyte factor 1, encodes a transmembrane noncanonical ligand in the Delta-Notch signaling pathway.[420]

Many neurotransmitters directly or indirectly modulate GnRH secretion, including norepinephrine, dopamine, serotonin, γ-aminobutyric acid (GABA), glutamate, opiates, NPY, and galanin, among others. Glutamate and norepinephrine provide stimulatory drive, whereas GABA and opioid peptides are inhibitory. Kisspeptins, encoded by the *KISS1* gene, and their cognate receptor, KISS1R, were identified as key GnRH secretagogues.[421,422] Administration of kisspeptin to normal men increases FSH, LH, and testosterone concentrations, and in normal women the peptide stimulates gonadotropin release most potently during the preovulatory surge.[423] Neurokinin B (NKB), a member of the substance P–related tachykinin family, is coexpressed with kisspeptin in the hypothalamus and appears to act through control of kisspeptin secretion to modulate GnRH release.[424,425] Substance P, another tachykinin family member, has also been shown to modulate GnRH secretion. Leptin, a product of peripheral adipose tissue, is a positive regulator of the hypothalamic-pituitary-gonadal (HPG) axis. This adipokine enables a pivotal link between body fat and reproduction, signaling energy availability centrally.[426] Nutritional, metabolic, stress, and circadian inputs all appear to act through these peptides to modulate GnRH, gonadotropin secretion, and activity of the HPG axis.[419]

The hallmark of hypothalamic GnRH secretion is the pulsatile rather than continuous release into the hypophyseal portal circulation, resulting in episodic stimulation of the gonadotroph.[427] In patients with GnRH deficiency, restoration of gonadotropin secretion can be achieved after exogenous pulsatile GnRH treatment, whereas continuous GnRH exposure suppresses gonadotropin secretion.[428] GnRH signaling initiates with recognition by its cognate receptor, GnRHR, which belongs to the rhodopsin G protein–coupled receptor family. GnRHR activation increases calcium mobilization and stimulates influx of extracellular calcium to induce pituitary LH and FSH secretion.[429] The pattern of GnRH signaling is important in determining the quantity and quality of gonadotropins secreted.[430,431] The amplitude, frequency, and contour of GnRH pulses can all vary, and each of these characteristics can influence gonadotroph responses, providing a mechanism for the differential synthesis and secretion of the two gonadotropins,



• **Fig. 8.32** Genetic and molecular basis of gonadotropin-releasing hormone (GnRH) neuronal development and migration, GnRH secretion, and GnRH action. (From Bianco SD, Kaiser UB. The genetic and molecular basis of idiopathic hypogonadotrophic hypogonadism. *Nat Rev Endocrinol.* 2009;5:569–576.)

LH and FSH. These alterations in GnRH pulse pattern are one mechanism by which two functionally distinct gonadotropins can be differentially regulated by a single hypothalamic-releasing hormone.

Inhibins and Activins

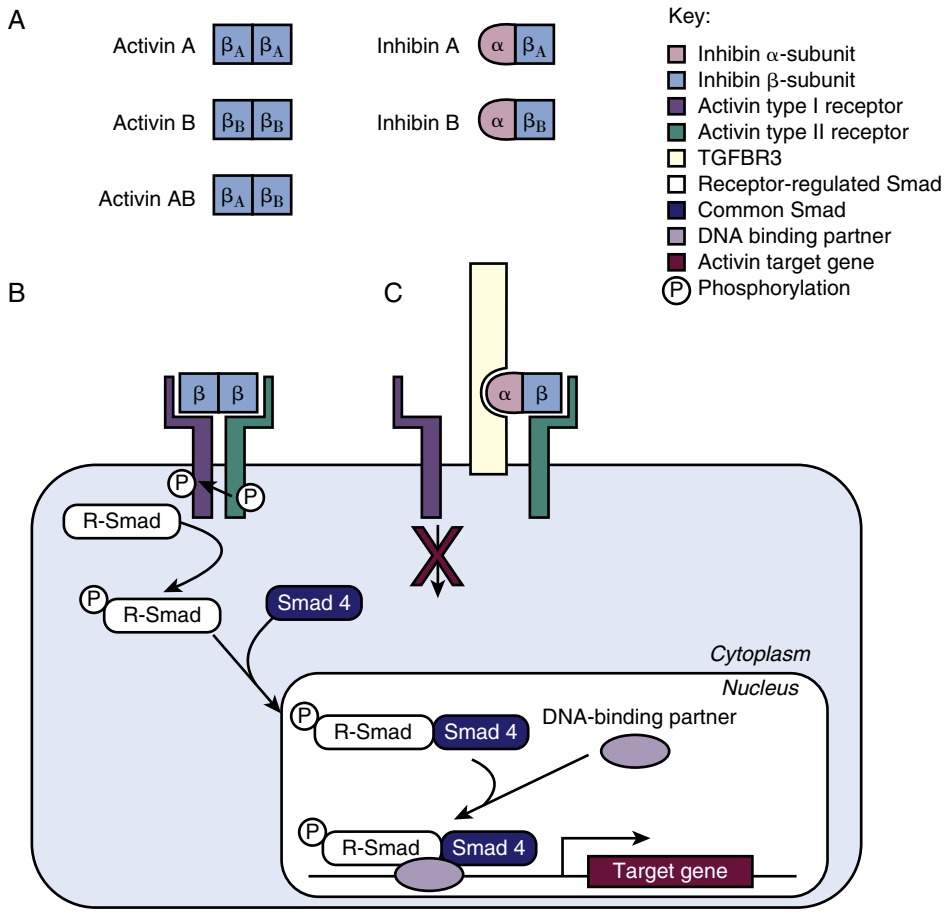
The hypothesis that a peptide of gonadal origin selectively regulates FSH secretion dates back to at least 1932.[432] It took over 50 years to isolate and characterize the structure of inhibin and its related peptides.[433] Inhibin-related peptides, members of the transforming growth factor-β family, are dimeric proteins covalently linked by a disulfide bridge and consisting of a common α-subunit and one of two highly homologous β-subunits, β_A or β_B (Fig. 8.33). In addition, the β-subunits can form dimers, called activins, to stimulate FSH synthesis and secretion.[434] A structurally unrelated, monomeric polypeptide, follistatin, was also identified, based on its ability to inhibit FSH.[434,435] These three peptides (inhibins, activins, and follistatin) are considered to be relatively selective for FSH in terms of their effects on gonadotropins and serve as an additional mechanism for the differential control of FSH and LH. Although inhibins act primarily as classic circulating endocrine hormones, originating in the gonads and acting on the pituitary to regulate FSH, activins play an important role as regulators of growth and differentiation in diverse tissues and are produced and act locally in the pituitary as autocrine/paracrine factors. In the human male, inhibin B is produced in the testes in response to FSH stimulation and circulates systemically to provide feedback inhibition of FSH. In women, inhibin A is secreted by dominant ovarian follicles and corpora lutea, contributing to the high circulating levels during the late follicular and luteal phases. Inhibin B is reciprocally elevated during the late luteal and early follicular phases of the menstrual cycle.

Activin receptors are heteromeric complexes comprising type I (ActRI) and type II (ActRII) serine-threonine kinase receptors. Activin binds to the type II receptors, thereby increasing association with the type I receptor and stimulating its phosphorylation, which in turn results in the activation by phosphorylation of intracellular signaling Smad proteins, resulting in translocation of the Smad complex to the nucleus, where it binds to gene regulatory elements and interacts with other transcription factors (such as FoxL2) to regulate gene transcription, thereby influencing cell fate and function.[34] Follistatin and inhibin act as extracellular modulators of activin through distinct mechanisms.[436] Follistatin, an activin-binding protein, inhibits activin action by interfering with activin binding to its receptor. Inhibins compete for binding to type II activin receptors, preventing recruitment of type I receptors and thereby blocking activin signaling. Additional extracellular and intracellular proteins and mechanisms also serve to modulate the local activin signal.

Sex Steroids

Gonadal steroid hormones include estrogens, progesterones, and androgens. Effects on gonadotropins occur both directly at the level of the gonadotroph and indirectly via effects at the hypothalamus that modulate GnRH secretion. Estrogen, androgen, and progesterone receptors have been identified in gonadotrophs, consistent with direct actions of these peripheral sex steroid hormones. Within the hypothalamus, these receptors have been identified in multiple neuronal cell types, suggesting that alterations in GnRH release largely occur indirectly through modulation of neuronal systems that impinge on GnRH neurons, particularly through kisspeptin neurons.[437,438]

In women, estrogens can exert dual feedback effects on gonadotropin secretion, depending on the reproductive state. The negative

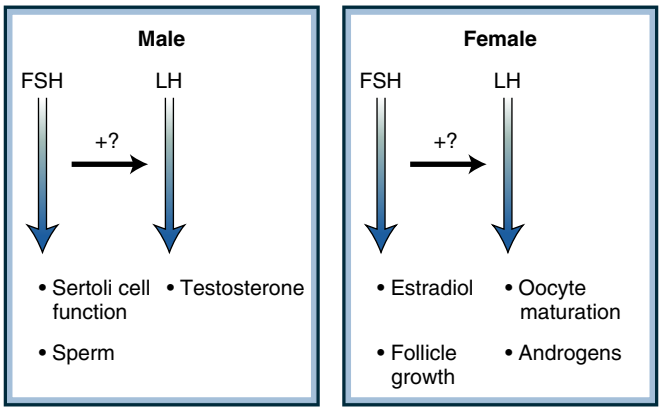


• **Fig. 8.33** Activins and inhibins and their mechanism of action. (A) Inhibins and activins are dimeric proteins made up of two subunits. Inhibins are made up of an α-subunit linked to one of two β-subunits, whereas activins are made up by dimerization of two β-subunits. (B) Activin signaling pathway. Activins bind to specific sets of serine–threonine kinase type I and type II receptors on the cell surface. Upon ligand binding, the type II receptor phosphorylates and thereby activates the type I receptor, which in turn phosphorylates downstream signaling molecules, the receptor-regulated S-Smads (R-Smads). Once phosphorylated, the R-Smads associate with the common co-Smad (Smad 4) and translocate to the nucleus where, in combination with cell type–specific binding partners, they bind to the promoter sequences of target genes to regulate gene transcription and cellular function. (C) Inhibins bind to activin type II receptors and block the recruitment of type I receptors, thereby blocking R-Smad activation and activin signaling. The presence of TGFBR3, a TGFβ superfamily accessory receptor also known as betaglycan, enhances the binding of inhibins to type II receptors, thereby enhancing the antagonistic actions of inhibins. (From Stenvers KL, Findlay JK. Inhibins: from reproductive hormones to tumor suppressors. *Trends Endocrinol Metab.* 2010;21:174–180.)

feedback effects of estrogens are clearly demonstrated by the elevated LH and FSH levels that follow ovariectomy or menopause but reverse with estrogen replacement. Negative feedback effects of estrogens are observed at the level of α-subunit and at LHβ and FSHβ mRNA levels through effects on gene transcription in addition to effects on LH and FSH secretion, mediated in part directly at the level of the pituitary gland. Estrogen also has negative feedback effects at the levels of the hypothalamus, mediated in large part through effects on kisspeptin neurons.[439] On the other hand, during the late follicular phase of the menstrual cycle, the feedback effects of estrogens shift from negative to positive, triggering the midcycle ovulatory surge of LH and FSH secretion. Positive feedback effects of estrogen are mediated in large part through effects on sexually dimorphic kisspeptin neurons in the anteroventral periventricular region of the hypothalamus in female mice.[440,441] Estrogens may also elicit direct positive effects at the pituitary level.

The principal effect of progesterone is to decrease the frequency of gonadotropin pulses, mediated by effects on GnRH pulse frequency. During the luteal phase of the human menstrual cycle, when progesterone concentrations are the highest, LH pulse frequency is markedly slowed.

Testosterone and its aromatized derivative estradiol are the two steroid hormones that exert negative feedback effects on gonadotropin secretion in the male. The net in vivo effect of testosterone administration to normal men is inhibition of serum LH and FSH levels. The available evidence suggests that 5α-reduction of testosterone is not essential for the inhibitory effects of testosterone on LH. Administration of a potent 5α-reductase inhibitor, finasteride, to normal men did not result in elevated LH and FSH levels.[442] Much like the effects of estrogen, these inhibitory effects are felt to occur largely at the hypothalamic level, by kisspeptin neurons, mediated by both androgen and estrogen receptors (AR



• **Fig. 8.34** Actions of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the male and female. The *plus signs* and *horizontal arrows* indicate potentially unrecognized new functions of FSH since the discovery of FSHβ gene mutations. (Modified from Layman LC. Genetics of human hypogonadotropic hypogonadism. *Am J Med Genet [Semin Med Genet].* 1999;89:240–248; Richards JS, Pangas SA. The ovary: basic biology and clinical implications. *J Clin Invest.* 2010;120:963–972; Bhasin S, Fisher CE, Sverdloff RS. Follicle-stimulating hormone and luteinizing hormone. In: Melmed S, ed. *The Pituitary.* 2nd ed. Malden, MA: Blackwell Science; 2002:216–278.)

and ERα). Mechanisms for testosterone feedback are complex, as testosterone also exerts a stimulatory effect on FSHβ mRNA levels.[443]

Secretion

In light of the episodic, pulsatile nature of LH and FSH secretion, there was a need to develop discrete pulse-detection algorithms. Santen and Bardin developed an algorithm that defined a peak as a 20% increase in the hormone concentration in a single sample over the preceding sample, which was subsequently modified to define the pulse based on a chosen multiple of the assay coefficient of variation. Because of its simplicity of use and apparent freedom from assumptions, this program is still widely used, although a number of refinements and alternative algorithms have also been developed. Application of deconvolution to pulse analysis made it possible to determine the instantaneous real-time secretory rates. The characteristic secretory episodes characterized for LH and FSH indicate daily production rates of 1000 IU and 200 IU, respectively, and a disappearance half-life of 90 minutes and 500 minutes for each respective β-subunit.[444,445] The longer circulating half-life of FSH makes the pulsatile secretory pattern of this gonadotropin less clear than that of LH, although differences in secretory pathways for FSH and LH also contribute.[446,446a]

Action

The primary targets of FSH and LH are the gonads, and thus targets and effects differ in the male and female (Fig. 8.34). The actions of FSH and LH in the male and female are presented briefly here but are discussed in greater detail in Chapters 17 and 19.

Female

FSH acts on FSH receptors in granulosa cells to facilitate follicular growth and estradiol biosynthesis (see Fig. 8.34).[447] The initiation of follicular growth can occur independently of gonadotropin stimulation, after which further maturation requires FSH. At these more advanced stages of development, follicles convert theca cell–derived androstenedione to estradiol (E_2) by the induction of aromatase activity in response to FSH.[448] FSH also controls granulosa cell production of inhibin during the follicular phase and induces LH receptor expression in granulosa cells of large preovulatory follicles. At the same time that FSH promotes the development of the dominant follicle, it also initiates the recruitment of the next generation of follicles that will enlarge during subsequent cycles.

LH, acting on LH receptors in ovarian theca cells, is a major regulator of ovarian steroid synthesis. LH stimulates estrogen production by promoting synthesis of androgen precursors in theca cells, which then diffuse into neighboring granulosa cells, where they are aromatized into estrogens under the control of FSH.[413] LH increases cholesterol availability for ovarian steroidogenesis by inducing the steroidogenic acute regulatory (StAR) protein,[449] which mediates cholesterol transfer from the outer to the inner membrane, where it becomes available for steroidogenesis. LH also enhances cytochrome P450–linked enzyme activity to synthesize pregnenolone and induces synthesis of 3β-hydroxysteroid dehydrogenase, 17α-hydroxylase, and 17,20-lyase. The midcycle LH surge stimulates resumption of oocyte meiosis and maturation in the preovulatory follicle, initiates the rupture of the ovulatory follicle and ovulation, and induces conversion of the follicle wall into the corpus luteum (luteinization).[447,448] LH stimulates the expression of progesterone receptors in the granulosa cells of the dominant follicle, which promotes luteinization. In addition, LH helps to sustain luteinization by stimulating progesterone synthesis.

Male

LH acts on LH receptors in Leydig cells to induce intratesticular testosterone synthesis, mediated by enhanced cAMP production. FSH in the male is involved in spermatogenesis (see Fig. 8.34), although the precise role in the spermatogenic process remains unclear.[450] FSH binds to FSH receptors on Sertoli cells and stimulates the production of inhibins, androgen-binding protein, androgen receptor, and other proteins. FSH mediates the maturation of spermatids into mature spermatozoa in concert with testosterone.[451]

Gonadotropin Measurements

Because of the high homology of the glycoprotein hormones, development of highly specific assays, especially those distinguishing free α-subunit from intact hormones, was necessary. Heterogeneity of circulating LH and FSH molecules, insufficient assay sensitivity to distinguish normal from low levels, and lack of rigorously pure reference preparations hampered assay development. Two-site–directed immunofluorometric and immunochemiluminescent LH and FSH assays have much improved sensitivity, are able to detect LH with a sensitivity of 0.1 mIU/mL, and have resolved prior challenges with cross-reactivity.[452] Differences in carbohydrate moieties result in isoelectric charge heterogeneity for LH, accounting for some observed disparities in biologic and immunoreactive LH ratios observed after GnRH agonist treatment, acute critical illness, or aging.

α-Subunit Assays

Both GnRH and TRH increase circulating levels of free α-subunit derived from either gonadotrophs or thyrotrophs, especially in patients with hypothyroidism, after castration, and during menopause. GnRH agonist treatment, TSH-secreting tumors, or nonfunctioning pituitary adenomas may result in discordant circulating ratios of free α-subunit from intact LH dimers.

GnRH Stimulation Test

A single bolus of GnRH (25–100 μg) dose-dependently evokes serum LH and FSH levels within 20 and 30 minutes. LH rises more abundantly than FSH, and peak values range from 8 to 34 mIU/mL. In contrast, patients with hypogonadotropic hypogonadism and no demonstrable hypothalamic-pituitary lesion have blunted LH responses and reversal of the LH:FSH ratio. The test, however, cannot adequately distinguish hypothalamic from pituitary lesions, and similar patterns are observed in patients with anorexia nervosa and hypothalamic amenorrhea. Repetitive GnRH pulses may in fact normalize responses, as would be expected from an intact hypothalamic-pituitary unit. GnRH responses may vary during the stages of puberty, reflecting altered pituitary sensitivity. This test is most frequently used in the assessment of pubertal status, for the diagnosis of hypogonadotropic hypogonadism or for evaluation of central precocious puberty.[453]

Clomiphene Stimulation Test

Clomiphene (100 mg), administered daily for up to 4 weeks, usually doubles LH levels, and FSH increases by about 50% over baseline. Because an abnormal or absent response does not distinguish hypothalamic from pituitary lesions, the utility of this test is limited.

Gonadotropin Deficiency

Causes

Gonadotropin deficiency may be congenital or acquired and may arise from hypothalamic or pituitary disorders. Congenital causes include gene mutations governing the processes of development and migration of GnRH neurons, the control of GnRH secretion, the development of the gonadotroph, or the regulation of LH and FSH secretion. Acquired HH may arise from functional or organic disorders. Functional causes are frequently encountered and include stress, malnutrition, chronic illness, depression, excessive exercise, and low body weight. Several centrally acting drugs, including opiates, glucocorticoids, sex steroids, GnRH agonists and antagonists, tranquilizers, antidepressants, and antipsychotic medications, suppress gonadotropin secretion either directly or indirectly via induction of hyperprolactinemia. Organic causes include malignant disease, developmental disorders, tumors, infiltrative disease, trauma, and hypothalamic or pituitary damage from surgery or radiotherapy. Congenital and acquired causes of central hypogonadism are considered more fully in Chapter 26.

Hypogonadotropic Hypogonadism. The genetic basis of HH was recognized over 60 years ago with the description by Kallmann of hypogonadism and anosmia in two families. It was not until 1991 that anosmin, a glycoprotein encoded by the *KAL1* gene, was identified as a cause of X-linked Kallmann syndrome.[454] The realization that *KAL1* gene defects only accounted for a small proportion of patients with classic Kallmann syndrome has led to genotype-phenotype studies that have provided important insights into the molecular genetics underlying HH. Gene mutations affecting the olfactory bulb and GnRH neuronal migration tend to give rise to the Kallmann phenotype. Multiple genetic defects have been associated with Kallmann syndrome, which in many cases can be distinguished clinically based on well-established nonreproductive phenotypes. On the other hand, defects in genes involved in regulating GnRH secretion or GnRH action are usually associated with normosmia, and in some cases the clinical phenotype is solely that of isolated HH (Table 8.10 provides details; see also Fig. 8.32).

Kallmann Syndrome. Kallmann syndrome consists of defective GnRH neuronal development, with olfactory nerve agenesis or hypoplasia and variable anosmia. Associated developmental disorders include optic atrophy, color blindness, sensorineural deafness, cleft palate, renal agenesis, cryptorchidism, and movement disorders.[455] This X-linked recessive disorder has been ascribed to a defective *KAL1* gene located on chromosome Xp22.3.[456] The encoded anosmin protein mediates hypothalamic migration of GnRH cells from the primitive olfactory placode, and its absence leads to defective GnRH synthesis and anosmia.[457,458] Both autosomal recessive and dominant forms of the disorder have since been described, with multiple additional genetic causes identified (see Table 8.10 and Fig. 8.32).

These patients are exposed to low or absent sex steroids from birth. Consequently, females are tall and present with primary amenorrhea and absent secondary sexual development, and males have delayed puberty and micropenis.[459] Absent GnRH secretory pulses result in characteristically low LH and FSH levels in the face of very low concentrations of estradiol or testosterone. Because the nonprimed normal pituitary may not respond initially to GnRH stimulation, this test is of little value in distinguishing a hypothalamic from a pituitary defect. In some patients, repetitive GnRH priming may elicit pituitary LH and FSH responses, indicating a hypothalamic defect in GnRH secretion.

Manifestations

Gonadotropin deficiency causes hypogonadism with decreased sex steroid production of varying degrees, depending upon the severity of the insult (Table 8.11). This disorder may occur at any stage of life. In its congenital form, primary amenorrhea and total absence of development of secondary sexual characteristics may occur. Onset later in life may present with a varying spectrum of reproductive dysfunction, ranging from luteal phase abnormalities with subfertility to oligomenorrhea or amenorrhea in women. Women exhibit secondary amenorrhea, infertility, decreased vaginal secretion, dyspareunia, hot flashes, decreased bone density, and breast tissue atrophy.

Males with congenital HH have small testes (<4 mL) and absence of secondary sexual characteristics. Tall stature with eunuchoid habitus may be present as a result of delayed or absent epiphyseal closure. Cryptorchidism and microphallus may be present, reflecting the absence of activity of the HPG axis during fetal development.[460] Men with gonadotropin deficiency later in life present with loss of libido, potency, and fertility. They may have impotence, testicular atrophy, decreased libido, low energy, infertility, loss of secondary sexual characteristics, decreased muscle strength and mass, decreased bone mass, decreased facial and body hair, and fine facial wrinkling.[461]

In both men and women, serum gonadotropin levels are inappropriately low in the face of decreased sex steroid levels. In women with amenorrhea or oligomenorrhea, serum LH, FSH, and estradiol levels should be measured. Endogenous estrogen sufficiency can also be assessed by the response to a progesterone challenge (100 mg intramuscularly or 10 mg medroxyprogesterone acetate orally daily for 5–10 days). Men should have serum LH, FSH, and testosterone levels measured in a morning sample.

Hypothalamic and pituitary disorders are the most common endocrine cause of male subfertility. Because normal secretion of both FSH and LH is required for quantitatively and qualitatively normal spermatogenesis, any disease that affects hypothalamic secretion of GnRH or pituitary secretion of FSH or LH will impair spermatogenesis.[462] As FSH is required for normal

TABLE 8.10 Genetics of Hypogonadotropic Hypogonadism

Phenotype/ Mechanism	Gene[a]	Inheritance	Function
Kallmann syndrome/neuronal development and migration	KAL1[454] KAL1[454]	X-linked	Anosmin required for cell surface signaling, adhesion, and migration
	FGFR1[561] FGFR1[561]	AD	Role in axonal development and guidance
	FGF8[562] FGF8[562]	AD	Endogenous ligand of FGFR1
	PROK2[563] PROK2[563]	AD, AR	Development of olfactory bulb and migration of GnRH neurons
	PROKR2[563] PROKR2[563]		
	NELF[564] NELF[564]	?	Encoding nasal embryonic LHRH factor
	SEMA3A, SEMA3E[460,565] SEMA3A[460]	AD	Axonal pathfinding
	HS6ST1[566] HS6ST1[566]	AD	Modifications of extracellular sugars
	CHD7[567] CHD7[567]	AD	Chromodomain helicase DNA binding protein 7, chromatin remodeling
	WDR11[568] WDR11[568]	AD	WD repeat domain protein interacts with transcription factor EMX1
	FGF17, IL17RD, DUSP6, SPRY4, FLRT3[569]		Components of fibroblast growth factor pathway
	KLB[570]	AD	FGF21/FGFR1 coreceptor
	SOX10[571]	AD	Transcription factor in olfactory ensheathing cells
	CCDC141[572]	AD	Coiled coil protein in GnRH neurons
	FEZF1[573]	AR	Protease
Normosmic hypogonadotropic hypogonadism/ GnRH synthesis, secretion, or action	KISS1[574] KISS1[574]	AR	Hypothalamic neuropeptide, stimulates GnRH secretion
	KISS1R[421,422] KISS1R[421,422]	AR	Receptor for kisspeptin
	TAC3[425] TAC3[425]	AR	Encodes neurokinin B, a neuropeptide that stimulates kisspeptin release
	TACR3[425] TACR3[425]	AR	Encodes neurokinin B receptor
	LEP[575] LEP[575]	AR	Encodes leptin, derived from adipose tissue to signal adequacy of nutritional status
	LEPR[575] LEPR[575]	AR	Encodes leptin receptor
	PCSK1[374] PCSK1[374]	AR	Processes GnRH
	GNRH1[535,576] GNRH1[535,576]	AR	Encodes GnRH
	GNRHR[421] GNRHR[421]	AR	Receptor for GnRH, stimulation of gonadotropin secretion

TABLE 8.10 Genetics of Hypogonadotropic Hypogonadism—cont'd

Phenotype/ Mechanism	Gene[a]	Inheritance	Function
	NR5A1	AD	Transcription factor for hypothalamic, pituitary, gonadal, and adrenal development
	NR0B1	X-linked	Transcriptional repressor; hypothalamic, pituitary, gonadal, and adrenal development
	SRA1[577]	AD	SF1 and nuclear receptor coregulator
	LHB	AR	Encodes luteinizing hormone β-subunit
	FSHB	AR	Encodes follicle-stimulating hormone β-subunit

[a]Superscript numbers refer to references at the end of this chapter.

AD, Autosomal dominant; *AR,* autosomal recessive; *FGF,* fibroblast growth factor; *GNRH,* gonadotropin-releasing hormone; *GPR,* orphan G protein–coupled receptor; *IHH,* isolated hypogonadotropic hypogonadism; *KAL,* Kallmann syndrome; *KISS,* KiSS metastasis suppressor; *LHRH,* luteinizing hormone–releasing hormone; *NELF,* nasal embryonic LHRH factor; *PROK,* prokineticin.

spermatogenesis, isolated FSH deficiency is associated with oligospermia or azoospermia. Men with inactivating FSHβ mutations are azoospermic, but some have normal puberty associated with normal to low-normal testosterone levels and high LH levels, whereas another presented with a low testosterone concentration and absent puberty.[463–465] Inactivating FSHβ mutations have also been characterized in women and have resulted in a phenotype of delayed puberty, absent or incomplete breast development, primary amenorrhea, and infertility, with low levels of estradiol and progesterone, undetectable FSH, and high LH.[463,464]

Mutations that abolish the activity of LH, resulting in isolated LH deficiency, have also been reported.[466,467] Isolated LH deficiency in males may manifest with delayed or absent puberty, with eunuchoidal body proportions as a result of low testosterone levels. Low LH levels in these patients lead to low intratesticular testosterone concentrations with resultant decreased spermatogenesis. A female with isolated LH deficiency due to an LH mutation had normal pubertal development and menarche but secondary amenorrhea and infertility.

Management

The goals of therapy are to replace or restore sex steroid hormones and to induce and maintain normal reproductive function. If fertility is not an immediate objective, sex steroid hormone replacement is usually sufficient. For induction of gametogenesis in those desiring fertility, therapy with gonadotropins or GnRH is usually required.

Evaluation. In evaluating hypogonadal patients in the absence of an obvious pituitary or gonadal disorder, the primary diagnostic challenge is to distinguish constitutional pubertal delay from other causes of hypogonadotropism.[341,468] When puberty is delayed after 14 years of age, a primary developmental disorder, HH, should be considered in the absence of acquired causes. Cryptorchidism or micropenis is suggestive of congenital HH, whereas low patient height relative to the parents' height suggests constitutional delay of puberty (CDP) rather than congenital HH, in which height tends to be normal or even increased, possibly with eunuchoidal proportions. No single test clearly distinguishes constitutional delayed puberty and true HH, and expectant follow-up is often helpful as many patients enter puberty spontaneously. To enable androgenization, testosterone replacement should be provided intermittently until age 18, with periodic interruptions to unmask physiologic pubertal advance.

TABLE 8.11 Clinical Features of Hypogonadotropism

Prepubertal Onset

High-pitched voice
Absent terminal facial hair
Decreased or absent body hair
Eunuchoidal body proportions
Female escutcheon
Testicular volume <6 cm^3
Testicular length <2.5 cm
Cryptorchidism may be present
Penile length <5 cm
Hypopigmented scrotum with absent rugae
Small prostate
Decreased libido
Decreased muscle and bone mass

Postpubertal Onset

Decreased libido
Decreased spontaneous erections
Slow beard growth
Decreased body hair
Testicular atrophy if long standing
Decreased muscle and bone mass

Normal

Voice pitch
Skeletal proportions
Penis length
Scrotal rugae
Prostate size

Sex Steroid Replacement Therapy. Estrogen or testosterone replacement is required for inducing and maintaining primary and secondary sexual characteristics and to maintain normal body composition and integrity of BMD and muscle mass. For patients not seeking fertility, sex steroid therapy is warranted to correct central hypogonadism. However, monitoring of LH and FSH responses does not accurately reflect adequate steroid hormonal replacement, as basal gonadotropin levels are already low or undetectable.

Estrogens are available as a tablet, patch, gel, or implant. Initiation of puberty can begin with any type or route of exogenous estrogen, oral or transdermal. Initial therapy should consist of estrogen alone to maximize breast growth and to induce uterine and endometrial proliferation. A progestin eventually needs to be added to prevent endometrial hyperplasia but should be avoided before completion of breast development, because it is likely to reduce ultimate breast size. In premenopausal women, oral estrogens or transdermal estradiol delivering 50 to 100 mg daily can be used, with concomitant cyclic progesterone therapy for women with an intact uterus to prevent unopposed endometrial proliferation. Although early sex steroid replacement lessens the risk of developing osteoporosis, effects of estrogen replacement on cardiovascular function are unresolved. In patients with hypopituitarism, estrogen replacement should be maintained until the age of 50, after which continuation should be determined on an individual basis by assessing risks and benefits, especially in terms of bone mineral integrity, cardiovascular function, and cancer risk. Estrogen treatment may be associated with thromboembolic disease, breast tenderness, and possibly enhanced risk for breast cancer.

For men, androgen replacement is available as intramuscular, gel, patch, implants, nasal, or oral preparations. Intramuscular injection of testosterone enanthate or testosterone cypionate is usually administered at doses of 200 to 300 mg every 2 or 3 weeks.[461] Administration of lower doses on a more frequent basis (e.g., 100 mg weekly) may stabilize fluctuations of hormone levels. Elderly males require lower doses, as do boys with delayed puberty. Testosterone undecanoate provides long-term replacement for 3 to 4 months after each injection with improved pharmacokinetic profiles, but rare cases of pulmonary oil microembolism and anaphylaxis have been reported; it must be administered in an office or hospital setting by a trained health care provider, and the patient should be monitored for 30 minutes afterward for adverse reactions. Transdermal testosterone patch and gel systems deliver 4 to 6 mg and sustained testosterone profiles. Patch sites may develop skin irritation, blisters, and vesicles in approximately 25% of patients.[469] Oral androgen replacement therapy is generally not recommended because of nonuniform absorption and hepatotoxicity. Nasal testosterone is a recently approved alternative with a shorter half-life, administered three times daily[470]; at the other end of the spectrum, longer acting implantable testosterone pellets are available. Testosterone may cause acne, gynecomastia, prostatic hypertrophy, and polycythemia. Although there is no compelling evidence that testosterone replacement causes prostate cancer, benign prostatic hypertrophy can be exacerbated, especially in elderly patients. Testosterone replacement should not be administered to men with diagnosed prostate cancer. Target serum hormone levels, which benefit lean mass, muscle strength, and sexual function without inducing undesirable adverse consequences, vary, and in the future, individual targets may guide the treatment of hypogonadism in men.[471]

Fertility. In patients with HH, fertility may be achieved with gonadotropin or GnRH therapy. In males, even relatively low sperm counts may be adequate for impregnation when fertility is induced by gonadotropins or GnRH. As testosterone therapy may suppress spermatogenesis, the steroid should be discontinued prior to initiating treatment. Partial rather than complete hypogonadism predicts a more favorable response to treatment, whereas persistence of cryptorchidism beyond the age of 1 year reduces the likelihood of successful fertility induction. hCG is administered subcutaneously or intramuscularly (1000–2000 IU two to three times weekly) to induce spermatogenesis, with the dose titrated according to testosterone levels.[472] If necessary, after 6 months, human menopausal gonadotropin (hMG) or purified or recombinant FSH should be added to improve sperm quantity, and doses may be doubled after a further 6 months. An increase in testicular volume correlates well with induction of spermatogenesis. If testosterone levels are increased, subsequent conversion to estradiol may result. Therefore both testosterone and estradiol levels should be monitored.

Pulsatile GnRH therapy is an alternative treatment for patients with normal pituitary function (i.e., those with idiopathic HH or Kallmann syndrome). GnRH is infused subcutaneously by continuous minipump (5 mg every 2 hours), with the dose titrated to maintain normal gonadotropin and testosterone levels. This approach may be marginally more effective than treatment with gonadotropins and may cause less gynecomastia. These approaches require strong patient commitment, as adequate spermatogenesis may not be attained for 2 years or longer despite normalized testosterone levels. Aliquots of successfully generated sperm samples should be frozen for future impregnation.

Clomiphene citrate is a weak estrogen receptor antagonist that stimulates gonadotropin secretion in normal women and men. Clomiphene has been used to increase spermatogenesis in men with partial hypogonadotropism with oligospermia or azoospermia and normal to mildly low serum testosterone concentrations, particularly in men with functional hypogonadism, with variable results.

In women with HH, fertility may be effectively achieved by pulsatile GnRH administration or by gonadotropin therapy (fully discussed in Chapters 17 and 26). Although ovulation is often induced and pregnancy achieved by gonadotropin treatment, a high rate of multiple follicle development remains a concern.[473] If residual pituitary gonadotroph reserve is sufficiently robust, GnRH therapy is more likely to result in ovulation of a single rather than multiple follicles, thereby reducing the chances of multiple gestation.[474] Therapy with kisspeptin or kisspeptin analogues is an area of current investigation and holds promise for further reducing the chances of multiple gestation or the risks of ovarian hyperstimulation[475,475a–475c] (see Chapter 17).

Thyroid-Stimulating Hormone

Physiology

The hypothalamic-pituitary-thyroid system plays a critical role in development, growth, and cellular metabolism through the action of thyroid hormones.

Thyrotroph Cells

Thyrotroph cells comprise approximately 5% of the functional anterior pituitary cells and are situated predominantly in the anteromedial areas of the gland. They are smaller than the other cell types, irregularly shaped with flattened nuclei and relatively small secretory granules, ranging from 120 to 150 μm, and they contain TSH that is released under TRH stimulation. Immunoreactive TSH cells are present in the fetal pituitary by 12 weeks. The differentiation of thyrotrophs is critically dependent on the expression of transcription factors GATA2, POU1F1, and PROP1 during development.

Structure

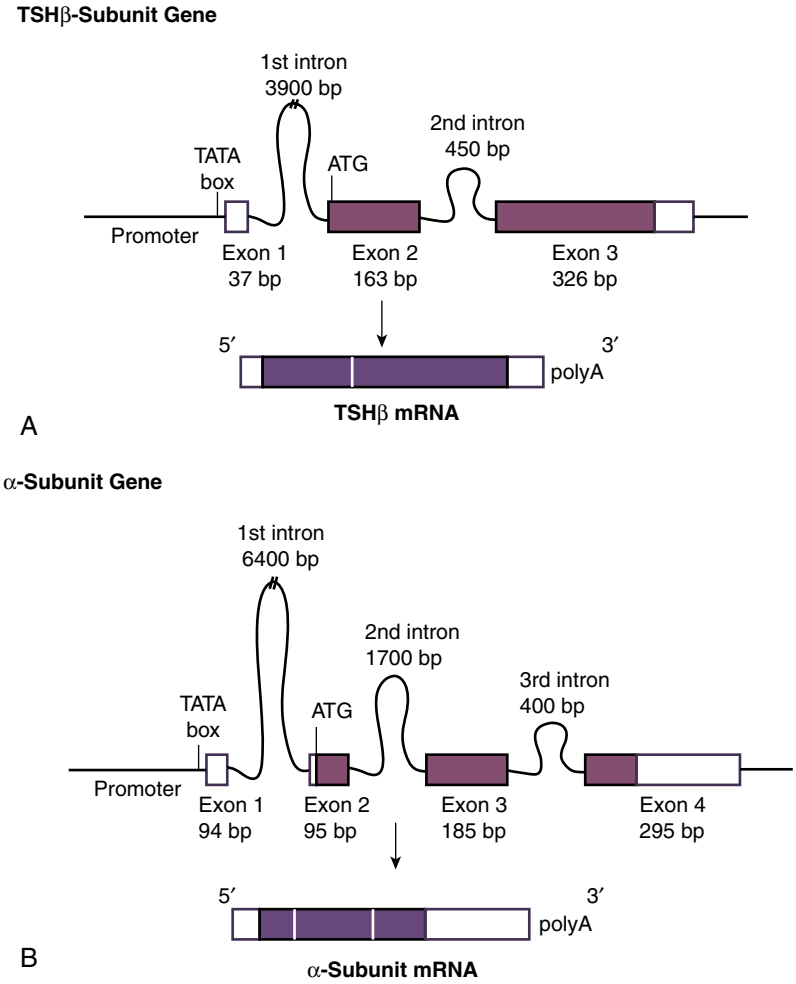
TSH is a glycoprotein hormone comprising a 28-kDa heterodimer of two noncovalently linked subunits (α and β).[476,477] The α-subunit and β-subunit are encoded by two separate genes located

on different chromosomes (Fig. 8.35). The tertiary TSH structure comprises three hairpin loops separated by central disulfide bonds, with the longer loop straddling one side.[477–479] The α-subunit is common to TSH, LH, FSH, and hCG, whereas the β-subunit is unique and confers specificity of action.[477] The α-subunit is the earliest hormone gene expressed embryonically, but activation of the β-subunit gene occurs later under the influence of GATA2 and POUF1F1.[480] The 13.5-kb α-subunit gene is located on chromosome 6 and comprises four exons and three introns (see Fig. 8.35).[481] Although the α-subunit gene is expressed in thyrotroph, gonadotroph, and placental cells, its regulation is uniquely cell specific. The downstream promoter region (−200 and below) is required for placental expression, intermediate sequences are required for gonadotroph expression, and upstream promoter elements are required for thyrotroph-specific expression.[482] The 4.9-kb TSH β-subunit gene located on chromosome 1 comprises three exons and two introns (see Fig. 8.35).[483] PIT1 binds directly to the gene promoter to confer tissue-specific expression.[484]

Production of the mature heterodimeric TSH molecule requires complex cotranslational glycosylation and folding of nascent α-subunit and β-subunit.[477] After subunit translation and signal peptide cleavage, glycosylation occurs at asparagine 23 on the β-subunit and at two asparagine residues, 52 and 78, on the α-subunit.[485] Appropriate glycosylation is required for accurate molecular folding and subsequent combination of α-subunit and β-subunit within the rough endoplasmic reticulum and Golgi apparatus.

Regulation

α-Subunit transcription is inhibited by the thyroid hormone receptor (TR) in concert with other nuclear corepressors.[486] TSHβ gene transcription is also suppressed by the TR acting directly on exon 1.[487] This potent suppression is evident within 30 minutes of T_3 exposure and is a critical determinant of ultimate TSH secretion. Both αTSH and βTSH subunit gene transcription are induced by TRH and suppressed by dopamine.[488] Both TRH and T_3 regulate TSH glycosylation, albeit in opposite directions.

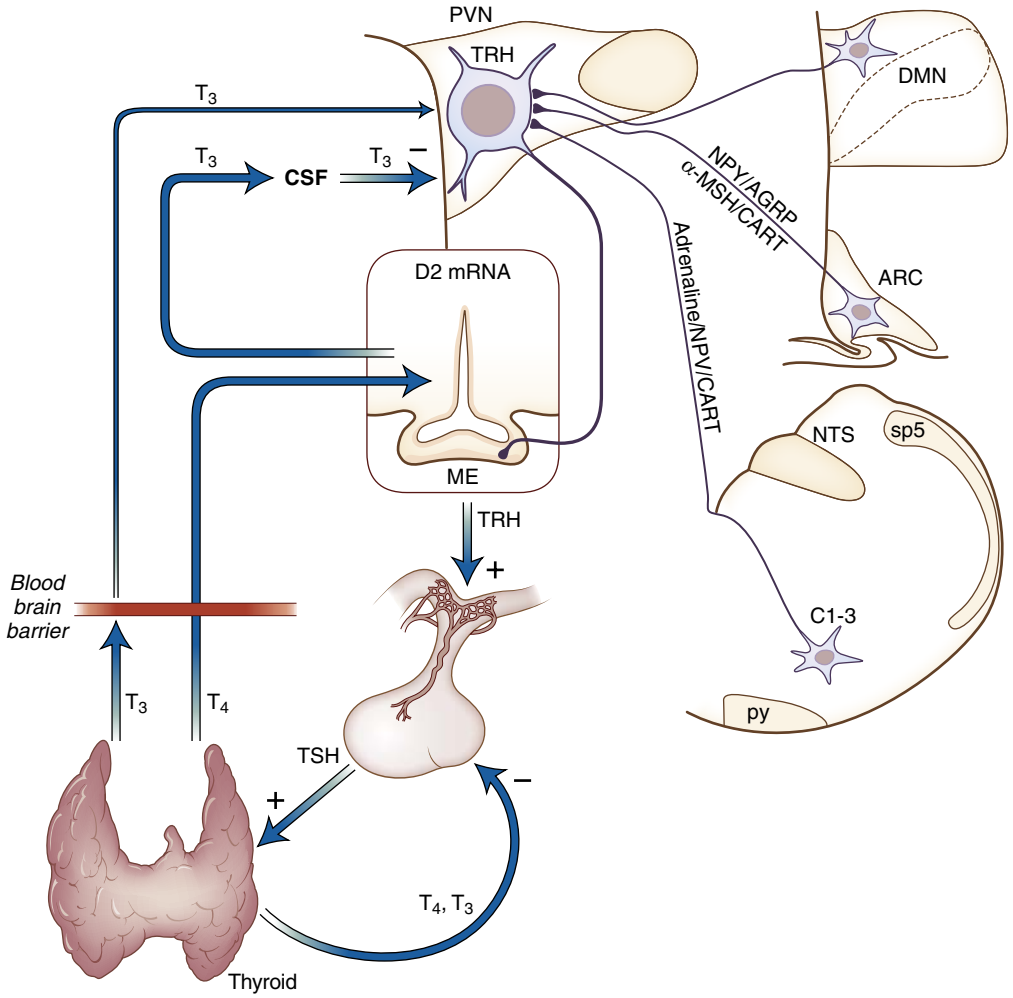


• **Fig. 8.35** Schematic representation of the TSHβ (A) and the α-subunit (B) genes. The relative locations and sizes of the exons and introns are shown within translated regions shown as *open boxes* and protein coding regions as *black boxes*. The TATA box position in the transcriptional start site is located in the promoter close to exon 1. The introns are spliced out during transcription and exons joined and a polyA tail added at the 3′ end. (From Gordon DF, Sarapura VD, Samuels MH, et al. Thyroid stimulating hormone: physiology and secretion. In Jameson JL, De Groot LJ, eds. *Endocrinology: Adult and Pediatric.* 6th ed. Philadelphia: Elsevier; 2010:1362–1383.)

TRH exposure or T_3 deprivation enhances oligosaccharide addition to the TSH molecule.[488,489]

The TRH neuron plays a central role in determining the set-point of the hypothalamic-pituitary-thyroid axis by regulating pituitary TSH release.[490] Hypothalamic TRH synthesis is in turn regulated by thyroid hormones. Three main neuronal groups mediate the effects of other physiologic stimuli on hypothalamic TRH neurons, which are located in the paraventricular nucleus. The first is adrenergic input from the medulla that mediates the stimulatory effects of cold exposure on the TRH neuron (Fig. 8.36). Second, TRH neurons receive projections from the arcuate nucleus that contain two leptin-responsive groups regulating energy homeostasis: The POMC system that promotes weight loss activates while the NPY/agouti-related protein (AGRP) system that promotes weight gain inhibits TRH neurons.[491] Fasting results in reduction of TRH expression, which is mediated by suppression of the POMC system and stimulation of the NPY/AGRP system. Third, the hypothalamic dorsomedial nucleus projects to the paraventricular nucleus and represents alternative pathways by which leptin acts to regulate TRH neurons.[491,492]

Feedback regulation by thyroid hormones on TRH and TSH are determined primarily by circulating thyroxine (T_4) entry into the blood-brain barrier elaborated through a complex system of paracrine control in the hypothalamus (see Fig. 8.36). The effects of thyroid hormones are mediated by thyroid hormone receptors, which are members of a superfamily of nuclear hormone receptors. TRs exist as two major isoforms, TRα and TRβ. TRα is the key TR isoform responsible for T_3-mediated negative-feedback



• **Fig. 8.36** Schematic illustration of the feedback system regulating the hypothalamic-pituitary-thyroid axis. Thyroid hormones exert negative feedback effect at the level of the pituitary and hypophysiotropic TRH neurons. The central feedback effect of thyroid hormones primarily depends on the circulating T_4 levels. In the hypothalamus, T_4 is converted to T_3 by deiodinase 2 (D2) in tanycytes. By volume transmission, T_3 secreted from tanycytes reaches the hypophysiotropic TRH neurons, where T_3 inhibits the proTRH gene expression via TRβ2 receptors. The set-point of the feedback regulation can be altered by two mechanisms: (1) regulation of D2 activity in tanycytes and (2) neuronal afferents altering CREB concentration in the hypophysiotropic TRH neurons. *ARC,* hypothalamic arcuate nucleus; *C1-3,* C1-3 adrenergic area of the brainstem; *CSF,* cerebrospinal fluid; *DMN,* hypothalamic dorsomedial nucleus; *ME,* median eminence; *NTS,* nucleus tractus solitarius; *PVN,* hypothalamic paraventricular nucleus; *py,* pyramidal tract; *sp5,* spinal trigeminal tract. (Redrawn from Fekete C, Lechan RM. Negative feedback regulation of hypophysiotropic thyrotropin-releasing hormone (TRH) synthesizing neurons: role of neuronal afferents and type 2 deiodinase. *Front Neuroendocrinol*. 2007;28:97–114.)

regulation by hypophysiotropic TRH neurons.[478] The local availability of T_3 is determined by deiodinase 2 and deiodinase 3, respectively, which provides and deactivates T_3.[490]

Deiodinase 2 is expressed in surrounding glial cells of the hypothalamus[491,493] and in tanycytes (lining the third ventricles), which generate T_3 from circulating T_4. Tanycytes appear to be the main contributor to the negative feedback regulation of the hypothalamic-pituitary-thyroid axis.[490] The expression by the TRH neuron of deiodinase 3, which inactivates T_3, points to the existence of an important local level of TRH regulation. T_3 suppresses hypothalamic TRH synthesis and decreases pituitary TRH receptor number.

In the anterior pituitary gland, deiodinase 2 is found in folliculostellate cells, whereas TRs and deiodinase 3 are expressed in thyrotrophs.[491,493] These findings indicate an important role for folliculostellate cells in processing and activating T_4. Production and action of local T_3 occur in separate cell types of the hypothalamus and anterior pituitary gland resulting in setting the level of TSH output.

Transporters of thyroid hormone play a key role in regulating the TRH neuron. The two most important transporter families that are involved in thyroid hormone transport in the brain are the organic anion transporting polypeptide (OATP) and the monocarboxylate transporter (MCT).[490] Among these, OATP14 is highly expressed in the paraventricular nucleus and OATP8 in brain neurons.[494] In humans, mutations in the MCT8 gene, located on the X chromosome, result in males with neurologic abnormalities with elevated T_3 levels and decreased T_4 levels in the presence of a normal TSH secretion.[495]

Secretion

Daily TSH production is approximately 100 to 400 mU[496] with a calculated circulating half-life of approximately 50 minutes. Secretion rates are enhanced up to 15-fold in hypothyroidism and are suppressed in hyperthyroidism. The degree of TSH glycosylation determines both metabolic clearance rate and bioactivity; in hypothyroidism, the molecule appears highly sialylated, enhancing bioactivity.[485] Immunoreactive fetal pituitary TSH is detectable by 12 weeks. Immediately after full-term birth, there is a brisk rise in TSH, which remains elevated for up to 5 days before stabilizing at adult levels.[497] TSH secretion is pulsatile; however, the low pulse amplitudes and long TSH half-life result in modest circulating variances that are amplified in hypothyroidism and abrogated in critical illness.[498] Secretory pulses every 2 to 3 hours are interspersed with periods of tonic, nonpulsatile TSH secretion.[499] Circadian TSH secretion peaks between 11 PM and 5 AM, mainly due to increased pulse amplitude that is not sleep entrained.[500] The 24-hour TSH secretion is stable and robust and not influenced by sex, body mass index, or age.[501] Thyroid hormones suppress tonic TSH secretion and pulse amplitude but not frequency. Free TSHα is secreted but not TSHβ.

Regulatory Factors

Several factors and drugs affect the secretion of TSH (Table 8.12). Somatostatin infusion inhibits TSH pulse amplitude and blocks the nocturnal TSH surge[502] directly at the pituitary level. Although SRIF analogues are used to treat TSH-secreting pituitary adenomas (see later), SRIF analogue treatment for acromegaly does not lead to hypothyroidism, although T_4 is lowered within the normal range. Dopamine infusions suppress TSH secretion and abrogate the nocturnal TSH surge.[502] Prolonged use of dopamine agonists, however, does not result in hypothyroidism. Glucocorticoids suppress TSH secretion; however, the effect does not result in hypothyroidism. Proinflammatory cytokines such as TNFα and IL1 inhibit TSH secretion.[503] Leptin increases TSH secretion from central stimulation of TRH. Acute opiate administration causes a mild transient increase of TSH levels.

A number of drugs such as nonsteroidal anti-inflammatory agents (e.g., meclofenamate, fenclofenac) displace thyroid hormone from thyroxine-binding globulin (TBG), increasing free hormone concentrations and thus causing transient decrease of TSH.[504]

TABLE 8.12 Factors Affecting the Secretion of TSH

Effect	Factors
Stimulatory	TRH Opioids Dopamine receptor antagonists Leptin
Inhibitory	Thyroid hormone Somatostatin analogues Dopamine agonists Proinflammatory cytokines Glucocorticoids

Action

TSH induces thyroid hormone synthesis and releases and maintains trophic thyroid cell integrity.[505] The TSH G protein–coupled receptor is located on the thyrocyte plasma membrane and is encoded by a gene on chromosome 11q31. Its regulation is comprehensively described in Chapters 11 and 12.

TSH Assays

The availability of ultrasensitive TSH measurements has revolutionized the biochemical and diagnostic evaluation of thyroid disorders. This is due to the widespread availability of immunoradiometric assays. The assay principle is based on TSH serving as a link between an immobilized antibody binding TSH at one epitope and a labeled (radioactive, chemiluminescent, or colorimetric) monoclonal directed against a second portion of the peptide. This technology substantially increases sensitivity and specificity with technical improvements leading to sequential generations of assays of higher sensitivity. Current commercial assays are mainly "third generation" with detection limits to 0.01 to 0.02 mU/L performed on automated platforms. They provide ready discrimination between hyperthyroid, euthyroid, and hyperthyroid states.[506,507] A major consequence of ultrasensitive TSH assay is the obsolescence of the TRH test for patients suspected of thyrotoxicosis. TSH measurement alone is not helpful in diagnosing central hypothyroidism, which is identified by concurrent measurement of thyroid hormone levels. Basal TSH levels are subnormal in only about one-third of patients with secondary hypothyroidism.[508] TSH deficiency is thus associated with low T_4 levels concomitant with low, normal, or even minimally elevated TSH levels. Importantly, this biochemical profile may also be encountered in critically ill patients with low TSH and T_4 levels without evidence of pituitary disease. The TRH test does not add to the diagnosis of central hypothyroidism or the sick-euthyroid state.[509] In a stimulation test, TRH (200–500 μg) is administered intravenously, and TSH levels are measured at –15, 0, 15, 30, 60, and 120 minutes. In euthyroid subjects,

peak TSH levels (up to 22-fold higher than basal) are observed after 30 minutes.[507] Because feedback suppression by elevated thyroid hormone levels on TSH overrides stimulation by the hypothalamus, basal TSH levels are undetectable and do rise to TRH in hyperthyroidism. The TRH test has a useful role in differentiating hyperthyroidism due to a TSH-secreting adenoma and elevated thyroid hormone levels due to thyroid hormone resistance. The TSH levels do not increase in response to TRH in most cases of TSH adenomas, but they do in thyroid hormone resistance.[509]

TSH Deficiency

Causes

Congenital isolated TSH deficiency may arise from mutational defects of the TSH and TRH receptor genes. Genetic disorders of pituitary gland development involved in cell differentiation give rise to TSH deficiency as a component of multiple pituitary hormone deficiencies. The developmental genes involved include *LHX3*, *PROP1*, and *POU1F1* (see Table 8.2). Pituitary damage may result in functional TSH deficiency.

Manifestations

The consequences of TSH deficiency are those of thyroid hormone deficiency, which causes childhood mental and growth retardation; in adults, it results in clinical features similar to primary hypothyroidism.

Treatment

L-Thyroxine is used for replacement therapy, in doses similar to those required for treating primary hypothyroidism ranging from 50 to 200 μg/day. Thyroxine is converted peripherally into the active T_3 and has a 7-day half-life with stable blood levels. There is strong evidence that patients with central hypothyroidism are frequently undertreated; it has been recommended that the dose be titrated to achieve midnormal free T_4 levels.[510] TSH cannot be used to guide dose titration in patients with secondary hypothyroidism because the damaged thyrotroph is unlikely to adequately reflect appropriate feedback suppression. As GH stimulates the conversion of T_4 to T_3, blood T_4 levels may fall into the subnormal range, unmasking central hypothyroidism during GH therapy for GH deficiency.[277,278] The principles of thyroid hormone replacement apply in this situation and T_4 supplementation is initiated. As many women with pituitary failure also likely receive estrogen replacement, free instead of total T_4 level is measured to avoid the confounding effects of increased TBG levels. Thyroid hormone replacement accelerates cortisol metabolism and requirements and may therefore exacerbate primary hypoadrenalism or precipitate adrenal crisis in patients with coexisting perturbed adrenal function. Therefore, in pituitary patients suspected of having ACTH deficiency, thyroid hormone replacement should only be initiated after adrenal status has been evaluated and treated.

TSH Excess

Other than primary hypothyroidism, TSH excess can rarely arise from TSH-secreting pituitary adenomas, thyroid hormone resistance, and TSH receptor resistance. The biochemical profiles of TSH adenoma and thyroid hormone resistance are similar, both conditions manifesting high TSH and thyroid hormone levels; however, the two are usually distinguished from familial history, imaging, clinical examination, and TRH testing.[508] Thyroid hormone resistance can arise from mutations of TRα and TRβ, with the latter being more common. TSH receptor mutations were first described in 1995 as a cause of congenital hypothyroidism.[511] However, mutations causing partial resistance may manifest as nonautoimmune subclinical hypothyroidism.[512]

Developmental, Genetic, and Acquired Causes of Pituitary Failure

Developmental Disorders

Congenital pituitary gland absence (aplasia), partial hypoplasia, or ectopic tissue rudiments are rarely encountered. Pituitary development follows midline cell migration from Rathke pouch, and impaired midline anomalies, including failed forebrain cleavage and anterior commissure and corpus collosum defects, lead to structural pituitary anomalies. Craniofacial developmental anomalies, including anencephaly, result in cleft lip and palate, basal encephalocele, hypertelorism, and optic nerve hypoplasia with varying degrees of pituitary dysplasia and aplasia. If these infants survive, lifelong appropriate pituitary hormone replacement is required. With sensitive MRI techniques for pituitary visualization, several anatomic features characteristic of hypopituitarism are now apparent. Evidence for acquired pituitary damage or destruction is often clearly visible on MRI, and patients presenting with hypopituitarism of undetermined cause may exhibit decreased gland volume, partial or complete empty sella, disturbed sella turcica architecture, absent or transected pituitary stalk, and an absent or ectopic posterior pituitary bright intensity signal.[513] Lesions of the pituitary stalk can arise from congenital maldevelopment causing stalk interruption (see later) or from acquired diseases involving the infundibulum. Among 92 patients with pituitary stalk lesions, 32% were found incidentally. About 15% were due to congenital causes (ectopic posterior pituitary, Rathke cyst), 33% to inflammatory causes (sarcoidosis, histiocytosis, hypophysitis), and over 50% to neoplastic causes (craniopharyngioma, pituitary adenoma, metastatic disease).[514] An absent infundibulum noted on MRI is associated with pituitary hormone deficits, and approximately 40% of patients with GHD of unclear cause show imaging evidence of mild stalk defects, reflecting a midline developmental anomaly.

Congenital basal encephalocele may result in the pituitary herniating through the sphenoid sinus roof, resulting in pituitary failure and diabetes insipidus.

Heritable Disorders

Mutations at each level of pituitary function, including hormones, receptors, and transcription factors that determine anterior pituitary development, may lead to pituitary deficiency syndromes[515] (see Table 8.1). Furthermore, mutations in specific pituitary genes, including those for GH, POMC, TSH, LH, and FSH, all lead to single hormone deficiencies. Patients heretofore diagnosed with idiopathic isolated or polyhormonal pituitary failure may in fact harbor a mutation, and as the transcriptional control of pituitary development is clarified, increasing numbers of mutant genes have become apparent (see Table 8.2).

HESX1, SOX2, SOX3, and OTX2

HESX1 (also known as RPX), one of the earliest transcriptional markers of the primitive pituitary, with expression restricted

to Rathke pouch, is a paired-like homeodomain transcription factor that acts as a transcriptional repressor.[516] Coincidentally, with appearance of specific pituitary cell types, HESX1 expression declines and is extinguished in the mature anterior pituitary,[517] leading to PROP1 activation. The heterogeneous syndrome of septo-optic dysplasia (hypoplastic optic nerves, absent corpus callosum and septum pellucidum, and hypopituitarism) is associated with mutations in HESX1.[518] Hypopituitarism ranges from panhypopituitarism to isolated GHD. Although the mutant molecule exhibits reduced DNA binding, panhypopituitarism may also occur secondary to profound anatomic defects in midline development. Mutations in HESX1 have also been associated with pituitary stalk interruption syndrome (PSIS), a congenital defect of the pituitary gland characterized by a very thin, interrupted pituitary stalk, an ectopic or absent posterior pituitary gland, and hypoplasia or aplasia of the anterior pituitary gland, visible on MRI.[519] Mutated SOX2, a member of the SRY-related high mobility group box (Sox) genes, is also associated with septo-optic dysplasia, anophthalmia, or microphthalmia, and anterior pituitary hypoplasia, frequently associated with HH and GHD, and other forebrain defects.[520] SOX3 mutations are associated with X-linked hypopituitarism and mental retardation. Affected males have GHD and may also have deficient gonadotropins, TSH, or ACTH. Both SOX3 duplications and loss of function mutations show similar phenotypes, suggesting that SOX3 dosage is critical for normal pituitary development.[521,522]

Mutations in OTX2 have also been implicated in anophthalmia/microphthalmia syndromes in humans. These mutations are associated with severe ocular and neurologic phenotypes, including developmental delay and seizures. Panhypopituitarism occurs, perhaps as a result of failure to activate transcription of HESX1 and POU1F1.[522]

LHX3 and LHX4

LHX3 is expressed early during anterior pituitary development, with strong uniform expression within Rathke pouch. Expression persists into adulthood. Missense and deletion mutations of LHX3, a LIM-type homeodomain transcription factor essential for pituitary development, are associated with failure of pituitary gland morphogenesis with reduced numbers of all cell types and multiple anterior pituitary hormone deficits, affecting all axes except for largely intact ACTH reserve.[436] Most missense mutations identified in patients have diminished capacity to activate transcription of the promoters of several potential LHX3 target genes, including those encoding αGSU, PRL, FSHβ, TSHβ, and POU1F1.[523] These patients also exhibit defective neck rotation ability due to a rigid cervical spine and variable sensorineural hearing loss.[524]

LHX4 is closely related to LHX3 and is also expressed throughout the invaginating Rathke pouch, but unlike LHX3, its expression is transient and not maintained in the adult pituitary. Patients with LHX4 mutations exhibit GHD and associated short stature, with variable additional endocrine deficits, particularly TSH and ACTH deficiency, and extrapituitary abnormalities. LHX4 mutations associated with anterior pituitary hypoplasia, an ectopic posterior pituitary, and with PSIS are unable to activate both PROP1 and POU1F1, and these result in pituitary failure.[522,525]

PITX1 and PITX2

PITX1 and PITX2, members of the class of bicoid homeodomain proteins, show a high degree of homology and are expressed in an overlapping pattern during pituitary development. PITX1 is expressed in all five anterior pituitary lineages in both the fetal and adult pituitary gland and is able to activate the expression of all six of the major anterior pituitary hormones, including LH and FSH, frequently acting in synergy with other pituitary transcription factors. Mutations in PITX2 cause Rieger syndrome, characterized by defects in the eyes and teeth and a protuberant umbilicus as well as pituitary hormone deficiencies.[27,28,526]

PROP1

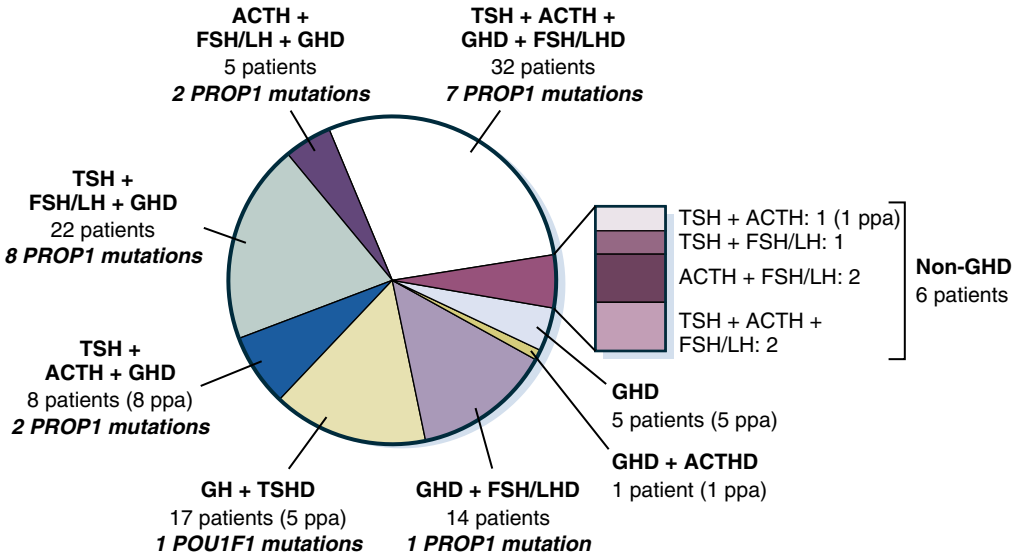
Mutations in PROP1 are the most common genetic cause of combined pituitary hormone deficiency (CPHD) (Fig. 8.37).[527] The role of this gene was first uncovered from studies of the Ames dwarf mouse, which harbors a missense PROP1 mutation and exhibits a hypoplastic pituitary gland with combined GH, PRL, and TSH deficiency. This mutation abrogates POU1F1 activation and results in failed development of POU1F1-dependent cell lineages.[528] Similarly, human PROP1 mutations are associated with deficiencies in POU1F1-dependent lineages (GH, PRL, and TSH). Impaired FSH and LH secretion, associated with delayed or absent puberty, HH, and infertility in females and in some males, is also frequently present.[529] ACTH deficiency can also occur, frequently with a later onset, suggesting a role in maintenance of corticotroph function and emphasizing the necessity for complete and continued clinical assessment of patients with PROP1 mutations.[263] Inheritance modes of PROP1 mutations usually reflect autosomal recessive patterns. Thus patients are usually homozygous for either deletion or missense frameshift mutations, leading to truncated PROP1 protein products devoid of functional activity.

The clinical spectra of CPHD associated with PROP1 mutations are variable and temporal. The phenotype varies with both the type of mutation and the age of the patient.[522] The onset of clinically evident pituitary failure is usually first manifest with slowing of linear growth (GHD, ~80%), then thyroid failure (TSH deficiency, ~20%), followed by hypogonadism, and later subclinical or overt adrenal insufficiency.[530] The pituitary gland size is usually small or normal. Combined hypothalamic hormone stimulation (GnRH, TRH, CRH, and GHRH) or insulin-evoked hypoglycemia reveals blunted responses consistent with varying degrees of pituitary hormone deficiencies. Serum IGF1 and IGFBP3 levels are usually low, but peripheral thyroid hormone levels are low or at the lower limits of normal ranges. In the face of low or absent TSH responses, these findings are consistent with secondary hypothyroidism. Most older patients also exhibit blunted cortisol responses to CRH and ACTH or insulin stimulation.[529]

POU1F1

The POU1F1 gene encodes a POU homeobox protein, PIT1, which activates transcription of the GH, PRL, TSHβ, and GHRH receptor genes.[531] PIT1 also partners with coactivators, including thyroid hormone, estrogen, and retinoic acid receptors, as well as other transcription factors, including CREB, LHX3, PITX1, HESX1, and ZN15. PIT1 autoregulates its own expression. Because of the absolute requirement of PIT1 for somatotroph, lactotroph, and thyrotroph development and specific gene expression, inactivating mutations of the gene result in a spectrum of pituitary hormone deficiencies.[532] The Snell and Jackson dwarf mouse strains both harbor POU1F1 gene mutations.[533] Both autosomal recessive (loss of function) and autosomal dominant (dominant negative action) POU1F1 mutations have been identified.

Some POU1F1 mutations exhibit characteristic clinical phenotypes, depending on the spectrum of loss of DNA binding,



• **Fig. 8.37** Pituitary hormone deficiencies repartition in 110 unrelated patients affected by congenital pituitary deficiency without stalk pituitary interruption or septo-optic dysplasia. Patients were studied for *PROP1*, *POUF1*, or *LHX3* according to hormonal deficit phenotype. Twenty mutations of *PROP1* and one mutation of *POU1F1* were found. Gonadotroph function was unavailable for prepubertal age *(ppa)* patients. *ACTH,* adrenocorticotropic hormone; *ACTHD,* ACTH deficiency; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GHD,* growth hormone deficiency; *LH,* luteinizing hormone; *LHD,* luteinizing hormone deficiency; *TSH,* thyroid-stimulating hormone; *TSHD,* thyroid-stimulating hormone deficiency. (From Reynaud R, Gueydan M, Saveanu A, et al. Genetic screening of combined pituitary hormone deficiency: experience in 195 patients. *J Clin Endocrinol Metab.* 2006;91:3326–3329.)

transcriptional activation, or interaction with partner proteins affected.[320,534] Both CBP/p300 protein recruitment as well as PIT1 dimerization are required for appropriate PIT1 activation of target hormone genes.[535] LHX4 appears to activate POU1F1, and mutations of LHX4 also lead to growth retardation.[525] Interestingly, adult-onset combined GH, PRL, and TSH deficiencies have also been reported in association with circulating autoantibodies directed against the PIT1 protein.[536]

IGSF1

Loss-of-function mutations have been identified in *IGSF1* in association with X-linked congenital central hypothyroidism, often in association with variable PRL deficiency and GHD and with testicular enlargement.[167] IGSF1 is a membrane glycoprotein highly expressed in the anterior pituitary gland, and the patients with mutations appear to have impaired pituitary TRH signaling.

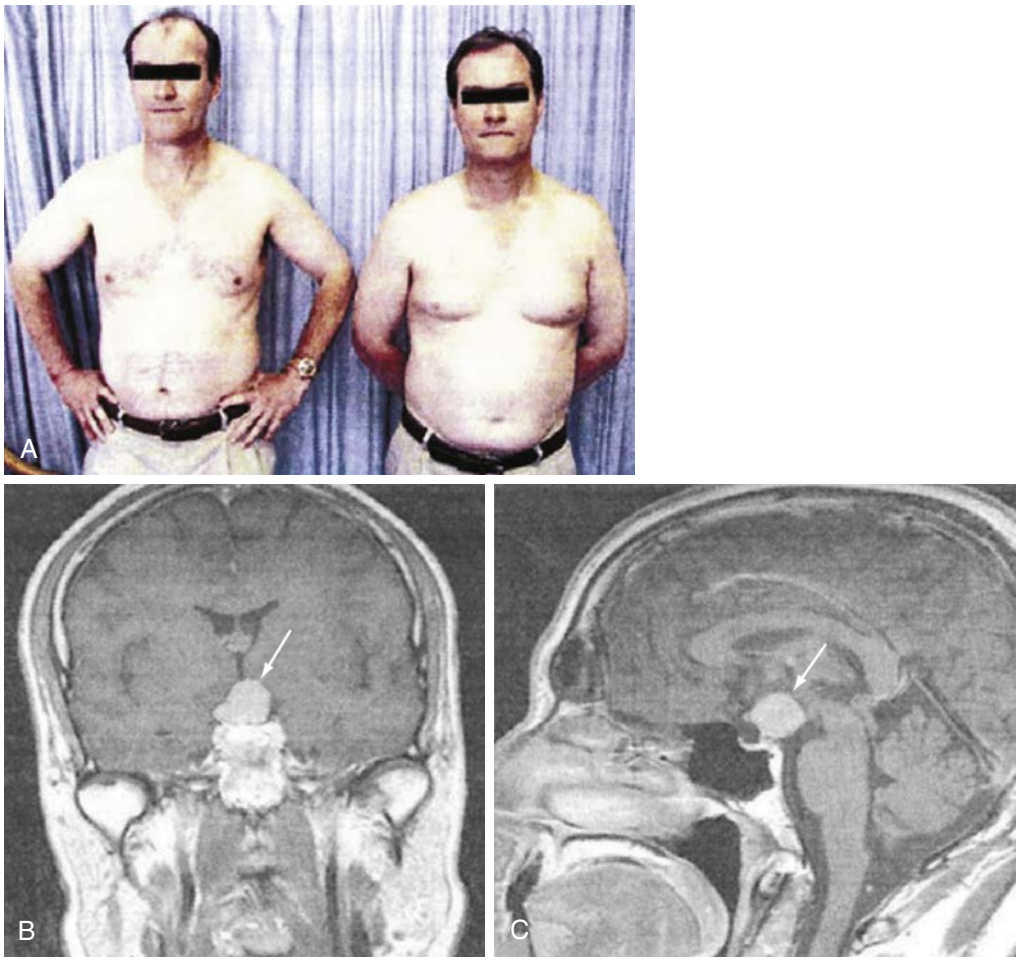
TBX19

TBX19 (also referred to as TPIT) mutations result in early-onset isolated ACTH deficiency and hypocortisolism[38] (see Table 8.2). Associated phenotypes, including those for POMC deficiency, may include obesity, red hair pigmentation, and other associated pituitary deficiencies. Patients are homozygous or compound heterozygous for TBX19 mutations, indicating an autosomal recessive mode of inheritance. Mutations in this gene appear to be the principal molecular cause of congenital neonatal isolated ACTH deficiency.[537] Interestingly, among 22 patients with isolated ACTH deficiency, diagnosed between ages 5 and 15 years, and no identified mutation of TBX19, three had common variable immunodeficiency (CVID), characterized by defective immunoglobulin production and recurrent infections, diagnosed at age 2 to 8 years. This has led to the proposal of a new syndrome linking these two rare disorders: deficient anterior pituitary function and variable immune deficiency (DAVID).[538] NFKB2, encoding a subunit of the transcription factor complex of nuclear factor-kappa-B, has recently been implicated as the transcription factor responsible for this syndrome.[539]

NR5A1 and NR0B1

NR5A1 encodes SF1, a member of the nuclear receptor family expressed throughout the reproductive axis (hypothalamus, pituitary, and gonads) and in the adrenal gland. It is a key transcriptional regulator of many genes involved in sexual differentiation, steroidogenesis, and reproduction, including the pituitary genes encoding αGSU, LHβ, FSHβ, and GnRHR. Patients with mutations in SF1 have been described with varying degrees of XY sex reversal, testicular dysgenesis, ovarian insufficiency, adrenal failure, and impaired pubertal maturation with HH.[61,540,541]

NR0B1 encodes DAX1 (*d*osage-sensitive sex-reversal *a*drenal hypoplasia critical region on the *X* chromosome protein *1*), a nuclear receptor transcription factor related to SF1, with a similar distribution pattern of expression. Mutations in NR0B1 are associated with X-linked HH and adrenal hypoplasia congenita,[31] with the majority of mutations causing truncations or frameshifts rendering the protein nonfunctional. HH is often mild, revealing itself as failure to undergo puberty or as incomplete puberty. The hypogonadism appears to be due to variable and combined hypothalamic and pituitary dysfunction. DAX1 is a transcriptional repressor and has been shown to inhibit SF1-mediated transcription of an array of target genes, including LHβ. How the loss of function in these two opposing genes, SF1 and DAX1, results in similar phenotypes is still not well understood. The HH is likely due to a developmental defect of the hypothalamus and pituitary, suggesting a role for DAX1 in proper development of these organs.[542]



• **Fig. 8.38** Features of hypopituitarism and hypogonadism, including central adiposity, proximal muscle wasting, loss of body hair, and gynecomastia. Note the contrast between the hypogonadal patient (A, *right side*) and his unaffected identical twin *(left side)*. Laboratory tests confirmed secondary hypogonadism, with testosterone level of 0.4 ng/mL (normal range, 2.9–8.0 ng/mL), follicle-stimulating hormone level of 2.8 IU/L (normal range, 1.5–12.4 IU/L), and luteinizing hormone level of 1.5 IU/L (normal range, 1.7–8.6 IU/L). Magnetic resonance imaging showed a lobulated, contrast-enhancing suprasellar mass (coronal view in B *[arrow]* and sagittal view in C *[arrow]*). Pathologic analysis confirmed the diagnosis of pituicytoma. (From Newnham HH, Rivera-Woll LM. Images in clinical medicine: hypogonadism due to pituicytoma in an identical twin. *N Engl J Med.* 2008;359:2824.)

Pituitary Stalk Interruption Syndrome

PSIS is a congenital defect of the pituitary gland characterized by a thin or interrupted pituitary stalk, anterior pituitary hypoplasia, and an ectopic posterior pituitary. Patients may present with an isolated pituitary hormone deficiency or with combined hypothalamic-pituitary hormone deficiencies. Accompanying midline defects and eye abnormalities suggest involvement of developmental processes. Mutations or single nucleotide variants in HESX1, LHX4, OTX2, SOX3, and PROKR2 have been associated with PSIS. A homozygous missense mutation was identified in a family with PSIS in GPR161, an orphan G protein–coupled receptor expressed in the hypothalamus and pituitary and implicated in the SHH signaling pathway.[543] A homozygous frameshift mutation in ARNT2, or aryl hydrocarbon receptor nuclear translocator 2, a helix-loop-helix transcription factor, has also been associated with anterior pituitary hypoplasia and pituitary stalk interruption, together with other CNS defects.[544] Mutations have been identified in less than 5% of patients with PSIS, suggesting that there is much more to be learned about the pathogenesis of this disorder.

Pituitary deficiencies have also been reported recently in patients with other syndromic disorders, including patients with CHARGE syndrome and CHD7 mutations; patients with PAX6 mutations, a well-known regulator of eye development; patients with septo-optic dysplasia or Kallmann syndrome and PROKR2, FGF8, and FGFR1 variants; and patients with holoprosencephaly and variants in SHH, GLI2, or TGIF.[539]

Acquired Disorders

In the absence of demonstrable hypothalamic-pituitary anatomic damage, and after excluding genetic and syndromic causes of pituitary insufficiencies, acquired, often transient, causes of pituitary failure should be considered (see Table 8.5). Causes of pituitary insufficiency, including pituitary tumors, parasellar masses, hypophysitis, aneurysms, and pituitary apoplexy, were discussed earlier. Hypothalamic damage reflected by the presence of a large parasellar mass, leading to decreased GnRH production, results in muscle wasting, obesity, and central hypogonadism with low levels of FSH and LH (Fig. 8.38). Marked caloric restriction, anorexia,[545] weight loss of other causes, and strenuous exercise may attenuate GnRH secretion and action. HH may occur in both men and women

TABLE 8.13 Hypopituitarism After Subarachnoid Hemorrhage

Reference	Number of Patients	Any Degree of Hypopituitarism (*n*)	Multiple Deficiencies (*n*)	GH (*n*)	LH/FSH (*n*)	ACTH (*n*)	TSH (*n*)	Remarks
Kelly et al, 2000[578]	2	2	0	2	0	0	0	
Brandt et al, 2004[579]	10	5	0	1	4	0	0	
Aimaretti et al, 2004[580]	40	15	4	10	5	1	3	No stimulation test for ACTH
Kreitschmann-Andermahr et al, 2004[581]	40	22	3	8	0	16	1	
Dimopoulou et al, 2004[582]	30	14	4	11	4	3	2	No stimulation test for GH (11 patients low IGF1)
Total number (%)	122 (100)	58 (48)	11 (9)	32 (26)	13 (11)	20 (16)	6 (5)	

ACTH, Adrenocorticotropic hormone; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *IGF1,* insulin-like growth factor 1; *LH,* luteinizing hormone; *TSH,* thyroid-stimulating hormone.

Modified from Schneider H, Aimaretti G, Kreitschmann-Andermahr I, et al. Hypopituitarism. *Lancet.* 2007;369:1461–1470.

(see Chapters 17 and 19). Exogenous anabolic steroid therapy and glucocorticoid therapy suppress the reproductive and adrenal axes, respectively. Patients with severe critical illnesses or chronic debilitating disease (including cirrhosis) may have impaired GH/IGF1, adrenal, and gonadal axes. Hypothyroidism, hypoadrenalism, or hypogonadism cause hyperplasia of specific trophic cells due to lack of negative feedback and sometimes actual pituitary tumor formation.[546] Acquired immunodeficiency syndrome (AIDS) is associated with suppressed pituitary function that is independent of other associated infections.[547] Drugs such as estrogens, which suppress FSH and LH, and GnRH analogues used for treating prostate cancer inhibit gonadotropin action. In addition to pituitary apoplexy, other vascular accidents such as aneurysms, strokes, cavernous sinus thrombosis, and arteritis can cause pituitary hormone insufficiency. Isolated hormone pituitary hormone deficiencies may also occur as a manifestation of vascular abnormalities, including arteritis or subarachnoid hemorrhage (Table 8.13).

Head Trauma

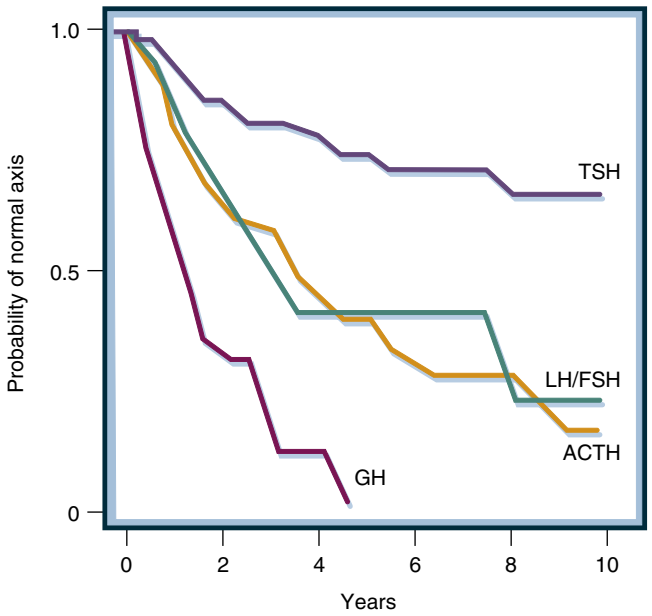
The pituitary may be partially or totally damaged by birth trauma, cranial hemorrhage, fetal asphyxia, or breech delivery. Head trauma may lead to direct pituitary damage by a sella turcica fracture, pituitary stalk section, trauma-induced vasospasm, or ischemic infarction following blunt trauma.[548] The most common traumatic cause of compromised pituitary function in the adult is iatrogenic neurosurgical trauma. Pituitary manipulation or damage during surgery leads to transient or permanent diabetes insipidus and varying degrees of anterior pituitary dysfunction. Hypopituitarism following head trauma usually manifests within a year after the insult. Seventy-five percent of patients with posttraumatic pituitary failure are young men under age 40 years involved in a motor vehicle accident. Virtually all patients with subsequent pituitary failure have a history of loss of consciousness following trauma, and half of all such patients have documented skull fracture.[548] One-third of these patients have demonstrable signs of hypothalamic or posterior pituitary hemorrhage or anterior lobe infarction on MRI. Diabetes insipidus is the most common endocrine disorder, encountered in about 30% of these patients.[549] As pituitary function can recover after trauma, the prevalence and extent of dysfunction vary and depend on the time of evaluation. A meta-analysis of more than 700 adult patients in 2007 reported that up to 35% of subjects acquired some impairment to pituitary function evaluated at least 5 months after major TBI.[550] GHD was found in 11%, gonadotropin deficiency in 13%, ACTH deficiency in 11%, TSH deficiency in 6%, and multiple deficits in 9%. There are sparse data of the pituitary consequences of TBI in children. A large prospective French study of 87 children, mean age of 6.7 years, reported a prevalence of 7% for GHD, 2% for thyroid, and 1% adrenal insufficiency evaluated 5 months after TBI.[551]

Klose and associates have questioned the accuracy of the frequency of adult GHD from previous studies because of methodologic issues arising from the use of different diagnostic tests, assays, and criteria based on published guidelines.[552] Using normative data drawn from over 100 healthy control subjects, the authors observed that the prevalence of GHD among 439 patients with TBI was highly dependent on choice of local or guideline-derived cutoffs, on diagnostic tests, and on the use of either single or confirmatory testing. They reported a prevalence of GHD of 4% with the ITT and 12% with the combined pyridostigmine-GHRH test and of isolated GHD in just 1% confirmed from these two tests. The nonspecific symptomology of hypopituitarism and the usually profound and variable CNS consequences of mixed brain and physical injuries pose a major challenge to the identification of pituitary dysfunction and its management. The level of evidence supporting who and when to investigate is low because endocrinopathy is one component of a much larger complex clinical problem. In the acute situation, assessment should focus on the HPA axis because of the vital role glucocorticoids play in the stress response. As pituitary impairment may recover, there is merit in deferring the assessment of the other axes to a time when the vital systems are stable.

Radiation

The progression and degree of hypopituitarism are dose and time dependent with pituitary exposure. Susceptibility is also

dependent on the age of irradiation.[553,554] The GH axis is the most radiosensitive, followed by the gonadotropin, adrenocorticotropic hormone (ACTH), and TSH axes[555,556] (Fig. 8.39). GH deficiency can develop with doses as low as 18 Gy with higher doses (30–50 Gy) that can reach 50% to 100% within 3 to 5 years. Gonadotrophin deficiency occurs with doses in excess of 30 Gy; however, lower doses in children cause precocious puberty. Pituitary irradiation induces hyperprolactinemia, a result of hypothalamic damage. This develops with exposure to 40 Gy or higher and occurs in both sexes in all age groups. Table 8.14 summarizes the conditions for which radiation is given, the exposure to the pituitary gland, and the impact on pituitary function.[557] Pituitary irradiation indicated for pituitary adenoma therapy will directly cause atrophy of the gland, in addition to damaging hypothalamic neurons.[554] Early anterior-pituitary dysfunction seems less likely after stereotactic radiosurgery for pituitary adenomas than after fractionated radiotherapy; however, the long-term, cumulative incidence of anterior-pituitary dysfunction is similar.[553] Previously irradiated patients should therefore undergo lifelong periodic anterior pituitary hormone testing to unmask incipient pituitary failure prior to onset of morbidity.[558]



• **Fig. 8.39** Life-table analysis indicating probabilities of initially normal hypothalamic-pituitary-target gland axes remaining normal after radiotherapy (3750–4250 cGy). Growth hormone (GH) secretion is the most sensitive of the anterior pituitary hormones to the effects of external radiotherapy, and thyroid-stimulating hormone (TSH) secretion is the most resistant. In two-thirds of patients, gonadotropin deficiency develops before adrenocorticotropic hormone (ACTH) deficiency. The reverse occurs in the remaining third. *FSH,* follicle-stimulating hormone, *LH,* lutenizing hormone. (From Littley MD, Shalet SM, Beardwell CG, et al. Hypopituitarism following external radiotherapy for pituitary tumors in adults. *Q J Med.* 1989;70:145–160.)

Empty Sella Syndrome

Damage to the sellar diaphragm may lead to arachnoid herniation into the sellar space. An empty sella may develop as a consequence of a primary congenital weakness of the diaphragm in those patients in whom no secondary cause is evident. Up to 50% of patients with primary empty sella have associated benign intracranial hypertension.[559] A secondary empty sella may develop subsequent to infarction of a pituitary adenoma or surgical or radiation-induced damage to the sellar diaphragm. These patients usually exhibit demonstrable pituitary tissue compressed against the sellar floor, with lateral stalk deviation visible on MRI. Although an empty sella is usually an incidental finding, if more than 90% of pituitary tissue is compressed or atrophied, pituitary failure usually occurs. About 10% of patients may develop small GH-secreting or PRL-secreting adenomas within the narrow rim of compressed pituitary tissue.

Clinical Features of Hypopituitarism

Patients with pituitary failure, regardless of cause, have excessive mortality rates, primarily due to vascular disease.[256] Age at diagnosis, female gender, and history of craniopharyngioma were the most striking determinants of increased fatality. The spectrum of clinical features of pituitary insufficiency depends on several factors. In acquired pituitary insufficiency the clinical spectrum depends upon the degree of hormone deficiency, the number of hormones impaired, and the rapidity of onset. In congenital forms, the earlier the age of onset, the greater the severity of thyroid, gonadal, adrenal, growth,

TABLE 8.14 Pituitary Dysfunction After Cranial Irradiation in Various Conditions

Condition Treated	Schedule	Dose (Gy)	Pituitary Dysfunction
Leukemia and lymphoma	Fractionated TBI Fractionated cranial	7–16 18–24	Isolated GHD, mostly pubertal children Isolated GHD, mostly pubertal children Precocious puberty girls only
Nonpituitary brain tumors	Conventional fractionated cranial	30–50	GHD (30–100%) Gonadotrophin, TSH, ACTH, Prl (3–20%) Precocious puberty both sexes
Nasopharyngeal carcinoma and skull-base tumors	Conventional fractionated cranial	50–70	GHD (100% within 5 yr) Gonadotrophin, TSH, ACTH, Prl (20–50%)
Pituitary tumors	Conventional fractionated cranial	30–50	GHD (100% within 5 yr) Gonadotrophin, TSH, ACTH, Prl (20–60%)

Prl, Prolactin; *TBI,* Total body irradiation.

Modified from Darzy KH. Radiation-induced hypopituitarism after cancer therapy: who, how and when to test. *Nat Clin Pract Endocrinol Metab.* 2009;5:88–99.

TABLE 8.15 Assessment of Anterior Pituitary Function

Test	Dose	Normal Response	Side Effects
ACTH			
Insulin tolerance	0.1–0.15 U/kg IV	Peak cortisol response >18 μg/dL, or increase by 7 μg/dL	Sweating, palpitation, tremor
Metyrapone	Oral administration of 30 mg/kg at 11 PM	Peak 11-DOC ≥7 μg/dL Peak cortisol ≤7 μg/dL Peak ACTH >75 pg/mL	Nausea, insomnia, adrenal crisis
CRH stimulation	100 μg IV	Peak ACTH ≥ twofold to fourfold Peak cortisol ≥20 μg/dL or ↑ ≥7 μg/dL	Flushing
ACTH stimulation	250 μg IV or IM or 1 μg IV	Peak cortisol ≥20 μg/dL	Rare
TSH			
Serum T_4 (free T_4) Total T_3 TSH—third generation TRH stimulation	200–500 μg IV	Peak TSH ≥2.5-fold or ↑ ≥5–6 mU/L (females), ↑ ≥2–3 mU/L (males)	Flushing, nausea, urge to micturate
PRL			
Serum PRL TRH stimulation	200–500 μg IV	PRL ≥2.5-fold	Flushing, nausea, urge to micturate
LH/FSH			
Serum LH and FSH Serum testosterone GnRH stimulation	100 μg IV	Elevated in menopause and in men with primary testicular failure 300–900 ng/mL (age-adjusted normal ranges) LH ≥ twofold to threefold, or by 10 IU/L FSH 1.5–2-fold, or by 2 IU/L	Rare
GH			
Insulin tolerance	0.1–0.15 U/kg	GH peak >5 μg/L	Sweating, palpitation, tremor
Glucagon	1–1.5 mg IM	GH peak >3 μg/L	Nausea, headaches
L-Arginine plus GHRH L-Arginine	0.5 g/kg (max 30 g) IV over 30 min	Peak GH >9 μg/L	Nausea
GHRH	1 μg/kg		Flushing

ACTH, Adrenocorticotropic hormone; *CRH,* corticotropin-releasing hormone; *11-DOC,* 11-deoxycorticosterone; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *GnRH,* gonadotropin-releasing hormone; *IM,* intramuscular; *IV,* intravenous; *LH,* luteinizing hormone; *PRL,* prolactin; T_3, triiodothyronine; T_4, thyroxine; *TSH,* thyroid-stimulating hormone; *TRH,* thyrotropin-releasing hormone.

or water disturbances. Heritable genetic disorders invariably exhibit the most severe phenotypic changes, although later changes may also occur in these disorders, as seen with PROP1 mutations. The resilience of the individual pituitary cell lineages to compressive, inflammatory, vascular, radiation, and invasive insults also differs. The lactotroph cell is often hyperfunctional as a result of decreased tonic inhibitory signals. PRL deficiency is thus exceedingly rare, except for complete pituitary destruction or genetic syndromes. The order of diminished trophic hormone reserve function by pituitary compression usually is as follows: GH > FSH > LH > TSH > ACTH. The corticotroph cell appears particularly resistant to hypothalamic or pituitary destruction and is usually the last cell to lose function. The qualitative phenotypic manifestations of pituitary failure are determined by which specific trophic hormones are lost (see earlier for description of individual hormone deficiencies).

Screening for Pituitary Failure

As the onset of hypopituitarism may be extremely slow, subclinical pituitary failure is often not apparent to the patient or physician. Screening for pituitary dysfunction should be undertaken in patients with hypothalamic or pituitary mass lesions, developmental craniofacial abnormalities, inflammatory disorders, brain granulomatous disease, prior head or neck irradiation, head trauma, prior skull base surgery, those with newly discovered empty sella, and those who have previously experienced pregnancy-associated hemorrhage or blood pressure changes.[560]

As hypopituitarism may develop insidiously and is often not readily clinically apparent, screening of appropriate patients is important to prevent long-term morbidity. Therefore all patients harboring hypothalamic or pituitary masses should be screened for hypopituitarism (Table 8.15). PRL should be measured because many patients with hypopituitarism may also present with secondary hyperprolactinemia. Up

TABLE 8.16 Replacement Therapy for Adult Hypopituitarism[a]

Deficient Hormone	Treatment
ACTH	Hydrocortisone, 10–20 mg daily in divided doses Cortisone acetate, 15–25 mg/day in divided doses
TSH	L-Thyroxine, 0.05–0.2 mg daily according to T_4 levels
FSH/LH	*Males:* Testosterone enanthate, 200 mg IM every 2–3 weeks Testosterone undecanoate 1000 mg IM every 3–6 months Testosterone skin patch, 2.5–5.0 mg/day—can increase dose up to 7.5 mg/day Testosterone gel, 5–10 gm/day (delivering 50–100 mg/day) For fertility: hCG three times weekly, or hCG + FSH or menopausal gonadotropin or GnRH *Females (nonoral route is recommended):* Estradiol skin patch, 4–8 mg, twice weekly Estradiol gel Conjugated estrogen, 0.65 mg daily Micronized estradiol, 1 mg daily Estradiol valerate, 1–2 mg Piperazine estrone sulfate, 1.25 mg All of the estrogens are administered with progesterone or progestin sequentially or in combination if uterus present For fertility: menopausal gonadotropin and hCG or GnRH
GH	*Adults:* Somatotropin, 0.2–1.0 mg SC daily *Children:* Somatotropin, 0.02–0.05 mg/kg/day
Vasopressin	Intranasal desmopressin, via rhinal tube, 5–20 μg twice daily Oral DDAVP, 300–600 μg daily, usually in divided doses

[a]Doses shown should be individualized and reassessed during stress, surgery, or pregnancy. Male and female fertility management is fully discussed in Chapter 26.

ACTH, Adrenocorticotropic hormone; *DDAVP,* desmopressin acetate; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GnRH,* gonadotropin-releasing hormone; *hCG,* human chorionic gonadotropin; *IM,* intramuscularly; *SC,* subcutaneously; T_4, thyroxine.

to two-thirds of patients harboring pituitary macroadenomas, craniopharyngiomas, and other parasellar lesions have compromised pituitary reserve function. Less commonly, patients with intrasellar aneurysms, pituitary metastases, parasellar meningiomas, optic gliomas, and hypothalamic astrocytomas may also have pituitary failure. Although about a third of patients with hypopituitarism undergoing pituitary surgery recover function after decompression, about 25% of patients experience further loss of pituitary function after surgery and therefore should be screened annually. Treatment regimens for pituitary failure are described in Table 8.16.

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The complete list of references is available online at ExpertConsult.com.

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9

Pituitary Masses and Tumors

SHLOMO MELMED

CHAPTER OUTLINE

KEY POINTS

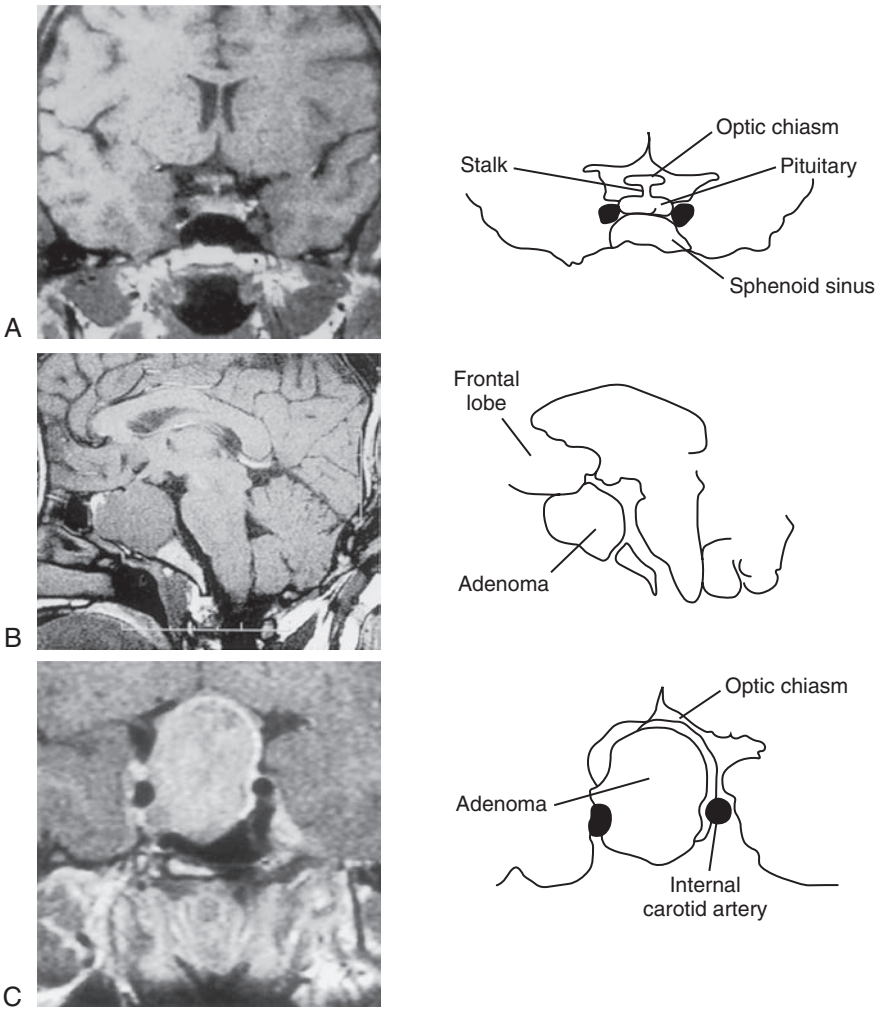
- Intrapituitary or parasellar masses are mostly benign pituitary adenomas.
- Nonpituitary sellar masses are rare and are usually inflammatory processes, infiltrations, or metastatic deposits, or may arise from adjacent structures, including aneurysms, meningiomas, or chordomas.
- Nonsecreting pituitary adenomas are usually of gonadotroph or null cell origin and usually present with compressive features or are diagnosed as incidentalomas.
- Secreting pituitary adenomas exhibit unique syndromes due to excess production of prolactin (prolactinomas), growth hormone (acromegaly/gigantism), adrenocorticotrophic hormone (Cushing disease), or very rarely, thyrotrophic hormone or gonadotrophins.
- Management of pituitary masses includes surgery, radiation, and specific targeted medical therapies.

Pituitary Masses

Pituitary Mass Effects

An expanding intrasellar mass may compress normal pituitary tissue and inexorably alter sellar size and shape by bony erosion and remodeling (Fig. 9.1). Although the time course for this process is unclear, it appears to be slowly progressive over years or decades. The tumor may invade soft tissue, and the dorsal sellar roof presents the least resistance to expansion from within the confines of the bony sella. Both suprasellar and parasellar compression and invasion may occur with an enlarging mass, with resultant clinical manifestations (Table 9.1). As sellar masses impinge upon the optic chiasm, they may interfere with vision. Because of the anatomy of the chiasm, pressure from below affects temporal visual fields, starting superiorly and ultimately extending to the entire temporal field. Continued growth and pressure on the optic apparatus can extend visual loss to the nasal field and may ultimately result in blindness. Long-standing optic chiasmal pressure results in optic disc atrophy. Extension of pituitary lesions laterally may also impinge on or invade the dural wall of the cavernous sinus. Despite invasion, these lesions only rarely compromise the third, fourth, and sixth cranial nerves, as well as the ophthalmic and maxillary branches of the fifth cranial nerve. Although tumors in the cavernous sinus often surround the internal carotid artery, clinical vascular sequelae are rarely encountered. Varying degrees of diplopia, ptosis, ophthalmoplegia, and decreased facial sensation may occur infrequently, depending on the extent of neural involvement by the cavernous sinus mass. In contrast to cavernous invasion by slow tumor progression, sudden insults to the cavernous sinus by hemorrhage or infarction of a pituitary tumor occur more frequently and may affect nerves coursing through the sinus. Downward extension into the sphenoid sinus indicates that the parasellar mass has eroded the bony sellar floor. Aggressive tumors may also invade the roof of the palate and cause nasopharyngeal obstruction, infection, and cerebrospinal fluid (CSF) leakage. Infrequently, temporal or frontal lobes may be invaded, causing uncinate seizures, personality disorders, and anosmia. In addition to the anatomic lesions caused by the expanding mass, direct hypothalamic involvement of the encroaching mass may lead to important metabolic sequelae discussed in Chapter 7.

Intrasellar tumors commonly present with headaches, even in the absence of demonstrable suprasellar extension. Small changes in intrasellar pressure caused by a microadenoma within the



• **Fig. 9.1** Magnetic resonance images of the pituitary. (A) Coronal section of a normal pituitary gland. (B) Sagittal view of a large pituitary adenoma lifting and distorting the optic chiasm and invading the sphenoid sinus and impinging the frontal lobe. (C) Coronal view of a large macroadenoma elevating the optic chiasm and invading the right cavernous sinus.

confined sella are sufficient to stretch the dural plate with resultant headache. Headache severity does not correlate with the size of the adenoma or the presence of suprasellar extension.[1] Relatively minor diaphragmatic distortions or dural impingement may be associated with persistent headache. Successful medical management of small functional pituitary tumors with dopamine agonists or somatostatin analogues is often accompanied by remarkable headache improvement. In a retrospective assessment of transsphenoidal surgery for microadenomas, headaches resolved or disappeared in 90% of patients with nonfunctioning tumors and in 56% of those with functioning tumors.[2] Regardless of cause or size, pituitary masses, including adenomas, may be associated with compression of surrounding healthy pituitary tissue and resultant hypopituitarism. In 49 patients undergoing transsphenoidal resection of pituitary adenomas, mean intrasellar pressure was elevated twofold to threefold in patients with associated pituitary failure. Furthermore, prevalence of headache and elevated prolactin (PRL) levels correlate positively with intrasellar pressure levels,[3] suggesting interrupted portal delivery of hypothalamic hormones. Thus, surgical decompression of a sellar mass may lead to recovery of compromised anterior pituitary function. In patients who do not recover pituitary function postoperatively, irreversible compromised pituitary reserve or ischemic necrosis of residual pituitary tissue is likely to have occurred. Stalk compression may result in pituitary failure caused by encroachment of the portal vessels that normally provide pituitary access to hypothalamic trophic hormones. Stalk compression also usually leads to hyperprolactinemia due to compromised dopamine access, with concomitant failure of other pituitary trophic hormones.

Evaluation of Pituitary Masses

Approach to the Patient Harboring a Pituitary Mass

Most pituitary masses are adenomas. Ninety-one percent of 1120 patients undergoing transsphenoidal surgery for sellar masses were diagnosed as harboring pituitary adenomas.[4] In a series of 2598 patients undergoing pituitary magnetic resonance imaging (MRI), pituitary adenomas accounted for 82% of visible lesions. Most commonly encountered nonadenomatous lesions include Rathke cleft cyst, craniopharyngioma, and meningioma,[5] with Rathke cysts accounting for up to 40% of all such masses.[6] Thus, the

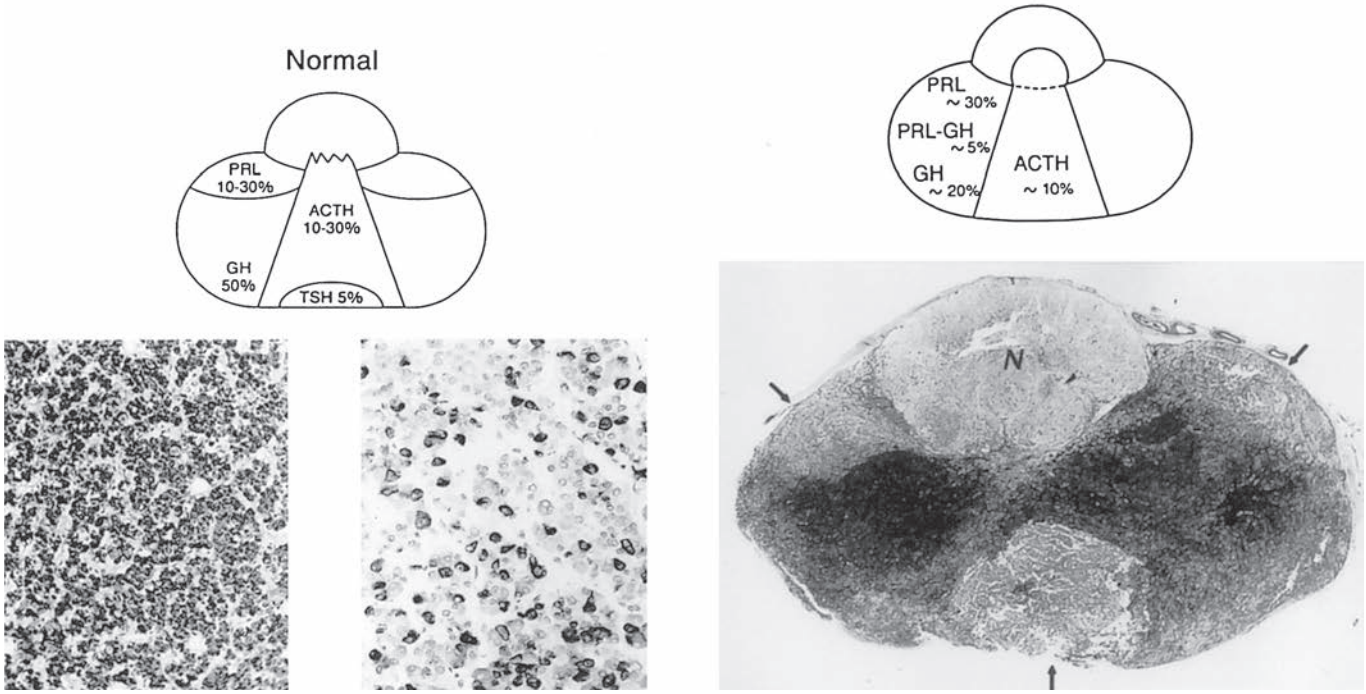
TABLE 9.1 Local Effects of an Expanding Pituitary, Parasellar, or Hypothalamic Mass

Impacted Structure	Clinical Effect
Pituitary	Growth failure, adult hyposomatotropism, hypogonadism, hypothyroidism, hypoadrenalism
Optic tract	Loss of red perception, bitemporal hemianopia, superior or bitemporal field defect, scotoma, blindness
Hypothalamus	Temperature dysregulation, obesity, diabetes insipidus; thirst, sleep; appetite, behavioral, and autonomic nervous system dysfunctions
Cavernous sinus	Ptosis, diplopia, ophthalmoplegia, facial numbness
Temporal lobe	Uncinate seizures
Frontal lobe	Personality disorder, anosmia
Central	Headache, hydrocephalus, psychosis, dementia, laughing seizures
Neuro-ophthalmologic tract	*Field defects:*
	Bitemporal hemianopia (50%), amaurosis with hemianopia (12%), contralateral or monocular hemianopia (7%)
	Scotomas—junctional; monocular central, arcuate, altitudinal; hemianopic
	Homonymous hemianopia
	Acuity loss:
	Snellen
	Contrast sensitivity
	Color vision
	Visual evoked potential
	Pupillary abnormality:
	Impaired light reactivity
	Afferent defect
	Optic atrophy:
	Papilledema
	Cranial nerve palsy—oculomotor, trochlear, abducens, sensory trigeminal
	Nystagmus
	Visual hallucinations
	Postfixation blindness

Adapted from Snyder PJ, Melmed S. Clinically nonfunctioning sellar masses. In: Jameson JL, DeGroot L, eds. *Endocrinology: Adult and Pediatric*, 7th ed. Philadelphia: Elsevier; 2016:256–265; Arnold A. Neuroophthalmologic evaluation of pituitary disorders. In: Melmed S, ed. *The Pituitary*, 2nd ed. Boston, MA: Blackwell; 2002.

differential diagnosis of a pituitary mass should be aimed at excluding the diagnosis of a pituitary adenoma before considering the presence of other rare sellar lesions. Pituitary adenomas arise from differentiated cells secreting trophic hormones, including growth hormone (GH), PRL, adrenocorticotrophic hormone (ACTH), thyroid-stimulating hormone (TSH, thyrotrophic hormone), or gonadotrophins. These tumors may hypersecrete respective hormones or may be clinically nonsecreting (Fig. 9.2). The management and prognosis of anterior pituitary adenomas differ markedly from those for other nonpituitary masses, and an important diagnostic challenge is to effectively distinguish a pituitary adenoma from other parasellar masses. Several physiologic states are associated with pituitary enlargement. Lactotroph hyperplasia occurs during pregnancy, and thyrotroph, gonadotroph, or rarely corticotroph hyperplasias occur in the presence of long-standing primary thyroid, gonadal, or adrenal failure, respectively.[7] Pituitary enlargement may also occur as a result of ectopic GH-releasing hormone (GHRH) or corticotrophin-releasing hormone (CRH) secretion, from pulmonary and pancreatic neuroendocrine tumors or hypothalamic gangliocytomas, with resultant hyperplasia of somatotroph or corticotroph cells. Autopsy series show that up to 20% of subjects harbor an incidental clinically silent pituitary adenoma (incidentaloma). With the widespread use of sensitive imaging techniques for nonpituitary indications, including head trauma, chronic sinusitis, or headaches, previously inapparent pituitary lesions are being identified with increasing frequency. Incidental pituitary cysts, hemorrhages, and infarctions are also discovered at autopsy. Pituitary abnormalities compatible with the diagnosis of microadenoma are detectable in about 10% of the normal adult population undergoing MRI studies.[8] Recognizing that approximately 90% of observed pituitary lesions represent pituitary adenomas, initial assessment should determine whether the mass is hormonally functional and whether local mass effects are apparent at the time of diagnosis or likely to develop in the future.

As the onset of clinical features associated with disordered hormone secretion is insidious and may be unnoticed for years or decades, endocrine function should always be tested at presentation (Table 9.2). Clinical evaluation for changes compatible with hypersecretion or hyposecretion of GH, gonadotrophins, PRL, or ACTH may reveal unique long-term sequelae requiring distinct therapies. In the absence of clinical features of a humoral hypersecretory syndrome, cost-effective laboratory screening should be performed. Serum PRL levels greater than 200 ng/mL strongly suggest the presence of a microprolactinoma or macroprolactinoma. Any elevation in serum PRL from minimal to high can occur when a microadenoma is present. A minimal to moderate elevation can also indicate secondary stalk interruption by a pituitary mass (usually a nonfunctioning macroadenoma). A PRL level greater than 500 ng/mL in a nonpregnant individual is considered pathognomonic of a prolactinoma, as significant PRL elevations can be caused by drugs such as risperidone and other second-generation antipsychotics.[9] Elevated age-matched and gender-matched insulin-like growth factor 1 (IGF1) levels indicate the presence of GH-secreting adenoma, and a high 24-hour urinary free cortisol level or elevated nighttime salivary cortisol[10] is an effective screen for most patients with Cushing disease. Nevertheless, the overall incidence of functional hormone-secreting tumors in asymptomatic subjects with incidentally discovered pituitary masses is low. The presence of, or the potential for, local compressive effects must also be considered. Because the risk for microadenoma progression to a compressive macroadenoma is low, no direct intervention may be warranted. For parasellar masses of uncertain origin, histologic examination of surgically excised tissue may be the best approach to yield an accurate diagnosis. Clearly, the benefits versus risks of biopsy or surgery should be considered in such cases, especially for lesions that are not growing or not causing a functional deficit. Although imaging features may



• **Fig. 9.2** Distribution of normal adenohypophyseal cells is reflected in pituitary adenomas. Nonfunctioning tumors, on the other hand, are typically macroadenomas that efface pituitary landmarks. The localization and frequency of functioning microadenomas reflect the maximal concentration of their corresponding normal pituitary cells. Left panels show normal pituitary with GH cells in lateral wings *(lower left)* compared with diseased pituitary *(lower right)*. Right panels depict adenoma distribution with multiple incidental adenomas shown by arrows. *ACTH,* adrenocorticotrophic hormone; *GH,* growth hormone; *N,* neurohypophysis; *PRL,* prolactin; *TSH,* thyroid-stimulating hormone. (From Scheithauer BW, Horvath E, Lloyd RV, et al. Pathology of pituitary adenomas and pituitary hyperplasia. In: Thapar K, Kovacs K, Scheithauer BW, et al, eds. *Diagnosis and Management of Pituitary Tumors.* Totowa, NJ: Humana Press; 2001.)

TABLE 9.2 Screening Tests for Functional Pituitary Adenomas

Disorder	Test	Comments
Acromegaly	IGF1 OGTT with GH obtained at 0, 30, and 60 minutes	Interpret IGF1 relative to age-matched and gender-matched control subjects. Normal subjects should suppress GH to <1 μg/L.
Prolactinoma	Serum PRL level	A level >500 ng/mL is pathognomonic for macroprolactinoma. If >200 ng/mL, prolactinoma is likely.[a]
Cushing disease	24-hour UFC Nighttime salivary cortisol Dexamethasone (1 mg) at 11 PM and fasting plasma cortisol measured at 8 AM ACTH assay	Ensure that urine collection is total and accurate by measuring urinary creatinine. Free salivary cortisol reflects circadian rhythm, and elevated levels may indicate Cushing disease. Normal subjects suppress to <1.8 μg/dL. Distinguishes adrenal adenoma from ectopic ACTH secretion or Cushing disease.
TSH-secreting tumor	TSH measurement Free T_4 by dialysis Total T_3	If T_4 or T_3 is elevated and TSH is measurable or elevated, a TSH-secreting tumor may be present.

[a]Risperidol may result in prolactin levels >200 ng/mL.

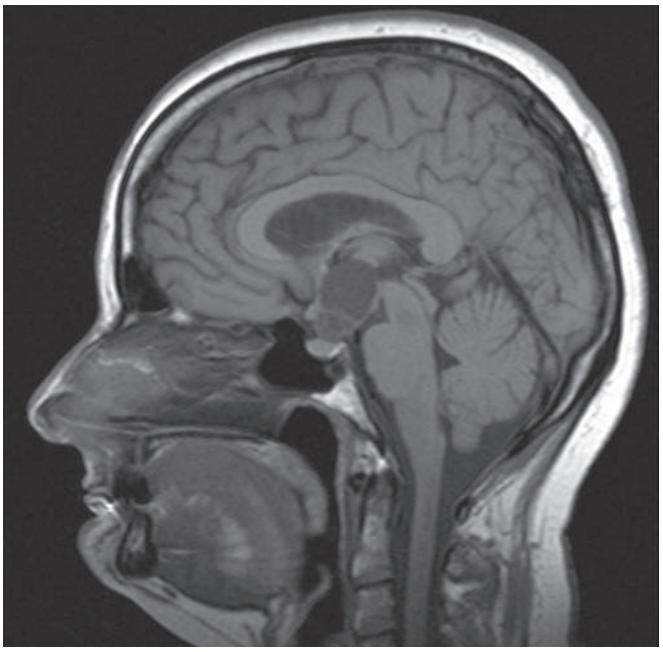
ACTH, Adrenocorticotrophic hormone; *GH,* growth hormone; *IGF1,* insulin-like growth factor type 1; *OGTT,* oral glucose tolerance test; *PRL,* prolactin; T_3, triiodothyronine; T_4, thyroxine; *TSH,* thyroid-stimulating hormone; *UFC,* urinary free cortisol.

be helpful in diagnosing the cause of a nonpituitary sellar mass, the final diagnosis may remain elusive until pathologic confirmation is obtained.

Parasellar masses include neoplastic and nonneoplastic lesions and manifest clinically by local compression of surrounding vital structures or as a result of metabolic or hormonal derangements. Rarely, sellar masses or infiltrative processes may be the presenting feature of a previously undiagnosed systemic disorder such as lymphoma, tuberculosis,[11] sarcoidosis, or histiocytosis.[12] Fever with or without associated sterile or septic meningitis may rarely be caused by fluid leakage into the subarachnoid space from Rathke cleft, dermoid, and epidermoid cysts, craniopharyngioma, and apoplexy.[13] Pituitary masses may present with hemorrhage and infarction, especially during pregnancy (see earlier discussion), when there is a pituitary tumor or when elderly individuals with unsuspected pituitary tumors become hypotensive because of another illness. Rarely, these adenomas present with CSF leak, which may predispose to meningitis. Pituitary masses may also undergo silent infarction leading to development of a partial or totally empty pituitary sella, with normal pituitary reserve, implying that the surrounding rim of pituitary tissue is fully functional. Rarely, functional pituitary adenomas may arise within the remnant pituitary tissue, and these tumors may not be visible by sensitive MRI (i.e., <2 mm in diameter), despite their endocrine hyperactivity. More than one kind of tumor may be found coincidentally in the same patient, such as a pituitary tumor and a meningioma[14] or pituitary adenoma with a craniopharyngioma component.[15] Acute or chronic infection with abscess formation may rarely occur within the mass. Compromised pituitary hormone hyposecretion may be due to direct pressure effects of the expanding mass on hormone-secreting cells or parasellar pressure effects that attenuate synthesis or secretion of hypothalamic hormones, with resultant pituitary failure.

Imaging

Tumors of the pituitary gland are best diagnosed with MRI because the technique has better resolution than other radiologic modalities for identifying soft tissue changes (see Fig. 9.1). When a pituitary tumor or other parasellar mass is suspected, an MRI specifically focused on the pituitary should be performed, as more widely spaced cuts during a routine brain MRI are often inadequate to visualize relatively small pituitary tumors.[16] This technique permits high-contrast detailed visualization of tumor mass effects on neighboring soft tissue structures, including the cavernous sinus or optic chiasm. A pituitary MRI includes images of the optic chiasm, hypothalamus, pituitary stalk, and cavernous and sphenoid sinuses.[17] High-resolution T1-weighted sections in the coronal and sagittal plane both before and after gadolinium-based contrast administration will distinguish most pituitary masses. Slice thickness should be less than 3 mm to obtain a pixel of 1 mm. Contiguous sections are therefore required to diagnose lesions of 1 to 3 mm. If necessary, especially for diagnosing high-signaling hemorrhage, T2-weighted images will provide additional diagnostic information. MRI thus clearly delineates the pituitary gland, stalk, optic tracts, and surrounding soft tissues. The gland may be concave, convex, or flat. The posterior pituitary lobe exhibits a discrete bright spot of high signal intensity on T1-weighted images, which declines with age and is absent in diabetes insipidus and most posterior pituitary lesions. This T1 shortening may reflect the presence of antidiuretic hormone (ADH) localized within neurosecretory vesicles.[18] The pituitary gland may transiently enlarge during adolescence, pregnancy, and postpartum, with teenage girls exhibiting increasing gland convexity during the menstrual cycle. During pregnancy, the gland should normally not exceed 10 to 12 mm, and the stalk should not exceed 4 mm in diameter. Pregnant women may rarely develop visual field deficits due to an enlarging pituitary gland even in the absence of a pituitary tumor. A thickened stalk may indicate the presence of hypophysitis, granuloma, germinoma, or chordoma. After gadolinium injection, microadenomas usually appear hypodense compared to the normal gland, especially when multiple thin section echo sequences are examined in the first few minutes after contrast agent injection. It has been suggested that intrasellar hypointensity may reflect compromised microadenoma vasculature.[19] Microadenomas may also cause gland asymmetry or stalk deviation. In contrast, macroadenomas, which are significantly more vascular than microadenomas, have a higher uptake of gadolinium. They often enlarge the sella turcica by remodeling the bony fossa, suggesting a gradual long-term process. These tumors can grow upward toward the optic apparatus and cause draping of the nerves over the tumor, often accompanied by visual field abnormalities. Tumors can also extend into the sphenoid sinus and not infrequently invade connective tissue separating the pituitary from the cavernous sinus. Radiologically, visible tumor tissue surrounding the carotid artery confirms cavernous sinus invasion. Infrequently, these patients may develop palsies of the third, fourth, or sixth cranial nerves. MRI may readily distinguish pituitary adenomas from other masses, including hyperplasias, craniopharyngiomas, meningiomas, chordomas, cysts, and metastatic lesions. Visualization of a distinct pituitary gland adjacent to a parasellar mass (Fig. 9.3) suggests that the mass is not of pituitary origin. Secondary distinguishing features such as visualization of noninvolved pituitary tissue, mass consistency, calcification, hemorrhage, and suprasellar involvement usually allow an imaging diagnosis of these masses, which can often only be confirmed by direct tissue histologic diagnosis. Preoperative



• **Fig. 9.3** Sagittal magnetic resonance image of a craniopharyngioma, with cystic and solid components. The tumor is in the suprasellar area located above a normal pituitary gland. The presence of a separate pituitary gland indicates that the suprasellar tumor is not of pituitary origin. (Courtesy N. Karavitaki.)

localization of carotid artery aneurysms can also be confirmed by MRI or MR angiography. Administration of gadolinium may be contraindicated in patients with impaired renal function because it may cause acute renal failure or be associated with nephrogenic systemic fibrosis.[20]

Pituitary computed tomography (CT) allows visualization of bony structures, including the sellar floor and clinoid bones, and identifies bony invasion. CT also recognizes calcifications that characterize craniopharyngiomas, meningiomas, and rarely aneurysms that are not evident on MRI. Rarely, pituitary adenomas may calcify. Pituitary CT scan is indicated for discovery of hemorrhagic lesions, metastatic deposits, chordomas, and evidence of calcification.

Receptor Imaging

As prolactinomas express dopamine 2 (D_2) receptors, they can be imaged with a radiolabeled D_2 receptor antagonist by using ^{123}I-iodobenzamide single-photon emission scanning. Failure to visualize nonfunctioning tumors by this technique has led some to advocate its use to distinguish the two tumor types.[21] Radiolabeled indium-pentetreotide has been used for in vivo tumor imaging. Most pituitary adenomas express somatostatin receptor subtypes to a varying degree, thus limiting the specificity of the procedure. As the sensitivity of single-photon emission CT (SPECT) is about 1 cm, and also detects normal pituitary tissue receptor expression, its use is limited for pituitary tumor detection, but it may be helpful for imaging ectopic ACTH-secreting tumors.

Neuro-Ophthalmologic Assessment of Pituitary Masses

The optic tracts are particularly vulnerable to compression by expanding pituitary masses. Accurate neuro-ophthalmologic evaluation is helpful for tumor diagnosis, determining pretreatment baseline visual status for posttreatment monitoring, or detection of mass recurrence.[22] The relationship of the optic chiasm and the intracranial components of the optic nerves with the pituitary gland and surrounding vessels are depicted in Fig. 9.4. A 10-mm posteriorly angled gap separates the optic chiasm and diaphragma sellae (Fig. 9.5). Therefore extensive suprasellar mass extension is required before visual function is compromised. Decussation of neural fibers originating from the nasal half of each retina occurs at the chiasm, and those originating from the temporal retinal halves are situated ipsilaterally.[23] Fibers from the superior and inferior retinal aspect are segregated in the corresponding chiasmal regions. Local vascular compromise and chiasmal stretching contribute to the pathogenesis of selective visual compromise. Reversibility of visual effects may correlate inversely with acuteness of the compressive insult, and long-standing visual tract compression from larger tumors invariably leads to adverse outcomes.

Visual Symptoms

An abnormal visual examination may unmask the presence of a pituitary mass in an asymptomatic patient. Prior to the availability of sophisticated assay and imaging techniques, virtually all pituitary masses presented with visual loss. Currently, fewer than 10% of patients present with visual loss and most harbor clinically nonfunctioning pituitary adenomas often detected by incidental imaging. Unilateral or bilateral temporal or central visual loss is often asymmetric and may be quite insidious, remitting, or recurring. Rarely, sudden visual loss occurs in a previously asymptomatic patient. Other symptoms include diplopia, impaired depth perception, and very rarely visual hallucinations.[24] The retinal nerve fiber layer may be decreased as a reflection of optic atrophy.[25]

Clinical Signs and Approach

Impingement of the inferior crossing chiasmal fibers leads to bitemporal visual loss, especially in the superior field portions, accounting for most pituitary-related visual defects (Fig. 9.6). Rarely, tumors compress the optic chiasm from above and cause inferior temporal compromise. As damage to the optic chiasm becomes more extreme, field cuts can extend into the nasal field and also cause optic atrophy. Pituitary-related defects preferentially marginate at the vertical field midline in contrast to other causes of bitemporal defects, which tend to occur away from the midline. Despite prominent field defects, many of which can be directly correlated with defined tumor location by MRI, visual acuity in the remaining fields is invariably normal in most patients. Anterior tumor extension may damage central visual acuity.[26] Rarely, pupillary abnormalities, optic atrophy, papilledema, cranial nerve palsies, and nystagmus may be encountered. Preoperatively, asymptomatic visual field deficits should be identified by visual field and acuity testing by automated quantitative perimetry, assessment of visual evoked potentials, and afferent pupillary defects, as well as disc appearance. Employing optic coherence tomography to assess retinal fiber layer thickness or damaged ganglion cell layer may provide an indication of potential for postoperative vision recovery.[22]

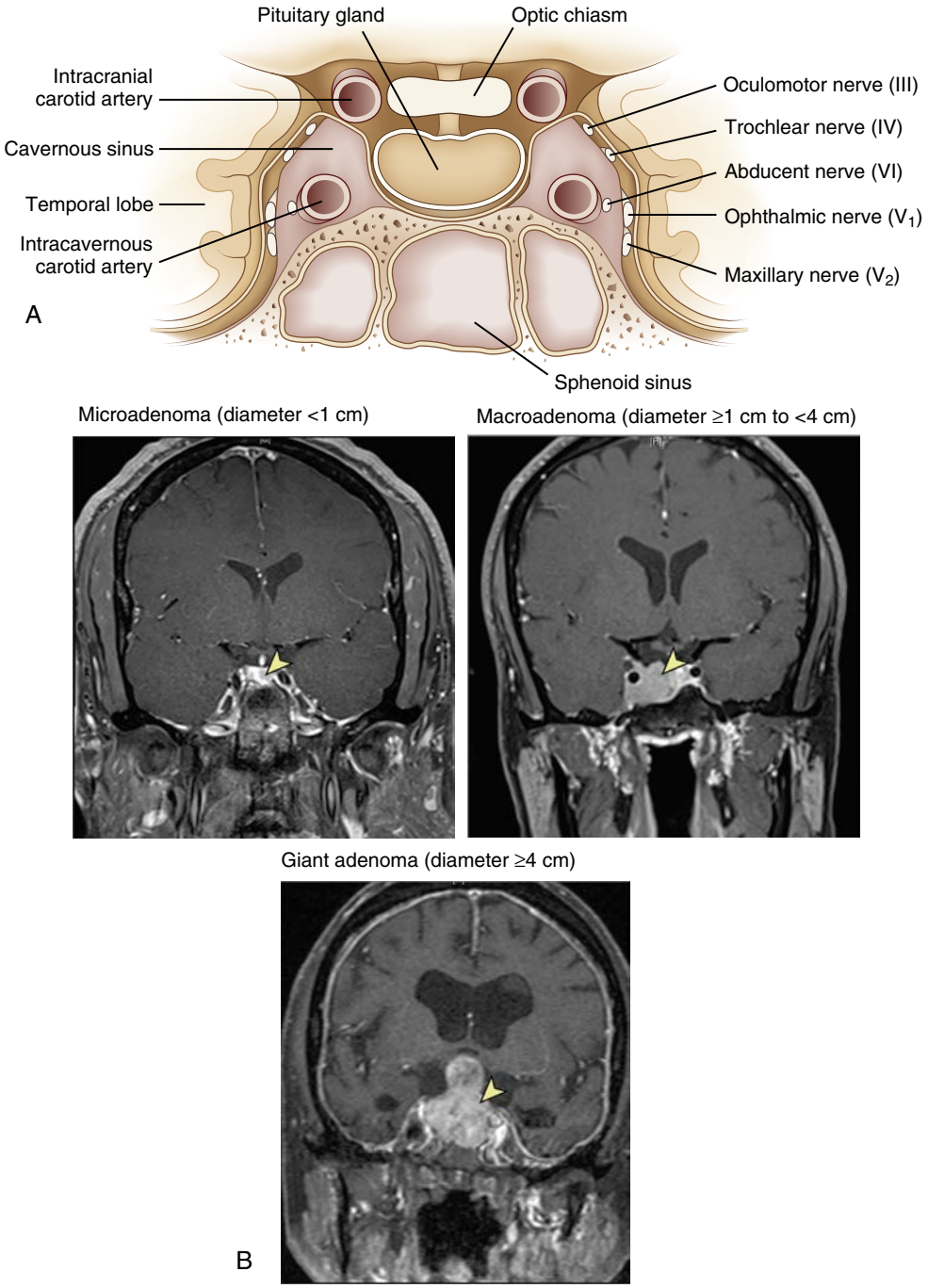
Management of Pituitary Masses

Although pituitary masses are usually benign, they may compress local structures or invade brain tissue (Fig. 9.7). The goals of therapy are to alleviate local compressive mass effects and to suppress hormone hypersecretion or relieve hormone hyposecretion, while maintaining intact pituitary trophic function. Three modes of available therapy include surgical, radiotherapeutic, and medical approaches. In general, the benefits of each type of therapy should be weighed against their respective risks, and comprehensive physician and patient awareness is required to individualize treatment approaches. Appropriate consolidated approaches from expert endocrinologists, neurosurgeons, radiologists, pathologists, and ophthalmologists have been advocated as ideal management teams in pituitary centers of excellence.[27]

Surgical Management of Pituitary Tumors and Sellar Masses

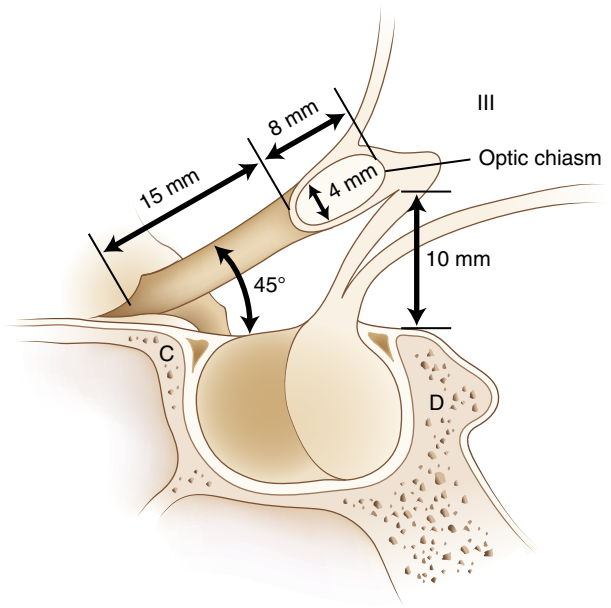
Pituitary surgery is indicated for excision of mass lesions causing central pressure effects, including visual compromise, primary correction of hormonal hypersecretion, or functional tumor resection in patients resistant or not immediately responsive to medical treatment. Unusual sellar lesions may require diagnostic tissue evaluation, and rarely, primary or secondary parasellar malignancies require wide excision.

In 1904, Horsley reported the surgical resection of a pituitary tumor by a lateral middle fossa approach.[28] The first successful transsphenoidal approach for pituitary tumor resection was reported by Schloffer in 1907[29] and subsequently refined by Cushing, who between 1910 and 1925 operated on 231 patients harboring pituitary tumors with a remarkably low 5.6% mortality rate.[28] Cushing used a sublabial incision to enable an endonasal approach for removing the septum and improved visualization using the Kanavel headlight. Hardy later improved the technique by using the operating microscope and intraoperative fluoroscopy, resulting in markedly reduced morbidity and mortality rates compared to those usually encountered with craniotomy, and his approach became the mainstay surgical technique for resecting these tumors.



• **Fig. 9.4** Coronal section of sellar structures and cavernous sinus. (A) Illustration showing the relationship of the oculomotor (III) and trochlear (IV) cranial nerves to the pituitary gland. (B) MRI of pituitary microadenoma *(left),* macroadenoma *(right),* and giant adenoma *(bottom). Arrows* indicate location of adenoma. (A, from Silver SI, Sharpe JA. Neuro-ophthalmologic evaluation of pituitary tumors. In: Thapar K, Kovacs K, Scheihauer BW, et al, eds. *Diagnosis and Management of Pituitary Tumors.* Totowa, NJ: Humana Press; 2001:173–200; B, from Molitch ME. Diagnosis and treatment of pituitary adenomas: a review. *JAMA.* 2017;317:516–524.)

The transsphenoidal approach avoids invasion of the cranial cavity and precludes the need for brain tissue manipulation required during a subfrontal surgical approach (Fig. 9.8). A ventral sphenoid approach for resection of pituitary masses likewise does not violate the cranial fossa. Thus, transsphenoidal surgery is associated with minimal morbidity and mortality rates, most patients are ambulatory within 6 to 9 hours, and the hospital stay is generally about 3 days. Furthermore, the transsphenoidal approach allows for a clearly visible operative field with high magnification and internal illumination. Normal pituitary can be clearly distinguished from tumor tissue, facilitating microdissection and small tumor resection (Fig. 9.9). The use of the transsphenoidal approach has been greatly enhanced by several technologic advances, including head immobilization techniques, microinstrumentation development,



• **Fig. 9.5** Relationship of the pituitary gland to the optic chiasm. The intracranial optic nerve/chiasmal complex lies up to 10 mm above the diaphragma sellae. *C,* anterior clinoid process; *D,* dorsum of the sella turcica. (From Miller NR. *Walsh and Hoyt's Clinical Neuro-Ophthalmology,* 4th ed, vol 1. Baltimore, MD: Williams & Wilkins; 1985:60–69.)

and novel angled endoscopes. Enhanced MRI sensitivity and precision, as well as intraoperative use of MRI, allow for clear delineation of tumor location, size, and invasiveness—all critical determinants of surgical success.

The endoscopic surgical technique has enabled an approach to both intrapituitary and some extrasellar masses.[30] Most approaches are endonasal[31] but some traverse the cranial base, and suprasellar lesions are reached by transposing the pituitary to access the lesion. In experienced hands, the endoscopic technique results in similar complication rates and outcomes, as compared to the traditional transsphenoidal approach.[32,33]

Craniotomy is indicated for the rare invasive suprasellar masses extending into the frontal or middle cranial fossa, optic nerves, or extensive posterior clival invasion. Suprasellar extension contained by a small diaphragmatic aperture ("hour-glass configuration") may also require a transcranial approach. Very rarely, tumors that are too solid to be removed transsphenoidally may require a combination of transsphenoidal and intracranial surgery.

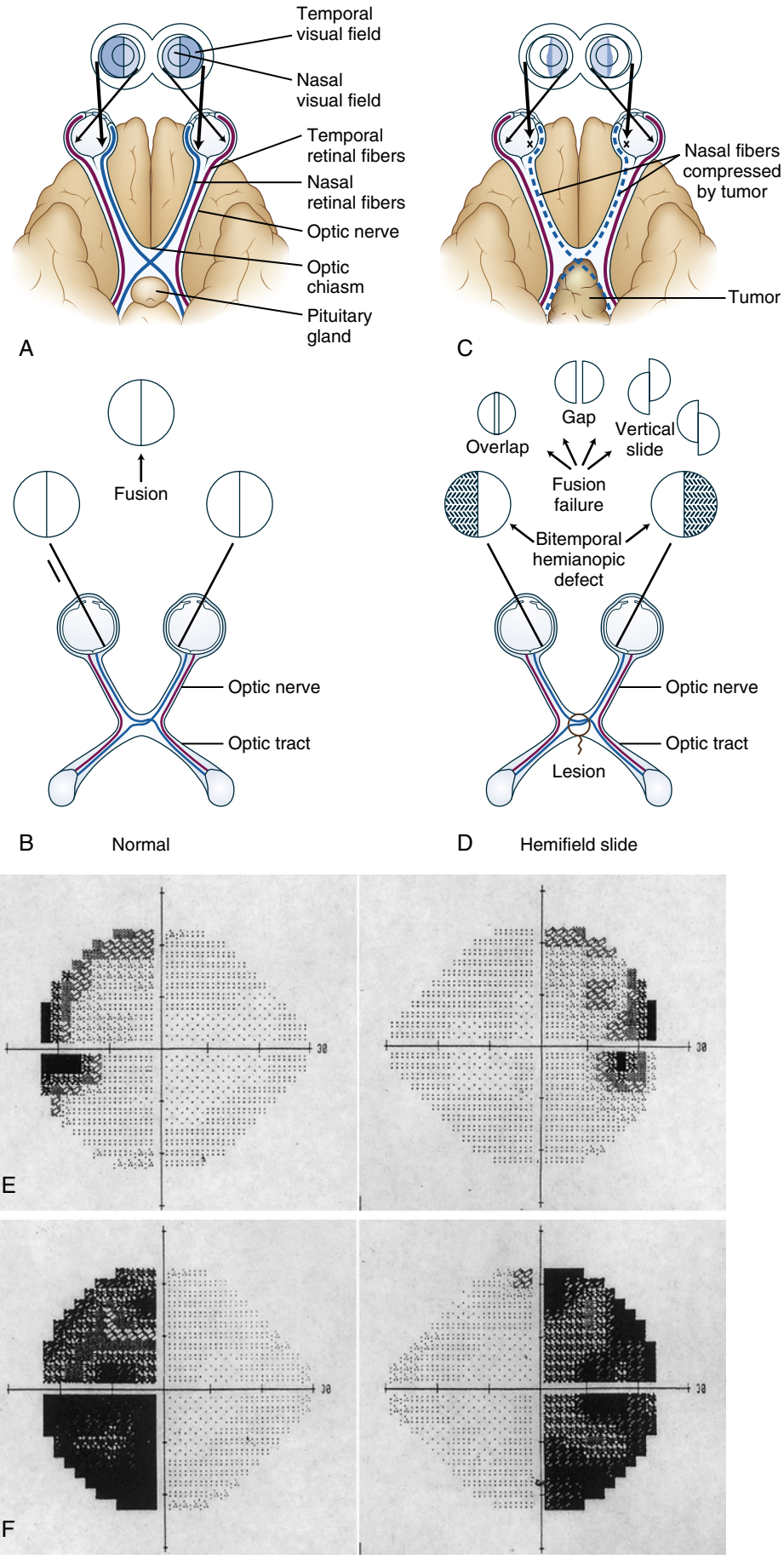
Goals of Surgery

The goal of pituitary surgery is for total resection limited to the lesion, without compromising postoperative endogenous pituitary function.[34] Careful selective mass resection may be difficult for poorly encapsulated lesions, those embedded deeply within the gland body, and those extending into the wall or the body of the cavernous sinuses or suprasellar lesions. However, suprasellar tumors (e.g., craniopharyngiomas) may also be successfully removed via a transnasal approach. Poor operative field visibility also limits precise resection. Excision of normal pituitary tissue and intraoperative gland manipulation should be avoided unless critical for effective tumor dissection. Occasionally, hemihypophysectomy or even nonselective total gland resection may be indicated for multifocal tumors if the surrounding normal gland is necrotic or if no mass lesion is discernible despite an accurate clinical and biochemical diagnosis (especially for ACTH-cell tumors). Successful surgery should decompress central visual defects and compromised trophic hormone secretion. For children and young adults, the consideration of adequate normal tissue for subsequent growth patterns and reproductive function is an important determinant for intraoperative decision making. Nevertheless, especially for functional tumors, small residual remnants attached to the dura are difficult to access but remain hypersecretory with persistent clinical progression. Thus the skilled neurosurgeon will carefully balance maximally effective tumor removal with the requirement to preserve nontumorous pituitary trophic function.

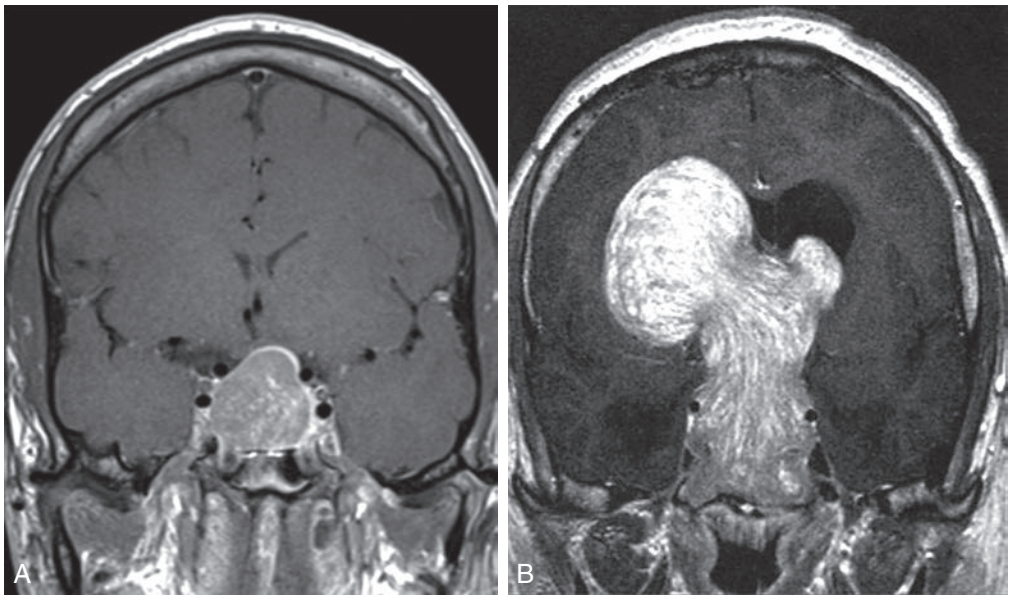
Recent advances have enabled improved surgical results, and long-term outcomes using new techniques have been rigorously compared to standard operations performed by skilled surgeons.[34,35] Image-guided approaches enable intraoperative surgical navigation by three-dimensional (3D) imaging. Intraoperative ultrasound and MRI technologies allow for real-time assessment of the dimensions and extent of the pituitary mass and the progress of surgery. Intraoperative MRI is performed while the surgical field is still open, thus allowing the surgeon to directly assess the need for further dissection, and provides an excellent baseline for postoperative follow-up. If there is suspicion of a vascular lesion, carotid and intracranial angiography is indicated prior to surgery. In contrast, postoperative image stabilization may not be evident for months after surgery, and MRI may be useful only after 1 year or longer, especially after resection of secretory tumors with measurable serum biomarkers.[36] Endonasal transsphenoidal endoscopy (Fig. 9.10) avoids use of a retractor or speculum, does not require nasal packing, and sometimes leads to a shorter operating time, allowing for reduced postoperative morbidity and a shorter hospital stay. The advantages of the technique include a clear panoramic view of bony landmarks and the ability to access suprasellar and parasellar tumor extensions into the cavernous sinuses.[32] Disadvantages of this approach include the management of perioperative intrasellar bleeding and CSF leaks. Combining both techniques may allow the advantages of both approaches.

Indications for Transsphenoidal Surgery

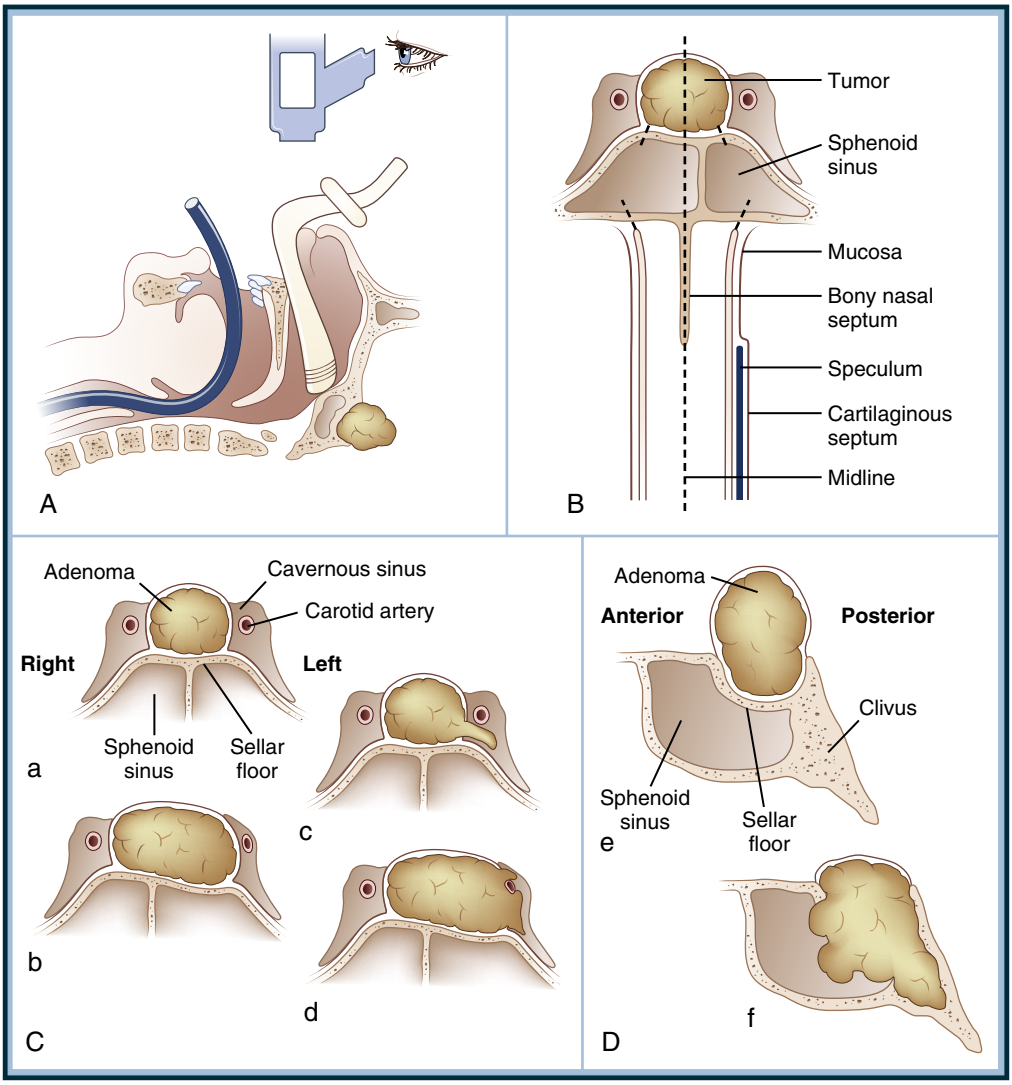
A pituitary mass that may or may not be compressing local vital structures should be evaluated for surgical resection (Table 9.3). Although surgical resection offers a rapid resolution of hormone hypersecretion and many of the resultant clinical features of functioning adenomas, indications for the procedure differ depending on tumor type (see upcoming discussion). In general, patients who are intolerant or resistant to medical therapy require surgery. Surgery is primarily indicated for patients with well-circumscribed GH-secreting adenomas, TSH-secreting adenomas, all ACTH-secreting tumors, and nonfunctioning macroadenomas that require surgery. Surgery may also be indicated when tissue histologic confirmation is required for diagnosing the nature of an enigmatic sellar mass. Progressive compressive features, including visual field loss, compromised pituitary function, or other central nervous system (CNS) functional change, are indications for surgical debulking and sellar decompression. Hemorrhage into the encased bony sella turcica, usually occurring within a known or previously unknown adenoma, may require immediate surgical decompression. Urgent surgical decompression is required for acute pituitary hemorrhage, especially in patients who have developed sudden visual field compromise. Hypopituitarism due to increased portal vessel pressure may recover shortly after



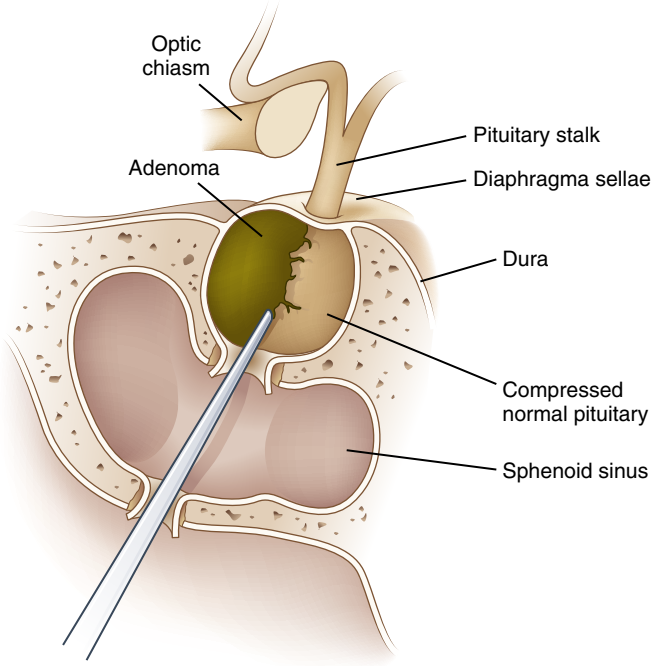
• **Fig. 9.6** (A–D) Local effects of an expanding pituitary tumor causing visual field defects. (A) Normal vision; (C) bitemporal hemianopia. (B–D) Hemifield slide phenomena arising in the setting of bitemporal hemianopia and from fusion instability. The nasal and temporal fields lose their linkage, resulting in overlap of the preserved visual fields. (E and F) Threshold field test showing superior bitemporal hemianopia in a patient with pituitary tumor compressing the optic chiasma (E) which later advanced to bitemporal hemianopia (F). (A and C, from Newell-Price J. Endocrine assessment. In: Sheaves R, Jenkins PJ, Wass JAH, eds. *Clinical Endocrinology Oncology.* Boston, MA: Blackwell Science; 1977; B and D, from Stiver SI, Sharpe JA. Neuro-ophthalmologic evaluation of pituitary tumors. In: Thapar K, Kovacs K, Scheithauer BW, et al, eds. *Diagnosis and Management of Pituitary Tumors.* Totowa, NJ: Humana Press; 2001.)



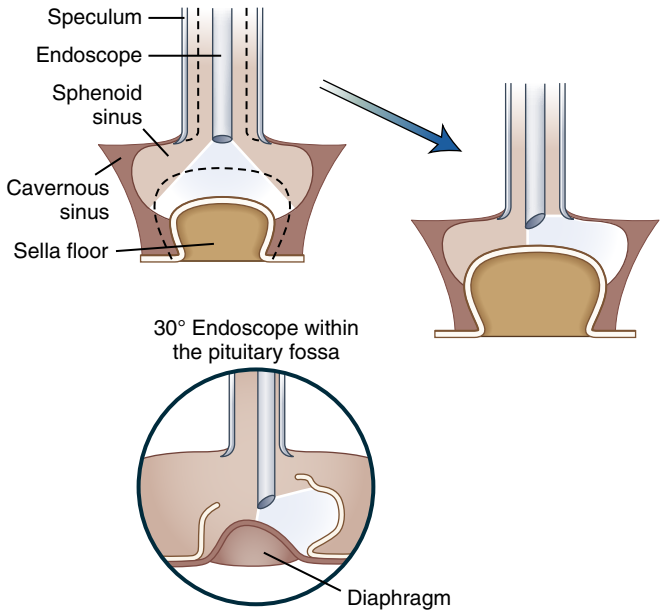
• **Fig. 9.7** (A) Pituitary macroadenoma invading laterally and elevating the optic chiasm dorsally. (B) Large invasive macroadenoma invading brain tissue. (B, from Li-Ng M, Sharma M. Invasive pituitary adenoma. *J Clin Endocrinol Metab.* 2008;93:3284–3285.)



• **Fig. 9.8** Transsphenoidal pituitary surgery. (A) Route of the transsphenoidal approach (lateral view) and surgical corridor of the transsphenoidal approach and positioning of the retractor. (B) The extent of removal of bone structures is indicated *(shaded areas).* (C) Parasellar extensions of pituitary adenomas (coronal sections) are shown: *a*, intrasellar adenoma; *b*, displacement of the cavernous sinus; *c*, focal invasion of the cavernous sinus; *d*, diffuse invasion of the cavernous sinus by the adenoma. (D) Extensions of a pituitary adenoma (sagittal sections): *e*, suprasellar extension; *f*, invasion of the sphenoid sinus and of the clivus. (Adapted from Honegger J, Buchfelder M, Fahlbusch R. Surgery for pituitary tumors. In: Sheaves R, Jenkins PJ, Wass JAH, eds. *Clinical Endocrinology Oncology.* Boston, MA: Blackwell Science; 1977.)



• **Fig. 9.9** Transsphenoidal resection of pituitary adenoma.



• **Fig. 9.10** Endoscope-assisted microsurgery provides a panoramic view of the sphenoid sinus. Using a 30-degree endoscope, a view "around the corner" is possible. Parasellar structures can be visualized and residual tumor detected and resected. (From Fahlbush R, Buchfelder M, Kreutzer J, et al. Surgical management of acromegaly. In: Wass J, ed. *Handbook of Acromegaly.* Bristol, UK: BioScientifica; 2001.)

TABLE 9.3 Transsphenoidal Pituitary Surgery

Primary Indications

General

Visual tract or central nervous compression arising from within sella
Relief of compressive hypopituitarism by presenting, residual, or recurrent tumor tissue
Tumor recurrence after surgery or irradiation
Pituitary hemorrhage
Cerebrospinal fluid leak
Resistance to medical therapy
Intolerance of medical therapy
Personal choice
Desire for immediate pregnancy with macroadenoma
Requirement for diagnostic tissue histology

Specific

Acromegaly
Cushing disease
Clinically nonfunctioning macroadenoma
Prolactinoma
Nelson syndrome
TSH-secreting adenoma

Side Effects

Transient

Diabetes insipidus
Cerebrospinal fluid leak and rhinorrhea
Inappropriate ADH secretion
Arachnoiditis
Meningitis
Postoperative psychosis
Local hematoma
Arterial wall damage
Epistaxis
Local abscess
Pulmonary embolism
Narcolepsy

Permanent (up to 10%)

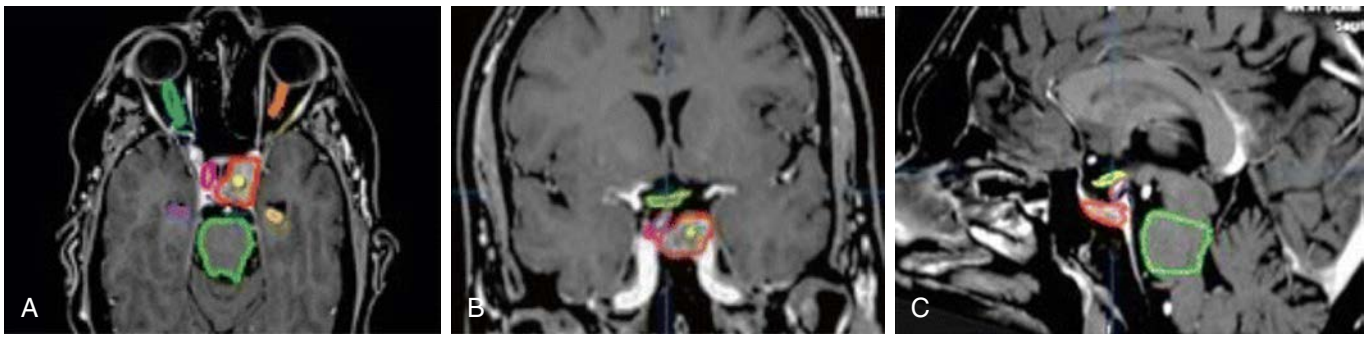
Diabetes insipidus
Total or partial hypopituitarism
Visual loss
Inappropriate ADH secretion
Vascular occlusion
CNS damage—oculomotor palsy, hemiparesis, encephalopathy
Nasal septum perforation

Surgery-Related Mortality (up to 1%)

Brain, hypothalamic injury
Vascular damage
Postoperative meningitis
Cerebrospinal fluid leak
Pneumocephalus
Acute cardiopulmonary disease
Anesthesia-related
Seizure

ADH, Antidiuretic hormone; *CNS,* central nervous system; *TSH,* thyroid-stimulating hormone.

decompressive surgery. When pituitary function after surgery was assessed in 234 patients, 52 patients developed new trophic hormone dysfunction, and 45 of 93 patients with preoperative evidence for hypopituitarism recovered between one and three previously suppressed axes. Significant factors determining restoration of postoperative pituitary function were no visible tumor remnants as assessed by MRI and no tumor invasion as determined both by the neurosurgeon and by pathologic examination of surrounding tissue.[37] Therefore, as some patients with preoperative pituitary failure recover function, depending on the clinical circumstance, patients should be considered for retesting of pituitary reserve prior to initiating postoperative substitution therapy, except for adrenal steroid replacement, which requires



• **Fig. 9.11** MR images showing radiation target delineation for a pituitary adenoma, axial (A), coronal (B), and sagittal (C). Gross tumor and planning target volumes are highlighted in *red*. Organs at risk include optic chiasm, left optic nerve *(orange),* right optic nerve *(cyan),* left optic lens *(light yellow),* right optic lens *(light blue),* brainstem *(green),* pituitary stalk *(pink),* right hippocampus *(purple),* and left hippocampus *(golden yellow).* (From Minniti G, Osti MF, Niyazi M. Target delineation and optimal radiosurgical dose for pituitary tumors. *Radiat Oncol*. 2016;11:135.)

greater caution. Indications for second surgery in the same patient include tumor recurrence, persistent hormonal hypersecretion by tumor remnants, and repair of a CSF leak.

After surgery, patients should be kept at bedrest at an angle of 30 to 45 degrees, and urine and serum osmolality and serum electrolytes are measured every 6 hours. Indications for postoperative vasopressin replacement include polyuria, especially with elevated serum sodium and osmolality and inappropriately low urine osmolality. Postoperative polyuria alone is not an indication for vasopressin replacement, unless it is a reflection of compromised posterior pituitary function. Excess fluid given intraoperatively may also result in postoperative polyuria. Requirements for fluid replacement should take into consideration both fluid intake and urine output.

Side Effects

The success of surgery is largely determined by the skill and experience of the neurosurgeon. Higher volume pituitary centers and experienced surgeons report superior postoperative outcomes and shorter hospital stays.[38,39] Pituitary surgery complications include sinus damage, disrupted nerve function, postoperative infections, vascular complications, or endocrine dysfunction. Importantly, tumor growth persistence or recurrence is a reflection of subsequent adverse outcome. Tumor size, degree of invasiveness, preoperative hormone levels, and previous pituitary surgery are determinants of surgical outcome.[40] Overall, the most significant predictors of postoperative recurrence of hormone-secreting tumors is the postoperative basal hormone level.[41,42] CSF leakage, transient diabetes insipidus, and inappropriate ADH secretion are the most commonly encountered transient side effects, occurring in up to 20% of patients (see Table 9.3). Local damage may result in arachnoiditis, vascular bleeding, hematoma formation, and epistaxis. Rarely, pulmonary embolism, narcolepsy, and local abscess have been reported. Iatrogenic hypopituitarism, diabetes insipidus, or syndrome of inappropriate antidiuretic hormone secretion (SIADH) is reported in up to 10% of patients. Rarely, the CNS may be permanently damaged with hemiparesis, cranial nerve palsies, or encephalopathy.

Three phases of postoperative diabetes insipidus have been described. The initial transient disorder is followed by an interphase on days 6 to 11 with no polydipsia or polyuria. During this second phase, hyponatremia with features of inappropriate ADH secretion have also been reported, even in patients with no signs and symptoms of diabetes insipidus after surgery.[43] The third phase is return of polyuria, polydipsia, and reduced ability to concentrate urine. Fatality has been reported in up to 1% of patients undergoing pituitary surgery and may be related to direct hypothalamic or cerebrovascular damage, meningitis, pneumocephalus formation, or anesthetic complications. Surgical failure may result from a non–pituitary-related event, including anesthesia-related complication or bleeding disorder. Incomplete tumor removal may also be due to inaccurate preoperative MRI localization or identification. Rarely, a previously undiagnosed functioning pituitary tumor or ectopic source of ACTH may be unmasked after initially unsuccessful pituitary surgery. Readmission rates following pituitary surgery are low. In a series of 466 consecutive cases, 29 were readmitted within 30 days, mainly for epistaxis, hyponatremia, or CSF leak.[44]

Pituitary Radiation

Principles

High-energy ionizing radiation can be delivered to deep tissues by megavoltage techniques.[45] The challenge is to provide maximal localized necrotizing radiation to the pituitary lesion while minimally exposing surrounding normal structures to radiation damage. Several advances have improved both efficacy and safety, including highly precise tumor localization and a high-voltage (6–15 MeV) linear accelerator. If required, accurate simulation models with isocentric rotational arcing allow repeat head positioning at the same exact points for recurrent patient visits. For fractionated approaches, up to a maximum of 5000 rads (= 50 Gy) are administered as 180-rad daily fractions for about 5 to 6 weeks. High-precision techniques such as stereotactic radiosurgery may be administered as either single or multiple fractions and may be delivered by cobalt-60 Gamma Knife or CyberKnife robotic surgery or linear accelerator. These allow delivery of high energy directly targeting the pituitary lesion, while minimizing radiation exposure to surrounding tissues[46] (Fig. 9.11). Radiosurgery is best suited for intrasellar and cavernous lesions distant from the optic nerves (Tables 9.4 and 9.5). In a long-term study with a mean 96-month follow-up of 76 patients, about half were in remission, 23% developed new-onset hypopituitarism, and 3 patients developed oculomotor palsies.[47] Whether or not stereotactic radiosurgery exhibits superior long-term efficacy or safety over fractionated treatments remains unresolved.[48]

TABLE 9.4 Pituitary Irradiation

INDICATIONS

Pituitary adenoma—adjuvant therapy for acromegaly, Cushing disease, nonfunctioning adenoma, prolactinoma
Craniopharyngioma
Nelson syndrome
Nonadenomatous invasive sellar mass
Tumor recurrence
Hormone hypersecretion recurrence
Resistance to surgery and medical treatments

SIDE EFFECTS

Hypopituitarism—deficient growth hormone, gonadotrophin, TSH, and ACTH reserve
Eye—visual loss, optic neuritis
Brain—brain necrosis, temporal lobe deficits, cognitive dysfunction

MALIGNANT BRAIN TUMORS AND MENINGIOMAS AFTER PITUITARY RADIATION

Treatment Period (calendar year)	PATIENTS		CASES		PATIENT-YEARS		INCIDENCE RATE PER 100,000 PATIENT-YEARS		
	RT	No RT	RT	No RT	RT	No RT	RT	No RT	RR: RT vs No RT
<1990	1497	1216	26	5	11,751	8227	221.0	60.8	3.64
1990–1999	1363	2090	8	3	9031	13,338	88.6	22.5	3.94
≥2000	376	1621	1	1	1561	6176	64.1	16.5	3.96

ACTH, Adrenocorticotrophic hormone; *RR,* risk ratio; *RT,* radiation therapy; *TSH,* thyroid-stimulating hormone.

Modified from Burman P, van Beek AP, Biller BMK, et al. Radiotherapy, especially ay young age, increases the risk for de novo brain tumors in patients treated for pituitary/sellar lesions. *J Clin Endocrinol Metab.* 2017;102:1051–1058.

TABLE 9.5 Effects of Stereotactic Radiosurgery in Patients With Hormone-Secreting and Nonsecreting Pituitary Adenomas

Study	Number of Patients	Median Marginal Dose (Gy)	Mean Follow-Up (months)	Remission Rate (%)	Time to Remission (months)	Predictive Factors of Remission	Hypopituitarism (%)
Acromegaly[a]							
Jezkova (2006)	96	32	54	50	NA	Initial hormone levels	27
Pollack (2007)	46	20	63	50	36	Initial hormone levels, SRL	33
Vik-Mo (2007)	53	26.5	66	17	NA	None	18
Jagannathan (2008)	95	22	57	53	29.8	SRL treatment	34
Losa (2008)	83	21.5	69	60	NA	Initial hormone levels	8.5
Pollock (2008)	27	20	47	67	NA	NA	27
Ronchi[459] (2009)	35	20	114	43	144	Initial hormone levels	8.5
Castinetti (2009)[b]	43	26	96	42	50	Initial hormone levels	21
Poon (2010)	40	Range, 20–35	74	75	NA	Cavernous sinus invasion	12
Sheehan (2011)	130	Range, 18–30	31	53	30	SRL treatment	34
Li (2012)	40	21	72	47.5	45	Initial hormone levels, cavernous sinus invasion	40

TABLE 9.5 Effects of Stereotactic Radiosurgery in Patients With Hormone-Secreting and Nonsecreting Pituitary Adenomas—cont'd

Ding (2018)	371	Range, 8.8–40	79	59	38	Cessation of IGF1 lowering therapy	22
Cushing Disease							
Hoybye (2001)	18	NA	204	83	NA	NA	66
Kobayashi (2002)	20	40	60	35	NA	NA	NA
Jagannathan (2007)	90	23	42	53	13	Tumor volume	22
Castinetti (2009)[b]	18	28.5	96	46	24	Initial hormone levels	28
Prolactinomas							
Pouratian (2006)	23	18.6	58	26	24.5	Use of dopamine agonists at time of surgery, tumor volume	29
Jezkova (2008)	35	34	75	37	96	None	14.3
Castinetti (2009)[b]	15	26	86	43	28	Initial hormone levels	13.3

Nonfunctioning Adenomas

Study	Number of Patients	Marginal Dose (Gy)	Mean Follow-Up (months)	Tumor Control Rate (%)	Rate of Visual Deficit (%)	Hypopituitarism (%)
Mingione (2006)	100	18.5	44.9	92.2	0	19.7
Liscak (2007)	140	20	60	100	0	2
Pollock (2008)	62	16	64	96.8 (95 at 5 years)	0	27
Kobayashi (2009)	71	14.1	50.2	96.7	2.8	8.2
Hayashi (2010)	43	18.2	36	100	0	0
Gopalan (2011)	48	18.4	95	83.3	0	39
Iwata (2011)	100	3×7/5×5	33	98	1	3
Park (2011)	125	13	62	90 (84 at 5 years)	0.8	24
Starke (2012)	140	18	50	89.6 (97 at 5 years)	0	30.3
Sheehan (2013)	512	16	36	93.4 (95 at 5 years)	7.9	21
Lee (2014)	41	12	48	92.7 (85 at 10 years)	2.4	24.4
Bir (2015)	57	15	45.5	93 (90 at 10 years)	0	8.8

[a]Remission after withdrawal of SRL.

[b]Only patients with a follow-up >60 months reported.

NA, Not available.

Data from Castinetti F, Regis J, Dufour H, et al. Role of stereotactic radiosurgery in the management of pituitary adenomas. *Nat Rev Endocrinol.* 2010;6:214–223; Minniti G, Flickinger J, Tolu B, et al. Management of nonfunctioning pituitary tumors: radiotherapy. *Pituitary.* 2018;21:154–161; Abu Dabrh AM, Asi N, Farah WH, et al. Radiotherapy versus radiosurgery in treating patients with acromegaly: a systematic review and meta-analysis. *Endocr Pract.* 2015;21:943–956; Ding D, Mehta GU, Patibandla MR, et al. Stereotactic radiosurgery for acromegaly: an international multicenter retrospective cohort study. *Neurosurgery.* 2019;84:717–725.

Indications

The use of radiation for treating pituitary tumors is highly individualized and depends on the expertise of the treating center, the conviction of the treating physician in weighing the potential benefits and risks of the procedure, and patient preference based upon informed choice (see Tables 9.4 and 9.5).[49] In general, radiation techniques are indicated for persistent hormone hypersecretion or residual mass effects after surgery or when surgery of a compressive mass is contraindicated. Overall, fractionated radiotherapy (45–50 Gy) halts growth in over 90% of nonsecreting tumors by 10 years, while secretory tumors are usually more resistant.[50] As GH-secreting and PRL-secreting tumors are generally amenable to medical therapy, indications for irradiation are rare for these adenomas. Most indications for radiation are adjuvant to either surgical or medical treatment. Radiation may be indicated after resection of a potentially recurring or inadequately resected pituitary mass, such as nonfunctioning pituitary adenoma, craniopharyngioma, or chordoma. In acromegaly, use of radiation as primary treatment is generally not recommended and is usually reserved as adjuvant to surgery or medical treatments,[51] but for aggressively growing prolactinomas that are resistant to medical therapy, the procedure may prevent further local invasion. Recurrent pituitary-dependent Cushing disease appears to be particularly suited for radiation, especially in younger patients.

Side Effects

Hypopituitarism. Pituitary failure occurs commonly in patients who have received pituitary irradiation (Table 9.6). Within 10 years after radiation, up to 80% of patients may have gonadotroph, somatotroph, thyrotroph, or corticotroph deficits.[47] The median time to develop pituitary failure after pituitary radiosurgery is about 5 years.[52] The mechanism for hypopituitarism appears to involve damage to hypothalamic hormone–releasing cells as well as direct pituitary damage. These patients require lifelong endocrine follow-up for pituitary reserve testing and hormone replacement when appropriate.

Second Brain Tumors. Glioma has been reported after conventional pituitary radiation for adenomas and craniopharyngioma with a mean latency period of 11.5 years[53] (see Table 9.4). In patients irradiated for pituitary tumors, it appears that the standardized incidence ratio (SIR) for second brain tumors is approximately 6 (confidence interval, 3.16–10.69), with a latency of 6 to 24 years in separate cohorts.[54,55] During 53,786 patient-years of surveillance following both stereotactic and conventional radiotherapy, the risk of developing a brain tumor following pituitary-directed radiotherapy increased 2.4-fold and meningioma risk increased 1.6-fold with every decade of younger age at therapy.[56] As patients harboring pituitary tumors are more likely to undergo routine brain imaging during follow-up, it is not entirely clear whether observed meningiomas are coincidental findings. This very rare complication appears to be dose and age related, and fractionated doses should not exceed 50 Gy. Use of confocal radiation techniques to irradiate a smaller tissue volume may minimize this adverse effect, but long-term, prospective controlled surveillance studies are required to further evaluate this important question.

Cerebrovascular Disease. The incidence of cerebrovascular events, but not mortality, was increased threefold, mainly in men irradiated for nonfunctioning adenomas.[57] However, in another study, mortality rate from cerebrovascular disease appeared higher in previously irradiated pituitary-deficient patients.[58,59] The direct causality of this relationship is unclear, but direct effects on cerebral vasculature, including atherosclerotic occlusive lesions, have been reported.[60]

TABLE 9.6 Complications After Stereotactic Radiosurgery for Nonfunctioning Pituitary Adenomas.

Complication	No. of Patients (%)
Patients w/ new cranial nerve (CN) dysfunction[a]	41 of 422 (9.3)
CN II	29 (6.6)
CN III	6 (1.36)
CN IV	1 (0.23)
CN V	4 (0.90)
CN VI	2 (0.45)
CN VII	1 (0.23)
New-onset or worsened hypopituitarism	92 of 435 (21.1)
Cortisol	29 of 293 (9.9)
Thyroid	40 of 246 (16.3)
Gonadotrophin	24 of 288 (8.3)
Growth hormone	31 of 269 (8.4)
Diabetes insipidus	6 of 422 (1.4)
Further tumor growth	31 of 469 (6.6)
Further surgery or radiation therapy	34 of 444 (7.7)

[a]Forty-one patients had 43 deficits.

From Sheehan JP, Starke RM, Mathieu D, et al. Gamma Knife radiosurgery for the management of nonfunctioning pituitary adenomas: a multicenter study. *J Neurosurg.* 2013;119(2):446–456.

Visual Damage. The risk of visual damage (and very rarely blindness) is minimized by fractionating dosages to less than 200 rads per treatment session for conventional radiotherapy. The incidence of reported new visual damage in patients undergoing radiosurgery is approximately 4%.[61]

Brain Necrosis. Dose-related radiation-induced brain necrosis was documented by MRI in 14 of 45 patients, with temporal lobe atrophy and cystic and diffuse cerebral atrophy reported. Cognitive dysfunction, especially memory loss, has also been reported.[62]

Medical Management

Pituitary tumors often express receptors mediating hypothalamic control of hormone secretion, and appropriate therapeutic ligands for dopamine D_2 receptor and the somatostatin receptor subtype 2 (SST2) are employed to effectively suppress PRL, GH, and TSH hypersecretion; to block tumor growth; and often to shrink tumor size. Medical ablation of target gland function, including thyroid and adrenal, may also be useful in mitigating the deleterious impact of pituitary tumor trophic hormone hypersecretion. Peripheral antagonists block GH or cortisol action without targeting the respective pituitary tumor source. These medical approaches are considered later in this chapter.

Parasellar Masses

Hypothalamic masses are described in Chapter 7, and causes of parasellar masses are depicted in Table 9.7.

TABLE 9.7 Diagnostic Pituitary Magnetic Resonance Imaging of Parasellar Masses.[a]

Diagnosis	Total
Anterior Pituitary Tumors	
Prolactinoma	395
Nonfunctioning adenoma	364
GH adenoma	127
ACTH adenoma	84
GH/prolactin mixed adenoma	4
Nelson syndrome	2
Pituitary carcinoma	2
LH/FSH functioning adenoma	1
TSH adenoma	1
GH/TSH mixed adenoma	1
Cysts	
Rathke cleft cyst	42
Craniopharyngioma	33
Arachnoid	2
Epidermoid	1
Pineal cyst	1
Nonadenomatous Neoplasms	
Meningioma	32
Chordoma	3
Pituitary lymphoma	2
Chondrosarcoma	1
Embryonal rhabdomyosarcoma	1
Germinoma	1
Granular cell tumor	1
Hemangiopericytoma, malignant	1
Leiomyosarcoma	1
Mucoepidermoid carcinoma	1
Pituicytoma	1
Xanthogranuloma	1
Inflammatory and Vasculitides	
Lymphocytic hypophysitis	3
Hypophysitis, unspecified type	2
Lymphocytic infundibulitis	1
Amyloidosis, primary	1
Sarcoidosis	1
Wegener granulomatosis	1
Infectious	
Pseudomonas aeruginosa	1
Syphilis	1
Metastases	
Breast	3
CNS lymphoma, to pituitary stalk	1
Nasopharyngeal lymphoma	1
Liver epithelioid hemangioendothelioma	1
Lung, adenocarcinoma	1
Pineal germinoma/dysgerminoma	1
Plasmacytoma	1
Prostate, adenocarcinoma	1
Sinusoidal squamous cell carcinoma	1
Vascular	
Apoplexy with masses	16
Carotid aneurysm	4
Hypothalamic cavernous angioma	1
Hypothalamic interpeduncular hematoma	1
Miscellaneous	
Empty sella	21
Hyperplasia	14
Ectopic pituitary gland	4
Fibrous dysplasia	3
Lipoma	1
Hypothalamic	
Astrocytoma	2
Germinoma	1
Hamartoma	1
Undiagnosed Masses	159
Normal Pituitary	1242

[a]Diagnosis in 2598 patients undergoing pituitary magnetic resonance imaging.

ACTH, Adrenocorticotrophic hormone; *CNS,* central nervous system; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *LH,* luteinizing hormone; *TSH,* thyroid-stimulating hormone.

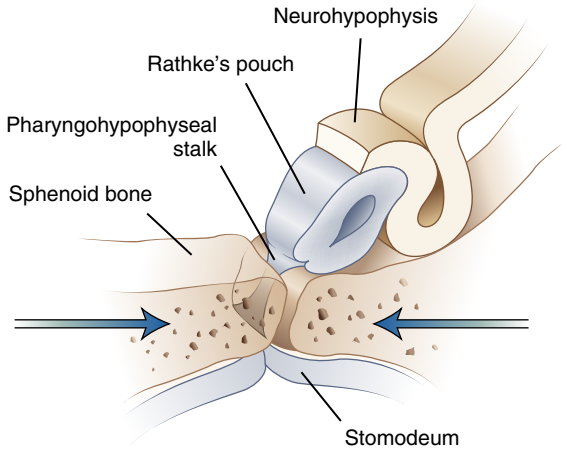
Modified from Famini P, Maya MM, Melmed S. Pituitary magnetic resonance imaging for sellar and parasellar masses: ten-year experience in 2598 patients. *J Clin Endocrinol Metab.* 2011;96:1633–1641.

Types of Parasellar Masses

Parasellar masses account for about 20% of all brain tumors, with a reported incidence of 13,340 new cases of pituitary tumors and craniopharyngiomas in the United States in 2016.[63]

Rathke Cysts

The anterior and intermediate lobes of the pituitary gland arise embryologically from Rathke pouch. Inadequate pouch obliteration results in cysts or cystic remnants at the interface between



• **Fig. 9.12** Pathogenesis of Rathke cysts. Schematic of the embryologic progenitors of sellar and parasellar structures. Rathke pouch arises from an outpocketing of stomodeum (ectoderm) and gives rise to the adenohypophysis. The pharyngohypophyseal stalk, which connects the stomodeum and Rathke pouch, is divided by the sphenoid bone as it grows together *(arrows),* isolating Rathke pouch and the neurohypophysis within the sella. (From Harrison MJ, Morgello S, Post KD. Epithelial cystic lesions of the sellular and parasellular region: a continuum of ectodermal derivates? *J Neurosurg.* 1994;80:1018–1025.)

the anterior and posterior pituitary lobes found in about 20% of pituitary glands at autopsy[64] (Fig. 9.12). Pituitary adenomas may also occasionally contain small cleft cysts. They are lined by cuboidal or columnar ciliated epithelium surrounding mucoid cyst fluid and arise from midline rudiments of failed Rathke cyst invagination and account for approximately 3% of pituitary mass lesions.[65] In contrast, pituitary epidermoid cysts are lined by squamous epithelium and rarely become malignant. Rathke cysts vary in size and may also extend to the suprasellar region. These lesions have heterogeneous MRI characteristics and may rarely present with panhypopituitarism with or without diabetes insipidus.[66] Most, however, are not symptomatic and in 43 of 75 patients followed for up to 126 months, cyst size did not change. Accordingly, most of these patients can be followed expectantly.[67] The extent of headache or visual disturbance is determined by the size and location of the cyst. Cyst formation is associated with sellar enlargement. MRI reveals hyperdense or hypodense masses on either T1-weighted or T2-weighted images, and CT scan shows homogeneous hypodense areas that may be distinguished from pituitary adenomas.[66] These patients should all be evaluated for hypopituitarism. After surgical resection or drainage, which may be required to alleviate severe headache,[68] MRI should be performed during long-term follow-up for signs of cyst recurrence.[64,65]

Arachnoid, epidermoid, and dermoid cysts develop mainly in the cerebellopontine angle but may also arise in the suprasellar region. Dermoid cysts containing greasy sebaceous products or hair follicles are rarely encountered in the pituitary, and the cyst lining may be calcified. Acquired pituitary cysts may arise secondarily to intrapituitary hemorrhage, usually associated with an underlying adenoma, and these rarely cause pituitary failure. Cyst compression causes internal hydrocephalus, visual disturbances, GH or ACTH deficiency, hyperprolactinemia, and diabetes insipidus. Rarely, squamous cell carcinoma may arise in the cyst.[69]

Granular Cell Tumors

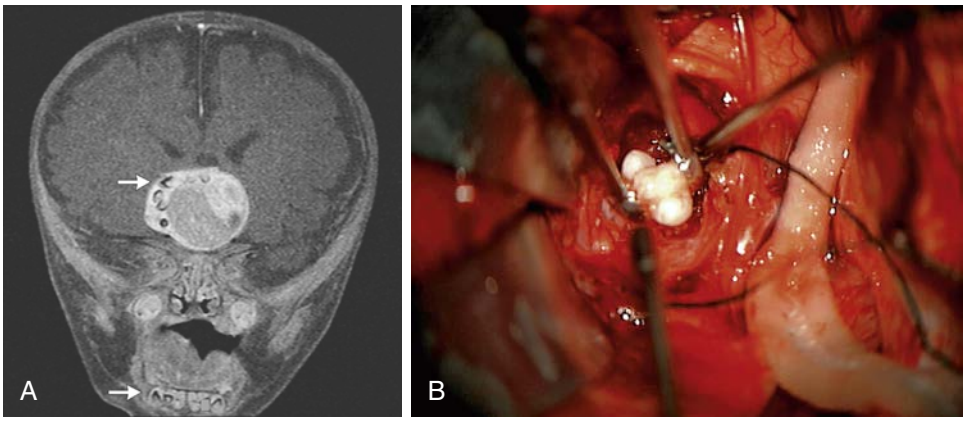
Pituitary choristomas, or schwannomas, usually present only after the age of 20. Their abundant cytoplasmic granules do not contain pituitary hormones, but these lesions may present with diabetes insipidus. Pituitary adenomas are occasionally coincidentally associated with these tumors.[70]

Chordomas

These slow-growing cartilaginous tumors arise from midline notochord remnants, are locally invasive, and may metastasize.[71] Most arise from the vertebrae and about one-third involve the clivus region. Chordomas contain a mucin-rich matrix that allows diagnosis by fine-needle aspiration. They present with headaches, asymmetric visual disturbances, hormone deficiency, and occasional nasopharyngeal obstruction. The tumor mass is associated with osteolytic bony erosion and calcification, and MRI may allow the normal pituitary gland to be distinguished from the very heterogeneous and often flocculent tumor mass. At surgery, the tumors are rough, heterogeneous, and lobular. Markers for epithelial cells, including cytokeratin and vimentin, are present. Recurrences commonly occur after surgical excision, with mean patient survival time of about 5 years. Rarely, chordomas undergo sarcomatous transformation with an aggressive natural history and require extensive surgical dissection.[72] Because of their anatomic location, the endoscopic endonasal approach may be preferable for chordoma surgical resection.[73]

Craniopharyngiomas

This parasellar tumor constitutes about 3% of all intracranial tumors and up to 10% of childhood brain tumors. The tumors are commonly diagnosed during childhood and adolescence.[74] However, they show a bimodal age distribution, occurring in children between 5 and 14 years and adults from 50 to 74 years of age.[75] Tumors arise from embryonic squamous remnants of Rathke pouch extending dorsally toward the diencephalon and may be large (>10 cm in diameter) and invade the third ventricle and associated brain structures. Over 60% arise from within the sella, and others arise from parasellar cell rests.[76,77] When intrasellar, they can often be distinguished from pituitary adenomas by separate visible rim of normal pituitary tissue seen on MRI (see Fig. 9.1A). The cystic mass is usually filled with cholesterol-rich viscous fluid, which may leak into the CSF, causing aseptic meningitis. These slow-growing tumors are composed of embryonic precursor cells and may also contain calcifications, teeth, and immunoreactive human chorionic gonadotropin (hCG) (Fig. 9.13).[78] Histologic appearance shows these tumors comprising two cell populations: Cysts are lined with a squamous epithelium containing islands characterized by columnar cells, and a mixed inflammatory reaction may also occur with calcification. Adamantinomatous craniopharyngiomas have a greater propensity to relapse than the less aggressive papillary variant.[79] Although large craniopharyngiomas may obstruct CSF flow, they rarely undergo malignant transformation. Increased intracranial pressure results in headache, projectile vomiting, papilledema, and somnolence, especially in children. Only about one-third of patients are over 40 years of age, and they commonly present with asymmetric visual disturbances, including papilledema, optic atrophy, and field deficits. If cavernous sinus invasion is present, other cranial nerves may also be involved. On CT imaging, most children and about half of all adults exhibit characteristic flocculent or convex calcifications. Rarely, however, pituitary adenomas, other parasellar tumors, and vascular lesions within the sella are also calcified.

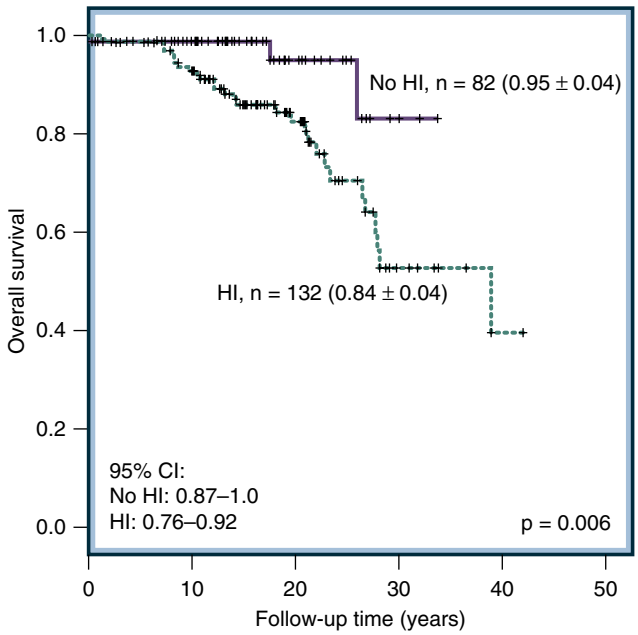


• **Fig. 9.13** Adamantinomatous craniopharyngioma containing teeth. (A) MRI showing tooth-like structures in the parasellar mass and mandible *(arrows).* (B) Fully formed teeth in the resected tumor. (From Beaty NB, Ahn E. Adamantinomatous craniopharyngioma containing teeth. *N Engl J Med*. 2014;370:860.)

In contrast to pituitary adenomas, where it is rarely encountered, diabetes insipidus is often the earliest feature of craniopharyngioma. These patients may also develop partial or complete pituitary deficiency. GH deficiency with short stature, diabetes insipidus, and gonadal failure are common. Pituitary stalk compression or damage to hypothalamic dopaminergic neurons results in hyperprolactinemia. Thus, craniopharyngioma may mimic a prolactinoma by intrapituitary imaging, presence of hyperprolactinemia, and favorable biochemical response to dopamine agonists.

Treatment of primary or recurrent craniopharyngiomas may involve radical surgery, radiotherapy, or a combination of these modalities.[75] A major side effect associated with such surgery is severe postoperative obesity, which can be mitigated by surgery that spares the hypothalamus.[80] The more complicated the surgical operation, the more visual problems are encountered. Patients with diabetes insipidus also have higher rates of anterior pituitary hormone deficits and subsequent obesity.[81] Treatment outcome appears to be related to hypothalamic involvement of the tumor, and careful hypothalamus-sparing surgery followed by local radiation therapy is recommended. Postoperative recurrences and tumor growth progressions are frequent.[74] Trans-sphenoidal microscopic surgery is successfully used for intrasellar craniopharyngiomas, and the expanded endoscopic transnasal approach has been successfully crafted to approach suprasellar tumors.[74] Adjuvant stereotactic irradiation is also used. Postoperative recurrence may occur in about 20% of patients undergoing radical surgical excision, and the degree of hypothalamic impairment is a major determinant of outcome (Fig. 9.14).[82] As several oncogenic mutations have been discovered in adamantinomatous (*CTNNB1*) and papillary (*BRAF* V600E) craniopharyngiomas, therapeutic approaches with BRAF/MEK targeting appear promising.[83]

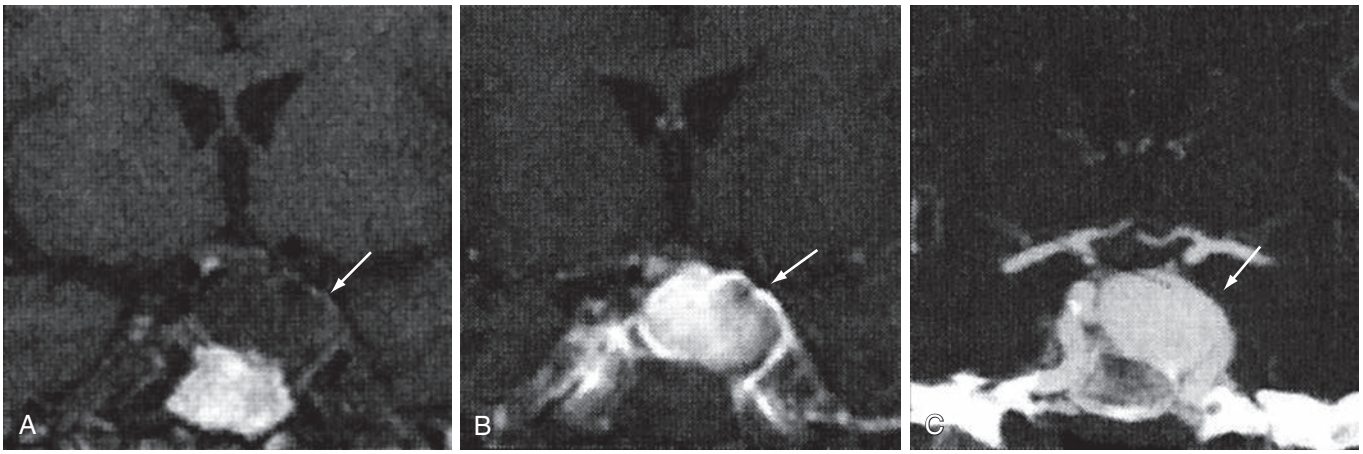
Life-complicating obesity that occurs after craniopharyngioma resection is associated with increased appetite (often insatiable), nonoptimal social development, as well as altered food intake–regulating hormones leptin and ghrelin.[82] Preoperative treatment with glucagon-like peptide-1 (GLP1) analogues led to weight loss.[84] These patients are at high risk for developing diabetes and cerebrovascular disease with increased mortality (standardized mortality ratio [SMR] 2.7; 95% confidence interval [CI]: 2.0–3.8) observed in 224 patients with 3153 years of follow-up.[85] Mortality from cerebrovascular disease is increased fivefold in those with childhood-onset craniopharyngiomas.[86]



• **Fig. 9.14** Overall survival for craniopharyngioma patients after surgical resection with and without hypothalamic involvement (HI). (Redrawn from Sterkenburg AS, Hoffmann A, Gebhardt U, et al. Survival, hypothalamic obesity, and neuropsychological/psychosocial status after childhood-onset craniopharyngioma: newly reported long-term outcomes. *Neuro Oncol*. 2015;17:1029–1038.)

Meningiomas

Meningiomas arise from arachnoid and meningioendothelial cells; those occurring in the sellar and parasellar region account for about one-fifth of all meningiomas.[87] Sellar meningiomas are usually well circumscribed and do not attain the size of craniopharyngiomas. Suprasellar meningiomas may invade the pituitary ventrally, and intrasellar tumor origins are rare.[88] Coexisting functional pituitary adenomas have been described in patients with parasellar meningiomas. Secondary hyperprolactinemia occurs in up to half of these patients, who usually present with local mass effects, including headache and progressive visual disturbances accompanied by optic atrophy. The differential distinction of a suprasellar meningioma with downward extension from an upwardly extending pituitary adenoma may be difficult. On MRI,



• **Fig. 9.15** Giant cavernous aneurysm of the left internal carotid artery. (A) Coronal T1-weighted MRI without intravenous contrast. A mildly hypointense mass is seen within the sella and left cavernous sinus. (B) Coronal T1-weighted MRI after gadolinium contrast. The mass enhances heterogeneously. (C) CT angiogram with maximum intensity reconstruction. *Arrows* indicate origin of the left internal carotid artery giant cavernous aneurysm. (From Lawson EA, Buchbinder BR, Daniels GH. Image in endocrinology: hypopituitarism associated with a giant aneurysm of the internal carotid artery. *J Clin Endocrinol Metab.* 2008;93:4616.)

meningiomas are isodense on both T1-weighted and T2-weighted imaging, in contrast to other parasellar lesions, which are usually hyperdense on T2-weighted imaging. Dural calcification may be evident on CT scanning. Because of their rich vascularization, these tumors pose an intraoperative risk for hemorrhage and a resultant higher surgical mortality rate than is usually encountered for pituitary adenoma resection.

Gliomas

Optic gliomas and low-grade astrocytomas arise from within the optic chiasm or optic tract, they often infiltrate the optic nerve, and less than one-third are intraorbital. Von Recklinghausen disease is the underlying cause in about one-third of these patients, and occasionally these tumors may be associated with growth retardation and delayed or precocious puberty and mass effects that include visual disturbances, diencephalic syndrome, diabetes insipidus, and hydrocephalus. Rarely, gliomas arise within the sella associated with hyperprolactinemia and should be considered in the uncommon differential diagnosis of a PRL-secreting pituitary adenoma.[89] Important distinguishing features include the young age of these patients (80% are <10 years old), relatively intact pituitary function, gross visual disturbances, and localization of the mass as visualized on MRI. Gliomas, unlike hamartomas, usually enhance after contrast injection.

Mucocele

Mucoceles are expanding accumulations of fluid within the sphenoid sinus and may compress parasellar structures. Headaches, visual disturbances (usually unilateral), and exophthalmos are characteristic features. On MRI, the homogeneous sphenoid mass may be quite prominent but may be distinguished from the pituitary gland dorsally.

Parasellar Aneurysms

A parasellar aneurysm may mimic a pituitary adenoma, and intraoperative rupture may be catastrophic, underlying the absolute need for preoperative diagnosis. Differentiating features of aneurysms from other pituitary masses may be subtle, including eye pain, very intense headaches, and relatively sudden onset of cranial nerve palsies (Fig. 9.15). Although imaging techniques usually distinguish blood and hemorrhage from solid tumor or tissue, a highly vascular meningioma may be confused with an aneurysm. Rarely encountered internal carotid artery aneurysms may cause hyperprolactinemia.[90]

Pituitary Infections

Acute pituitary abscesses and perisellar arachnoiditis are encountered with sinus infections, especially after transsphenoidal surgery. Pituitary abscess may develop from hematogenous or direct local spread of infectious agents or may arise within a preexisting pituitary adenoma and may be difficult to distinguish from an adenoma, as these patients may not have fever or signs of meningitis. They often present with diabetes insipidus and headache, and over 80% of a series of 66 patients presented with pituitary insufficiency.[91] On MRI, an isointense central cavity with surrounding ring enhancement is characteristic for an abscess.[4,91] In 33 consecutive patients with pituitary abscess, most presented with a mass associated with pituitary failure, and 5 had undergone previous surgery. Typical MRI features included a sellar cystic mass with an enhanced rim.[92] Gram-positive streptococci or staphylococci may originate from nasopharyngeal passages. Disseminated *Entamoeba histolytica*, *Pneumocystis jirovecii,* or *Klebsiella* may also seed to the pituitary.[93,94] Immunosuppressed patients may develop pituitary infections, including cytomegalovirus, toxoplasmosis, aspergillosis, histoplasmosis, and coccidiosis. Syphilitic gumma may also lead to pituitary damage, insufficiency, and painful hypophysitis.[95] Common viral infections, including influenza, measles, mumps, and herpes, are rarely associated with pituitary damage and insufficiency. Although tuberculosis is rarely confined to the pituitary gland, most of the fewer than 20 reported patients exhibited suprasellar extension of the pituitary mass, compromised pituitary function, and visual defects. Although systemic tuberculosis is usually present, isolated sellar tuberculomas have been described.[96]

Hematologic Malignancies

Primary CNS lymphomas are usually B-cell non-Hodgkin types, and to date, 33 such patients with pituitary lymphoma have been described.[97–99] The pituitary mass may be an isolated presentation of the underlying disease. The disorder is usually diagnosed by histologic finding of tissue obtained by excision biopsy. Presentation includes headache and cranial nerve abnormalities with varying degrees of hypopituitarism. MRI reveals cavernous sinus invasion and isotense T1-weighted and T2-weighted images, which enhance after gadolinium. About 38% of patients with solitary pituitary plasmacytomas may develop classic multiple myeloma.[100] Acute lymphoblastic leukemia may be associated with periglandular pituitary infiltrates with minimal pituitary dysfunction.

Pituicytoma

Pituicytoma is a rare benign central noninvasive suprasellar glial cell tumor that presents with mass effects or hypopituitarism.[101] The tumor, which is isointense or hypointense on T1-weighted MR and hyperintense on T2, arises from cells in the neurohypophysis and stains for vimentin, S100 protein, and glial fibrillar acidic protein.[102] The preferred effective resection is by an expanded endoscopic endonasal transsphenoidal and transplanum approach.[103]

Sarcoidosis

Hypothalamic granulomatous involvement is commonly encountered in patients with CNS sarcoidosis and may be the sole manifestation of the disease.[104] The hypothalamus, pituitary stalk, and posterior pituitary are diffusely invaded by noncaseating granulomas, consisting of giant cells, macrophages, and lymphocytes. These patients may present with varying degrees of anterior pituitary failure with or without diabetes insipidus.[105] Onset of diabetes insipidus with no obvious features of a pituitary disorder should alert the physician to exclude hypothalamic sarcoid deposits especially in the face of a thickened stalk on MRI.[106] In 24 patients with hypothalamic-pituitary sarcoid, all but 2 had anterior pituitary dysfunction (gonadotrophin deficiency 21/24, TSH deficiency 15/24, and hyperprolactinemia in 12/24 cases), and 12 had diabetes insipidus. Imaging studies showed pituitary stalk thickness and involvement of the infundibulum and pituitary gland, which improved or disappeared in 50% of patients. After glucocorticoid treatment, two patients experienced reversal of hypopituitarism.[107]

Langerhans Cell Histiocytosis

The disorder may be associated with granulomatous damage to the hypothalamus and/or posterior pituitary, with characteristic diabetes insipidus occurring in about 25% of children with this disorder.[108] Sleep disorders, adipsia, morbid obesity, axillary skin rash, history of recurrent pneumothorax, and classic bony lesions can occur.[109] Pituitary lesions comprise dendritic Langerhans cells, and pituitary MRI may reveal stalk thickening or a diminished posterior pituitary bright spot. Adults with the disorder should be carefully evaluated for anterior pituitary hormone deficits and appropriately replaced. Multisystem Langerhans cell histiocytosis causes long-term morbidity extending into adulthood.[110] Although surgery and radiation were, for many years, the mainstay treatments, a chemotherapeutic approach using cladribine has been successful in some patients.[111]

Hereditary Iron Storage Diseases

Hemochromatosis and hemosiderosis result in predominantly gonadotroph cell damage.

Idiopathic Retroperitoneal Fibrosis

Idiopathic retroperitoneal fibrosis may also be associated with a suprasellar mass and hypothalamic panhypopituitarism.[112]

Metastases to the Pituitary Region

Pituitary metastases are found in up to 3.5% of cancer patients,[113] especially in older patients with diffuse malignant disease. As the vascular supply to the posterior pituitary is derived directly from the systemic circulation via the internal carotid arteries, the posterior pituitary is the preferred site for bloodborne metastatic spread.[114] Over one-third of carcinomas that metastasize to the pituitary are breast metastases (37.2% of 425 reported cases), followed by lung (24.2%), prostate (5.2%), renal (4.9%), and 28 other sites.[115] Diabetes insipidus is a common presenting sign, and cranial nerve palsies and hypopituitarism may occur as well.[116] If extensive bony erosion is present and disease onset is rapid, the diagnosis is more readily apparent. However, pituitary imaging may not clearly distinguish metastatic deposits from a pituitary adenoma; these lesions may masquerade as an adenoma and the diagnosis only made by histologic study of the resected specimen.[117] When the diagnosis is clear cut in the presence of a primary cancer, relatively low-dose pituitary radiation may be sufficient to shrink the metastasis and improve morbidity.

Evaluation of Parasellar Lesions

Because the differential diagnosis of parasellar lesions covers a wide spectrum of neoplastic, vascular, inflammatory, and infectious processes, patient age and sex, relevant clinical history and symptoms, and other comorbid conditions can often help narrow down the differential diagnosis. MRI with gadolinium is essential in defining the exact location of the lesion, the gadolinium enhancement pattern, the presence of vascular voids or cystic regions, and associated vasogenic edema in surrounding brain tissue. Other imaging studies are often used in select cases: CT scan identifies calcification in craniopharyngiomas; CT angiogram, MR angiogram, or other angiography may be used when vascular lesions are suspected; and positron emission tomography may occasionally be used to identify metabolically active and rapidly growing lesions.

In a subset of lesions, serum molecular markers may be helpful in the diagnostic workup. For example, nongerminomatous germ cell tumors, which often localize to the suprasellar space, can be diagnosed by elevation of characteristic tumor markers, including β-hCG or α-fetoprotein. Furthermore, if the lesion is suspected to cause pituitary or hypothalamic dysfunction, pituitary function testing is useful in characterizing potential endocrine dysfunction. As parasellar lesions often impinge on the optic apparatus, due to their anatomic proximity, neuro-ophthalmologic evaluation, including Humphrey visual fields test, is required. The decision to resect a parasellar lesion depends on factors related to the patient (age, neurologic status, medical comorbid conditions) and the lesion itself (size, anatomic location, vascular pattern, benign or malignant growth pattern, sensitivity to radiotherapy or chemotherapy, sensitivity to medical therapy).

If resection is pursued, a number of surgical approaches can be used, including craniotomies (pterional, supraorbital, subfrontal), as well as endoscopic transnasal approaches. Minimally invasive transnasal endoscopy is useful for resection of select parasellar lesions, with excellent surgical outcomes and potentially fewer complications than those observed after conventional craniotomy.[30]

Primary Hypophysitis

Pituitary mass lesions composed of inflammatory cells may arise as primary disorders of the anterior and posterior pituitary glands or the neurohypophysis.[118,119] At least five clinicopathologic forms have been described, and the definitive diagnosis may require pituitary biopsy.

Lymphocytic Hypophysitis

This autoimmune inflammatory disorder may occur during or shortly after parturition[120] and has also been reported after menopause[119]; approximately 15% of reported cases occur in males. Of the 57% of patients developing the disorder in association with pregnancy, most often the disorder occurs during the last month of pregnancy or during the first 2 months postpartum.[119] It is characterized by a lymphocytic and plasma cell pituitary infiltrate, which may be isolated or associated with other recognized endocrinopathies. Circulating antipituitary antibodies have occasionally been reported, and the presence of isolated pituitary hormone deficiency may imply a selectively targeted autoimmune process to pituitary cell types. Although the natural history is often short lived, the few comprehensive pathologic evaluations suggest that secondary adenohypophyseal cell atrophy, with a resultant empty sella, is a frequent outcome. Pathologic criteria for diagnosis include islands of anterior pituitary cells surrounded by diffuse lymphocytic (T-cell and B-cell) infiltrates. The defining feature is lymphocytic infiltration comprising T and B lymphocytes; plasma cells were found in 53%, eosinophils in 12%, and macrophage histiocytes and neutrophils in 6% of cases,[119] and mast cells have also been identified.

Clinical Features

Over half of patients with lymphocytic hypophysitis present with headache, visual field impairment, and hyperprolactinemia,[119] and pituitary deficiency accounts for the remaining cases. Fifty-six percent of patients have secondary hypoadrenalism, followed in frequency by hypothyroidism, hypogonadism, and GH or PRL deficiency. Hypothyroidism may occur later, even after 9 months. In 76 patients reported by the German Pituitary Working Group, headache and obesity were prominent, and only 11% were associated with pregnancy. Hypogonadism was the most frequent feature of pituitary failure.[121] MRI reveals a pituitary mass, often indistinguishable from an adenoma. Both intrasellar and suprasellar pituitary enlargement occur, and the pituitary stalk may be thickened, especially when diabetes insipidus is present. The inflammatory process often resolves with time, and initially abnormal pituitary function may be restored as well or may remain chronically compromised. Diabetes insipidus, which is encountered in up to 20% of patients, has been attributed to posterior pituitary or stalk infiltration of the inflammatory process.[122] In a study of 95 patients with autoimmune hypopituitarism and positive antipituitary antibodies, those with central diabetes insipidus had antihypothalamic antibodies, suggesting autoimmune involvement of the hypothalamus rather than expansion of the pituitary inflammatory process.[123] In one-third of patients, other autoimmune conditions, including thyroiditis, hypoadrenalism, parathyroid failure, atrophic gastritis, systemic lupus erythematosus, or Sjögren syndrome, may also be present. The differential diagnosis includes prolactinoma and other sellar masses, and careful history and demonstrated loss of the posterior pituitary "bright spot" on MRI are useful for supporting the diagnosis.

Laboratory Findings

The erythrocyte sedimentation rate is often elevated; antibodies to a 49-kDa cytosolic protein were detected in 70% of patients with histologically confirmed lymphocytic hypophysitis and in 10% of control subjects.[124] PRL levels are usually elevated in both female and male patients. Hyperprolactinemia is expected during pregnancy and during the early postpartum period, and the mass effect may contribute to stalk compression and secondary hyperprolactinemia in the others. GH and ACTH responses to hypothalamic hormone challenges may be blunted. Rarely, the disorder may be associated with isolated ACTH or TSH deficiencies.

Treatment

If the diagnosis is convincingly supported, then in the absence of compressive visual field disturbances, surgical therapy should be withheld, pituitary hormone deficits appropriately replaced, and spontaneous resolution of the inflammatory mass expectantly followed. Treatment with high-dose glucocorticoids is the mainstay, often resolving the sellar mass and improving endocrine dysfunction, although recurrences are reported in over 40% of those responding to glucocorticoids.[125] Glucocorticoids are also indicated if adrenal reserve is compromised. Transsphenoidal or endoscopic surgical resection may be required to confirm a tissue diagnosis and may also relieve compression symptoms, but the degree of surgical resection should be constrained by the need to conserve viable pituitary tissue, particularly in view of frequent spontaneous resolution.[125]

Granulomatous Hypophysitis

Granulomatous hypophysitis is not usually associated with pregnancy, but in a systematic review comprising 82 patients, females predominated.[126] Rarely, the condition may coexist with lymphocytic hypophysitis in the same gland. Pituitary histologic appearance shows features of chronic inflammation and granuloma with histiocytes and multinucleated giant cells. Patients present with headache and may have aseptic meningitis. Fever, nausea, or vomiting at presentation and histologic evidence of necrosis correlate with reduced time to presentation. Panhypopituitarism at presentation is predictive of the requirement for long-term replacement therapy.[126] MRI shows pituitary enlargement. Suprasellar extension occurs in about 60% of patients, often with extension to or compression of the optic chiasm (25.7%). The condition may reflect an underlying systemic disorder such as sarcoidosis[127] or Takayasu disease.[128]

Xanthomatous Hypophysitis

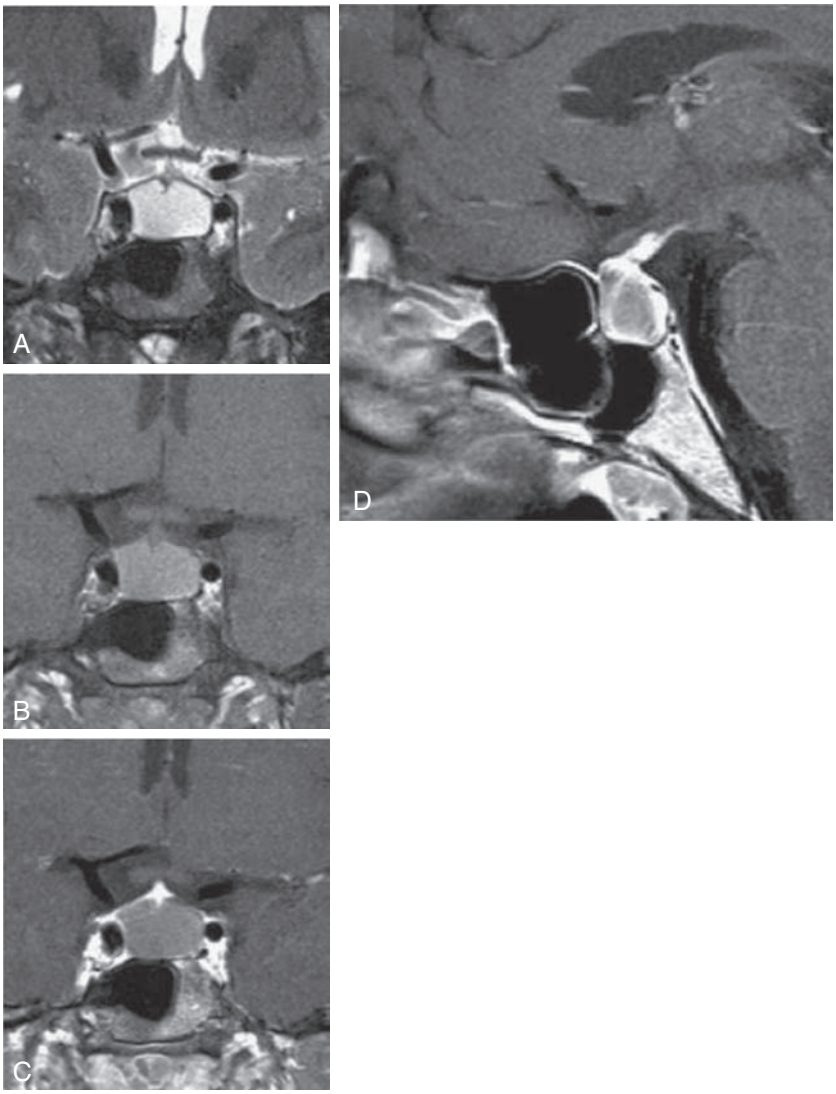
This rare primary pituitary inflammatory process occurs at equal frequency in both sexes and comprises lipid-laden macrophages. MRI often reveals a highly cystic lesion, possibly reflecting an inflammatory response to a ruptured pituitary cyst.

Necrotizing Infundibulo-Hypophysitis

This rare form of hypophysitis has been reported in patients with an enlarged sellar mass. Patients present with diabetes insipidus, hypopituitarism, and severe headache[129] (Fig. 9.16).

IgG4-Related Hypophysitis

Patients with these rarely encountered pituitary lesions may also have retroperitoneal fibrosis, pancreatitis, and thyroid, lung, and meningeal involvement.[130] Only 34 such patients have been reported,[131] and they may present with pituitary failure, diabetes insipidus, and elevated IgG4 levels.



• **Fig. 9.16** Infundibulo-hypophysitis depicted by MRI. The 22 × 20 × 16-mm pituitary mass is isointense to hyperintense on T2-weighted image with suprasellar extension (A) and hypointense in T1-weighted, non-enhanced image (B). After intravenous contrast (C), only slight to moderate and inhomogeneous enhancement occurs, most pronounced at the periphery of the mass. The pituitary stalk and infundibulum are slightly thickened, showing avid enhancement (D). (From Gutenberg A, Caturegli P, Metz I, et al. Necrotizing infundibulo-hypophysitis: an entity too rare to be true? *Pituitary.* 2012;15:202–208.)

Immune Checkpoint Inhibitor-Induced Hypophysitis

This form of hypophysitis is caused by exposure to an antibody, ipilimumab, used to treat metastatic cancer. The drug blocks the cytotoxic T-lymphocyte antigen 4 (CTLA4), which is also expressed on pituitary tissue, leading to local complement activation[132] (Table 9.8). In a single-center analysis of 211 patients with advanced melanoma, an 8% overall incidence of hypophysitis was reported. Presenting symptoms include headache, nausea, vomiting, extreme fatigue, diarrhea, arthralgias, and mental status changes.[133] The median time to onset following drug administration was 4 months, but a delay in up to 19 months after treatment was also observed. The most common endocrine deficit encountered was secondary adrenal insufficiency (84%). In contrast to other forms of hypophysitis, no patients receiving ipilimumab developed diabetes insipidus.[134] Many also had evidence of hypothyroidism/thyroiditis (6%). Eleven of 19 patients had biochemical evidence consistent with central hypothyroidism (low free T_4 [thyroxine] and normal or low TSH). Diabetes has also been observed in these patients. Pituitary MRIs were clearly abnormal in most patients with diffuse enlargement. Treatment with high-dose steroids initially, followed by replacement doses in those with sustained secondary adrenal insufficiency, is effective. However, few patients exhibit return of normal endocrine function.[135] Azathioprine has been found effective in some patients.[136] Less commonly, the antibodies pembrolizumab, nivolumab, and their respective combinations may also induce hypophysitis.

Hemorrhage and Infarction

Intrapituitary hemorrhage and infarction are usually caused by ischemic damage to the hypophyseal-portal system and may be catastrophic. These acute events cause significant damage to the

TABLE 9.8 Features of Ipilimumab-Induced Hypophysitis

Measurement	Result
Incidence	10%
Ipilimumab dose	3 or 10 mg/kg
M:F ratio	2:1
Time until hypophysitis diagnosis	7–16 weeks
Anterior hypopituitarism	All affected patients
Central hypothyroidism	60–100%
Adrenal insufficiency	50–84%
Thyroiditis	Up to 25%
Hyponatremia	Up to 50%
Prolactin	Usually low
Testosterone	Usually low

Data from retrospective analyses of 365 patients treated for melanoma in Faje AT, Sullivan R, Lawrence D, et al. Ipilimumab-induced hypophysitis: a detailed longitudinal analysis in a large cohort of patients with metastatic melanoma. *J Clin Endocrinol Metab*. 2014;99:4078–4085; Ryder M, Callahan M, Postow MA, et al. Endocrine-related adverse events following ipilimumab in patients with advanced melanoma: a comprehensive retrospective review from a single institution. *Endocr Relat Cancer*. 2014;21:371–381.

pituitary gland, and small clinically silent microinfarcts are found in up to 5% of unselected autopsies. Pituitary cells are relatively resilient to vascular insult, and pituitary insufficiency is only clinically apparent when approximately 75% of the gland is ischemically damaged. Ten percent residual functional pituitary cell mass appears sufficient to mask complete pituitary failure. Ischemic damage is limited to the anterior lobe, and posterior pituitary function usually remains intact, reflecting the predominant neural control of oxytocin and ADH secretion. Acute intrapituitary hemorrhage can cause significant life-threatening damage to the pituitary and its surrounding vital structures.[137]

Postpartum Pituitary Infarction

During pregnancy, the pituitary gland normally enlarges in response to estrogen stimulation. The hypervascular gland is thus particularly vulnerable to arterial pressure changes and prone to hemorrhage. Sheehan syndrome classically described after severe postpartum hemorrhage is now less commonly encountered with the advent of modern obstetric care,[138] but it occurs much more frequently in developing countries.[139,140] The presentation varies from development of hypovolemic shock resulting in adenohypophyseal vessel vasospasm and pituitary necrosis to the gradual onset of partial to complete pituitary insufficiency over months to years. Initial presentations also include hyponatremia, asthenia, and weight loss. Most prominent among symptoms are inability to nurse and postpartum amenorrhea.[139] Pituitary autoimmunity has been implicated in gland failure after postpartum hemorrhage.[141]

Pituitary Apoplexy

Pituitary apoplexy may result from spontaneous hemorrhage into a pituitary adenoma (pituitary tumor apoplexy) or may occur after head trauma, with skull base fracture, or in association with hypertension and diabetes mellitus, sickle cell anemia, or acute hypovolemic shock[142] (Table 9.9). Precipitating factors include major surgery, pregnancy, stereotactic irradiation, anticoagulant therapy,[143] coagulopathy secondary to liver failure,[144] and administration of thyrotrophin-releasing hormone (TRH), gonadotrophin-releasing hormone (GnRH) agonists, bromocriptine, and cabergoline.[145,146]

Clinical Features

Pituitary apoplexy is often an endocrine emergency.[147] The condition may evolve over 1 to 2 days, usually presenting with severe headache and ocular palsies or visual field defects. Cardiovascular collapse, change in consciousness, neck stiffness, and sometimes hypoglycemia may occur. Bilateral cerebral infarction may also occur.[148] Acute adrenal insufficiency is a frequent occurrence due to loss of ACTH. It may also be superimposed owing to disordered intravascular clotting disorders, heparin administration, or acute effects of CNS hemorrhage. Pituitary imaging without contrast (CT or MRI) usually reveals signs of intrapituitary or intra-adenoma hemorrhage, stalk deviation, compression of normal pituitary tissue, and in severe cases signs of parasellar hemorrhage[149] (Fig. 9.17). In a study of 13 consecutive patients with pituitary apoplexy, baseline serum cortisol was below 5 μg/dL in 7, between 5 and 15 μg/dL in 4, and above 15 μg/dL in 2 patients. Five patients also had low T_4 levels, and all 13 had evidence of gonadal dysfunction. Thus, these patients with underlying pituitary tumors likely had preexisting pituitary insufficiency.[150] Apoplexy, like Sheehan syndrome, is one of the few pituitary tumor presentations in which hyperprolactinemia is not a feature unless the infarction occurs within a prolactinoma. Patient characteristics, signs and symptoms, and outcomes in 207 patients in five series are portrayed in Table 9.9.[13]

Management

Patients with visual field compromise require emergency transsphenoidal surgery. Others may recover spontaneously but may develop long-term pituitary insufficiency. Patients who are fully alert and conscious with no visual symptoms may be observed. The decision to initiate therapy with high-dose glucocorticoids depends on the clinical status,[137] but the high incidence of adrenal dysfunction either before or after treatment indicates a need for replacement or stress doses of cortisone in most. Ophthalmoplegia, which is common, may resolve spontaneously over time.[137] Postoperative recovery of visual function correlates inversely with the time elapsed subsequent to the acute hemorrhage.[151] Cranial nerve palsies, however, often improve whether or not surgery is undertaken. Pituitary function does not commonly recover after resolution of the acute hemorrhage, and adrenal, thyroid, and gonadal steroid hormone replacement may be required. The subsequent atrophy of infarcted pituitary tissue often results in the development of a complete or partially empty sella evident on MRI.

Pituitary Adenomas

Pituitary Trophic Activity

Benign Adenomas

Transgenic animal models have been developed in which pituitary growth factors or genes have been overexpressed or deleted to recapitulate both functional and nonfunctional pituitary adenomas. For example, a transgenic zebrafish expressing corticotroph-targeted pituitary tumor-transforming gene (*PTTG*) recapitulates a Cushing phenotype with pituitary adenoma growth

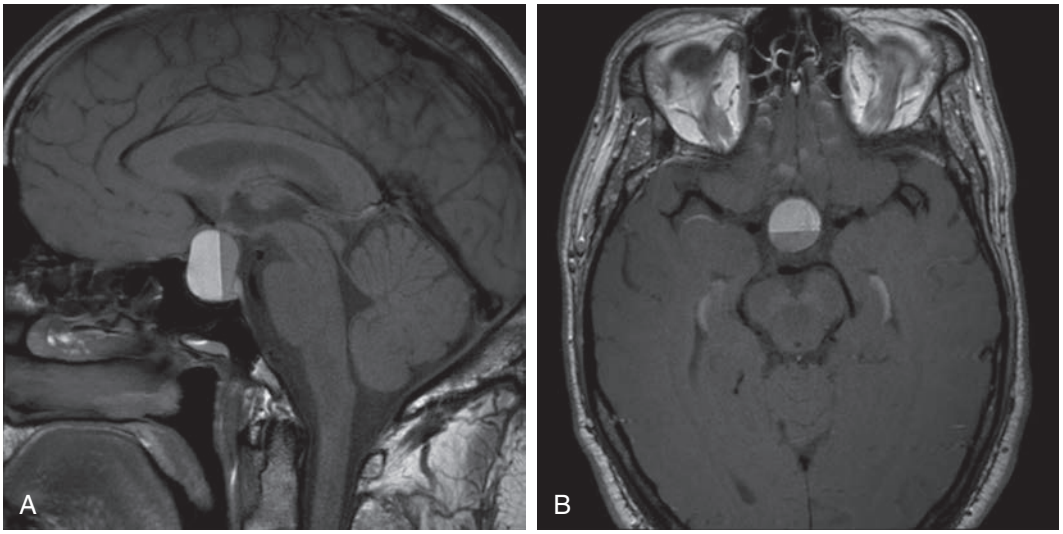
TABLE 9.9 Presentation and Outcomes After Conservative or Surgical Management of Pituitary Apoplexy

	AYUK (2004)		GRUBER (2006)		SIBAL (2004)		LEYER (2011)		BUJAWANSA (2014)	
	Conservative	Surgery	Conservative	Surgery	Conservative	Surgery	Conservative	Surgery	Conservative	Surgery
N	18	15	20	10	18	27	25	19	22	33
Presentation										
Decreased visual acuity, n (%)	NA	NA	11 (55)	7 (70)	4/15 (26)	14/24 (58)[a]	8 (32)	16 (84)	NA	NA
Visual field defect, n (%)	6 (33)	7 (46)	4 (20)	6 (60)	4/17 (24)	16/25 (64)[a]	5 (20)	14 (74)	10 (45)	13 (39)
Ocular palsy, n (%)	7 (39)	8 (53)	12 (60)	3 (37)	8/17 (47)	14/26 (54)	12 (48)	10 (53)	15 (68)	18 (54)
Hypopituitarism, n (%)	13 (87)	15 (83)	15 (75)	9 (90)	13/18 (72)	21/24 (87)	20/23 (87)	15/17 (88)	NA	NA
Outcome										
Decreased visual acuity										
Complete resolution, n (%)	NA	NA	5/11 (45)	4/7 (57)	3/4 (75)	8/14 (57)	6/8 (75)	7/16 (44)	NA	NA
Partial resolution, n (%)	NA	NA	4/11 (36)	2/7 (28)	1/4 (25)	5/14 (36)	1/8 (12)	1/16 (6)	NA	NA
No improvement, n (%)	NA	NA	2/11 (19)	1/7 (15)	0	1/14 (7)	1/8 (12)	6/16 (37)	NA	NA
Visual field defect										
Complete resolution, n (%)	6/6 (100)	4/7 (57)	2/4 (50)	2/6 (33)	3/4 (75)	7/16 (43)	4/5 (80)	8/14 (57)	6/10 (60)	4/13 (31)
Partial resolution, n (%)	0	NA	1/4 (25)	3/6 (50)	1/4 (25)	8/16 (50)	NA	1/14 (7)	NA	NA
No improvement, n (%)	0	NA	1/4 (25)	1/6 (17)	0	1/16 (7)	NA	4/14 (29)	NA	NA
Ocular palsy										
Complete resolution, n (%)	7/7 (100)	5/8 (63)	10/12 (83)	2/3 (66)	6/8 (75)	9/14 (64)	11/12 (92)	6/10 (60)	15/15 (100)	15/18 (83)
Partial resolution, n (%)	0	NA	2/12 (17)	1/3 (33)	2/8 (25)	4/14 (29)	1/12 (9)	1/10 (10)	0	3/18 (17)
No improvement, n (%)	0	NA	0	0	0	1/14 (7)	0	2/10 (20)	0	0
Endocrine function										
Normal	NA	NA	1 (5)	2 (20)	2 (11)	5 (19)	9 (37)	3 (16)	2/22 (9)	3/33 (9)
ACTH deficiency, n (%)	13/18 (72)	13/15 (87)	(68)	(60)	NA	NA	NA	NA	NA	NA
TSH deficiency, n (%)	9/15 (60)	13/15 (87)	(70)	(68)	NA	NA	NA	NA	NA	NA
LH/FSH deficiency, n (%)	15/18 (83)	10/15 (67)	(80)	(86)	NA	NA	NA	NA	NA	NA

[a] $P = 0.01$.

ACTH, Adrenocorticotrophic hormone *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone; *NA,* not available; *TSH,* thyrotrophic hormone.

Modified from Briet C, Salneve S, Bonneville JF, et al. Pituitary apoplexy. *Endocr Rev.* 2015;36:622–645.



• **Fig. 9.17** Pituitary apoplexy depicted by MRI showing intrapituitary fluid levels. T1-weighted sagittal (A) and (B) axial images show hyperintensity in the upper compartment and isointensity in the lower compartment. (From Briet C, Salenave S, Bonneville JF, et al. Pituitary apoplexy. *Endocr Rev*. 2015;36:622–645.)

and hypercortisolism.[152] In humans, benign monoclonal pituitary adenomas arise from differentiated pituitary cells (see Fig. 9.2). Pituitary trophic signals may enhance or restrain expansion of a monoclonal tumor cell population by regulating the intrapituitary milieu.[153,154]

Normal and hyperplastic pituitary tissues are polyclonal, whereas pituitary adenomas arise as the result of monoclonal pituitary cell proliferation. Using X-chromosomal inactivation analysis, the monoclonal origin of GH-secreting, PRL-secreting,[155] and ACTH-secreting adenomas[156,157] and nonfunctioning pituitary tumors was confirmed in female patients heterozygous for variant alleles of the X-linked genes hypoxanthine phosphoribosyltransferase (*HPRT*) and phosphoglycerate kinase (*PGK*). Thus an intrinsic somatic pituitary cell genetic alteration likely gives rise to clonal expansion of a single cell, resulting in adenoma formation (Table 9.10).

The pituitary gland responds to central and peripheral signals that regulate both hormone production and cell proliferation ranging from microadenoma to aggressive adenoma (Fig. 9.18). For example, during pregnancy hypothalamic and peripheral hormones act to regulate pituitary trophic activity resulting in increased pituitary volume, and prolonged target gland failure (e.g., hypothyroidism) can cause pituitary hyperplasia by releasing the gland from negative feedback inhibition. There is, however, no direct evidence in humans that pituitary hyperplasia is a prerequisite for tumor development. Thus lactotroph hyperplasia occurring with pregnancy and lactation does not lead to increased frequency of prolactinomas. Oral contraception use is also not associated with pituitary adenoma development, and somatotroph hyperplasia caused by ectopic GHRH production[158] is not commonly associated with true adenoma formation. Adenohypophyseal tissue surrounding pituitary tumors is usually not hyperplastic, supporting the notion that hypothalamic hormones, pituitary growth factors, and sex steroid hormones enable a permissive environment, which potentiates cell mutation and subsequent tumor growth.

Hormonal Factors

Hypothalamic factors may have a specific role in the pathogenesis of pituitary cell proliferation, in addition to regulating pituitary hormone gene expression and secretion (see Table 9.10). For example, ectopic GHRH-secreting tumors (bronchial neuroendocrine tumors, pancreatic islet cell tumors, or small cell lung carcinomas) result in GH hypersecretion and acromegaly with somatotroph hyperplasia.[158,159] In transgenic mice overexpressing a GHRH transgene, the pituitary size increases dramatically due to somatotroph hyperplasia, and older mice develop GH-secreting adenomas.[160] However, adenomatous hormonal secretion is usually independent of physiologic hypothalamic control, and the surgical resection of small well-defined adenomas usually results in definitive cure of hormonal hypersecretion. These observations imply that these tumors do not arise because of excessive polyclonal pituitary cell proliferation due to generalized hypothalamic stimulation. However, hypothalamic factors may promote and maintain growth of already transformed adenomatous pituitary cells (Fig. 9.19).

Genetic Factors

Activating mutations of the stimulatory G protein (G_s) are present in up to 40% of human GH-secreting adenomas (Table 9.11). These somatic heterozygous activating point mutations of the G protein α-subunit ($G\alpha_s$) gene involving either arginine 201 (replaced by cysteine or histidine) or glutamine 227 (replaced by arginine or leucine) constitutively activate the $G\alpha_s$ protein and convert it into an oncogene (*GSP*). This G protein activation increases cyclic adenosine monophosphate (cAMP) levels; activates protein kinase A, which in turn phosphorylates the cAMP response element-binding protein (CREB); and leads to sustained constitutive GH hypersecretion and cell proliferation. *GSP*-bearing adenomas are smaller, have mildly lower GH levels and enhanced intratumoral cAMP, do not respond briskly to GHRH, and are sensitive to the inhibitory effect of somatostatin.[161] *GSP*-activating mutations do not occur in PRL-secreting and in TSH-producing adenomas and are very rarely present in nonfunctioning pituitary tumors or ACTH-secreting tumors (<10%). Similar early postzygotic somatic mutations in codon 201 of the $G\alpha_s$ were identified in tissues derived from patients with McCune-Albright syndrome.[162] As transgenic mice overexpressing an inactive pituitary CREB mutant exhibit a dwarf phenotype and somatotroph hypoplasia,[163] cAMP likely stimulates

TABLE 9.10 Factors Involved in Pituitary Tumor Pathogenesis

Hereditary

MEN1
Carney complex
AIP mutations

Hypothalamic

Excess GHRH or CRH production
Receptor activation
Dopamine deprivation

Pituitary

Signal transduction mutations or constitutive activation (*GSP, USP8, GPR101, CREB,* McCune-Albright syndrome)
Disrupted paracrine growth factor or cytokine action (EGFR, FGF2, FGF4, LIF, BMP, STAT3)
Activated oncogene or cell cycle disruption (PTTG, RAS, P27, HMG)
Intrapituitary paracrine hypothalamic hormone action (GHRH, TRH)
Loss of tumor suppressor gene function with LOH (11q13, GADD45γ)

Environmental

Estrogens
Irradiation

Peripheral

Target failure (ovary, thyroid, adrenal)
Ectopic hypothalamic hormone secretion

Evidence for an Intrinsic Pituitary Defect in the Pathogenesis of Pituitary Tumors

Pituitary adenomas are monoclonal.
There is no hyperplasia surrounding the adenomas.
Surgical resection of well-circumscribed small adenomas leads to biochemical control in ~75% of patients.
Unrestrained pituitary hormonal hypersecretion persists independent of feedback suppression by elevated target hormones.
Hormonal pulsatility pattern is often restored after adenoma resection.

AIP, Aryl hydrocarbon receptor–interacting protein; *BMP,* bone morphogenetic protein; *CREB,* cyclic adenosine monophosphate response element–binding protein; *CRH,* corticotrophin-releasing hormone; *EGF,* epidermal growth factor; *FGF,* fibroblast growth factor; *GADD45γ,* growth arrest and DNA damage–inducible gamma gene; *GHRH,* growth hormone–releasing hormone; *GSP,* stimulatory G protein α-subunit oncogene; *HMG,* high mobility group; *LIF,* leukemia inhibitory factor; *LOH,* loss of heterozygosity; *MEN1,* multiple endocrine neoplasia type 1; *P27,* cyclin-dependent kinase inhibitor 1B; *PROP1,* prophet of Pit1 (paired-like homeodomain transcription factor); *PTTG,* pituitary tumor-transforming gene; *RAS,* RAS family of oncogenes; *TRH,* thyrotrophin-releasing hormone.

Data from Melmed S. Acromegaly pathogenesis and treatment. *J Clin Invest.* 2009;119:3189–3202; Melmed S. Pathogenesis of pituitary tumors. *Nat Rev Endocrinol.* 2011;7:257–266.

somatotroph proliferation mediated by CREB phosphorylation. This was borne out by the observation that 15 human GH-secreting pituitary adenomas were shown to express elevated levels of phosphorylated CREB.[164] However, only four of these tumors also contained the mutant *GSP* oncogene, and CREB phosphorylation was also demonstrated in adenomas overexpressing wild-type $G\alpha_s$ protein, suggesting a trophic role of CREB independent of G protein actions. Other signaling pathways overexpressed in pituitary tumors include those for AKT and mitogen-activated protein kinase (MAPK).[165]

Mice with heterozygous *Rb1* inactivation develop pituitary tumors with high penetrance, but mice with deregulated pituitary E2F activity develop tissue hyperplasia without progression to tumor formation, likely because sustained E2F activity ultimately triggers premature senescence in a pRB-dependent, p16-dependent, and p19-dependent fashion.[166] About 15% of spontaneous pituitary adenomas exhibit loss of heterozygosity (LOH) for chromosomes 11q13, 13, and 9, often correlating with tumor size and invasiveness. Although highly invasive pituitary tumors and pituitary metastases exhibit LOH of region 13q14 (*RB1* locus), no distinct tumor suppressor gene has yet been identified for sporadic pituitary tumors.[167] Nevertheless approximately 25% of GH-secreting adenomas do exhibit loss of pRB expression, likely associated with promoter hypermethylation.[168] *TP53* gene mutations (encoding the p53 protein) have not been detected in pituitary adenomas or in pituitary carcinomas and their metastases.[169]

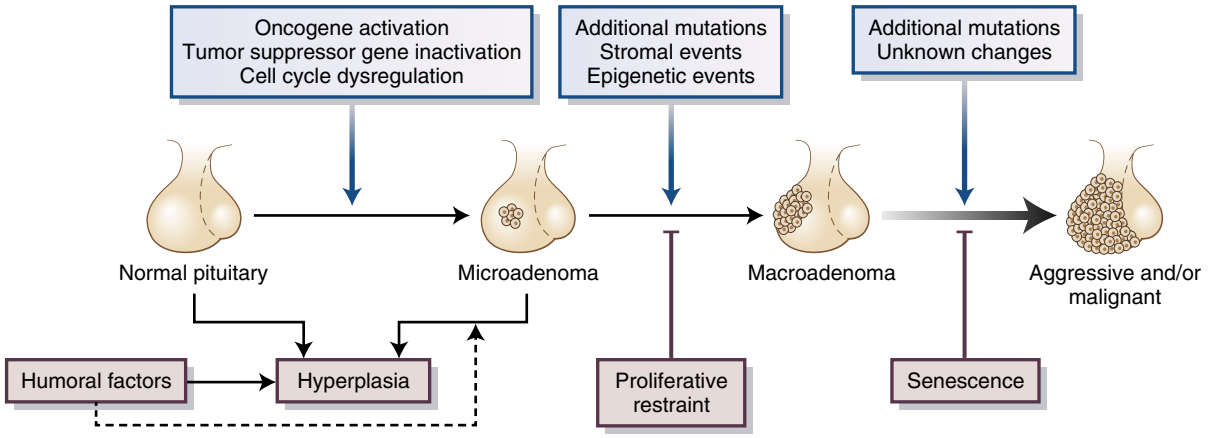
PTTG, isolated from experimental pituitary tumors, is abundant in all pituitary tumor types, especially prolactinomas.[170,171] *PTTG*, a mammalian securin homolog, also induces fibroblast growth factor (FGF) production and angiogenesis and is upregulated by estrogen.[172] *PTTG* overexpression may lead to dysregulated chromatid separation and cell aneuploidy,[173,174] and pituitary targeted *PTTG* transgene expression in mice leads to hormone-secreting adenomas.[175] *PTTG* mutations have not been identified in pituitary tumors, but tumor *PTTG* abundance is induced by the RWD-containing sumoylation enhancer (RSUME).[176] In a meta-analysis of 1464 adenomas in 24 studies, *PTTG* abundance was shown to correlate with pituitary tumor invasiveness.[177]

Cyclin D1, D2, and D3 are upregulated when quiescent cells enter the cell cycle and cyclin-dependent kinase (CDK) complexes lead to pRB phosphorylation, releasing E2F to promote cell cycle progression. Allelic imbalance at the *CCND1* locus encoding cyclin D1 is frequently observed in invasive, nonfunctioning pituitary adenomas.[178] The CDK4 and CDK6 inhibitor p16INK4a, encoded by the *CDKN2A* gene, maintains RB in the unphosphorylated state. In nonfunctioning pituitary tumors, the *CDKN2A* promoter is hypermethylated and consequently not expressed; less frequently the *CDKN2B* gene that encodes p15INK4b is also silenced. Mice deficient in p18INK4c develop features of gigantism with intermediate lobe pituitary hyperplasia and tumors.[179] When the CDK1 and CDK2 inhibitor p27Kip1 is knocked out, multiorgan hyperplasia occurs with development of intermediate lobe pro-opiomelanocortin (POMC) cell pituitary tumors.[180]

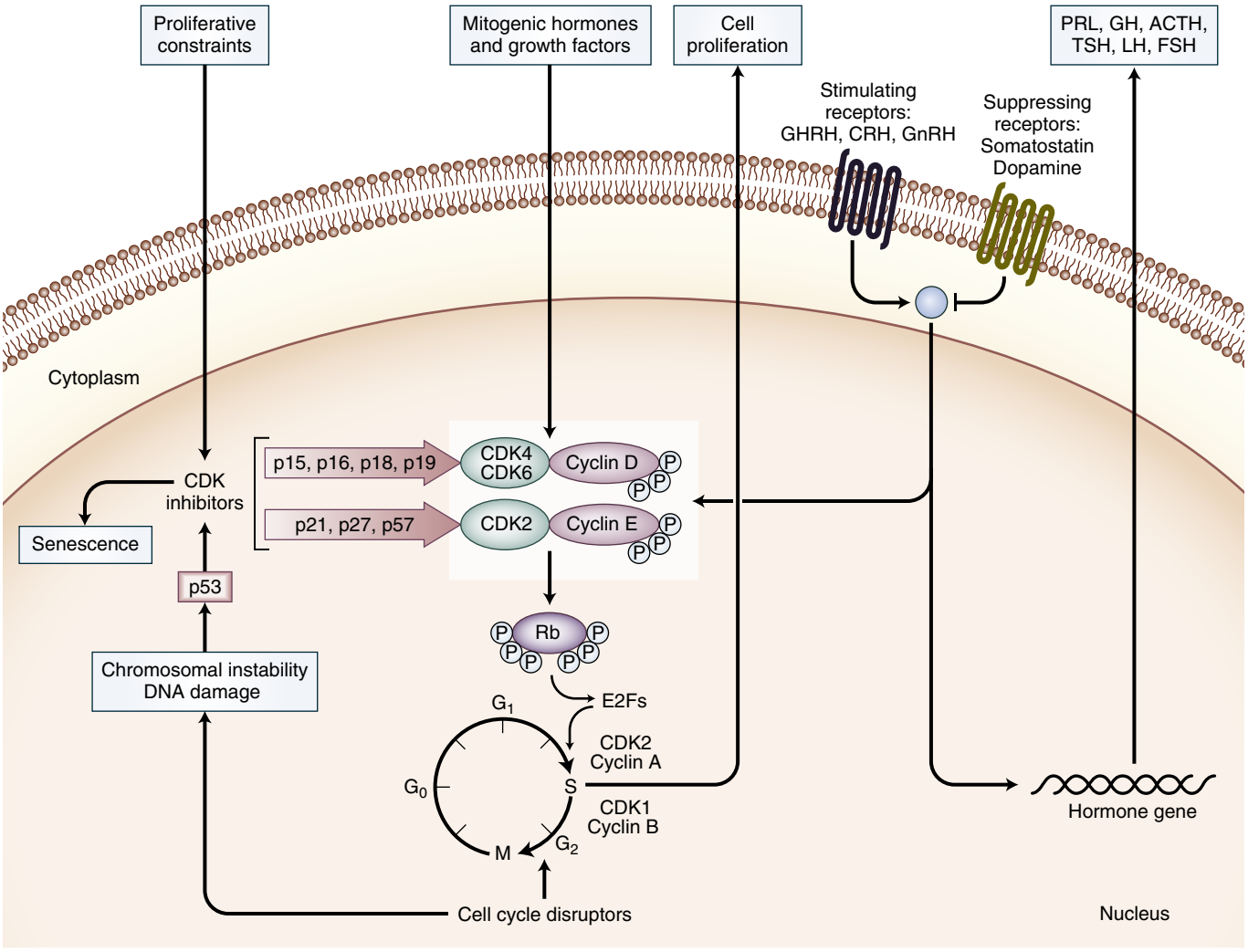
Basic FGF (FGF2) is abundantly expressed in the pituitary, and pituitary PTTG and FGF2 expression is increased in a time-dependent and dose-dependent manner in estrogen-treated rats.[172] Human prolactinomas express FGF4, and transfected FGF4 enhances PRL secretion and tumor vascularity.[181]

Epidermal growth factor (EGF) exhibits potent mitogenic activity in pituitary cells, and both EGF and its receptor EGFR are overexpressed in pituitary tumors, particularly in ACTH-secreting and nonfunctioning adenomas. EGFR abundance in a subset of ACTH-secreting tumors is induced by *USP8* mutations leading to deubiquitination.[182] Both ErbB2 and ErbB3 are expressed in aggressive, recurrent prolactinomas.[183] Gefitinib, the EGFR antagonist, decreases experimental prolactinoma cell proliferation and PRL secretion in vitro and in vivo, associated with abrogated EGFR/ERK signaling.[184] Two patients with dopamine-resistant aggressive prolactinomas showed attenuation of PRL levels and stabilization of tumor growth after treatment with lapatinib, a tyrosine kinase inhibitor.[185]

The growth arrest and DNA damage–inducible protein γ (GADD45γ), a tumor growth suppressor, is silenced in most pituitary adenomas, likely by methylation of CpG islands in



• **Fig. 9.18** Cascade of pituitary tumorigenesis. Pituitary hyperplasia is usually reversible, as exemplified by the situation that occurs during pregnancy. (Modified from Melmed S. Pathogenesis of pituitary tumors. *Nat Rev Endocrinol.* 2011;7:257–266; Di Ieva A, Rotondo F, Syro LV, et al. Aggressive pituitary adenomas: diagnosis and emerging treatments. *Nat Rev Endocrinol.* 2014;10:423–435, used with permission.)



• **Fig. 9.19** Pituitary tumorigenesis. Transcription of hormone genes in a differentiated cell and cell proliferation are mostly induced by pituitary mitogenic factors, including hypothalamic hormones and transcription factors, as well as endocrine hormones. Pituitary proliferative constraints include somatotrophin release-inhibiting factor and tumor suppressor genes. Cell cycle progression through G1 to S phase is mediated by CDK-cyclin complexes that phosphorylate Rb and cause it to release E2F, which drives cell proliferation. CDK inhibitors block kinase phosphorylation, thereby restraining the cell cycle. Chromosomal instability, DNA damage, and senescence may act to constrain malignant transformation of pituitary tumors. *ACTH,* adrenocorticotrophic hormone; *CDK,* cyclin-dependent kinase; *CRH,* corticotrophin-releasing hormone; *FSH,* follicle-stimulating hormone; *GHRH,* gonadotrophin-releasing hormone; *GH,* growth hormone; *GnRH,* growth hormone–releasing hormone; *LH,* luteinizing hormone; *PRL,* prolactin; *TSH,* thyroid-stimulating hormone. (Modified from the American Society for Clinical Investigation © Melmed S. Acromegaly pathogenesis and treatment. *J Clin Invest.* 2009;119:3189–3202, used with permission.)

TABLE 9.11 Selected Genes Associated With Molecular Pathogenesis of Pituitary Adenomas

Gene	Function	Mode of Activation or Inactivation	Clinical Context
GNAS	Oncogene	Activating, imprinting	Nonfamilial, syndromic or sporadic
CREB	Transcription factor	Constitutive phosphorylation	Sporadic
AIP	Tumor suppressor	Inactivating	Familial, syndromic
MEN1	Tumor suppressor	Inactivating	Familial, syndromic
PRKAR1A	Tumor suppressor	Inactivating	Familial, syndromic
H-Ras	Oncogene	Activating	Invasive or malignant
CCNB2	Cyclin	Induced by HMGA	Sporadic
CCND1	Oncogene	Overexpression	Sporadic
CDKNIB	Cyclin-dependent kinase inhibitor	Inactivating	Sporadic, syndromic
HMGA2	Oncogene	Overexpression	Sporadic
FGFR4	Oncogene	Alternative transcription	Sporadic
PTTG	Securin	Overexpression	Sporadic
Rb	Tumor suppressor	Epigenetic silencing	Sporadic
CDKN2A	Cyclin-dependent kinase inhibitor	Epigenetic silencing	Sporadic
BRG1	Tumor suppressor	Glucocorticoid receptor function	Sporadic
GADD45G	Proliferation inhibitor	Epigenetic silencing	Sporadic
MEG3	Proliferation inhibitor	Epigenetic silencing	Sporadic
USP8	Pro-proliferative (corticotrophs)	Ubiquitination	Sporadic
GPR101	Hypersecretion (GH)	Duplication	Sporadic
STAT3	Hypersecretion (GH)	Transcriptional	Sporadic

Modified from Melmed S. Acromegaly pathogenesis and treatment. *J Clin Invest.* 2009;119:3189–3202; used with permission of the American Society for Clinical Investigation.

the gene promoter.[186] An isoform of *MEG3* containing an extra exon (*MEG3a*) is undetectable in both nonfunctioning and GH-secreting pituitary adenomas, conferring a tumor growth advantage, likely due to hypermethylation of the promoter region.[187]

Transgenic *HMGA1* and *HMGA2* overexpression results in murine GH-secreting adenomas and prolactinomas, and chromosome 12 trisomy, the locus for *HMGA2*, is frequently encountered in human PRL-secreting pituitary adenomas. *HMGA2* was overexpressed in 38 of 98 (39%) pituitary adenomas, being found in 15 of 22 FSH/LH-secreting adenomas (68%), 5 of 15 prolactinomas (31%), and 12 of 18 ACTH-secreting adenomas (18%).[188] *HMGA2* levels correlate with tumor size, invasiveness, and proliferation markers. *HMGA2* tumorigenic effects may also be mediated by inducing cyclin B2 expression[189] and activation of the E2F pathway. *HMGA2* is suppressed by *Let-7* microRNA, and *HMGA2* and *Let-7* expression correlates inversely in human pituitary adenoma samples.[188] Pituitary adenomas exhibit a high degree of aneuploidy, and when 127 adenomas were subjected to multiplexed next-generation sequencing, copy number variations were found to be more prominent in hormone-secreting adenomas.[190]

Frequent epigenetic changes are encountered in all pituitary tumor types, and novel targeted therapies to reverse disrupted epigenomic events have been proposed.[191]

Pituitary Senescence

Cellular senescence, or cell growth arrest, is induced by age-linked telomere shortening, DNA damage, oxidative stress, and oncogene activation. Oncogene-induced premature cell cycle arrest is protective of the cellular response to oncogenic events, is largely irreversible, and is mediated through upregulation of cell cycle inhibitors, including p16INK4A, p15INK4B, p21CIP1, and p53. p21CIP1 induction and senescence markers were elevated in all of the 38 GH-secreting adenomas tested.[192] In contrast, p21CIP1 was undetectable in pituitary carcinomas, nonsecreting pituitary oncocytomas, and null cell adenomas. Senescence-associated β-galactosidase activity, a marker of senescence, is also strongly positive in GH-secreting adenomas. As cellular senescence is protective for malignant transformation,[193] this process may underlie the invariably benign nature of pituitary adenomas.[166]

Familial Syndromes

These rare syndromes are summarized in Table 9.12 and include the following types.

Multiple Endocrine Neoplasia Type 1

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant hereditary disorder characterized by tumor formation or hyperfunction of parathyroid, pancreatic islets, anterior

TABLE 9.12 Genes Associated With Familial Pituitary Tumor Syndromes

Syndrome	Gene (Locus)	Most Frequent Mutation(s)	Pituitary Features	Other Features
MEN1	*MEN1* (11q13)	c.249-252delGTCT, an exon 2 predicted frameshift, in 4.5%	Pituitary adenoma in 30–40% (PRL 60%, NFA 15%, GH 10%, ACTH 5%, TSH rare)	Primary hyperparathyroidism, pancreatic tumors, foregut carcinoid tumors, adrenocortical tumors (usually nonfunctional), rarely pheochromocytomas, skin lesions (facial angiomas, collagenomas, and lipomas)
MEN1-like (MEN4)	*CDKN1B* (12p13)	Only two reported cases	Pituitary adenoma[a]	Primary hyperparathyroidism and single cases reported of renal angiomyolipoma, neuroendocrine cervical carcinoma
Carney complex	*PRKAR1A* (17q23-24)	c.491-492delTG in exon 5	Pituitary hyperplasia in most patients Adenoma in ~10% (GH and PRL)	Atrial myxomas, lentigines, Schwann cell tumors, adrenal hyperplasia
Familial, isolated pituitary adenomas	*AIP*[b] (11q13.3)	Gln14X nonsense mutation[c]	Pituitary adenoma (majority GH, PRL, or mixed GH and PRL)	Young patients, often macroadenomas with gigantism

[a]Only two reported cases to date: one GH-secreting adenoma and one ACTH-secreting adenoma.

[b]*AIP* mutations reported in 15% of individuals with familial isolated pituitary adenoma and 50% of those with isolated familial somatotropinomas.

[c]This is the most commonly identified mutation but is likely to be overrepresented secondary to a Finnish founder effect.

ACTH, Adrenocorticotrophic hormone; *GH,* growth hormone; *MEN1,* multiple endocrine neoplasia type 1; *NFA,* nonfunctioning adenoma; *NR,* not reported; *PRL,* prolactin.

From Elston MS, McDonald KL, Clifton-Bligh RJ, et al. Familial pituitary tumor syndromes. *Nat Rev Endocrinol.* 2009;5:453–461.

pituitary, and less commonly, other neuroendocrine, thyroid, and adrenal tumors.[194] Pituitary tumors are nonfunctioning or prolactin-secreting or GH-secreting microadenomas.[195] The MEN1 syndrome (fully described in Chapter 42), is associated with germ cell inactivation of the *MEN1* gene (encoding the menin protein) located on chromosome 11q13.[196] Unlike pituitary tumors in the MEN1 syndrome, *MEN1* gene mutations were not identified in non-MEN1 familial pituitary adenomas.[197] Mutations of other genes may also confer a MEN1 clinical syndrome. Approximately 20% of patients with a clinical diagnosis of MEN1 do not exhibit identifiable *MEN1* mutations, and rarely the gene for p27Kip1 (*CDKN1B*) is mutated in patients with clinical features of MEN1 but with no *MEN1* mutations.[198]

Familial Isolated Pituitary Adenomas

Less than 5% of prolactinomas and GH-secreting tumors are inherited on a familial basis.[199] In familial acromegaly, about 25% of afflicted individuals are diagnosed as teenagers or young adults, usually with gigantism. These patients have been linked to LOH at the 11q13.1-11q13.3 locus.[200] Germline mutations of the aryl hydrocarbon receptor–interacting protein (AIP) were found to predispose to familial pituitary tumors with autosomal dominant inheritance.[199,201] Eleven of 73 families with familial isolated pituitary adenomas were found to have 10 germline *AIP* gene mutations associated mainly with GH-secreting and PRL-secreting tumors.[202,203] *AIP* mutations are rarely encountered in patients with sporadic tumors and have been reported mainly in the youngest patients with acromegaly or gigantism.[204,205] When patients under the age of 30 with sporadic macroadenomas were tested, 11.7% were found to harbor germline *AIP* mutations. In young acromegaly patients, the frequency of *AIP* mutations ranged from 2.3% to 5.5% with no apparent differences in patients with or without mutations.[206]

Carney Complex

Carney complex is an autosomal dominant disorder comprising benign mesenchymal tumors, including cardiac myxomas, schwannomas, thyroid adenomas, and pituitary adenomas associated with spotty skin pigmentation.[207] The disorder has been mapped to chromosome 17q24 and results from a mutated type 1α regulatory subunit (R1α) of the cAMP-dependent protein kinase A (PRKAR1A).[208] These patients may have elevated levels of GH, IGF1, or PRL, and 10% exhibit clinical acromegaly with GH-secreting tumor formation associated with inactivating mutations of *PRKAR1A*, leading to constitutive protein kinase A catalytic subunit activation. Excess GH levels in those patients with concomitant acromegaly may, in fact, be a growth factor in development of cardiac myxomas.[209] In some patients, the wild-type *PRKAR1A* allele is retained in tumor tissue, and haploinsufficiency may be sufficient for tumorigenesis. A 17-miRNA signature for pituitary tumors was identified on the background of pituitary hyperplasia and *PRKARIA* mutations. In GH-secreting tumors, miR-26b and miR-128 appear to regulate the PTEN-AKT pathway.[210] These defects provide attractive potential subcellular therapeutic targets.

In summary, multifactorial mechanisms subserve the multistep pathogenetic process of pituitary adenoma formation, including early initiating chromosomal mutations that result in mutated pituitary stem or progenitor cells. The transformed pituitary cell is subjected to signals facilitating clonal expansion, and permissive factors, including hypothalamic hormone receptor signals, intrapituitary growth factors, and disordered cell

TABLE 9.13 Pituitary Adenoma Prevalence[a]

	Belgium (n = 68)	Finland (n = 164)	Iceland (n = 471)	Malta (n = 316)	Sweden (n = 592)	Switzerland (n = 44)	United Kingdom (n = 63)
Population prevalence	1/1064	1/1471	1/865	1/1321	1/2688	1/1241	1/1289
ACTH secreting, %	6	3	6	2	4	4	2
GH secreting, %	13	8	11	16	9	9	11
PRL secreting, %	66	51	40	46	32	56	57
Nonfunctioning, %	15	37	43	34	54	30	28

[a]Includes three adenomas not classified, nine TSH-secreting adenomas, and one gonadotrophin-secreting adenoma.

Data from Molitch ME. Diagnosis and treatment of pituitary adenomas: a review. *JAMA*. 2017;317:516–524.

cycle regulation, may determine the ultimate biologic fate of the tumor. Autonomous anterior pituitary hormone production and secretion and cell proliferation, which are the hallmarks of pituitary adenomas, result. However, proximal subcellular events initiating the formation of most sporadic pituitary adenomas have yet to be elucidated.

Pathogenesis

Pituitary tumors account for about 15% of all intracranial neoplasms and are commonly encountered at autopsy[211] (Table 9.13). The Brain Tumor Registry of Japan reports that 15.8% of 28,424 cases were histologically confirmed pituitary adenomas.[212] The prevalence of pituitary tumors in Belgium is 1 in 1064 inhabitants,[213] and in Banbury, United Kingdom, 63 pituitary tumors were noted in 89,334 inhabitants, a population prevalence of approximately 77 cases per 100,000. Of these, 57% were prolactinomas, 28% were nonfunctioning adenomas, 11% were GH-secreting adenomas, and 2% were Cushing adenomas. The median age of onset was 37 years, but nonfunctioning tumors were most commonly encountered in patients older than 60 years.[214] A recent population-based study in Iceland showed a rising prevalence over time. The total prevalence of adenomas from 2003 to 2012 was 115 per 100,000, up from 72 per 100,000 in the period from 1993 to 2002. Prolactinomas accounted for 54/100,000 and nonfunctioning adenomas accounted for 43/100,000.[215] Using large and comprehensive population-based cancer registries, the annual pituitary tumor incidence rate in the United States was reported to increase from 2.52 per 100,000 population in 2004 to 3.13 in 2009 (Table 9.14).[216] An increase in incidence was similarly seen in a study in Sweden.[217] Whether or not this increase is due to an inherent actual tumor increase or to enhanced reporting, awareness, and diagnosis is yet unclear. These benign monoclonal adenomas may express and secrete hormones autonomously, leading to hyperprolactinemia, acromegaly, Cushing disease, and hyperthyroidism, or they may be functionally silent and initially diagnosed as a sellar mass. Although almost invariably benign, the neoplastic features of these adenomas represent a unique tumor biology, which is reflected in their important local and systemic manifestations. These neoplasms have a slow doubling time and rarely resolve spontaneously. Nevertheless, they can be aggressive and locally invasive or compressive to vital central structures. Two classifications, Hardy and Knosp, are used to characterize adenoma mass characteristics (Fig. 9.20). Although pituitary adenomas usually express a single differentiated gene product, polyhormonal expression may reflect a primitive stem cell or mature bimorphous cellular origin. In children, ACTH-secreting and PRL-secreting adenomas account for about 40% of surgical series, with a 65% surgical remission rate.[218] These tumors are particularly challenging to manage due to high recurrence rates and the need for rigorous pituitary hormone replacement.

Classification of Pituitary Tumors

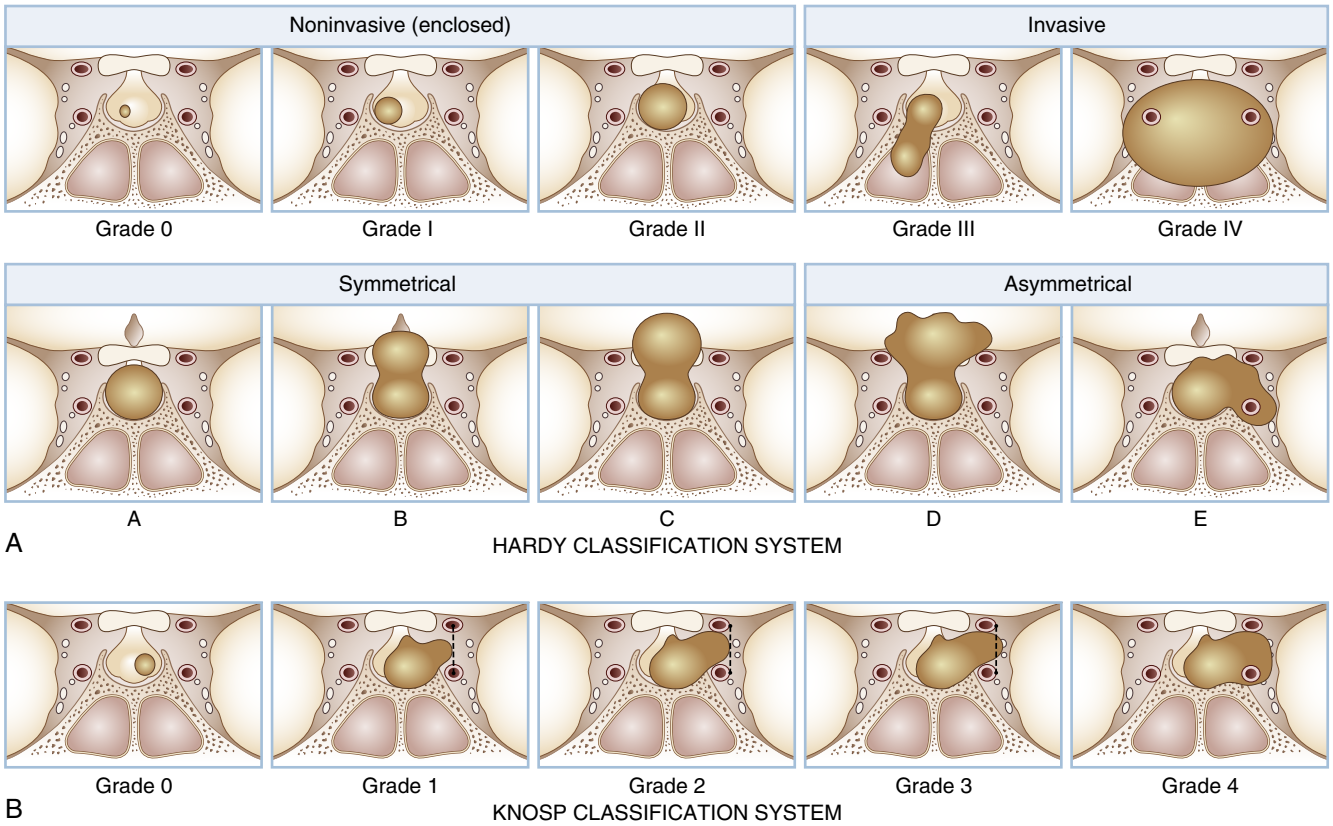
Pituitary tumors arise from hormone-secreting adenohypophyseal cells, and their secretory products depend upon the cell of origin (Table 9.15). Pathologic classification enables accurate clinical correlates and identifies cell-type origin.[219–221] Previously, clinically inapparent pituitary adenomas were found in about 11% of autopsies. They localize to unique areas of the gland, reflecting relative cell-type abundance and intragland distribution (see Fig. 9.2). Although 46% of a subset of these immunostain for PRL,[222] expectant management may still be indicated.[223] In a study on 100 normal volunteers, 10 were found to have focal abnormalities on MRI consistent with microadenomas; they measured from 3 to 6 mm in diameter.[8] Such tumors have been termed *incidentalomas*. In a survey of 506 patients harboring incidentalomas, 20% were nonfunctioning; of these, 20% increased in size during a mean follow-up of 50 months.[222] When larger, particularly nonfunctioning tumors are encountered inadvertently, pituitary function should be assessed, including measuring PRL, IGF1, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex steroids. A 24-hour urinary free cortisol or salivary cortisol measurement may exclude Cushing disease. In a study of 52 patients with macroadenomas that were incidentally discovered by CT or MRI, 22 were gonadotroph cell adenomas, 21 were null cell adenomas, and 9 were clinically nonfunctioning but immunostained for various pituitary hormones.[224] Radiologic and surgical classifications are based upon tumor localization, size, and degree of invasiveness (see Fig. 9.20). Microadenomas are

TABLE 9.14 Pituitary Tumor Age-Adjusted Incidence and Annual Percent Change in Incidence in the United States

Year	No. of Cases	Age-Adjusted Incidence Rate (95% CI)	Annual Percent Change (95% CI)
2004–2009	51.125	2.87 (2.85–2.90)	4.25 (2.91–5.61)
2004	7243	2.52 (2.46–2.58)	
2005	7811	2.69 (2.63–2.75)	
2006	8485	2.88 (2.82–2.94)	
2007	8722	2.92 (2.86–2.98)	
2008	9266	3.07 (3.01–3.14)	
2009	9598	3.13 (3.07–3.20)	

Modified from Gittleman H, Ostrom QT, Farah PD, et al. Descriptive epidemiology of pituitary tumors in the United States, 2004-2009. *J Neurosurg.* 2014;121:527–535.

intrasellar and generally smaller than 10 mm in widest diameter. Macroadenomas are 10 mm or larger and usually impinge upon adjacent sellar structures. Specific tumor types are considered later for each respective cell type. Immunocytochemistry detects pituitary cell gene products at both the light and electron microscopic levels and allows classification of pituitary tumors based on their function. Unlike the corticotroph, somatotroph, lactotroph, and thyrotroph cell tumors, which hypersecrete their respective hormones, gonadotroph cell tumors are usually clinically silent and do not efficiently secrete their gene products. Double immunostaining identifies mixed tumors expressing combinations of hormones; they are often macroadenomas secreting GH concomitantly with PRL, TSH, or ACTH. Generally, immunohistochemical identification of pituitary hormones correlates with tumor-specific messenger RNA (mRNA) hormone transcription factor markers measured either in whole tissue extracts or at the single cell level.[225] With the exception of the glycoprotein α-subunit, immunohistochemical positivity of greater than 5% of cells composing the tumor is usually reflective of peripheral circulating hormone levels. Quantification of immunostaining intensity is subjective, and a scale of intensity



• **Fig. 9.20** Classification systems characterizing pituitary adenomas. (A) *Hardy classification system.* Sella turcica tumors can be noninvasive (grade 0, intact with normal contour; grade I, intact with bulging floor; or grade II, intact, enlarged fossa) or invasive (grade III, localized sellar destruction; or grade IV, diffuse destruction). Suprasellar tumors can be symmetrical (grade A, suprasellar cistern only; grade B, recess of the third ventricle; or grade C, whole anterior third ventricle) or asymmetrical (grade D, intracranial extradural; or grade E, extracranial extradural [cavernous sinus]). (B) *Knosp classification system* used to quantify invasion of the cavernous sinus, in which only grades 3 and 4 define true invasion of the tumor into the cavernous sinus. Grade 0, no cavernous sinus involvement; grades 1 and 2, the tumor pushes into the medial wall of the cavernous sinus but does not go beyond a hypothetical line extending between the centers of the two segments of the internal carotid artery (grade 1) or it goes beyond such a line but without passing a line tangent to the lateral margins of the artery itself (grade 2); grade 3, the tumor extends laterally to the internal carotid artery within the cavernous sinus; grade 4, total encasement of the intracavernous carotid artery. (Modified from Di Ieva A, Rotondo F, Syro LV, et al. Aggressive pituitary adenomas: diagnosis and emerging treatments. *Nat Rev Endocrinol.* 2014;10:423–435, used with permission.)

TABLE 9.15 The 2017 WHO Pathologic Classification of Pituitary Adenomas

Adenoma Type	Morphologic Variants	Pituitary Hormones by Immunohistochemistry	Transcription Factors and Other Cofactors	Clinical Syndrome
Somatotroph	Densely granulated	GH, α-subunit	Pit1	Acromegaly, gigantism
	Sparsely granulated	GH	Pit1	
	Mammosomatotroph	GH + PRL (in same cells) ± α-subunit	Pit1, ERα	
	Mixed somatotroph-lactotroph	GH + PRL (in different cells) ± α-subunit	Pit1, ERα	
Lactotroph	Sparsely granulated	PRL	Pit1, ERα	Hypogonadism, galactorrhea
	Densely granulated	PRL	Pit1, ERα	
	Acidophil stem cell	PRL, GH (focal and variable)	Pit1, ERα	
Thyrotroph		βTSH, α-subunit	Pit1, GATA2	Hyperthyroidism
Corticotroph	Densely granulated	ACTH	Tpit	Cushing disease
	Sparsely granulated	ACTH	Tpit	
	Silent[a]	ACTH	Tpit	
	Crooke cell	ACTH	Tpit	
Gonadotroph		βFSH, βLH, α-subunit (various combinations)	SF1, GATA2, ERα	Hypogonadism, local signs
Null cell[a]		None	None	None or pituitary failure
Plurihormonal	Pit1 positive[a]	GH, PRL, βTSH ± α-subunit	Pit1	Mixed, local signs
	Unusual immunohistochemical combinations	Various combinations		

[a]Usually nonsecreting and clinically silent.

Modified from Mete O, Lopes MB. Overview of the 2017 WHO classification of pituitary tumors. *Endocr Pathol.* 2017;28:228–243.

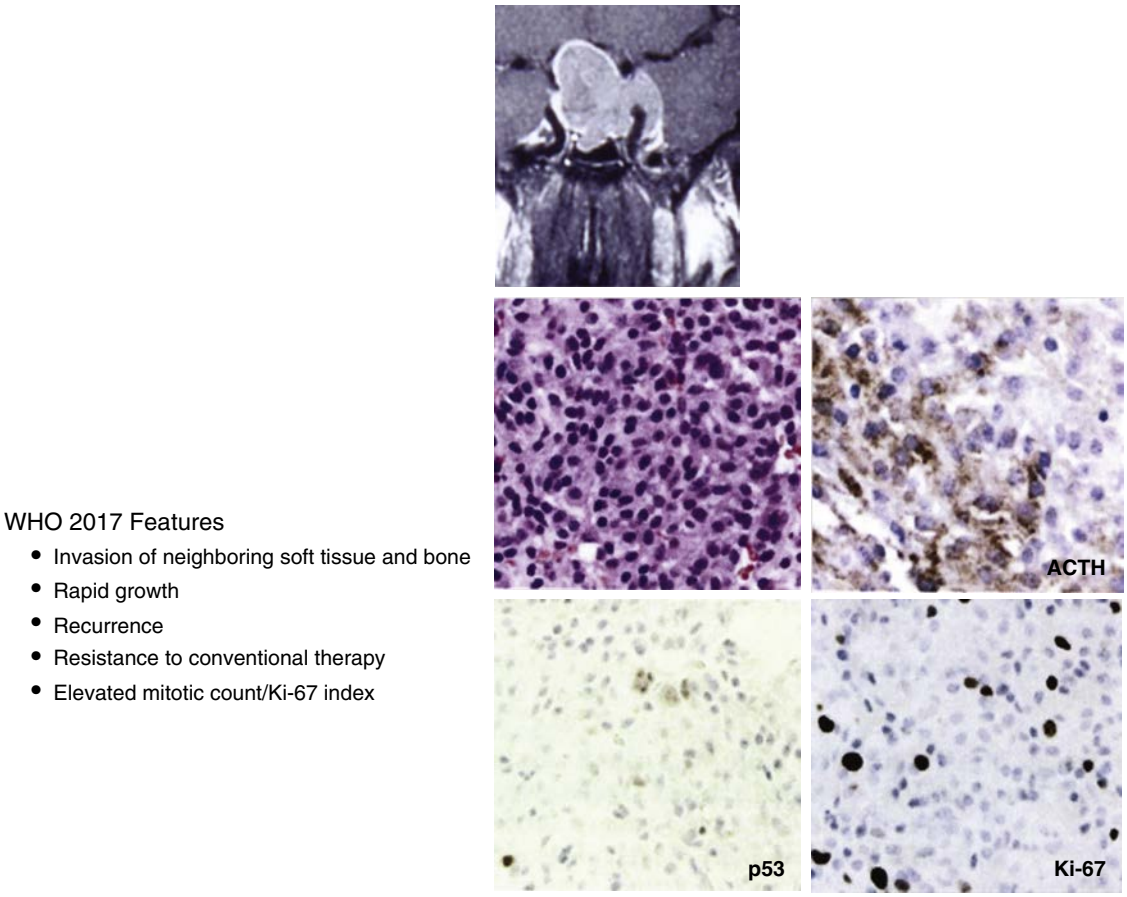
should also include a description of the extent of staining, such as whether occasional, scattered, or most tumor cells express the immunodetectable protein. Electron microscopy is useful for assessing the ultrastructure of hormone secretory granules, their size, and distribution. Other subcellular features important for diagnosis include visualizing large mitochondria in nonfunctioning oncocytomas and the secretory nature of Golgi and endoplasmic reticulum, especially for prolactinomas. Peroxidase or colloid gold particles of different diameters are also sensitive electron microscopic markers for identifying and localizing intracellular hormone signals. Because of the cost, other markers have superseded routine electron microscope usage. Invasive pituitary tumors are slow growing, and mitotic markers, including proliferating cell nuclear antigen (PCNA) and Ki-67, are useful.[226,227]

Aggressive Adenomas

True pituitary carcinoma with demonstrable extrapituitary metastases is exceedingly rare.[228,229] However, about 30% of patients undergoing adenoma resection exhibit persistent tumor growth or progressive growth for up to 37 years after surgery.[230] Tumors are regarded as aggressive if they are resistant to medical therapy and exhibit multiple recurrences despite rigorous surgical, medical, and radiation approaches.[231] Aggressive adenomas, previously referred to as atypical adenomas, are invasive, with elevated Ki-67 proliferative index and increased mitotic activity[231,232] (Fig. 9.21). In a series of 121 consecutive patients undergoing transsphenoidal pituitary tumor resection, 18 were classified as atypical.[233] These tumors grow aggressively and often persist or recur after surgery. In a study of 50 aggressive adenomas, more than 80% were macroadenomas and 42% had invaded the cavernous sinus.[234] They may arise from any of the pituitary cell types, and about 20% actively secrete GH or PRL; they rarely secrete ACTH. Several histologic types are particularly prone to aggressive growth, such as silent corticotroph adenomas.[186] No controlled prospective studies have rigorously assessed the postoperative recurrence rates of these tumors, and the utility of p53 as a prognostic marker has been questioned. There is yet no compelling evidence that these tumors undergo true malignant transformation. Nevertheless, frequently recurring or persistent invasive adenomas with high Ki-67 indices should raise awareness to consider the rare diagnosis of malignancy by excluding the presence of metastasis.[235]

Malignant Pituitary Tumors

Very rarely, pituitary tumors may metastasize either outside the central nervous system or as a separate focus within the brain; they account for 0.2% of pituitary adenomas.[236] Because no cell markers clearly distinguish aggressive invasiveness from malignancy, demonstration of extracranial metastasis is a prerequisite for diagnosis of pituitary malignancy.[237] When they occur, these cancers most often secrete either ACTH or PRL.



• **Fig. 9.21** Aggressive pituitary adenomas. Criteria for diagnosis as proposed by World Health Organization (WHO). Histologic panels depict atypical adrenocorticotrophic hormone (ACTH)–expressing adenoma. (From Zada G, Woodmansee WW, Ramkissoon S, et al. Atypical pituitary adenomas: incidence, clinical characteristics, and implications. *J Neurosurg.* 2011;114:336–344; Lopes MBS. The 2017 World Health Organization classification of tumors of the pituitary gland: a summary. *Acta Neuropathol.* 2017;134:521–535.)

As *HRAS* mutations are rarely encountered in distant metastatic pituitary carcinomas, but not in their respective primary pituitary tumors or in noninvasive adenomas,[169,238] these mutations may be important in the very rare progression to malignancy.

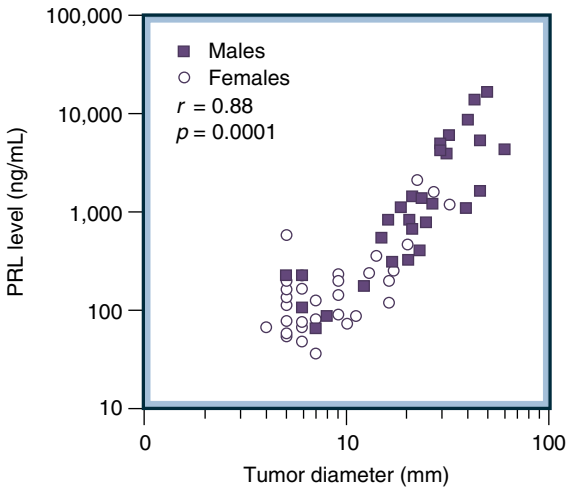
Temozolomide, an alkylating agent that induces DNA damage to disrupt gene transcription, has been used to treat aggressive pituitary tumors in patients who have failed to respond to other therapies or who have evidence of pituitary carcinoma. Results of tumor shrinkage or hormone suppression have been highly variable, with about half of patients showing initial responses.[239] It has been recommended that drug therapy be reevaluated after three treatment cycles. Although O^6-methylguanine-DNA methyltransferase (MGMT) may interfere with drug efficacy, assessing MGMT expression in pituitary tumor samples has been reported to have variable use.[240–243]

Prolactin-Secreting Adenomas

Prolactinomas are the most frequently encountered secretory pituitary tumors, occurring with an annual incidence of approximately 30 per 100,000 population.[214] This incidence would be much higher if the estimate included microadenomas discovered in approximately 11% of pituitaries at autopsy, 46% of which immunostain positively for PRL.[222] The female:male ratio for microprolactinomas is 20:1, and for macroadenomas the sex ratio is roughly equivalent. Both PRL levels and tumor size generally remain stable, although in some patients, PRL levels fall over time. Microadenomas may disappear after discontinuing dopamine agonist therapy, although 7% to 14% of microadenomas continue to grow.[244] Additionally, smaller prolactinomas may sometimes regress after pregnancy and lactation.[245]

Macroprolactinomas have a greater propensity to grow and tumor size correlates with serum PRL levels (Fig. 9.22), so a PRL level higher than 200 ng/mL is strongly indicative of a PRL-secreting pituitary tumor. Although prolactinomas account for more than 75% of all female pituitary adenomas,[214] men harbor larger tumors. In 45 men and 51 women with prolactinomas, mean serum PRL was 2789 ± 572 ng/mL versus 292 ± 74 ng/mL, respectively. Prolactinomas are larger in men than in women (26 ± 2 mm vs. 10 ± 1 mm), are more invasive, and show histologic evidence of more rapid growth.

PRL levels higher than 200 ng/mL may reflect use of a drug such as risperidone, but levels higher than 500 ng/mL are



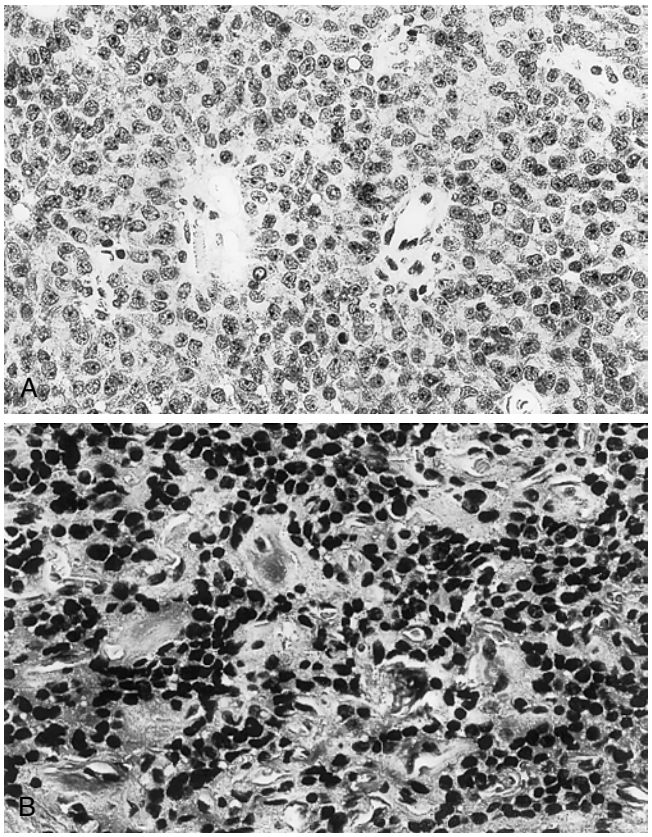
• **Fig. 9.22** Prolactin (PRL)–secreting tumors are more often macroadenomas in men (n = 31) versus women (n = 45). Serum prolactin levels highly correlate with tumor size. (Adapted from Danila DC, Klibanski A. Prolactin secreting pituitary tumors in men. *Endocrinologist.* 2001;11:105–111.)

exclusively observed in patients with prolactinomas.[246] In contrast, a lower PRL concentration of less than 200 ng/mL in a patient harboring a macroadenoma indicates that the tumor is likely not producing PRL, but hyperprolactinemia may occur as a result of mass pressure on the pituitary stalk or portal circulation, likely interrupting inhibitory control by dopamine.[247] Importantly, microprolactinomas can be associated with PRL levels ranging from minimal elevations to hundreds of nanograms per milliliter. However, when a patient with a small macroadenoma and a PRL level approximately 200 ng/mL is first encountered, it is prudent to first treat medically. If the tumor is indeed a prolactinoma, dopamine agonist treatment should lower PRL levels and shrink the tumor. If the tumor does not shrink, the mass is likely not secretory, and the hyperprolactinoma is caused by compressive stalk effect.

Pathology and Pathogenesis

Although more than 99% of prolactinomas are not malignant, and often sharply demarcated without evidence of invasion, about half may invade local structures[248] (Fig. 9.23). Invasive tumors may have higher mitotic activity and are more cellular and pleomorphic. Invasion into adjacent dura, bone, or venous structures may represent an intermediate form of prolactinoma between the sharply demarcated benign variety and the exceedingly rare malignant tumor. Invasive tumors that do not metastasize are considered benign. Immunostaining for PRL confirms the diagnosis of prolactinoma, which is usually distinct from the adjacent normal pituitary but is not truly encapsulated. These tumors have a "pseudocapsule" composed of compressed adenohypophyseal cells and a reticulin fiber network. About 20% of macroprolactinomas contain areas of hemorrhage not usually associated with features of apoplexy, and these areas may resolve.[249]

Prolactinomas are mostly slow growing, arise sporadically, usually occur singly, and are the most common pituitary tumors associated with MEN1, occurring in approximately 20% of a large kindred.[250,251] Familial prolactinomas have been described



• **Fig. 9.23** (A) Densely granulated prolactin-secreting adenoma. (B) Prolactin-producing pituitary adenoma removed by surgery from a patient treated with dopamine agonist in the preoperative period. The adenoma cells are small, possessing dark nuclei and a narrow rim of cytoplasm. Mild accumulation of interstitial connective tissue is apparent. (Hematoxylin-eosin stain; original magnification ×400.) (Photomicrograph kindly provided by Dr. Kalman Kovacs. University of Toronto, Toronto, Canada.)

with no other features of MEN1,[252] and rarely prolactinomas also occur in patients with germline *AIP* mutations.[253] Hyperprolactinemia derived from ectopic secretion from a perivascular epithelioid tumor resolved after resection of the abdominal mass.[254]

The entity of giant prolactinomas has been ascribed to those aggressive tumors representing <5% of prolactinomas that exhibit very high PRL levels (>1000 ng/mL) with a diameter greater than 40 mm, and with a high male preponderance (9:1).[255] Although these invasive tumors usually respond well to dopamine agonists, a subset is highly invasive and surgery is required, especially if mass effects do not resolve.[251,256] PRL levels may drop with cabergoline therapy or surgery, but recovery of gonadal function is only seen in about 30% of these patients. Recurrence rates are high and repeat surgery and/or radiation in addition to high-dose cabergoline are required for these aggressive tumors.

Clinical Features

Prolactinomas usually come to attention because of symptoms or signs associated with hyperprolactinemia or with tumor size or invasiveness (Table 9.16).

TABLE 9.16 Signs and Symptoms of Prolactinomas

Associated With Tumor Mass	Associated With Hyperprolactinemia
Visual field abnormalities	Amenorrhea, oligomenorrhea, infertility
Blurred vision or decreased visual acuity	Decreased libido, impotence, premature ejaculation, oligospermia
Symptoms of hypopituitarism	Galactorrhea
Headaches	Osteoporosis
Cranial nerve palsies	
Pituitary apoplexy	
Seizures (temporal lobe)	
Hydrocephalus (rare)	
Unilateral exophthalmos (rare)	

Hyperprolactinemia

Both large and small PRL-secreting tumors can present with signs and symptoms of hyperprolactinemia. Menstrual irregularities, sexual dysfunction, galactorrhea,[257] osteopenia,[258] and impaired quality of life[259] are attributable to elevated PRL levels and presence of a tumor. Elevated PRL causes sexual dysfunction via a short loop feedback effect on gonadotrophin pulsatility, presumably inhibiting GnRH[260] and LH pulse frequency and amplitude. High PRL also directly inhibits ovarian and testicular function. Women with prolactinomas may present with primary or secondary amenorrhea, oligomenorrhea, menorrhagia, delayed menarche, or regular menses with a short luteal phase that may cause infertility. Patients may also report changes in libido and vaginal dryness. Sexual dysfunction in men usually manifests as loss or decrease in libido, impotence, premature ejaculation or intracoital erection loss, oligospermia, or azospermia.[261]

Up to 50% of women and 35% of men with prolactinomas have galactorrhea; this difference may occur because male mammary tissue is less susceptible to lactogenic effects of hyperprolactinemia. Galactorrhea can be overlooked unless actively elicited. Bone density may decrease in both men and women as a result of hyperprolactinemia-induced sex steroid deficiency, and an increase in vertebral fractures detected radiologically has been reported in women.[262]

Tumor Mass Effects

Prolactinomas may present as a result of tumor size or invasiveness. Microadenomas range from entirely asymptomatic tumors found at autopsy as small as 2 to 3 mm in diameter to larger ones that are still less than 10 mm in diameter. These tumors can be invasive despite their small size. In contrast, macroadenomas range in size from noninvasive or diffuse tumors approximately 1 cm in diameter to huge tumors that may impinge upon parasellar structures. Signs and symptoms caused by large or invasive tumors are often related to compressive effects on visual structures. Objective findings are superior bitemporal defects, bitemporal hemianopsia, and decreased visual acuity. Headaches are common, but seizures (a result of extension into the temporal lobe) and hydrocephalus[263] are rarely encountered, as is unilateral exophthalmos. Tumors may invade the cavernous sinuses and yet cranial nerve palsies are rare. A sudden insult, such as pituitary apoplexy, is the more common cause of such palsies and may be a presenting symptom. Prolactinomas can also be discovered incidentally on an MRI or CT scan performed for another clinically unrelated purpose.

Evaluation

Patients with pituitary tumors should all have serum PRL levels measured. Conversely, patients with elevated serum PRL levels not fully explicable by an obvious cause (such as pregnancy or exposure to neuroleptic medications) should be evaluated for the presence of a pituitary tumor. Prolactinomas may also coexist with another cause of hyperprolactinemia, such as neuroleptic drug administration (see Chapter 8). Even minimal to moderate PRL elevations are important to investigate because they may indicate the presence of a large pituitary tumor that does not secrete PRL. PRL levels correlate with tumor size and are usually higher in male patients. Occasionally, a patient with a very high serum PRL level might have a "normal" result reported if serum dilutions are not assayed, a phenomenon called the "high-dose hook effect."[264] In contrast, serum PRL may be elevated by the presence of high-molecular-weight PRL, which is a weaker lactogen than the monomeric PRL molecule. Although usually clinically inactive, macroprolactinemia can also occur in patients with pituitary tumors. Pituitary adenomas are diagnosed in approximately 20% of patients with macroprolactinemia, some of which are associated with galactorrhea, oligomenorrhea or amenorrhea, or erectile dysfunction and decreased libido. Thus, assessment of macroprolactinemia by polyethylene glycol precipitation should be performed in patients with reported high levels of PRL and few or absent clinical features of hyperprolactinemia.[265]

A careful history will often unmask symptoms or signs of a space-related mass such as visual field abnormalities, impaired visual acuity, blurred or double vision, CSF rhinorrhea, headaches, diabetes insipidus, and hypopituitarism. Patients should also be questioned carefully about sexual history, including onset of menarche, regularity of menses, fertility, libido, potency, and ability to maintain an erection. A history of galactorrhea should also be ascertained. The coexistence of galactorrhea and amenorrhea suggests a diagnosis of pituitary adenoma until otherwise proven.

PRL is also elevated in up to 50% of patients with acromegaly.[266] Patients in the early stages of acromegaly or with mild disease or patients harboring acidophilic stem cell adenomas may have few obvious signs of GH excess. Because the human GH molecule has lactogenic properties similar to those of PRL,[267] signs and symptoms of a prolactinoma may be mimicked by a purely GH-secreting tumor, and serum IGF1 should be measured. Elevated PRL levels are occasionally encountered in patients with TSH-secreting tumors. Other pituitary hormone functions should be ascertained to determine the presence of hypopituitarism. An MRI is required to establish a definitive diagnosis of prolactinoma.

Treatment

Optimal treatment outcomes for a prolactinoma include normalization of PRL levels (and associated signs and symptoms) and complete tumor removal or shrinkage with a reversal of tumor mass effects. Specifically, previously abnormal sexual function and fertility should be restored, galactorrhea stopped, impaired bone density improved, tumor eliminated or reduced in size without impairing pituitary or hypothalamic function, and vision normalized, if impaired.

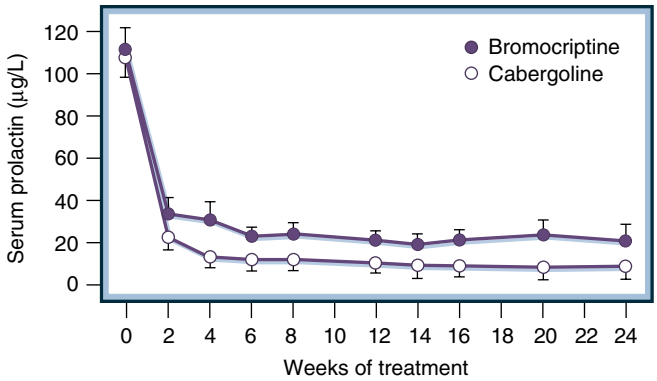
TABLE 9.17 Dopamine Agonist Treatment of Prolactinomas[a]

	Bromocriptine[b] (2.5–7.5 mg/day)	Cabergoline[b] (0.5–1 mg twice weekly)
PRL Normalized (range, 40–100%)		
Macroadenomas	70	80
Microadenomas	65	70
Menses Resumed (range, 40–100%)		
Macroadenomas	70	80
Microadenomas	85	80
Tumor Shrinkage (range, 20–100%)		
None	20	20
Up to 50%	40	55
50% or more	40	25
Visual Field Improvement (range, 33–100%)	90	70
Drug Intolerance	15	5

[a]Long-acting cabergoline has improved patient compliance and has fewer gastrointestinal side effects. For fertility, bromocriptine is preferred as it is short acting and can be discontinued immediately on pregnancy confirmation.

[b]Values = % of patients.

Data from Webster J, Piscatelli G, Polli A, et al. A comparison of cabergoline and bromocriptine in the treatment of hyperprolactinemic amenorrhea. Cabergoline Comparative Study Group. *N Engl J Med.* 1994;331:904–909; Verhelst J, Abs R, Maiter D, et al. Cabergoline in the treatment of hyperprolactinemia: a study in 455 patients. *J Clin Endocrinol Metab.* 1999;84:2518–2522; Melmed S, Casanueva FF, Hoffman AR, et al. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2011;96:273–288.



• **Fig. 9.24** Comparison of bromocriptine and cabergoline in suppressing prolactin levels in women with hyperprolactinemia. (From Webster J, Piscitelli G, Polli A, et al. A comparison of cabergoline and bromocriptine in the treatment of hyperprolactinemic amenorrhea. *N Engl J Med.* 1996;331:904–909.)

Medical Treatment

Medical management of prolactinomas with dopamine agonist drugs has been widely recommended as the treatment of choice[268] (Table 9.17).

Bromocriptine

Bromocriptine, a semisynthetic ergot alkaloid dopamine agonist, lowers elevated PRL levels, restores abnormal menstrual function in 80% to 90% of patients, shrinks prolactinomas, restores impaired sexual function, and resolves galactorrhea.[269] Improvement in visual field abnormalities occurs in approximately 90% of affected patients.[270] Drug withdrawal can result in rapid tumor expansion.[271] Occasionally, tumors that shrink during bromocriptine therapy do not enlarge following drug withdrawal. In a subset of patients, hyperprolactinemia disappears spontaneously after long-term observation. Very occasionally, bromocriptine lowers PRL levels despite continued tumor expansion, although when tumors grow during dopamine agonist therapy there is usually a simultaneous PRL elevation.

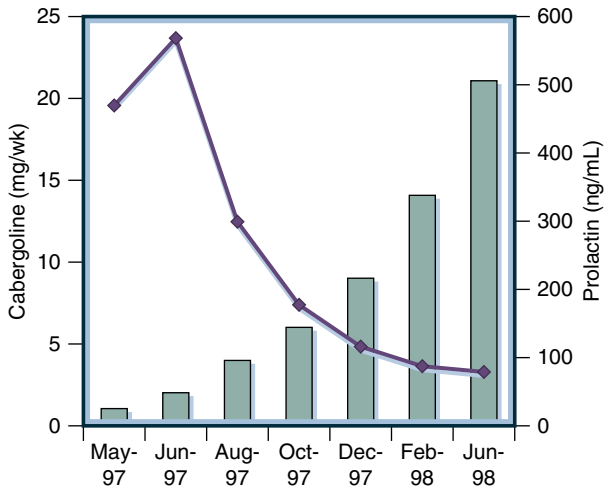
Despite high doses of bromocriptine, some patients are entirely or partially resistant to its effects and to those of cabergoline as well.[272] Not infrequently, it is difficult to completely normalize PRL levels in patients with initially very high levels, although these patients respond to treatment with impressive tumor shrinkage and sometimes improved sexual function. Although higher doses or a change in the form of dopamine agonist has been reported to further normalize PRL in some cases,[272] many such patients remain with elevated PRL levels regardless of treatment used. Resistance to dopamine agonists may reflect reduced D_2 receptor binding sites or receptor gene polymorphisms.[273]

Bromocriptine shrinks prolactinomas by shrinking tumor cell size, including cytoplasmic, nuclear, and nucleolar areas.[274] Histologic sections appear very dense as a result of small cell size and clumping of nuclei (see Fig. 9.23). PRL mRNA and PRL synthesis are inhibited, exocytoses are reduced, PRL secretory granules decrease, and rough endoplasmic reticulum and Golgi apparatus involute. The net effect is reduced cell volume. Tumor necrosis may also occur.[275]

Perivascular fibrosis observed in prolactinomas derived from patients treated with bromocriptine has been attributed to difficulty in tumor resection. However, there is no effect of prior treatment with bromocriptine on surgical success rates,[276] and bromocriptine was a helpful adjunct to transsphenoidal microsurgery for macroprolactinomas.[277] Even the largest tumors or those with the highest PRL levels respond well to treatment with up to 2.5 mg bromocriptine three times daily. Higher doses are often not more effective. Once positive effects on tumor size and amenorrhea and galactorrhea are established, some patients can be satisfactorily maintained with lower doses but rarely without medication.

Cabergoline

Because cabergoline has a longer duration of action than bromocriptine and is usually administered once or twice weekly, it has surpassed bromocriptine as the first-line therapeutic choice for most patients, unless pregnancy is desired.[270] The long half-life of cabergoline is a result of its high affinity for lactotroph D_2 receptors and a greater propensity of the drug to remain in pituitary tissue. In pharmacokinetic studies, cabergoline lowered PRL levels in a dose-related manner.[278] PRL levels were normalized in 83% of 459 women with hyperprolactinemia treated with cabergoline (0.5–1 mg twice weekly) and in 52% of women on bromocriptine (2.5–5 mg twice daily). Cabergoline was also more effective than bromocriptine in restoring ovulatory cycles and fertility (72% vs. 52%; $p < 0.001$), was better tolerated than bromocriptine, and caused fewer but similar side effects (Fig. 9.24). Tumor size decreased in 11 of 15 patients with macroadenomas, and menses resumed in 3 of 4 premenopausal women.[279] In 85 patients with



• **Fig. 9.25** Stepwise decrease in PRL levels *(diamonds)* concordant with stepwise increase in cabergoline doses in a patient with a prolactin-secreting macoadenoma *(bars)*. (Redrawn from Molitch ME. Pharmacologic resistance in prolactinoma patients. *Pituitary*. 2005;8:43–52.)

macroprolactinomas treated with cabergoline (0.25–10.5 mg/week), PRL concentrations were normalized in 61% of patients and decreased by at least 75% in an additional 24 patients, and tumor size decreased in 66% of patients. Nine patients were resistant to cabergoline despite doses of up to 7 mg per week.[272] Cabergoline also may result in dramatic improvement of prolactinoma-associated headache.[280]

Prolactinomas completely or substantially resistant to medication are infrequently encountered. Up to 15% of patients receiving optimal cabergoline doses fail to normalize PRL levels or exhibit less than 50% tumor mass shrinkage. Many of these patients respond to higher cabergoline doses (Fig. 9.25).[281] Most "resistant" patients may have partial resistance (i.e., tumors shrink and PRL levels are lowered but do not normalize). These patients may require surgery or radiation therapy. With tumor growth controlled on treatment, persistently elevated PRL levels should be addressed by evaluating and treating specific reproductive and bone disorders caused by hyperprolactinemia.

Administration

Attention to the mode of dopamine agonist administration may avoid or minimize potential adverse effects (Fig. 9.26). Usual starting doses are 1.25 mg bromocriptine (daily) or 0.25 mg cabergoline (weekly). Doses of medication are either increased gradually, as tolerated, or decreased depending on tolerability and should be initiated with a small dose with food before bedtime. Patients should initially avoid activities that cause peripheral vasodilatation (e.g., hot baths), thereby decreasing the risk of postural hypotension. If side effects are troublesome, the subsequent dose should be halved, then doses increased gradually thereafter to achieve effective levels. Switching from one medication to another may be beneficial. Application of intravaginal bromocriptine administration has been advocated to alleviate adverse gastrointestinal events.[282] Dopamine agonists are also effective in persistently shrinking cystic prolactinoma size.[283]

Remission of hyperprolactinemia may occur in about 20% of patients in whom dopamine agonists are tapered and then discontinued. Generally, withdrawal should only be attempted after at least 2 years of therapy and in those patients with no evidence of tumor invasion.[268,284] Tapering of cabergoline doses prior to complete therapy withdrawal has been recommended. Predictors of achieving normoprolactinemia after withdrawal include lower cabergoline doses required with evident tumor mass shrinkage and mass resolution.[285] Recurrences after withdrawal are more likely to occur for macroadenomas and are reported in about 30% of patients.[244,284,286,287]

In contrast to adults, about 75% of childhood prolactinomas are large macroadenomas.[288] In a cohort of 77 children and adolescents with macroprolactinomas, factors associated with dopamine agonist resistance encountered in 25% of patients included younger age of onset, higher PRL levels, and larger tumors.[289]

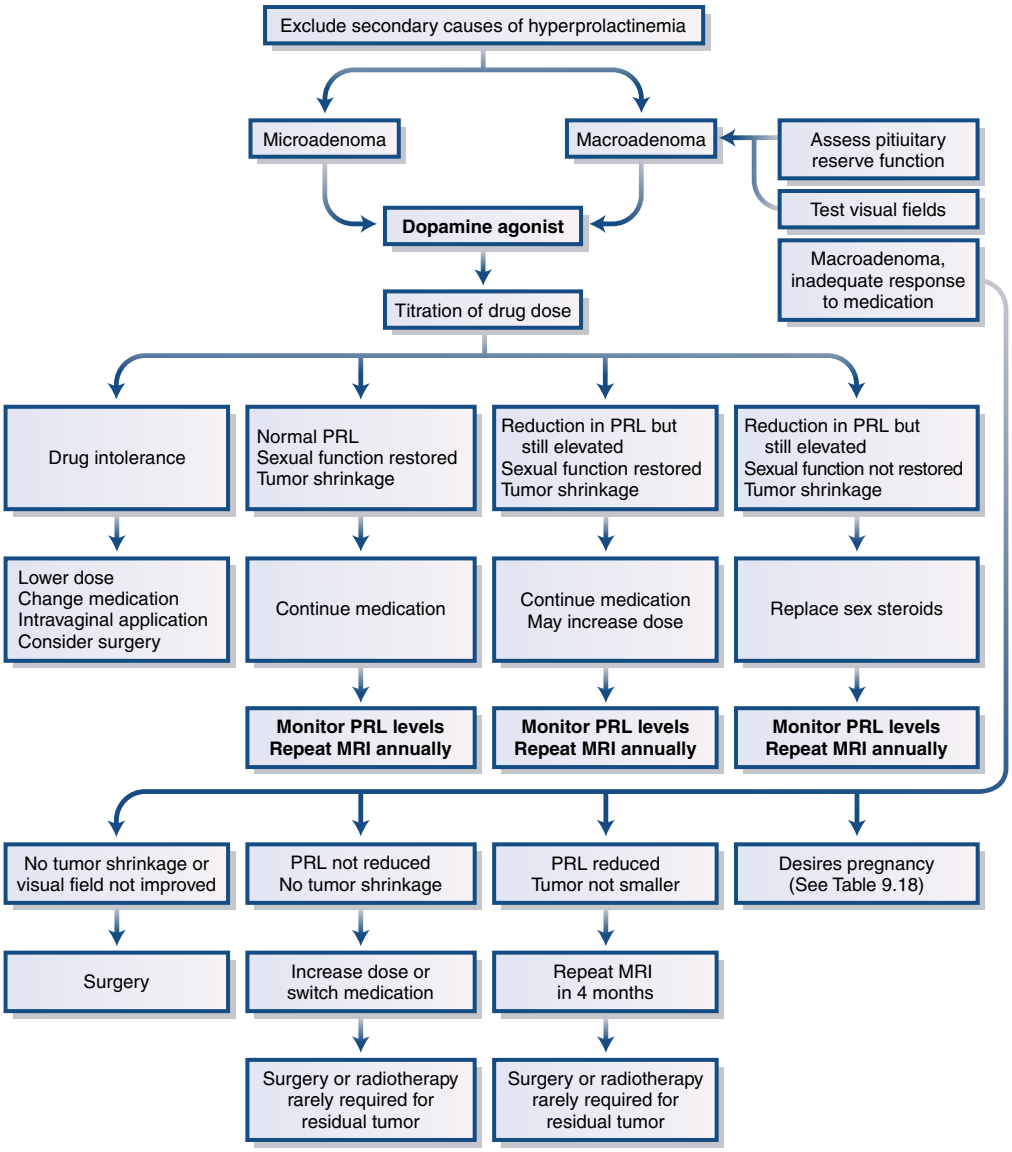
Adverse Effects of Dopamine Agonists

Side effects of dopamine agonists are common. Nausea occurs in up to 50% of patients; nasal stuffiness, depression, and digital vasospasm occur, the latter more frequently with higher doses, as seen in patients with Parkinson disease. Postural hypotension can cause loss of consciousness, occurs infrequently, and is usually avoided by careful dosing. Signs and symptoms of psychosis or exacerbation of preexisting psychosis can be encountered in up to 1.3% of patients receiving bromocriptine.[290] A history of psychotic symptoms should raise concerns about using these medications. If psychosis occurs in a patient in whom dopamine agonists are clearly the treatment of choice, the judicious combination of this agent and antipsychotic medication can be effective. A neuroleptic that is not a potent PRL stimulator, such as olanzapine, is preferred. CSF rhinorrhea occurs during dopamine agonist treatment in up to 6.1% of patients with macroadenomas, some of which are more resistant to dopamine agonists.[291] Other rarely reported serious side effects include hepatic dysfunction and cardiac arrhythmias. Retroperitoneal fibrosis, pleural effusions and thickening, and restrictive mitral regurgitation have been reported in patients taking high doses of bromocriptine.[292,293] Hypersexuality with disordered impulse control may occur with restoration of eugonadism or may be a direct dopamine effect.[294]

In patients with Parkinson disease high doses of ergot-derived dopamine agonists may lead to increase of moderate to severe regurgitation in at least one valve.[295] These observations with high ergot doses raise concern for patients with pituitary tumors who mostly require far lower drug doses. Several reports have shown no evidence that low doses of cabergoline place patients at risk for significant valve disease,[296] while some have raised the possibility that heart valve disease might be associated with standard endocrine doses of cabergoline used for patients with prolactinomas. One hundred prospectively studied patients with hyperprolactinemia who were exposed to dopamine agonists for a median of 124 months and received a median cumulative dose of 277 mg cabergoline showed no altered cardiac valve function or calcification.[297] In a cross-sectional study of 747 patients with hyperprolactinemia, there were no associations of cumulative doses of dopamine agonists and cardiac valvulopathy.[298] These results were affirmed in a follow-up of 192 of these patients.[299]

Radiation Therapy

Linear accelerator radiotherapy is effective in controlling or reducing the size of prolactinomas. However, this therapy takes years to achieve maximal effect. The usual recommended radiation dose is 4500 to 4600 cGy, and normalization of PRL was achieved in 18 of 36 patients at a mean of 7.3 years after treatment.[300] Hypopituitarism occurs as a side effect of radiation. In 36 patients with prolactinomas, of whom 83% had normal GH responses to insulin-induced hypoglycemia before therapy, 34 were GH deficient



• **Fig. 9.26** Prolactinoma management. After secondary causes of hyperprolactinemia have been excluded, subsequent management decisions are based on clinical imaging and biochemical criteria. *MRI,* magnetic resonance imaging; *PRL,* prolactin.

at 9 to 12 years after radiotherapy.[300] Stereotactic radiosurgery is often effective in treating prolactinomas resistant to or intolerant of dopamine agonists.[301]

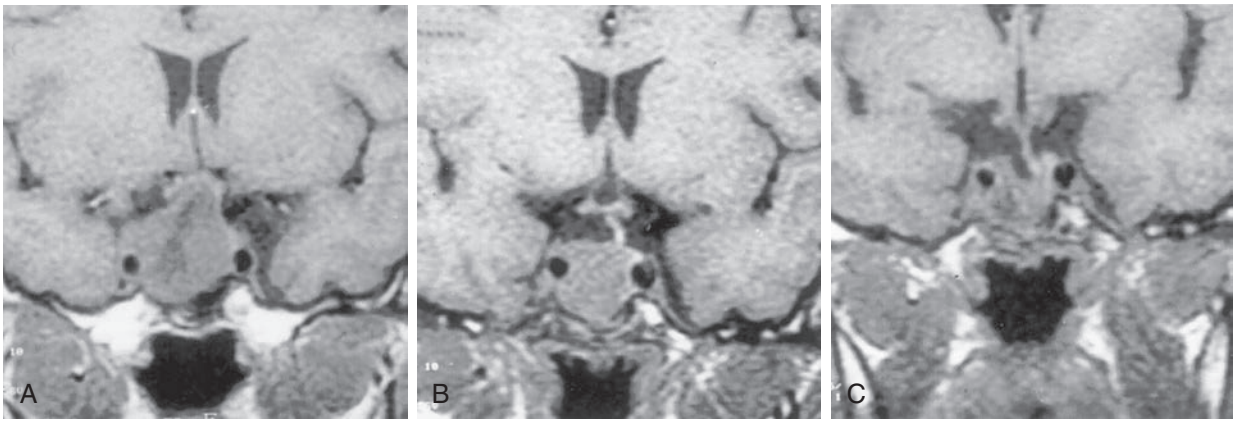
Surgery

Endoscopic endonasal transsphenoidal surgery has been used to resect prolactinomas. Most patients with microprolactinomas experience normalization of PRL levels, and overall about 50% of patients with macroprolactinoma are in remission after surgery.[302] The success rate of pituitary surgery is determined by experience of the surgeon and correlates inversely with tumor size and serum PRL concentrations.[276] In a compilation of results in 31 published surgical series, serum PRL was normalized in 71% of 1224 patients with microprolactinomas. More recent studies report up to 100% cure rates for microprolactinomas achieved by high-volume surgeons.[302] Nevertheless, a high rate of hyperprolactinemia recurrence is commonly encountered postoperatively. Complete resection of macroprolactinomas, especially large invasive tumors, is difficult to achieve, and postoperative serum PRL levels were initially normalized in only 32% of patients, with a 19% to 45% recurrence rate.[303]

Although results of medical therapy are superior to those of surgery, there remains a role for surgery, especially in those resistant to dopamine agonist therapy who are particularly well suited for surgery. If tumor removal is only partial, adjunctive radiation therapy could be considered, as second surgery is usually associated with a higher complication rate. Prophylactic transsphenoidal surgery should be considered in women whose prolactinomas are large enough to potentially threaten vision during pregnancy. A subset of patients cannot tolerate dopamine agonists, and others prefer surgery and refuse medication.

Chemotherapy

For aggressive PRL-secreting tumors unresponsive to other therapies, temozolomide, an alkylating compound that readily crosses the blood-brain barrier, may control tumor growth.[304–306] The



• **Fig. 9.27** Shrinkage of a macroadenoma by cabergoline in a woman harboring a macroadenoma at 22 weeks of gestation (A), when prolactin was 488 μg/L (B), and further reduction at 3 weeks postpartum (C). (Reproduced from Liu C, Tyrrell JB. Successful treatment of large macroprolactinoma with cabergoline during pregnancy. *Pituitary.* 2001;4:179–185.)

TABLE 9.18 Management of Patients with Prolactinomas Planning Pregnancy

Microadenoma	Macroadenoma
Discontinue dopamine agonist when pregnancy test is positive	Consider surgery before pregnancy
Periodic visual field examinations during pregnancy	Ensure bromocriptine sensitivity before pregnancy
Postpartum magnetic resonance imaging (MRI) after 6 weeks[a]	Monitor visual fields expectantly and frequently
	Administer bromocriptine if vision becomes compromised
	Or continue bromocriptine throughout pregnancy if tumor previously affected vision
	Consider high-dose steroids or surgery during pregnancy if vision is threatened or adenoma hemorrhage occurs
	Postpartum MRI after 6 weeks

[a]Pituitary MRI may be performed during pregnancy if deemed necessary.

response to temozolomide may sometimes be predicted by low tumor staining for MGMT.[307,308]

Pregnancy

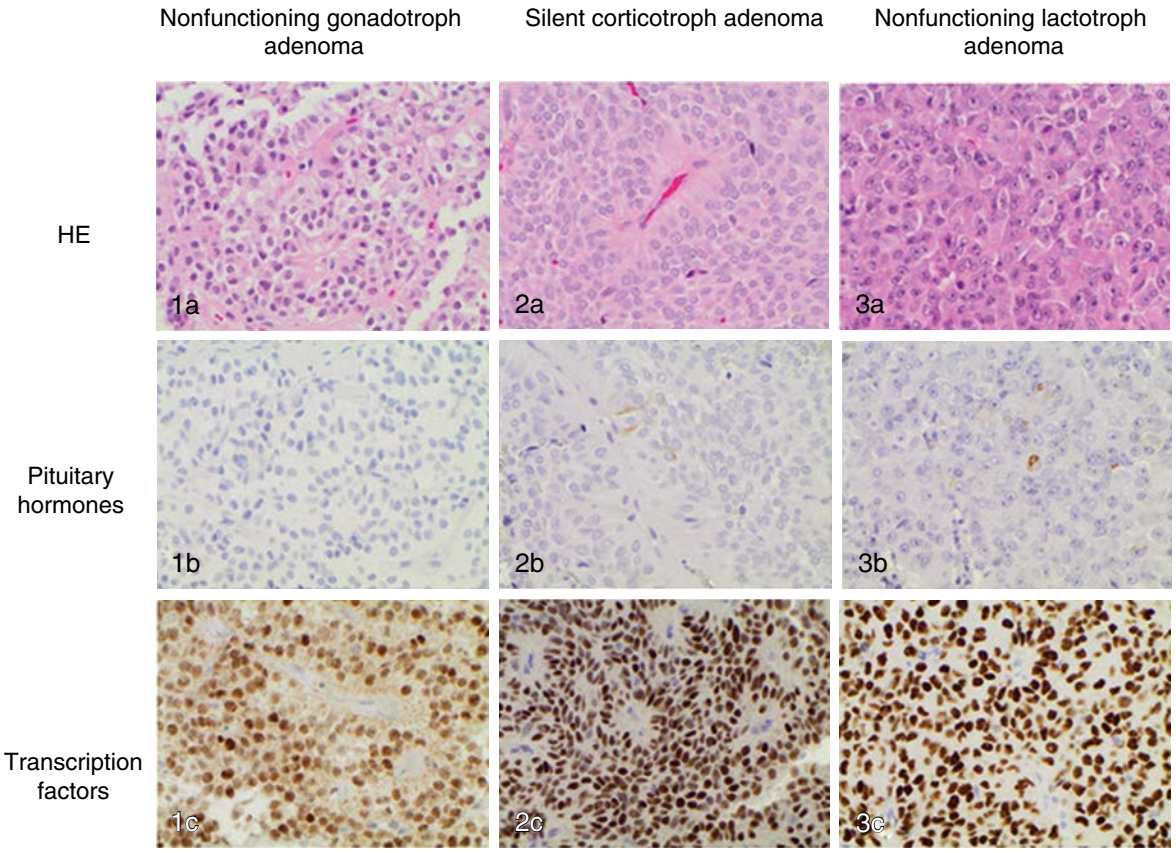
The normal pituitary enlarges during pregnancy and prolactinomas may also increase in size during pregnancy. The incidence of pregnancy-associated tumor enlargement, as determined by development of abnormal visual fields, has been estimated to occur in 1.4% of women with microadenomas and 16% of women with macroadenomas.[308] In other reports, the risk for macroadenoma enlargement has been estimated to be as high as 36%. In a prospective analysis in which 57 patients with microprolactinomas were followed by formal visual field examinations during pregnancy, none developed visual disturbances.[309]

Although dopamine agonists have been used during pregnancy to prevent tumor growth (Fig. 9.27),[310] it seems prudent to reduce fetal exposure to medication if possible.[311] It is recommended that menstrual periods be allowed to occur naturally for a sufficient period of time (3–4 months) to predict that a missed period might be a result of pregnancy (Table 9.18). Barrier contraception is recommended during this period. Within several days to a week of obtaining a positive hCG test, medication should be discontinued. Of 6239 pregnancies managed in this manner, bromocriptine therapy was not associated with increased abortions or terminations, prematurity, multiple births, or infant malformations above that expected in the control population. There is no compelling evidence that other dopamine agonists are less safe, but pregnancy exposure to the other agonist forms is less comprehensively documented. Treatment options for patients harboring prolactinomas whose vision becomes impaired during pregnancy include administering bromocriptine during pregnancy, high-dose steroids, and surgical resection.[308] Of 53 pregnant women receiving bromocriptine, mean offspring birth weight was normal, congenital abnormalities occurred in four babies, and physical and intellectual development of children was normal for up to 9 years. In an observational report on 380 pregnancies in women treated with cabergoline, early fetal exposure did not increase the risk of miscarriage or fetal malformation.[312] In 91 patients with hyperprolactinemia treated with cabergoline prior to pregnancy, the medication was discontinued at 6 weeks of gestation, and no increase in miscarriages or fetal malformations was observed when monitored for up to 60 months.[313]

To avoid neurologic complications of tumor enlargement during pregnancy, it is recommended that women with prolactinomas be tested for prolactin sensitivity to dopamine agonists, as well as tolerance of these drugs, before proceeding with a pregnancy. If tumors are insensitive to dopamine agonist–related tumor shrinkage, prophylactic surgery could be appropriate. If the tumor is a macroadenoma approximating the optic chiasm, the likelihood of visual difficulties is greater and therefore undertaking surgery prior to pregnancy could be prudent.[314]

Nonfunctioning Pituitary Tumors

Nonfunctioning pituitary tumors comprise approximately 25% to 35% of pituitary tumors.[315] These tumors are clinically silent (i.e., do not actively secrete hormones), but they arise from pituitary cells capable of expressing hormone genes, including LH and FSH, and more rarely, ACTH and TSH. Diagnostic markers useful for determining pituitary tumor classification and behavior include hormone immunohistochemistry and cell-specific transcription factors to define differentiated cell types, as well as



• **Fig. 9.28** Transcription factors determine cell-specific diagnosis of pituitary adenoma subtype when hormone expression is sparse or absent. Nonfunctioning gonadotroph adenoma showing hematoxylin-eosin staining *(1a),* negative FSH and LH immunoreactivity *(1b);* and nuclear expression of SF1 *(1c).* Silent corticotroph adenoma showing hematoxylin-eosin staining *(2a),* sparse ACTH immunoreactivity *(2b);* and nuclear expression of Tpit *(2c).* Nonfunctioning lactotroph adenoma showing hematoxylin-eosin staining *(3a),* sparse prolactin immunoreactivity *(3b);* and nuclear expression of Pit1 *(3c).* (Magnification ×400.) (From Manojlovic-Gacic, Engstrom BE, Casar-Borota O. Histopathological classification of nonfunctioning pituitary neuroendocrine tumors. *Pituitary*. 2018;21:119–129.)

proliferative indices (Fig. 9.28). Gonadotroph and corticotroph cell tumors predominate amongst the nonfunctioning tumors.[316]

Gonadotroph Cell Tumors

Most nonfunctioning or hormonally silent tumors arise from gonadotroph cells, and they most frequently present as clinically nonfunctioning masses not associated with elevated serum gonadotrophins. Yet, they usually express gonadotrophin subunits detectable by immunohistochemistry. In a series of nonfunctioning adenomas, 42% of tumors immunostained for TSH β-subunit, 83% for LH β-subunit, 75% for FSH β-subunit, and 92% for α-subunit.[317] Although LH, FSH, and α-subunit are released from these nonfunctioning tumors when maintained in culture, production is usually not sufficient to elevate blood levels. A small subset of gonadotroph tumors do secrete sufficient hormone to elevate serum gonadotrophin or α-subunit levels, which occasionally cause clinical syndromes.

Presentation

Clinically nonfunctioning tumors generally come to attention because of their large size, or they are detected incidentally (incidentaloma) (Table 9.19). Of 506 incidentally discovered pituitary masses, 324 were clinically nonfunctioning tumors, and the remainder were cystic or parasellar masses.[318] A gradual visual deficit arising from optic chiasmal compression is common, and patients are often unaware of the disturbance. Recognition of visual field deficits is often delayed because formal visual fields are not routinely evaluated unless a defect is suspected clinically. In the absence of associated space-occupying or hormonal disorders these large tumors may go unrecognized for many years and be inadvertently detected on MRI performed for other purposes. Sinusitis evaluation, pituitary apoplexy, or performance of a brain MRI for an unrelated indication (e.g., head trauma) may bring these tumors to clinical attention. Although not often the initial presenting complaint, these patients are commonly deficient in one or more pituitary hormones, as noted in two-thirds of 56 patients with nonfunctioning macroadenomas.[37] Although the most commonly encountered endocrine symptoms are related to gonadotrophin deficiency, quality of life may be decreased, and daytime somnolence has also been reported.[319]

A very small subset of gonadotroph adenomas producing elevated serum FSH, LH, and α-subunit concentrations are considered functioning gonadotroph adenomas and may be associated with specific endocrine syndromes. Most of these are macroadenomas, and imaging may also reveal ovarian cysts or increased testicular volumes.[320] High serum FSH level, usually with a low LH level, is usually the only sign that a pituitary

TABLE 9.19 Clinical Presentation of Silent (Nonsecreting) Pituitary Adenomas

	Frequency (%)
Incidental finding on MRI	8–38
Neurologic symptoms	
Visual field deficits	61
Extraocular muscle palsy	14
Headache	10–61
Endocrine symptoms	
Amenorrhea	10
Decreased libido	26
Apoplexy	2–12
Hormonal deficiencies	
GH	36–61
LH/FSH	40
TSH	36
ACTH	33
Diabetes insipidus	2
Pituitary apoplexy	4–10
Immunostaining	
Gonadotrophin subunits	44
POMC/ACTH	5–19
GH	2–4
PRL	2
TSH	1

Data from Mayson SE, Snyder PJ. Silent pituitary adenomas. *Endocrinol Metab Clin North Am.* 2015;44:79–87; Saeger W, Ludecke DK, Buchfelder M, et al. Pathohistological classification of pituitary tumors: 10 years of experience with the German Pituitary Tumor Registry. *Eur J Endocrinol.* 2007;156:203–216; Brochier S, Galland F, Kujas M, et al. Factors predicting relapse of nonfunctioning pituitary macroadenomas after neurosurgery: a study of 142 patients. *Eur J Endocrinol.* 2010;163:193–200; Yamada S, Ohyama K, Taguchi M, et al. A study of the correlation between morphological findings and biological activities in clinically nonfunctioning pituitary adenomas. *Neurosurgery.* 2007;61:580–584; Nishioka H, Inoshita N, Mete O, et al. The complementary role of transcription factors in the accurate diagnosis of clinically nonfunctioning pituitary adenomas. *Endocr Pathol.* 2015;26:349–355.

TABLE 9.20 Characteristics of Gonadotroph Adenomas and Primary Hypogonadism in Men

	Gonadotroph Adenoma	Primary Hypogonadism
Puberty	Normal	Often incomplete
Fertility history	Normal	Decreased
Testicular size	Normal	Small
Serum testosterone level	Low to high	Low to normal
Testosterone response to hCG (when basal value is subnormal)	Brisk to well within normal range	Subnormal
Serum FSH level	High	High
Serum LH level	Usually normal or slightly high	High if testosterone is low
α-Subunit level	High to very high	High
FSH response to TRH	Common	Absent
LHβ response to TRH	Common	Absent

FSH, Follicle-stimulating hormone; *hCG,* human chorionic gonadotropin; *LH,* luteinizing hormone; *TRH,* thyrotrophin-releasing hormone.

Modified from Wass JAH, Karavitaki N. Nonfunctioning and gonadotrophin-secreting adenomas. In Melmed S, ed. *The Pituitary,* 4th ed. Philadelphia, PA: Elsevier; 2017:589–603.

tumor secretes FSH. Female patients with such tumors may present with pelvic pain due to ovarian hyperstimulation.[321] High gonadotrophin levels associated with menopause or testicular failure may complicate interpretation of gonadotrophin levels, but both LH and FSH are high in primary gonadal failure (Table 9.20). LH-producing tumors are exceedingly rare and in males may cause elevations in serum testosterone with acne and skin oiliness. Very rarely, isosexual precocious puberty may be a presenting feature in children. Paradoxically, these patients may sometimes present with hypogonadism due to gonadal downregulation.

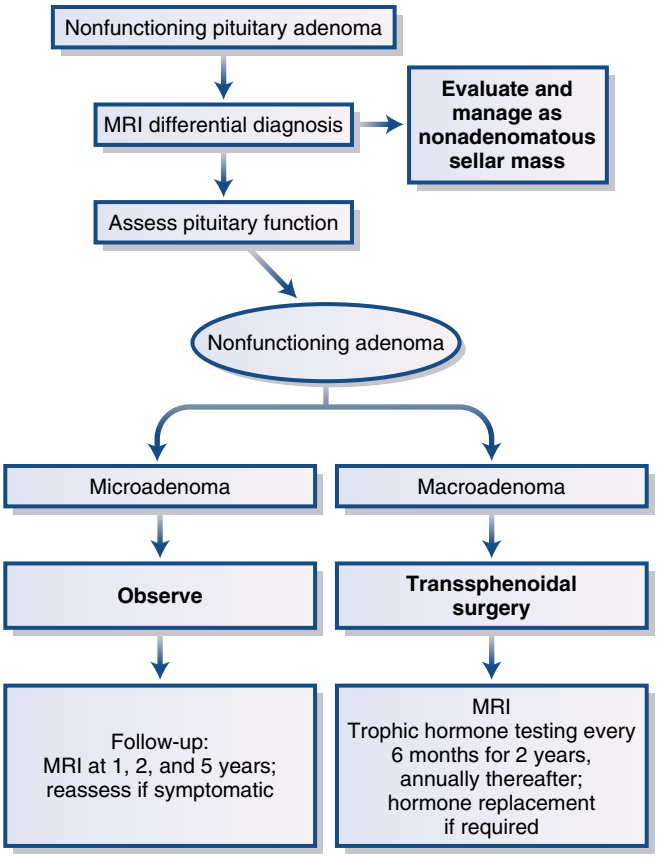
Evaluation

Patients with incidentally discovered adenomas (incidentalomas) should be comprehensively evaluated for hormone hypersecretion, pituitary failure, and local mass effects.[322] Subsequent decisions on whether to undertake surgery are determined by the size (>10 mm) of the mass, evidence of continued growth, visual effects, central compressive features, or signs of hemorrhage. If hormone hypersecretion is detected, specific therapeutic approaches are required.

MRI, visual field examination, and pituitary hormones should be evaluated, the latter not only to detect hypopituitarism but also to exclude hormone overproduction that may not be clinically apparent. This distinction of nonfunctioning versus secretory tumors is important for determining subsequent management.[323] LH, FSH, α-subunit, PRL, T_4, triiodothyronine (T_3), TSH, cortisol, and IGF1 levels should be measured. A serum cortisol at 8 AM, cortisol response to cosyntropin, or an insulin tolerance test is used to exclude secondary adrenal insufficiency. The extent of hormonal evaluation requires clinical judgment. Elevated LH or FSH levels should be interpreted in light of the patient's physiologic state. Elevated serum FSH in a woman with regular menstrual cycles would be interpreted differently from those detected in a menopausal patient; gonadotrophin elevations in patients with primary gonadal failure are not generally limited to one hormone, and circulating α-subunit elevation is consistent with a pituitary tumor but not gonadal failure. TRH stimulation may differentiate elevated gonadotrophin levels ascribed to end-organ failure or to independent tumor production. In patients harboring gonadotroph cell adenomas, increased FSH, LH, LH β-subunit, or α-subunit are evoked in response to TRH.[324]

Treatment

Clinical judgment should be used in determining appropriate therapy, including surgery, surgery followed by radiotherapy, radiotherapy alone, or expectant observation (Fig. 9.29). No reliable tumor marker is predictive of mass growth or recurrence.



• **Fig. 9.29** Management of nonfunctioning pituitary adenomas. Skilled magnetic resonance imaging *(MRI)* interpretation is crucial to diagnose nonadenomatous mass (e.g., meningioma, aneurysm, or other sellar lesion).

Surgery

Surgical resection of nonfunctioning adenomas is recommended for symptomatic patients, if vision is threatened, or for macroadenomas whose size threatens vital structures.[325] Transsphenoidal microscopic or endoscopic endonasal transsphenoidal surgery is the recommended approach[326] (Table 9.21). Gross total mass removal was reported after surgery in 137 of 173 patients with nonfunctioning pituitary adenomas,[326] and in another study gross total resection was achieved in 65.3% of 359 patients with improved vision or normalization of visual loss in 80% of affected patients.[327] In a large prospective study, visual function was restored in 75% to 90%, while hypopituitarism was rescued in up to 50% of patients.[328] Progression-free survival after surgery in 365 patients was strongly determined by extent of tumor invasion and grade.[329]

Postoperative Radiotherapy

To lower the postoperative risk of tumor growth progression, postoperative radiotherapy may be recommended.[330] An expectant follow-up of 65 patients after pituitary surgery for nonfunctioning adenomas showed that 32% of patients not receiving postoperative radiotherapy exhibited tumor regrowth during a mean follow-up period of 76 months.[331] Similar recurrence rates were observed in a retrospective follow-up of 212 patients.[332] In another study, tumor recurrence or regrowth occurred in 6% to 46% of patients not receiving radiotherapy after transsphenoidal surgery, and patients having received radiotherapy had a recurrence rate of 0% to 36%.[333] Nevertheless, despite the relatively high incidence of postoperative tumor regrowth, even after apparently complete resection, many neurosurgeons avoid routine postoperative radiation therapy. This approach requires advising careful follow-up, with periodic annual MRIs and visual evaluations, and encouraging patients to maintain medical follow-up.

Radiation can be offered if the tumor mass reexpands. In a retrospective study of 62 patients with nonfunctioning pituitary tumors treated with stereotactic radiosurgery, 60% experienced decreased tumor size and 37% of tumors remained unchanged. However, the risk of developing new anterior pituitary hormone deficits at 5 years was 32%.[334] In 140 consecutive patients undergoing stereotactic radiosurgery, the tumor mass was stabilized or decreased in 90% and median time to tumor progression was 14.5 years, with delayed hypopituitarism observed in 30% of patients.[335] In a large multicenter

TABLE 9.21 Outcomes of Transsphenoidal Surgery for Nonfunctioning Pituitary Tumors

Outcomes	Risk (95% CI)	No. of Patients	I^2 (%)
Complete removal	0.20 (0.09–0.38)	1207	95
Surgical death	0.01 (0.01–0.02)	1232	0
CSF leakage/ fistula	0.03 (0.02–0.06)	868	44
Meningitis	0.01 (0.01–0.03)	547	0
Transient diabetes insipidus	0.11 (0.04–0.27)	774	95
Persistent diabetes insipidus	0.05 (0.03–0.07)	622	14
New anterior pituitary deficits	0.09 (0.03–0.23)	850	87
Pituitary function improvement	0.30 (0.12–0.57)	714	89
New visual field defects	0.03 (0.02–0.04)	1032	0
Visual field defect improvement	0.78 (0.62–0.89)	795	93
Recurrence after surgery alone	0.18 (0.12–0.26)	734	79
ACTH improvement	0.37 (0.22–0.54)	145	64
ACTH worsening	0.39 (0.26–0.53)	49	0
TSH improvement	0.22 (0.07–0.51)	46	58
TSH worsening	0.17 (0.09–0.28)	160	31
LH/FSH improvement	0.23 (0.13–0.28)	190	71
LH/FSH worsening	0.10 (0.01–0.71)	143	89

Median follow-up was 4.29 years after surgery. *ACTH,* Adrenocorticotrophic hormone; *CSF,* cerebrospinal fluid; *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone; *TSH,* thyroid-stimulating hormone.

From Murad MH, Fernández-Balsells MM, Barwise A, et al. Outcomes of surgical treatment for nonfunctioning pituitary adenomas: a systematic review and meta-analysis. *Clin Endocrinol.* 2010;73:777–791.

TABLE 9.22 Changes in Pituitary Incidentaloma Size During Observation

	MICROADENOMAS				MACROADENOMAS				
	Total	Increased	Decreased	No change	Total	Enlarged	Decreased	No change	Years Followed
Reincke	7	1	1	5	7	2	0	5	8
Donovan	15	0	0	15	16	4[a]	0	12	6-7
Nishizawa					28	2[a]	0	26	5.6
Feldkamp	31	1	1	29	19	5	1	13	2.7
Igarashi	1	0	0	1	22	6	10	6	5.1
Sanno	74	10	7	57	165	20[a]	22	123	2.3
Day	11	1	0	10	7	1	0	6	3.2
Arita	5	2	0	3	37	19[a]	0	18	5.2
Karavitaki	16	2	1	13	24	12	4	8	3.6
Dekkers					28	14	8	6	7.1
Anagnostis	6	0	1	5	3	1	0	2	4.0
Lenders[b]	27	2[a]	3	22	23	9	2	12	3.0
Esteves	14	0	2	12	12	1	3	8	3.2
Iglesias[b]	22	4	1	17	28	0	1	27	1.2
Total (n = 648)	229	23 (10%)	17 (7%)	189 (83%)	419	96 (23%)	51 (12%)	272 (65%)	

[a]Eight cases had tumor enlargement due to apoplexy.

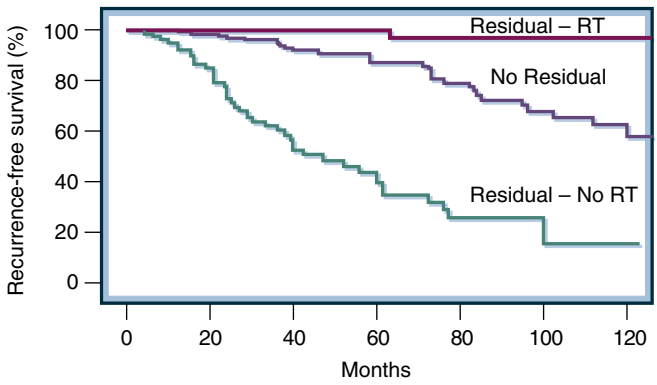
[b]Personal communication.

From Huang W, Molitch ME. Management of nonfunctioning pituitary adenomas (NFAs): observation. *Pituitary.* 2018;21:162–167.

outcomes analysis of 512 patients undergoing stereotactic radiosurgery for nonfunctioning pituitary adenomas, median follow-up was 36 months. Progression-free tumor survival was associated with smaller tumor size and absence of suprasellar extension. New or aggravated pituitary failure was encountered in 21% of patients.[336] Because patients may experience tumor regrowth even after radiation therapy, they too should undergo periodic posttreatment MRIs, albeit less frequently. In a retrospective study of 237 patients followed for a median of 5.9 years, the 5-year second regrowth rate was 36% after surgery, 12.5% after radiotherapy, and 12.7% after surgery with adjuvant radiotherapy.

Expectant Observation for Incidentalomas

Given the slow growth rate for nonfunctioning microadenomas or small macroadenomas, patients may be followed expectantly.[337] Some tumors do not grow over years or even decades. Overall, only 10% of incidentally discovered microadenomas will continue to enlarge. However, over 20% of incidental macroadenomas are reported to continue growing (Table 9.22). After 5 years, the rate of growth appears to increase.[337] Overall, about 10% of incidentalomas may decrease in size when followed for up to 8 years. Serial MRIs should be undertaken at 1, 2, and 5 years. Nonfunctioning adenomas may grow insidiously postoperatively and are usually asymptomatic until such time that they are large enough to affect vision (Fig. 9.30). As 20% of tumor regrowth occurs more than 10 years after surgery, performing serial MRIs, perhaps indefinitely, is suggested.[338] Periodic, but less frequent,



• **Fig. 9.30** Recurrence-free survival by Kaplan-Meier plots in 436 patients with silent (clinically nonfunctioning) pituitary adenomas according to the presence or absence of residual adenoma after surgery and with or without radiation therapy *(RT).* (Adapted from Losa M, Mortini P, Barzaghi R, et al. Early results of surgery in patients with nonfunctioning pituitary adenoma and analysis of the risk of tumor recurrence. *J Neurosurg.* 2008;108(3):525–532. As adapted in Mayson SE, Snyder PJ. Silent (clinically nonfunctioning) pituitary adenomas. *J Neurooncol.* 2014;117:429–436.)

endocrine evaluation is also suggested because hypopituitarism occurs frequently and may be challenging to detect. Over half of these patients exhibit pituitary hormone deficits (especially GH and gonadotrophins) prior to surgery, and postoperatively the condition may persist, recover, or become newly manifest. In

a nationwide follow-up of 2795 patients with nonfunctioning tumors in Sweden, overall excess mortality was reported, especially for patients younger than 40 years of age and women with hypopituitarism.[339] As ACTH and gonadotrophin deficiency are important determinants of mortality in patients with nonfunctioning adenomas (about a twofold to fivefold increase), careful hormone replacement regimens are required.[340]

Pregnancy

Microadenomas only very rarely lead to impaired vision during pregnancy, as opposed to macroadenomas, which do so with greater frequency.[341] Because macroadenomas do not usually shrink on medical therapy, the risks of visual impairment arising during pregnancy must be weighed carefully, and tumor resection may be indicated prior to pregnancy.

Medications

Medications are not usually effective in reducing tumor size and visual compromise. As most nonfunctioning adenomas express D_2 receptors, treatment with cabergoline has been advocated to shrink some nonfunctioning tumors or to prevent regrowth of postoperative remnants.[342] Treatment of 79 patients with cabergoline (mean weekly dose 1.5 mg) for a mean of 8.8 years resulted in overall control of tumor growth in 87%, with 38% experiencing tumor shrinkage. Overall, 13% of treated versus 42% of untreated patients required further surgery or radiotherapy.[343]

Silent Corticotroph Tumors

These tumors are usually resected with the clinical diagnosis of nonfunctioning macroadenomas and the diagnosis is subsequently made by pathologic assessment. They immunostain for ACTH and POMC but are clinically silent and more aggressive than nonsecreting tumors of pure gonadotroph cell origin.[344] ACTH secretion is unaltered, with no associated clinical or biochemical features of hypercortisolism, although these tumors are morphologically indistinguishable from adenomas associated with Cushing disease. A mixed corticotroph-gonadotroph cell origin may underlie the primitive tumor cell type, with associated aggressive growth.[345] Silent corticotroph tumors may represent up to 7% of all surgically removed adenomas and are usually hemorrhagic and invariably macroadenomas. Unlike Cushing disease, they have a 2:1 male preponderance, often present with mass effects, and about one-third have preoperative evidence for pituitary insufficiency. About half exhibit cavernous sinus or bony invasion, hemorrhage, necrosis, and cyst formation. They often recur and postoperative radiation and reoperation are required to eradicate tumor regrowth or residual mass.[346]

A review of 20 cases of silent ACTH-producing macroadenomas reported visual dysfunction as the most common presenting complaint (38%), and 13% presented with pituitary apoplexy.[346] In a study of 50 patients undergoing surgical resection and stereotactic radiosurgery for silent ACTH-secreting tumors and followed for a median of 40 months, 82% exhibited tumor growth control versus 91% control achieved for nonfunctioning adenomas.[347] Regular MRI follow-up is required because of the high risk for recurrence. Unless appropriate immunostaining is performed, many of these tumors remain undiagnosed and are inadvertently classified as recurrent nonfunctioning macroadenomas.

Silent Subtype 3 Tumors

This entity usually expresses GH, PRL, and/or TSH (i.e., cells of the Pit1 lineage). They are clinically aggressive, nonsecreting adenomas that exhibit cytologic features of nuclear atypia.[348]

Silent GH-Expressing Tumors

Silent GH-expressing tumors occur with a prevalence of about 2% of all pituitary adenomas. These patients should be followed prospectively for recurrences and for potential progression to acromegaly.[349]

Acromegaly

In 1886, Pierre Marie published the first clinical description of disordered somatic growth and proportion and proposed the name "acromegaly."[350] When relation of this syndrome to a pituitary tumor was later recognized, Benda showed in 1900 that these tumors comprise mainly adenohypophyseal eosinophilic cells, which he proposed to be hyperfunctioning.[351] Cushing, Davidoff, and Bailey documented the clinicopathologic features of acromegaly and demonstrated clinical remission of soft tissue signs after adenoma resection.[352] Evans and Long induced gigantism in rats injected with anterior pituitary extracts, confirming the association of a pituitary factor with somatic growth.[353] Establishment of the unequivocal pathophysiologic link between hyperfunctioning adenoma and acromegaly was the earliest example of a pituitary disorder to be clinically and pathologically recognized and appropriately managed by surgical excision of a hypersecreting source.

Incidence

The prevalence of acromegaly is estimated to range from 28 to 137 cases per million. Recent surveys indicate an increase in the annual incidence to about 10 cases per million[213,215,217,354–363] (Table 9.23). In the United States, over 3000 new cases of acromegaly are diagnosed annually, with an estimated population prevalence of 25,000 patients.[363]

Pathogenesis

GH and IGF1 act both independently and dependently to induce features of hypersomatotropism. Acromegaly is caused by pituitary tumors secreting GH or very rarely by extrapituitary disorders[364] (Fig. 9.31). Regardless of the cause, the disease is characterized by elevated levels of GH and IGF1 with resultant signs and symptoms of hypersomatotropism.

Pituitary Acromegaly

Over 95% of patients with acromegaly harbor a GH-secreting pituitary adenoma (Fig. 9.32 and Table 9.24). Pure GH-cell adenomas contain either densely or sparsely staining cytoplasmic GH granules, and these two variants are either slow (densely granulated) or rapidly growing (sparsely granulated).[365] The former type arises insidiously and presents during or after middle age, but the latter type arises in younger subjects with more aggressive tumor growth and florid disease. Mixed GH cell and PRL cell adenomas are composed of distinct somatotrophs expressing GH and lactotrophs expressing PRL. Monomorphous acidophilic stem cell adenomas arise from the common

TABLE 9.23 Epidemiology of Acromegaly

Study	Population Covered	Prevalence (per 100,000)	Annual Incidence (per 100,000)
Mestron (2004)	Population of Spain in 2001	3.4	0.2
Daly (2006)	72,792	12.5	NA
Bex (2007)	10,850,000	4	0.2
Fernandez (2010)	81,449	8.6	NA
Raappana (2010)	722,000–733,000	NA	0.3
Gruppetta (2013)	417,608	12.4	0.3
Kwon (2013)	48,456,369	2.8	0.4
Tjornstrand (2014)	1,590,640	3.3	0.4
Agustsson (2015)	321,857	13.7	NA
Hoskuldsdottir (2015)	316,075	13.3	0.8
Broder (2016)	24,508,019 (prevalence) 14,785,312 (incidence)	8.78	1.17
Burton (2016)	50,170,946	7.8	1.1
Dal (2016)	5,534,738	8.5	0.4

Modified from Lavrentaki A, Paluzzi A, Wass JAH, et al. Epidemiology of acromegaly: review of population studies. *Pituitary.* 2017;20:4–9; Broder MS, Change E, Cherepanov D, et al. Incidence and prevalence of acromegaly in the United States: a claims-based analysis. *Endocr Pract.* 2016;22:1327–1335.

GH and PRL stem cell and often contain giant mitochondria and misplaced GH granule exocytosis. They grow rapidly, are invasive, and present with predominant features of hyperprolactinemia. Monomorphous mammosomatotroph cell adenomas express both GH and PRL from a single cell, but plurihormonal tumors may express GH with any combination of PRL, TSH, ACTH, and α-subunit. These patients present with clinical features of acromegaly as well as hyperprolactinemia, Cushing disease, or very rarely hyperthyroxinemia. Somatotroph hyperplasia is difficult to distinguish from a GH cell adenoma, and silver staining displays a well-preserved reticulin network without a surrounding pseudocapsule. The rigorous morphologic diagnosis of GH cell hyperplasia is usually associated with stimulation by ectopic GHRH derived from an extrapituitary tumor causing acromegaly.

Both pituitary and hypothalamic factors influence pituitary tumor pathogenesis. Even when exhibiting marked nuclear pleomorphism, mitotic activity, and invasiveness, these tumors are invariably benign.

Disordered GHRH Secretion or Action

Adenomas express receptors for GHRH, ghrelin,[366] and somatostatin, but functional mutations of either the GHRH or somatostatin receptor have not been reported. GHRH directly stimulates GH gene expression and also induces somatotroph mitotic activity. Clinically, GHRH production by hypothalamic, abdominal, or chest neuroendocrine tumors causes somatotroph hyperplasia, and rarely adenoma, with resultant unrestrained GH secretion and acromegaly.[159] However, histologic examination of most pituitary GH cell adenoma tissue specimens does not show hyperplastic somatotroph tissue surrounding the adenoma, implying no generalized hypothalamic overstimulation. Failure to downregulate GH secretion during prolonged GHRH stimulation also points to a role for GHRH in maintaining persistent GH hypersecretion. Furthermore, a GHRH antagonist reduced human growth hormone production in 50 patients with acromegaly, suggesting a role for endogenous GHRH.[367] Expression of intra-adenomatous GHRH abundance correlates with tumor size and activity, implying a paracrine role for GHRH in mediating adenoma growth.[368] However, complete surgical resection of well-defined GH-secreting microadenomas usually results in a definitive cure of excess hormone secretion with very low postoperative tumor recurrence rates, strongly suggestive of intact hypothalamic function in these patients.

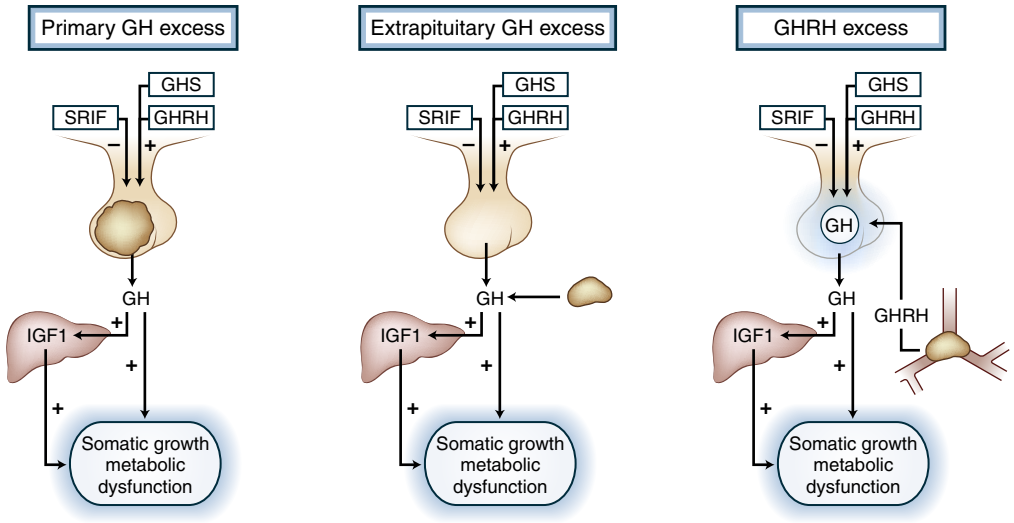
Disordered Somatotroph Cell Function

The sequence of events leading to somatotroph clonal expansions appears multifactorial (see earlier discussion). An activated oncogene may be required for initiating tumorigenesis, and promotion of tumor growth may require GHRH and other growth factor stimulation. The cellular mutation may not be sufficient to provide a growth advantage for a GH-secreting adenoma without additional disordered hypothalamic or paracrine growth factor signaling.

Monoclonal origin of somatotroph adenomas was determined by X-chromosome inactivation analysis of somatotroph tumor DNA.[155] An altered $G\alpha_s$ protein identified in a subset of GH-secreting pituitary adenomas leads to high levels of intracellular cAMP and GH hypersecretion.[161] Point mutations in two critical sites, Arg201, the site for adenosine diphosphate ribosylation, and Gly227, the guanosine triphosphate (GTP)–binding domain of $G\alpha_s$ proteins, prevent GTPase activity and result in constitutive adenyl cyclase activation. This dominant *GSP* mutant mimics GHRH effects, results in elevated cAMP levels, and is present in about 30% of GH-secreting tumors. Germline inactivating mutations of *AIP* have been found in a subset of familial somatotropinomas,[203,253] especially in younger patients with acromegaly or gigantism.

McCune-Albright Syndrome

This rare hypersecretory syndrome consists of polyostotic fibrous dysplasia, cutaneous pigmentation, sexual precocity, hyperthyroidism, hypercortisolism, hyperprolactinemia, and acromegaly due to somatotroph hyperplasia. In a comprehensive review of 112 patients, acromegaly was reported in up to 30% of patients with the syndrome and was invariably associated with skull base fibrous dysplasia.[369] About half of these patients have definitive imaging evidence for a pituitary adenoma. $G\alpha_s$ mutations have been detected in both endocrine and nonendocrine tissues.[162] GH hypersecretion is rarely controlled by surgery, and these patients require somatostatin receptor ligands, GH receptor antagonist, or pituitary irradiation.



Pituitary adenoma
Densely granulated GH cell
Sparsely granulated
Mixed GH cell and PRL
Mammosomatotroph cell
Acidophil stem cell
Plurihormonal
Silent somatotroph

Pituitary carcinoma

Ectopic

Familial syndromes
Multiple endocrine neoplasia-type I
McCune-Albright syndrome
Familial acromegaly
Carney complex

Pancreatic islet cell tumor
Lymphoma

Iatrogenic

Central
Hypothalamic tumor

Peripheral
Bronchial carcinoid
Pancreatic islet cell tumor
Small cell lung cancer
Adrenal adenoma
Medullary thyroid carcinoma
Pheochromocytoma

• **Fig. 9.31** Pathogenesis of acromegaly. *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *GHS,* growth hormone secretagogue; *IGF1,* insulin-like growth factor type 1; *PRL,* prolactin; *SRIF,* somatostatin (somatotropin release–inhibiting factor). (From Melmed S. Medical progress: acromegaly. *N Engl J Med.* 2006;355:2558–2573.)

Extrapituitary Acromegaly

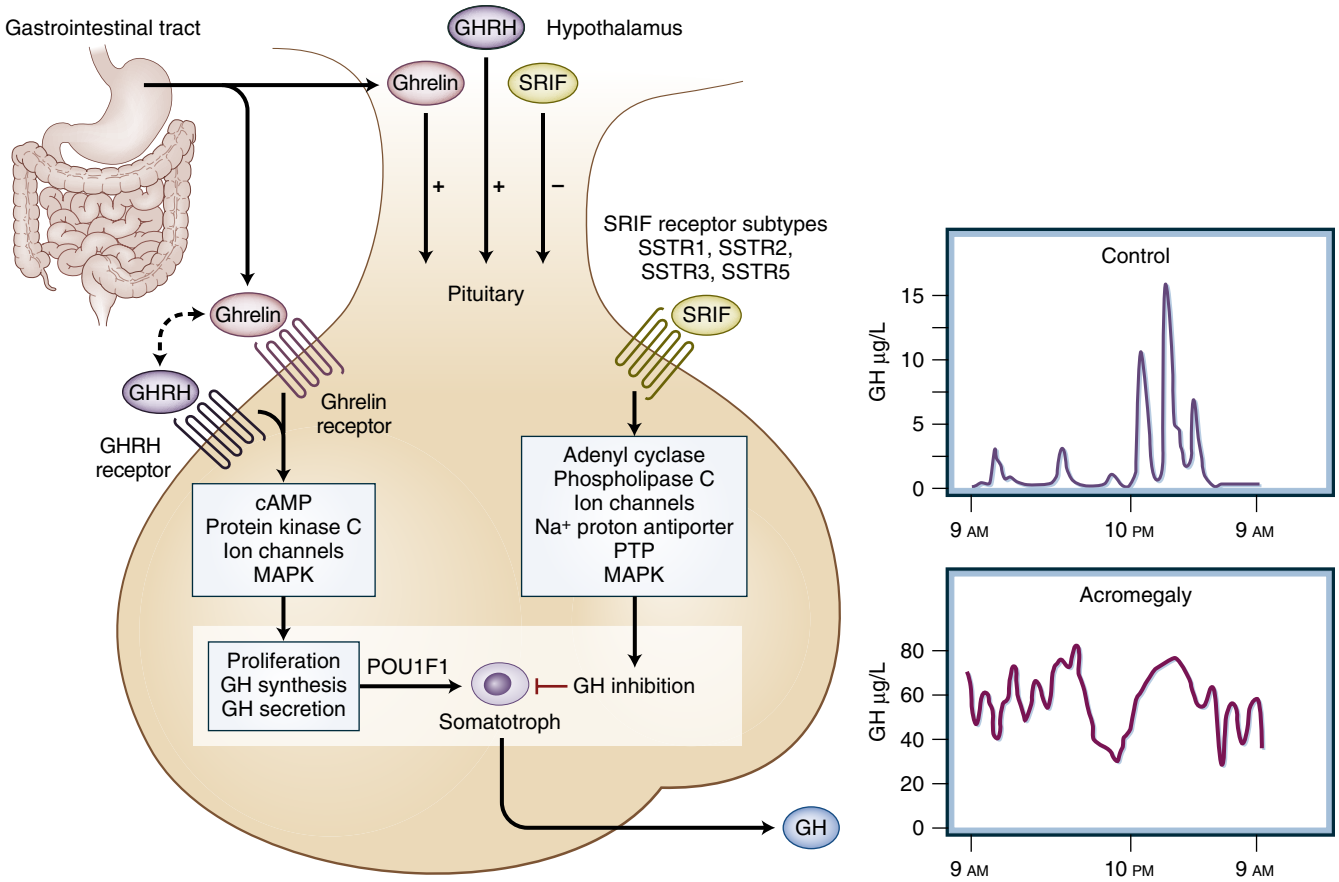
The source of excess GH secretion in acromegaly may not necessarily be of pituitary origin. Because management of ectopic acromegaly differs from that for pituitary-dependent GH hypersecretion, rigorous clinical and biochemical criteria should be fulfilled to confirm the diagnosis of ectopic acromegaly.[370] These include demonstration of elevated circulating GHRH or GH levels in the absence of a primary pituitary lesion, a significant arteriovenous hormone gradient across the ectopic tumor source, biochemical and clinical cure of acromegaly after resection of the ectopic hormone-producing tumor, as well as normalization of the GHRH/GH/IGF1 axis. Finally, GHRH or GH gene product expression should be shown. Patients with nonconclusive imaging or biochemical or clinical features of pituitary acromegaly may inadvertently be diagnosed as harboring a nonpituitary source of excess GH secretion and hence be inappropriately treated.

GHRH Hypersecretion

Hypothalamic tumors, including hamartomas, choristomas, gliomas, and gangliocytomas, may produce GHRH with subsequent somatotroph hyperplasia and resultant acromegaly[159] (see Fig. 9.31). Primary mammosomatotroph hyperplasia with no evidence for pituitary adenoma or an extrapituitary tumor source of GHRH has been described in gigantism.[371] The structure of hypothalamic GHRH was in fact elucidated from material extracted from pancreatic GHRH-secreting neuroendocrine tumors in patients with acromegaly.[158] GHRH immunoreactivity is detectable in about 25% of neuroendocrine tumor samples, and those of bronchial (foregut) origin comprise most tumors associated with ectopic GHRH secretion.[372] Acromegaly in neuroendocrine tumor patients, however, is uncommon. In a retrospective survey of 177 patients with acromegaly, only a single patient was identified with elevated plasma GHRH levels.[373] Rare pancreatic cell tumors, small cell lung cancers, adrenal adenoma, pheochromocytoma, and medullary thyroid, endometrial, and breast cancer express GHRH and may cause acromegaly.[374,375] Surgical resection of the tumor secreting ectopic GHRH should reverse GH hypersecretion, and pituitary surgery is not required in these patients. Carcinoid syndrome with ectopic GHRH secretion can also be managed with somatostatin receptor ligands (SRL), which lower GH and IGF1 levels and suppress ectopic tumor elaboration of GHRH.[376]

Ectopic Pituitary Adenomas

GH-secreting adenomas may arise from ectopic pituitary remnants in the sphenoid sinus, petrous temporal bone, or nasopharyngeal



• **Fig. 9.32** Normal and disrupted GHRH-GH-IGF1 axis and molecular targets for therapy. Normal and disrupted GHRH-GH-IGF1 axis in GH-secreting somatotroph adenomas, pituitary somatotroph cell development, and gene expression are determined by the POU1F1 transcription factor. Net GH secretion is determined by integration of hypothalamic, nutritional, hormonal, and intrapituitary signals. GH synthesis and secretion are induced by hypothalamic GHRH and gut-derived ghrelin. Hypothalamic somatostatin *(SRIF)* suppresses GH secretion mainly by high-affinity binding to SSTR2 and SSTR5 receptor subtypes expressed on somatotrophs. SRIF or analogs signal through SSTR2 and SSTR5 to control GH hypersecretion and shrink tumor mass. GH secretion patterns in a normal subject and in acromegaly are depicted in the insets showing secretory bursts (mainly at night) and daytime troughs. *cAMP,* cyclic adenosine monophosphate; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *IGF1,* insulin-like growth factor type 1; *MAPK,* mitogen-activated protein kinase; *PTP,* protein tyrosine phosphatase; *SRIF,* somatostatin (somatotropin release–inhibiting factor); *SSTR,* SRIF receptor subtypes. (Modified from Melmed S. Acromegaly pathogenesis and treatment. *J Clin Invest.* 2009;119:3189–3202, used with permission.)

cavity.[377] Very rarely, pituitary carcinoma may spread to the meninges, CSF, or cervical lymph nodes, resulting in functional GH-secreting metastases, which may be diagnosed by radiolabeled octreotide imaging (Octreoscan).[378]

Peripheral Growth Hormone–Secreting Tumors

Lung adenocarcinoma, breast cancer, and ovarian tissues contain immunoreactive GH without clinical evidence of acromegaly. Rarely, GH-secreting intramesenteric pancreatic islet cell tumor[370] or a non-Hodgkin lymphoma[379] may cause acromegaly. These patients have a normal-sized or small pituitary gland on MRI, absent GH response to TRH injection, and normal levels of circulating plasma GHRH.

Acromegaloidism

Rarely, patients exhibit soft tissue and skin changes usually associated with acromegaly but normal baseline and dynamic GH and IGF1 with no demonstrable pituitary or extrapituitary tumor, termed *acromegaloid*. Pachydermoperiostosis should be considered in the differential diagnosis. Insulin resistance and defective IGF1 binding demonstrated in cells derived from some patients with acanthosis nigricans, and treatment is symptomatic.

Gigantism

Tall stature may be caused by a GH-secreting pituitary tumor or hyperplasia associated with several specific syndromes, including the McCune-Albright syndrome, with somatotroph hyperplasia or rarely pituitary adenomas.[380] *AIP* mutations have been traced in the lineage of some patients with gigantism.[381] Somatotroph hyperplasia and acidophilic stem cell adenomas (with hyperprolactinemia) may rarely cause gigantism during infancy or early childhood, suggesting early hypersecretion of GHRH or disordered pituitary cell differentiation.[371] Eighteen patients were reported with X-linked gigantism (X-LAG syndrome), with Xq26.3 chromosomal microduplications (Fig. 9.33). These patients exhibit features of very early accelerated gigantism. Tumors derived from these patients overexpress *GPR101*, a putative regulator of GH.[382] Pituitary gigantism

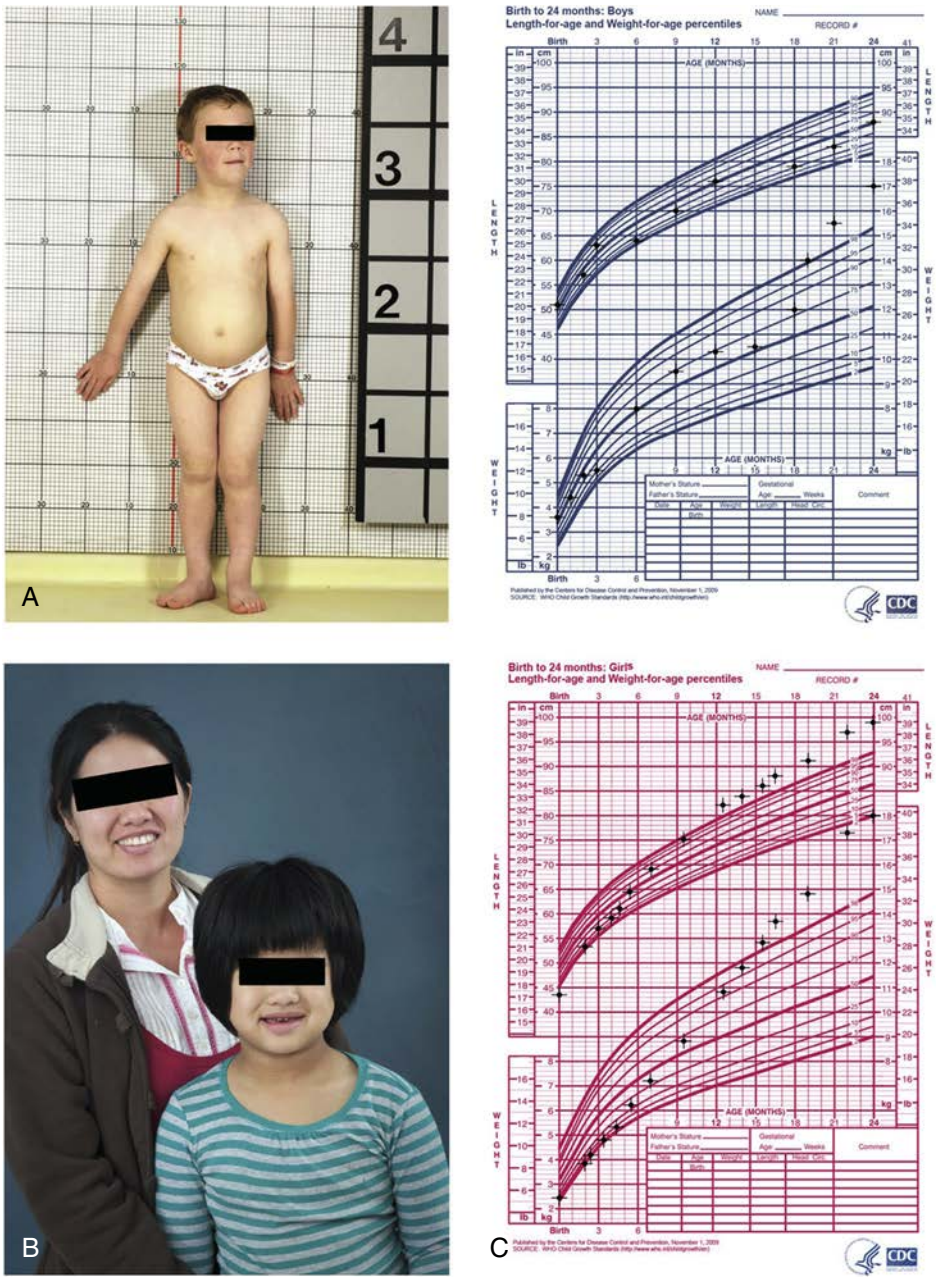
TABLE 9.24 Causes of Acromegaly

Cause	Prevalence (%)	Hormonal Products	Clinical Features	Pathologic Characteristics
Excess GH Secretion				
Pituitary	98			
Densely granulated GH cell adenoma	30	GH	Slow growing, clinically insidious	Resemble normal somatotrophs, numerous large secretory granules
Sparsely granulated adenoma	30	GH	Rapidly growing, often invasive	Cellular pleomorphism, characteristic ultrastructure
Mixed GH cell and PRL cell adenoma	25	GH and PRL	Variable	Densely granulated somatotrophs, sparsely granulated lactotrophs
Mammosomatotroph cell adenoma	10	GH and PRL	Common in children; gigantism, mild hyperprolactinemia	Both GH and PRL in same cell, often same secretory granule
Acidophil stem cell adenoma		PRL and GH	Rapidly growing, invasive, hyperprolactinemia dominant	Distinctive ultrastructure, giant mitochondria
Plurihormonal adenoma		GH (PRL with αGSU, FSH/LH, TSH, or ACTH)	Often secondary hormonal products are clinically silent	Variable; either monomorphous or plurimorphous
GH cell carcinoma or metastases		GH	Usually aggressive	Documented metastasis
MEN1 (adenoma)		GH or PRL	Pancreatic, parathyroid, or pituitary tumors	Adenoma
McCune-Albright syndrome		GH, PRL	Classic triad	Hyperplasia
Ectopic sphenoid or parapharyngeal sinus pituitary adenoma		GH	Ectopic mass	Adenoma
Familial acromegaly		GH	Young patients	Large adenomas
Carney syndrome		GH	Classic syndrome	Adenoma
Extrapituitary Tumor				
Pancreatic islet cell tumor	<1			Small pituitary
Excess GHRH Secretion				
Central—hypothalamic hamartoma, choristoma, ganglioneuroma	<1		Hypothalamic mass	Somatotroph hyperplasia
Peripheral—bronchial carcinoid, pancreatic islet cell tumor, small cell lung cancer, adrenal adenoma, medullary thyroid carcinoma, pheochromocytoma	1	GH, PRL	Systemic features	Somatotroph hyperplasia, rarely adenoma

ACTH, Adrenocorticotrophic hormone; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *αGSU,* glycoprotein α-subunit; *LH,* luteinizing hormone; *MEN1,* multiple endocrine neoplasia type 1; *PRL,* prolactin; *TSH,* thyroid-stimulating hormone.

Adapted from Melmed S. Medical progress: Acromegaly. *N Engl J Med.* 2006;355:2558–2573; Melmed S, Braunstein GD, Horvath E, et al. Pathophysiology of acromegaly. *Endocr Rev.* 1983;4:271–290.

should be considered in children who are more than 3 standard deviations above normal mean height for age or more than 2 standard deviations over their adjusted mean parental height. The biochemical diagnosis is similar to that for acromegaly (i.e., GH levels >1 ng/mL after a glucose load, elevated concentrations of serum IGF1). In children undergoing pubertal growth spurts, GH responses to glucose may be paradoxical, and serum IGF1 concentrations are often physiologically elevated. Thus, the diagnosis requires clear-cut MRI evidence for a pituitary lesion. The differential diagnosis includes familial tall stature, redundancy of Y chromosomes, Marfan syndrome, and homocystinuria. Aggressive control of the tumor mass as well as hormone hypersecretion is important to mitigate long-term tissue damage from excess GH and IGF1. Surgery is recommended as primary therapy, and postoperative SRLs and the GH receptor antagonist have been used effectively.[383,384] Radiation therapy should be followed by lifelong serial evaluation of pituitary function.



• **Fig. 9.33** Growth patterns due to Xq26.3 microduplication. (A) Patient age 3 years and growth chart up to 24 months of age depicting early rapid acceleration in weight; acceleration in height did not begin until after age 2. (B) Patient age 3 years at a height of 120 cm and her unaffected mother. (C) Growth chart for another female patient depicting early increase in height and weight starting at 6 months. (From Trivellin G, Daly AF, Faucz FR, et al. Gigantism and acromegaly due to Xq26 microduplications and GPR101 mutation. *N Engl J Med*. 2014; 371:2363–2374.)

Clinical Features of Acromegaly

Manifestations of acromegaly are caused by either central pressure effects of the pituitary mass or peripheral actions of excess GH and IGF1. Headache is often severe and debilitating. Local signs are especially important presenting features because a higher preponderance of macroadenomas (>65%) is encountered in acromegaly as compared to mostly microadenomas for PRL-secreting tumors.[385]

Effects of hypersomatotropism on acral and soft tissue growth and metabolic function occur insidiously over several years[386](Table 9.25) (Figs. 9.34 and 9.35). As a result, acromegaly remains underrecognized; the slow onset and elusive symptomatology often result in delayed diagnosis, with a mean delay of almost 10 years, especially in females.[387] In a comparison of acromegaly features observed between 1981 and 1994, and between 1995 and 2006, the delay in diagnosis was quite similar (5.9 vs. 5.2 years). Although biochemical features were also remarkably similar, sleep apnea and colon polyps were more frequently encountered in the latter time frames, likely reflecting enhanced awareness.[388] Patients may seek care for dental, orthopedic, rheumatologic, or cardiac disorders. Only 13% of 256 patients diagnosed during a 20-year period

TABLE 9.25 Clinical Features of Acromegaly[a]

Local Tumor Effects
Pituitary enlargement
Visual field defects
Cranial nerve palsy
Headache

Somatic Effects

Acral Enlargement
Thickening of soft tissues in hands and feet

Musculoskeletal
Gigantism
Prognathism
Jaw malocclusion
Arthralgias and arthritis
Carpal tunnel syndrome
Acroparesthesia
Proximal myopathy
Hypertrophy of frontal bones

Skin
Hyperhidrosis
Oily
Skin tags

Colon
Polyps

Cardiovascular
Left ventricular hypertrophy
Asymmetric septal hypertrophy
Cardiomyopathy
Hypertension
Congestive heart failure

Pulmonary
Sleep disturbances
Sleep apnea—central and obstructive
Narcolepsy

Visceromegaly
Tongue
Thyroid
Salivary gland
Liver
Spleen
Kidney
Prostate

Endocrine-Metabolic Effects

Reproductive
Menstrual abnormalities

Galactorrhea
Decreased libido, impotence, low sex hormone–blinding globulin

Multiple Endocrine Neoplasia Type 1 (MEN1)
Hyperparathyroidism
Pancreatic islet cell tumors

TABLE 9.25 Clinical Features of Acromegaly[a]—cont'd

Carbohydrates
Impaired glucose tolerance
Insulin resistance and hyperinsulinemia
Diabetes mellitus

Lipids
Hypertriglyceridemia

Minerals
Hypercalciuria, increased 1,25-hydroxyvitamin D_3
Urinary hydroxyproline

Electrolytes
Low renin
Increased aldosterone

Thyroid
Low thyroxine-binding globulin
Goiter

[a]Most soft tissue and metabolic changes are reversible by tight hormonal control. Bony changes, hypertension, and central sleep apnea are generally not reversible.

Modified from Bonert V, Melmed S. Acromegaly. In: Bar S, ed. *Contemporary Endocrinology*. Totowa, NJ: Humana Press; 2002:201–228.

presented with primary symptoms of altered facial appearance or enlarged extremities.[389] In a review of several hundred patients presenting with acromegaly worldwide, 98% had acral enlargement, and hyperhidrosis was prominent in 70%.[386] When presenting early, facial and peripheral features are usually not obvious, and serial review of old photographs often accentuates the progress of subtle physical changes. Characteristic features include large fleshy lips and nose, spade-like hands, frontal skull bossing, and cranial ridges. Enlarged tongue, bones, salivary glands, thyroid, heart, liver, and spleen are the effects of generalized visceromegaly. Increase in shoe, ring, or hat size is commonly reported. Progressive acral changes may lead to facial coarsening and skeletal disfigurement especially if excess GH secretion begins prior to epiphyseal closure.[390] These changes include mandibular overgrowth with prognathism, maxillary widening, teeth separation, jaw malocclusion overbite, large nose, and coarse oily skin. Sonorous voice deepening occurs in association with laryngeal hypertrophy and enlarged paranasal sinuses. Up to half of patients may experience joint symptoms severe enough to limit daily activities. Arthropathy occurs in about 70% of patients, most of whom exhibit joint swelling, hypermobility, and cartilaginous thickening.[391] Cartilage T2 relaxation times on MRI scans are high, reflecting increased water content.[392] These signs may often persist after complete remission.[393] Local periarticular fibrous tissue thickening may cause joint stiffening or deformities and nerve entrapment. Knees, hips, shoulders, lumbosacral joints, elbows, and ankles are affected as monoarticular or polyarticular arthritides, but joint effusions rarely develop. Spinal involvement includes osteophytosis, disk space widening, and increased anteroposterior vertebral length, which may result in dorsal kyphosis. Neural enlargement and wrist tissue swelling may lead to carpal tunnel syndrome in up to half of all patients.

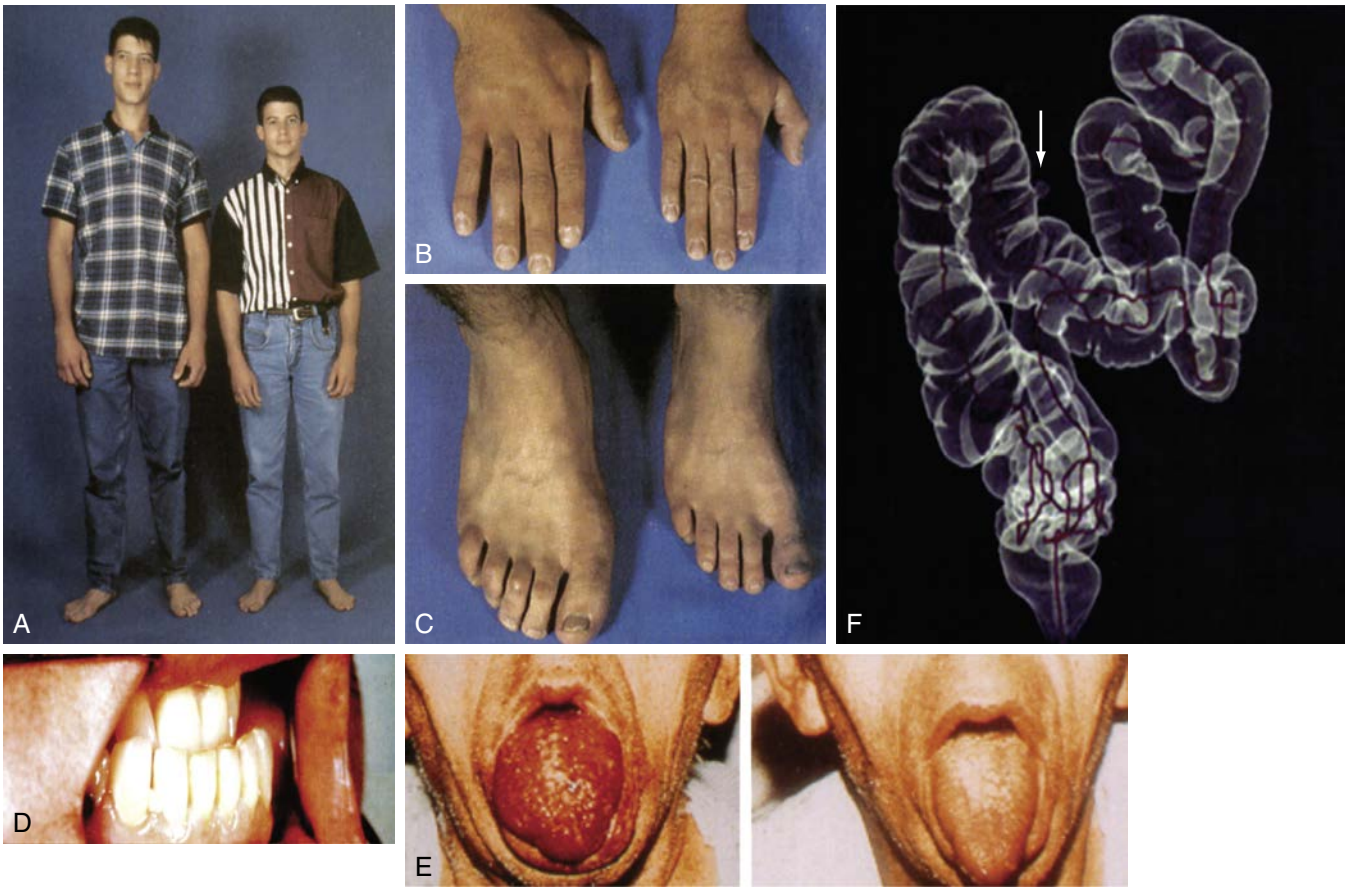
• **Fig. 9.34** Harvey Cushing's first acromegaly patient. (A) Some years before presentation and (B) at admission. (From Jane JA, Laws ER. History of acromegaly. In: Wass J, ed. *Handbook of Acromegaly.* Bristol, UK: BioScientifica; 2001:3–15.)

Both median and ulnar nerve cross-sectional areas increase, and nerve conduction is abnormal.[394] Chondrocyte proliferation with increased joint space occurs early, and ulcerations and fissures of weight-bearing cartilage areas are often accompanied by new bone formation. Debilitating osteoarthritis may result in bone remodeling, osteophyte formation, subchondral cysts, narrowed joint spaces, and lax periarticular ligaments. Vertebral fractures occur with increasing frequency, and osteophytes commonly occur at the phalangeal tufts and over the anterior aspects of spinal vertebrae.[395–397] Synovial edema leads to hyperplastic wrist ligaments and tendons that contribute to painful median nerve compression. Peripheral acroparesthesias and symmetric peripheral neuropathy should be distinguished from diabetic neuropathy, which may occur secondarily to acromegaly.[398] Proximal myopathy may also be accompanied by myalgias and cramps. Ligaments may ossify, and periarticular calcium pyrophosphate deposition occurs. Hyperhidrosis and malodorous oily skin are common early signs, occurring in up to 70% of patients. Facial wrinkles, nasolabial folds, heel pad thickening, and body hair coarsening[399] are attributed to glycosaminoglycan deposition and increased connective tissue collagen production. Skin tags are common and may be markers for the adenomatous colonic polyps. Raynaud phenomenon is reported in up to one-third of patients.

Symptomatic cardiac disease is present in about 20% of patients at diagnosis and is a major cause of morbidity and mortality.[400] Hypertension is present in about 50% of patients with active acromegaly, and half of these patients have evidence of left ventricular (LV) dysfunction with or without regurgitant valve disease.[401] Asymmetric septal hypertrophy and cardiac failure with increased ventricular ejection fraction may occur, and subclinical LV diastolic dysfunction is due to myocardial hypertrophy, interstitial fibrosis, and lymphocytic myocardial infiltrates. Increased aortic root diameter and aortic ectasia were reported in 26% of patients.[402] Resting electrocardiograms are abnormal in about 50% of patients, with ST-segment depression, T-wave abnormalities, conduction defects, and arrhythmias. Plasma renin levels are suppressed, and renal sodium channel activity is induced by GH at the aldosterone-sensitive distal nephron.[403] In a prospective study of 30 patients, cardiovascular responses to SRL therapy were shown to be highly variable, despite attainment of biochemical control.[404] Despite increased cardiovascular risk factors,[405] there is no reported increased myocardial infarction in acromegaly.[406]

Prognathism, thick lips, macroglossia, and hypertrophied nasal structures may obstruct airways. Irregular laryngeal mucosa, cartilage hypertrophy, tracheal calcification, and cricoarytenoid joint arthropathy lead to unilateral or bilateral vocal cord fixation or laryngeal stenosis with voice changes. Tracheal intubation may be particularly difficult in patients undergoing anesthesia, and tracheostomy may be required. Both central respiratory depression and airway obstruction lead to paroxysmal daytime sleep (narcolepsy), sleep apnea, and habitual excessive snoring. Obstructive sleep apnea with daytime somnolence occurs especially in men with acromegaly, who also may have a ventilation-perfusion defect with hypoxemia. Sleep apnea may also be central in origin and associated with higher GH and IGF1 levels.[407]

Hypertrophied tissue surrounding the canal of Schlemm may impede aqueous filtration, leading to open-angle glaucoma. Progressive facial and bodily disfigurement often leads to lowered



• **Fig. 9.35** Clinical features of acromegaly. (A–C) Features of acromegaly/gigantism in identical twins. A 22-year-old man with gigantism due to excess growth hormone is shown to the left of his identical twin. The increased height and prognathism (A) and enlarged hand (B) and foot (C) of the affected twin are apparent. Their clinical features began to diverge at the age of approximately 13 years. (D) Increased incisor spacing and prognathism in patient with acromegaly. (E) Macroglossia *(left)* and a normal tongue *(right).* (F) Dolicomegacolon in acromegaly as visualized by computed tomography colonography. (A–C, from Gagel R, McCutcheon IE. Images in clinical medicine: pituitary gigantism. *N Engl J Med.* 1999;324:524, used with permission; D and E, from Turner HE. Clinical features, investigation and complications of acromegaly. In: Wass J, ed. *Handbook of Acromegaly.* Bristol, UK: BioScientifica; 2001, used with permission; F, from Resmini E, Tagliafico A, Bacigalupo L, et al. Computed tomography colonography in acromegaly. *J Clin Endocrinol Metab.* 2009;94:218–222, used with permission.)

self-esteem. Depression and mood swings may occur secondarily to physical deformity, with impaired quality of life.[408]

Growth Hormone and Tumor Formation

GH and IGF1 as well as insulin may exhibit direct or indirect mitogenic effects on mammalian cells and act as permissive cell growth stimulators.[409] In a meta-analysis, the risks of adenomatous and hyperplastic polyps and colorectal cancer were increased 2.5-fold, 3.6-fold, and 4.4-fold, respectively.[410] The prevalence of polyps was 32% in 165 patients in a case control study, with an estimated relative risk of 6.21 (95% CI, 4.08–9.48). Higher IGF1 levels at diagnosis were more likely associated with distal colon lesions.[411] Hypertrophic mucosal folds and colonic hypertrophy as well as diverticulae[412] are commonly present, and dolicomegacolon may be visualized by CT colonography.[413] Increased incidence of gallbladder polyps[414] and benign prostate hypertrophy[415] have been reported. Although the German Acromegaly Registry does not report higher cancer incidence,[416] a survey of 1512 patients in Italy revealed a moderately increased cancer incidence.[417] Similarly, a nationwide study in Denmark of 529 acromegaly patients revealed an overall cancer SIR of 1.5 (95% CI, 1.2–1.8). Incidence of colorectal, kidney, and thyroid cancers appear to be the most significantly elevated.[418–420] Thus, although disordered cell proliferation and increased risk for growth promotion of coexisting neoplasms as well as mortality could be anticipated from elevated GH and IGF1 levels, a significantly enhanced cancer incidence has not been consistently observed. Colon cancer appears to be of particular concern, and screening colonoscopy should be performed at diagnosis in all patients. Thereafter, screening should follow established guidelines. As acromegaly patients are now living longer as a result of improved biochemical and cardiac control, long-term prospective studies are required to resolve this question in an aging population.

Endocrine Complications

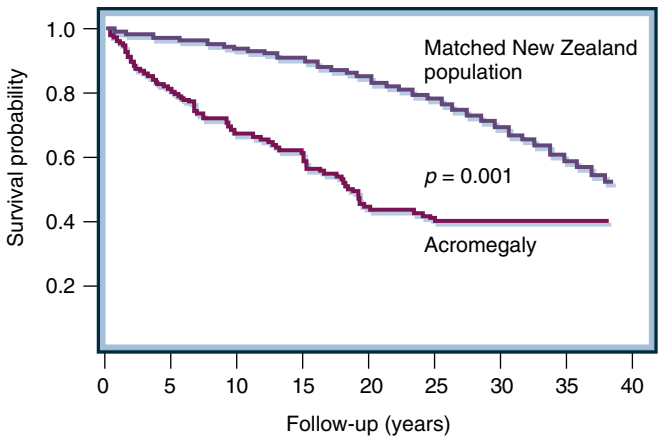
About 30% of patients exhibit elevated serum PRL levels (up to 100 ng/mL or more), with or without galactorrhea. Functional pituitary stalk compression by a pituitary mass prevents lactotroph access of hypothalamic dopamine, releasing the cell from tonic hypothalamic inhibition. GH-secreting adenoma

subtypes may also concomitantly secrete PRL. As GH behaves as an agonist for breast PRL-binding sites, the tumor may cause galactorrhea in the face of normal PRL levels. Tumor mass compressing surrounding normal pituitary tissue may also cause hypopituitarism. Over half of all patients have amenorrhea or impotence,[385–421] and secondary thyroid or adrenal failure is present in about 20% of patients. Gonadal dysfunction may exacerbate bone loss and vertebral fractures.[396,397] In a prospective controlled study of 22 patients, active acromegaly was associated with PTH-independent calcium-phosphate balance, with low PTH and high phosphorus levels associated with increased coupled bone formation and resorption.[422] Skeletal fragility may persist despite long-term biochemical control.[423,424]

GH suppresses beta cell function and antagonizes insulin action, and acromegaly is associated with increased insulin resistance, lipolysis, and gluconeogenesis,[425,426] despite increased lean body mass, decreased fat mass, and elevated IGF1 concentrations that would be expected to enhance insulin sensitivity. Accordingly, fasting glucose intolerance and diabetes mellitus occur in about 50% of patients.[426,427] Increased diabetes risk with a hazard ratio of 4.0 (95% CI: 2.7–5.8) was reported in a nationwide cohort study in Denmark.[361] Unique cardiomyopathy features are also associated with diabetes and acromegaly.[425] Carbohydrate intolerance and insulin requirements improve rapidly on lowering GH after surgery or somatostatin analogue therapy; however, diabetes may persist in some patients after biochemical remission.[428] Hypertriglyceridemia (type IV), hypercalciuria, and hypercalcemia also occur. Thyroid dysfunction may be associated with diffuse or nodular toxic or nontoxic goiter or Graves disease, especially as IGF1 is a major determinant of thyroid cell growth.[429] Associated MEN1 features may be present in affected individuals, including hypercalcemia with hyperparathyroidism or pancreatic tumors.

Morbidity and Mortality

In a meta-analysis of 16 studies, overall mortality rate was reported to be increased in acromegaly with a standardized mortality ratio of 1.72. Lower recent mortality rates reported likely reflect the positive impact of introduction of somatostatin analogues, improved surgical technique, and enhanced cardiac therapies[430] (Fig. 9.36). Cardiovascular disease is the leading cause of death followed by respiratory disease (18%) and cerebrovascular disease (14%). Diabetes mellitus, occurring in 20% of patients, is associated with 2.5 times the predicted mortality rate, and hypertension is present in about half of all patients.[431] The most significant mortality determinants are GH levels greater than 2.5 μg/L, elevated IGF1 levels, the presence of coexisting hypertension and cardiac disease, older age, a history of pituitary irradiation, and overreplaced ACTH-dependent adrenal insufficiency.[59] Overtreatment of adrenal insufficiency with doses of hydrocortisone more than 25 mg daily also is predictive of mortality.[59] In the large UK West Midlands acromegaly registry comprising 501 patients, a last available recorded GH level of 1 ng/mL or less versus 1 ng/mL or more was highly predictive of mortality rate. However, when summated over time in an unbiased fashion, the relative risk of fatality appeared to be associated with a GH cutoff level of 5 ng/mL.[432] Moreover, control of GH levels to less than 2.5 μg/L and normal IGF1 levels after surgery or medical treatments is associated with significant reductions of both morbidity and mortality.[431,433] However, in a 20-year follow-up controlled study, all-cause mortality rates remained elevated (odds ratio [OR] 1.6; 95% CI, 1.2–2.2) and was more evident in women (Table 9.26).



• **Fig. 9.36** Mortality rate outcome in retrospective studies of acromegaly. (Data integrated from Holdaway IM, Rajasoorya RC, Wong J, et al. The natural history of treated functional pituitary adenomas. In: Webb S, ed. *Pituitary Tumors*. Bristol, UK: BioScientifica; 1998:31–42.)

TABLE 9.26 Factors Associated With All-Cause Acromegaly Mortality on Multivariate Analysis[a]

Variable	HR	95% CI	*p* Value
Age at diagnosis (years)	1.1	1.08–1.13	<0.001
Gender (M/F)			
Up to 20 years from diagnosis	2.5	1.46–4.17	<0.001
After 20 years from diagnosis	0.9	0.42–1.91	0.78
Serum basal GH at diagnosis (μg/L)	1.0	0.99–1.01	0.91
Primary operation (no vs yes)	1.5	0.86–2.61	0.15
Radiotherapy (no vs yes)	0.5	0.34–0.88	0.012
Last known basal serum GH <2.5 μg/L after primary treatment (no vs yes)[b]	1.8	1.21–2.83	0.048

[a]Piecewise Cox model.

[b]Measured a median of 5 years after diagnosis.

CI, Confidence interval; *F*, female; *GH*, growth hormone; *HR*, hazard ratio; *M*, male.

Modified from Ritvonen E, Löyttyniemi E, Jaatinen P, et al. Mortality in acromegaly: a 20-year follow-up study. *Endocr Relat Cancer*. 2016;23:469–480.

Causes of death shifted with time from predominantly cardiovascular to cancer diagnoses.[434]

Diagnosis

Measurement of Growth Hormone and IGF1 Levels

The diagnosis of acromegaly requires measurement of a GH nadir during a 75-g glucose load of less than 0.4 μg/L using ultrasensitive GH assays or GH less than 1.0 μg/L using standard assays with an accompanying elevated IGF1 level[435,436] (Fig. 9.37).

In healthy subjects, serum GH levels initially fall after oral glucose administration and subsequently increase as plasma glucose declines. In patients with acromegaly, oral glucose fails to suppress GH; GH levels may increase, remain unchanged, or fall modestly in approximately one-third of patients. Basal morning (AM) and random GH levels are usually elevated in acromegaly. Because of the episodic nature of GH secretion, however, serum concentrations may normally fluctuate from "undetectable" up to 30 μg/L. Unlike the largely undetectable nadir GH levels in normal subjects, those with acromegaly sampled over 24 hours have mean GH levels above 2 μg/L.[436] Evoked GH responses to GHRH administration are not of diagnostic use. Random GH levels measured with sensitive assays in acromegaly may be as low as 0.37 μg/L with persistently elevated postoperative IGF1 levels.[437] Serum IGF1 levels are high[438,439] and correlate with clinical features (Fig. 9.38). Basal GH levels determine ambient circulating IGF1 levels in a log-linear fashion.[440] Age-matched and gender-matched IGF1 elevations may persist for several months after GH levels are biochemically controlled after surgery. Although elevated IGF1 levels may be encountered during pregnancy and late puberty, an appropriately corrected high IGF1 level is highly specific for acromegaly and correlates with clinical indices of disease activity. IGF-binding protein 3 (IGFBP3) levels are also elevated but provide little added diagnostic value. GH-secreting adenomas exhibit discordant GH responses to TRH and GnRH administration in up to 50% of patients, but these adjunctive tests are rarely required to confirm the diagnosis.

Differential Diagnosis

The overwhelming majority of patients with acromegaly harbor a GH cell pituitary adenoma; rarely extrapituitary acromegaly should be considered. Regardless of the cause of unrestrained GH secretion, IGF1 levels are invariably elevated, and GH levels fail to suppress after an oral glucose load. When clinical features of acromegaly are associated with normal GH and IGF1 levels, "burned out" or "silent" acromegaly associated with an infarcted pituitary adenoma, often with a secondary empty sella, should be considered.[441] About 5% of consecutive patients with proven GH cell adenomas have "normal" GH and elevated IGF1 levels. Plasma GHRH levels are invariably elevated in patients with peripheral GHRH-secreting tumors but are normal or low in those with hypothalamic GHRH-secreting tumors.[373] Presumably, excess eutopic hypothalamic GHRH secretion into the hypophyseal portal system does not appreciably enter the systemic circulation.

MRI and CT scanning are used to localize pituitary or extrapituitary tumor. Routine abdominal or chest imaging of all acromegaly patients will yield a very low incidence of ectopic tumors and is not recommended. A normal-sized or small pituitary gland, or clinical and biochemical features of other tumors known to be associated with extrapituitary acromegaly and elevated circulating GHRH levels, are indications for extrapituitary imaging. An enlarged hyperplastic pituitary gland, however, is often present in patients with peripheral GHRH-secreting tumors, and the radiologic diagnosis of a pituitary adenoma may be difficult to exclude. The very rare McCune-Albright syndrome should be considered after definitive exclusion of pituitary and extrapituitary tumors.

Treatment

Aims

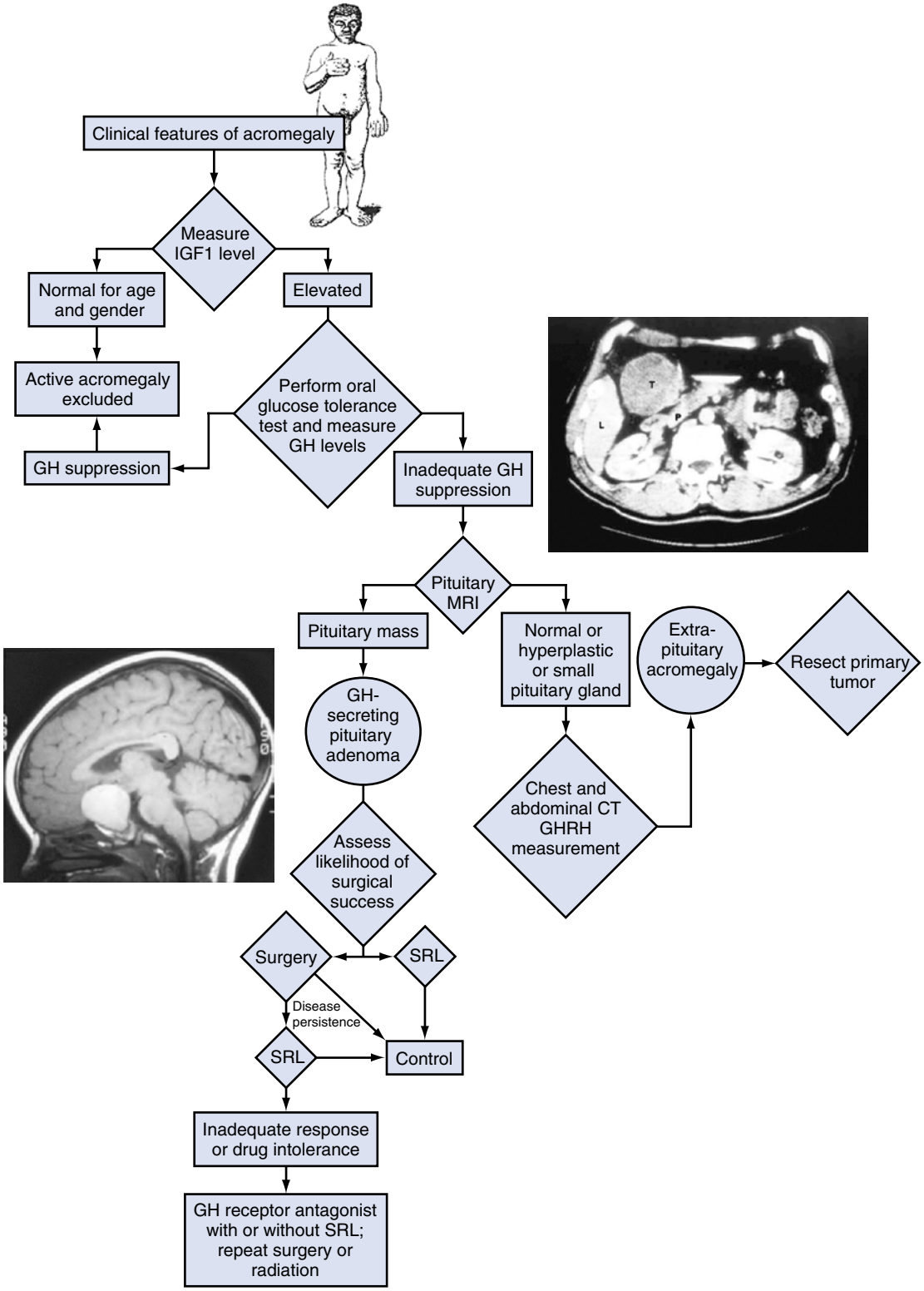
A comprehensive strategy for treating patients with acromegaly should aim to manage the pituitary mass, suppress GH and IGF1 hypersecretion, and prevent long-term clinical sequelae of hypersomatotropism while maintaining normal anterior pituitary function.[442,443] Serum GH levels should be suppressed to at least 1 μg/L or less after an oral glucose load, and age-matched and gender-matched serum IGF1 levels should be normalized. Ideally, a controlled patient should also have a "normal" 24-hour integrated secretion of GH (<2.5 μg/L), but these measurements are impractical. As current therapeutic modes for acromegaly management, including surgery, irradiation, and medical treatment, do not uniformly fulfill these goals,[444] personalized approaches have been proposed (discussion to come).

Surgical Management

Well-circumscribed somatotroph cell adenomas should preferably be resected by transsphenoidal surgery using either microscopic or laparoscopic techniques.[445–447] Successful resection alleviates preoperative compression effects and compromised trophic hormone secretion, and the skilled surgeon balances the extent of maximal tumor tissue removal while preserving anterior pituitary function. Within 2 hours of successful resection, metabolic dysfunction and soft tissue swelling start improving, and GH levels are sometimes controlled within an hour. Overall, 38% to 83% of patients achieve normalized IGF1 levels. Fewer patients with macroadenomas are controlled. Of 1018 patients reported in 13 studies, 73% with microadenomas and 61% harboring macroadenomas achieved GH levels lower than 1.0 μg/L during glucose loading and normal serum IGF1 levels (Table 9.27).[448–450] When remission was rigorously defined as a normal IGF1 and either a nadir glucose-suppressed GH level less than 0.4 μg/L or a random GH level less than 1 μg/L, all 14 of 14 patients with microadenomas and 28 of 46 patients with macroadenomas achieved remission.[445] As over one-third of premenopausal women exhibit cavernous sinus tumor invasion, remission rates are lower than in men under 50 years of age.[451] Experience of the neurosurgeon,[38] smaller tumor size, lower Knosp score, and lower preoperative IGF1 and GH levels are predictive of postsurgical remission (Table 9.28). Postoperative GH level measured within 24 hours of surgery is a significant outcome predictor (Fig. 9.39).[42] Difficulties in endotracheal intubation due to macroglossia or severe kyphosis may rarely necessitate tracheostomy for anesthesia.

Side Effects

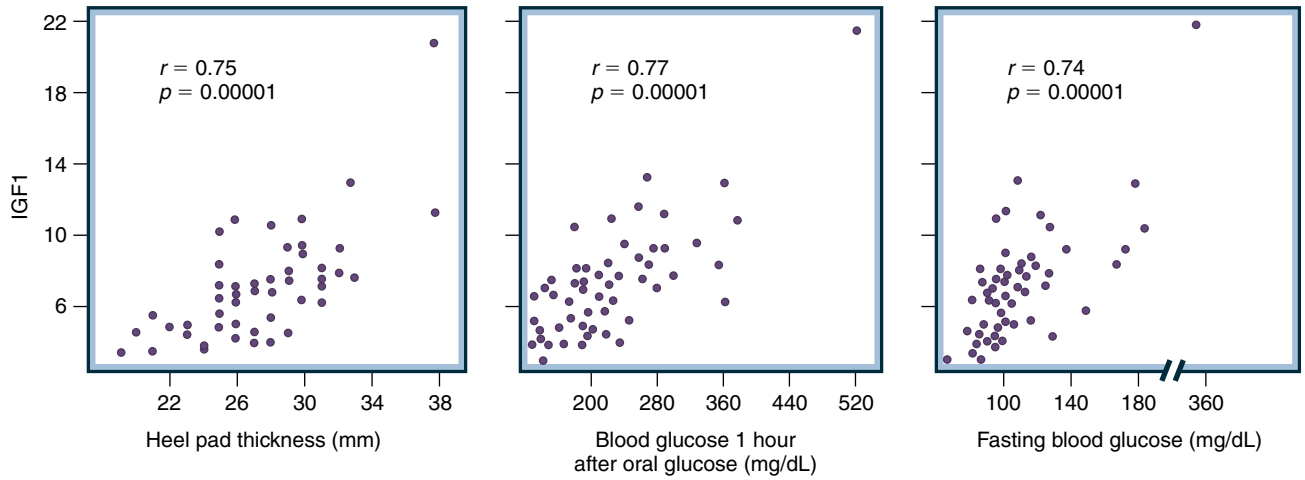
Although often transient, surgical complications may require lifelong pituitary hormone replacement. New hypopituitarism develops in up to approximately 20% of patients, reflecting operative damage to the surrounding normal pituitary tissue. Permanent diabetes insipidus, CSF leaks, hemorrhage, and meningitis occur in up to 10% of patients (see Table 9.3). Postoperative pituitary deficits assessed in a meta-analysis occur in ~15% of patients overall. GH deficiency may require careful replacement if clinical features of GH deficiency become apparent.[452] Secondary adrenal insufficiency is an important determinant of mortality,[453] and the incidence of postoperative adrenal failure is 2/1000 person-years in acromegaly patients in remission.[454] The extent and prevalence of local complications depend upon tumor size and invasiveness. Experienced pituitary surgeons report more favorable postoperative complication rates.[38,39] Biochemical or anatomic acromegaly recurrence (~7% over 10 years) or postoperative tumor persistence may indicate incomplete resection of adenomatous tissue, surgically inaccessible cavernous sinus tissue, or nesting of functional tumor tissue within the dura.



• **Fig. 9.37** Diagnosis and treatment of acromegaly. Oral glucose tolerance test (OGTT) is performed with 75 g glucose and growth hormone (GH) measured during 2 hours. Disease control implies nadir GH level of less than 1 μg/L after OGTT using standard assays and less than 0.4 μg/L using ultrasensitive assays, and age-matched and gender-matched normal insulin-like growth factor type 1 (IGF1) level. *CT,* computed tomography; *GHRH,* growth hormone–releasing hormone; *MRI,* magnetic resonance imaging; *SRL,* somatostatin receptor ligand. Insets depict pituitary adenoma *(left)* and extrapituitary acromegaly *(right): L,* liver; *P,* pancreas; *T,* tumorsecreting GH. (From Melmed S. Acromegaly. *N Engl J Med.* 2006;355:2558–2573. Clinical features figure from Minkowski O. Ueber einen Fal von Akromegalie Berliner. *Klin Wochenschr.* 1887;21:371–374.)

Radiation Therapy

Primary or adjuvant radiation of GH-secreting tumors may be achieved by conventional external deep x-ray therapy as well as heavy-particle (proton-beam) or stereotactic radiosurgery. Maximal tumor radiation should ideally be attained with minimal soft tissue damage. Radiation is a highly individualized choice, depending on the expertise and experience of the treating radiotherapist, and includes careful physician and patient consideration of the benefits of therapy weighed against potential risks. For conventional therapy, up to 5000 rads are administered in split doses of 180-rad fractions divided over 6 weeks. Radiation arrests tumor growth, and most adenomas ultimately shrink. As GH levels fall gradually during the first year after treatment, most patients are still exposed to unacceptably high levels of circulating GH and



• **Fig. 9.38** Correlation between insulin-like growth factor type 1 (IGF1) and clinical indexes linear regression analysis between fasting somatomedin C (IGF1) and growth hormone levels 1 hour after oral glucose administration. (Adapted from Clemmons DR, Van Wyk JJ, Ridgway EC, et al. Evaluation of acromegaly by radioimmunoassay of somatomedin-C. *N Engl J Med.* 1979;301:1138–1142.)

TABLE 9.27 Remission After Transsphenoidal Surgery for GH-Secreting Pituitary Adenomas Published Since 2010

		REMISSION				
Study	*n*	Overall	Microadenomas	Macroadenomas	Remission Criteria (GH Nadir), (μg/L)	Technique
Hofstetter (2010)	24	38	NA	NA	<1.0	Endoscope
Gondim (2010)	67	75	86	72	<1.0	Endoscope
Campbell (2010)	26	58	75	55	<1.0	Endoscope
Jane (2011)	60	70	100	61	<1.0	Endoscope
Wang (2012)	43	67	77	63	<1.0	Endoscope
Starke (2013)	43	70	82	66	<1.0	Microscope
Starke (2013)	72	71	88	66	<1.0	Endoscope
Shin (2013)	53	49	83	46	<1.0	Endoscope
Hazer (2013)	214	63	63	63	<1.0	Endoscope
Yilidirim (2014)	56	66	80	67	<1.0	Endoscope
Fathalla (2015)	41	45	NA	NA	<1.0	Endoscope
Fathalla (2015)	23	34	NA	NA	<1.0	Microscope
Netuka (2016)	105	61	75	58	<1.0	Endoscope
Babu (2017)	55	78	35	65	<1.0	Endoscope
Sarkar (2017)	66	29	13	44	<1.0	Endoscope
Kim (2017)	134	73	87	72	<0.4	Endoscope

Data from Buchfelder M, Schlaffer SM. The surgical treatment of acromegaly. *Pituitary.* 2017;20:76–83; Babu H, Ortega A, Nuno M, et al. Long-term endocrine outcomes following endoscopic endonasal transsphenoidal surgery for acromegaly and associated prognostic factors. *Neurosurgery.* 2017;81:357–366; Kim JH, Hur KY, Lee JH, et al. Outcome of endoscopic transsphenoidal surgery for acromegaly. *World Neurosurg.* 2017;104:272–278.

IGF1 during the initial years if not receiving concomitant medical therapy. Stereotactic radiosurgery effectively focuses on the adenoma, thereby sparing normal surrounding structures.[51,455–457] Of 136 patients followed for a median 61.5 months, 65% achieved normalized IGF1 levels or nadir GH level less than 1 ng/mL after an oral glucose load.[51] Higher radiation doses to the tumor margin as well as maximal doses and lower initial IGF1 levels portend a significantly more favorable outcome.[51] In a comprehensive meta-analysis, stereotactic radiosurgery was shown to be modestly more effective than conventional radiotherapy, with significantly lower IGF1 levels achieved.[458] In a multicenter study comprising 371 patients receiving stereotactic radiosurgery, endocrine remission was achieved in 59% of patients, with a mean time to remission of 38 months. Biochemical relapse was observed in 9%, with a mean time to recurrence of 17 months.[459]

TABLE 9.28 Significant Predictors of Postoperative Biochemical Remission in Acromegaly Patients

Older age
Smaller tumor size
Lower Knosp grade
Lower preoperative GH level
Lower preoperative IGF1 level

GH, Growth hormone; *IGF1,* insulin-like growth factor 1.

Modified from Sun H, Brzana J, Yedinak CG, et al. Factors associated with biochemical remission after microscopic transsphenoidal surgery for acromegaly. *J Neurol Surg B Skull Base.* 2014;75:47–52.

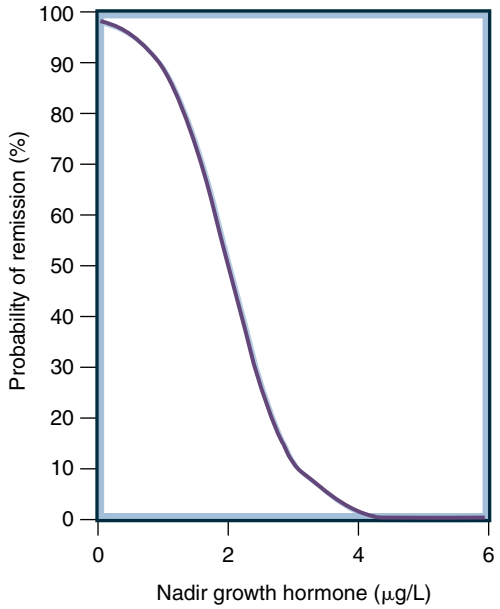
Side Effects

After 10 years, about half of all patients receiving radiotherapy have signs of pituitary trophic hormone disruption, and this prevalence increases annually thereafter, requiring gonadal steroids, thyroid hormone, or corticosteroid replacement. Side effects of conventional radiation, including hair loss, cranial nerve palsies, tumor necrosis with hemorrhage, and rarely loss of vision or pituitary apoplexy, have been documented in up to 2% of patients. Lethargy, impaired memory, and personality changes may also occur. In a 10-year follow-up study of 35 patients treated with Gamma Knife radiosurgery, half the patients developed pituitary hormone deficiencies (40% hypoadrenalism, 11% hypothyroidism, 13% hypogonadism, and 6% GH deficiency).[458] In another study, new pituitary hormone deficits were reported in 31% of patients within 61.5 months; however, visual deterioration and new cranial nerve palsies were rarely observed.[51] Because of side effects and slow onset of efficacy, radiation therapy should be used as an adjuvant for patients not controlled by surgery or medical management or for those who do not consent to these therapies. Prior radiation has been associated with increased acromegaly mortality, especially with a 4.42 (95% CI, 2.71–7.22) standardized mortality ratio for cerebrovascular deaths.[453]

Medical Management

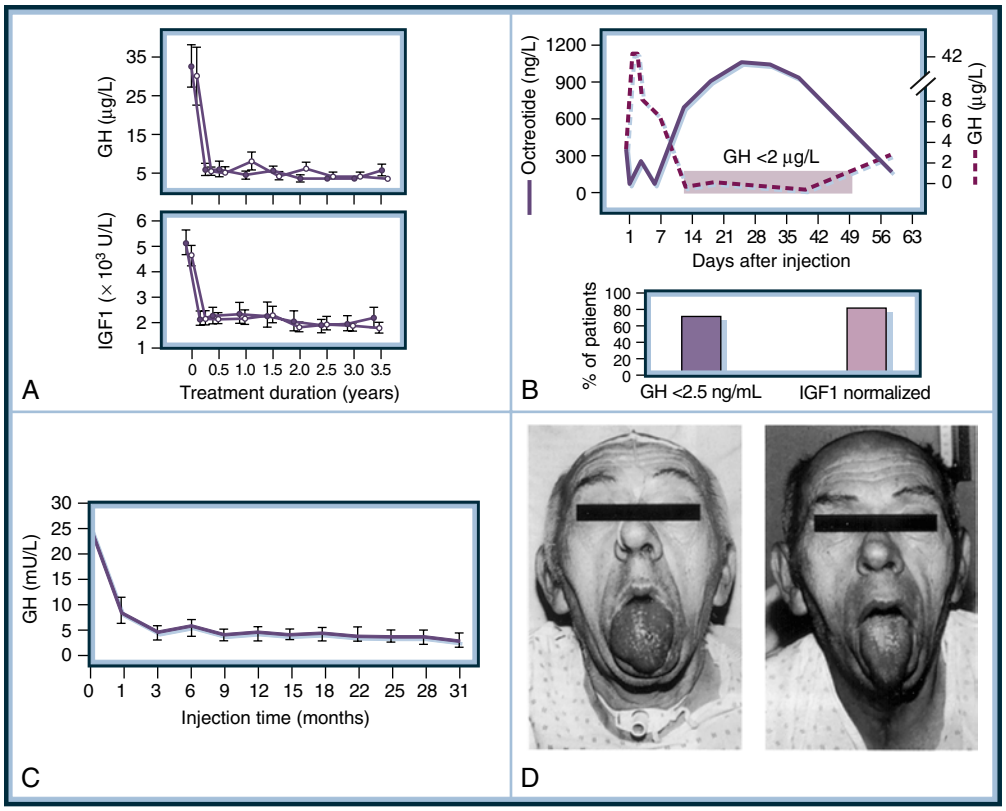
Dopamine Agonists

D_2 receptor agonists have been used as either primary or adjuvant therapy for acromegaly, especially in patients with mildly elevated GH and IGF1 levels.[460] In open nonrandomized studies, cabergoline is reported to suppress GH to less than 2 μg/L and normalize IGF1 in up to a third of patients with relatively mild IGF1 elevations.[460] In patients resistant to SRLs, the addition of cabergoline may normalize IGF1 levels in about 50% of patients.[461] Dopamine agonist efficacy appears to be



Nadir GH level (μg/L)	Remission (%)
0.1	98
0.3	97.1
0.5	95.7
0.7	93.7
0.9	90.8
1	88.9
1.2	84.2
1.4	78
1.6	70.2
1.8	61.1
2	51.1
2.1	46
2.3	36.2
2.5	27.4
2.7	20
2.9	14.3
3	12
4	1.7
5	0.2

• **Fig. 9.39** Postoperative GH monitoring. The probability of surgical remission according to the nadir growth hormone (GH) level in a 1-week postoperative oral glucose tolerance test (OGTT). When the nadir GH level on the 1-week postoperative OGTT is around 1 μg/L, an 88.9% probability of surgical remission is expected. However, if it is greater than 4 μg/L, it is unlikely that the patients will achieve delayed surgical remission. (Derived from Kim, Oh MC, Lee EJ, et al. Predicting long-term remission by measuring immediate postoperative growth hormone levels and oral glucose tolerance test in acromegaly. *Neurosurgery.* 2012;70:1106–1113.)



• **Fig. 9.40** (A) Growth hormone (GH) and insulin-like growth factor 1 (IGF1) concentrations with long-term octreotide treatment. Comparison of primary octreotide treatment in 25 previously untreated patients and in 80 patients who had previously undergone surgical resection or irradiation. (B) Pharmacodynamics of octreotide long-acting release (LAR) 12-hour mean serum octreotide and GH concentrations in a representative patient treated with a single 30-mg injection of octreotide LAR and followed for 60 days. After injection, drug levels peak at 28 days, and nadir GH levels are sustained for 4 weeks. (C) Mean GH concentration with octreotide (long-acting release) long-term treatment. Serum GH levels in acromegaly following monthly LAR octreotide injections in 12 patients for 1 year and 8 patients for 31 months. (D) Clinical impact of octreotide in reducing soft tissue swelling. Acromegaly in a patient suffering from obstructive sleep apnea before octreotide. Note the macroglossia, tracheotomy for airway obstruction, and intranasal feeding tube. After 6 months' treatment with octreotide, tongue size was reduced by half. Tracheotomy and nasal tube have been removed and sleep apnea has resolved. (A, from Newman C, Melmed S, George A, et al. Octreotide as primary therapy for acromegaly. *J Clin Endocrinol Metab.* 1998;83:3034–3040; B, adapted from Lancranjan I, Bruns C, Grass P, et al. Sandostatin LAR: a promising therapeutic tool in the management of acromegalic patients. *Metabolism.* 1996;45:S67–S71; C, from Davies PH, Stewart SE, Lancranjan I, et al: Long-term therapy with long-acting octreotide [Sandostatin-LAR] for the management of acromegaly. *Clin Endocrinol.* 1998;48:311–316; D, courtesy Seymour Reichlin, University of Arizona, Treson.)

independent of PRL concentrations.[462] Side effects of cabergoline include gastrointestinal symptoms, dizziness, headache, and mood disorders.

SRLs

Of the five somatostatin receptor subtypes, SST2 and SST5 are preferentially expressed on somatotroph and thyrotroph cell surfaces and mediate suppression of GH and TSH secretion.[463,464] Several SRLs have been safely used as approved or investigational drugs for acromegaly (Fig. 9.40). Octreotide (D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-OH), an octapeptide somatostatin analogue, binds predominantly to SST2 and less avidly to SST5[465] and inhibits GH secretion with a potency 45 times greater than native somatostatin, but its potency for inhibiting insulin release is only 1.3-fold that of somatostatin. The in vivo half-life of the analogue is prolonged (up to 2 hours) because of its relative resistance to enzymatic degradation. These properties are highly advantageous for long-term use in acromegaly.[466] GH responsiveness directly correlates with the abundance of pituitary SST2 receptors.

Subcutaneous octreotide (50 or 100 μg) suppresses GH secretion for up to 5 hours. In patients harboring microadenomas, integrated GH and IGF1 levels are almost invariably normalized, but the response in larger tumors is less pronounced.

Long-acting SRL formulations are convenient and allow for enhanced compliance and sustained biochemical control. Serum levels of long-acting release (LAR) octreotide (20–30 mg/intramuscularly), a sustained-release octreotide depot preparation,[467] peak at 28 days, with integrated GH levels effectively suppressed for up to 49 days. Monthly injections for 9 years reduced integrated serum GH levels to less than 2 μg/L in over 75% of patients.[468]

A long-acting preparation of lanreotide (autogel) is administered by monthly deep subcutaneous injection. In a randomized, placebo-controlled, multicenter study with a 52-week open extension, serum GH levels decreased by more than 50% from baseline in 63% of patients receiving lanreotide autogel.[469] When administered (60, 90, or 120 mg) every 28 to 42 days, GH was suppressed to less than 2.5 μg/L in 130 patients at 1 year.[470]

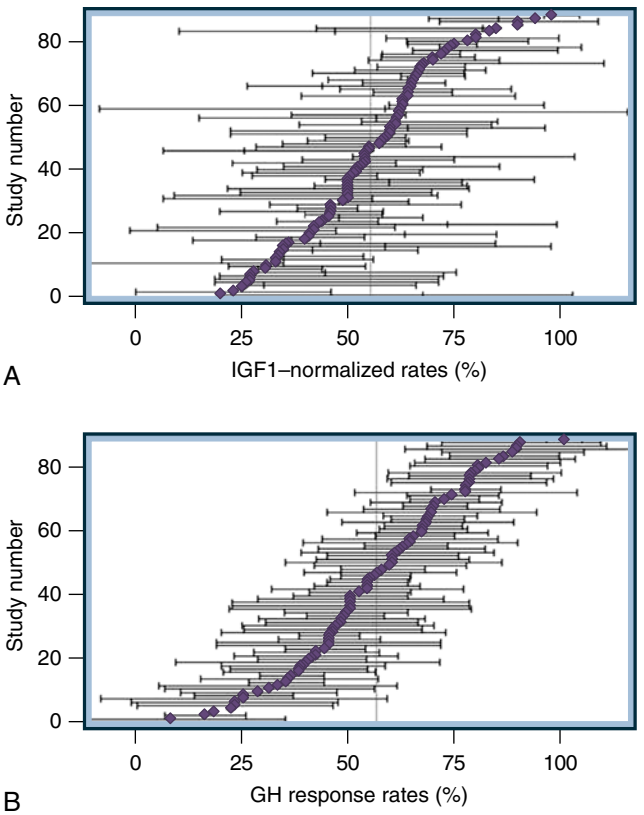
Pasireotide, a multireceptor-targeting SRL, exhibits highest affinity for SST5 more than SST2[471] and exhibits superior efficacy over octreotide.[472] In a randomized 12-month study in 358 patients, pasireotide LAR (40 mg) or octreotide (20 mg) resulted in biochemical control in 31% and 19% of patients, respectively.[472] Of those patients resistant to maximal doses of octreotide or lanreotide, 20% subsequently achieved control when placed on 60 mg pasireotide.[473] Reported side effects included those expected for SRLs as well as hyperglycemia noted in 57% of patients.

Oral octreotide formulated with a transient permeability enhancer attenuates basal and GHRH-elicited GH levels in healthy volunteers.[474] When administered to acromegaly patients, GH and IGF1 levels were controlled in 65% after being switched from injectable SRLs, and about 90% maintained responsiveness for up to 13 months. Although not yet approved, this formulation may offer an alternative future treatment option.[475]

Effects of SRLs on Pituitary Adenoma. Tumors rarely grow while patients receive depot SRL preparations, and a significant decrease in tumor size has been reported. In a comprehensive meta-analysis of 64 studies, 59% of patients were reported to experience a 50% reduction of tumor mass; this effect generally correlates with controlled GH and IGF1 levels[476] and may be observed within 6 months.[477] As the magnitude of shrinkage is variable, the use of preoperative SRL to improve subsequent surgical outcomes has been debated[478–480]; however, enthusiasm for this approach is dampened by the short prospective follow-up duration.

Effects on Clinical Features. Over 70% of patients experience improved general well-being, and soft tissue swelling dissipates within several days of treatment.[481] Headache, a common symptom, usually resolves within minutes of octreotide injection,[482] likely reflecting a specific central analgesic effect. Decreased heart rate and diminished LV wall thickness, reduced systemic arterial resistance and fluid volume, and restored functional activity are reported. Control of IGF1 and GH levels is associated with improved LV ejection function.[483] Joint function improves and crepitus is reduced, ultrasound shows evidence of bone or cartilage repair, and after several months sleep apnea resolves.[415] Although most soft tissue disease comorbidities are ameliorated once biochemical control has been achieved, low quality-of-life measures, including anxiety and depression, may persist.[484] Hypertension, joint space narrowing, and new-onset vertebral fractures do not appear to be ameliorated despite controlled GH and IGF1 levels.[424]

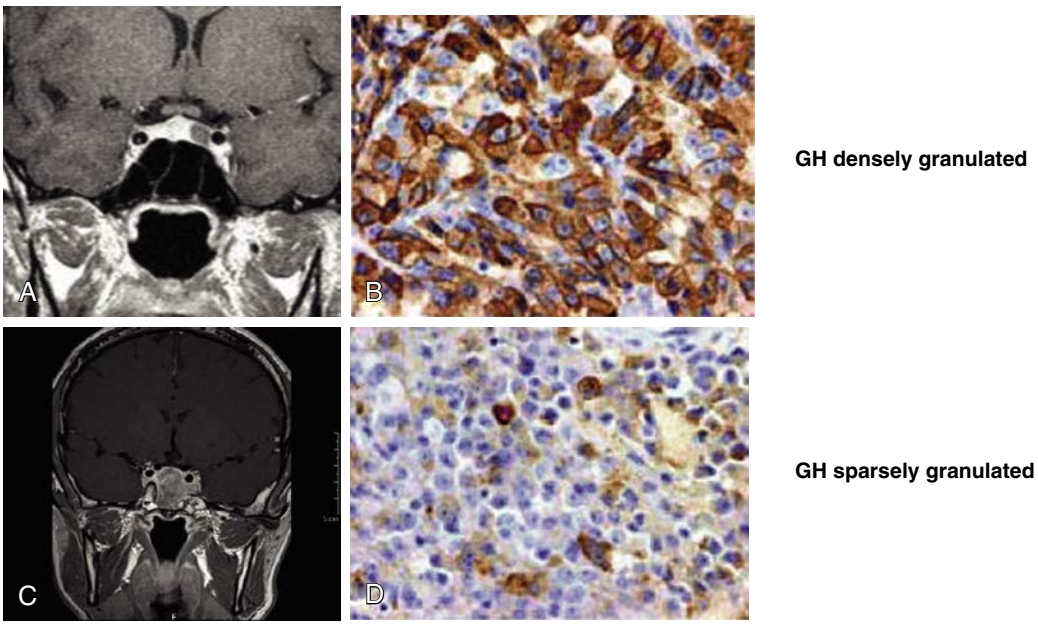
Determinants of SRL Responsiveness. In a meta-analysis of 4464 patients treated with an SRL, average GH control rates and IGF1 normalization rates were 56% and 55%, respectively (Fig. 9.41).[485] Lanreotide autogel and octreotide LAR exhibit similar clinical efficacy and side effect profiles, and they were shown equivalent in control of acromegaly symptoms and biochemical markers.[486] In 166 patients with acromegaly, results of oral glucose tolerance tests were concordant for appropriate diagnosis and establishing efficacy of surgery and radiotherapy but were not helpful in evaluating effectiveness of medical SRL therapy.



• **Fig. 9.41** Meta-analysis of somatostatin receptor ligand (*SRL*) responsiveness in acromegaly. (A) Insulin-like growth factor 1 (IGF1) response rates and 95% confidence intervals for 90 analyzed cohorts. (B) Growth hormone (GH) response rates and 95% confidence intervals for the 90 analyzed cohorts. Median response rates for GH and IGF1 noted by the vertical lines. Figures are sorted from least to greatest percent response rate. (From Carmichael J, Bonert VS, Nuno M, et al. Acromegaly clinical trial methodology impact on reported biochemical efficacy rates of somatostatin receptor ligand treatments: a meta-analysis. *J Clin Endocrinol Metab.* 2014;99:1825–1833.)

Measurement of serum IGF1 levels was the most rigorous marker to assess effectiveness of SRL therapy.[487] Results of controlled prospective studies show IGF1 levels normalized in up to 35% of patients.[469,472,488,489]

Understanding mechanisms driving SRL responsiveness and resistance, including both individual patient and tumor characteristics, has enabled a personalized approach to acromegaly classification and management.[365] Negative predictors of SRL therapeutic responses as well as markers of aggressive disease correlate with more adverse long-term outcomes. These determinants include patient age, treatment duration, frequency of drug administration, total daily dose, tumor size, degree of tumor GH granularity, and pretreatment GH and IGF1 levels. More favorable results reported in earlier studies may reflect clinical trial design, subject heterogeneity, and possibly selection of responsive patients. Increasing the monthly dose of octreotide LAR to 40 to 60 mg or the monthly lanreotide autogel dose to 180 mg, or, alternatively, increasing the dose frequency of lanreotide to 120 mg every 21 days, may improve efficacy.[490,491] Control rates also improve with time.[492] The most important determinant of therapeutic responsiveness is tumor SST2 expression.[493] Tumors immunopositive for SST2 expression are more likely to respond to octreotide and lanreotide,[494] and those with a higher SST2 to SST5 ratio show



• **Fig. 9.42** Growth hormone (GH) granulation pattern and clinical characteristics of GH-secreting adenomas. (A) Small noninvasive microadenomas usually contain (B) densely packed cytoplasmic GH granules (type 1 acromegaly).[369] (C) Larger and invasive macroadenomas are usually (D) very sparsely granulated, and these features portend a more adverse outcome (type 3 acromegaly). (A, from author's personal collection; B through D, courtesy Luis V. Syro, MD.)

improved therapeutic outcomes.[495] Tumors lacking SST5 immunoreactivity are resistant to pasireotide, while those immunoreactive for SST5 had superior biochemical responses.[496] Truncated SST5 variants may correlate inversely with GH responses to SRLs.[497]

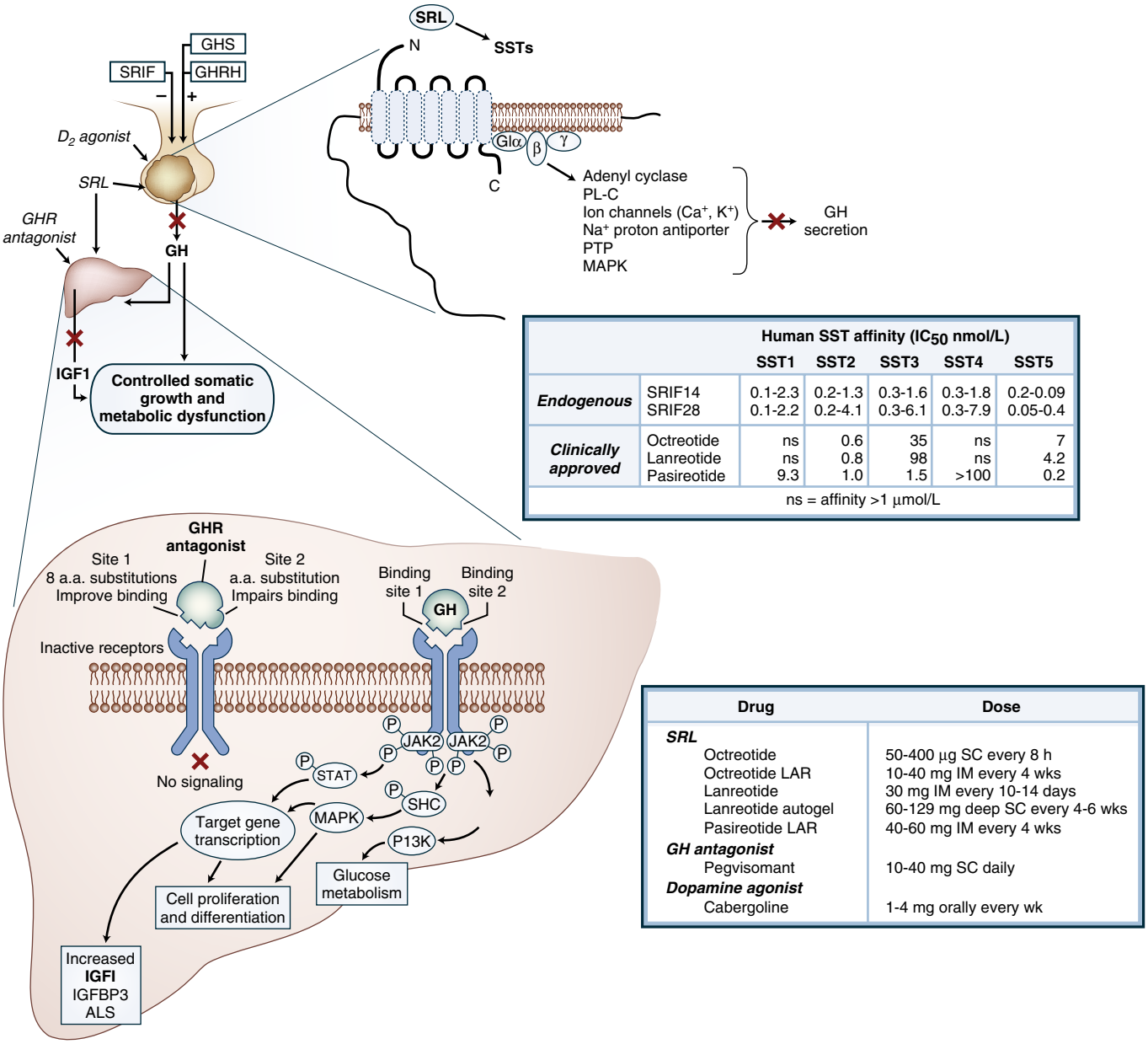
Tumors containing large, dense intratumoral GH granules diffusely distributed throughout the cytosol are more responsive to SRLs than are those with sparse GH granules[498] (Fig. 9.42). Sparsely granulated adenomas express SST2 less abundantly and more SST5, and are more resistant to SRLs.[495,499–501] Sparse granules are also associated with larger and more invasive tumors, especially in younger patients,[502] which display MRI features of T2-weighted hyperintensity. Hypointense adenomas are smaller and less invasive than hyperintense and isointense adenomas and exhibit higher IGF1 levels. These MRI and histologic features are helpful in predicting both biochemical responses and tumor shrinkage in response to SRLs.[503,504]

Molecular tumor markers associated with SRL responsiveness include p21 expression, low AIP expression,[505–507] high β-arrestin,[508] and presence of the *gsp* oncogene mutation.[509]

Side Effects. SRLs are generally safe and well tolerated. Gastrointestinal side effects predominate, occur early, and include transient loose stools, nausea, cramps, mild malabsorption, and flatulence, reported in about one-third of patients. Hypoglycemia or hyperglycemia is not commonly encountered, and insulin requirements in diabetic patients with acromegaly are reduced within hours of receiving octreotide, concomitant with GH lowering. Overall, SRLs do not cause major effects on glucose homeostasis, and hyperglycemia may also be associated with uncontrolled underlying disease.[510] Pasireotide, however, is associated with hyperglycemia and new-onset diabetes in about 50% of patients.[511] The drugs attenuate gallbladder contractility, and delayed emptying leads to reversible sludge formation evidenced by ultrasonography in up to 25% of patients.[512] Cholecystitis is very rarely reported.[467] Octreotide may interact with cyclosporine, enhancing transplant rejection risk. SRL dose adjustments should be carefully titrated in patients requiring insulin or oral hypoglycemic agents, calcium channel blockers, and beta blockers. Asymptomatic sinus bradycardia has also been recognized.

Growth Hormone Receptor Antagonist

GH action through the surface membrane GH receptor is mediated by ligand-induced receptor signaling[513] (Fig. 9.43). The postreceptor GH signal is not elicited if the receptor is bound by pegvisomant, a GH-receptor antagonist, which blocks subsequent IGF1 generation.[514] The drug blocks peripheral GH action and does not target the pituitary tumor. IGF1 measurement is the appropriate marker of patient responsiveness, and measuring GH is therefore not an efficacy marker. Earlier studies showed that daily injections (up to 40 mg daily) normalize IGF1 levels in about 90% of patients, and they dose-dependently improve fatigue, decrease soft tissue swelling as assessed by ring size, and diminish perspiration.[515] In a subsequent drug surveillance report of 1288 patients, control of IGF1 was reported in 63% of patients,[516] likely reflecting submaximal dose titration as compared to controlled dosing of a clinical trial. Accordingly, in a 91-month follow-up (median 18 months), IGF1 levels were controlled in 95% of patients treated in two centers.[517] Similar results were obtained in a 9-year therapeutic follow-up.[518] The drug may be used as primary monotherapy[519] and is particularly useful in patients resistant to SRL therapy because it effectively normalizes IGF1 levels when added in these patients.[520,521] Significant drug response determinants include age, body mass index, and baseline IGF1 levels.[522] There is a concern that loss of negative IGF1 feedback control on somatotroph tumor proliferation may theoretically



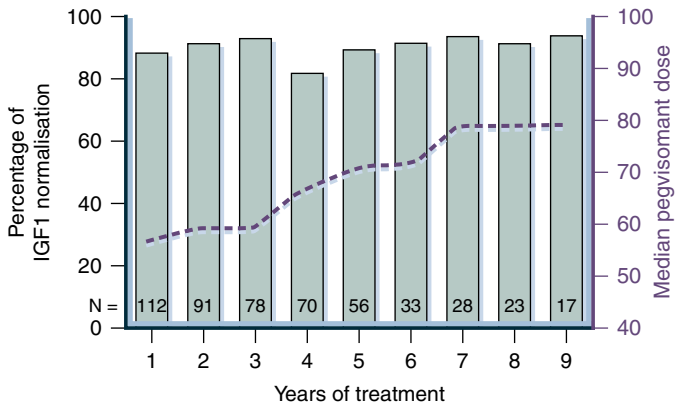
		Human SST affinity (IC_{50} nmol/L)				
		SST1	SST2	SST3	SST4	SST5
Endogenous	SRIF14	0.1-2.3	0.2-1.3	0.3-1.6	0.3-1.8	0.2-0.09
	SRIF28	0.1-2.2	0.2-4.1	0.3-6.1	0.3-7.9	0.05-0.4
Clinically approved	Octreotide	ns	0.6	35	ns	7
	Lanreotide	ns	0.8	98	ns	4.2
	Pasireotide	9.3	1.0	1.5	>100	0.2
ns = affinity >1 µmol/L						

Drug	Dose
SRL	
Octreotide	50-400 µg SC every 8 h
Octreotide LAR	10-40 mg IM every 4 wks
Lanreotide	30 mg IM every 10-14 days
Lanreotide autogel	60-129 mg deep SC every 4-6 wks
Pasireotide LAR	40-60 mg IM every 4 wks
GH antagonist	
Pegvisomant	10-40 mg SC daily
Dopamine agonist	
Cabergoline	1-4 mg orally every wk

• **Fig. 9.43** Action of growth hormone receptor (GHR) antagonist, somatostatin receptor ligands (SRLs), and dopamine (D_2) agonists. Normally a single molecule of GH binds two GH receptors through sites 1 and 2, and the GH signal transduction pathway is activated. Pegvisomant increases binding of GHR to site 1 and blocks binding at site 2 to prevent functional GHR dimerization, initiation of GH action, and induction of insulin-like growth factor type 1 (IGF1) synthesis and secretion. The peripheral effects of excess GH are antagonized at the cellular level, independent of the presence of somatostatin (SST) or dopamine receptors on the pituitary tumor. SRLs inhibit GH secretion and IGF1 synthesis and suppress pituitary tumor growth. GH-secreting adenomas express predominantly SST2 and SST5. Figure depicts affinities for SST receptor subtypes and for D_2 receptor. *a.a.,* amino acid; *ALS,* acid-labile subunit; *C,* carboxyl terminal; *GHRH,* gonadotrophin hormone–releasing hormone; *GHS,* gonadotrophin hormone secretagogue; *IC_{50},* 50% inhibitory concentration; *IGFBP3,* insulin-like growth factor–binding protein 3; *JAK2,* Janus kinase 2 tyrosine kinase; *LAR,* long-acting release; *MAPK,* mitogen-activated protein kinase; *N,* amino terminal; *ns,* not significant; *P,* elemental phosphorus; *PI3K,* phosphoinositide 3 kinase; *PL-C,* phospholipase C; *PTP,* protein tyrosine phosphatase; *SHC,* Src homology–containing protein; *SRIF,* somatostatin; *STAT,* signal transducer and activator or transcription. (Adapted from Melmed S. Acromegaly. *N Engl J Med.* 2006;355:2558–2573; Heaney AP, Melmed S. Molecular targets in pituitary tumours. *Nat Rev Cancer.* 2004;4:285–295.)

lead to rebound mass expansion. However, rebound tumor enlargement rarely occurs and may reflect discontinuing SRL, yet tumor growth while receiving pegvisomant should be monitored by MRI, especially if the residual tumor mass abuts visual tracts.[523–526] The GH receptor antagonist enhances insulin sensitivity and is therefore particularly suited for patients with coexisting diabetes.[517,527]

Side Effects. Because elevated (threefold or higher) hepatic transaminases have been reported,[528] liver enzymes should be



• **Fig. 9.44** Percentages of patients with insulin-like growth factor type 1 (IGF1) < 1.2 × ULN *(left axis)* and median pegvisomant doses *(dotted line, right axis)* each year during 9 years of combined somatostatin receptor ligand (SRL) and pegvisomant treatment. Number of patients each treatment year is depicted at the bottom of the bar. (Redrawn from Neggers SJ, Franck SE, de Rooij FW, et al. Long-term efficacy and safety of pegvisomant in combination with long-acting somatostatin analogs in acromegaly. *J Clin Endocrinol Metab*. 2014;99:3644–3652.)

measured every 6 months. Local injection site inflammation and lipodystrophy have been reported.[529] Levels of GH rise as IGF1 negative feedback on the pituitary is lost.

SRLs and GH Receptor Antagonist Combination

Combination treatment for acromegaly is most effective for patients in whom there has been tumor shrinkage on somatostatin analogue with reduction, albeit inadequate, in GH or IGF1 levels (Fig. 9.44). In 11 such uncontrolled patients, dual blockade of the GH axis with pegvisomant and SRLs has been shown to exhibit greater efficacy than either drug alone.[530] Monthly doses of long-acting somatostatin analogue have been successfully combined with weekly doses of pegvisomant.[531] A study in 63 patients showed that 4 years of combination treatment was safe, but 23 patients developed elevated liver enzymes, especially if they were diabetic.[532]

Management Approach

Employing an integrated classification base on clinical, biochemical, pathologic, and imaging features, acromegaly may be classified to enable evidence-based personalized management (Table 9.29).[365] Type I acromegaly accounts for over 50% of patients. These are usually older patients with smaller tumors containing dense GH granularity and abundant SST2 and p21 expression. IGF1 levels are lower and therapeutic outcomes are most favorable. In contrast, type 3 acromegaly is encountered in younger patients with smaller, sparsely granulated tumors and low p21 and SST2 expression. IGF1 levels are higher, and both surgical and medical outcomes are less favorable. Type 2 patients exhibit intermediate features.[365] Given the challenge of choosing the most appropriate therapy, use of classification systems, as well as clinical outcome instruments, may enable a rigorous personalized approach for evidence-based care.[365,533] Using these parameters prospectively will enable a personalized approach to determining optimal management for these patients.

Ideally, the goal of therapy should be tight control of GH secretion and normalization of IGF1 levels, as adverse comorbidities and mortality rates correlate with GH levels. Discordant GH and IGF1 results after treatment should necessitate repeating the respective assay(s) in a reputable laboratory using rigorous assay standardizations and appropriate sensitivity cutoffs.[439] Normal or low IGF1 levels may be encountered with systemic disease or malnutrition and GH elevations reflecting persistent disease activity. Each treatment modality has respective advantages and disadvantages that should be assessed to individualize patient care (Fig. 9.45 and Tables 9.30 and 9.31).[534] Counterintuitively, features of GH deficiency may be encountered after effective therapy, and judicious GH replacement may be required to reverse adverse quality of life and lipid disorders.[535]

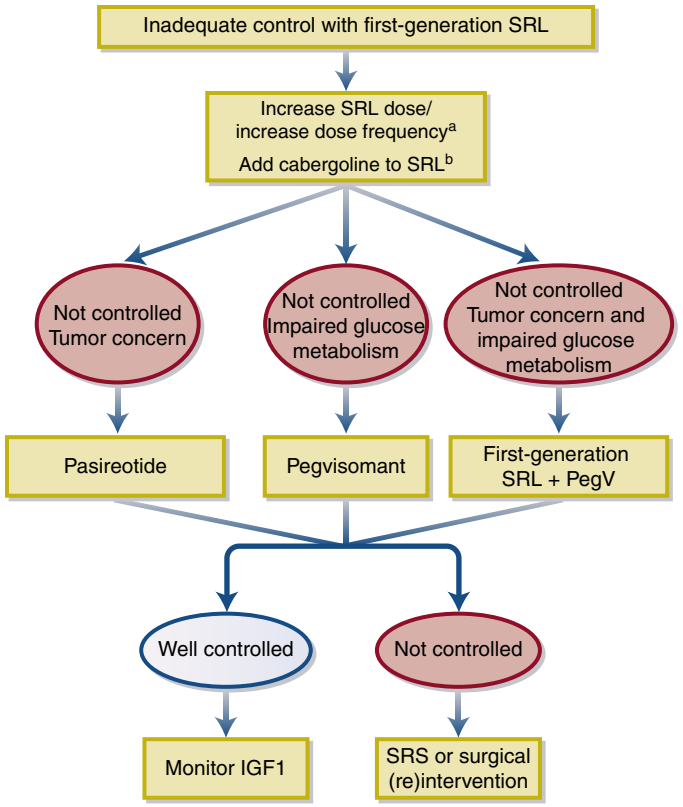
Selective surgical excision of a well-defined pituitary mass is recommended for most patients with microadenomas.[536] As remission rates are lower for macroadenomas and locally invasive tumors, attempted medical debulking of the sellar mass prior to surgery would be intuitively desirable, and limited controlled prospective studies appear to confirm the validity of this approach to improve outcomes, especially for patients with surgically inaccessible tumor tissue and cavernous sinus invasion.[537] Surgical debulking may also improve subsequent responsiveness to SRL therapy.[538,539] For uncontrolled patients after surgery, a long-acting SRL should be administered.[443] Patients with mild GH

TABLE 9.29 Acromegaly Subclassification for Precise, Personalized Approach to Therapy

		ACROMEGALY TYPES		
		Type 1	Type 2	Type 3
Frequency		50%	19%	31%
Tumor	Tumor shape and CSI	Concave	Flat	Peanut
	Size	Micro or macro	Macro	Macro
	Invasiveness by MRI	Intermediate	Rarely	Always
	GH granulation	Dense	Both	Sparse
Immunoreactivity	α-Subunit	Positive	Positive or negative	Negative
	Ki-67 index <3%	90%	33%	42%
	SST2	58%	30%	22%
	p21	38%	15%	4%
Biochemistry	IGF1 at diagnosis	Lower	Intermediate	Higher
Management and outcomes	No. of medications	≤2	≤2	≥2
	No. of surgeries	1	1 or 2	≥2
	Disease control	Frequent	Intermediate	Rare

CSI, Cavernous sinus invasion; *GH,* growth hormone; *IGF1,* insulin-like growth factor type 1; *MRI,* magnetic resonance imaging.

Modified from Cuevas-Ramos D, Carmichael JD, Cooper O, et al. A structural and functional acromegaly classification. *J Clin Endocrinol Metab*. 2015;100:122–131.



[a]Only in partial responders (>50% decrease in GH/IGF1);
[b]If IGF1 remains modestly elevated during SRL administration.

• **Fig. 9.45** A proposed algorithm for the treatment of acromegaly in patients inadequately controlled with first-generation SRLs lanreotide autogel and octreotide LAR. Tumor concern is defined as clinically relevant residual tumor on imaging and/or clinical concern for tumor growth. *GH,* growth hormone; *IGF1,* insulin-like growth factor 1; *PegV,* pegvisomant; *SRL,* somatostatin receptor ligand; *SRS,* stereotactic radiosurgery. (Redrawn from Melmed S, Bronstein MD, Chanson P, et al. A consensus statement on acromegaly therapeutic outcomes. *Nat Rev Endocrinol.* 2018;14:552–561.)

elevations can also be treated with cabergoline; although efficacy of this drug is low, it is relatively inexpensive and free of major side effects.

Primary therapy with SRLs may be offered to those patients in whom complete tumor removal is not likely or to those who refuse surgery or in whom the risks of surgery or anesthesia are unacceptable (Fig. 9.46). As invasive macroadenomas invariably hypersecrete GH postoperatively in patients whose pituitary lesion does not compress vital structures, primary medical management may be an appropriate therapeutic option.[489,540] Pegvisomant, either alone or in combination with an SRL, should be offered to resistant patients. Radiation should be administered to patients who are resistant to or cannot tolerate medications, who prefer not to receive long-term injections, or those who cannot afford medication. After irradiation, medications are required for several years until GH levels are effectively controlled. Tumors recurring despite medical therapy or irradiation may rarely require reoperation.

Acromegaly patients also require counseling for anxiety engendered by disfigurements and interpretation of laboratory test results. Patients should be followed until biochemical control is achieved; thereafter, hormone evaluation is performed semiannually. In those who are biochemically in remission and in whom no residual tumor tissue is present, MRI should be repeated every 1 to 2 years. Follow-up evaluation includes treating new soft tissue overgrowth, nerve entrapments, and jaw overbites; rheumatologic, dental, and cardiac evaluations; and metabolic assessment. Maximal and sustained long-term GH and IGF1 control should ameliorate most deleterious comorbidities by judicious use of available treatment modalities.

ACTH-Secreting Tumors (Cushing Disease)

The evaluation and management of patients with ACTH-secreting pituitary tumor (Cushing disease) is fully described in Chapter 15. Briefly, the diagnosis of an ACTH-secreting pituitary tumor is suggested by features of hypercortisolism, elevated 24-hour urinary free cortisol levels, and elevated late night salivary cortisol values, together with nonsuppressed serum ACTH levels. Failure to suppress morning cortisol levels to less than 1.8 μg/dL after 1 mg dexamethasone administered at 11 PM supports the diagnosis.[541] In healthy subjects, glucocorticoid feedback suppresses CRH and ACTH, attenuating cortisol secretion. Surgical resection of an ACTH-secreting adenoma is the treatment of choice. As these tumors are usually small, sometimes less than 2 mm diameter, they may not be visible by sensitive MRI and venous sampling for ACTH. Therefore these tumors pose a significant challenge even for the experienced surgeon. Bilateral petrosal venous sampling for ACTH levels and cavernous sinus venography should ideally be performed prior to surgery. However, if sellar venous sinus drainage is predominantly unilateral, left-right ACTH gradients may not reliably lateralize the lesion. If an ACTH gradient is indeed detected with normal venous drainage patterns, hemihypophysectomy may in fact be curative in most patients with clearly defined biochemical features of ACTH-dependent Cushing disease. Meticulous surgical exploration of both anterior and posterior lobes is required for these tiny tumors, which are often off-white and speckled by petechiae, and may be inadvertently suctioned. Even carefully performed preoperative lateralization is not infallible, and the "normal" side should also be carefully explored.

Assessment of Surgical Outcome

Transsphenoidal adenoma resection is the preferred treatment for these adenomas.[542] After selective adenomectomy of a clearly identifiable adenoma, remission was achieved in 75% of 295 patients. However, partial hypophysectomy performed in patients in whom an adenoma cannot be identified may result in biochemical remission in some patients.[543] On the third postoperative day, 1 mg dexamethasone can be given at 10 PM, and cortisol levels are measured the following morning prior to initiating hydrocortisone therapy. If the immediate postoperative cortisol level is less than 3 μg/dL, a 95% 5-year remission rate can be expected. In 21 of 27 patients tested prior to glucocorticoid administration, postoperative cortisol levels lower than those obtained from preoperative midnight sampling and values less than 10 μg/dL were predictive of remission.[544]

Medical Treatment

Pasireotide, an SRL, has been approved for treating ACTH hypersecretion associated with Cushing disease.[545] About 20% of patients exhibit normalized urinary free cortisol levels, associated with improved clinical features. Up to 50% of patients may develop hyperglycemia, and blood sugars should be aggressively monitored. Mifepristone, a glucocorticoid receptor antagonist, is

TABLE 9.30 Management of Acromegaly

Goals

Control GH and IGF1 secretion
Control tumor growth
Relieve central compressive effects, if present
Preserve or restore pituitary trophic hormone function
Treat comorbidities (hypertension, cardiac failure, hyperglycemia, sleep apnea, arthritis)
Normalize mortality rates
Prevent biochemical recurrence

TREATMENTS

Characteristic	Surgery	Radiotherapy	SRL	GHR Antagonist	Dopamine Agonist
Advantages					
mode	Transsphenoidal resection	Noninvasive	Monthly injection	Daily injection	Oral
Biochemical control					
GH <2.5 μg/L	Macroadenomas, <50% Microadenomas, >80%	~35% in 10 years	~55–65%	Increases	<15%
IGF1 normalized		<30%	~55–65%	>65%	<15%
Onset	Rapid	Slow (years)	Rapid	Rapid	Slow (weeks)
Patient compliance	One-time consent	Good	Must be sustained	Must be sustained	Good
Tumor mass	Debulked or resected	Ablated	Growth constrained or shrinks ~50%	Unknown	Unchanged
Disadvantages					
Cost	One time	One time	Ongoing	Ongoing	Ongoing
Hypopituitarism	~10%	>50%	None	Very low IGF1 if overtreated	None
Other	Tumor persistence or recurrence, 6% Diabetes insipidus, 3% Local complications, 5%	Local nerve damage Second brain tumor Visual and CNS disorders, ~2% Cerebrovascular risk	Gallstones, 20% Nausea, diarrhea	Elevated liver enzymes (rare)	Nausea, ~30% Sinusitis High dose required

OUTCOMES

Feature	Evaluation	Treatment
Safe Biochemical Activity		
Nadir GH <0.4 μg/L Age-matched normal IGF1 Asymptomatic No comorbidities	Assess GH/IGF1 axis Evaluate adrenal, thyroid, and gonadal axes Periodic but less frequent MRI	None or no change in current treatment
Unsafe Biochemical Activity		
Nadir GH >0.4 μg/L Elevated IGF1 Discordant GH and IGF1 Asymptomatic No comorbidities	Assess GH/IGF1 axis Evaluate pituitary function Periodic MRI	Weigh treatment benefit vs. risks Consider new treatment if being treated
Unsafe Biochemical and Clinical Activity		
Nadir GH >1 μg/L Elevated IGF1 Clinically active tumor growing	Assess GH/IGF1 axis Evaluate pituitary function Assess cardiovascular, metabolic, and tumoral comorbidity Periodic MRI	Actively treat or change treatment

CNS, Central nervous system; *GH,* growth hormone; *GHR,* growth hormone receptor; *IGF1,* insulin-like growth factor type 1; *MRI,* magnetic resonance imaging; *SRL,* somatostatin receptor ligand.

Modified from Melmed S. Medical progress: acromegaly. *N Engl J Med.* 2006;355:2558–2573.

TABLE 9.31 Medical Therapy of Acromegaly

Therapy	Receptor Target	Route of Administration	Dose	Frequency	Side Effects	Efficacy (GH/IGF1 Normalization)
Cabergoline	D2 receptor	Oral	1–4 mg	Biweekly up to daily	Nausea, dizziness, orthostatic hypotension	30–40%
Octreotide	SST2, SST5	SC	50–400 μg/day	One to three times daily	Nausea, vomiting, diarrhea, constipation, abdominal pain, cholelithiasis/biliary sludge, bloating, bradycardia, fatigue, headache, alopecia, dysglycemia	50–60%
Octreotide LAR	SST2, SST5	IM	20–40 mg	Monthly		
Lanreotide Autogel	SST2, SST5	Deep SC	6–120 mg	Every 4–6 weeks		
Pasireotide LAR	SST1, SST2, SST3, SST5	IM	40–60 mg	Monthly	Same as above, with more hyperglycemia	Up to 80%
Oral octreotide[a]	SST2, SST5	Oral	40–80 mg	Twice daily	Nausea, vomiting, diarrhea, dyspepsia, cholelithiasis, headache, dizziness, dysglycemia	65%
Pegvisomant	GH receptor	SC	10–40 mg	Daily to once weekly (less frequent when used in combination)	Transaminase elevation, lipodystrophy, arthralgias	60–90%

[a]Investigational

D2, Dopamine type 2; *GH,* growth hormone; *IGF1,* insulin-like growth factor type 1; *IM,* intramuscular; *LAR,* long-acting release; *SC,* subcutaneous; *SSTR,* somatostatin receptor.

Modified from Langlois F, McCartney S, Fleseriu M. Recent progress in the medical therapy of pituitary tumors. *Endocrinol Metab (Seoul).* 2017;32:162–170; Melmed S. New therapeutic agents for acromegaly. *Nat Rev Endocrinol.* 2016;12:90–98.

approved to ameliorate hyperglycemia associated with Cushing disease.[546] Side effects include adrenal dysfunction due to cortisol withdrawal, hypokalemia, and excessive vaginal bleeding.

Thyrotrophic Hormone-Secreting Tumors

TSH-producing pituitary tumors are rare. During 1979 to 1992, Mindermann and Wilson analyzed tumor type by immunohistochemistry and found that the overall prevalence of TSH-secreting tumors was 19/2225 (0.85%). Between 1989 and 1991, the same group found a prevalence of 2.8% among pituitary adenomas.[547] More recent studies have confirmed a progressive increased prevalence, accounting for 4% of pituitary tumor resections.[548] It is not clear whether newer, more sensitive TSH assays account for this or whether the tumor type frequency is increasing. TSH-secreting tumors can also cosecrete other hormones, including GH, PRL, and rarely ACTH, and can cause elevated serum IGF1 or PRL levels.[549]

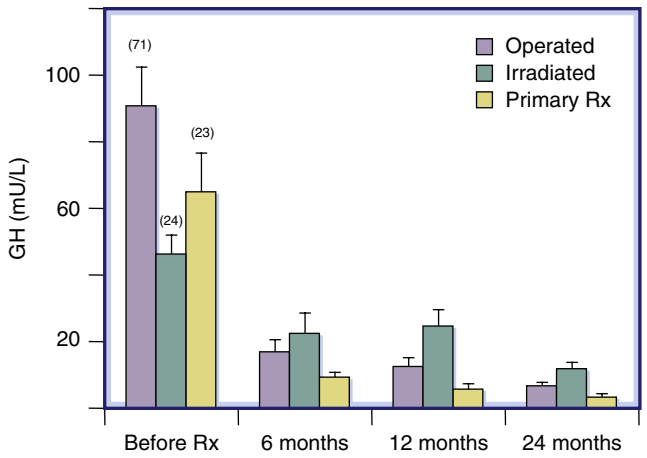
The hallmark of these rare tumors is an elevation of thyroid hormone levels in the face of inappropriately suppressed TSH levels. These biochemical features are reflective of autonomous tumor TSH production that is not suppressed by negative thyroid hormone feedback.

Pathology

These tumors are invasive but for the most part benign, and distant metastases are extremely rare. The expression pattern is often multihormonal, with immunoreactivity to TSHβ, α-subunit, GH, PRL, and sometimes ACTH.[550] Twenty-four-hour sampling indicates that pulse frequency of TSH is increased, yet diurnal rhythm was preserved at a higher mean hormone level.[551] TSH-secreting tumors exhibit positive immunostaining for α-subunit and TSHβ in up to 75% of cells and for the pituitary-specific transcription factor Pit1. These tumors express SST2 mRNA and, in some cases, SST3 and SST5 mRNA.[552]

Presentation

Patients may present with thyroid abnormalities, including goiter, nodules, and features of hyperthyroidism. As most TSH-secreting tumors are already macroadenomas at presentation,[553] they may present with symptoms due to tumor growth (including visual field abnormalities, cranial nerve palsies, or headache). Signs and symptoms of hyperthyroidism, including palpitations, arrhythmias, weight loss, tremor and nervousness, or goiter, are common. Very rarely, periodic paralysis and postoperative thyroid storm have been reported.[554,555] Serum TSH is often but not invariably elevated. In those cases, the combination of abnormally high thyroid hormone levels and a TSH value within the normal range points to a TSH-producing pituitary tumor. A relatively long period of hyperthyroidism, initially thought to be Graves disease and treated accordingly, often predates the realization that the hyperthyroidism is indeed a result of a TSH-secreting pituitary tumor. Alternatively, thyroid hormone insensitivity can also present with similar laboratory profiles.[556] TSH-secreting tumors are usually large, and 70% to 90% are macroadenomas, with most locally invasive.[557] From analysis of 10 reports on a total of 153 patients, it appears that TSH is frankly elevated in 58% of patients, with the remainder having normal albeit inappropriately elevated TSH levels. Patients previously treated with radioactive iodine for presumed Graves disease present with significantly higher TSH levels than patients not previously radioablated (mean: 56 mU/L vs. 9 mU/L, respectively). An



• **Fig. 9.46** Primary medical therapy in acromegaly patients. Control with medical therapy compared to surgery or radiation. Plasma growth hormone (GH) levels (mean ± SEM) in the group of patients (n = 118) before and during long-term treatment with lanreotide. *Rx,* treatment; *SEM,* standard error of the mean. (Data from Baldelli R, Colao A, Razzore P, et al. Two-year follow-up of acromegalic patients treated with slow release lanreotide [30 mg]. *J Clin Endocrinol Metab.* 2000;85[11]:4099–4103.)

ectopic TSH-producing tumor has also been reported.[558] Serum T_4 is high in most patients, as is the glycoprotein hormone α-subunit. Approximately two-thirds of patients with TSH-producing pituitary tumors have goiters with elevated radioactive iodine uptake. Rarely, patients with TSHomas have been reported with concurrent differentiated thyroid cancer.[559] About 30% of TSHomas cosecrete GH or PRL.[560] Consequently, features of acromegaly or hyperprolactinemia may also be present.

Evaluation

Serum T_4, T_3, TSH (by high sensitivity assay), and α-subunit should be measured. The combination of high T_4, T_3, and α-subunit; high or inappropriately normal TSH; and a pituitary tumor strongly confirms the diagnosis of a TSH-producing pituitary adenoma. TRH stimulation can differentiate between TSH overproduction by a TSH-secreting tumor and thyroid hormone insensitivity. In TSH-secreting tumors, the TSH response elicited by TRH is blunted. In contrast, TSH usually rises in response to TRH in patients with thyroid hormone insensitivity and in normal subjects. Concomitant measurement of α-subunit at each point during the TRH test is helpful because the molar ratio of α-subunit to TRH is high (>1) in almost 85% of patients with TSH-secreting tumors. A T_3 suppression test is helpful in that complete inhibition of TSH does not occur in patients with TSH-secreting tumors. This test can also differentiate subclinical hypothyroidism in patients previously treated with radioactive iodine for hyperthyroidism but found to have an incidental pituitary tumor. TSH elevation may also result from inadequate thyroid hormone replacement. A pituitary MRI should be performed, and IGF1 and PRL levels determined, to exclude acromegaly or hyperprolactinemia. Expression of other pituitary hormones in immunostained histologic sections does not necessarily imply elevated serum levels.

Importantly, the degree of hyperthyroidism should be assessed to determine whether control of these signs and symptoms should be undertaken prior to further evaluation or treatment of the pituitary tumor. Hyperthyroidism may be clinically severe and present in most patients for years before the diagnosis is made.

Management

Surgery

The European Thyroid Association guidelines suggest surgery as first-line therapy. Cures are achieved for most patients with microadenomas, but remission is achieved in about 60% of patients with macroadenomas.[560] After a mean follow-up of 64.4 months, 75% of 70 patients achieved biochemical control and 58% normalized both pituitary imaging and thyroid function, but complete surgical cures are achieved in fewer than 40% of patients[557] (Table 9.32 and Fig. 9.47). However, the rarity of this tumor type has precluded large controlled studies. Most patients have cavernous or sphenoid sinus invasion, and tumors are often fibrous and unusually hard. About one-third of patients require adjuvant radiotherapy to achieve biochemical normalization. Nevertheless, 9% developed pituitary hormone deficiencies, and 3% had recurrence of tumor or hyperthyroidism within the first 2 years of radiotherapy. After 18 to 96 months of follow-up, 32% of patients had developed new-onset pituitary deficits.[557]

Radiation Therapy

There are no large series reporting treatment of TSH-secreting tumors with radiotherapy alone. Radiation has mostly been used as adjunctive therapy to surgery, especially when the latter was not curative.

SRLs

Octreotide, used as either primary or adjunctive treatment, normalizes T_4 and T_3 and reduces TSH levels by half.[561] Overall, tumors shrink in about a third of patients. In 18 patients with TSH-secreting adenomas, lanreotide (30 mg every 10 or 14 days) significantly decreased TSH levels from 2.72 to 1.89 mU/L, decreased T_4 levels, but did not shrink tumors. Octreotide LAR (up to 30 mg monthly) responsiveness appeared similar to that observed for the subcutaneous preparation in seven patients.[562] In another report, octreotide suppressed TSH in 90% of patients with TSH-secreting tumors and reduced tumor size in 50% of these patients.[563] SRLs are effective in more than 90% of patients with TSHomas,[560] but tachyphylaxis may occasionally occur.

Preoperative Management

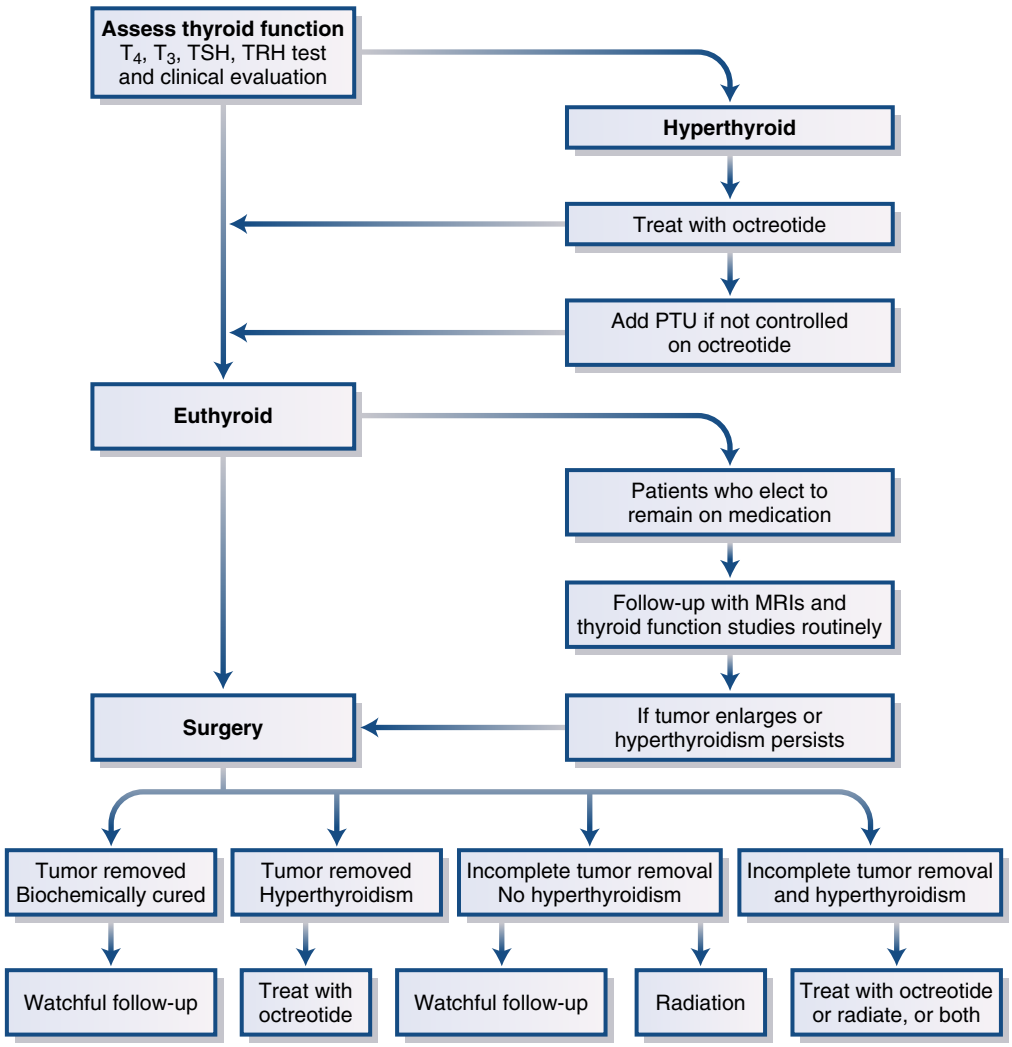
Unless vision is threatened, patients should be evaluated to determine whether clinical signs of hyperthyroidism warrant immediate treatment. Propranolol, radioactive iodine thyroid ablation, thyroidectomy, antithyroid medications (including methimazole), and SRLs are used. Both radioactive iodine and antithyroid medications are targeted to the thyroid gland rather than the pituitary seat of the disorder. This approach also inhibits the negative feedback of T_3 on TSH and leads to increased tumor TSH production. Surgery and SRLs simultaneously treat hyperthyroidism and tumor TSH hypersecretion. SRLs lower TSH, α-subunit, and T_4 and are recommended as first-line drugs in the initial control of hyperthyroidism due to TSH-secreting tumors because their onset of action is faster than other therapeutic approaches and tumor shrinkage occurs in up to 40% of patients. When invasive tumor tissue persists, patients continue to have abnormal TSH responses to TRH and require SRL therapy.

TABLE 9.32 TSH-Secreting Adenomas: Results of Surgical Treatment

Study	Country	Study Period	Prevalence Among All Resected Pituitary Adenomas (%)	N	Macroadenoma (%)	Postoperative Euthyroidism (%)	Median Follow-Up (Months)
Kirkman (2014)	United Kingdom	2002–2012	3.5	32	88	69	80 (10–204)
Malchiodi (2014)	Italy	1982–2012	NA	68	NA	58	NA
Yamada (2014)	Japan	1991–2013		90	82	84	33.6
Azzalin (2015)	United States	1993–2013	1.2	20	95	66[a]	10.4 (1.2–150)

[a]In patients with active tumors and central hyperthyroidism.

Modified from Azzalin A, Appin CL, Schniederjan MJ, et al. Comprehensive evaluation of thyrotropinomas: single-center 20-year experience. *Pituitary.* 2016;19:183–193.



• **Fig. 9.47** Management of thyrotrophic hormone (TSH)–secreting pituitary tumors. *MRI,* magnetic resonance imaging; *PTU,* propylthiouracil; T_3, triiodothyronine; T_4, thyroxine; *TRH,* thyrotrophin-releasing hormone.

Silent TSH-Secreting Tumors

A subset of TSH-secreting tumors immunostain positively for but do not hypersecrete TSH or cause thyrotoxicosis. Of 29 tumors that immunostained positively for TSH, 9 were not associated with hyperthyroidism.[564]

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The complete list of references is available online at ExpertConsult.com.

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10

Posterior Pituitary

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CHAPTER OUTLINE

KEY POINTS

- The posterior pituitary is composed of neural tissue and consists of distal axon terminals of the hypothalamic magnocellular vasopressin and oxytocin neurons that constitute the neurohypophysis.
- Regulation of neurohypophyseal hormone synthesis occurs at the level of transcription. Stimuli for the secretion of vasopressin or oxytocin also induce transcription and increase the hormone messenger ribonucleic acid (mRNA) content in the magnocellular neurons.
- Physiologic regulation of vasopressin synthesis and secretion involves two systems: osmotic and pressure/volume. Each are controlled by separate neural inputs to the neurohypophysis and have different thresholds for vasopressin secretion.
- Once released, vasopressin elicits end-organ effects via activation of vasopressin receptors. Renal vasopressin V_2 receptors induce antidiuresis via a signal transduction cascade that results in insertion of aquaporin 2 water channels into the apical membrane of principal cells in the renal collecting duct.
- Diabetes insipidus is a disorder of a large volume of urine (diabetes) that is hypotonic (dilute) and tasteless (insipid), leading to polyuria and polydipsia. It can be caused by deficient vasopressin section, increased vasopressin catabolism, or decreased vasopressin effect in the kidneys.
- The syndrome of inappropriate antidiuresis (SIAD) occurs when plasma levels of vasopressin are elevated at times when physiologic vasopressin secretion from the posterior pituitary would normally be osmotically suppressed, leading to water retention and hypoosmolality. It can be caused by several diseases and drugs.

Anatomy

Normal

The posterior pituitary is neural tissue and consists only of the distal axons of the hypothalamic magnocellular neurons that make up the neurohypophysis. The perikarya (cell bodies) of these axons are located in paired paraventricular and supraoptic nuclei of the hypothalamus. During embryogenesis neuroepithelial cells of the lining of the third ventricle mature into magnocellular neurons while migrating laterally to and above the optic chiasm to form the supraoptic nuclei and to the walls of the third ventricle to form the paraventricular nuclei.[1] In the posterior pituitary the axon terminals of the magnocellular neurons contain neurosecretory granules, membrane-bound packets of hormones stored for subsequent release. The blood supply for the anterior pituitary is via the hypothalamic/pituitary portal system, but the posterior pituitary is supplied directly from the inferior hypophyseal arteries, which are branches of the posterior communicating and internal carotid arteries. The drainage is into the cavernous sinus and internal jugular vein.

The hormones of the posterior pituitary, oxytocin and vasopressin, are for the most part synthesized in individual hormone-specific magnocellular neurons although a small number of neurons (~3%) express both peptides.[2] The supraoptic nucleus (SON) is relatively simple with 80% to 90% of the neurons producing vasopressin[3] and virtually all axons projecting to the posterior pituitary.[1] The organization of the paraventricular nucleus

(PVN), however, is much more complex and varies among species. In the human there are five subnuclei,[3] and parvocellular (smaller cell) divisions synthesize other peptides such as corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), and somatostatin[4] as well as opioids.[5] The parvocellular neurons project to the median eminence, brainstem, and spinal cord[6] where they play a role in a variety of neuroendocrine autonomic functions. The suprachiasmatic nucleus, which is located in the midline at the base of and anterior to the third ventricle, also synthesizes vasopressin and controls both circadian and seasonal rhythms.[3]

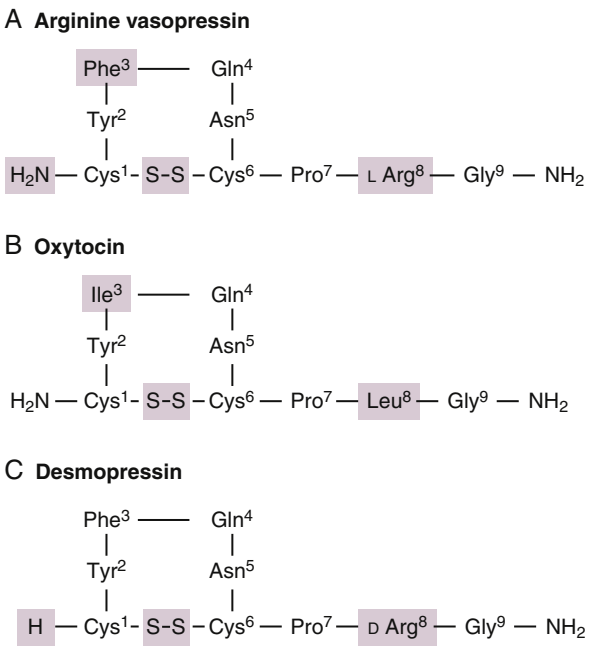
The major stimulatory neurotransmitter in the neurohypophysis is glutamate with noradrenergic stimulatory inputs acting by stimulation of glutamate.[7,8] Glutamate receptors account for 25% of synapses on magnocellular neurons.[7] The major inhibitory input is γ-aminobutyric acid (GABA), which accounts for 20% to 40% of the synaptic input to the magnocellular neurons.[9] Phasic firing of vasopressin neurons is the most efficient activity pattern for release of vasopressin from axon terminals. Phasic activity is controlled by glutamate stimulation and opioid inhibition. Dynorphin is synthesized in vasopressin neurons and co-released with vasopressin from dendrites at the somatic level where it acts in an autocrine fashion to inhibit the activity of the vasopressin neurons, contributing to the phasic firing pattern.[10,11] One of the most remarkable aspects of the magnocellular system is the plasticity of the system in response to prolonged stimulation. Plasticity is of most import in humans during parturition and lactation.[9]

Ectopic Posterior Pituitary

With the development of magnetic resonance imaging (MRI) scans of the brain it was discovered that T1-weighted images with MRI produced a bright signal in the posterior pituitary.[12] This allowed the identification of children in whom there was abnormal anatomy of the posterior pituitary when the "bright spot" was recognized in the base of the hypothalamus. These cases are referred to as *ectopic posterior pituitary* or *pituitary stalk interruption*. Etiologies include traumatic delivery (these patients have a higher incidence of breech delivery and perinatal injuries) and genetic abnormalities of the transcription factors that regulate pituitary embryogenesis.[13] The latter is supported in cases where abnormalities of the posterior pituitary and/or stalk are associated with extrapituitary malformations such as septal optic dysplasia. Cases with malformations are more likely to have diabetes insipidus or other osmotic dysfunction than simple ectopic posterior pituitary.[14,15] Cases are recognized in children with growth retardation and anterior pituitary deficiency rather than posterior pituitary deficiency. The degree of anterior pituitary deficit depends on the persistence of a pituitary stalk and a retained portal vasculature from the hypothalamus to the anterior pituitary.[16–18] Deficiency of adrenocorticotropic hormone (ACTH) is common and should be investigated as the patients may not respond appropriately to stress.[13]

Synthesis and Release of Neurohypophyseal Hormones

Vasopressin and oxytocin are nonapeptides consisting of a six–amino acid ring with a cysteine-to-cysteine bridge and a three–amino acid tail. All mammals have arginine vasopressin and oxytocin, as illustrated in Fig. 10.1, with the exception of the pig



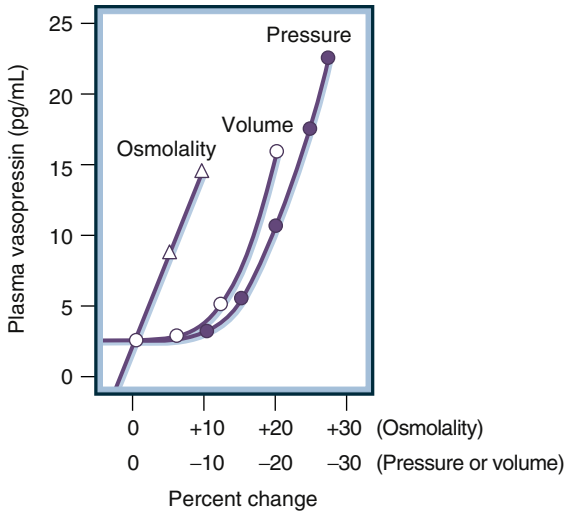
• **Fig. 10.1** Comparison of the chemical structures of arginine vasopressin (A), oxytocin (B), and desmopressin (C). The differences are illustrated by the shaded areas. Oxytocin differs from vasopressin in position 3 (Ile for Phe) and position 8 (Leu for Arg). Desmopressin differs from arginine vasopressin in that the terminal cystine is deaminated and the arginine in position 8 is a D-isomer rather than an L-isomer. (From A.G. Robinson, University of California at Los Angeles, used with permission.)

in which lysine is substituted for arginine in position 8 producing lysine vasopressin. Both genes are found on chromosome 20[19] although they are situated in a tail-to-tail position and transcribed in opposite directions.[20] The hormones are synthesized as part of a precursor molecule consisting of the nonapeptide and a hormone-specific neurophysin and, for vasopressin, a 39–amino acid peptide named copeptin.[21] The precursor is packaged in neurosecretory granules and cleaved to the products during transport to the posterior pituitary.

When a stimulus for secretion of vasopressin or oxytocin acts on the appropriate magnocellular cell body, an action potential is generated and propagates down the long axon to the posterior pituitary. The action potential causes an influx of calcium, which induces neurosecretory granules to fuse with the cell membrane and extrude the entire contents of the neurosecretory granule into the perivascular space and subsequently into the capillary system of the posterior pituitary. At physiologic pH of plasma there is no binding of hormone (vasopressin or oxytocin) to their respective neurophysins, so each peptide circulates independently in the bloodstream.

The control of hormone synthesis is at the level of transcription. Stimuli for secretion of vasopressin or oxytocin also stimulate transcription and increase the mRNA content in the magnocellular neurons. This has been studied in most detail in rats where dehydration[22] accelerates transcription and increases the levels of vasopressin (and oxytocin) mRNA[23–25] and where hypoosmolality produces a decrease in the content of vasopressin mRNA.[26]

The transport of neurosecretory vesicles from the site of synthesis to the posterior pituitary along microtubule tracks[27] is also regulated. When synthesis is turned off, transport stops; when synthesis is increased, transport is upregulated.[27] Thus



• **Fig. 10.2** Comparison in humans of the release of vasopressin in response to increased osmolality *(open triangles)* or to decreased blood pressure *(filled circles)* or blood volume *(open circles)*. Plasma vasopressin is much more sensitive to change in osmolality, responding to as little as a 1% increase, whereas a change of 10% to 15% or greater in volume or pressure is required to stimulate release of vasopressin. (Redrawn from Robertson GL, Berl T. Water metabolism. In: Brenner B, Rector F Jr, eds. *The Kidney*. 3rd ed, vol 1. Philadelphia, PA: Elsevier; 1986:385. Figure by A.G. Robinson, University of California at Los Angeles, used with permission.)

there is coordination of stimulated release of hormone, transport of hormone, and synthesis of new hormone. There is, however, asynchrony in the timing of these events. The asynchrony is demonstrated by changes in the content of vasopressin stored in the posterior pituitary. The absolute content varies considerably among species but is a remarkable store, generally equivalent to the amount of hormone required to sustain basal release for 30 to 50 days or maximum release for 5 to 10 days.[28] In animals, prolonged and intense stimulation of vasopressin release such as dehydration or salt loading produces a depletion of stored hormone in the posterior pituitary.[25,29,30] Then when animals are returned to normal water intake, there is in 7 to 14 days a gradual recovery of pituitary content back to baseline. Modeling of this phenomenon has provided experimental evidence that a long half-life of the vasopressin message, approximately 2 days, is (from a minimalist point of view) a plausible explanation.[31] When a strong and/or sustained stimulus releases vasopressin there is an immediate stimulus to transcription of new mRNA, but it requires several days for the peak level of mRNA to be reached; thus while release of hormone is rapid, translation increases slowly. When the stimulus is removed, the elevated mRNA slowly declines while continuing to synthesize hormone that repletes the store in the posterior pituitary.

Physiology of Secretion of Vasopressin and Thirst

The physiologic regulation of vasopressin synthesis and secretion involves two systems: osmotic and pressure/volume (Fig. 10.2). Functions of these two systems are so distinct that historically it was thought there were two hormones, an antidiuretic hormone and a vasopressor hormone. Hence the two names that are used interchangeably for (8-arginine) vasopressin. There are separate systems at the level of the receptors on the end organs of response. The V_{1a} receptors on blood vessels are distinct from V_2 receptors on renal collecting duct epithelia. These two vasopressin receptor subtypes are responsible for the main physiologic actions of vasopressin. A third receptor, V_{1b}, is responsible for the nontraditional biologic action of vasopressin to stimulate ACTH secretion from the anterior pituitary and has been found in numerous peripheral tissues and areas of the brain.[32] V_2 receptors also regulate the nontraditional action of vasopressin to stimulate factor VIII and von Willebrand factor production. Vasopressin is the main hormone involved in the regulation of water homeostasis and osmolality, while the renin-angiotensin-aldosterone system (RAAS) is mainly responsible for regulation of blood pressure and volume. Pathologic disorders of the neurohypophysis are primarily expressed as abnormalities of osmolality produced by abnormal excretion or retention of water. In the case of osmoregulation vasopressin secretion is relatively uncomplicated with small increases in osmolality producing a parallel increase in vasopressin secretion and small decreases in osmolality causing a parallel decrease in vasopressin secretion. The regulation of volume and blood pressure is significantly more complicated (see review by Thrasher[33]) and experimental models of vasopressin and baroreceptor regulation in animals often involve inhibiting and/or measuring other concurrent sympathetic inputs to the system to ascertain direct effects of any stimulus on secretion of vasopressin (see Fig. 10.2). Other influences on secretion of vasopressin such as the inhibiting influence of glucocorticoids and the potent stimulus of nausea and vomiting are less important as physiologic regulators of vasopressin but might contribute during pathologic situations.

Volume and Pressure Regulation

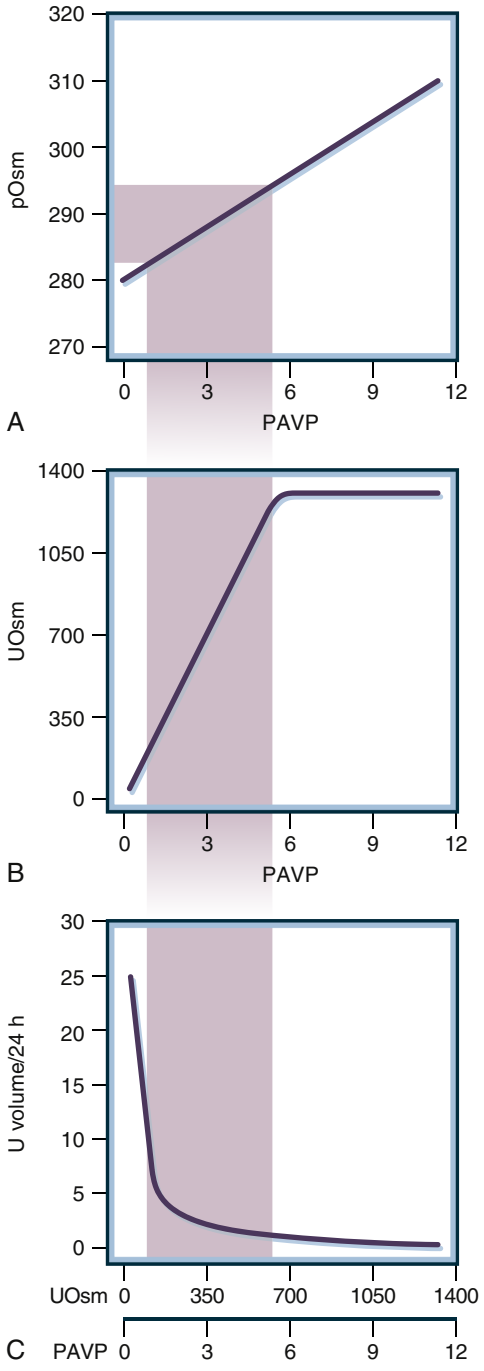
High-pressure arterial baroreceptors are located in the carotid sinus and aortic arch; low-pressure volume receptors are located in the atria and pulmonary venous system.[33] The afferent signals from these receptors are carried from the chest to the brainstem through cranial nerves 9 and 10. Interruption of the vagal input by vagal cold block in dogs[34,35] and destruction in rabbits of the A1 area of the medulla, which receives input from cranial nerves 9 and 10,[36–38] leads to an increase in vasopressin secretion. These and other data led to the concept that baroreceptors and volume receptors normally inhibit the magnocellular neurons and that decreases in this tonic inhibition result in release of vasopressin. Arterial and venous constriction induced by vasopressin action on V_{1a} receptors will contract the vessels around the existing plasma volume to effectively "increase" plasma volume and reestablish the inhibition of secretion of vasopressin. Vasopressin's action at the kidney to retain water will help replace volume but, in fact, the major hormonal regulation to control volume is the RAAS, which stimulates sodium reabsorption in the kidney (see Chapter 15). The concept of tonic inhibition of vasopressin secretion by baroreceptors has been questioned,[33,39] but there is agreement that the volume/baroreceptor responses are much less sensitive than are the osmoreceptors (see Fig. 10.2). The lesser response has been attributed to the fact that changes in blood volume and central venous pressure have little effect to increase vasopressin in humans as long as arterial pressure can be maintained by alternative regulatory mechanisms such as RAAS and sympathetic reflexes.[33] When the hypovolemia is sufficient to cause a decrease in blood pressure there is an exponential increase in the level of vasopressin in plasma[33,40] (see Fig. 10.2). There is also agreement that changes in

volume or pressure that are insufficient to cause direct increases in vasopressin can nonetheless modify the response of the vasopressin system to osmoregulatory inputs.[40,41] Increases in pressure and central volume will decrease the secretion of vasopressin,[42] but the response of the RAAS to cause sodium excretion is much more sensitive to increases of pressure and volume than is the response to decrease secretion of vasopressin.[33] Consequently, changes in blood pressure and volume involve both excitatory and inhibitory influences from the brainstem to magnocellular neurons, with the dominant effect depending on the physiologic circumstances.

Osmotic Regulation

The primary receptors for sensing changes in osmolality are located in the brain. Most of the brain is within the blood-brain barrier, which is generally impermeable to polar solutes. The osmostat is insensitive to urea and glucose, which readily cross cellular membranes but not the blood-brain barrier; this provides strong evidence that the osmoreceptors must be outside the blood-brain barrier. Experimental brain lesions in animals have implicated cells in the organum vasculosum of the lamina terminalis (OVLT) and in areas of the anterior hypothalamus near the anterior wall of the third cerebral ventricle as the primary osmoreceptors. Because these and other circumventricular organs are perfused by fenestrated capillaries, they are outside the blood-brain barrier. Surgical destruction of the OVLT abolishes vasopressin secretion and thirst responses to hyperosmolality but not their responses to hypovolemia.[43] Patients with brain damage that destroys the region around the OVLT cannot maintain normal plasma osmolalities even under basal conditions.[44] In contrast, destruction of the magnocellular neurons of the SON and PVN eliminates dehydration-induced secretion of vasopressin but does not alter thirst, clearly indicating that osmotically stimulated thirst must be generated at a site proximal to the magnocellular cells. Because the osmoreceptors are relatively solute specific, they are most sensitive to alterations in plasma sodium concentrations but respond less well to elevations in blood urea and seem insensitive to changes in plasma glucose concentration.[45]

Extracellular fluid osmolality (predominantly determined by sodium concentration) varies from 280 to 295 mOsm/kg H_2O in normal subjects, but in any individual it is maintained within a more narrow range. The ability to maintain this narrow range is dependent upon the sensitive response of plasma vasopressin to changes in plasma osmolality, the sensitive response of urine osmolality to changes in plasma vasopressin, and the gain in the system by the response of urine volume to change in plasma vasopressin (Fig. 10.3). Basal plasma vasopressin is in the range of 0.5 to 2 pg/mL. As little as a 1% increase or decrease in plasma osmolality will cause a rapid increase or decrease of vasopressin released from the store of hormone in the posterior pituitary.[40] Rapid metabolism of vasopressin is also characteristic of the hormone that circulates in plasma with a half-life of approximately 15 minutes, which allows rapid changes in levels of vasopressin in plasma. Thus small increases in plasma osmolality produce a concentrated urine and small decreases produce a water diuresis. Fig. 10.3A illustrates the linear relationship between plasma osmolality and plasma vasopressin that has been described in humans.[40] This linear relationship for osmolalities persists well above the normal excursion of osmolalities as demonstrated when the increase is induced by infusion of hypertonic saline or is observed during dehydration of patients with nephrogenic diabetes insipidus.[46] Similarly, Fig. 10.3B illustrates that there is a sensitive and linear relationship between the level of vasopressin in plasma and



• **Fig. 10.3** Effect of change in plasma osmolality (pOsm, in mOsm/kg of H_2O) on plasma arginine vasopressin (PAVP, in pg/mL) and consequent effects on urine osmolality (UOsm, in mOsm/kg of H_2O) and urine volume (L/day). The *shaded area* represents the normal range. (A) Small changes in pOsm induce changes in PAVP, typically between less than 0.5 and 5 to 6 pg/mL. (B) Changes in PVAP induce changes in UOsm through the full range, from maximally dilute to maximally concentrated urine. Although PAVP can rise to higher levels than 6 pg/mL, this does not translate into increased UOsm, which has a maximum determined by the osmolality of an inner medulla of the kidney. (C) The relationship of urine volume to UOsm is logarithmic, assuming a constant osmolar load and the urine volume that would excrete that osmolar load at the UOsm indicated. As a result, urine volume changes relatively little with small changes in the other parameters until there is almost complete absence of PAVP, after which the urine volume increases dramatically. (Calculated from a formula presented in Robertson G, Shelton R, Athar S. The osmoregulation of vasopressin. *Kidney Int*. 1976;10:25–37. Figure by A.G. Robinson, University of California at Los Angeles, used with permission of Macmillan Publishers, Ltd.)

the induced osmolality of the urine. Although plasma vasopressin might increase above the normal physiologic range, the urine osmolality plateaus at approximately 800 to 1200 mOsm/kg H_2O because the maximum concentration of fluid in the renal collecting duct is the osmolality of the inner medulla. Fig. 10.3C shows the relationship of plasma vasopressin to urine volume. This is a calculated relationship based on the urine volume necessary to excrete a fixed quantity of osmolytes (800 mOsm) at the urine osmolality produced by the change in plasma vasopressin. These graphs demonstrate the gain in the system when considering the changes in urine volume relative to plasma vasopressin. When vasopressin is absent, 18 to 20 L of urine are excreted daily; with an increase of vasopressin by as little as 0.5 to 1 pg/mL, however, urine volume is reduced to less than 4 L/day. This illustrates the important point that at low plasma levels of vasopressin small changes of vasopressin are much larger determinants of polyuria than are greater changes at higher plasma levels.

In the kidney, water is conserved by the combined functions of the loop of Henle and the collecting duct. The loop of Henle generates a high osmolality in the renal medulla via the countercurrent multiplier system. Vasopressin acts in the collecting duct to increase water (and urea) permeability, thereby allowing osmotic equilibration between the urine and the hypertonic medullary interstitium. The net effect of this process is to extract water from the urine (which is removed from the medulla by interstitial blood vessels, vasa recta), resulting in increased urine concentration and decreased urine volume (antidiuresis). Vasopressin produces antidiuresis by binding to V_2 receptors on the epithelial principal cells of the renal collecting tubule. Binding activates adenylate cyclase, increasing cyclic adenosine monophosphate (cAMP), which then stimulates protein kinase A. This leads to phosphorylation and activation of aquaporin 2 and movement of the water channels into the luminal membrane.[47] Aquaporin 2 is one of the widely expressed family of water channels that mediate rapid water transport across cell membranes.[48] In the kidney, water moves from the collecting duct into the hypertonic inner medulla producing concentrated urine.[49] In addition to moving constitutively synthesized aquaporin 2 from the cytoplasm to the luminal membrane, activation of the V_2 receptor also increases the synthesis of aquaporin 2 and the permeability of aquaporin 2 to water.[50] Aquaporin 3 and 4 are constitutively synthesized and are expressed at high levels in the basolateral plasma membranes of principal cells, where they are responsible for the high water permeability of the basolateral plasma membrane.[48,49] Dissociation of vasopressin from the V_2 receptor allows intracellular cAMP levels to decrease; the water channels are then reinternalized, terminating the increased water permeability. The aquaporin-containing vesicles remain just below the apical membrane and can be quickly "shuttled" into and out of the membrane in response to changes in intracellular cAMP levels. This mechanism allows minute-to-minute regulation of renal water excretion in response to changes in ambient levels of vasopressin in plasma. There is also long-term regulation of collecting duct water permeability in response to prolonged high levels of circulating vasopressin. Chronically high levels of vasopressin induce increased synthesis of aquaporin 2 and 3 water channels in the collecting duct principal cells and hence high levels of these proteins. This response requires at least 24 hours and is not rapidly reversible. Increased numbers of aquaporin 2 and 3 water channels combined with the effect of vasopressin to insert aquaporin 2 into the apical plasma membrane allows the collecting ducts to achieve extremely high water permeabilities and water conservation during prolonged dehydration.[48,49]

Thirst

Renal free water clearance can be reduced to a minimum by the antidiuretic actions of vasopressin, but water loss is not completely eliminated, and insensible water loss from respiration and sweating is a continuous process. To maintain water homeostasis, water must also be consumed to replace the obligate urinary and insensible fluid losses. This is regulated by thirst. The sensation of thirst can be stimulated by increases in plasma osmolality or by decreases in intravascular volume, in a manner identical to vasopressin. Furthermore, there is evidence that the receptors are similar (i.e., osmoreceptors in the anterior hypothalamus and low-pressure and/or high-pressure baroreceptors in the chest [with a likely contribution from circulating angiotensin II to stimulate thirst during more severe degrees of intravascular hypovolemia and hypotension]).[51] Careful physiologic studies in healthy humans, using quantitative estimates of subjective symptoms of thirst, have confirmed that the characteristics of osmotically stimulated thirst are similar to those of vasopressin secretion; the osmotic thresholds are similar, and there is a close linear relationship between thirst scores and plasma osmolality throughout a wide range of plasma tonicity.[52] The threshold for producing thirst by hypovolemia is significantly higher than that for osmotic stimulation.

Although osmotic changes clearly are effective stimulants of thirst, most humans consume the bulk of their ingested water as a result of the relatively unregulated components of fluid intake—that is, beverages are consumed with food for reasons of palatability, taken for desired secondary effects (e.g., caffeine), or taken for social or habitual reasons (e.g., sodas or alcoholic beverages). As a result, humans generally ingest volumes in excess of what can be considered an actual need for fluid. In humans, plasma osmolality does not vary by more than 1% to 2% of basal levels. However, in adipsic patients the absence of thirst is associated with profound alterations of plasma water homeostasis, which emphasizes that even in humans who mostly drink for pleasure, thirst still plays a fundamental role in physiologic osmoregulation.

Clinical Consequences of Osmotic and Volume Regulation

Under physiologic situations and during most pathophysiologic conditions, the integration of osmotic and baroregulated vasopressin release and thirst are closely integrated and maintain plasma osmolality and sodium concentrations within normal ranges. During dehydration, for example, plasma osmolality increases and blood volume decreases, and vasopressin secretion and thirst sensation occur as a composite result of the two separate stimuli. There is good evidence that a decrease in blood volume shifts the plasma vasopressin/plasma osmolality response curve to the left, resulting in a greater release of vasopressin per unit rise osmolality; in other words, hypovolemia enhances the vasopressin response to an osmotic stimulus.[33,53] Similarly, an excess of fluid produces a decrease in osmolality and an increase in volume, and both will cause a decrease in vasopressin secretion and thirst.

The clinical manifestations of osmoregulatory diseases are largely explainable on the basis of the physiology of vasopressin secretion and its antidiuretic actions. Fig. 10.3 shows that loss of vasopressin neurons sufficient to decrease the vasopressin secretory capacity from that able to produce plasma concentrations of 10 to 20 pg/mL down to a secretory capacity able to generate plasma concentrations of only 5 pg/mL would not compromise the ability to maximally concentrate urine. Further losses of vasopressin neurons such that maximal plasma vasopressin concentrations are

in the 1 to 5 pg/mL range are associated with a linear decrease in the ability to maximally concentrate the urine; however, from the urine volume curve it can be seen that this results in only modest increases in urine volume. But when loss of vasopressin neurons to produce maximal plasma vasopressin concentrations below 1 pg/mL occurs, there is a large increase in urine volume, as urine concentration is severely compromised. Water conservation is therefore protected despite minimal ability to secrete vasopressin. In addition, the phenomenon of recovery of diabetes insipidus after transsphenoidal surgery or traumatic brain injury, even after prolonged symptoms, might reflect a comparatively minor recovery of vasopressin neurons sufficient to abolish polyuric symptoms.

In contrast, in the syndrome of inappropriate antidiuresis, a patient who is unable to suppress plasma vasopressin levels to less than 1 pg/mL might be unable to excrete more than 2 to 4 L of urine a day at a standard osmolar load. However, because osmoregulated thirst is not suppressed despite hyponatremia in SIAD,[54] if fluid intake increases above these levels the antidiuretic effects of nonsuppressible vasopressin secretion lead to dilutional hyponatremia. Thus, although the regulation of vasopressin secretion and thirst demonstrates a simple but elegant human system to maintain water balance, abnormal functioning of these complex systems leads to the development of the pathophysiologic states that will be described in this chapter.

Reset Osmostat During Pregnancy

Major shifts of fluid during normal pregnancy produce a fall in plasma osmolality of about 10 mmol/kg and an increase in plasma volume[55]; this is the best physiologic example of a true resetting of the osmostat. The shift in osmotic threshold for vasopressin appears at about 5 to 8 weeks of gestation and persists throughout pregnancy, returning to normal by 2 weeks after delivery.[55] The physiology of the reset osmostat has been considered in relation to the expanded plasma volume. Total body water in pregnant women is increased by 7 to 8 L due to profound vasodilatation.[56] This volume is sensed as normal and vasopressin responds appropriately to decreases and increases of the expanded volume.[55,57] Both the changes in volume and the changes in osmolality have been reproduced by infusion of relaxin (a normal hormone of pregnancy that is a member of the insulin-like growth factor [IGF] family) into virgin female and normal rats,[58,59] and reversed in pregnant rats by immunoneutralization of relaxin.[60] Increased nitric oxide (NO) by relaxin is reported to increase vasodilatation, and estrogens also increase NO synthesis.[61]

In women the placenta produces an enzyme, cysteine aminopeptidase, which is released into the plasma and is known as oxytocinase.[55,62] This enzyme is equally potent in degrading oxytocin and vasopressin. The activity of oxytocinase (vasopressinase) increases markedly around 20 weeks of gestation and increases further to 40 weeks, returning slowly to normal over a few weeks after delivery.[63] The potential pathology produced by oxytocinase is described in "Diabetes Insipidus."

Osmotic Regulation in Aging

Numerous studies have reported that elderly humans are at risk for both hypernatremia and hyponatremia.[64,65] In older subjects there is a decrease in glomerular filtration rate,[64] and the collecting duct in the aged kidney is less responsive to vasopressin-stimulated increases in aquaporin 2 water channels, thus limiting the ability to excrete free water.[66] Elderly subjects are reported to have lower nocturnal plasma levels of arginine vasopressin (AVP) and a prolonged effect of administered desmopressin.[67] Many other abnormalities of fluid and electrolyte balance in the elderly are due to comorbid conditions and/or the numerous pharmacologic agents these patients are often taking.[68] Studies of responses to dehydration, osmolar stimulation, or volume stimulation in the elderly are complicated by the fact that by age 75 to 80 years total body water declines to 50% of the level of normal young adults.[69] The elderly have a decreased thirst with dehydration and a lesser fluid intake during recovery from dehydration.[70,71] At the other end of the spectrum, elderly patients have been found to excrete a water load less well than younger subjects, and at least part of this is due to decreased suppression of vasopressin.[72] In summary, there are age-related changes in body volumes and renal function that predispose the elderly to abnormalities in water and electrolyte balance.[73] Diseases that are more common in the elderly aggravate this; in addition, the therapy for these diseases affects water balance. Healthy elderly humans probably have at least a normal (or increased) ability to secrete vasopressin but a decreased appreciation for thirst and a decreased ability to achieve either a maximum concentration of urine to retain water or a maximum dilution of urine to excrete water. This demonstrates the necessity of paying attention to fluid balance problems in the elderly as undetected hypernatremia or hyponatremia can lead to increased morbidity and mortality.[74]

Diabetes Insipidus

Diabetes insipidus is a clinical disorder that is characterized by the excretion of large volumes of urine (diabetes) that is hypotonic, dilute, and tasteless (i.e., insipid). This is in contrast to the hypertonic and sweet urine of diabetes mellitus (i.e., honey). Diabetes insipidus can result from four abnormal pathophysiologic processes:

1. Primary polydipsia, caused by excess fluid intake
2. Hypothalamic or central diabetes insipidus, caused by diminished synthesis or secretion of vasopressin
3. Diabetes insipidus of pregnancy, caused by increased enzymatic metabolism of vasopressin
4. Nephrogenic diabetes insipidus, caused by renal resistance to vasopressin

Causes of Diabetes Insipidus

Diabetes Insipidus Due to Excess Fluid Intake (Primary Polydipsia)

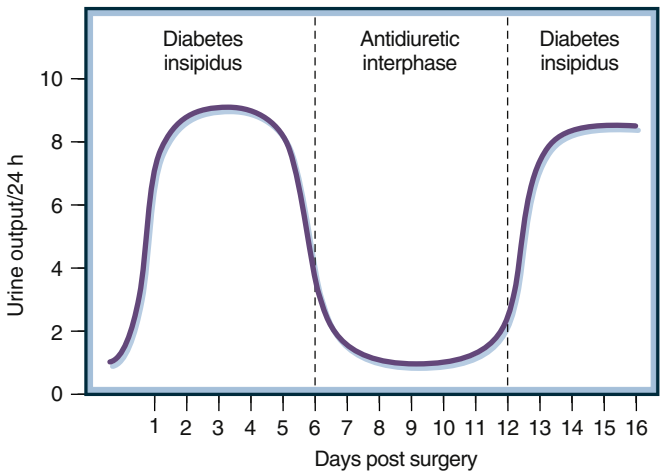
Primary polydipsia is associated with a wide variety of organic structural brain lesions, including sarcoidosis of the hypothalamus[75] and craniopharyngioma.[76] It can also be produced by drugs that cause a dry mouth or by any peripheral disorder causing an elevation of renin and/or angiotensin.[77] However, there is often no identifiable pathologic etiology; in this circumstance the disorder can be associated with psychiatric syndromes or be habitual throughout a lifetime. Series of patients in psychiatric hospitals have shown that as many as 42% have some form of polydipsia, and for over half of those there was no obvious explanation for the polydipsia.[78,79]

Hypothalamic/Central Diabetes Insipidus

Hypothalamic or central diabetes insipidus (CDI) can occur secondary to a large variety of genetic, immunologic, and structural conditions. The relative frequency of the causes of CDI depends on

the nature of the unit where the endocrinologist works. However, as most pituitary endocrinologists work in units where their work is integrated with that of a neurosurgical unit, neurosurgical conditions are usually the commonest encountered causes of CDI.[80]

Pituitary adenomas almost never cause CDI to such an extent that if a patient presents with a pituitary mass and polyuria and polydipsia, it is safe to assume that he or she does not have a pituitary adenoma. However, neurosurgical intervention with transsphenoidal or transcranial surgery causes CDI in as many as 50% to 60% of patients, most of whom will recover, with only a small number having permanent diabetes insipidus.[81] Permanent CDI is more common after craniotomy for large tumors. If there is complete stalk section, patients can exhibit a pattern known as triphasic diabetes insipidus (Fig. 10.4). The first phase is diabetes insipidus with onset within the first 24 hours of surgery and is thought to be due to axon shock and inability of action potentials to be propagated from the cell body to the neurons terminating in the posterior pituitary. The second phase is an antidiuretic phase, which is due to unregulated release of vasopressin from damaged neurohypophyseal neurons; this phase, which occurs typically 5 to 7 days following surgery, is often marked by hyponatremia, particularly if there is injudicious administration of intravenous hypotonic fluids. This phase terminates in permanent CDI, as the damaged neurons undergo gliosis, and loss of secretory function. An important observation is that the second phase of the triphasic response (i.e., uncontrolled release of vasopressin due to axon trauma) can occur without preceding or subsequent diabetes insipidus.[82,83] This has been reported clinically and has been produced experimentally in the rat by unilateral lesion of the supraopticohypophyseal track.[83] The interpretation is that if the trauma is to only some of the axons coursing to the posterior pituitary then the remaining intact axons will have sufficient vasopressin function to avoid clinically apparent diabetes insipidus characteristic of the first and third phases of the triphasic response. However, the store of hormone in the posterior pituitary is sufficiently large that necrosis of even a fraction of these vasopressin neurons will cause enough uncontrolled release of vasopressin to produce hyponatremia if excess fluid is administered. The hyponatremia



• **Fig. 10.4** Typical triphasic response of urine volume after sectioning of the pituitary stalk induced by surgery or head trauma. The first phase of diabetes insipidus occurs immediately postoperatively and continues to day 6. The second phase of antidiuresis occurs from day 7 and continues to day 12. The third stage is the recurrence of diabetes insipidus on day 13. Durations vary; see text for detailed discussion. (From A.G. Robinson, University of California at Los Angeles, used with permission.)

is often symptomatic and patients present with headache, nausea and emesis, or seizure.[84] When all the vasopressin from the damaged neurons has been secreted, the stimulus for water retention resolves and the retained water is excreted producing recovery from the hyponatremia. Thus the clinical picture is one of hyponatremia occurring around 7 to 10 days after pituitary surgery, persisting for a few days, and then returning to normal. This syndrome of transient hyponatremia has been referred to as "isolated second phase"[83] to emphasize the pathophysiologic etiology. Isolated hyponatremia has been reported in 10% to 25% of patients after pituitary surgery.[85–87]

A patient presenting with a sellar mass is more likely to have another tumor in the pituitary area or, alternatively, a granulomatous disorder. Craniopharyngioma is particularly associated with CDI, particularly after extensive suprasellar surgery; it is hoped that the new recommendations for a more limited surgical approach in conjunction with postoperative radiotherapy for craniopharyngioma will result in fewer hypothalamic complications such as CDI, but there is a paucity of randomized controlled data to address this possibility. Other tumors, such as germinoma, pinealoma, or a metastasis from a distant solid organ tumor, can also present with CDI. Metastatic disease involving the pituitary is usually found in association with widespread metastatic disease and may be asymptomatic and only reported at autopsy. Metastases are twice as likely to involve the posterior pituitary as the anterior pituitary,[88,89] which is thought to be due to a more direct arterial blood supply to the posterior pituitary.[90] Most primary tumors in the hypothalamic/pituitary area that cause diabetes insipidus are relatively slow growing, and any tumor in this area that shows rapid growth in a short period of time should be considered as a possible metastatic tumor.[91,92] Pituitary abscess is a rare cause of a pituitary mass and diabetes insipidus.[93,94] Diabetes insipidus has been reported with lymphomas and leukemia in the hypothalamic/pituitary area.[95–97] Diabetes insipidus is distinctly more common in nonlymphocytic leukemia.[98–100] MRI studies in leukemia can show infiltration or an infundibular mass[98] but often are normal even when leukemic cells are found in the cerebrospinal fluid (CSF).[100]

A variety of granulomatous diseases, such as sarcoidosis and histiocytosis, have been associated with CDI; usually there is evidence of characteristic disease elsewhere in the body.[101–103] The MRI often shows involvement of the hypothalamus and absence of the posterior pituitary bright spot on T1-weighted images with widening of the pituitary stalk (Table 10.1). Although there are occasional reports of resolution of the diabetes insipidus with

TABLE 10.1 Diseases Associated With Enlarged Infundibular Stalk

1. Germinoma
2. Craniopharyngioma
3. Metastases to the hypothalamus and long portal vessels (e.g., carcinoma of the breast or lung)
4. Granulomatosis diseases
   a. Langerhans cell histiocytosis
   b. Sarcoidosis
   c. Wegener granulomatosis
   d. Non–Langerhans cell histiocytosis (e.g., Erdheim-Chester disease)
5. Tuberculosis
6. Lymphocytic infundibulo-hypophysitis

appropriate therapy primary disease, in most cases once established, diabetes insipidus is permanent.[104–106] Lymphocytic infundibuloneurohypophysitis, associated with a thickened stalk and loss of the pituitary bright spot on T1-weighted MRI, also presents commonly with CDI. Lymphocytic adenohypophysitis presents classically in females around the time of a pregnancy, whereas infundibuloneurohypophysitis occurs in either sex. Infundibuloneurohypophysitis occurring in middle-aged to elderly males in association with IgG4-related systemic disease has recently been reported. IgG hypophysitis is often associated with other organ involvement, including the pancreas, and other endocrine glands. The diagnosis can be established by elevated serum IgG4 and characteristic histology of biopsies. Response to steroids or other immunosuppressive drugs is characteristic.[107,108]

CDI with no obvious underlying cause has traditionally been considered to be idiopathic, but evidence suggests that a significant proportion of idiopathic CDI cases are in fact autoimmune in origin.[109,110] Several groups have reported the presence of antibodies to vasopressin cells in the plasma of patients with "idiopathic" CDI, and one series reported an incidence of associated autoimmune endocrine disease in 30% of patients with idiopathic CDI,[111] which is higher than the rate reported in, for example, type 1 diabetes mellitus.

CDI has been reported in a number of vascular and traumatic brain disorders. Approximately 15% of patients who have sustained subarachnoid hemorrhage develop acute CDI,[112] which nearly always resolves if the patient survives the initial insult. Very few survivors of subarachnoid hemorrhage have permanent CDI; however, some patients who have surgical clipping of anterior communicating artery aneurysms can develop permanent adipsic CDI due to vascular damage to the perforating arteries of the anterior communicating artery, which provide vascular input to the anterior hypothalamus where the osmoreceptors are situated (see upcoming discussion). Patients who have moderate or severe traumatic brain injury (TBI) also develop CDI quite commonly in the immediate aftermath of injury: 15% to 20% develop CDI, and if close attention is not paid to fluid balance when the patient is obtunded or has cognitive decline, hypernatremia can develop. Hypernatremia and persistent CDI have been shown to be a poor prognostic indicator in acute TBI, often heralding a rise in intracerebral pressure as a preterminal event. Patients who develop CDI secondary to TBI also occasionally go through the triphasic response of early polyuria, followed by transient hyponatremia due to unregulated AVP release, before gliosis produces permanent CDI. There may be a risk in TBI patients during the second phase of SIAD, as hyponatremia will produce cerebral edema, which leads to raised intracranial pressure. It is important therefore to treat early CDI with intermittent dosing of parenteral desmopressin; the effect of one dose should be allowed to wane before administering another dose to ensure that the patient has not developed second phase SIAD. Patients with moderate/severe TBI can also develop anterior pituitary deficiency, including ACTH and cortisol deficiency, which can affect the ability to excrete water. Cortisol deficiency should therefore be considered if diabetes insipidus appears to resolve spontaneously with reversal of polyuria. Cortisol deficiency alone decreases the ability to excrete water even in the absence of vasopressin.[113] Lastly, in a long-term follow-up of these patients partial diabetes insipidus can occur,[114,115] but there may be return of sufficient vasopressin function that under basal conditions the patient is no longer symptomatic from polyuria.[113,114]

Most patients with CDI have normal osmoregulated thirst; as a result, they do not develop hypernatremia unless they develop diminished consciousness or cognitive impairment or are in an environment where water supply is insufficient.[116] However, damage to the osmoreceptors in the anterior hypothalamus[117] may lead to the dangerous combination of CDI and adipsia. Adipsic DI can be seen where surgical damage is so severe that the damage is not only to the neurohypophysis but also to the central anteriorly placed osmostat,[117] such as following extensive surgery for large craniopharyngioma.[76] Adipsic DI can also be caused by isolated damage to the hypothalamic osmoreceptors, with intact baroreceptors, as seen after surgery to an anterior communicating artery aneurysm.[118] In the former case, there is no release of vasopressin in response to either osmotic or baroreceptor stimulation; in the latter case, however, there is adequate synthesis of vasopressin with normal baroregulated but attenuated osmotic release of AVP.[119] Adipsic DI is sometimes associated with widespread damage to other parts of the hypothalamus and can be associated with other manifestations of hypothalamic syndrome, such as hyperphagia, sleep apnea, thermoregulation, seizures, and high mortality.[117,120]

Although rare, genetic abnormalities of the vasopressin gene are a recognized cause of CDI in childhood. Familial CDI is characterized by the onset of classic diabetes insipidus with polydipsia and polyuria in childhood or as a young adult, but during infancy they may be asymptomatic.[121,122] This is in contrast to cases of familial nephrogenic diabetes insipidus in which the defect is expressed as a polyuric disease at birth (see later description). A rare type of familial CDI that can be present at birth is in infants with homozygotes mutation of the AVP hormone region of the pre-prohormone. This can produce excretion of an inactive vasopressin but no difficulty in folding of the pre-prohormone.[123] In the usual familial CDI, MRI findings are variable even within affected family members, but the most constant finding is the presence of a posterior pituitary bright spot in children, which progressively disappears with time.[124] The genetic defect is usually in the biologically inactive neurophysin or in the signal peptide of the pre-prohormone. Although genetically heterozygous with the defect expressed in only one allele, the clinical phenotype is autosomal dominant. Lack of normal cleavage of the signal peptide from the prohormone and abnormal folding of the vasopressin/neurophysin precursor are thought to produce fibrillar aggregations in the endoplasmic reticulum, which is cytotoxic to the neuron, explaining the dominant phenotype.[125,126] Autopsy studies have confirmed neuronal cell death.[127] Genetic testing of asymptomatic children in affected families will negate the need for repeated dehydration testing and allow early treatment.[128] Wolfram syndrome is a rare autosomal recessive disease with diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (DIDMOAD). The genetic defect is for the protein wolframin that is found in the endoplasmic reticulum and is important for folding proteins.[129] Wolframin is localized to chromosome 4. It is involved in beta-cell proliferation and intracellular protein processing and calcium homeostasis, producing a wide spectrum of endocrine and central nervous system (CNS) disorders. Diabetes insipidus is usually a late manifestation and is associated with decreased magnocellular neurons in the paraventricular and supraoptic nuclei.[124,130]

Diabetes insipidus is reported as a premorbid development in 50% to 90% of patients with brain death.[131,132] This is most likely to represent a reaction to rising intracranial pressure. Although some aspects of hormonal treatment of organ donors

are controversial, a consensus has emerged that treatment of diabetes insipidus should be standard in donors who develop CDI.[133]

Diabetes Insipidus Due to Accelerated Metabolism of Vasopressin (Diabetes Insipidus of Pregnancy)

The physiologic adaptations associated with normal pregnancy include expansion of blood volume and decreased plasma osmolality and serum sodium. Although thirst and increased fluid intake are commonly reported in pregnancy, in some patients the increased thirst is driven by marked polyuria, which could be indicative of diabetes insipidus. There are two types of transient diabetes insipidus in pregnancy, both caused by the placental enzyme cysteine aminopeptidase, named oxytocinase, which enzymatically degrades oxytocin, which protects the fetal unit from early labor.[134] Because of the close structural homology between AVP and oxytocin, this enzyme also metabolizes AVP. In the first type of pregnancy-associated DI, the activity of oxytocinase is abnormally elevated. This syndrome has been referred to as "vasopressin resistant diabetes insipidus of pregnancy,"[135] and has been reported with preeclampsia, acute fatty liver, and coagulopathies. Affected patients have decreased metabolism of vasopressinase by the liver.[56,136–138] Characteristically, subsequent pregnancies are not complicated by diabetes insipidus or acute fatty liver. In the second type of pregnancy-associated DI, the accelerated metabolic clearance of vasopressin produces diabetes insipidus in a patient with borderline vasopressin function from a specific disease (e.g., mild nephrogenic diabetes insipidus or partial hypothalamic/neurohypophyseal diabetes insipidus).[62,139,140] Vasopressin is rapidly destroyed and the neurohypophysis is unable to keep up with the increased demand. Labor and parturition usually proceed normally and patients have no trouble with lactation.[141] There is the threat of chronic and severe dehydration when diabetes insipidus is unrecognized, and this can pose a threat to a pregnant woman.[142] Patients with Sheehan syndrome have been reported to have asymptomatic partial diabetes insipidus[143] but rarely develop overt diabetes insipidus.[144]

Nephrogenic Diabetes Insipidus

Genetic variants of nephrogenic diabetes insipidus (NDI) usually present in infancy, with vomiting, failure to thrive, and polyuria.[145,146] NDI can occur as a result of mutations in the V_2 receptor and mutations of the aquaporin 2 water channels.[145–147] Over 90% of cases are X-linked recessive in males, and over 200 different mutations of the V_2 receptor have been reported.[148] Three general categories of V_2 receptor mutations have been described[148]:

1. Type 1, characterized by impaired AVP binding
2. Type 2, typified by defective transport
3. Type 3, where unstable receptors are rapidly degraded

Most of the reported cases are of type 2.[149] Ten percent of the V_2 receptor defects causing congenital nephrogenic diabetes insipidus occur de novo. The majority of female carriers of the X-linked V_2 receptor mutation are asymptomatic, though careful physiologic testing reveals that some might have attenuated maximum urine osmolality in response to vasopressin.[150] In rare cases where heterozygous females have a defect as severe as males, it is thought that there is coexisting inactivation of the function of the X chromosome.[151,152]

When the proband is a female it is likely the defect is a mutation of the aquaporin 2 water channel gene due to an autosomal recessive disease.[153] This should be especially considered where consanguinity is known in the family and the disease is expressed in males and females. The patients can be heterozygous for two different recessive mutations[154] or be homozygous for the same abnormality from both parents.[155] Mutations of the aquaporin 2 protein can produce an autosomal dominant nephrogenic diabetes insipidus when the mutant aquaporin 2 protein associates with the wild-type normal protein to inhibit normal intracellular routing and function of the wild type.[156]

The development of NDI in an adult is less likely to reflect a genetic cause. The commonest cause of acquired NDI in clinical practice is lithium therapy, with other causes (including hypokalemia, hypercalcemia, and release of bilateral urinary tract obstruction) associated with downregulation of aquaporin 2 and decreased function of vasopressin.[153,156] Lithium produces a decrease in urea transporters, reducing vasopressin-stimulated urea uptake and decreasing urea recycling, which reduces intermedullary osmolality.[153,157] Even more dramatic is the reduction in aquaporin 2 levels to decrease water transport in the collecting duct.[156] There is as much as a 95% decrease in aquaporin 2 content and even the 5% of aquaporin 2 that persists is not normally transported to the renal principal cell membrane.[158] The defect of aquaporins with lithium is slow to correct both in experimental animals and in humans and can be permanent.[156,159] Demeclocycline is another commonly recognized drug to cause nephrogenic diabetes insipidus and is sometimes used clinically to treat SIAD. A comprehensive review[160] has documented the large number of drugs that can cause NDI.

Approach to the Differential Diagnosis of Polyuric States

The first diagnostic step is to confirm polyuria, because up to 15% of patients referred for investigation of polyuria have urinary frequency due to bladder wall defects, infection, or prostate disease, with normal urine volume. A 24-hour urine volume greater than 50 mL/kg body weight is worthy of further investigation. Diabetes mellitus, hypercalcemia, hypokalemia, and chronic renal failure are excluded by biochemical tests. Urine osmolality should be low in all polyuric states; however, a random urine osmolality above 700 mOsm/kg excludes diabetes insipidus and makes the diagnosis of primary polydipsia certain. Presenting serum sodium concentration is almost always normal in diabetes insipidus, but results at the higher part of the reference range are more suggestive of DI than primary polydipsia. However, it is difficult to confidently distinguish between CDI, NDI, and thirst disorders solely on baseline laboratory measurements, so osmotic stimulation of the posterior pituitary is needed to secure a firm diagnosis.

The water deprivation test, which incorporates a dehydration step followed by a desmopressin challenge, is usually the first line of investigation. The interpretation of the initial period of water deprivation is based on the understanding that a functioning osmoregulatory system will respond to elevation of plasma osmolality with the secretion of vasopressin and subsequent concentration of the urine. As the test progresses, urine volume decreases and urine osmolality rises usually to over 750 mOsm/kg H_2O. Importantly, an adequate osmotic stimulus to AVP secretion is necessary; a plasma osmolality of over 295 mOsm/kg H_2O is needed to stimulate sufficient AVP to maximally concentrate the urine. In primary polydipsia urine should concentrate relatively normally, as AVP secretion is normal, whereas in patients with either CDI or NDI, urine remains dilute at the end of dehydration. The second part of the test, the administration of desmopressin, is designed to differentiate CDI from NDI. As patients with

CDI are deficient in AVP, exogenous desmopressin concentrates urine osmolality, whereas patients with NDI do not respond to the antidiuretic effects of desmopressin and do not concentrate urine appropriately. The water deprivation test is best performed in a specialized center. Careful patient supervision to monitor for surreptitious drinking is important, and body weight is checked as patients with severe CDI can develop hypernatremia when fluid is withheld due to excess urination. In addition, there are subtleties of interpretation of results that must be considered. Although the water deprivation test differentiates between primary polydipsia, CDI, and NDI in classic and complete cases, published data have shown that accurate diagnosis was made in only 70% of polyuric patients, and the correct diagnosis in primary polydipsia was concluded in only 41% of cases.[161] A number of confounding factors can produce misleading or uninterpretable results:

1. Patients with prolonged severe polyuria may not concentrate urine in response to endogenous or exogenous vasopressin. Chronic hypoosmolality suppresses AVP secretion, and in the absence of V_2 receptor stimulation intracellular aquaporin 2 is not generated. Patients with thirst disorders may therefore be classified as partial DI on the basis of a subnormal rise in urine osmolality during the water deprivation step, despite normal AVP responses. For the same reason, receptor stimulation with desmopressin in step 2 might not stimulate increased urine concentration in patients with CDI, leading to an erroneous diagnosis of NDI.
2. In patients with partial CDI, upregulation of V_2 receptors can result in urine concentration with relatively low plasma AVP concentrations.
3. High plasma AVP concentrations achieved by fluid deprivation in NDI can partially overcome renal resistance; urine osmolality can rise to over 300 mOsm/kg H_2O, leading to diagnostic confusion with partial CDI.[162]

Several studies have demonstrated that the diagnostic accuracy of the water deprivation test was enhanced by the inclusion of measurement of plasma AVP concentration using a sensitive radioimmunoassay.[163,164] However, AVP is unstable after venipuncture and requires careful sample handling, immediate centrifugation, and storage at -70°C. In addition, commercially available AVP antibodies for use in clinical radioimmunoassays are poorly sensitive and have lower limits of detection for the hormone that exceeds low physiologic plasma concentrations. Good-quality, sensitive and specific assays are available in a few specialized centers, but results are often only available weeks after testing has been performed. A recent analysis claimed a diagnostic accuracy of only 46% when direct AVP measurement was prospectively tested in 50 patients with polyuria,[161] but the results may have reflected suboptimal performance of the AVP assay that was used. Alternatively, measurement of the AVP responses during the intravenous infusion of hypertonic (3–5%) sodium chloride solution at the end of a water deprivation test enables more confident distinction between CDI and NDI or primary polydipsia.

Because the logistic difficulty of accurate and sensitive plasma AVP measurements make routine use of the assay impractical for most clinical laboratories, there has been much interest in the measurement of plasma copeptin, which offers a biologic surrogate for the measurement of plasma AVP. Copeptin is the C-terminal locus of the AVP precursor provasopressin, and it is co-secreted from the neurohypophysis in equimolar amounts with AVP in response to the same stimuli. It is much more stable ex vivo, so sample handling is straightforward. A sandwich immunoluminometric assay is used rather than a radioimmunoassay, which enables quick, reliable turnover of results. Careful studies have revealed that the copeptin responses to changes in plasma osmolality are similar to those seen with plasma AVP levels when measured during water deprivation.[165,166] In a prospective multicenter study, a postoperative copeptin concentration of less than 2.5 pmol/L had a post-transsphenoidal surgery predictive value of 81% for CDI, with a specificity of 97%. Conversely, high plasma copeptin concentrations effectively excluded the diagnosis of central DI; a plasma copeptin concentration over 30 pmol/L on day 1 following transsphenoidal surgery was associated with a negative predictive value of 95%, with a sensitivity of 94%.[167] In addition, a single copeptin concentration of over 21.4 pmol/L has been shown to differentiate NDI from other causes of polyuria, with a sensitivity and specificity of 100%, thus mitigating the need for water deprivation testing.[168] Conversely, a baseline copeptin less than 2.6 pmol/L diagnoses complete CDI with 95% sensitivity and 100% specificity. Copeptin is therefore a promising marker of plasma AVP secretion, which offers a convenient, rapid, and sensitive diagnostic tool for polyuric states. The assay should be manageable in most clinical laboratories, and it is likely to replace measurement of plasma AVP in routine diagnostic practice.

Measurement of thirst, using a simple visual analogue scale, in osmotic studies has demonstrated that thirst onset occurs at the same osmotic threshold as AVP secretion[52] and is rapidly switched off by drinking.[169] The characteristics of osmoregulated thirst are surprisingly reproducible.[170] When patients with CDI are studied during water deprivation testing or hypertonic saline infusion, they show a similar pattern of linear elevation during osmotic stimulation, and they show suppression after drinking.[116]

Measurement of thirst is valuable diagnostically in two clinical situations. In adipsic DI, absent osmoregulated thirst during water deprivation or hypertonic saline infusion is the gold standard for diagnosis of the condition.[116,117] Patients with primary polydipsia have three clearly defined abnormalities of thirst: (1) a low osmotic threshold for thirst, which is disconnected from that of AVP release; (2) exaggerated thirst responses to elevation of plasma osmolality; and (3) failure to suppress thirst during drinking after osmotic stimulation.[171] Failure to suppress thirst after drinking by more than 50% of stimulated levels is the most useful of these abnormalities, as it is a strong diagnostic indicator of primary polydipsia.

Further Investigations of Diabetes Insipidus

Once a diagnosis of CDI is established, MRI of the hypothalamo-neurohypophyseal region is indicated. T1-weighted MRI images show a classic bright spot in the posterior pituitary[12] caused by AVP stored in neurosecretory granules.[29,172–175] The bright spot is present in over 80% of normal subjects[176,177] and it is absent in most patients with central diabetes insipidus.[178] Some patients with familial hypothalamic/neurohypophyseal diabetes insipidus can have a posterior pituitary bright spot early in the disease, when diabetes insipidus is partial; however, the bright spot disappears as AVP-secreting neurons diminish in numbers and AVP deficiency gets worse.[179]

The posterior pituitary bright spot decreases with a prolonged stimulus to vasopressin secretion[180] and has been variably reported in other polyuric disorders. In primary polydipsia the bright spot usually is seen.[174,181] In nephrogenic diabetes insipidus the bright spot has been reported to be absent in some patients[174] but present in others.[174,182] Patients with nephrogenic diabetes insipidus have high levels of vasopressin in plasma and are chronically

dehydrated, so the posterior pituitary might be depleted of vasopressin stores. Similarly, with the osmotic stress of untreated diabetes mellitus or the transient diabetes insipidus of pregnancy the posterior pituitary can be depleted of vasopressin, and the bright spot is transiently lost only to reappear with resolution of the underlying condition.[180,183]

Imaging of the hypothalamus is also important to establish whether structural abnormalities of the neurohypophysis are responsible for CDI. Because 90% of the vasopressin neurons must be destroyed to produce symptomatic CDI,[113,184] a mass lesion must either destroy a large area of the hypothalamus or be located where the tracks of the supraoptic and paraventricular nuclei converge at the base of the hypothalamus, superior to the pituitary stalk. Pituitary adenomas confined within the sella do not cause diabetes insipidus.[113] Diseases of the pituitary stalk commonly cause CDI and are listed in Table 10.1. When there is a diagnosis of CDI, thickening of the stalk is usually associated with absence of the posterior pituitary bright spot, and a search for systemic diseases is indicated.[101] A thickened stalk with coexistent anterior pituitary deficiency is especially suggestive of etiologic systemic disease.[185,186] A recent retrospective study has suggested that T2 DRIVE sequence images of the pituitary stalk are so reliable that the need for gadolinium enhancement is unnecessary.[187]

In the absence of structural causes of CDI on MRI, it is important to recognize that in some cases of germinoma the tumor may not be detectable on the initial scans. Therefore, particularly in children and young adults who are more vulnerable to germ cell tumors, markers such as beta human chorionic gonadotropin (βhCG) and alpha-fetoprotein (AFP) should be measured in the blood and in the cerebrospinal fluid if the index of clinical suspicion is high enough. In addition, repeat MRI should be obtained every 3 to 6 months for the first 2 years of follow-up.[185,186,188,189] If follow-up imaging shows a decrease in size of the stalk, a likely diagnosis is infundibulo-neurohypophysitis.[190] Increases in size often occur initially with infundibuloneurohypophysitis, but sustained increases over a 2-year period necessitate biopsy of the pituitary stalk.

Other investigations are dictated by clinical circumstances; if histiocytosis X is suspected, a radiologic skeletal survey should be performed. Sarcoidosis can be revealed by classic radiographic or computed tomography (CT) thorax changes or elevation of serum angiotensin-converting enzyme activity. When MRI of the pituitary is normal, autoimmune variants of CDI should be considered; studies of patients classified as "idiopathic" CDI have revealed the presence of antibodies to AVP-neurons and a high incidence of related autoimmune conditions, most commonly thyroid disease.[111]

Treatment of Polyuric Conditions

Central Diabetes Insipidus in Ambulatory Patients

Treatment of CDI depends on regulation of water intake and excretion. Because thirst is normal in CDI, water intake self-regulates. Replacement of absent AVP is generally done with desmopressin,[191,192] a synthetic analog of vasopressin in which the substitution of D-arginine markedly reduces pressor activity and removing the terminal amine increases the half-life (see Fig. 10.1). These two changes produce an agent nearly 2000 times more specific for antidiuresis than naturally occurring L-arginine vasopressin.[193] Desmopressin is available as tablets for oral administration, a lyophilate for sublingual administration (oral melt), a solution for intranasal administration, and a solution for parenteral use.[194] Most patients prefer desmopressin tablets (0.1 and 0.2 mg); the oral preparations are effective, and unlike nasal sprays absorption is not impaired with nasal infections. However, intestinal peptidase activity can result in increased degradation before absorption, so the tablets should be taken 1 hour before or 2 hours after meals. Desmopressin melt (60, 120, and 240 μg) is reported to be more acceptable in some children.[195] As the degree of AVP deficiency is variable among patients, the dosage and frequency of desmopressin are also highly variable.[196–198] Some patients with partial CDI need a single dose before bed to prevent nocturia, whereas some patients with complete AVP deficiency need desmopressin two to four times daily. The total duration of action of desmopressin will usually be 6 to 18 hours depending on the route of administration.

When a dose of desmopressin is sufficient to elicit a stable therapeutic response, further increasing (e.g., doubling) the dose produces a moderate increase in duration of only a few hours,[196,197] consistent with the half-life of desmopressin in plasma.[196] The maximum dose rarely exceeds 0.2 mg orally or 20 μg intranasally (two sprays) given two or three times a day (usually three for tablets and two for intranasal).[197] For greater flexibility with intranasal administration, the patient can be taught to use the rhinal catheter.[192] Parenterally administered desmopressin (2-mL vials of 4 μg/mL) is only needed for ambulatory patients during an intercurrent illness.[197] Parenterally administered desmopressin gives virtually identical therapeutic response when given as an intravenous bolus, intramuscularly or subcutaneously,[197] and the parenteral administration is 5 to 20 times as potent as an intranasally administrated dose.[192,197] Published studies have reported an increased antidiuretic response to desmopressin in women and in the elderly.[199]

Hyponatremia is a common complication of desmopressin therapy. Mild hyponatremia (plasma sodium 131–134 mmol/L) occurs in 27% of ambulatory blood samples in patients with intact thirst, and in 15% of blood samples serum sodium is less than 130 mmol/L.[80] This reflects the tendency in humans to drink for pleasure or to drink socially; as desmopressin produces a continuous antidiuretic effect, the usual physiologic suppression of endogenous AVP that occurs during drinking does not occur, with consequent dilutional hyponatremia. The hyponatremia seen due to desmopressin is severe enough to precipitate hospital admission in 6% of patients.[80] Hyponatremia is particularly an issue in infants, who consume a large part of their calories as liquid formula or breast milk, and in whom treatment with desmopressin can cause erratic serum sodium consequences and the risk of symptomatic hyponatremia.[200] The risk of dilutional hyponatremia can be reduced by any one of three desmopressin dosing schedules:

1. Omitting a full dose once a week to allow an aquaresis to occur; this is effective, but unpleasant for the patient
2. Delaying a desmopressin dose once or twice weekly until the patient urinates two or three times
3. Delaying each dose of desmopressin until the patient begins to urinate

In practice, individual patients vary as to which of these methods they prefer.

Hypernatremia is much less common in ambulant patients, as the intact thirst mechanism ensures that fluid intake is sufficient to supply physiologic needs; only 1% of ambulant plasma sodium concentrations are above the normal reference range.[80] In patients with adipsic DI, ambulatory hypernatremia is much more common, occurring in up to 20% of blood samples.

Central Diabetes Insipidus in Hospitalized Patients

Although hypernatremia is rare in ambulatory patients with normal thirst, the incidence is much higher during intercurrent illness, particularly if the patient is vomiting, and unable to absorb oral desmopressin. As a result, close attention must be paid to fluid balance when patients are admitted as emergencies. If the patient develops severe hypernatremic dehydration, consideration should be given to anticoagulation to prevent thrombotic complications. Equally, if a patient is on intravenous fluids, regular electrolyte estimations are necessary to ensure that dilutional hyponatremia does not develop, particularly if hypotonic fluids such as dextrose solutions are used. In patients with abnormal thirst, hypernatremia and hyponatremia are both much more common, and the need for careful monitoring of electrolytes and fluid balance is more crucial.[80]

Central Diabetes Insipidus in Neurosurgical Patients

Transient polyuria due to CDI is not uncommon after pituitary surgery; it is more common after extensive surgery for larger pituitary adenomas or for craniopharyngioma. The diagnosis is made by the presence of the classic triad of polyuria, hypernatremia, and dilute urine after exclusion of other possibilities such as diabetes mellitus and mannitol therapy. Sometimes diuresis after surgery is the result of water retention during the procedure. Vasopressin is released during surgical procedures and administered fluid is retained. When the stress of surgery abates, the vasopressin level falls and retained fluid is excreted. If an attempt is made to match the urine output with further fluid infusion, persistent polyuria might be mistaken for diabetes insipidus. If in doubt, fluid should be withheld until there is a modest increase in serum sodium. If the urine output decreases and the serum sodium remains normal, the polyuria was due to excretion of physiologically retained fluid. If the serum sodium begins to rise while urine osmolality is low and there is a positive response to administered desmopressin, the diagnosis of diabetes insipidus can be established.[201] If the duration of diabetes insipidus is quite transient, it is acceptable to treat this simply with fluid replacement, parenterally or orally. However, most patients who develop diabetes insipidus require desmopressin 0.5 to 2 μg subcutaneously, intramuscularly, or intravenously. Urine output will be reduced in 1 to 2 hours and the duration of effect is 6 to 24 hours. Care should be taken that hypotonic intravenous fluids are not given excessively after administering desmopressin, as the combination can lead to profound hyponatremia.[202] Because there is always the possibility of developing the triphasic response due to pituitary stalk damage, it is recommended that polyuria should be recurrent before a decision to administer subsequent doses of desmopressin is made.[203]

Acute diabetes insipidus after traumatic brain injury can be treated similarly to that recommended for patients following pituitary surgery; as the patient with head injury is more likely to be comatose or cognitively impaired, and thus unable to depend on thirst, hypernatremia is more likely to develop. Because a comatose patient must be given fluids parenterally, it is important to monitor serum sodium concentration regularly to check for dilutional hyponatremia. Persistent diabetes insipidus has been demonstrated in prospective studies to be a predictor of fatal outcome after traumatic brain injury, perhaps because it is a manifestation of rising intracranial pressure and imminent herniation.[204]

Adipsic Diabetes Insipidus

The combination of attenuated thirst and diabetes insipidus confers high risk of severe hypernatremia. As well, when treated with desmopressin they are at risk for developing hyponatremia. A carefully monitored regimen of a fixed dose of desmopressin to maintain chronic antidiuresis and a prescribed volume of fluid intake is preferable.[205,206] Daily weight can be used to guide intake, and regular follow-up with measurement of serum sodium is essential to ensure that these patients do not develop water intoxication with hyponatremia or recurrent dehydration with hypernatremia. Patients with adipsic DI have been reported to develop thrombotic complications when severely dehydrated, and they should be prophylactically anticoagulated when admitted with severe intercurrent illness. Although management of water balance is the clinical priority for endocrinologists, it is important to holistically approach the hypothalamic complication associated with adipsic DI. Sleep apnea can respond to treatment with continuous positive airway pressure (CPAP) or modafinil, and treatment for associated hypothalamic obesity is also important.[117] This group of patients has high mortality rates and requires very careful management.

Treatment of Diabetes Insipidus in Pregnancy

Desmopressin is the only therapy recommended for treatment of diabetes insipidus during pregnancy. Desmopressin has 2% to 25% the oxytocic activity of lysine vasopressin or arginine vasopressin[193] and can be used with minimal stimulation of the oxytocin receptors in the uterus.[141,207] Desmopressin is not destroyed by the cysteine aminopeptidase (oxytocinase) of pregnancy[141,208] and is reported to be safe for both the mother and the child.[209,210] During delivery, patients can maintain adequate oral intake and are therefore safe to continue administration of desmopressin. Physicians should be cautious about overadministration of fluid parenterally during delivery because these patients will not be able to excrete the fluid and can develop water intoxication and hyponatremia. After delivery, plasma oxytocinase decreases and patients can recover completely or be asymptomatic with regard to fluid intake and urine excretion.

Treatment of Nephrogenic Diabetes Insipidus

Adequate water intake is the mainstay of treatment of NDI as pharmacologic reversal of the renal resistance to AVP is rarely possible. Nephrogenic diabetes insipidus does not respond to desmopressin therapy, although occasionally partial defects have limited response to high doses of desmopressin.[211,212] In congenital NDI, therapy aimed at reducing symptomatic polyuria is addressed primarily by inducing plasma volume contraction via a low sodium diet and a thiazide diuretic. It has been hypothesized that contraction of extracellular fluid volume, decreased glomerular filtration rate, proximal tubular sodium and water reabsorption, and decreased delivery of fluid to the collecting duct result in a decreased volume of urine.[213] Studies have also demonstrated that thiazide diuretics can increase aquaporin 2 independent of vasopressin.[214] All the thiazide diuretics appear to have similar effects. Potassium replacement and/or coadministration of a potassium-sparing antidiuretic may be necessary, as hypokalemia can worsen renal resistance to AVP. The effect of thiazides can be augmented by coadministration of nonsteroidal anti-inflammatory drugs (NSAIDs), but this combination is nephrotoxic, and careful monitoring of renal function is mandatory. Selective cyclooxygenase 2 (COX2) inhibitors with less gastrointestinal (GI) effect have been reported to decrease water loss, but long-term safety has not been documented.[156]

Drug-induced nephrogenic diabetes insipidus should be treated by stopping the offending agent if possible. Persistence

of nephrogenic diabetes insipidus can be treated by hydrochlorothiazide and amiloride. Plasma volume contraction produced by thiazide diuretics can decrease lithium excretion and predispose to lithium toxicity.[160,215] The diuretic amiloride blocks sodium channels in the luminal membrane of the collecting duct cells and inhibits lithium reabsorption, a unique advantage in treating lithium-induced nephrogenic diabetes insipidus.[216] In animal studies of lithium-induced nephrogenic diabetes insipidus, treatment with amiloride increased levels of both aquaporin 2 and urea transporters.[157]

Studies have reported the possibility of rescuing mutant receptors in NDI. In autosomal dominant type 2 NDI, the misfolded receptor protein is trapped in the quality control system of the endoplasmic reticulum, but in some cases the defect involves transport of the receptor, and were the receptor to reach the cell membrane it would respond to vasopressin. V_2 receptor antagonists (vaptans) have been reported to act as pharmacologic chaperones that combine with the misfolded receptor, changing the confirmation to allow maturation and transport to the plasma membrane where vasopressin (in excess of the vaptans) would cause the receptor to be activated.[217–219] Similar studies of rescue have recently been reported with nonpeptide V_2 receptor agonists. These agonists combine with the mutant receptor trapped in the endoplasmic reticulum and allow maturation of the mutant receptor. The rescued receptor is then inserted into the cell membrane and when stimulated by vasopressin or desmopressin generates sufficient cAMP to move aquaporin 2 from the cytoplasm to the cell membrane to enhance water transport.[218,219] Nonpeptide antagonists working as chaperones is a potential new treatment of nephrogenic diabetes insipidus, especially in patients with a partial disorder.[220] Sequencing of genes in all families with NDI is recommended because of the small size of the genes to be sequenced and because of the value of the information.[149] In X-linked disorders, carrier females can be distinguished from noncarrier females, so it will be known which sibling's children are at risk and require special observation at birth. Molecular testing of newborns will confirm the need for long-term treatment to avoid complications in the affected children and obviate the need for water deprivation or other testing in unaffected children.[152,221]

Treatment of Primary Polydipsia

Treatment of primary polydipsia entails reduction of excessive fluid intakes, best done in a graded fashion to allow patients to slowly achieve a level of intake that reduced urine volume below polyuric levels (50 mL/kg BW). Measures to reduce mouth dryness (e.g., ice chips, hard candy to stimulate salivary flow) are useful adjuncts to reduce thirst. Pharmacologic therapies have been tried but without consistent evidence of success.

Diabetes Insipidus in Association With Other Therapeutic Decisions

Routine Surgical Procedures

In all cases there should be preoperative consultation of the surgeon, the anesthesiologist, and the endocrinologist/nephrologist. For most routine surgical procedures the patient is not unconscious for a sufficiently long period of time to require anything more that administration of the usual dose of desmopressin and careful monitoring of fluids during the surgery to prevent overhydration. If the patient is taking desmopressin orally and is NPO, a parenteral dose can be administered before the procedure. Close monitoring of serum sodium is essential. In NDI there should be a greater emphasis on fluid replacement to avoid dehydration and hypernatremia.[222]

Panhypopituitarism

Patients with coexistent diabetes and anterior pituitary deficiency are at risk of developing hyponatremia, as cortisol deficiency is associated with impaired water excretion. Patients who miss their hydrocortisone doses are vulnerable to desmopressin-induced hyponatremia.

Hypertonic Encephalopathy

Hypertonic encephalopathy is uncommon in diabetes insipidus and is only seen when there is inadequate fluid intake in an adipsic patient or in a patient who is unconscious and not receiving adequate fluid supplementation. Conditions other than diabetes insipidus are more common causes of hypernatremic encephalopathy. The condition is associated with loss of hypotonic fluids by the kidney or the intestines, or from insensible losses; or, it might occur following administration of hypertonic sodium containing fluids or hyperalimentation.[223] Hypernatremia leads to osmotic movement of water out of cells and cellular dehydration. Studies indicate that in the brain idiogenic osmoles, mainly polyols, trimethylamines, and amino acids, are generated intracellularly so the degree of cell shrinkage is less than would occur based on the degree of hypernatremia.[224] Loss of water from the brain occurs in minutes and electrolytes enter the brain in a few hours, but the increase in organic osmoles occurs over several days.[225] When fluid is replaced, intracellular organic osmoles dissipate more slowly than the decrease in osmolality of extracellular fluid. This asynchrony increases the potential for cerebral edema and worsening of the neurologic condition with overzealous treatment of hypernatremia.[225] In most cases of diabetes insipidus seen following neurosurgery or head injury the diagnosis will be made within a few hours and therapy should be instituted promptly. In cases where the duration of the hypernatremia is not known, the degree of correction of hypernatremia should not exceed 0.5 mEq/L/hour to prevent cerebral edema and convulsions.[223,225]

The Syndrome of Inappropriate Antidiuresis

The SIAD is produced when plasma levels of vasopressin are elevated at times when the physiologic secretion of vasopressin from the posterior pituitary would normally be osmotically suppressed. The clinical abnormality is a decrease in the osmotic pressure of body fluids, so the hallmark of SIAD is hypoosmolality. This led to the identification of the first well-described cases of this disorder in 1957[226] and the subsequent clinical investigations that resulted in delineation of the essential characteristics of the syndrome.[227] Although initially called the syndrome of inappropriate antidiuretic hormone (SIADH) secretion, the term SIAD is more appropriate in view of the inability to measure elevated vasopressin levels in some patients with this disorder. It is necessary to first review hypoosmolality and hyponatremia before discussing details that are specific to SIAD.

Hypoosmolality and Hyponatremia

Incidence

Hypoosmolality is the most common disorder of fluid and electrolyte balance encountered in hospitalized patients. The incidence and prevalence of hypoosmolar disorders depend on the nature of the patient population studied as well as on the

laboratory methods and criteria used to diagnose hyponatremia. Most investigators have used the serum sodium concentration ($[Na^+]$) to determine the clinical incidence of hypoosmolality. When hyponatremia is defined as a serum $[Na^+]$ of less than 135 mEq/L, prevalences as high as 15% to 38% have been observed in studies of both acutely and chronically hospitalized patients.[228,229] However, incidences decrease to the range of 1% to 4% when only patients with serum $[Na^+]$ under 130 to 131 mEq/L are included, which represents a more appropriate level at which to define the occurrence of clinically significant cases of this disorder.[230] Even using these more stringent criteria, incidences from 7% to 53% have been reported in institutionalized geriatric patients.[231] Although hyponatremia and hypoosmolality are quite common, the majority of cases are relatively mild and most are acquired during the course of hospitalization. Nonetheless, hyponatremia is important clinically because (1) severe hypoosmolality (serum $[Na^+]$ levels <120 mEq/L) is associated with substantial morbidity and mortality,[232] (2) even relatively mild hypoosmolality can quickly progress to more dangerous levels during the course of therapeutic management of other disorders, (3) overly rapid correction of hyponatremia can itself cause severe neurologic morbidity and mortality,[233] and (4) it has been observed that mortality rates are from 3-fold to 60-fold higher in patients with even asymptomatic degrees of hypoosmolality compared to normonatremic patients.[234,235]

Osmolality, Tonicity, and Serum $[Na^+]$

As discussed, the osmolality of body fluid normally is maintained within narrow limits for each individual by osmotically regulated vasopressin secretion and thirst. Plasma osmolality can be determined directly by measuring the freezing-point depression or the vapor pressure of plasma. Alternatively, it can be calculated indirectly from the concentrations of the three major solutes in plasma:

$$\text{pOsm}\ (\text{mOsm/kg}\ H_2O) = 2 \times [Na^+]\ (\text{mEq/L}) + \text{glucose}\ (\text{mg/dL})\ /\ 18 + \text{blood urea nitrogen}\ (\text{mg/dL})\ /\ 2.8$$

Direct measure and calculation produce comparable results under most conditions. However, while either of these methods produce valid measures of *total* osmolality, this is not always equivalent to the *effective* osmolality, which is commonly referred to as the "tonicity" of the plasma. Only solutes such as Na^+ and Cl^- that are impermeable to the cell membrane and remain relatively compartmentalized within the extracellular fluid (ECF) space are effective solutes, because these solutes create osmotic gradients across cell membranes and regulate the osmotic movement of water between the intracellular fluid (ICF) compartment and the ECF compartment. Solutes that readily permeate cell membranes (e.g., urea, ethanol, and methanol) are not effective solutes. Therefore only the concentrations of effective solutes in plasma should be used to ascertain whether clinically significant hyperosmolality or hypoosmolality is present.

Sodium and its accompanying anions represent the major effective plasma solutes, so hyponatremia and hypoosmolality are usually synonymous. However, there are two situations in which hyponatremia will not reflect true hypoosmolality. The first is *pseudohyponatremia*, which is produced by marked elevations of either lipids or proteins in plasma. If serum $[Na^+]$ is measured by flame photometry, the concentration of sodium per liter of plasma is artifactually decreased because of the larger relative proportion of plasma volume that is occupied by the excess lipids or proteins.[236] However, the increased protein or lipid will not appreciably change the total number of solute particles in solution, so the directly measured plasma osmolality will not be significantly affected. Measurement of serum $[Na^+]$ by ion-specific electrodes, which is now commonly used by most clinical laboratories, is less influenced by high concentrations of lipids or proteins than is measurement of serum $[Na^+]$ by flame photometry. However, this can still occur if the electrode measurement is done using a diluted sample of the serum. The second situation in which hyponatremia does not reflect true plasma hypoosmolality occurs when high concentrations of effective solutes other than Na^+ are present in the plasma. The initial hyperosmolality produced by the additional solute causes an osmotic shift of water from the ICF to the ECF, which in turn produces a dilutional decrease in serum $[Na^+]$. Once equilibrium between both fluid compartments is achieved, the total effective osmolality remains relatively unchanged. This situation most commonly occurs with hyperglycemia and represents a frequent cause of hyponatremia in hospitalized patients, accounting for up to 10% to 20% of all cases.[234] Misdiagnosis of true hypoosmolality in such cases can be avoided by measuring plasma osmolality directly or, alternatively, by correcting the measured serum $[Na^+]$ for the glucose elevation. Traditionally this correction factor has been 1.6 mEq/L for each 100-mg/dL increase in serum glucose concentration above normal levels,[237] but some studies have shown a more complex relation between hyperglycemia and serum $[Na^+]$ and reported that a more accurate correction factor is closer to 2.4 mEq/L.[238] When the plasma contains significant amounts of unmeasured solutes, such as osmotic diuretics, radiographic contrast agents, and some toxins (ethanol, methanol, and ethylene glycol), plasma osmolality cannot be calculated accurately, and in these situations osmolality must be ascertained by direct measurement.

Pathogenesis of Hypoosmolality

Water moves freely between the ICF and ECF, consequently osmolality will always be equivalent in both of these fluid compartments. Since the bulk of body solute is comprised of electrolytes, namely the exchangeable Na^+ (Na^+_E) in the ECF and the exchangeable K^+ (K^+_E) in the ICF along with their associated anions, total body osmolality (OSM_T) will largely be a function of these parameters[239]:

$$OSM_T = OSM_{ECF} = OSM_{ICF}$$

$$OSM_T = (\text{ECF solute} + \text{ICF solute})\ /\ \text{body water}$$

$$OSM_T\ (2 \times Na^+_E + 2 \times K^+_E + \text{nonelectrolyte solute})\ /\ \text{body water}$$

According to this definition, the spresence of plasma hypoosmolality indicates a relative excess of water to solute in the ECF. This can be produced either by an excess of body water, resulting in a *dilution* of remaining body solute, or by a *depletion* of body solute, either Na^+ or K^+, relative to body water. This classification is an oversimplification, because most hypoosmolar states involve significant components of both solute depletion and water retention. Nonetheless, it is conceptually useful for understanding the mechanisms underlying the pathogenesis of hypoosmolality and as a framework for therapy of hypoosmolar disorders.

Solute Depletion

Depletion of body solute can result from any significant losses of ECF. Body fluid losses by themselves rarely cause hypoosmolality because excreted or secreted body fluids are usually isotonic or hypotonic relative to plasma and therefore tend to increase plasma osmolality. When hypoosmolality accompanies ECF losses, it is the result of replacement of body fluid losses by more hypotonic solutions either by drinking or by infusion, thereby diluting the remaining body solutes. If the solute losses are marked, these patients show signs of volume depletion (e.g., addisonian crisis). However, such patients often have a more deceptive clinical presentation because the volume deficits have been partially replaced. Moreover, they may not manifest signs or symptoms of cellular dehydration because osmotic gradients will draw water into the ICF, which is relatively hypertonic to the solute-depleted ECF. Therefore clinical evidence of hypovolemia strongly supports solute depletion as the cause of plasma hypoosmolality, but absence of clinically evident hypovolemia never completely eliminates this as a possibility. Although ECF solute losses are responsible for most cases of depletion-induced hypoosmolality, ICF solute loss can also cause hypoosmolality as a result of osmotic water shifts from the ICF into the ECF. This mechanism contributes to some cases of diuretic-induced hypoosmolality in which depletion of total body K^+ often occurs.[240]

Water Retention

Despite the importance of solute depletion in some patients, most cases of clinically significant hypoosmolality are caused by increases in total body water rather than by primary losses of extracellular solute. This can occur because of either impaired renal free water excretion or excessive free water intake. The former accounts for most hypoosmolar disorders because normal kidneys have sufficient diluting capacity to allow excretion of 18 to 24 L/day of free water. Intakes of this magnitude are occasionally seen in some psychiatric patients but not in most patients with SIAD in whom fluid intakes average 2 to 3 L/day.[241] Consequently, dilutional hypoosmolality usually is the result of an abnormality of renal free water excretion. The renal mechanisms responsible for impairments in free water excretion can be subgrouped according to whether the *major* impairment in free water excretion occurs in proximal or distal parts of the nephron, or both. Any disorder that leads to a decrease in glomerular filtration rate causes increased reabsorption of both Na^+ and water in the proximal tubule. As a result, the ability to excrete free water is limited because of decreased delivery of tubular fluid to the distal nephron. Disorders that cause a decreased glomerular filtration rate in the absence of significant ECF fluid losses are, for the most part, edema-forming states associated with decreased effective arterial blood volume (EABV) and secondary hyperaldosteronism.[242] Even though these conditions are characterized by increased proximal reabsorption of both Na^+ and fluid, water retention also results from increased distal reabsorption caused by nonosmotic baroreceptor-mediated stimulated increases in plasma vasopressin levels. Distal nephron impairments in free water excretion are characterized by an inability to dilute tubular fluid maximally. These disorders are usually associated with abnormalities in the secretion of vasopressin. Just as depletion-induced hypoosmolar disorders usually include an important component of secondary impairments of free water excretion, most dilution-induced hypoosmolar disorders also involve significant degrees of secondary solute depletion. This is described later with SIAD.

Some dilutional disorders do not fit well into either category, specifically the hyponatremia that sometimes occurs in patients who ingest large volumes of beer with little food intake for prolonged periods, called "beer potomania."[243] Even though the volume of fluid ingested may not seem sufficiently excessive to overwhelm renal diluting mechanisms, free water excretion is limited by very low urinary solute excretion, thereby causing water retention and dilutional hyponatremia.

Adaptation to Hyponatremia: ICF and ECF Volume Regulation

Many past studies have suggested that the combined effects of water retention plus urinary solute excretion cannot adequately explain the degree of plasma hypoosmolality observed in patients.[227,244] This observation led to the theory of *cellular inactivation of solute,* which suggested that as ECF osmolality falls, water moves into cells along osmotic gradients, thereby causing the cells to swell; at some point during this volume expansion, the cells theoretically osmotically "inactivate" some of their intracellular solutes as a defense mechanism to prevent continued cellular swelling with subsequent detrimental effects on cell function and survival. This effect would decrease the intracellular osmolality allowing water to shift back out of the ICF into the ECF, thereby further worsening the dilution-induced hypoosmolality. Despite the appeal of this theory, its validity has never been demonstrated conclusively in either human or animal studies. An alternative theory is that cell volume is maintained under hypoosmolar conditions by extrusion of intracellular solutes such as potassium.[245] Whole brain volume regulation via electrolyte losses was first described by Yannet[246] and has long been recognized as the mechanism by which the brain is able to adapt to hyponatremia and limit brain edema to sublethal levels.[247] Following the recognition that low molecular weight organic compounds, called *organic osmolytes,* also constituted a significant osmotic component of a wide variety of cells, studies demonstrated the accumulation of these compounds in response to hyperosmolality in both kidney[248] and brain[249] tissue and conversely that the brain also loses organic osmolytes in addition to electrolytes during volume regulation to hypoosmolar conditions in experimental animals[250,251] and human patients.[252] These losses occur relatively quickly (within 24–48 hours in rats) and can account for as much as one-third of the brain solute losses during hyponatremia.[253] Such coordinate losses of both electrolytes and organic osmolytes from brain cells allow effective regulation of brain volume during chronic hyponatremia.

Although recent studies of volume regulation during hyponatremia have focused on the brain, all cells regulate volume by cellular losses of both electrolyte and organic solutes to varying degrees. However, volume regulatory processes are not limited to cells. In most cases of hyponatremia induced by stimulated antidiuresis and water retention, natriuresis also regulates the volumes of the ECF and intravascular spaces. Both experimental and clinical observations are consistent with ECF volume regulation via secondary solute losses. First, the concentrations of most blood constituents other than Na^+ and Cl^- are not decreased in patients with SIAD,[254] suggesting that plasma volume is not nearly as expanded as would be predicted simply by the measured decreases in serum $[Na^+]$. Second, an increased incidence of hypertension has never been observed in patients with SIAD, again evidence against significant expansion of the arterial blood volume. Third, results of animal studies in both dogs[255] and rats[256] have indicated that a significant component of chronic hyponatremia is attributable to secondary Na^+ losses rather than water retention; the relative

contributions from water retention versus sodium loss vary with the duration and severity of the hyponatremia: Water retention was found to be the major cause of decreased serum [Na^+] in the first 24 hours of induced hyponatremia in rats, but Na^+ depletion then became the predominant etiologic factor after longer periods (7–14 days) of sustained hyponatremia, particularly at very low (<115 mEq/L) serum [Na^+] levels.[256] Finally, multiple studies of body fluid compartment volumes in hyponatremic patients have not demonstrated either plasma or ECF expansion. For example, a report of body fluid space measurements using isotope dilution techniques in hyponatremic and normonatremic patients with small cell lung carcinoma showed no differences between the two groups with regard to exchangeable sodium space, ECF volume by $^{35}SO_4$ distribution, or total body water.[257] Fig. 10.5 schematically illustrates the volume regulatory processes that occur in response to water retention induced by inappropriate antidiuresis.

Differential Diagnosis of Hyponatremia and Hypoosmolality

Because of the multiplicity of disorders causing hypoosmolality and the fact that many involve more than one pathologic mechanism, a definitive diagnosis is not always possible at the time of initial presentation. Nonetheless, an approach based on clinical parameters of ECF volume status and urine sodium concentration generally allows a sufficient categorization for appropriate decisions regarding initial therapy and further evaluation.

Decreased Extracellular Fluid Volume

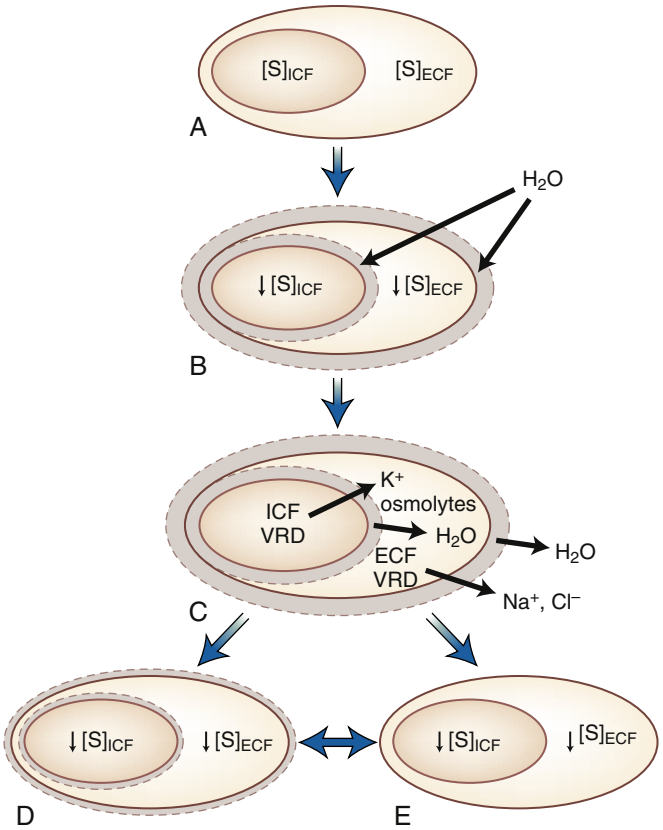
Clinically detectable hypovolemia always signifies total body solute depletion. A low urine [Na^+] indicates a nonrenal cause and an appropriate renal response. A high urine [Na^+] indicates renal causes of solute depletion are more likely. Therapy with thiazide diuretics is the most common cause of renal solute losses,[240] particularly in the elderly,[258] but mineralocorticoid deficiency as a result of adrenal insufficiency or mineralocorticoid resistance must be considered as well as (less commonly) renal solute losses due to salt-wasting nephropathy (e.g., polycystic kidney disease, interstitial nephritis, or chemotherapy).

Increased Extracellular Fluid Volume

Clinically detectable hypervolemia always signifies total body Na^+ excess. In these patients hypoosmolality results from an even greater expansion of total body water caused by a marked reduction in the rate of water excretion (and sometimes an increased rate of water ingestion). The impairment in water excretion is secondary to a decreased EABV,[242] which increases the reabsorption of glomerular filtrate not only in the proximal nephron but also in the distal and collecting tubules by stimulated secretion of vasopressin. These patients generally have a low urine [Na^+] because of secondary hyperaldosteronism. However, under certain conditions urine [Na^+] may be elevated if there is concurrent diuretic therapy, a solute diuresis (e.g., glucosuria in diabetics), or after successful treatment of the underlying disease (e.g., improved cardiac output in patients with congestive heart failure).

Normal Extracellular Fluid Volume

Many different hypoosmolar disorders present with euvolemia, and measure of urinary [Na^+] is an especially important first step.[259] A high urine [Na^+] usually implies a distally mediated, dilution-induced hypoosmolality such as SIAD. However, glucocorticoid deficiency can mimic SIAD so closely that these two disorders are often indistinguishable in terms of water balance.



• **Fig. 10.5** Schematic illustration of potential changes in whole-body fluid compartment volumes at various times during adaptation to hyponatremia. (A) Under basal conditions, the concentrations of effective solutes in the extracellular fluid ($[S]_{ECF}$) and in the intracellular fluid ($[S]_{ICF}$) are in osmotic balance. (B) During the first phase of water retention resulting from inappropriate antidiuresis, the excess water distributes across total body water, causing expansion of both ECF and ICF volumes *(dotted lines)*, with equivalent dilutional decreases in both $[S]_{ICF}$ and $[S]_{ECF}$. (C) In response to the volume expansion, compensatory volume regulatory decreases *(VRD)* occur to reduce the effective solute content of the ECF (via pressure diuresis and natriuretic factors) and the ICF (via increased electrolyte and osmolyte extrusion mediated by stretch-activated channels and downregulation of synthesis of osmolytes and osmolyte uptake transporters). (D and E) If both processes go to completion, such as under conditions of fluid restriction, a final steady state can be reached in which ICF and ECF volumes have returned to normal levels but $[S]_{ICF}$ and $[S]_{ECF}$ remain low. In most cases, this final steady state is not reached, and moderate degrees of ECF and ICF expansion persist, although they are significantly less than would be predicted from the decrease in body osmolality (D). Consequently, the degree to which hyponatremia is the result of dilution due to water retention versus solute depletion from volume regulatory processes can vary markedly, depending on which phase of adaptation the patient is in and the relative rates at which the different compensatory processes occur. For example, delayed ICF VRD can worsen hyponatremia because of shifts of intracellular water into the ECF as intracellular organic osmolytes are extruded and subsequently metabolized; this likely accounts for some component of the hyponatremia that was unexplained by the combination of water retention and sodium excretion in early clinical studies. (From Verbalis JG. Hyponatremia: epidemiology, pathophysiology, and therapy. *Curr Opin Nephrol Hypertens*. 1993;2:626–652.)

Hyponatremia from diuretic use also can present without clinically evident hypovolemia, and the urine [Na^+] will usually be elevated.[240] A low urine [Na^+] suggests a depletion-induced hypoosmolality from ECF losses with subsequent volume replacement by water or other hypotonic fluids. The solute loss often

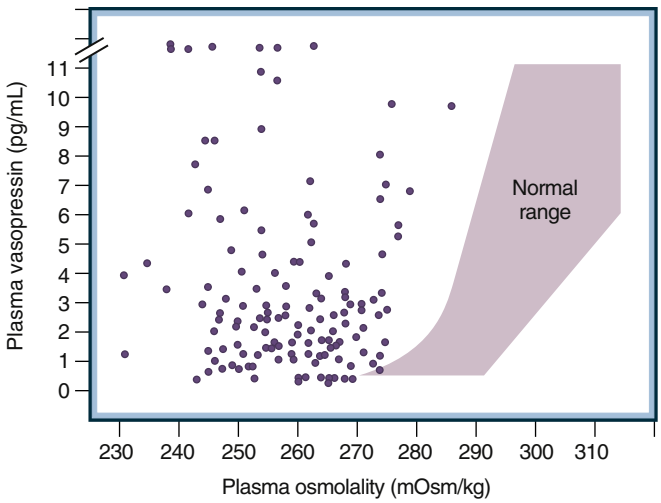
is nonrenal, but an important exception is recent cessation of diuretic therapy, because urine [Na^+] can decrease to low values within 12 to 24 hours after discontinuation of the drug. A low urine [Na^+] also can also be seen during the recovery phase from SIAD.

Syndrome of Inappropriate Antidiuresis

SIAD is the most common cause of euvolemic hypoosmolality and it is also the single most common cause of hypoosmolality of all etiologies encountered in clinical practice with prevalence rates from 20% to 40% among all hypoosmolar patients.[234,241] The clinical criteria necessary to diagnose SIAD remain basically as set forth by Schwartz and Bartter in 1967:[227]

1. Decreased effective osmolality of the extracellular fluid (P_{osm} <275 mOsm/kg H_2O). Pseudohyponatremia or hyperglycemia alone must be excluded.
2. Inappropriate urinary concentration at some level of hypoosmolality. This does not mean that urine osmolality is greater than plasma osmolality, only less than maximally dilute (i.e., urine osmolality >100 mOsm/kg H_2O). Also, urine osmolality need not be elevated inappropriately at all levels of plasma osmolality, because in the reset osmostat variant form of SIAD, vasopressin secretion can be suppressed with resultant maximal urinary dilution if plasma osmolality is decreased to sufficiently low levels.[260]
3. Clinical euvolemia, as defined by the absence of signs of hypovolemia (orthostasis, tachycardia, decreased skin turgor, dry mucous membranes) or hypervolemia (subcutaneous edema, ascites). Hypovolemia or hypervolemia strongly suggests different causes of hypoosmolality. Patients with SIAD can become hypovolemic or hypervolemic for other reasons, but in such cases it is impossible to diagnose the underlying inappropriate antidiuresis until the patient is rendered euvolemic and is found to have persistent hypoosmolality.
4. Elevated urinary sodium excretion while on a normal salt and water intake. This criterion is included because of its utility in differentiating between hypoosmolality caused by a decreased EABV, in which case renal Na^+ conservation occurs, and distal dilution-induced disorders, in which urine Na^+ excretion is normal or increased secondary to ECF volume expansion. Patients with SIAD can have low urine Na^+ excretion if they subsequently become hypovolemic or solute depleted, conditions that sometimes follow severe salt and water restriction. Consequently, a high urine Na^+ excretion is the rule in most patients with SIAD; its presence does not guarantee this diagnosis, and its absence does not rule out the diagnosis.
5. Absence of other potential causes of euvolemic hypoosmolality. Most notably are hypothyroidism, hypocortisolism (Addison disease or pituitary ACTH insufficiency), and diuretic use.

Several other criteria support but are not essential for a diagnosis of SIAD. Volume expansion and vasopressin acting on V_1 receptors in the kidney increase the clearance of uric acid, so hypouricemia is found with SIAD. When patients are hyponatremic, values of uric acid are generally <4 mg/dL (<0.24 mmol/L).[261] A water-loading test is of value when there is uncertainty regarding the etiology of modest degrees of hypoosmolality in euvolemic patients, but it does not add useful information if the plasma osmolality is already under 275 mOsm/kg H_2O. Inability to excrete a standard water load normally (with normal excretion defined as a cumulative urine output of at least 90% of the administered water load within 4 hours and suppression of urine osmolality to <100 mOsm/kg H_2O) confirms the presence of an underlying defect in free water excretion. However, water excretion is abnormal in almost all disorders that cause hypoosmolality, whether dilutional or depletion induced with secondary impairments in free water excretion. Two exceptions are primary polydipsia, in which hypoosmolality can rarely be secondary to excessive water intake alone, and the reset osmostat variant of SIAD, in which normal excretion of a water load can occur once plasma osmolality falls below the new set-point for vasopressin secretion.



• **Fig. 10.6** Plasma arginine vasopressin (AVP) levels in patients with the syndrome of inappropriate antidiuretic hormone (SIADH) secretion as a function of plasma osmolality. Each point depicts one patient at a single point in time. The *shaded area* represents AVP levels in normal subjects over physiologic ranges of plasma osmolality. The lowest measurable plasma AVP level that could be detected with this radioimmunoassay was 0.5 pg/mL. (From Robertson GL, Aycinena P, Zerbe RL. Neurogenic disorders of osmoregulation. *Am J Med.* 1982;2:339–353.)

Another supportive criterion is an inappropriately elevated plasma vasopressin level in relation to plasma osmolality. However, several factors limit the utility of vasopressin measurements to diagnose SIAD. First, although plasma vasopressin levels are elevated in most patients with this syndrome, the elevations generally remain within the normal physiologic range and are abnormal only in relation to plasma osmolality (Fig. 10.6). Second, 10% to 20% of patients with SIAD do not have measurably elevated plasma vasopressin levels and are at the limits of detection by radioimmunoassay (see Fig. 10.6).[262] Third, and perhaps most importantly, most disorders causing solute and volume depletion or decreased EABV are associated with elevations of plasma vasopressin levels secondary to nonosmotic hemodynamic stimuli.

Etiology

Although the list of disorders associated with SIAD is long (Table 10.2), they can be divided into several major etiologic groups.

Tumors

The most common association of SIAD is with tumors. Many different types of tumors have been associated with SIAD, but bronchogenic carcinoma of the lung has been uniquely associated with SIAD since the first description of this disorder in 1957.[226] In virtually all cases, the bronchogenic carcinomas causing this syndrome have been of the small cell variety. Incidence

TABLE 10.2 Common Causes of the Syndrome of Inappropriate Antidiuretic Hormone (SIADH) Secretion

Tumors

Pulmonary/mediastinal (bronchogenic carcinoma, mesothelioma, thymoma)
Nonchest (duodenal carcinoma, pancreatic carcinoma, ureteral/prostate carcinoma, uterine carcinoma, nasopharyngeal carcinoma, leukemia)

Central Nervous System Disorders

Mass lesions (tumors, brain abscesses, subdural hematoma)
Inflammatory diseases (encephalitis, meningitis, systemic lupus erythematosus, acute intermittent porphyria, multiple sclerosis)
Degenerative/demyelinative diseases (Guillain-Barré syndrome, spinal cord lesions)
Miscellaneous (subarachnoid hemorrhage, head trauma, acute psychosis, delirium tremens, pituitary stalk section, transsphenoidal adenomectomy, hydrocephalus)

Drug Related

Stimulated release of AVP (nicotine, phenothiazines, tricyclics)
Direct renal effects or potentiation of AVP antidiuretic effects (dDAVP, oxytocin, prostaglandin synthesis inhibitors)
Mixed or uncertain actions (ACE inhibitors, carbamazepine and oxcarbazepine, chlorpropamide, clofibrate, clozapine, cyclophosphamide, 3,4-methylenedioxymethamphetamine [ecstasy], omeprazole; serotonin reuptake inhibitors, vincristine)

Pulmonary

Infections (tuberculosis, acute bacterial and viral pneumonia, aspergillosis, empyema)
Mechanical/ventilatory causes (acute respiratory failure, COPD, positive-pressure ventilation)

Other Causes

Acquired immunodeficiency syndrome (AIDS) and AIDS-related complex
Prolonged strenuous exercise (marathon, triathlon, ultramarathon, hot-weather hiking)
Senile atrophy
Idiopathic

ACE, Angiotensin-converting enzyme; *AVP,* arginine vasopressin; *COPD,* chronic obstructive pulmonary disease; *dDAVP,* desmopressin.

of hyponatremia is as high as 11% of all patients with small cell carcinoma,[263] or 33% of cases with more extensive disease,[264] have been reported. The high incidence of small cell carcinoma of the lung makes it imperative that all adult patients presenting with an otherwise unexplained SIAD be investigated thoroughly and aggressively for a possible lung tumor. Head and neck cancers account for another group of malignancies associated with relatively higher incidences of SIAD,[265] and some of these tumors have clearly been shown to synthesize vasopressin.[266] A report from a large cancer hospital showed an incidence of hyponatremia for all malignancies of 3.7%, with approximately one-third of these due to SIAD.[267]

Central Nervous System Disorders

A large number of different CNS disorders are associated with SIAD, but without a common denominator linking them. This is not surprising when one considers the neuroanatomy described earlier. Magnocellular vasopressin neurons receive excitatory inputs from osmoreceptive cells located in the anterior hypothalamus, but also a major innervation from brainstem cardiovascular regulatory and emetic centers. Although various components of these pathways have yet to be elucidated fully, many of them appear to have inhibitory as well as excitatory components. Consequently, any CNS disorder can potentially cause vasopressin hypersecretion either by nonspecifically exciting these pathways via irritative foci or alternatively by disrupting them and thereby decreasing the level of inhibition.

Drugs

Drug-induced hyponatremia is a common cause of hypoosmolality.[268] Table 10.2 lists some of the agents that have been associated with SIAD, but new drugs are added continually. Pharmacologic agents may stimulate secretion of vasopressin, activate renal V_2 receptors, or potentiate the antidiuretic effect of vasopressin. Not all of the drug effects are fully understood, and many appear to work through a combination of mechanisms. A particularly interesting and clinically important class of agents is the selective serotonin reuptake inhibitors (SSRIs). Hyponatremia following SSRI administration has been reported almost exclusively in the elderly, with rates as high as 22% to 28%, although in larger series the incidence was closer to 1 in 200.[269] A similar effect is likely also responsible for the recent reports of severe fatal hyponatremia caused by use of the recreational drug 3,4-methylenedioxymethamphetamine, "ecstasy," which possesses substantial serotonergic activity.[270]

Pulmonary Disorders

A variety of pulmonary disorders have been associated with this syndrome, but other than tuberculosis, acute pneumonia, and advanced chronic obstructive lung disease, the occurrence of hypoosmolality has been noted only sporadically. Hypoxia stimulates secretion of vasopressin in animals,[271] but in humans hypercarbia is more associated with abnormal water retention. Elevated vasopressin may be limited to the initial days of hospitalization, when respiratory failure is most marked. Therefore, with SIAD in nontumor pulmonary disease, the pulmonary disease is obvious with severe dyspnea or extensive radiographically evident infiltrates, and the inappropriate antidiuresis will usually be limited to the period of respiratory failure. Mechanical ventilation can cause SIAD via inappropriate secretion of vasopressin via decreased venous return.

Other Causes

In acquired immunodeficiency syndrome (AIDS) or AIDS-related complex (ARC) and in patients with human immunodeficiency virus (HIV) infection, hyponatremia has been reported as high as 30% to 38% in adults and children.[272] Although there are many potential etiologies, including dehydration, adrenal insufficiency, and pneumonitis, from 12% to 68% of AIDS patients who develop hyponatremia appear to meet criteria for a diagnosis of SIAD.[272] Not unexpectedly, some of the medications used to treat these patients may cause the hyponatremia, either via direct renal tubular toxicity or induced SIAD.[273]

Elderly patients often develop SIAD without any apparent underlying etiology, and the high incidence of hyponatremia in geriatric patients[231,274] suggests that the normal aging process may be accompanied by abnormalities of regulation of water balance and of secretion of vasopressin as noted earlier. Such an effect could potentially account for the fact that drug-induced hyponatremia occurs much more frequently in elderly patients. In a

series of 50 consecutive elderly patients meeting criteria for SIAD, 60% remained idiopathic despite rigorous evaluation, leading the authors to conclude that extensive diagnostic procedures were not warranted in such elderly patients if routine history, physical examination, and laboratory evaluation failed to suggest an underlying etiology.[275]

Pathophysiology

Sources of Vasopressin Secretion

Elevated plasma levels of vasopressin can be broadly divided into those associated with paraneoplastic ("ectopic") secretion of vasopressin or pituitary hypersecretion of vasopressin. There is substantial cumulative evidence that tumor tissue can in fact synthesize vasopressin,[276] but it is not certain that all tumors associated with SIAD do so because only about half of small cell carcinomas have been found to contain vasopressin immunoreactivity, and many of the tumors listed in Table 10.2 have not been carefully studied.

Pituitary Vasopressin Secretion—Inappropriate Versus Appropriate

In the majority of cases of SIAD, the vasopressin secretion originates from the posterior pituitary. This is also true of over 90% of all cases of hyponatremia, including patients with hypovolemic and hypervolemic hyponatremia.[234] This raises the question of what constitutes "inappropriate" secretion of vasopressin. Secretion of vasopressin in response to a hypovolemic stimulus is clearly physiologically "appropriate," but when it leads to symptomatic hyponatremia it could be considered inappropriate for the ECF osmolality. Despite these semantic conundrums, the diagnosis of SIAD should be based upon the original Schwartz-Bartter criteria and specifically exclude other clinical conditions that cause known impairments in free water excretion *even when* these are mediated by a secondary nonosmotic physiologic stimulation of vasopressin secretion. Without maintaining these distinctions, arguable as some may be, the definition of SIAD becomes too broad to retain any practical clinical utility.

Patterns of Vasopressin Secretion

Studies of plasma vasopressin levels in patients with SIAD during graded increases in plasma osmolality produced by hypertonic saline administration have defined four patterns of secretion: (1) random hypersecretion of vasopressin; (2) inappropriate nonsuppressible basal vasopressin release, but normal secretion in response to osmolar changes above basal plasma osmolality; (3) a reset osmostat system, whereby vasopressin is secreted at an abnormally low threshold of plasma osmolality but otherwise displays a normal response to relative changes in osmolality; and (4) low or even undetectable plasma vasopressin levels despite classic clinical characteristics of SIAD.[262] The pattern of SIAD that occurs without measurable vasopressin secretion is not yet well understood, but the positive response of one such patient to a vasopressin V_2-receptor antagonist would suggest that this may represent increased renal sensitivity to low circulating levels of vasopressin.[277] It is surprising that no correlation has been found between any of these patterns of secretion of vasopressin and the various etiologies of SIAD.[262] Recent studies of pediatric patients with hyponatremia and unmeasurable plasma vasopressin levels led to the discovery of an activating mutation of the vasopressin V_2 receptor as the cause of their inappropriate antidiuresis.[278] It is more appropriate to call these cases the *nephrogenic syndrome of inappropriate antidiuresis*, reserving SIAD only for those cases where measured plasma vasopressin levels are really inappropriate. Although the incidence of nephrogenic syndrome of inappropriate antidiuresis in the general population is unknown, the description of Belgian kindred with this mutation suggests that it can present later in life as well as in childhood.

Contribution of Natriuresis to the Hyponatremia of SIAD

Since the original cases studied by Schwartz and Bartter, increased renal Na^+ excretion has been one of the cardinal manifestations of SIAD, indeed one which later became embedded in the requirements for its diagnosis.[227] Demonstration that the natriuresis accompanying administration of antidiuretic hormone is not due to vasopressin itself but to the volume expansion produced as a result of water retention was unequivocally shown by Leaf and associates even before the description of the disorder.[279] Although a negative Na^+ balance occurs during the development of hyponatremia in patients with SIAD, eventually urinary sodium excretion simply reflects daily sodium intake.[226] Thus the term *renal sodium wasting* is used to describe continued excretion of sodium despite being hyponatremic, but in reality there is a new steady state in which patients are in neutral sodium balance. Studies of long-term antidiuretic-induced hyponatremia in both dogs and rats have indicated that a large proportion of the hyponatremia was attributable to secondary Na^+ losses rather than to water retention,[255,256] but the natriuresis did not actually worsen the hyponatremia; rather, it allowed volume regulation of ECF.[280] Secondary natriuresis in patients with SIAD likely explains the failure to find expanded plasma or ECF volumes using tracer dilution techniques (see Fig. 10.5).[257]

Cerebral Salt Wasting

The degree to which hyponatremia might occur primarily as a result of primary natriuresis is controversial. Cerebral salt wasting syndrome (CSWS) was proposed by Peters and associates in 1950[281] as an explanation for the natriuresis and hyponatremia that sometimes accompanies intracranial disease, particularly subarachnoid hemorrhage in which up to one-third of patients often develop hyponatremia. After the description of SIAD in 1957, such patients were generally assumed to have hyponatremia secondary to vasopressin hypersecretion with a secondary natriuresis. However, over the last decade clinical and experimental data have been interpreted to indicate that some patients with subarachnoid hemorrhage and other intracranial diseases indeed have a primary natriuresis leading to volume contraction rather than SIAD,[282,283] and the elevated plasma vasopressin levels may be physiologically appropriate for the degree of volume contraction. With regard to the potential mechanisms of natriuresis, both plasma and CSF levels of atrial natriuretic peptide are elevated in many patients with subarachnoid hemorrhage and have been found to correlate variably with hyponatremia in patients with intracranial diseases.[284] However, clearly documented SIAD also is frequently associated with elevated plasma levels of atrial natriuretic peptide, so this finding does not prove causality. In other disorders of hyponatremia due to Na^+ wasting (e.g., Addison disease) and diuretic-induced hyponatremia, infusion of saline restores normal ECF volume and plasma tonicity by shutting off the secondary vasopressin secretion. In subarachnoid hemorrhage, however, large volumes of isotonic saline sufficient to maintain plasma volume did not change the incidence of hyponatremia.[285]

Those authors who have distinguished CSWS from SIAD have emphasized that in CSWS the primary disorder, salt

wasting, produces convincing evidence of decreased extracellular fluid volume.[286,287] There are only a few case reports of patients after traumatic brain injury or neurosurgery who while being observed in the hospital have acute onset of massive diuresis and natriuresis with clear evidence of volume contraction by weight loss, decreased central venous pressure, increased blood urea nitrogen (BUN), or increased hematocrit. Most of these cases have been in children[288,289] and have responded to replacement with normal or hypertonic saline, but concurrent treatment with fludrocortisone has also been advocated.[288,290] However, a recent study of 100 consecutive adult patients with acute nontraumatic aneurysmal subarachnoid hemorrhage found that the cause of the hyponatremia was attributable to SIAD in 71.4% and acute glucocorticoid deficiency in 8.2%, with the remaining cases caused by incorrect intravenous fluid administration or hypovolemia. Most significantly, no cases were found that met historically accepted criteria for a diagnosis of CSWS.[112] This suggests that CSWS is an exceedingly rare cause of the hyponatremia with intracranial disorders.

Renal Escape From Antidiuresis

In addition to excreting osmoles to bring volumes back toward normal, there are intrarenal adaptations that allow excretion of more water. Chronic stimulation by vasopressin in SIAD produces dramatic increases of aquaporin 2 content and insertion into the collecting duct principal cell membranes, which increases the efficiency of water retention and aggravates the pathology. However, the induced volume expansion and hypotonicity act on the tubular cells of the collecting duct to decrease the content and action of aquaporin 2 substantially, thus decreasing the amount of water resorbed in spite of high vasopressin levels. Experimental studies have suggested this may be due to downregulation of vasopressin V_2-receptor expression in the kidney.[291] This renal "escape" therefore represents another (in addition to natriuresis) adaptation that allows patients with persistent SIAD to come into a new steady state of Na^+ and water balance despite low serum sodium concentrations.[292]

Hypoosmolar Symptoms, Morbidity, and Mortality

Regardless of the etiology of hypoosmolality, most clinical manifestations are similar. Nonneurologic symptoms are relatively uncommon, although a number of cases of rhabdomyolysis have been reported presumably secondary to osmotically induced swelling of muscle fibers. Hypoosmolality is primarily associated with a broad spectrum of neurologic manifestations ranging from mild nonspecific symptoms (e.g., headache, nausea) to more significant disorders (e.g., disorientation, confusion, obtundation, focal neurologic deficits, and seizures).[293] This neurologic symptom complex has been termed "hyponatremic encephalopathy"[294] and primarily reflects brain edema resulting from osmotic water shifts into the brain because of decreased effective plasma osmolality. Significant neurologic symptoms generally do not occur until serum $[Na^+]$ falls below 125 mmol/L, and the severity of symptoms are roughly correlated with the degree of hypoosmolality.[293,295] However, individual variability is marked, and for any single patient the level of serum $[Na^+]$ at which symptoms appear cannot be predicted. The rate of fall of serum $[Na^+]$ is often more strongly correlated with morbidity and mortality than is the actual magnitude of the decrease.[293,295] This is due to the fact that the volume-adaptation process takes a finite period of time to complete, and the more rapid the fall in serum $[Na^+]$ the more brain edema will be accumulated before the brain is able to volume regulate. Thus there is a much higher incidence of neurologic symptoms as well as a higher mortality in patients with acute hyponatremia than in those with chronic hyponatremia.[293] For example, the most dramatic cases of death due to hyponatremic encephalopathy have generally been reported in postoperative patients in whom hyponatremia develops rapidly as a result of intravenous infusion of hypotonic fluids.[293,296] In such cases nausea and vomiting are frequently overlooked as potential early signs of increased intracranial pressure. Critically ill patients with unexplained seizures also should be immediately evaluated for possible hyponatremia, since as many as one-third of such patients have a serum $[Na^+]$ below 125 mEq/L as the cause of the seizure activity.[297] Underlying neurologic disease and nonneurologic metabolic disorders (e.g., hypoxia,[298] acidosis, hypercalcemia) can raise the level of plasma osmolality at which CNS symptoms occur.

In the most severe cases of hyponatremic encephalopathy, death results from respiratory failure after tentorial cerebral herniation and brainstem compression. One-quarter of patients with severe postoperative hyponatremic encephalopathy manifested hypercapneic respiratory failure, the expected result of brainstem compression, but three-quarters had pulmonary edema as the apparent cause of the hypoxia.[299] Studies of acute hyponatremia after marathon races have shown hypoxia and pulmonary edema in association with brain edema.[300] These results suggest the possibility that hypoxia from noncardiogenic pulmonary edema may represent an early sign of developing cerebral edema even before brainstem compression and tentorial herniation. Clinical studies have suggested that menstruating women[296] and young children[301] may be particularly susceptible to the development of neurologic morbidity and mortality during hyponatremia, especially in the acute postoperative setting.[294] However, other studies have failed to corroborate these findings.[302,303]

Although some studies have suggested that hyponatremia is an indicator of severe underlying disease and poor prognosis rather than a cause of the increased mortality in such patients, a meta-analysis of studies in which some patients had correction of their hyponatremia indicated that improvement in serum $[Na^+]$ was associated with a 50% reduction in mortality of the corrected groups compared to patients in whom the hyponatremia remained uncorrected, suggesting that hyponatremia may in fact be causally related to increased mortality.[304] Recent studies have further indicated that even mild hyponatremia is an independent predictor of higher mortality across a wide variety of disorders, including patients with acute ST-elevation myocardial infarction, heart failure, and liver disease.[228,235]A large study of over 55,000 electronic heath records from a single Boston hospital showed that the association of hyponatremia with inpatient mortality was significant across all levels of hyponatremia and even began at serum $[Na^+]$ levels in the lower part of the normal range.[229] These findings were corroborated in studies of 249,000 Danish patients hospitalized over a 5-year period that showed increased 30-day and 1-year mortality associated with all levels of hyponatremia, including the range of 130 to 134.9 mmol/L,[305] and analyses of 2.3 million hospitalized patients enrolled in the Cerner Health Facts database.[306] The mortality associated with chronic hyponatremia is much less well studied, but results of the Rotterdam Longitudinal Aging Study noted significantly decreased survival of elderly patients with hyponatremia over a 12-year period of observation.[307]

Once the brain has volume regulated via solute losses, thereby reducing brain edema, neurologic symptoms are not as prominent and may even be virtually absent. This accounts for the fairly common finding of relatively asymptomatic patients even with severe levels of hyponatremia.[295,308] Despite this powerful adaptation process, chronic hyponatremia is frequently associated with neurocognitive symptomatology, albeit milder and more subtle in nature, such as headaches, nausea, mood disturbances, depression, difficulty concentrating, slowed reaction times, unstable gait, increased falls, confusion, and disorientation.[309,310] Even in patients adjudged to be "asymptomatic" by virtue of a normal neurologic examination, accumulating evidence suggests that there may be previously unrecognized adverse effects as a result of chronic hyponatremia. In one study, 16 patients with hyponatremia secondary to SIAD, in the range of 124 to 130 mmol/L, demonstrated a significant gait instability that normalized after correction of the hyponatremia to a normal range.[309] The functional significance of the gait instability was illustrated in a study of 122 Belgian patients with a variety of levels of hyponatremia all judged to be asymptomatic at the time of visit to an emergency department (ED). These patients were compared with 244 age-matched, gender-matched, and disease-matched controls also presenting to the ED during the same time period. Researchers found that 21% of the hyponatremic patients came to the ED because of a recent fall, compared to only 5% of the controls; this difference was highly significant and remained so after multivariable adjustment.[309] Analogous results were found in a study of admissions to a US hospital geriatric trauma unit over a 3-year period; when patients admitted because of a fall (n = 1841) were analyzed for risk factors associated with fall, the odds ratio for serum [Na^+] below 135 mmol/L was 1.81 (p <0.001), which was greater than all other risk factors except age over 85 years.[311] Consequently, these studies clearly document an increased incidence of falls in so-called asymptomatic hyponatremic patients. Recent studies in both experimental animals[312] and humans[313] have demonstrated decreases in nerve conduction associated with hyponatremia as a potential cause of the gait disturbances.

The clinical significance of the gait instability and fall data have been indicated by multiple independent international studies that have demonstrated increased rates of bone fractures in patients with hyponatremia.[307,314–316] Other studies have shown that hyponatremia is associated with increased bone loss in experimental animals and a significantly increased odds ratio for osteoporosis of the femoral neck in those older than 50 years in the Third National Health and Nutrition Examination Survey (NHANES III) database.[317] These findings have been corroborated by multiple epidemiologic studies that have demonstrated decreased bone mineral density in human subjects.[318,319] In the largest epidemiologic analysis to date (2.9 million independent electronic health records), the odds ratios for osteoporosis and fractures were significantly greater for hyponatremia (3.99 and 3.05, respectively) than for any other diseases or medications associated with increased bone loss and fracture risk.[320] Of particular note, the odds ratios for both osteoporosis and fractures were the highest in patients with chronic persistent hyponatremia, indicating that duration of hyponatremia represents an important risk factor for bone disease and fractures.[320] These findings were supported by a subsequent clinical study of hip fractures in Argentina.[321]

Recent studies of a cohort of 5435 community dwelling men over the age of 65 in the United States have found that hyponatremia is independently associated with greater cognitive impairment and cognitive decline in this population,[322] and a retrospective study of 4900 hyponatremic patients in Taiwan found that the hyponatremic patients had a 2.36-fold higher hazard ratio of developing dementias compared to matched controls, which increased to a hazard ratio of 4.29 for patients with more severe hyponatremia. Thus the major clinical significance of chronic hyponatremia may lie in the increased morbidity and mortality associated with falls, fractures, neurocognitive impairments, and dementias in our older population as well as potential adverse effects not yet studied in humans.[323]

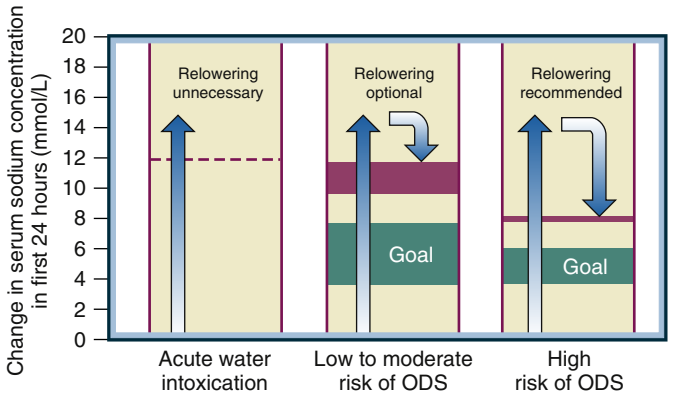
Therapy of SIAD and Other Hypoosmolar Disorders

General Principles

Correction of hyponatremia is associated with markedly improved neurologic outcomes in patients with severely symptomatic hyponatremia. In a retrospective review of patients who presented with severe neurologic symptoms and serum [Na^+] below 125 mmol/L, prompt therapy with isotonic or hypertonic saline resulted in a correction in the range of 20 mEq/L over several days and neurologic recovery in almost all cases; in contrast, in patients who were treated with fluid restriction alone, there was very little correction over the study period (<5 mmol/L over 72 hours), and the neurologic outcomes were much worse, with most of these patients either dying or entering a persistently vegetative state.[324] Based on this and similar retrospective analyses, prompt therapy to rapidly increase the serum [Na^+] represents the standard of care for treatment of patients presenting with severe symptoms of hyponatremia.

Brain herniation, the most dreaded complication of hyponatremia, is seen almost exclusively in patients with acute hyponatremia (usually <24 hours) or in patients with intracranial pathology.[325] In postoperative patients and in patients with self-induced water intoxication associated with marathon running, psychosis, or use of "ecstasy" (3,4-methylenedioxy-N-methamphetamine [MDMA]), nonspecific symptoms such as headache, nausea and vomiting, or confusion can rapidly progress to seizures, respiratory arrest, and ultimately death or a permanent vegetative state as a complication of cerebral edema.[232] Hypoxia from noncardiogenic pulmonary edema and/or hypoventilation can exacerbate brain swelling caused by the low serum [Na^+].[299,300] Although usually self-limited, hyponatremic seizures may be refractory to anticonvulsants.

As discussed earlier, chronic hyponatremia is much less symptomatic as a result of the process of brain volume regulation. Because of this adaptation process, chronic hyponatremia is arguably a condition that clinicians feel less concerned about, which has been reinforced by the common usage of the descriptor *asymptomatic hyponatremia* for many such patients. However, as discussed, it is clear that many such patients very often have neurologic symptoms, even if milder and more subtle in nature. Consequently, all patients with hyponatremia who manifest any neurologic symptoms that could possibly be related to the hyponatremia should be considered candidates for treatment of the hyponatremia, regardless of the chronicity of the hyponatremia or the level of serum [Na^+]. An additional reason to treat even asymptomatic hyponatremia effectively is to prevent a lowering of the serum [Na^+] to more symptomatic and dangerous levels during treatment of underlying conditions (e.g., increased fluid administration via parenteral nutrition, treatment of heart failure with diuretics).



• **Fig. 10.7** Recommended goals *(green)* and limits *(red)* for correction of hyponatremia based on risk of producing ODS and recommendations for relowering of serum sodium concentration ($[Na^+]$) to goals for patients presenting with serum $[Na^+]$ lower than 120 mmol/L who exceed the recommended limits of correction in the first 24 hours. *ODS,* osmotic demyelination syndrome. (From Verbalis JG, Goldsmith SR, Greenberg A, et al. Diagnosis, evaluation, and treatment of hyponatremia: expert panel recommendations. *Am J Med.* 2013;126:S1–S42.)

Therapies for Treatment of Hyponatremia

Conventional management strategies for hyponatremia range from saline infusion and fluid restriction to pharmacologic measures to adjust fluid balance. Although the number of available treatments for hyponatremia is large, some are not appropriate for correction of symptomatic hyponatremia because they work too slowly or inconsistently to be effective in hospitalized patients (e.g., demeclocycline, mineralocorticoids). Consideration of treatment options should always include an evaluation of the benefits as well as the potential toxicities of any therapy and must be individualized for each patient.[310,326] For all therapies, careful attention should be paid to recommendations for goals and limits of correction of the serum $[Na^+]$ to reduce the risk of the osmotic demyelination syndrome (ODS)[310] (Fig. 10.7). It should also be remembered that sometimes simply stopping treatment with an agent that is associated with causing hyponatremia is sufficient to correct a low serum $[Na^+]$.

Hypertonic Saline

Acute hyponatremia presenting with severe neurologic symptoms is life threatening and should be treated promptly with hypertonic solutions, typically 3% NaCl ($[Na^+]$ = 513 mmol/L), as this represents the most reliable method to quickly raise the serum $[Na^+]$. A continuous infusion of hypertonic NaCl is usually utilized in inpatient settings. Various formulae have been suggested for calculating the initial rate of infusion of hypertonic solutions,[325] but until now there has been no consensus regarding optimal infusion rates of 3% NaCl. One of the simplest methods to estimate an initial 3% NaCl infusion rate utilizes the following relationship[310]:

$$\text{Patient's weight (kg)} \times \text{desired correction rate (mEq/L/hr)} = \text{infusion rate of 3\% NaCl (mL/hr)}$$

This may not achieve the desired correction rate, but frequent monitoring of the serum $[Na^+]$ will inform the clinician whether the rate should be increased or decreased, similar to using measurement of serum glucose to guide the infusion rate of insulin drips. Depending on individual hospital policies, the administration of hypertonic solutions may require special considerations (e.g., placement in the intensive care unit [ICU], sign-off by a consultant), which each clinician needs to take into account to optimize patient care. One barrier to the use of hypertonic saline that appears to be overstated and unfounded is the frequent requirement for a central intravenous catheter for chronic infusion. A recent study demonstrated a low rate of complications using peripheral infusions of 3% NaCl (6% infiltration and 3% thrombophlebitis) and concluded that peripheral administration of 3% NaCl carries a low risk of minor nonlimb or life-threatening complications.[327]

An alternative option for more emergent situations is administration of a 100-mL bolus of 3% NaCl, repeated twice if there is no clinical improvement in 30 minutes, which has been recommended by a consensus conference organized to develop guidelines for the prevention and treatment of exercise-induced hyponatremia[328] and adopted as a general recommendation by several expert panels.[310,329] Injecting this amount of hypertonic saline intravenously raises the serum $[Na^+]$ by an average of 2 to 4 mmol/L, which is well below the recommended maximal daily rate of change of 10 to 12 mmol/L/24 hours or 8 mmol/L/24 hours for patients with increased risk factors for ODS (serum $[Na^+] \leq 105$ mmol/L, hypokalemia, advanced liver disease, malnutrition, or a history of alcoholism)[310,330] (see Fig. 10.7). Because the adult brain can only accommodate an average increase of approximately 8% in brain volume before herniation occurs, quickly increasing the serum $[Na^+]$ by as little as 2 to 4 mmol/L in acute hyponatremia can effectively reduce brain swelling and intracranial pressure.[331]

Isotonic Saline

The treatment of choice for depletional hyponatremia (i.e., hypovolemic hyponatremia) is isotonic saline ($[Na^+]$ = 154 mmol/L) to restore ECF volume and ensure adequate organ perfusion. This initial therapy is appropriate for patients who either have clinical signs of hypovolemia or in whom a spot urine $[Na^+]$ concentration is below 20 to 30 mEq/L.[310] Such patients often develop a free water diuresis (aquaresis) as their ECF volume is corrected, potentially leading to an overly rapid correction with increased risk of ODS, so the serum $[Na^+]$ and urine output should be followed carefully during the first 24 to 48 hours of therapy. However, isotonic saline is ineffective for dilutional hyponatremias such as SIAD,[226] and continued administration of isotonic saline to a euvolemic patient may worsen their hyponatremia[332] and/or cause fluid overload. Although saline may improve the serum $[Na^+]$ in some patients with hypervolemic hyponatremia, their volume status will generally worsen with this therapy, so unless the hyponatremia is profound both hypertonic and isotonic saline should be avoided.

Fluid Restriction

For patients with chronic hyponatremia, fluid restriction has been the most popular and most widely accepted treatment. When SIAD is present, fluids should generally be limited to 500 to 1000 mL/24 hours. Because fluid restriction increases the serum $[Na^+]$ by underreplacing the excretion of fluid by the kidneys, some have advocated an initial restriction to 500 mL less than the 24-hour urine output.[333] When instituting a fluid restriction, it is important for the nursing staff and the patient to understand that this includes all fluids that are consumed, not just water (Table 10.3). Generally the water content of ingested food is not included in the restriction because this is balanced by insensible water losses

TABLE 10.3 General Recommendations for Use of Fluid Restriction and Predictors of the Increased Likelihood of Failure of Fluid Restriction

General Recommendations

- Restrict *all* intake that is consumed by drinking, not just water.
- Aim for a fluid restriction that is 500 mL/day *below* the 24-hour urine volume.
- Do *not* restrict sodium or protein intake unless indicated.

Predictors of the Likely Failure of Fluid Restriction

- High urine osmolality (≥500 mOsm/kg H_2O).
- Sum of the urine [Na^+] and [K^+] concentrations exceeds the serum [Na^+] concentration.
- 24-hour urine volume <1500 mL/day.
- Increase in serum [Na^+] concentration <2 mmol/L/day in 24–48 hours on a fluid restriction of ≤1 L/day.

(perspiration, exhaled air, feces, etc.), but caution should be exercised with foods that have high fluid concentrations (such as fruits and soups). Restricting fluid intake can be effective when properly applied and managed in selected patients, but serum [Na^+] is generally increased only slowly (1–2 mmol/L/day) even with severe fluid restriction.[226] In addition, this therapy is often poorly tolerated because of an associated increase in thirst leading to poor compliance with long-term therapy.

Fluid restriction should not be used with hypovolemic patients and is particularly difficult to maintain in hospitalized patients with very elevated urine osmolalities secondary to high vasopressin levels; similarly, if the sum of urine [Na^+] and [K^+] exceeds the serum [Na^+], most patients will not respond to a fluid restriction since an electrolyte-free water clearance will be difficult to achieve.[334,335] These and other known predictors of failure of fluid restriction are summarized in Table 10.3; the presence of any of these factors in hospitalized patients with symptomatic hyponatremia make this therapy less than ideal. In addition, fluid restriction is not practical for some patients, particularly including patients in intensive care settings who often require administration of significant volumes of fluids as part of their therapies. Such patients are candidates for more effective pharmacologic or saline treatment strategies.

Arginine Vasopressin Receptor Antagonists

Conventional therapies for hyponatremia, although effective in specific circumstances, are suboptimal for many different reasons, including variable efficacy, slow responses, intolerable side effects, and serious toxicities. But perhaps the greatest deficiency of most conventional therapies is that they do not directly target the underlying cause of most dilutional hyponatremias, namely inappropriately elevated plasma vasopressin levels. A new class of pharmacologic agents (vasopressin receptor antagonists [vaptans]) that directly block vasopressin-mediated receptor activation have been approved for the treatment of euvolemic hyponatremia and hypervolemic hyponatremia in many countries.[326,336]

Conivaptan has been approved by the US Food and Drug Administration (FDA) for euvolemic and hypervolemic hyponatremia in hospitalized patients. It is available only as an intravenous preparation and is given as a 20-mg loading dose over 30 minutes, followed by a continuous infusion of 20 or 40 mg/day. Generally, the 20-mg continuous infusion is used for the first 24 hours to gauge the initial response. If the correction of serum [Na^+] is thought to be inadequate (e.g., <5 mmol/L), the infusion rate can be increased to 40 mg/day. Clinical studies have supported the efficacy of bolus infusions of conivaptan rather than continuous infusions.[337] Therapy is limited to a maximum duration of 4 days because of drug interaction effects with other agents metabolized by the CYP3A4 hepatic isoenzyme. Importantly, for conivaptan and all other vaptans, it is critical that the serum [Na^+] be measured frequently during the active phase of correction of the hyponatremia—a minimum of every 6 to 8 hours for conivaptan but more frequently in patients with risk factors for the ODS.[310] If the correction exceeds 10 to 12 mmol/L in the first 24 hours, the infusion should be stopped and the patient monitored closely. Consideration should be given to administering sufficient water, orally or as intravenous 5% dextrose in water, to avoid a correction of more than 12 mmol/L/day. The maximum correction limit should be reduced to 8 mmol/L over the first 24 hours in patients with risk factors for ODS[310] (see Fig. 10.7). The most common side effects of conivaptan include headache, thirst, and hypokalemia.[338]

Tolvaptan, an oral vasopressin receptor antagonist, is also FDA approved for the treatment of euvolemic and hypervolemic hyponatremia. In contrast to conivaptan, the availability of tolvaptan in tablet form allows short-term and long-term use.[339] Similar to conivaptan, tolvaptan treatment must be initiated in the hospital so that the rate of correction can be monitored carefully. In the United States, patients with a serum [Na^+] lower than 125 mmol/L are eligible for therapy with tolvaptan as primary therapy; if the serum [Na^+] is 125 mmol/L or higher, tolvaptan therapy is only indicated if the patient has symptoms that could be attributable to the hyponatremia and is resistant to attempts at fluid restriction.[340] In the European Union, tolvaptan is approved only for the treatment of euvolemic hyponatremia due to SIAD, but any symptomatic euvolemic patient is eligible for tolvaptan therapy regardless of the level of hyponatremia or response to previous fluid restriction. The starting dose of tolvaptan is 15 mg on the first day, although in clinical practice some clinicians recommend starting with a lower dose of 7.5 mg,[341] and the dose can be titrated to 30 and 60 mg at 24-hour intervals if the serum [Na^+] remains lower than 135 mmol/L or the increase in serum [Na^+] has been less than 5 mmol/L in the previous 24 hours. As with conivaptan, it is essential that the serum [Na^+] be measured frequently during the active phase of correction of the hyponatremia at a minimum of every 6 to 8 hours, particularly in patients with risk factors for ODS. Goals and limits for the safe correction of hyponatremia and methods to compensate for overly rapid corrections are the same as described previously for conivaptan (see Fig. 10.7). One additional factor that helps avoid overly rapid correction with tolvaptan is the recommendation that fluid restriction should not be used during the active phase of correction, thereby allowing the patient's thirst to compensate for an overly vigorous aquaresis. Common side effects of tolvaptan include dry mouth, thirst, increased urinary frequency, dizziness, nausea, and orthostatic hypotension.[340]

Vaptans are not needed in the treatment of hypovolemic hyponatremia because simple volume expansion would be expected to abolish the nonosmotic stimulus to AVP secretion and lead to a prompt aquaresis. Furthermore, inducing increased renal fluid excretion via diuresis or aquaresis can cause or worsen hypotension in such patients. This possibility has resulted in the labeling of these drugs as contraindicated for hypovolemic hyponatremia. Importantly, clinically significant hypotension was not observed in the conivaptan or tolvaptan clinical trials in euvolemic and

hypervolemic hyponatremic patients. Although vaptans are not contraindicated with decreased renal function, these agents generally will not be effective if the serum creatinine level is more than 3 mg/dL.

The FDA has issued a caution about hepatic injury that was noted in patients who received tolvaptan in a 3-year clinical trial examining the effect of tolvaptan on autosomal dominant polycystic kidney disease, the Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and Its Outcomes (TEMPO) study.[342] Although the doses used in the TEMPO study were up to twice the maximum dose approved for hyponatremia (e.g., tolvaptan, 120 mg/day) and in clinical trials of tolvaptan at doses approved by the FDA for treatment of clinically significant euvolemic or hypervolemic hyponatremia liver damage was not reported, the FDA recommended that "Samsca treatment should be stopped if the patient develops signs of liver disease. Treatment duration should be limited to 30 days or less, and use should be avoided in patients with underlying liver disease, including cirrhosis." The European Medicines Agency (EMA) has approved the use of tolvaptan for SIAD but not for hyponatremia due to heart failure or cirrhosis. Based on the TEMPO trial results, the EMA also issued a warning about the possible occurrence of hepatic injury in patients treated with tolvaptan but did not recommend any restriction on the duration of treatment of SIAD patients with tolvaptan. Accordingly, appropriate caution should be exercised in patients treated with tolvaptan for hyponatremia for extended periods (e.g., >30 days), but this decision should be based on the clinical judgment of the treating physician. Patients who are refractory to or unable to tolerate or obtain other therapies for hyponatremia, and for whom the benefit of tolvaptan treatment outweighs the risks, remain candidates for long-term therapy with tolvaptan.

An additional barrier to the use of vasopressin antagonists for treatment of hyponatremia is the high cost of the drug. This is true in the United States and the European Union but interestingly not in Asian countries. Despite this pronounced geographic disparity, many economic analyses have confirmed the increased economic burden of hyponatremia, which is largely driven by longer hospital and intensive care stays.[343,344] An analysis of use of tolvaptan compared with fluid restriction showed a favorable cost savings that offset the high cost of tolvaptan, suggesting that selective use of these agents in appropriate inpatients may in fact be cost effective in hospitalized patients in the United States and the European Union.[345]

Urea

Urea has been described as an alternative oral treatment for SIAD and other hyponatremic disorders. The mode of action is to correct hypoosmolality not only by increasing solute-free water excretion but also by decreasing urinary sodium excretion. Doses of 15 to 60 g/day are generally effective; the dose can be titrated in increments of 15 g/day at weekly intervals as necessary to achieve normalization of the serum [Na^+]. It is advisable to dissolve the urea in orange juice or some other strongly flavored liquid to camouflage the bitter taste. Even if completely normal water balance is not achieved, it is often possible to allow the patient to maintain a less strict regimen of fluid restriction while receiving urea. The disadvantages associated with the use of urea include poor palatability, the development of azotemia at higher doses, and the unavailability of a convenient or FDA-approved form of the agent. Data suggest that blood urea concentrations may double during treatment,[346] but it is important to remember that this does not represent renal impairment.

Reports of retrospective, uncontrolled studies suggest that the use of urea has been effective in treating SIAD in patients with hyponatremia due to subarachnoid hemorrhage and in critical care patients,[347] and case reports have documented success in infants with chronic SIAD[348] and the nephrogenic syndrome of inappropriate antidiuresis.[349] More recent evidence from a short study in a small cohort of SIAD patients suggests that urea may have a comparable efficacy to vaptans in reversing hyponatremia due to chronic SIAD.[350] Although these reports suggest that urea might be an acceptable alternative for treatment of chronic hyponatremia, data regarding efficacy and safety of long-term urea treatment of hyponatremia are lacking.[351]

Furosemide and NaCl

The use of furosemide (20–40 mg/day) coupled with a high sodium intake (200 mEq/day) represents an extension of the treatment of acute symptomatic hyponatremia[352] in selected cases.[329,353] However, similar to urea, the efficacy of this approach to correct symptomatic hyponatremia promptly and within accepted goals limits is unknown.

Efficacy of Hyponatremia Treatment

There have been no adequately powered randomized controlled trials to compare the efficacy and safety of different treatments utilized to correct hyponatremia. However, results of a prospective observational study in a large number of hospitalized patients in the United States and the European Union provide useful data about the success rates of different therapies in euvolemic hyponatremic patients.[354,355] In this study, success was defined by three different criteria, from least to most stringent: (1) an increase in serum [Na^+] of at least 5 mmol/L, (2) correction to a serum [Na^+] of 130 mmol/L or above, and (3) correction to a normal serum [Na^+] of 135 mmol/L or above. Only 3% NaCl and tolvaptan had success rates significantly greater than 50% for the least stringent criterion, and only tolvaptan achieved this level for the next most stringent criteria and a significantly higher rate for the most stringent criteria of normalization of the serum [Na^+]. Of particular note, fluid restriction, the most frequently prescribed therapy in the Hyponatremia Registry patients, achieved a correction of serum [Na^+] in only 44% of patients treated with this therapy and isotonic saline in only 36% of patients. This is consistent with a prospective study of 183 hyponatremic patients that demonstrated that up to 60% of patients with SIAD had one or more criteria predicting nonresponse to fluid restriction (see Table 10.3).[356] These data underscore the importance of carefully selecting therapy for individual patients to meet predefined goals for correction of serum [Na^+].

Hyponatremia Treatment Guidelines Based on Symptom Severity

Although many authors have published recommendations on the treatment of hyponatremia,[310,329,357–361] no standardized treatment algorithms have yet been universally accepted, and some major differences between the various guidelines and expert recommendations exist.[326,362] For almost all treatment recommendations, the initial evaluation includes an assessment of the ECF volume status of the patient because treatment recommendations differ for hypovolemic, euvolemic, and hypervolemic hyponatremic patients.[310] Euvolemic patients, mainly including those with SIAD, represent a unique challenge because of the multiplicity

of causes and presentations of patients with SIAD. Recent expert opinion recommendations are based primarily on the neurologic symptomatology of hyponatremic patients rather than the serum [Na^+] or the chronicity of the hyponatremia, because the latter is often difficult to ascertain accurately.[310] A careful neurologic history and assessment should always be conducted to identify potential causes for the patient's symptoms other than hyponatremia, although it will not always be possible to exclude an additive contribution from the hyponatremia to an underlying neurologic condition. In this algorithm, patients are divided into three major groups based on their presenting symptoms.

Severe Symptoms. Coma, obtundation, seizures, respiratory distress or arrest, and unexplained vomiting usually imply a more acute onset or worsening of hyponatremia that requires immediate active treatment. Therapies that will quickly raise serum [Na^+] are necessary to reduce cerebral edema and decrease the risk of potentially fatal brain herniation.

Moderate Symptoms. Altered mental status, disorientation, confusion, unexplained nausea, gait instability, and falls generally indicate some degree of brain volume regulation and absence of clinically significant cerebral edema. These symptoms can be chronic or acute, but allow more time to elaborate a deliberate approach to the choice of therapies.

Mild or Absent Symptoms. Minimal symptoms, such as difficulty concentrating, irritability, altered mood, depression, or unexplained headache, or a virtual absence of discernible symptoms, indicate that the patient may have chronic or slowly evolving hyponatremia. These symptoms necessitate a cautious approach, especially when patients have underlying comorbidities, to prevent both worsening of the hyponatremia and overly rapid correction with production of ODS.

Patients with severe neurologic symptoms should be treated with hypertonic (3%) NaCl as first-line therapy, followed after 24 to 48 hours by fluid restriction and/or vaptan therapy. Because overly rapid correction of serum [Na^+] occurs in more than 10% of patients treated with hypertonic NaCl,[363] such patients are at risk for ODS unless carefully monitored. For this reason, some authors have proposed simultaneous treatment with desmopressin to reduce the rate of correction to only that produced by the hypertonic NaCl infusion itself.[364] Whether sufficient clinical data eventually prove that this approach is effective and safe in larger numbers of patients remains to be determined.[365,366] Only one case of ODS has been reported in a patient receiving vaptan monotherapy,[367] and two abstracts have reported ODS when vaptans were used directly following hypertonic saline administration within the same 24-hour period. Consequently, no additional active hyponatremia therapy should be administered until at least 24 hours following successful increases in serum [Na^+] using hypertonic NaCl.

The choice of treatment for patients with moderate symptoms will depend on their ECF volume status. Hypovolemic patients should be treated with solute repletion via isotonic NaCl infusion or oral sodium replacement. Euvolemic patients, typically with SIAD, will benefit from vaptan therapy, limited hypertonic saline administration, or in some cases urea (where available). This can be followed by fluid restriction or long-term vaptan therapy when the cause of the SIAD is expected to be chronic. In hypervolemic patients with heart failure, vaptans are usually the best choice because fluid restriction is rarely successful in this group,[368] saline administration can cause fluid retention with increased edema, and urea can lead to ammonia buildup in the GI tract if hepatic function is impaired. Although moderate neurologic symptoms can indicate that a patient is in an early stage of acute hyponatremia, they more often indicate a chronically hyponatremic state with sufficient brain volume adaptation to prevent marked symptomatology from cerebral edema. Most patients with moderate hyponatremic symptoms have a more chronic form of hyponatremia, so guidelines for goals and limits of correction should be followed closely (see Fig. 10.7), and close monitoring of these patients in a hospital setting is warranted until the symptoms improve or stabilize.

Patients with no or minimal symptoms should be managed initially with fluid restriction, although treatment with pharmacologic therapy, such as vaptans or urea, may be appropriate for a wide range of specific clinical conditions. Foremost of these is a failure to improve the serum [Na^+], despite reasonable attempts at fluid restriction, or the presence of clinical characteristics associated with poor responses to fluid restriction (see Table 10.3).

A special case is when spontaneous correction of hyponatremia occurs at an undesirably rapid rate because of the onset of water diuresis. This can occur following cessation of desmopressin therapy in a patient who has become hyponatremic, replacement of glucocorticoids in a patient with adrenal insufficiency, replacement of solutes in a patient with diuretic-induced hyponatremia, or spontaneous resolution of transient SIAD. Brain damage from ODS can clearly ensue in this setting if the preceding period of hyponatremia has been long enough (usually ≥48 hours) to allow brain volume regulation to occur. If the correction parameters discussed earlier have been exceeded and the correction is proceeding more rapidly than planned (usually because of continued excretion of hypotonic urine), the pathologic events leading to demyelination can be reversed by administration of hypotonic fluids, with or without desmopressin. The efficacy of this approach has been suggested from animal studies[369] and case reports in humans,[370] even when patients are overtly symptomatic.[371] However, relowering the serum [Na^+] after an initial, overly rapid correction is only strongly recommended for patients at high risk of ODS; it is considered optional for patients with a low-to-moderate risk of ODS, and unnecessary for patients with acute water intoxication (see Fig. 10.7).

Monitoring the Serum [Na^+] in Hyponatremic Patients

The frequency of serum [Na^+] monitoring is dependent on both the severity of the hyponatremia and the therapy chosen. All patients undergoing active treatment with hypertonic saline for symptomatic hyponatremia should have frequent monitoring of serum [Na^+], urine output, and ECF volume status (every 2–4 hours) to ensure that the serum [Na^+] does not exceed the limits of safe correction during the active phase of correction[310] since overly rapid correction of serum [Na^+] will increase the risk of ODS.[233] Patients treated with vaptans for moderate or mild symptoms should have serum [Na^+] monitored every 6 to 8 hours during the active phase of correction, which will generally be the first 24 to 48 hours of therapy. Active treatment with any therapy should be stopped when the patient's symptoms are no longer present, a safe serum [Na^+] (usually >125 mmol/L) has been achieved, or the rate of correction has reached maximum limits of 10 to 12 mmol/L within 24 hours, 18 mmol/L within 48 hours,[310,330] or 8 mmol/L over any 24-hour period in patients at high risk of ODS (see Fig. 10.7). In patients with a stable level of serum [Na^+] treated with fluid restriction or therapies other than hypertonic saline, measurement of serum [Na^+] daily is generally sufficient since levels will not change that quickly in the absence of active therapy or large changes in fluid intake or administration.

Long-Term Treatment of Chronic Hyponatremia

Some patients will benefit from continued treatment of hyponatremia following discharge from the hospital. In many cases, this will consist of a continued fluid restriction, but long-term compliance with this therapy is poor because of the increased thirst that occurs with more severe degrees of fluid restriction. Thus for select patients who have responded to tolvaptan in the hospital, consideration should be given to continuing the treatment as an outpatient after discharge. In patients with established chronic hyponatremia, tolvaptan has shown to be effective for maintaining a normal [Na^+] for as long as 3 years of continued daily therapy.[372] However, many patients with inpatient hyponatremia have a transient form of SIAD without the need for long-term therapy. Selection of which patients with inpatient hyponatremia are candidates for long-term therapy should be based on the cause of the SIAD. In all cases, consideration should be given to a trial of stopping the drug 2 to 4 weeks after discharge to determine if hyponatremia is still present. A reasonable period of tolvaptan cessation to evaluate the presence of continued SIAD is 7 days because this period was found to be sufficient for demonstration of a recurrence of hyponatremia in the tolvaptan SALT[339] and SALT-WATER trials.[372]

Findings of hepatotoxicity in a small number of patients on high doses of tolvaptan in a clinical trial of polycystic kidney disease led to a recent FDA recommendation that tolvaptan not be used for longer than 30 days. If tolvaptan is used for longer than 30 days, liver function should be assessed at regular intervals (e.g., every 3 months) at least for the first year of therapy. As is always the case, decisions about appropriate treatment of hyponatremia should be based on clinical judgment and risk/benefit analysis that is individualized for specific patients, because there is no single treatment that represents the "best" therapy for all patients with SIAD.[326]

Oxytocin

Study of the normal physiologic regulation of oxytocin secretion and action is complicated by the fact that secretion and function of oxytocin varies markedly among different experimental mammals. There are varying sites of synthesis in the ovary and in tissues of the uterus that are different among species. It is difficult to study pregnant women and human tissue, so physiologic regulation of oxytocin secretion and function is less well known in humans than other species. The classic roles of oxytocin are smooth muscle activation promoting milk letdown with nursing and uterine myometrial contraction at parturition.

Lactation

A characteristic of all mammals is lactation, and all mammals secrete oxytocin to stimulate milk letdown associated with nursing.[373] The other hormone critical to lactation is prolactin. Each of these pituitary/hypothalamic hormones is importantly influenced and regulated by gonadal steroid hormones. The milk-producing unit of the breast is the alveolar system with multiple clusters of milk-producing cells surrounded by specialized myoepithelial cells. The alveoli are directly connected to ductules, and then ducts converge and lead to the nipple. Milk is synthesized in the glandular cells of the alveoli.[374] Oxytocin receptors are localized on glandular cells, and oxytocin in the systemic circulation acts on these receptors to cause myoepithelial contraction. Oxytocin also acts on myoepithelial cells along the duct to shorten and widen the ducts to enhance milk flow through the ducts to the nipple.[375]

When an infant begins sucking at the breast, an afferent signal is transmitted from the mechanoreceptors or tactile receptors in the breast to the spinal cord and eventually ascend to the oxytocin magnocellular neurons in the supraoptic nucleus and the paraventricular nucleus.[375,376] Pulsatile release of oxytocin produces a pulsatile pumping action on the alveoli, which promotes maximum emptying of milk from the alveoli.[374] The importance of oxytocin in maintaining milk secretion is demonstrated by transgenic mice with a knockout that inhibits oxytocin synthesis. These animals deliver their young normally and have normal milk production, but there is no milk release in spite of normal suckling. The pups die of dehydration with no milk in the stomach.[377] Administration of oxytocin to these oxytocin-deficient mice rescues the ability to secrete milk and allows the pups to survive. Similarly oxytocin may promote successful lactation in women who have lactational insufficiency.[378]

As breastfeeding continues in humans, the basal levels of oxytocin decrease but pulses of oxytocin in response to suckling continue and may increase.[379] Humans with diabetes insipidus have been able to successfully breastfeed infants, and this has caused some to question the importance of oxytocin in humans.[380] However, oxytocin secretion may be preserved in the absence of vasopressin in patients with diabetes insipidus.

Parturition

The isolation of oxytocin was followed quickly by the description of oxytocin to stimulate uterine contractions, and this was followed shortly by clinical use of oxytocin as a uterotonic agent.[381] Parturition in humans is much more complex than just the role of oxytocin.[382] In all species the uterus must grow during pregnancy, and estrogen is a promoter of this growth. Levels of oxytocin in humans are not well defined in pregnancy but are not reported to increase until the expulsive stage at term.[382–384] The uterine myometrial cells have intrinsic contractile activity, but during pregnancy the uterus is maintained in a quiescent state by the actions of progesterone and relaxin (produced by the corpus luteum and decidual tissue).[385–387] The initiation of labor is accomplished by a relative increase in estrogen activation and a decrease in progesterone activation. Changes in oxytocin receptors and oxytocin produced by the placenta may be more important than levels of oxytocin in the circulation. During early labor there is an upregulation in the uterus of oxytocin receptor mRNA, and oxytocin receptor numbers increase.[384,388] Oxytocin receptors are prominent in the fundus of the uterus where they stimulate myometrial contraction and in decidual cells where they stimulate the production of prostaglandins. At parturition increased oxytocin activity in the fundus will push the fetus toward the cervix that is thinned and relaxed by the effects of prostaglandins.[383,389] Prostaglandins play a key role in an inflammatory process that is important in the uterus at parturition. Cytokines induce enzymes that digest extracellular matrix to soften and ripen the cervix.[383] The role of progesterone in maintaining uterine quiescence is not only the action on oxytocin receptors but also the antagonizing of the inflammatory response that softens the lower uterus and cervix.[384]

There are three situations in pregnancy where a pharmacologic role of oxytocin is of interest. The first situation and most widely used role of oxytocin is to induce and augment labor.[390] This has received increased interest in an effort to decrease the number of and morbidity of cesarean sections.[391] Oxytocin may be delivered alone or in combination with another pharmacologic agent such as propranolol or prostaglandins.[391,392] The second area of interest

is preterm labor with an effort toward prevention by decreasing contractile activity of the uterus and/or inhibiting the inflammatory response.[393] Peptide and nonpeptide oxytocin antagonists have been of special interest to inhibit myometrial contractions, but widespread clinical use awaits the development of antagonist with better risk/benefit activity.[394] The third pharmacologic interest in oxytocin is as a uterotonic to decrease postpartum hemorrhage associated with uterine atony.[395] Postpartum hemorrhage is the major cause of maternal deaths worldwide and ranks second to embolism as a cause of maternal death in the United States.[396] Mechanical options in the active management of the third stage of labor include cord traction to reduce the risk of retained placenta and uterine massage, which has been augmented by pharmacologic agents, most commonly oxytocin and/or ergotamine.[395] Maternal death by postpartum hemorrhage is most significant in developing countries. Oxytocin is heat labile and requires a trained staff for appropriate administration,[397] prompting a search for other agents. Promising results have been reported with prostaglandin analogues (e.g., misoprostol).[394,395,398]

Behavior

This chapter is about functions of vasopressin and oxytocin as traditional endocrine hormones secreted by the posterior pituitary. For further discussion related to these hormones in purported functions as neurotransmitters, especially with regard to influencing social and ingestive behaviors, the reader is referred to Chapters 9 and 20.

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The complete list of references is available online at ExpertConsult.com.

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11

Thyroid Pathophysiology and Diagnostic Evaluation

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CHAPTER OUTLINE

KEY POINTS

- This chapter illustrates the principal events involved in the ontogeny and development of the thyroid gland in many life forms, ranging from invertebrates to humans.
- The thyroid gland anatomy and function and the pivotal role played by iodine in thyroid economy are described.
- The key molecular elements and mechanisms involved in thyroid hormone action and metabolism in peripheral tissues are reviewed.
- This chapter also dissects the mechanisms responsible for thyroid hormone homeostasis and function under physiologic and pathologic conditions.
- In addition, this chapter provides the physiologic rationale and expected results for the various tests that can be used in the biochemical evaluation of patients with clinical thyroid dysfunction or disease.

Dysfunction and anatomic abnormalities of the thyroid are among the most common diseases of the endocrine glands. This chapter provides the physiologic and biochemical background and describes the various tests for evaluating patients with suspected thyroid dysfunction based on the pathophysiology of these conditions.

Phylogeny, Embryology, and Ontogeny

Phylogeny

The phylogeny, embryogenesis, and certain aspects of thyroid function are closely interlinked with the gastrointestinal tract. The capacity of the thyroid to metabolize iodine and incorporate it into a variety of organic compounds occurs widely throughout the animal and plant kingdoms. However, the anatomy of the thyroid gland differs considerably among the vertebrate classes. Monoiodotyrosine (3′-monoiodo-L-tyrosine [MIT]) and diiodotyrosine (3,5-diiodo-L-tyrosine [DIT]) are present in a variety of invertebrate species, including mollusks, crustaceans, coelenterates, annelids, insects, and certain marine algae. In these lower forms, however, no recognizable thyroid tissue is present. Thyroid tissue is confined to, and is present in, all vertebrates. A close link to the thyroid of higher vertebrates is evident in the ammocoete, the larval form of the lamprey, where the ventral part of the pharynx is the origin of a structure present only during larval life, the endostyle. The epithelium of the endostyle is capable of carrying out iodinations, and these cells are fated to become follicular cells only after metamorphosis, when they will form classic thyroid follicles.[1]

The phylogenetic association of the thyroid gland and the gastrointestinal tract is evident in several functions. The salivary and gastric glands, like the thyroid, are capable of concentrating iodide in their secretions, although iodide transport in these sites is not responsive to stimulation by thyrotropin (TSH, thyroid-stimulating hormone). The salivary gland contains enzymes that are capable of iodinating tyrosine in the presence of hydrogen peroxide, although it forms insignificant quantities of iodoproteins under normal circumstances.

Structural Embryology

The morphogenesis of the thyroid gland, the anterior-most organ that buds from the gut tube, begins with a thickening of the endodermal epithelium in the foregut, which is referred to as the *thyroid anlage*. The human thyroid anlage is first recognizable

at embryonic day 16 or 17. This median thickening deepens and forms first a small pit and then an outpouching of the endoderm adjacent to the developing myocardial cells. With continuing development, the median diverticulum is migrating caudally, following the myocardial cells in their descent. The primitive stalk connecting the primordium with the pharyngeal floor elongates into the thyroglossal duct. During its caudal migration, the primordium assumes a bilobate shape, coming into contact and fusing with the ventral aspect of the fourth pharyngeal pouch when it reaches its final position at about embryonic day 50. Normally the thyroglossal duct undergoes dissolution and fragmentation by about the second month after conception, leaving at its point of origin a small dimple at the junction of the middle and posterior thirds of the tongue, the *foramen caecum.* Cells of the lower portion of the duct differentiate into thyroid tissue, forming the pyramidal lobe of the gland. At this time, the lobes contact the ultimobranchial glands, leading to the incorporation of C cells into the thyroid. Concomitantly, histologic alterations occur throughout the gland. Complex interconnecting cordlike arrangements of cells interspersed with vascular connective tissue replace the solid epithelial mass and become tubule-like structures at about the third month of fetal life; shortly thereafter, follicular arrangements devoid of colloid appear, and by 13 to 14 weeks the follicles begin to fill with colloid. Investigations of thyroid gland development in mice using gene-targeting techniques are beginning to identify the critical factors that are required for normal thyroid gland development.[2,3] The role of these various proteins is currently being evaluated with respect to the potential for defects in the synthesis or formation of the thyroid gland (see Chapter 13).

Functional Ontogeny

The ontogeny of thyroid function and its regulation in the human fetus are fairly well defined.[4,5] Future follicular cells acquire the capacity to form thyroglobulin (Tg) as early as the 29th day of gestation, whereas the capacities to concentrate iodide and synthesize thyroxine (T_4) are delayed until about the 11th week. Radioactive iodine inadvertently given to the mother would be accumulated by the fetal thyroid soon thereafter. Early growth and development of the thyroid do not seem to be TSH dependent because the capacity of the pituitary to synthesize and secrete TSH is not apparent until the 14th week. Subsequently, rapid changes in pituitary and thyroid function take place. Probably as a consequence of hypothalamic maturation and increasing secretion of thyrotropin-releasing hormone (TRH), the serum TSH concentration increases between 18 and 26 weeks of gestation, after which levels remain higher than those in the mother.[4] The higher levels may reflect a higher set-point of the negative feedback control of TSH secretion during fetal life than at maturity. Thyroxine-binding globulin (TBG), the major thyroid hormone–binding protein in plasma, is detectable in the serum by the 10th gestational week and increases in concentration progressively to term. This increase in TBG concentration accounts in part for the progressive increase in the serum T_4 concentration during the second and third trimesters, but increased secretion of T_4 must also play a role because the concentration of free T_4 also rises.

Several aspects of thyroid development are of note from a clinical standpoint. Rarely, thyroid tissue may develop from remnants of the thyroglossal duct near the base of the tongue. Such lingual thyroid tissue may be the sole functioning thyroid present, and thus its surgical removal will lead to hypothyroidism. More commonly, elements of the thyroglossal duct may persist and later give rise to thyroglossal duct cysts, or ectopic thyroid tissue may be present at any location in the mediastinum or, rarely, even in the heart.

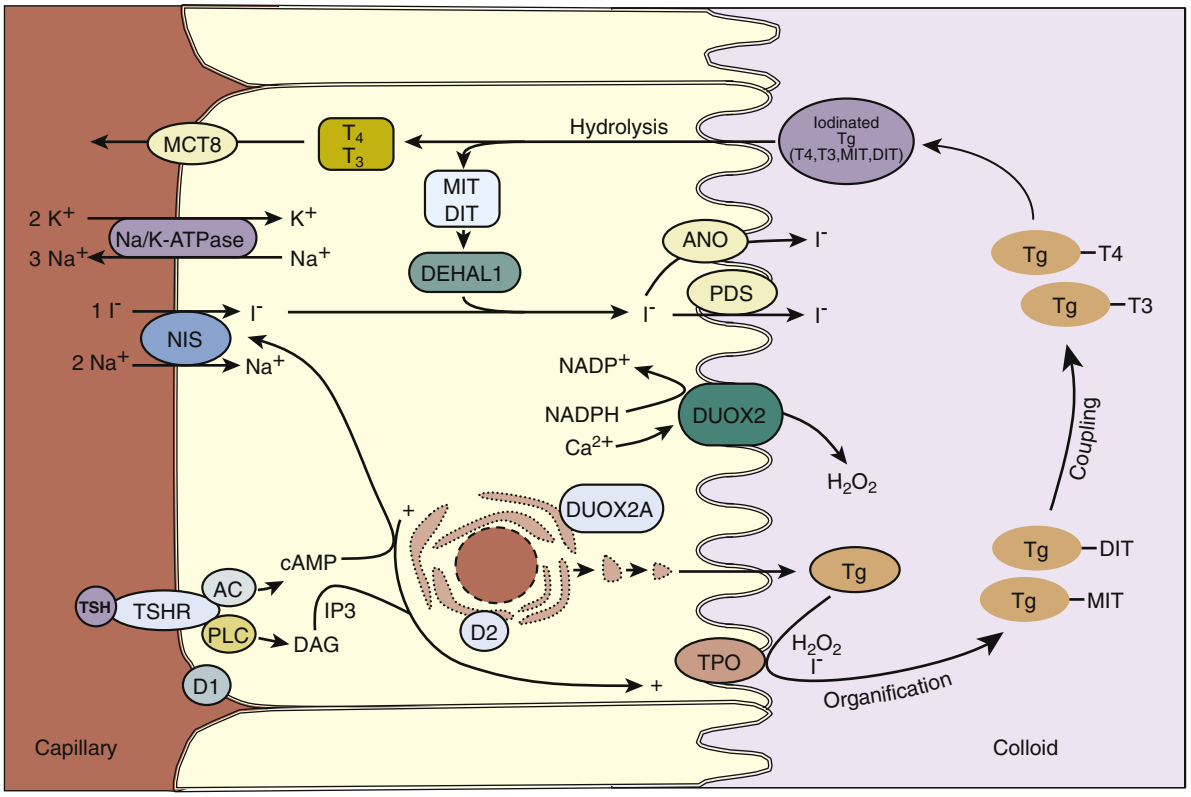
Anatomy and Histology

The thyroid is one of the largest of the endocrine organs, weighing approximately 15 to 20 g in North American adults. Moreover, the potential of the thyroid for growth is tremendous. The enlarged thyroid, commonly termed *goiter,* can weigh many hundreds of grams. The normal thyroid is made up of two lobes joined by a thin band of tissue, the isthmus, which is approximately 0.5 cm thick, 2 cm wide, and 1 to 2 cm high. The individual lobes normally have a pointed superior pole and a poorly defined blunt inferior pole that merges medially with the isthmus. Each lobe is approximately 2 to 2.5 cm in thickness and width at its largest diameter and is approximately 4 cm in length. Occasionally, especially when the remainder of the gland is enlarged, a pyramidal lobe is discernible as a finger-like projection directed upward from the isthmus, generally just lateral to the midline, usually on the left. The right lobe is normally more vascular than the left, is often the larger of the two, and tends to enlarge more in disorders associated with a diffuse increase in gland size. Two pairs of vessels constitute the major arterial blood supply: the superior thyroid artery, arising from the external carotid artery, and the inferior thyroid artery, arising from the subclavian artery. Estimates of thyroid blood flow range from 4 to 6 mL/minute/g, well in excess of the blood flow to the kidney (3 mL/minute/g). In a diffuse toxic goiter due to Graves disease, blood flow may exceed 1 L/minute and be associated with an audible bruit or even a palpable thrill.

The gland is composed of closely packed spherical units termed *follicles,* which are invested with a rich capillary network. The interior of the follicle is filled with the clear proteinaceous colloid that normally is the major constituent of the total thyroid mass. On cross section, thyroid tissue appears as closely packed, ring-shaped structures consisting of a single layer of thyroid cells surrounding a lumen. The diameter of the follicles varies considerably, even within a single gland, but averages about 200 nm. The follicular cells vary in height with the degree of glandular stimulation, becoming columnar when active and cuboidal when inactive. The epithelium rests on a basement membrane that is rich with glycoproteins separating the follicular cells from the surrounding capillaries. From 20 to 40 follicles are demarcated by connective tissue septa to form a lobule supplied by a single artery. The function of a given lobule may differ from that of its neighbors.

On electron microscopy, the thyroid follicular epithelium has many features in common with other secretory cells and some peculiar to the thyroid. From the apex of the follicular cell, numerous microvilli extend into the colloid. It is at or near this surface of the cell that iodination, exocytosis, and the initial phase of hormone secretion, *colloid resorption,* occur (Fig. 11.1). The nucleus has no distinctive features, and the cytoplasm contains an extensive endoplasmic reticulum (ER) laden with microsomes. The ER is composed of a network of wide irregular tubules that contain the precursor of Tg. The carbohydrate component of Tg is added to this precursor in the Golgi apparatus, which is located apically. Lysosomes and mitochondria are scattered throughout the cytoplasm. Stimulation by TSH results in enlargement of the Golgi apparatus, formation of pseudopodia at the apical surface, and the appearance in the apical portion of the cell of many droplets that contain colloid taken up from the follicular lumen (see Fig. 11.1).

The thyroid also contains parafollicular cells, or C cells, which are the source of calcitonin. The traditional view was that these



• **Fig. 11.1** Schematic illustration of a follicular cell showing the key aspects of thyroid iodine transport and thyroid hormone synthesis. *AC,* adenyl cyclase; *ATPase,* adenosine triphosphatase; *cAMP,* cyclic adenosine monophosphate; *D1,* thyroidal deiodinase type 1; *D2,* thyroidal deiodinase type 2; *DAG,* diacylglycerol; *DEHAL1,* iodotyrosine dehalogenase 1 (IYD); *DIT,* diiodotyrosine; *DUOX,* dual oxidase; *IP3,* inositol triphosphate; *MIT,* monoiodotyrosine; *$NADP^+$,* oxidized form of nicotinamide adenosine dinucleotide phosphate; *NADPH,* reduced nicotinamide adenosine dinucleotide phosphate; *NIS,* sodium-iodide symporter; *PDS,* pendrin (SLC26A4); *PLC,* phospholipase C; *T_3,* triiodothyronine; *T_4,* thyroxine; *Tg,* thyroglobulin; *TPO,* thyroid peroxidase; *TSH,* thyrotropin; *TSHR,* thyrotropin receptor.

cells derive from the neural crest, but newer lineage tracing data suggest that they arise also in the endoderm.[6] Ultimately they come to rest either among the cells of the follicular epithelium or in the thyroid interstitium. They differ from the cells of the follicular epithelium in never bordering on the follicular lumen and in being rich in mitochondria. The C cells, detected also in human lingual thyroids,[7] undergo hyperplasia early in the syndrome of familial medullary carcinoma of the thyroid (multiple endocrine neoplasia type 2 [MEN2]) and give rise to this tumor in both its familial and its sporadic forms (see Chapter 42).

Iodine and the Synthesis and Secretion of Thyroid Hormones

The function of the thyroid is to generate the quantity of thyroid hormone necessary to meet the demands of the peripheral tissues. This action requires iodide uptake by the thyroidal sodium-iodide symporter (NIS), its transfer to the colloid, and its oxidation by thyroid peroxidase (TPO) to allow the synthesis of approximately 110 nmol/L (85 μg) of T_4, which is 65% iodine by weight. This requires the synthesis of an approximately 330-kDa glycoprotein, Tg. Specific tyrosine residues of Tg homodimers are then iodinated at the apical border of the thyroid cell to form MIT and DIT (see Fig. 11.1). This requires formation of hydrogen peroxide (H_2O_2) by dual oxidases (DUOX1 and 2) and TPO, which catalyzes the oxidation of iodide and its transfer to tyrosine. TPO also catalyzes the coupling of two molecules of DIT or one of DIT and one of MIT, leading to formation of T_4 and triiodothyronine (T_3), respectively, which are then stored within the colloid, still as part of the Tg molecule. Pinocytosis of stored colloid leads to the formation of phagolysosomes, the colloid droplets in which Tg is digested by specific proteases to release T_4, T_3, DIT, and MIT as the droplet is translocated toward the basal portion of the cell. T_4 and T_3 are transported out of the phagolysosomes and across the basolateral cell membrane, at least in part by MCT8, to exit the cell and enter circulation, whereas DIT and MIT are deiodinated by iodotyrosine dehalogenase (DEHAL1) to allow recycling of the iodide. The synthesis of thyroid hormones requires the expression of a number of thyroid cell–specific proteins. In addition to Tg and TPO, the TSH receptor (TSHR) is also required to transduce the effects of extracellular TSH for efficient hormone synthesis. A number of transcription factors, including thyroid transcription factors TTF1 (NKX2-1) and TTF2 (FOXE1), PAX8, and hepatocyte nuclear factor 3 (HNF3 [FOXE2]), as well as TSH, are necessary to achieve functional differentiation of the thyroid follicular cells and the onset of hormonogenesis.[1,3] Transient overexpression of the transcription factors NKX2-1 and PAX8 is sufficient to direct mouse embryonic stem cell (mESC) differentiation into thyroid follicular cells that organize into three-dimensional follicular structures when treated with TSH, and follicles show significant iodide organification activity.[8] Although the biochemical

details of these processes are beyond the scope of this discussion, those aspects with clinical relevance are reviewed in greater detail in the following section.

Dietary Iodine

Formation of normal quantities of thyroid hormone requires the availability of adequate quantities of exogenous iodine to allow thyroidal uptake of approximately 60 to 75 μg daily, taking into account the fecal losses of about 10 to 20 μg iodine of iodothyronines as glucuronides and about 100 to 150 μg as urinary iodine in iodine-sufficient populations.[9] Plasma iodide (I^-), the form of the element in biologic solutions, is completely filterable with about 60% to 70% of the filtered load reabsorbed. At least 100 μg of iodine per day is required to eliminate all signs of iodine deficiency (Table 11.1). In healthy adults, the absorption of iodide is greater than 90%. In North America, the daily dietary iodine intake is in the range of 150 to 300 μg, largely owing to the iodination of salt; in Japan, however, where large quantities of foods rich in iodine are consumed, intakes may be as high as several milligrams per day. Notably, iodine intake in the United States is decreasing due to a reduction in salt consumption, with median urinary iodine of 160 μg/L but a low urinary iodine (<5 μg/dL) in close to 10% of the population.[10] The daily dietary intake of iodine varies widely throughout the world, depending on the iodine content of soil and water and on dietary practice (see Table 11.1) (Iodine Global Network; http://www.ign.org/). Even in a single area, iodine intake varies among different individuals and in the same individual from day to day. Iodine may also enter the body via medications, diagnostic agents, dietary supplements, and food additives. As discussed more extensively under "Regulation of Thyroid Function," iodine deficiency is common, especially in mountainous and formerly glaciated regions of the earth.[11] An estimated 1 billion individuals live in iodine-deficient areas of the world, and these people often develop TSH-induced compensatory enlargement of the thyroid *(endemic goiter)*. If iodine deficiency is severe during pregnancy, fetal thyroid hormone production falls with irreparable damage to the developing central nervous system (CNS). This damage is manifested by varying degrees of mental retardation and is termed *endemic cretinism*. Thus iodine-deficiency disorders (IDDs), including endemic goiter and cretinism, are the most common thyroid-related human illnesses; indeed, they are the most common endocrine disorders worldwide. Iodine deficiency is also the most prevalent preventable cause of mental impairment.

Plasma iodide is partly replenished by that lost from the thyroid into the blood and by iodide liberated through deiodination of iodothyronines in peripheral tissues. Ultimately, however, the diet is its most important source. Iodine is ingested in both inorganic and organically bound forms. Iodide per se is rapidly and efficiently absorbed from the gastrointestinal tract (within 30 minutes), and little is lost in the stool. In the body, iodide is confined largely to the extracellular fluid. It is also found, however, in red blood cells and is concentrated in the intraluminal fluids of the gastrointestinal tract, notably the saliva and gastric juice, from which it is reabsorbed, thus reentering the extracellular fluid. Iodide is also concentrated in milk. Until oxidized and organified on tyrosyl residues in Tg, iodide entering the thyroid by active transport is in rapid equilibrium with the main iodide pool. The concentration of iodide in the extracellular fluid is normally 10 to 15 μg/L (~10^{-7} mol/L), and the content of the peripheral pool is approximately 250 μg. The thyroid contains the largest pool of body iodine, under normal circumstances approximately 8000 μg, most of which is in the form of DIT and MIT. Normally this pool of iodine turns over slowly (about 1% per day).

TABLE 11.1 Recommended and Typical Values for Dietary Iodine Intake

Recommended Daily Intake	
Adults	150 μg
During pregnancy[a]	220 μg
Children	90–120 μg
Median Urinary Iodine Concentrations[b]	
United States (2010)	213 μg
China (2017)	239 μg
Belgium (2011)	113 μg
Switzerland (2015)	137 μg
Russia (2004)	78 μg

[a]Recommendations by the Institute of Medicine, Food and Nutrition Board.

The World Health Organization (WHO), United Nations Children's Fund (UNICEF), and the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) recommend a slightly higher iodine intake for pregnant women of 250 μg/day.

[b]Data from Iodine Global Network, http://www.ign.org/.

Iodide Metabolism by the Thyroid Cell

The spherical thyroid follicle, which is formed by a single layer epithelium of thyroid cells surrounding the lumen, is the functional unit of the thyroid and its integrity is essential for the synthesis of thyroid hormones. Because the concentration of iodide in plasma is so low, a mechanism is required for the thyroid cell to concentrate the required amounts of this element. This process, iodide trapping, is accomplished by a membrane protein, the sodium-iodide symporter NIS, encoded by the *SLC5A* gene. Human NIS is a 643–amino acid glycoprotein with 13 membrane-spanning domains. The transport of iodide is an active process dependent on the presence of a sodium gradient across the basal membrane of the thyroid cell such that downhill transport of two Na^+ ions results in the entry of one iodide atom against an electrochemical gradient (see Fig. 11.1). In addition to being expressed in the basolateral membrane of the thyroid cell, NIS has also been identified in other iodide-concentrating cells, including salivary and lactating mammary glands, choroid plexus, and gastric mucosa, and in the cytotrophoblast and syncytiotrophoblast.[12,13] NIS is also expressed in the ovary and testis and in ovarian cancer and the majority of seminomas and embryonal testicular carcinomas.[14] In the lactating mammary gland, NIS plays an important role by concentrating iodide in the milk, thereby supplying newborns with iodide for thyroid hormone synthesis. The iodide transport system generates an iodide gradient of 20 to 40 across the cell membrane, and NIS also transports pertechnetate (TcO_4^-), perchlorate (ClO_4^-), and thiocyanate (SCN^-), accounting for the utility of radioactive TcO_4^- as a thyroid scanning tool and the capacity of $KClO_4^-$ to block iodide uptake as a competitive inhibitor.[15,16] The affinity of NIS for iodide is much higher than it is for the other inorganic halide anions, such as bromide and chloride, accounting for the selectivity of the thyroid transport mechanism.[17]

Transcription of the *NIS* gene is increased by TSH, and TSH also prolongs NIS protein half-life and targets the protein to the cell membrane. Iodide uptake by NIS and the organification process are inversely regulated by high intracellular iodide concentrations (Wolff-Chaikoff effect; see later).[18]

That the iodide-concentrating mechanism is required for normal thyroid function has been known for decades in that its absence is associated with congenital hypothyroidism and goiter unless large quantities of inorganic iodide are provided.[19] A number of families have now been identified in which biallelic mutations in the *NIS* gene are associated with an iodide transport defect, resulting in congenital hypothyroidism. Importantly, several studies have documented decreases in NIS expression in human thyroid adenomas and carcinomas that contribute to the loss of iodine uptake in neoplastic thyroid cells, which therefore present as "cold" nodules on radioisotopic imaging.[19] However, changes in the subcellular location of NIS may also explain this phenomenon. At the apical membrane, iodide must enter the follicular lumen. This process is thought to involve pendrin, a highly hydrophobic membrane glycoprotein and multianion exchanger located at the apical membrane of thyrocytes.[20,21] In addition to the thyroid, pendrin is also expressed in the kidney and in the inner ear.[22] In the kidney, pendrin plays an important role in acid-base metabolism as a chloride/bicarbonate exchanger.[23] In the inner ear, pendrin is important for generation of the endocochlear potential.[19] Pendrin belongs to the SLC26A family and is encoded by the *SLC26A4* gene. Mutations in the *SLC26A4* gene lead to Pendred syndrome, an autosomal recessive disorder characterized by sensorineural deafness, goiter, and a partial defect in iodide organification.[24,25] Deafness or hearing impairment is the major phenotypic manifestation in Pendred syndrome. Goiter usually develops during childhood, and under conditions of scarce iodine intake, some individuals with biallelic mutations in pendrin present with congenital hypothyroidism. There is, however, a substantial variation within and between families and different geographic regions. This observation along with the absence of thyroid dysfunction in mice with targeted inactivation of the *Slc26A4* gene suggest that iodide efflux can also occur in the absence of pendrin.[21] Anoctamin 1 (ANO1/TMEM16A), a calcium-activated anion channel, which is also expressed at the apical membrane of thyrocytes, may also be involved in mediating apical efflux.[26,27]

Iodide Oxidation and Organification

After reaching the follicular lumen, iodide participates in a series of reactions that lead to the synthesis of the active thyroid hormones. The first of these involves oxidation of iodide and incorporation of the resulting intermediate into the hormonally inactive iodotyrosines MIT and DIT, a process termed *organification*. Iodide is normally oxidized rapidly, immediately appearing in organic combination with Tg. The iodinations that lead to formation of iodotyrosines occur within Tg, rather than on the free amino acids. Oxidation of thyroidal iodide is mediated by the heme-containing protein TPO and requires the H_2O_2 generated by the calcium-dependent DUOX1 and DUOX2 enzymes. The protein contains a membrane-spanning region near the carboxy-terminus, and it is oriented in the apical membrane of the thyroid cell with residues 1-844 in the follicular lumen in which iodination occurs (see Fig. 11.1). TPO is the major thyroid microsomal antigen, and recombinant human TPO is now used for the detection of antithyroid microsomal antibodies commonly present in the serum of patients with Hashimoto thyroiditis. The evanescent product of the peroxidation of iodide (i.e., the active iodinating form) remains unclear and hypoiodite (OI^-), hypoiodous acid (HOI), or iodonium (I^+) have been proposed.[28] The *DUOX1* and *DUOX2* genes encode glycoflavoproteins predominantly expressed at the apical thyrocyte membrane where they constitute the catalytic core of the H_2O_2 generator required for thyroid hormone synthesis (see Fig. 11.1).[29] They are Ca^{2+}, NADPH-dependent oxidases that catalyze the formation of the H_2O_2 required for TPO-catalyzed Tg iodination. The maturation factor DUOXA2, a resident ER protein, is required for the maturation, plasma membrane localization of DUOX2, and H_2O_2 generation.[30] Iodide excess inhibits DUOX2 glycosylation, which may be an additional mechanism contributing to the Wolff-Chaikoff effect.[31]

The rate of organic iodination is dependent on the degree of thyroid stimulation by TSH (see later). Congenital defects in the organification process cause goitrous congenital hypothyroidism or, if less severe, goiter without hypothyroidism (complete or partial iodide organification defect).[32] They can be caused by usually biallelic mutations in the *TG, SLC26A4, TPO, DUOX2,* and *DUOX2A* genes. Complete or partial TPO defects are among the most frequent causes of abnormal thyroid hormone biosynthesis.[33] Mutations in the *DUOX2* and *DUOXA2* genes have been identified in patients with permanent and transient congenital hypothyroidism, and digenic mutations in *DUOX1* and *DUOX2* have been found in a family with very severe congenital hypothyroidism (see Chapter 13).[34,35]

Iodothyronine Synthesis

The MIT and DIT are precursors of the hormonally active iodothyronines T_4 and T_3. Synthesis of T_4 from DIT requires the TPO-catalyzed fusion of two DIT molecules to yield a structure with two diiodinated rings linked by an ether bridge (the coupling reaction). Concomitantly, a residual dehydroalanine is formed at the site of the DIT residue contributing to the phenolic hydroxyl group.

Efficient synthesis of T_4 and T_3 in the thyroid requires Tg.[36] The Tg messenger RNA (mRNA) is approximately 8.5 kb in length and encodes a 330-kDa (12S) subunit that is 10% carbohydrate by weight. There are 134 tyrosyl residues in the 660-kDa homodimer. Only 25 to 30 of these are iodinated, but only residues 5, 1290, and 2553 form T_4 and residue 2746, T_3.[37] The T_4-forming, readily iodinated, and iodothyronine-forming acceptor residues of Tg from different species are in a Glu/AspTyr or a Thr/SerTyrSer sequence, suggesting an important role of primary sequence in these reactions. There are three to four T_4 molecules in each molecule of human Tg under conditions of normal iodination (25 atoms per Tg molecule, approximately 0.5% iodine by weight), but only about one in five molecules of human Tg contains a T_3 residue. In Tg from patients with untreated Graves disease, the content of T_4 residues remains approximately the same, but the number of T_3 residues doubles to an average of 0.4 per molecule. This difference is independent of the iodination state of the Tg and is a consequence of thyroidal stimulation. Because the coupling reaction is catalyzed by TPO, virtually all agents that inhibit organic binding (e.g., the thiourea drugs) also inhibit coupling.

Storage and Release of Thyroid Hormone

The thyroid is unique among the endocrine glands by virtue of the large store of hormone it contains and the low rate at which the

hormone turns over (1% per day). This aspect of thyroid hormone economy has homeostatic value in that the reservoir provides prolonged protection against depletion of circulating hormone in case synthesis ceases. In normal humans, the administration of antithyroid agents for as long as 2 weeks has little effect on serum T_4 concentrations. There are approximately 250 μg T_4 per gram of wet weight in normal human thyroid, or 5000 μg of T_4 in a 20-g gland.[38] This amount is sufficient to maintain a euthyroid state for at least 50 days. When released rapidly in an uncontrolled fashion during subacute or painless thyroiditis, this quantity of T_4 will cause significant transient thyrotoxicosis. Tg is present in the plasma of normal individuals at concentrations up to 50 ng/mL, probably leaving the thyroid through the lymphatics. However, peripheral hydrolysis of Tg does not contribute significantly to the thyroid hormones in the circulation, even during thyroiditis, when large quantities of this protein are present.

The first step in thyroid hormone release is the endocytosis of colloid from the follicular lumen by two processes: macropinocytosis by pseudopods formed at the apical membrane and micropinocytosis by small coated vesicles that form at the apical surface (see Fig. 11.1). Both processes are stimulated by TSH, but the relative importance of the two pathways varies among species, with micropinocytosis thought to predominate in humans. Following endocytosis, endocytotic vesicles fuse with lysosomes, and proteolysis is catalyzed by cathepsin D and D-like thiol proteases, all of which are active at the acidic pH of the lysosome. The iodotyrosines MIT and DIT released from Tg are rapidly deiodinated by the NADPH-dependent iodotyrosine deiodinase DEHAL1/IYD, and the released iodine is recycled.[39] Thyroid hormones are released from Tg in the lysosome, but it is not entirely clear how their transfer into the cytosol and subsequently the plasma is affected. Exit at the basolateral membrane involves, at least in part, the thyroid hormone transporter MCT8.[40] It has been shown that T_4 can be released from Tg within the thyroid cell with minimal disruption of its molecular weight. This presumably is a consequence of selective proteolysis, which is facilitated by the fact that the major hormonogenic peptides of the Tg molecule are located at the amino-terminus and the carboxy-terminus of the Tg monomer.

Presumably the T_4 becomes accessible to the thyroidal type 1 and 2 deiodinases (D1 and D2) because basal and TSH-stimulated conversion of T_4 to T_3 is readily demonstrated in the perfused dog thyroid. Because this conversion is inhibited by propylthiouracil (PTU), it is catalyzed by D1 (Table 11.2). The contribution of thyroidal T_4 deiodination to T_3 secretion in humans under physiologic conditions is not known. The fact that the ratio of T_4 to T_3 in human Tg is 15:1, although estimates of the molar ratio of T_4 to T_3 in thyroid secretion is approximately 10:1, suggests that this does occur. Stimulation of D1-catalyzed and D2-catalyzed 5′-deiodination of T_4 in the thyroid gland of patients with Graves disease may enhance that pathway and contribute to the marked increase of the ratio of T_3 to T_4 production in that condition.[41] An inhibition of the D1-catalyzed T_4 to T_3 conversion may contribute to the rapid effect of PTU to reduce circulating T_3 in patients with Graves disease (see Chapter 12).[41,42] That deiodinases in thyroid-derived cells can modulate the systemic conversion of T_4 to T_3 has been shown in several patients with metastatic thyroid carcinoma. The high expression of D2 in one large mediastinal tumor mass was associated with a high normal T_3 and reduced T_4 with a normal TSH. Removal of the tumor reversed these abnormalities.[43]

T_4 release from the thyroid cells is inhibited by several agents, the most important of which is iodide. Inhibition of hormone release is responsible for the rapid improvement that iodide causes in hyperthyroid patients. The mechanism by which this effect is mediated is uncertain, but iodide inhibits the stimulation of thyroid adenylate cyclase by TSH and by the stimulatory immunoglobulins of Graves disease. Increasing iodination of Tg also increases its resistance to hydrolysis by acid proteases in the lysosomes. Lithium inhibits thyroid hormone release, although its mechanism of action is poorly understood and may differ from that of iodide.[44]

Deiodination of Iodotyrosines

In addition to the active transport of iodide from the extracellular fluid, intracellular iodide is also generated by the action of the DEHAL1 or iodotyrosine deiodinase (IYD) enzyme. IYD catalyzes nicotinamide adenosine dinucleotide phosphate (NADPH)–dependent deiodination of MIT and DIT, with greater activity against MIT.[45] *Dhal1* transcription is stimulated by cyclic adenosine monophosphate (cAMP) and encodes a membrane protein concentrated at the apical cell surface, which catalyzes NADPH-dependent deiodination of MIT and DIT and recycles iodide. The iodide thereby released is immediately reconjugated to newly synthesized Tg after exiting the apical membrane of the cell. This process is interrupted by the thiourea class of antithyroid drugs that inhibit TPO, such as methimazole (MMI), carbimazole (CBZ), and PTU, thus causing intrathyroidal iodine deficiency in patients receiving these agents.[38] Biallelic mutations in the *IYD* gene have been identified in patients with hypothyroidism, goiter, and an elevated DIT level.[39] Functional studies revealed that the mutations abolished the capacity of IYD to deiodinate MIT and DIT.

Role and Mechanism of Thyrotropin Effects

All steps in the formation and release of thyroid hormones are stimulated by TSH secreted by the pituitary thyrotrophs (see Chapter 8). Thyroid cells express the TSHR, a member of the glycoprotein G protein–coupled receptor family. This protein contains a large extracellular amino-terminal domain, seven membrane-spanning domains, and an intracellular domain that transduces the signal by promoting exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on the α-subunit of G proteins.[46] In fact, the TSHR has been reported to couple to 11 different G protein α-subunits in vitro, and therefore much remains to be learned about signaling through it. Although the TSHR mainly couples to G_s, when activated by high concentrations of TSH (100 times physiologic levels), it couples also to G_q/G_{11}, activating the inositol-phosphate diacylglycerol cascade. The induction of signal via the phospholipase C (PLC) and intracellular Ca^{2+} pathways regulates iodide efflux, H_2O_2 production, and Tg iodination, whereas the signal via the protein kinase A (PKA) pathways mediated by cAMP stimulates growth and regulates iodine uptake and transcription of Tg, TPO, and the NIS mRNAs leading to thyroid hormone production (Table 11.3).[47] Although the discovery that different mutations in various regions of the TSHR molecule result in intrinsic activation and the identification of important domains for intramolecular TSHR signal transduction (see Chapter 12), the precise mechanisms of receptor activation and the early events of TSHR signal transduction are not fully understood.[46] Remarkably, the wild-type TSHR itself displays constitutive activity, a phenomenon that is not shared by the closely related

TABLE 11.2 Human Iodothyronine Selenodeiodinases

Parameter	Type 1 (Outer and Inner Ring)	Type 2 (Outer Ring)	Type 3 (Inner Ring)
Physiologic role	rT_3 and T_3S degradation, the source of plasma T_3 in thyrotoxic patients	Provide intracellular T_3 in specific tissues, a source of plasma T_3	Inactivate T_3 and T_4
Tissue location	Liver, kidney, thyroid, pituitary (?) (not CNS)	CNS, pituitary, BAT, placenta thyroid, skeletal muscle, heart	Placenta, CNS, fetal or adult liver, skeletal muscle
Subcellular location	Plasma membrane	Endoplasmic reticulum	Plasma membrane
Preferred substrates (position deiodinated)	rT_3 (5′), T_3S (5)	T_4, rT_3 (5′)	T_3, T_4 (5)
K_m	rT_3, 10^{-7}; T_4, 10^{-6}	10^{-9}	10^{-9}
Susceptibility to PTU	High	Absent	Absent
Response to increased T_4	↑	↓	↑

BAT, Brown adipose tissue; *CNS,* central nervous system; K_m, Michaelis-Menten constant; *PTU,* 6-*N*-propylthiouracil; rT_3, reverse triiodothyronine; T_3, triiodothyronine; T_3S, T_3SO_4; T_4, thyroxine.

TABLE 11.3 Thyroid Cell Functions Stimulated by Thyrotropin

Function Affected	General Mechanism
Iodide Metabolism	
Increase I^- in follicular lumen	PLC
Delayed increase in NIS expression	cAMP
Increase in thyroid blood flow	↑ Nitric oxide synthesis (↓ cellular iodide)
Increase in I^- efflux from thyroid cell	?
Thyroid Hormone Synthesis	
Hydrogen peroxide	PLC
Thyroglobulin and TPO synthesis	cAMP
NADPH via pentose-phosphate	?
Pathway	
Thyroid Hormone Secretion	
Pinocytosis of thyroglobulin	cAMP
Release of thyroglobulin into plasma via basolateral membrane	cAMP (?)
Thyrocyte Proliferation and Differentiation	
Mitogenesis, differentiation	cAMP, PLC, and $IGFI^-$ and FGF-mediated kinase activation

cAMP, Cyclic adenosine monophosphate; *FGF,* follicular growth factor; *IGF,* insulin-like growth factor; I^-, plasma iodide; *NADPH,* nicotinamide adenosine dinucleotide phosphate; *NIS,* sodium-iodide symporter; *PLC,* phospholipase C; *TPO,* thyroid peroxidase.

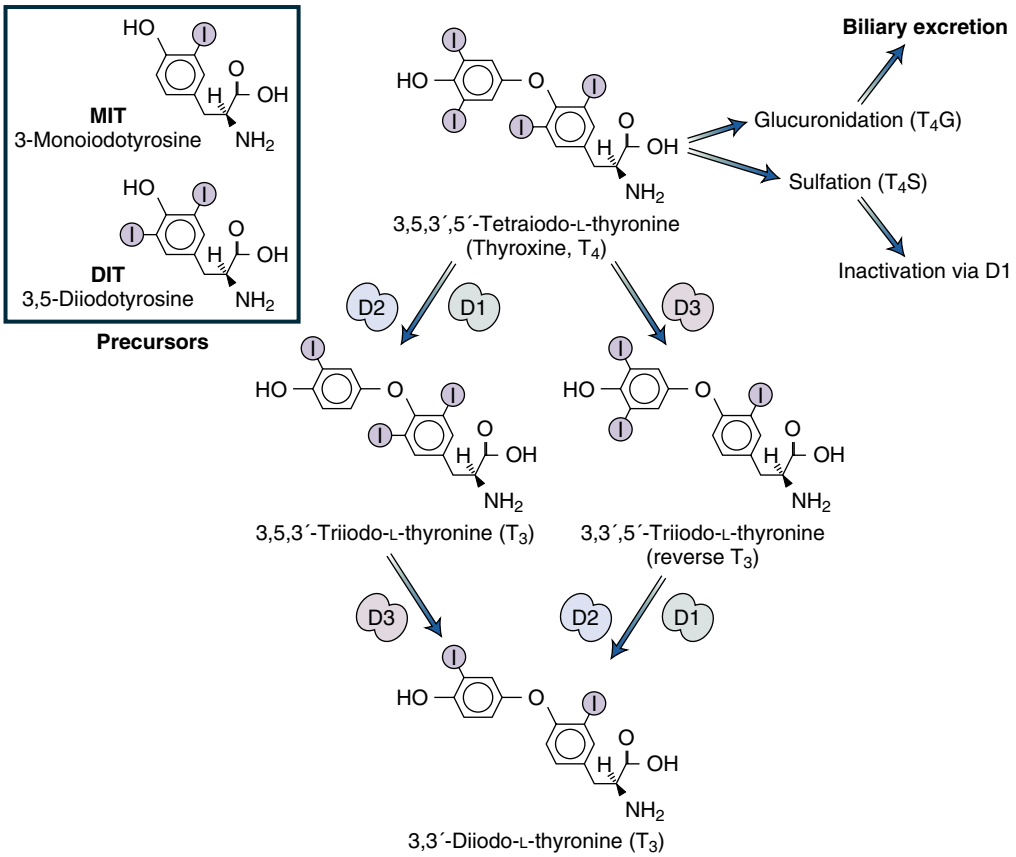
receptors for luteinizing hormone/chorionic gonadotropin (LH/CG) and follicle-stimulating hormone (FSH). This suggests that the unliganded TSHR might be less constrained than other G protein–coupled seven-transmembrane receptors.[46]

The TSHR, in addition to the TSH, also binds TSHR-stimulating antibody (TRAb), thyroid-blocking antibodies (TBAb), and neutral antibodies to the TSHR (see Chapter 12). The closely related luteinizing hormone and chorionic gonadotropin also bind to and activate TSHR signaling when present at high levels.[48] The latter accounts for the physiologic hyperthyroidism of early pregnancy. Besides the thyrocyte, the TSHR is also expressed in a variety of tissues such as osteoclasts, fibroblasts, and adipocytes, as well as retroorbital adipocytes and skin.[48,49] Activating mutations in the TSHR in the germline cause congenital nonautoimmune hyperthyroidism; somatic gain-of-function mutations result in toxic adenomas.[50] In contrast, biallelic inactivating mutations in the TSHR result in congenital hypothyroidism with thyroid hypoplasia or, in the case of partial inactivation, euthyroid hyperthyrotropinemia.[46]

Thyroid Hormones in Peripheral Tissues

Plasma Transport

The metabolic transformations of thyroid hormones in peripheral tissues determine their biologic potency and regulate their biologic effects. Consequently, an understanding of thyroid physiopathology requires knowledge of the pathways of thyroid hormone metabolism. A wide variety of iodothyronines and their metabolic derivatives exist in plasma. Of these, T_4 is highest in concentration and the only one that arises solely from direct secretion by the thyroid gland. In normal humans, T_3 is also released from the thyroid, but approximately 80% is derived from the peripheral tissues by the enzymatic removal of a single 5′ iodine atom (outer ring or 5′ monodeiodination) from T_4.[51] The remaining iodothyronines and their derivatives are generated in the peripheral tissues from T_4 and T_3. Principal among them are 3,3′,5′-triiodothyronine (reverse T_3, or rT_3) and 3,3′-diiodo-L-thyronine (3,3′-T_2) (Fig. 11.2). Trace concentrations of other diiodothyronines, monoiodothyronines, and conjugates thereof with glucuronic or sulfuric acid are also present.[52] Deaminated derivatives of T_4 and T_3 that bear an acetic acid rather than an alanine side chain (tetrac and triac) are also present in low concentrations. 3-Iodothyronamine (T1AM) is an endogenous thyroid hormone derivative with unknown biosynthetic origins.[53] Structural similarities have led to the hypothesis that T1AM is an extrathyroidal metabolite of T_4.



• **Fig. 11.2** Major deiodinative and nondeiodinative pathways of thyroid hormone metabolism. The iodothyronine deiodinases are abbreviated *D1, D2,* and *D3* for type 1, 2, and 3 deiodinases, respectively. *Arrows* refer to monodeiodination of the outer or inner ring of the iodothyronine nucleus, which are termed 5′ or 5 by convention. T_4 is activated by monodeiodination of the phenolic thyronine ring by D1 or D2 to form T_3. Deiodination of the tyrosyl ring by D1 or D3 inactivates T_4 and T_3. This inactivation pathway is markedly favored by sulfation of the phenolic hydroxyl to form T_4SO_4 (T_4S) or T_3SO_4 (T_3S). Glucuronidated T_4 and T_3 (T_4G and T_3G) are excreted into the bile but may be partially reabsorbed after deglucuronidation in the intestine.

The major iodothyronines are poorly soluble in water and thus bind reversibly to plasma proteins. The plasma proteins with which T_4 is mainly associated are TBG and transthyretin (TTR; formerly termed T_4-binding prealbumin [TBPA]) and albumin (Table 11.4). About 75% to 80% of T_3 is bound by TBG and the remainder by TTR and albumin.

Thyroxine-Binding Globulin

TBG is a glycoprotein with a molecular mass of about 54 kDa, about 20% of which is carbohydrate. It is encoded by a 3.8-kb transcript generated by the X chromosomal *TBG (SERPINA7)* gene.[54] The protein sequence of TBG resembles that of the serpin family of serine antiproteases. Because there is one iodothyronine binding site per TBG molecule, the T_4 or T_3 binding capacity of TBG in normal human serum is equivalent to its concentration, which is approximately 270 nmol/L (21 μg/dL). The half-life of the protein in plasma is about 5 days.

Congenital deficiency of TBG is common, occurring in 1 in 5000 newborns, and is associated with the complete absence of the protein in males. L-Asparaginase blocks the synthesis of TBG, which accounts for the low T_4 concentrations in patients receiving this agent.

The glycosylation of TBG influences its clearance from the plasma and its behavior during isoelectric focusing. In estrogen-treated patients, there is an increase in the prevalence of the more acidic bands of TBG. The more highly sialylated TBG is cleared more slowly from plasma than is the more positively charged TBG, because sialylation inhibits the hepatic uptake of glycoproteins. Sera from pregnant patients, women receiving oral contraceptives, and patients with acute hepatitis have increased fractions of acidic TBG. Patients with inherited TBG excess have normal amounts of highly sialylated TBG, as do men and nonpregnant women. Because TBG is the principal T_4-binding and T_3-binding protein, changes in TBG or its binding are paralleled by changes in total plasma T_4 and T_3 even though T_4 and T_3 production is little changed.

Another post-translational modification affecting TBG occurs in septic patients or following cardiopulmonary bypass surgery.[55] TBG is subjected to cleavage by a serine protease released from polymorphonuclear leukocytes, resulting in the release of a 5-kDa carboxy-terminal loop with a consequent decrease in affinity for T_4. An analogous reaction has been described for cortisol-binding globulin, which releases cortisol at the site of inflammation.[56] It has been postulated that the released T_4 might play a critical role

in the response to injury, perhaps by providing a supply of iodine for antibacterial purposes.[55] The cleaved TBG of approximately 49 kDa circulates, and because it binds T_4 with lower avidity, it may explain the increased ratio of free to bound T_4 in acute illness even when TBG saturation studies or immunoassays indicate TBG concentration is normal (see "Thyroid Function During Fasting or Illness").

Transthyretin

TTR is a transport protein for T_4 and retinol-binding protein bound to retinol (vitamin A), hence its name. It consists of four identical polypeptide chains with a total molecular mass of approximately 55 kDa and is not glycosylated. Its concentration in plasma is approximately 4 mmol/L (250 µg/mL). Each mole of TTR binds 1 mole of T_4 with high affinity, and a second T_4 molecule is bound with lower affinity at high concentrations of T_4. Its half-life in plasma is normally about 2 days, but this decreases during illness. TTR is expressed in the choroid plexus, and it is the major thyroid hormone–binding protein in the cerebrospinal fluid (CSF).[57] High levels of TTR have been detected in fetal serum, probably directly produced by placental cells.[58] Targeted TTR gene disruption in mice shows that there is no impairment of uptake of T_4 into the brain, leaving the role of TTR in CSF undefined in regard to thyroid physiology.[59,60]

Variant forms of TTR are associated with familial amyloidotic polyneuropathy.[61] In affected families, the TTR monomer has one of several different point mutations, and TTR accumulates in the amyloid tissue deposits. Neither thyroid dysfunction nor altered vitamin A metabolism has been reported, although there is altered affinity of some of the mutant proteins for T_4. Families with both high-affinity TTR and a few with increased TTR levels have been reported.

TABLE 11.4 Comparison of the Major Human Thyroid Hormone–Binding Proteins

Parameter	Thyroxine-Binding Globulin	Transthyretin	Albumin
Molecular weight of holoprotein (kDa)	54,000	54,000 (4 subunits)	66,000
Plasma concentrations (µmol/L)	0.27	4.6	640
T_4 binding capacity as µg T_4/dL	21	350	50,000
Association constants of the major binding site (L/mol)			
T_4	1×10^{10}	7×10^7	7×10^5
T_3	5×10^8	1.4×10^7	1×10^5
Fraction of sites occupied by T_4 in euthyroid plasma	0.31	0.02	<0.001
Distribution volume (L)	7	5.7	7.8
Turnover rate (% day)	13	59	5
Distribution of iodothyronines (% protein)			
T_4	68	11	20
T_3	80	9	11

T_3, Triiodothyronine; *T_4*, thyroxine.

Competition for T_4 and T_3 Binding to TBG and TTR by Therapeutic Agents

The TBG binding site has an affinity for T_3 that is about 20-fold less than that for T_4 (see Table 11.4). Binding of T_4 and T_3 by TBG is inhibited by phenytoin,[62] salicylate,[63] salsalate, furosemide, fenclofenac, and mitotane. The affinity of these compounds for TBG is much weaker than is that of the iodothyronines, but their concentration in plasma is sufficiently high to compete with T_4 and T_3 binding and reduce total hormone levels, although free T_4 remains normal. Because all methods used for estimating the free fractions of T_4 and T_3 in human serum except ultrafiltration dilute the serum, euthyroid patients receiving these drugs may appear to have low total and free T_4 or T_3, whereas in vivo the free fraction is normal.

Albumin

The affinity of albumin for T_4 and T_3 binding is much lower than that of either TBG or TTR, but the high concentration of this protein results in the binding of 10% of the plasma thyroid hormones (see Table 11.4). Changes in albumin concentration per se have little influence on total hormone levels, unless accompanied by alterations in TBG and TTR, all three of which are synthesized in the liver. Hepatic failure or nephrotic syndrome leads to decreases in the plasma concentration of all three, and the serum albumin concentration in patients with these illnesses may serve as a surrogate for estimating TBG concentrations.

The role of albumin in thyroid physiology becomes clinically important in patients with familial dysalbuminemic hyperthyroxinemia ($FDHT_4$) and hypertriiodothyroninemia ($FDHT_3$).[64,65] Both are dominantly inherited and characterized by a high concentration of total T_4 or T_3 (FDH). However, the free hormone concentrations remain normal and the patients are euthyroid. Recognition of these variants is important because affected individuals are at risk of erroneous treatment. Such patients may have a confusing pattern of test results, especially when analogue methods or labeled T_3 are used to estimate the free T_4 or T_3 (see Chapter 4).

Other Plasma Thyroid Hormone–Binding Proteins

Between 3% and 6% of plasma T_4 and T_3 are bound to lipoproteins. The T_4-binding lipoprotein is a 27-kDa homodimer with an affinity for T_4 that is lower than that of TBG. This binding is of uncertain physiologic significance but could play a role in targeting T_4 delivery to specific tissues.

Free Thyroid Hormones

Because most of the circulating T_4 and T_3 is bound to TBG, its concentration and degree of saturation are the major determinants of the free fraction of T_4. Binding of the thyroid hormones to the plasma proteins alters their metabolism. The negligible urinary excretion of T_3 and T_4 is due to the limited filterability of the hormone-binding protein complexes at the glomerulus. In vitro, the interaction between the thyroid hormones and their binding proteins conforms to a reversible binding equilibrium that can be expressed by conventional equilibrium equations. For the formulations that follow, T_4 is used as the prototype,

with the understanding that similar interactions apply in the case of T_3. The interaction between T_4 and TBG can be expressed as follows:

$$T_4 + TBG \overset{K_2}{\rightarrow} T_4 \bullet TBG$$

where TBG represents the *unoccupied* binding protein, k_a the equilibrium association constant for the interaction, and T_4 the concentration of *free* T_4; T_4 • TBG is T_4 bound to TBG (~68% of total T_4 is bound to TBG).

Rearranging, we can express this as:

$$\frac{T_4 \bullet TBG}{(T_4)\,(TBG)} = k_a$$

$$\frac{T_4}{T_4 \bullet TBG} = \frac{1}{(TBG)\,k_2}$$

Thus the free fraction of T_4 (T_4/T_4 • TBG) is inversely proportional to the concentration of *unoccupied* TBG binding sites. Estimates of the free T_4 concentration in serum can be generated by direct or indirect assays. In normal serum, the free T_4 is approximately 0.02% of the total (about 20 pmol/L, 1.5 ng/dL). The approximately 20-fold lower affinity of TBG for T_3 results in a higher proportion of unbound T_3 (0.30%) (Table 11.5; also see Table 11.4).

It is the free hormone that is available to the tissues for cellular uptake and feedback regulation that induces its metabolic effects and that undergoes deiodination or degradation. The bound hormone acts merely as a reservoir. It follows that the concentration of the free hormone is the determinant of the metabolic state, and it is this concentration that is defended by homeostatic mechanisms. If a change in TBG occurs, the free T_4 concentrations and T_3 concentrations can be maintained at normal levels only if the bound hormone changes in the same direction. For example, when TBG concentrations are increased by administration of estrogen, the free T_4 reduction reduces T_4 clearance, allowing an increase in the plasma total T_4 concentration. This is an iterative process that eventually would normalize the free T_4 at a new equilibrium without a change in T_4 secretion rate. The transient decrease in free thyroid hormones also slightly reduces the negative feedback on the hypothalamic-pituitary-thyroid axis, which causes an increase in thyroid hormone production as an additional compensation.[66]

The preceding formulation is termed the *free thyroid hormone hypothesis*.[67,68] If it is free hormone that is available for cellular entry, what is the role, if any, of the hormone-binding proteins? Protein binding facilitates the distribution of the hydrophobic thyroid hormones throughout the vascular system. For example, if a protein-free solution containing tracer T_3 is perfused through rat liver via the portal vein, there is a steep concentration gradient with a decreasing quantity of T_3 in the solution as the distance from the center of the portal lobule increases. In fact, virtually all of the T_3 is taken up by the first cells to be contacted by the bolus. In contrast, if albumin is added to the perfusate, the distribution of tracer is uniform throughout the lobule. Both influx and efflux of thyroid hormone from tissues are rapid. Thus intracellular free T_3 and T_4 are in equilibrium with the free hormone pool in plasma although transporter activity and metabolism will influence the magnitude of the ratio. In the steady state, the rate of T_3 and T_4 metabolism, not the dissociation rate from plasma proteins, is rate limiting in the exit of hormones from the plasma.

TABLE 11.5 Comparison of Triiodothyronine (T_3) and Thyroxine (T_4) in Humans

Parameter	T_3	T_4
Production rate (nmol/day)	50	110
Fraction from thyroid	0.2	1.0
Relative metabolic potency	1.0	0.3
Serum concentration		
Total (nmol/L)	1.8	100
Free (pmol/L)	5	20
Fraction of total hormone in free form ($\times 10^{-2}$)	0.3	0.02
Distribution volume (L)	40	10
Fraction intracellular	0.64	0.15
Half-life (days)	0.75	6.7

To convert T_4 from nmol/L to μg/dL (total) or pmol/L to ng/dL (free), divide by 12.87. To convert T_3 from nmol/L to ng/dL (total) or pmol/L to pg/dL (free), multiply by 65.1.

T_4 and T_3 Transport Across Cell Membranes and Intracellular T_3 Binding

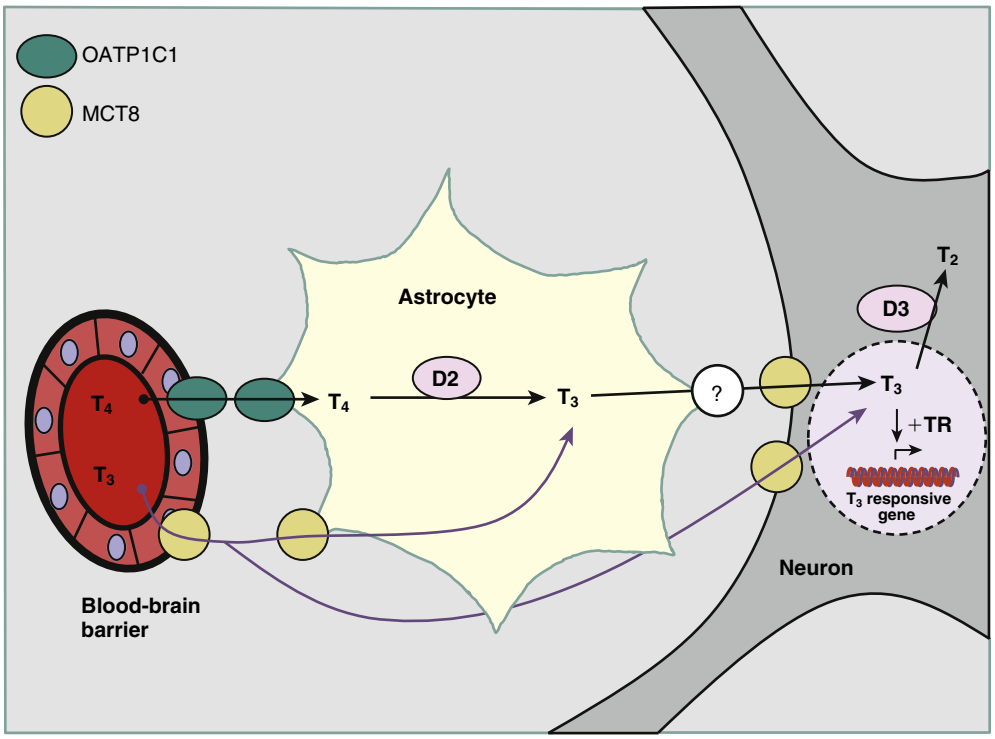
It has been assumed for a long time that transport of iodothyronines across the plasma membrane occurs by passive diffusion, but it has become increasingly clear that cellular uptake and efflux of thyroid hormone are mediated by transporter proteins.[69,70] Several specific thyroid hormone transporters have been identified, including monocarboxylate transporter 8 (MCT8), MCT10, organic anion transporting polypeptide 1C1 (OATP1C1), and L-type amino acid transporters LAT1 and LAT2. MCT8 and MCT10 are expressed in multiple tissues, where they facilitate transport of T_3, T_4, rT_3, and T_2 across cell membranes; OATP1C1 is expressed predominantly in the brain and transports preferentially T_4, wherein it may mediate the entry of T_4 into the astrocytes. A defect in a single thyroid hormone transporter molecule, MCT8, has been shown to cause a severe developmental neurologic phenotype.[71] The Allan-Herndon-Dudley syndrome (AHDS) is an X-linked condition characterized by severe mental retardation, dysarthria, athetoid movements, muscle hypoplasia, and spastic paraplegia associated with an elevated serum T_3.[72] All patients tested with this syndrome have mutations in the *MCT8* gene. More than 200 individuals belonging to some 100 families of all races and diverse ethnic origins harboring more than 70 different mutations have been identified.[73] Although most mutations result in a complete functional inactivation of the MCT8 protein, significant residual activity was observed with a number of *MCT8* mutations, some of which are associated with a milder clinical phenotype.[74] MCT8-null mice, despite the presence of marked increased T_3 levels, lack any overt neurologic abnormalities, a rather unexpected finding in light of the severe human phenotype.[75,76] The discrepancy with the human phenotype is now explained by the fact that mice express OATP1C1 at the blood-brain barrier, which compensates for the lack of transport by MCT8.[77] Coexistence of thyroid hormone excess and deprivation in different tissues is a distinct characteristic of this syndrome. Tissues expressing transporters other than

MCT8, such as liver and kidney, respond to the high circulating T_3 levels resulting in a local hyperthyroid state, whereas tissues depending on MCT8 for thyroid hormone entry into cells, such as the brain, are hypothyroid.[73,78] Two therapeutic options, PTU combined with levothyroxine T_4 (L-T_4)[73] and a thyromimetic compound, diiodothyropropionic acid (DITPA), which is not dependent on MCT8 for cellular entry, have been used to treat several patients harboring *MCT8* gene mutations.[79,80]

Another transporter specific for T_4, OATP1C1 (a member of the organic anion transporting polypeptide family), is expressed in capillaries throughout the brain, suggesting it may be involved in the transport of T_4 across the blood-brain barrier.[71] Taken together, these results suggest that the supply of T_3 to neurons may occur according to the schema shown in Fig. 11.3.[81] T_4 is transferred into the choroid plexus or into tanycytes via the action of OATP1C1, which is negatively regulated in brain capillaries by thyroid hormone. In the tanycyte or astrocyte, T_4 is converted to T_3 by the type 2 iodothyronine deiodinase (D2) and exits the cell, possibly via the MCT8/MCT10 transporters, where it becomes available for neuronal uptake, also via MCT8.[82,83] Neurons express the type 3 deiodinase (D3), which prevents activation of T_4 and catalyzes degradation of T_3 (see "Iodothyronine Deiodination"). This would provide a logical explanation of the association of the mutations in MCT8 with attention-deficit/hyperactivity disorder (ADHD), although it remains puzzling why the neurologic manifestations of this condition are so different from those seen in patients with untreated congenital hypothyroidism or severe iodine deficiency (see Chapter 13).

The transport field has become more complex as evidence accumulates of tissue-specific as well as generalized iodothyronine transporters belonging to a number of different transporter protein families. Each of these has many members with small variations in structure, which alter the specificity of the target substance. A thorough review of this topic is beyond the scope of this chapter, and the interested reader is referred to excellent reviews for further information.[69,70]

In most cells, about 90% of the intracellular T_3 is located in the cytosol. The known exception is in the pituitary, where approximately 50% of the intracellular T_3 is present in the nucleus. The mechanisms determining this distribution are still unknown, but it would not be surprising if there were active transport of thyroid hormones in and out of the nucleus and between other intracellular compartments. An intracellular T_3-binding protein (mu-crystallin) has been identified, which is expressed at high levels in the human brain, cochlea, and heart but is widely distributed. Disruption of the *Crym* gene encoding mu-crystallin leads to congenital deafness.[84] This or similar proteins may also play a role in the subcellular localization of the active hormone.



• **Fig. 11.3** Potential pathways for entry of T_3 into the central nervous system. Thyroid hormones are transported through the blood-brain barrier (organic anion transporting polypeptide [OATP]) or the blood-CSF barrier (OATP and MCT8). In the astrocytes and tanycytes, T_4 is converted to T_3, which then enters the neurons, possibly through MCT8. In the neurons, both T_4 and T_3 are degraded by D3. T_3 from the tanycytes may reach the portal vessels in the median eminence. Other transporters may be present on the astrocyte or tanycyte membranes. In most cases, the transport could be bidirectional, although only one direction is shown. The interaction of T_3 with the thyroid hormone receptor (TR) bound as a heterodimer with retinoid X receptor to the thyroid hormone–response element, often in the 5′ flanking region of a T_3-responsive gene, causes either an increase or a decrease in the transcription of that gene. This leads to parallel changes in the concentrations of critical proteins, thus producing the thyroid hormone response characteristic of a given cell. *D2* and *D3,* type 2 and type 3 iodothyronine deiodinases; *MCT8,* the monocarboxylate transporter 8; *T_2,* diiodothyronine; *T_3,* triiodothyronine; *T_4,* thyroxine.

Iodothyronine Deiodination

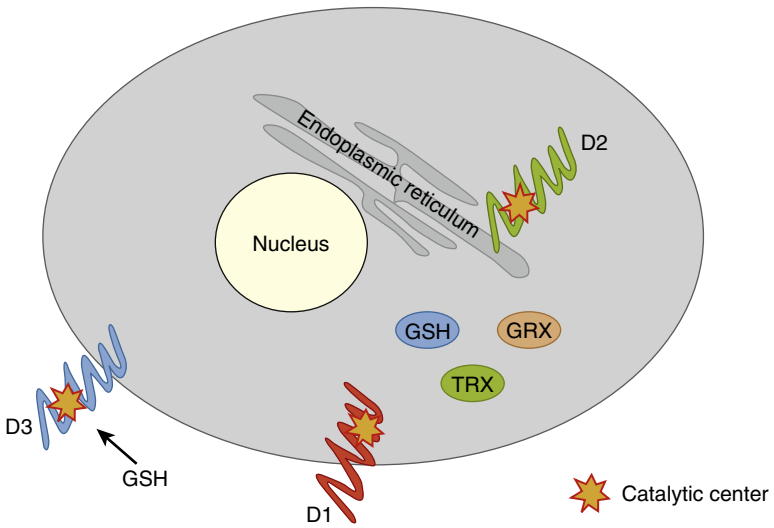
The most important pathway for T_4 metabolism is its outer ring (5′) monodeiodination to the active thyroid hormone, T_3. This reaction is catalyzed by the type 1 and 2 deiodinases (D1 and D2) and is the source of more than 80% of the circulating T_3 in humans (see Fig. 11.2). Inner ring deiodination, an inactivating step, is catalyzed primarily by D3, which inactivates T_3 and prevents activation of T_4 by converting it to rT_3 (see Fig. 11.2).[51,85] The structures of the three human deiodinases are similar, all being homodimers and integral membrane proteins and all requiring a thiol cofactor for successful catalysis (Fig. 11.4). They contain the rare amino acid selenocysteine in the active catalytic center (see Table 11.2). Selenocysteine has nucleophilic properties that make it ideal for catalysis of oxidoreductive reactions such as iodothyronine deiodination and the reduction of H_2O_2 by another family of selenoenzymes, the glutathione peroxidases.[86,87] The crystal structure of the catalytic domain of mouse deiodinase 3 (Dio3) revealed a close structural similarity to atypical 2-cysteine peroxiredoxin(s).[88] Selenium is thought to be the iodine acceptor during deiodination reactions. Mutagenesis of selenocysteine in D1 to cysteine, that is, replacing selenium with sulfur, reduces the enzyme velocity by approximately 200-fold. The presence of selenocysteine has implications beyond catalytic activity, considering that the cellular processes for synthesizing selenoproteins are complex and inefficient.[51] This is accomplished by a combination of a specific structural feature, the selenocysteine insertion sequence (SECIS) element, in the 3′ untranslated region of the mRNAs, encoding these proteins together with a specific group of selenocysteine incorporating gene products. All these elements are required for the complex cellular function by which the normal STOP codon UGA is recognized as the specific codon for the insertion of the selenocysteine residue during protein translation.[89,90]

Biallelic mutations in SECIS-binding protein 2 (SBP2) lead to abnormal thyroid function tests with elevated TSH, T_4, FT_4, and reverse T_3 but low total and free T_3 concentrations and can be associated with short stature and delayed bone age.[91]

Enzymology and Regulation of the Selenodeiodinases

Although both D1 and D2 activate T_4, they have several important differences (see Table 11.2). D1 catalyzes both 5′ and 5 deiodination of T_4 to form T_3 and rT_3, respectively, although the Michaelis-Menten constant (K_m) for these reactions is approximately 3 orders of magnitude greater than that of D2 and D3 for this substrate. The preferred substrates of D1 are rT_3 (5′ deiodination) and T_3SO_4 (5 deiodination). D1 is inhibited by PTU, unlike D2 or D3. D1 also differs from D2 in being markedly increased by excess thyroid hormone through increased gene transcription, whereas D2 mRNA and protein are reduced by thyroid hormones. D2 has a half-life of only 20 to 30 minutes but that of D1 and D3 is more than 12 hours. This is due to the rapid ubiquitination of D2, a process that is accelerated by interaction with its substrates T_4 or rT_3. D1 and D3 are not thought to be ubiquitinated.

The intracellular location of D2 close to the nucleus gives the T_3 formed by its catalytic action better access to the nucleus than that formed by D1.[92] The T_3 produced by D2 is especially effective in entering the nucleus and binding to thyroid hormone receptors (TRs), a property explained by its location in the endoplasmic reticulum (ER) (see Fig. 11.4). D1, on the other hand, is located in the plasma membrane, and the T_3 produced by this enzyme preferentially enters the plasma pool.[51] Studies with deiodinase inhibitors and cofactors differentially able to cross the cell membrane indicate that the D3 active center is outside the cell and that of D2 and D1 are intracellular (see Fig. 11.4).[93] This makes D2 especially important for regulating the hypothalamic-pituitary-thyroid axis, where its activity increases in response to a decrease in serum T_4 concentrations, such as occurs in iodine deficiency or early autoimmune thyroid disease, well before the serum T_3 falls. If the decrease in plasma T_4 is too great to be compensated for by the increase in D2 activity in the hypothalamus and thyrotrophs, an increase in TRH and TSH will occur to stimulate the thyroid. For this reason, D2 has principally been thought of as an enzyme that provides intracellular T_3, but there is increasing evidence that



• **Fig. 11.4** Predicted topologies of the three iodothyronine deiodinases. The deiodinases are integral membrane proteins that require a thiol cofactor for catalytic activity. The type 1 deiodinase (D1) is in the plasma membrane and type 2 (D2) is localized in the endoplasmic reticulum. The active centers of D1 and D2 are in the cytosol and depend on intracellular thiols such as reduced glutathione (GSH), thioredoxin (TRX), and glutaredoxin (GRX) for catalytic activity. The type 3 deiodinase (D3) is also anchored in the plasma membrane but has access to extracellular thiols.

D2 could also contribute to plasma T_3. Recent studies in rodents suggest that D2 ubiquitination in the hypothalamus is relatively less than in other D2-expressing tissues. As a result, the hypothalamus is wired to have increased sensitivity to T_4 and allows the physiologic TSH response to minimal variation in serum T_4.[94] On the other hand, in thyrotoxicosis, the threefold to fourfold increase in D1, particularly in the thyroid, and the reduced D2 make D1 the major extrathyroidal source of T_3. This explains why the D1-inhibitor PTU causes a much more rapid fall in circulating T_3 than does methimazole in the patient with Graves disease.[41,42]

A single-nucleotide polymorphism, A/G, in the *DIO2* gene, predicts a threonine (Thr) to alanine (Ala) substitution at codon 92 (D2 Thr92Ala).[95] This is present in about 20% of the Caucasian population. This polymorphism has been found, in some studies, to be associated with insulin resistance in obese patients, bipolar mood disorder, psychologic well-being, mental retardation, hypertension, and risk for osteoarthritis, although other studies failed to find such association. It is not yet clear whether this D2 polymorphism impairs its catalytic efficiency in vivo.[96,97] A recent study indicates that the D2 Thr92Ala polymorphism reduces D2 enzyme activity and serum T_3 levels in thyroid-deficient patients.[98]

In the opposite direction, D2 has been found overexpressed in a few follicular carcinomas. In these patients, a reduced T_4:T_3 ratio has been found and is likely due to the increased D2-mediated T_4 to T_3 conversion.[43]

D3 is the most important thyroid hormone–inactivating enzyme catalyzing deiodination of the inner ring of both T_3 and T_4.[99,100] D3 activity has been identified in only a limited number of postnatal tissues, including placenta and uterine endometrium, the CNS (in which it is primarily in neurons), and skin.

Much higher D3 expression has been demonstrated in various fetal tissues such as liver, brain, placenta, uterus, and umbilical arteries and veins. However, in adult tissues, D3 expression may be reinduced under conditions in which cellular proliferation is required.[101] In the adult, D3 has been identified in some malignant cell lines and in a number of human tumors, including astrocytomas, oligodendromas, gliosarcomas, glioblastomas, TSH-secreting pituitary adenomas, colon cancers,[102] and basal cell carcinomas.[103] Tumoral D3 activity can be robust, and the highest D3 activity reported in any human tissue to date has been in infantile hemangiomas. In infants with extensive hepatic hemangiomas, D3 may overwhelm the secretory capacity of the infant's thyroid, causing hypothyroidism, a syndrome termed *consumptive hypothyroidism*.[104] Although most patients with consumptive hypothyroidism have hemangiomas, it now has become evident that it can occur in other types of tumors, including gastrointestinal stromal tumors.[105] Patients with consumptive hypothyroidism may only represent the extreme of a clinical spectrum of hypothyroidism due to dysregulated thyroid hormone metabolism in malignant tissues. *DIO3* gene expression is increased by thyroid hormone at a transcriptional level.[106]

Gene-targeting studies have begun to provide further insights into the physiologic roles of the deiodinases in mammals.[107] Inactivation of the *Dio2* gene results in a phenotypically normal mouse with an elevated serum T_4, normal serum T_3, and elevated serum TSH.[85,108] These animals have hypothalamic-pituitary resistance to T_4, impaired auditory function, impaired thermogenesis in response to cold stress, impaired muscle regeneration,[109] and relatively subtle defects in neurologic function. These findings are all consistent with the expectations based on earlier studies indicating an important role for D2 in brown fat cell function, cochlear maturation, and neurologic development. The intracellular balance between D2 and D3 is dynamically regulated and plays a central role in controlling muscle homeostasis and regenerative potential.[110] Mice with targeted inactivation of *Dio1* are phenotypically normal but also have an elevated serum T_4 and a normal serum T_3, but TSH is normal.[111] The most striking finding in the D1-deficient mouse is a marked shift in the T_4 clearance pathway from deiodinative to biliary/fecal clearance. Interestingly, mice lacking both of the activating deiodinases D1 and D2 are still capable (by increasing TSH and thyroidal T_3 secretion) of maintaining normal T_3 concentrations in serum and do not suffer from systemic hypothyroidism, indicating that thyroidal T_3 production can guarantee T_3 homeostasis at least in rodents.[85,108] Mice with targeted inactivation of the *Dio3* gene show profound abnormalities. They have impaired fertility and develop central hypothyroidism in adult life, presumably due to hypothalamic thyrotoxicosis during developmental programming.[112]

Quantitative and Qualitative Aspects of Thyroid Hormone Metabolism

Thyroid Hormone Turnover

In the normal adult, T_4 has a distribution volume of approximately 10 L (see Table 11.5). Because the concentration of total T_4 in plasma is approximately 100 nmol/L (~8 μg/dL), the extrathyroidal T_4 pool is approximately 1 μmol (800 μg). In the adult, the fractional rate of turnover of T_4 in the periphery is about 10% per day (half-life, 6.7 days). Thus about 1.1 L of the peripheral T_4 distribution space is cleared of hormone daily, a volume containing approximately 110 nmol (85 μg) of T_4.

The kinetics of T_3 metabolism differ from those of T_4, partly because of its 10-fold to 15-fold lower affinity for TBG. The volume of distribution of T_3 in the normal adult is about 40 L, about four times that of T_4, and its fractional turnover rate is approximately 60% per day. At a mean normal serum T_3 concentration of 1.8 nmol/L (120 ng/dL), 50-fold lower than T_4, the daily production of T_3 is approximately 50 nmol (33 μg) or about 46% that of T_4 (see Table 11.5). The rapid metabolic clearance rate of the product of inner ring T_4 deiodination, rT_3, and the low concentration in plasma (0.25 nmol/L, 15 ng/dL) combine to yield daily production rates for rT_3 of about 45 nmol. Thus about 80% of T_3 and all of rT_3 production in humans can be accounted for by peripheral deiodination of T_4, findings consonant with the high ratio of T_4 to T_3 (15:1) and rT_3 (100:1) in human Tg. Of the T_3 generated via T_4 5′ deiodination in euthyroid humans, only about 70% is inhibited by PTU, consistent with a significant contribution of D2-dependent T_3 production.[113] Although much of the T_3 and rT_3 produced from T_4 in peripheral tissues exits those tissues and enters the blood, an uncertain fraction of both are degraded intracellularly before their exit. As discussed later, in some D2-containing tissues, such as the pituitary, a significant fraction of T_3 in the cell nucleus is derived from intracellular T_4 deiodination to T_3, rather than from the plasma. This is particularly true in the thyrotroph.[114]

Other pathways are also involved in T_4 and T_3 metabolism. In humans, T_4 undergoes glucuronidation of the phenolic hydroxyl by the uridine diphosphate glucuronyl transferases (UDPGTs), but only minimal amounts of T_3 undergo this process (see Fig. 11.2). This pathway is clinically significant because certain pharmacotherapeutic agents may enhance glucuronide conjugation through induction of UDPGT, leading to biliary excretion of T_4-glucuronide (T_4-G) into the intestine.[115] These agents include

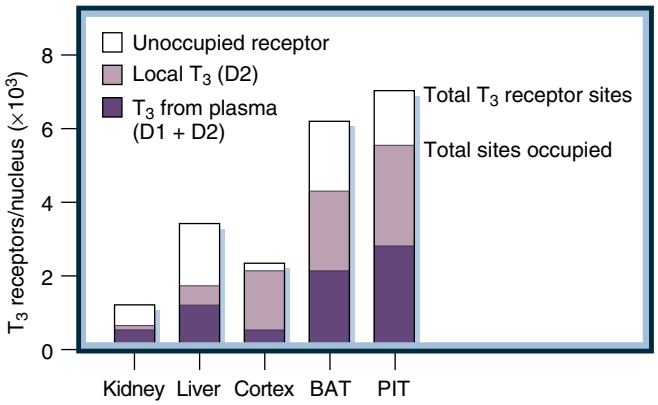
phenobarbital, phenytoin, rifampin, and possibly certain of the synaptosomal serotonin reuptake inhibitors, such as sertraline. Because T_4-glucuronide may not be easily reabsorbed from intestinal contents, the clinical significance of this pathway is that therapy with such agents will generally increase levothyroxine requirements. In patients with an intact thyroid, this will not be apparent because internal adjustments will increase the T_4 production rate to compensate for the accelerated biliary excretion. In patients with hypothyroidism, however, an increase in levothyroxine dosage will often be required.

Sources of Intracellular T_3

In view of the differential tissue distribution of the various deiodinases, their different K_m values, and differential regulation, it is not surprising that tissues may derive intracellular T_3 via different pathways (Fig. 11.5). In several rat tissues, including tissues expressing D1 such as kidney and liver, most of the nuclear T_3 is derived from plasma T_3. In D2-containing tissues, such as the rat cerebral cortex, pituitary, brown fat, and skeletal muscle, D2 functions as an additional intracellular source of T_3, such that the nuclear T_3 concentration will be higher given the combination of T_3 from the plasma and the T_3 that is locally converted from T_4. In these tissues, half or more of intracellular T_3 is generated locally from T_4 within the tissue. In the CNS, the D2-generated T_3 in neurons is likely to derive from paracrine sources in tanycytes and astrocytes (see Fig. 11.3). In the rat, the tissues that depend on D2 for nuclear T_3 are those in which a constant supply of thyroid hormone is critical for either normal development (cerebral cortex), thyroid gland regulation (pituitary), or survival during cold stress (brown adipose tissue). These tissues are also characterized by a high degree of saturation of the nuclear T_3 receptors in comparison to tissues such as the liver and kidney in which nuclear T_3 receptor sites are only about 50% occupied at normal serum T_3 concentrations (see Fig. 11.5).

Intracellular D2-catalyzed T_3 production has important implications for thyroid hormone physiology. First, because the T_3 produced from T_4 occupies a significant fraction of the receptors in those tissues, changes in either serum T_4 or serum T_3 can change receptor occupancy. However, because a fall in T_4 will also increase D2 protein half-life by decreasing the rate of ubiquitination and its proteasomal degradation, a rise in D2 activity mitigates the impact of a reduction of serum T_4 in D2-expressing tissues, helping to maintain T_3 homeostasis.[51] The requirement for both T_3 and T_4 for normal saturation of pituitary and CNS T_3 receptors permits a response of the hypothalamic-pituitary axis to a reduction in plasma T_4, which is the earliest manifestation of iodine deficiency or primary hypothyroidism (see "Regulation of Thyroid Function"). Because the *Dio2* gene is positively regulated by cAMP, D2 activity and T_3 production increase rapidly in brown adipose tissue under stimulation by the sympathetic nervous system. This response is critical to adaptive thermogenesis during cold exposure in the human neonate and lifelong in the rodent.[116]

At the same time, tissues expressing D3 have lower T_3 concentrations than would be expected from the plasma contribution (Fig. 11.6); thus D3-expressing tissues have a gene expression profile similar to hypothyroid cells. This is explained by the inactivation of T_3 and T_4 that takes place immediately after these hormones enter the cell. The D3-mediated reduction in T_3 levels likely occurs in several physiologic (development, regeneration) or pathologic settings (cancer cells, inflammation, myocardial infarction) in which D3 is upregulated.[117]



• **Fig. 11.5** Schematic diagram of the origin of the specifically bound nuclear T_3 in various rat tissues. Data are derived from studies in which the sources of specifically bound nuclear T_3 in rat tissues were estimated using double-isotope labeling techniques. In tissues in which the receptor saturation is significantly greater than 50%, the additional T_3 is provided by D2-catalyzed conversion of thyroxine (T_4) to T_3. T_3 in rat plasma is derived from thyroid secretion (~40%) with the remainder from D1-catalyzed and D2-catalyzed T_4 to T_3 conversion. *BAT,* brown adipose tissue; *D1* and *D2,* type 1 and 2 iodothyronine deiodinases; *PIT,* pituitary; *T_3,* triiodothyronine.

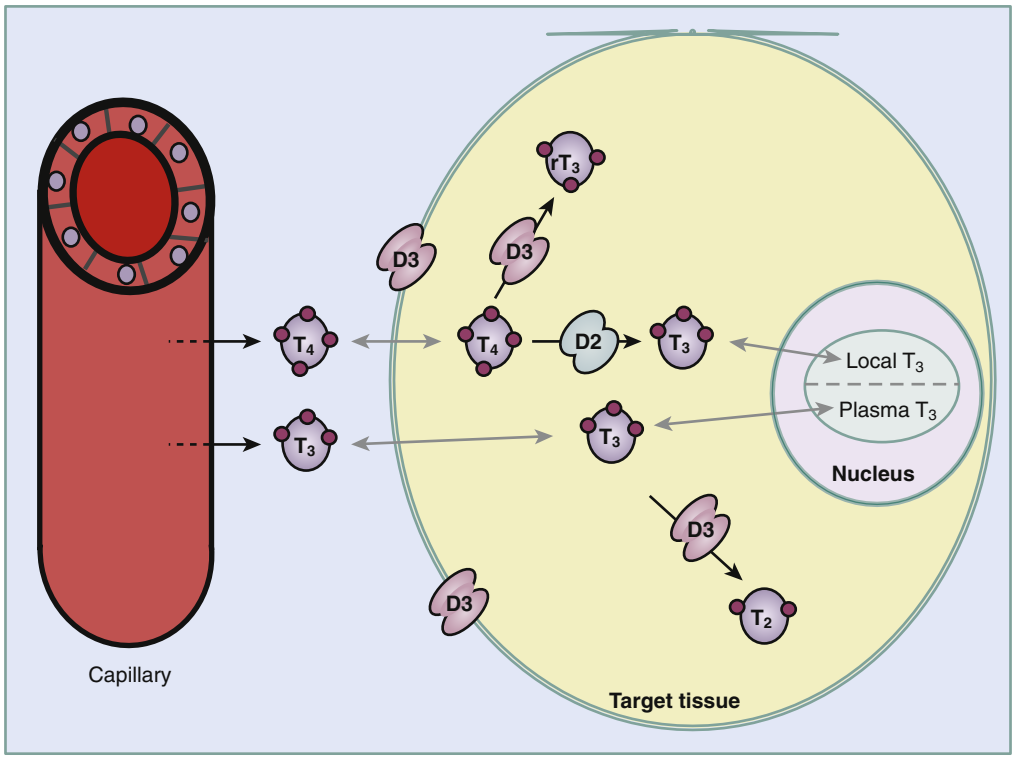
Pharmacologic Agents Inhibiting Thyroid Hormone Deiodination

A number of commonly used pharmacologic agents have significant effects on thyroid hormone deiodination. PTU inhibition of D1 has been mentioned earlier. The antiarrhythmic drug amiodarone shares sufficient structural similarity with T_4 that it can inhibit deiodination of T_4 and rT_3 by D1 and possibly by D2 (Fig. 11.7). This causes an increase in plasma T_4 to maintain serum T_3 in the normal range. Also, levels of TSH increase within the first weeks of therapy but gradually return to normal as the thyroid axis reequilibrates.[118] The T_4 and rT_3 metabolic clearance rates are reduced by 20% to 25%, with a reduction in the fractional T_4 to T_3 conversion rate of about 50%. Amiodarone also inhibits the active transport of T_4 and T_3 into hepatocytes, and the drug or one of its degradation products may interfere with T_3 binding to TRs.[119]

The effects of amiodarone resemble those observed with the iodoaniline derivatives formerly used for gallbladder visualization (see Fig. 11.7). Iopanoic and iopodipic acid inhibit the deiodinases by competing with the iodothyronine substrates.[51] These agents are useful in the acute treatment of patients with severe hyperthyroidism, but they are no longer available for clinical use in the United States.

High dosages of glucocorticoids (10 times replacement) will acutely reduce the ratio of T_3 to T_4 in plasma, suggesting that conversion of T_4 to T_3 is blocked. The ratio of rT_3 to T_4 increases, raising the possibility that D3 action is also increased.[120] These effects resolve during long-term therapy such that thyroid function is little affected and thyroid hormone requirements are not increased by chronic glucocorticoid therapy.

Recombinant growth hormone increases the circulating T_3:T_4 ratio. Growth hormone deficiency is associated with a decrease in the ratio of T_3 to T_4 in serum, possibly associated with a decrease in outer ring deiodination. As expected, dietary selenium deficiency also inhibits the synthesis of D1 in humans.[121]



• **Fig. 11.6** Schematic diagram of thyroid hormone activation and inactivation in a cell expressing the iodothyronine deiodinases D2 and D3. The triiodothyronine (T_3) that enters the cell can either be deiodinated to 3,3′-diiodothyronine (T_2) or enter the nucleus and bind to the thyroid hormone receptor. An additional source of T_3 is that generated by outer ring deiodination of thyroxine (T_4) within the cell. *rT_3*, reverse T_3.

Mechanism of Thyroid Hormone Action

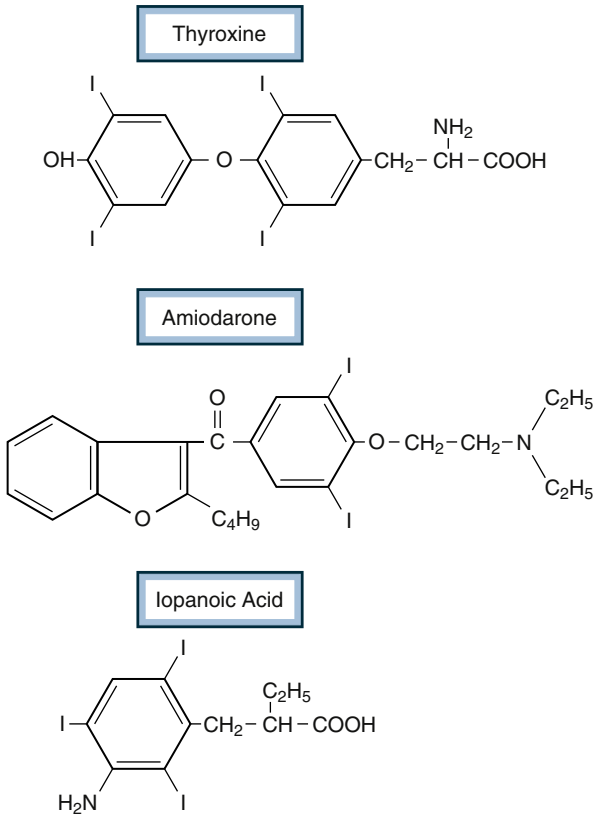
Thyroid hormone acts by binding to specific nuclear TRs, which in turn bind to DNA, usually as a heterodimer with retinoid X receptor (RXR) at specific sequences (thyroid hormone response elements [TREs]) dictated by the DNA binding-site preferences of the RXR-TR (or TR-TR) complex. In humans, there are two TR genes, α and β, found on different chromosomes (TRα, chromosome 17; TRβ, chromosome 3). Several alternatively spliced gene products from each of these genes form both active and inactive gene products. The active proteins are $TR\alpha_1$ and TRs β_1, β_2, and β_3.[122] The protein structure of TRs includes three major functional domains, one binding DNA, one binding ligand, and a major transcriptional activation domain in the carboxy-terminus.

The general mechanism by which nuclear receptor–activating ligands such as T_3 produce their effects is discussed in Chapter 2. T_3 has a 15-fold higher binding affinity for TRs than does T_4, explaining its function as the active thyroid hormone.

There are tissue-specific preferences in expression of the various TRs, suggesting that they subserve different functions in different tissues.[123] $TR\alpha_1$ mRNA is expressed in the brain and brown adipose tissue and also in skeletal muscle, the gastrointestinal tract, lungs, and heart. In general, TRβ, particularly $TR\beta_2$, is thought to be important in the hypothalamus and pituitary, in which regulation of thyroid function occurs.[124] $TR\beta_1$ is expressed in all tissues, although its mRNA is especially highly expressed in the kidney and liver. $TR\beta_2$ is also expressed in the cochlea and the retina. $TR\beta_3$ mRNA is expressed at very low levels but is more abundant in the liver, kidneys, and lungs in comparison with other tissues. In addition to differences in the amino-terminus between $TR\beta_1$ and $TR\alpha_1$, the two proteins are encoded by distinct genes and hence under the regulation of different promoters, which can function in tissue-specific patterns. $TR\beta_2$ is downregulated by T_3, whereas $TR\alpha_1$ mRNA expression is not affected.[125]

Experiments in which TRα and TRβ have been inactivated illuminate their different physiologic roles. Disruption of the *Thrb* gene (encoding both $TR\beta_1$ and $TR\beta_2$) in mice causes deafness, a marked reduction in feedback sensitivity of the hypothalamic-pituitary-thyroid axis, and a decrease in hepatic D1. Thus these mice have significant elevations in both TSH and thyroid hormones similar to those in families with resistance to thyroid hormone (RTH), in which TRβ mutations markedly reduce its binding affinity for T_3. RTH is dominantly inherited and characterized by resistance to thyroid hormone in tissues predominantly expressing TRβ (e.g., the liver and the pituitary) but signs of thyrotoxicosis in tissues with predominant expression of TRα (e.g., the heart).[126] Peripheral thyroid hormones are elevated and TSH remains inadequately normal or elevated. Clinical signs include goiter, tachycardia, and hyperactivity. The missense mutations in $TR\beta_1$ or $TR\beta_2$ disrupt binding of T3, and the mutated allele acts as a dominant negative inhibitor of the intact TRβ proteins encoded by the wild-type allele (see Chapters 2 and 13).

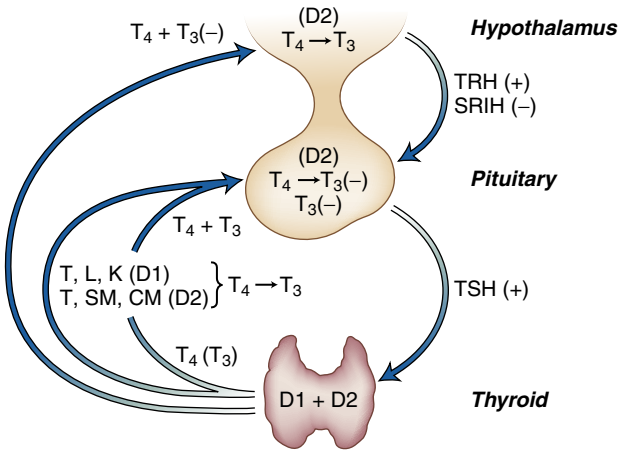
The effect of a $TR\alpha_1$ disruption in the mouse is quite different. The predominant phenotypic effects are modest bradycardia and hypothermia. Several patients with mutations in *THRA* have now been identified.[127,128] All patients are heterozygous, suggesting dominant negative effects of the mutant receptors on wild-type TRα. They presented with low serum T_4 and high serum T_3 levels, growth retardation, delayed mental and bone development, bradycardia, and decreased colonic motility with severe constipation.[127–129]



• **Fig. 11.7** Comparison of the chemical structure of thyroxine (T_4) with that of two agents that block the deiodination of the iodothyronines. The inhibition of T_4-to-T_3 conversion, which occurs in patients receiving amiodarone, may be due to the drug itself or to a metabolic product. Iopanoic acid and related iodoanilines are competitive inhibitors of all three iodothyronine deiodinases.

It is likely that small differences in the ligand-binding domains of TRα and TRβ will allow design of thyroid hormone analogues selective for one or the other of these receptors. This may result in agents that could, for example, suppress TSH in patients with thyroid cancer, without inducing tachycardia, such as GC1, a potential treatment for obesity via stimulation of metabolic rate and oxygen consumption.[130]

The ability of thyroid hormone to cause rapid effects, at least under certain experimental situations, has led to the investigation of other potential mechanisms for thyroid hormone action that are referred to as extranuclear, noncanonic, or nongenomic actions. Several examples are provided here. Studies of mice in which the endogenous thyroid hormone receptor genes have been mutated to prevent thyroid hormone receptor–DNA binding have provided strong evidence that heart rate, body temperature, blood glucose, and serum triglyceride levels can be regulated by nongenomic actions of TRs, whereas negative regulation of the hypothalamic-pituitary-thyroid axis requires DNA binding of TRβ.[131] T_3 has been shown to rapidly activate phosphatidylinositol 3-kinase at the cell membrane via interaction with TRβ, and this action enhances maturation of synapses in the mouse hippocampus in vivo.[132] In addition, integrin $\alpha_V\beta_3$ has been identified as a putative plasma membrane thyroid hormone–binding site and has been proposed as a mediator of rapid effects of T_4.[133] The effect of T_4 per se to initiate the ubiquitination of D2 is perhaps the most important nongenomic effect of physiologic concentrations of free T_4.[51]



• **Fig. 11.8** Roles of thyroxine (T_4) and triiodothyronine (T_3) in the feedback regulation of secretion of thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH). Secreted T_4 must be converted to T_3 to produce its effects. This conversion may take place in tissues such as the liver (L), kidney (K), and thyroid (T) catalyzed by the type 1 iodothyronine deiodinase, D1. Type 2 (D2) is present in human thyroid (T), skeletal muscle (SM), possibly cardiac muscle (CM), and the pituitary and hypothalamus. *SRIH,* somatotropin release-inhibiting factor (somatostatin hormone).

Regulation of Thyroid Function

The Hypothalamic-Pituitary-Thyroid Axis

The thyroid participates with the hypothalamus and pituitary in a classic feedback control loop (Fig. 11.8). In addition, there is an inverse relationship between the iodine level in the thyroid and the fractional rate of hormone formation. Such autoregulatory mechanisms stabilize the rate of hormone synthesis despite fluctuations in the availability of iodine. Stability in hormone production is achieved in part because the large intraglandular store of hormone buffers the effect of acute increases or decreases in hormone synthesis. Autoregulatory mechanisms within the gland in turn tend to maintain a constant thyroid hormone pool. Finally, the hypothalamic-pituitary feedback mechanism senses variations in the availability of free thyroid hormones, however small, and acts to correct them. Hypothalamic tanycytes play a role in the homeostatic regulation of the HPT axis to maintain circulating thyroid hormone levels in a narrow physiologic range.[134]

There is a close relationship between the hypothalamus, the anterior pituitary, the thyroid gland, and still higher centers in the brain, the function of the entire complex being modified in a typical negative-feedback manner by the availability of the thyroid hormones. In addition, other hormones and neuropeptides also influence this axis (see Chapters 7 and 8).

Thyrotropin-Releasing Hormone Synthesis and Secretion

TRH, a modified tripeptide (pyroglutamyl-histidyl-proline amide), is derived from a large prepro-TRH molecule of 29 kDa that contains five progenitor sequences. The TRH peptides are released from the prepro molecule by a peptidase that acts at flanking lysine/arginine residues. TRH is expressed in the hypothalamus, the brain, the C cells of the thyroid gland, the beta cells of the pancreas, the myocardium, the reproductive organs (including prostate and testis), and the spinal cord. The parvocellular region of the paraventricular nuclei (PVN) of the hypothalamus is the source of the TRH that regulates TSH secretion. The 5′ flanking region of the gene encoding TRH has sequences for mediating

responses to glucocorticoids and cAMP. In addition, at least two elements in this region are responsible for the negative regulation of this gene by thyroid hormone.[135] TRH travels in the axons of the peptidergic neurons through the median eminence and is released close to the hypothalamic-pituitary portal plexus. The neuron bodies producing TRH are innervated by catecholamine, leptin, neuropeptide Y (NPY), agouti-related protein (AgRP) or melanocyte-stimulating hormone (MSH), and somatostatin-containing axons, all of which potentially influence the rate of synthesis of the prepro-TRH molecule (see Chapter 7). T_3 suppresses the levels of prepro-TRH mRNA in the hypothalamus,[136,137] but normal feedback regulation of prepro-TRH mRNA synthesis by thyroid hormone requires a combination of T_3 and T_4 in the circulation, the latter giving rise to T_3 via T_4 5′ deiodination in the CNS in astrocytes and tanycytes (see Fig. 11.3). Another event in this feedback regulation may be the thyroid hormone–mediated induction of the TRH-inactivating pyroglutamyl peptidase II (PPII) in the hypothalamic tanycytes. This regulation is observed in vivo exclusively in the parvocellular division of the PVN, but in tissues outside the CNS expressing the *TRH* gene, negative regulation by thyroid hormone is absent. Thus part of the negative feedback induced by T_4 may be generated at the median eminence/arcuate nucleus at a point where neuropeptides and T_3 enter the pituitary portal system.[138] Although D2 is also present in astrocytes in the median eminence and arcuate nucleus region, selective ablation of D2 from astrocytes in transgenic mice has no significant effect on feedback regulation of the hypophysiotropic TRH neurons, indicating that astrocytes have little or no role in the regulation of this response.[139] In addition to inhibiting the synthesis of prepro-TRH mRNA, thyroid hormone also blocks the capacity of TRH to stimulate TSH release from the thyrotroph.

TRH is rapidly inactivated within the CNS by a cell-surface peptidase called TRH-degrading ectoenzyme (TRH-DE), also termed protein peptidase II. TRH-DE is very specific: There is no other ectopeptidase known capable of degrading TRH, and TRH is the only known substrate of this unique enzyme.[140]

Thyrotropin Synthesis and Secretion

TSH is the major regulator of the morphologic and functional states of the thyroid. It is a glycoprotein secreted by the thyrotrophs in the anteromedial portion of the adenohypophysis (see Chapter 8). TSH is composed of an α-subunit of 14 kDa (92 amino acids) that is common to luteinizing hormone, follicle-stimulating hormone, human chorionic gonadotropin (hCG), and a specific β-subunit synthesized only in thyrotrophs, which is a 112–amino acid protein. In normal thyrotrophs and in thyrotroph tumors, synthesis of α-subunit is in excess, indicating that the quantity of β-subunit is rate limiting for TSH secretion. Levels of the α-subunit in serum range from 0.5 to 5 μg/L but are increased in postmenopausal women and patients with pituitary tumors. TRH increases and thyroid hormone suppresses the transcription of both subunits; these are the two most important influences on TSH synthesis.

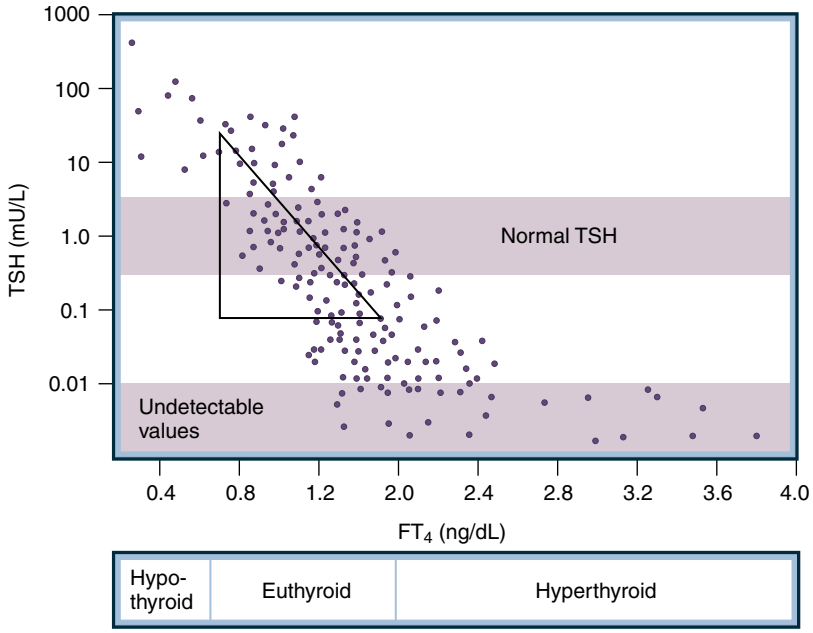
The pretranslational regulation of TSH synthesis and secretion is a complex process. The physiologic glycosylation of TSH involves several post-translational steps, including the excision of signal peptides from both subunits and cotranslational glycosylation with high mannose oligosaccharides.[141] The glycosylation of the subunits protects them from intracellular degradation and permits normal folding of the protein chains so that internal disulfide linkages are correctly formed. Glycosylation is required for full biologic activity.[142,143] TRH is required for this process as illustrated by the inappropriately low biologic activity of the TSH in the serum of patients with pituitary tumors or hypothalamic disorders. In animals that breed seasonally, alternatively glycosylated TSH secreted from the pars tuberalis (PT) of the anterior pituitary gland activates thyroid hormone within the hypothalamus, which in turn induces gonadotropin-releasing hormone (GnRH), leading to gonadal growth in a seasonal fashion.[144]

In normal serum, TSH is present at concentrations between 0.4 and 4.2 mU/L. The level is increased in primary hypothyroidism and reduced in thyrotoxicosis. The plasma TSH half-life is about 30 minutes, and production rates in humans are 40 to 150 mU/day. Circulating TSH displays both pulsatile and circadian variations. The former are characterized by fluctuations at intervals of 1 to 2 hours. The magnitude of TSH pulsations is decreased during fasting, during illness, or after surgery. There is an acute reduction of TSH in fasting humans, associated with a fall in leptin levels. This is due to a decrease in the amplitude of the TSH pulses.[145] The circadian variation is characterized by a nocturnal surge that precedes the onset of sleep and appears to be independent of the cortisol rhythm and fluctuations in the serum and T_4 and T_3 concentrations. The circadian rhythm shows parallelism with changes in T_3 levels.[146] When the onset of sleep is delayed, the nocturnal TSH surge is enhanced and prolonged, and the early onset of sleep results in a surge of lesser magnitude and shorter duration. TSH levels also show seasonal changes in humans with a decrease during the summer and an increase during winter, changes that correlate with daily temperatures.[147]

The degree of thyroid hypofunction after destruction of the hypothalamus is less severe than that which follows hypophysectomy, and residual thyroid function in the former circumstance can be altered by raising or lowering the concentration of thyroid hormones in the blood. Thus both T_4 and T_3 mediate the feedback regulation of TSH secretion, and TRH determines its set-point (see Fig. 11.8). There is a linear inverse relationship between the serum free T_4 concentration and the log of the TSH (Fig. 11.9), making the serum TSH concentration an exquisitely sensitive indicator of the thyroid state of patients with an intact hypothalamic-pituitary axis. Gene-targeting studies show that TRH secretion is likely to be the dominant factor mediating the thyroid hormone feedback regulation of TSH secretion because the markedly elevated TSH secretion of mice with inactivation of TRβ cannot be sustained in mice lacking the TRH gene.[148] This is somewhat surprising given the less severe hypothyroidism associated with hypothalamic (as opposed to primary) hypothyroidism but may be explained by the absolute nature of the TRH deficiency achieved by the genetic manipulation as opposed to the clinical situation in humans with central hypothyroidism, wherein the TRH deficiency is not likely to be complete.

Somatostatin (somatotropin release–inhibiting hormone [SRIH]), acting through inhibitory G protein (G_i), decreases TSH secretion in vitro and in vivo, but prolonged treatment with a somatostatin analogue does not cause hypothyroidism.[149,150] Similar acute effects occur during dopamine infusion and the administration of bromocriptine, a dopamine agonist. Both of these agents inhibit adenylate cyclase.

A number of drugs or hormones may suppress or stimulate TSH secretion (Table 11.6). Glucocorticoids given in high doses transiently suppress TSH secretion, although prolonged therapy is not associated with central hypothyroidism.[149] Patients with Cushing disease have subnormal TSH production but with minimal effects on T_4 production.[149] Bexarotene, a RXR agonist used for treatment of T-cell lymphoma, suppresses TSH sufficiently



• **Fig. 11.9** The log/linear relationship between thyroid-stimulating hormone (TSH) *(on the vertical axis)* and the free T_4 concentrations (FT_4). Typical free T_4 concentrations in hypothyroid, euthyroid, and hyperthyroid patients are shown.

TABLE 11.6 Endogenous and Exogenous Agents That May Stimulate or Inhibit Thyrotropin Secretion

Stimulatory Agents	Inhibitory Agents
Thyrotropin-releasing hormone (TRH)	Thyroid hormones and analogues
Prostaglandins (?)	Dopamine and dopamine agonists
α-Adrenergic agonist (? via TRH)	Biotin
Opioids (humans)	Opioids (rat)
Arginine vasopressin (AVP)	Glucocorticoids (in vivo, high dose)
Glucagon-like peptide 1 (GLP1)	Serotonin
Galanin	Cholecystokinin (CCK)
Leptin	Gastrin or gastrin-releasing peptide (GRP)
Glucocorticoids (in vitro)	Arginine vasopressin (AVP)
	Neuropeptide Y (NPY)
	Interleukin 1β and 6
	Tumor necrosis factor α (TNFα)
	Bexarotene (retinoid receptor agonist)
	Phenytoin
	Somatostatin and somatostatin analogues
	Mitotane

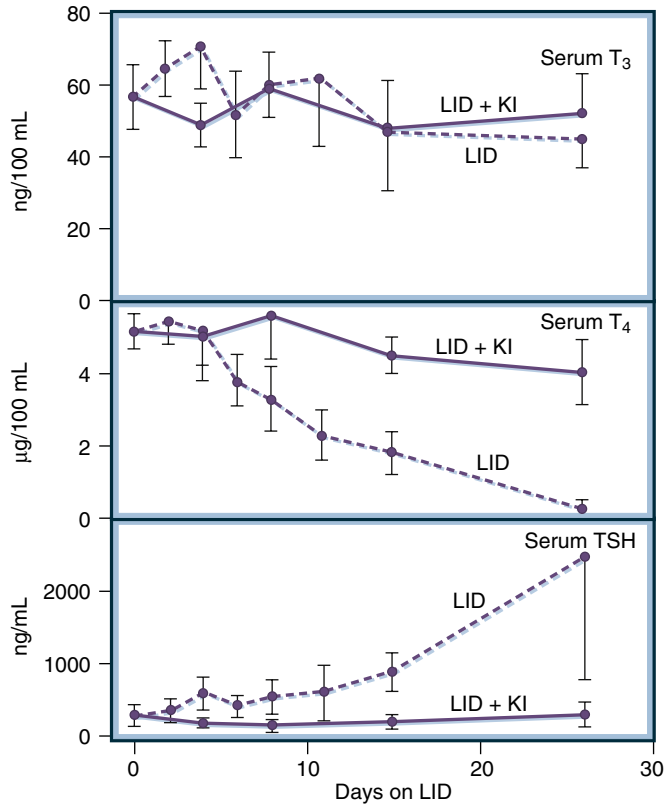
to cause central hypothyroidism, presumably by reducing TSHβ gene transcription.[151,152]

Neurotransmitters are important direct and indirect modulators in TSH synthesis and secretion. A complex network of neurotransmitter neurons terminates on cell bodies of hypophysiotropic neurons, and several neurotransmitters (such as dopamine) are directly released into hypophyseal portal blood, exerting direct effects on anterior pituitary cells. Furthermore, many dopaminergic, serotoninergic, histaminergic, catecholaminergic, opioidergic, and GABAergic systems project from other hypothalamic/brain regions to the hypophysiotropic neurons involved in TSH regulation. These projections are important for a normal TSH circadian rhythm and response to stress and cold exposure, but basal TSH secretion is mainly regulated by intrinsic hypothalamic activity.

Iodine Deficiency

The response of vertebrates to iodine deficiency is designed to conserve this limited resource and improve the efficiency of its utilization. These adjustments occur at the hypothalamic, pituitary, thyroid, and peripheral tissue levels. Removal of iodine from the diet causes a rapid decrease in serum T_4 concentrations and a simultaneous increase in serum TSH (Fig. 11.10).[153] Interestingly, no detectable decrease in T_3 occurs, suggesting that the signal to increase TSH must derive from a decrease in the T_3 generated intracellularly from T_4 in the pituitary, the hypothalamus, or both. TSH increases NIS, Tg, and TPO synthesis and iodine organification and Tg turnover (see Fig. 11.1). Because of the decrease in iodide supply and the ratio of DIT/MIT, the ratio of T_4 to T_3 in Tg decreases and the rate of thyroidal T_3 secretion may increase despite a fall in T_4 secretion. TSH also stimulates cell division, leading to goiter. In the rat model, the fall in plasma T_4 increases D2 from 5-fold to 20-fold in the CNS, hypothalamus, and pituitary, increasing the efficiency of T_4 conversion to T_3. With moderately severe iodine deficiency, D3 in the CNS is also reduced, prolonging the mean residence time of T_3 in that organ.[154] This permits serum T_3 to remain normal and the CNS T_3 to be only moderately reduced despite up to a 10-fold decrease in circulating T_4.

Despite the TSH elevation and nearly undetectable serum T_4 in acutely iodine-deficient rodents, growth, O_2 consumption, and thermal homeostasis can be maintained.[155] However, if iodine deficiency is prolonged and severe, hypothyroidism will supervene. In humans, these compensatory alterations in thyroid function come into operation when total iodine intake falls below



• **Fig. 11.10** Effects of acute depletion of dietary iodine on serum triiodothyronine (T_3), thyroxine (T_4), and thyroid-stimulating hormone (TSH) in rats. Animals received a low iodine diet (LID) without or with supplementation of potassium iodide (KI) in drinking water. (From Riesco G, Taurog A, Larsen PR, et al. Acute and chronic responses to iodine deficiency in rats. *Endocrinology* 1977;100:303–313.)

75 μg/day (see Table 11.1). This situation can occur even after the implementation of iodine supplementation programs in the absence of adequate longitudinal monitoring.[156]

Changes in serum hormones seen in experimental animals have been well documented in humans in areas of iodine deficiency and in patients with NIS mutations.[157] However, they may not be seen in older members of the population when thyroid autonomy often develops. The physiologic response to iodine deficiency is similar to that which occurs during the development of primary hypothyroidism in humans. It is also reproduced when the efficiency of iodide trapping and organification is reduced in Hashimoto disease or in the patient with Graves disease receiving thiourea drugs.[38] The physiologic effects of this series of events are clear. T_3 has approximately 10 times the potency of the prohormone T_4 and contains only three iodine atoms. This results in a more efficient use of the iodine atom. Maintenance of normal circulating T_3 independent of serum T_4 concentrations should provide hormone for those tissues in which the nuclear T_3 is completely derived from the plasma such as liver and kidney (see Fig. 11.5).

Iodine Excess

The thyroid is also protected against an excess of iodide that might otherwise lead to hyperthyroidism. As with the response to iodine deficiency, there are multiple levels of defense against this eventuality. The usual source of excess iodine is pharmaceutic,

TABLE 11.7 Iodine Content of Various Iodinated Pharmaceuticals [a]

Agent	Iodine Content
Saturated solution of potassium iodide	38 mg/drop
Lugol solution	6 mg/drop
Iodized salt (1 part KI/10,000 NaCl)	760 μg/10 g
Amiodarone (200-mg tablet)	75 mg organic iodine, 8–17% is released as iodide
Iopanoate, ipodate	350 mg/tablet
Angiographic and CT dyes	400–4000 mg/dose
Povidone-iodine	10 mg/mL
Kelp tablets	150 μg/tablet
Prenatal vitamins	150 μg/tablet
Iodinated glycerol	25 mg/mL
Quantity of iodine required to suppress radioactive iodine uptake to <2%	>30 mg/day

[a]Typical iodide intake in the United States is 100–400 μg/day.

CT, Computed tomography; *KI*, potassium iodide; *NaCl*, sodium chloride.

with radiographic dyes, amiodarone, and povidone-iodine being the most common sources (Table 11.7).

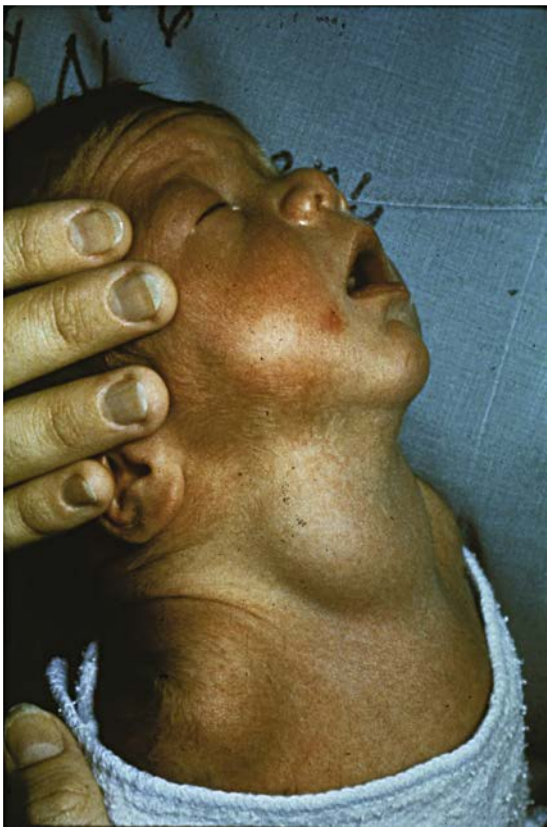
Effects of Increased Iodine Intake on Thyroid Hormone Synthesis

The quantity of iodine organified in Tg, which includes T_4 and T_3, displays a biphasic response to increasing doses of iodide, at first increasing and then decreasing as a result of a relative blockade of organic binding. This decreasing yield of organic iodine from increasing doses of iodide, the Wolff-Chaikoff effect, results from a high concentration of inorganic iodide within the thyroid cell.[18] The susceptibility to the Wolff-Chaikoff effect can be increased either by stimulation of iodide trapping, as occurs in patients with Graves disease, or during persistent TSH stimulation by impairment of iodine organification in the human fetus, in patients with Hashimoto disease, or in thyroids previously irradiated by either ^{131}I or external beam therapy. In such situations, goiter and hypothyroidism (iodide myxedema) can develop if excess iodide is given for long periods. The mechanism for organification inhibition may involve inhibitory effects of high iodide concentrations on TPO and DUOX2.

In normal subjects given iodide, the inhibition of iodothyronine formation is reversible over time. This escape or adaptation phenomenon occurs because iodide transport activity decreases probably through a decrease in NIS expression. Consequently, thyroidal iodide falls to levels insufficient to maintain the full Wolff-Chaikoff effect.[19,158] Importantly, it does *not* occur in the third-trimester fetus, so chronic high iodine intake during pregnancy must be avoided because it will cause fetal hypothyroidism and compensatory potentially obstructive goiter in the newborn (Fig. 11.11).

Effects on Thyroid Hormone Release

An important practical effect of pharmacologic doses of iodide is the prompt inhibition of thyroid hormone release. This occurs to



• **Fig. 11.11** Newborn infant with iodide-induced goiter due to Lugol solution treatment of the mother during the third trimester. This illustrates the danger of chronic excess iodide administration during pregnancy.

some extent normally but is especially apparent in patients with Graves disease or toxic nodules (see Chapter 12). The mechanism is unknown, but the effect is mediated at the thyroid cell level rather than through an action on TSH. Iodine also diminishes the hypervascularity and hyperplasia that characterize the diffuse toxic goiter of Graves disease. The pharmacologic use of iodide is therefore useful in patients with severe hyperthyroidsm *(thyroid storm)* or in the preparation of thyrotoxic patients for surgery.[159]

Thyroid Function in Pregnancy and in the Fetus and Newborn

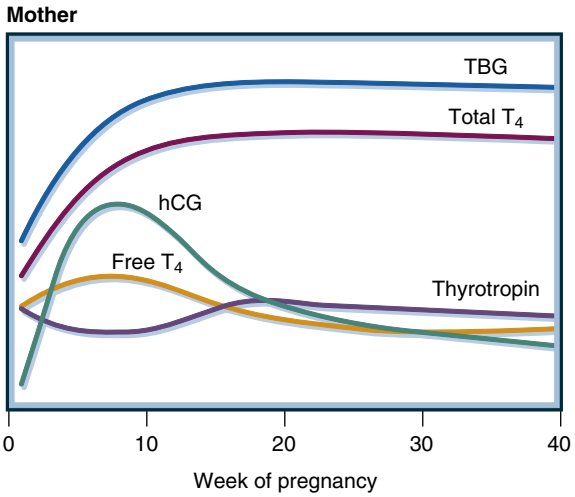
Pregnancy affects virtually all aspects of thyroid hormone economy (Table 11.8).[160] The total serum T_4 and T_3 concentrations rise to levels about 1.5-fold those of nonpregnant women, owing to the increase in TBG concentration in the first trimester (Fig. 11.12). The markedly increased TBG extracellular pool must be filled steadily with increasing amounts of T_4 until a new equilibrium is reached. Human chorionic gonadotropin (hCG) cross-reacts with the TSHR, which results in a small and transient increase in free T_4 levels near the end of the first trimester (peak circulating hCG), resulting in a partial TSH suppression. In some women, hCG can induce transient gestational thyrotoxicosis, and trophoblast tumors (hydatidiform moles, choriocarcinomas) can rarely lead to severe hyperthyroidism. A mutation in the TSHR resulting in increased affinity for hCG has been found in familial gestational hyperthyroidism.[161] When tested in bioassays, normal hCG is only about 1/100 as potent as TSH. This weak thyrotropic activity explains why, in normal conditions, the effects of hCG remain largely unnoticed.[162] In addition to the increase in serum TBG, there is also an increased plasma volume as well as accelerated inactivation of T_3 and T_4 by D3 expression in the fetal-placental-uterine unit.[163] Based on the changes in requirements for levothyroxine during gestation in women with primary hypothyroidism, the estimated increase in T_4 production required during this period is 20% to 40%.

The requirement for increased T_4 secretion increases iodine requirements during pregnancy.[164] This need is compounded by the fact that the higher glomerular filtration rate during gestation enhances renal iodide clearance, leading to higher fractional

TABLE 11.8 Effects of Pregnancy on Thyroid Physiology

Physiologic Change	Thyroid-Related Consequences
↑ Serum thyroxine-binding globulin	↑ Total T_4 and T_3; ↑ T_4 production
↑ Plasma volume	↑ T_4 and T_3 pool size; ↑ T_4 production; ↑ cardiac output
D3 expression in placenta and (?) uterus	↑ T_4 production
First trimester ↑ in hCG	↑Free T_4; ↓ basal thyrotropin; ↑ T_4 production
↑ Renal I^- clearance	↑ Iodine requirements
↑ T_4 production; fetal T_4 synthesis during second and third trimesters	
↑ Oxygen consumption by fetoplacental unit, gravid uterus, and mother	↑ Basal metabolic rate; ↑ cardiac output

D3, Type 3 iodothyronine deiodinase; *I^-*, plasma iodide; *hCG*, human chorionic gonadotropin; *T_3*, triiodothyronine; *T_4*, thyroxine.



• **Fig. 11.12** Changes in various critical components of the thyroid-pituitary axis during pregnancy. Note the early increase in free thyroxine (T_4), probably due to thyroidal stimulation by human chorionic gonadotropin (hCG), which causes a reciprocal modest suppression of serum thyrotropin (TSH) during the late first trimester. (From Burrow GN, Fisher DA, Larsen PR. Mechanisms of disease: maternal and fetal thyroid function. *N Engl J Med* 1994;331:1072–1078.)

urinary excretion of circulating iodide. In addition, maternal iodine intake must be increased to supply the requirements of the fetal thyroid during the second and third trimesters (see Table 11.8). If these increased requirements for iodide are not met, serum T_4 falls and TSH rises. This series of events is well documented in areas of endemic iodine deficiency or borderline iodine supply, such as Brussels, Belgium.[9] In that city, 70% of pregnant women carefully followed throughout pregnancy had a 20% or greater increase in thyroid volume during gestation due to increased TSH. After delivery, the changes in thyroid function gradually return to normal, and serum TBG values reach normal levels 6 to 8 weeks postpartum.

During pregnancy, autoimmunity is suppressed, affecting patients with Graves and Hashimoto diseases (see Chapters 12 and 13). In general, TSHR antibody (TRAb)–mediated thyroid stimulation in the Graves disease patient is exacerbated during the first trimester and is attenuated during the second and third trimesters only to exacerbate in the first several months postpartum. Thyroid autoantibody titers fall during gestation in patients with Hashimoto disease only to rise sharply postpartum in association with a phase of acute T-cell–mediated thyroid cell destruction—postpartum thyroid disease (PPTD)—which occurs in about 30% of patients with Hashimoto disease and significant residual thyroid tissue.[165]

The basal metabolic rate (BMR) increases during the second trimester owing to the increase in the total mass of body tissue consequent to the pregnancy. The changes of pregnancy, together with the decreased peripheral vascular resistance, vasodilatation, and modest tachycardia, may suggest thyrotoxicosis (see Table 11.8). It is important to appreciate that such changes are physiologic in pregnancy, especially when managing the hyperthyroid pregnant patient.

Fetal Thyroid Function

The peripheral metabolism of T_4 in the human fetus differs markedly from that in the adult, both quantitatively and qualitatively. Overall, rates of production and degradation of T_4 in terms of units per body mass exceed those in the adult by 10-fold. In addition, D1 catalysis is reduced and D3 is enhanced, favoring the formation of the inactive rT_3 at the expense of T_3. D3 is highly expressed in fetal tissues, including the liver, skin, tracheobronchial, urothelial, and gastrointestinal epithelia.[163] This condition results in a persistently low serum T_3 concentration and an elevated serum rT_3. This change permits the highly regulatable conversion of T_4 to T_3 by D2 to be the major pathway for generating tissue T_3.[166]

Fetal thyroid function begins at about the end of the first trimester.[5] Thereafter, there are steady increases in fetal TBG and total T_4 and T_3.[9,167] Throughout gestation, the serum TSH values are greater than are present in maternal circulation and higher than would be expected in adults with normal thyroid function. This indicates that there is increasing hypothalamic-pituitary resistance to T_4 during fetal development, which is speculated to be a consequence of increased TRH secretion.[168] Despite the low circulating T_3, the fetal free T_4 concentrations approximate those in the maternal circulation from the gestational age of 28 weeks and onward.

Maternal-Fetal Interactions

The fetal pituitary-thyroid axis functions as a unit that is essentially independent from the mother.[4,168] Transplacental passage of TSH from mother to fetus is negligible, but the same is not true of maternal T_4. In infants with congenital hypothyroidism caused by either genetic TPO deficiency or athyreosis, serum concentrations of T_4 in cord blood are usually one-third to one-half of normal.[169] Thus at least when the maternal-fetal concentration gradient is high, significant transfer of maternal T_4 to the fetal circulation can occur. This transfer may be significant, given the capacity of the fetal brain to increase the efficiency of T_4-to-T_3 conversion. Furthermore, T_4 can be found in coelomic and amniotic fluids before the onset of thyroid function.[170] The major factor limiting T_4 and T_3 transfer from mother to fetus is the D3 expressed in the uterus, placenta, and fetal epithelium.

Thyroid Function in the Newborn

Mean total T_4 level in cord sera is 150 nmol/L (12 μg/dL). Serum TBG concentrations are elevated, but are not as high as in the maternal serum. At term, free T_4 concentrations are slightly lower than those in the mother. Cord serum T_3 concentrations are low (0.8 nmol/L, 50 ng/dL), and rT_3 and T_3SO_4 are elevated.[4,171,172] After delivery, the serum TSH level in the neonate increases rapidly to a peak at about 2 to 4 hours after birth, returning to its initial value within 48 hours.[171] Levels above 60 mU/L are typical. This neonatal TSH surge is thought to occur in response to the rapid reduction in environmental temperature after delivery. In response, the serum T_4, T_3, and Tg concentrations increase rapidly during the first few hours after delivery and are in the hyperthyroid range by 24 hours of life.[173] The TSH surge doubtless contributes to the increase in serum T_3 concentration, but enhancement of extrathyroidal conversion of T_4 to T_3 by D1 or D2 is thought to be a major factor as well.[51] The adrenergic stimulation of the *Dio2* gene and the reactivation of D2 by its deubiquitination in brown adipose tissue are likely to be major contributors to this increase.[174]

Premature infants have an immature hypothalamic-pituitary-thyroid axis with low T_4, T_3, and TSH.[171,175] Serum T_4, TBG, and free T_4 all tend to correlate with gestational age. Preterm infants also have an attenuated TSH surge after delivery. In addition, when prematurity is accompanied by complications, such as respiratory distress syndrome or nutritional problems, serum T_4, and especially T_3, may fall to low levels as a result of a combination of reduced TBG production, immaturity of the thyroid gland, suppression of the hypothalamic-pituitary axis due to illness, impairment of T_4-to-T_3 conversion, and increases in D3 activity.[176,177] These changes are in many respects similar to those in adults with severe illness. All of these issues need to be taken into account when evaluating the thyroid status of the preterm infant, particularly given the increased prevalence of congenital hypothyroidism in this age group.[175]

Thyroid hormone production rates are higher per unit of body weight in neonatal infants and children than in adults. The daily levothyroxine requirement is about 10 μg/kg in the newborn, decreasing progressively to about 1.6 μg/kg in the adult.[175]

Aging and the Thyroid

The thyroid gland undergoes several anatomic changes with age. There is a reduction in weight of the gland, in the size of follicles, and in the content of colloid, and there is increased fibrosis, often with marked lymphocytic infiltration. However, these changes do not correlate with thyroid function.[176] In the healthy elderly patient, there is a normal level of free T_4, but serum T_3 levels appear to be lower, although studies of selected healthy people indicated that T_3 levels are unaffected by aging.[179] TSH may increase or decrease with age in relation to the iodine intake.[180] Population studies in

humans and animal models show negative correlations between thyroid hormone levels and longevity, a finding that led to the hypothesis that constraints on thyroid hormone signaling at certain life stages, notably during maturity, are advantageous for optimal aging.[181] However, some other studies have suggested that both subclinical hypothyroidism and subclinical hyperthyroidism might be associated with increased mortality in the elderly.[182]

Thyroid Function During Fasting or Illness

A number of changes take place in thyroid function during nutritional deprivation or illness. These changes consist of a central reduction in TSH secretion and a decrease in plasma T_3 levels and T_4 and T_3 binding in serum. This constellation of findings is termed the *low T_3 syndrome,* the *euthyroid sick syndrome,* or *nonthyroidal illness*.[183] The patterns of changes in circulating thyroid hormones and TSH during fasting and illness are quite similar. During fasting, there is a reduction of 50% or more in serum T_3 and an increase in serum rT_3 without initial changes in serum total or free T_4 (Table 11.9).[145,184] Although the role of specific deiodinases in causing these changes has not been documented at a tissue level in humans during fasting, several lines of evidence suggest that decreases in peripheral T_4-to-T_3 conversion by both D1 and D2 and a reduced clearance of rT_3 by D1 play a role in this process. The finding of normal T_3 plasma levels in mice with the genetic absence of both D1 and D2 enzymes suggests that, under normal conditions, the thyroid gland by itself is able to compensate for impaired peripheral conversion to normalize serum T_3. This observation suggests that very powerful mechanisms are in place to maintain serum T_3 levels within the normal range, with the notable exceptions of when it is not meant to be in that range (i.e., during fasting or illness). In these circumstances, by a mechanism probably regulated by the hypothalamus, all compensatory mechanisms are reduced and serum T_3 may drop to almost undetectable levels.[92] Deiodination by D3 increases the generation of rT_3 from T_4 and converts T_3 to 3,3′-diiodothyronine (see Fig. 11.2), which exaggerates the changes resulting from the decrease in D1 and D2. The finding that D3-null mice can still develop the low T_3 syndrome suggests that D3 upregulation is not the only event occurring in this clinical state. It is not yet known if such an increase in D3 also occurs during caloric restriction. The attenuation of TSH secretion despite a fall in serum T_3 levels during fasting is discussed earlier (see "The Hypothalamic-Pituitary-Thyroid Axis").

TABLE 11.9 Changes in Thyroid Hormone Levels During Illness

Severity of Illness	Free T_3	Free T_4	Reverse T_3	TSH	Probable Cause
Mild	↓	N	↑	N	↓ D2, D1
Moderate	↓↓	N, ↑↓	↑↑	N, ↓	↓↓ D2, D1, ? ↑ D3
Severe	↓↓↓	↓	↑	↓↓	↓↓ D2, D1, ↑ D3
Recovery	↓	↓	↑	↑	?

D1 through *D3,* Iodothyronine deiodinases; *N,* no change; *T_3,* triiodothyronine; *T_4,* thyroxine; *TSH,* thyroid-stimulating hormone (thyrotropin).

During fasting, basal oxygen consumption and heart rate decline, and nitrogen balance, initially negative, returns toward normal.[185] In some studies, these changes in overall metabolism are partially reversed by replacement of exogenous T_3 while fasting continues. Thus the decrease in T_3 during fasting (and presumably illness) can be viewed as a beneficial energy-sparing and nitrogen-sparing adaptation. Chronic malnutrition such as occurs in anorexia nervosa is also associated with a reduction in serum T_3 and rarely in free T_4.[186] TSH concentrations remain in the reference range, although again, they are inappropriately low in the context of the reductions in circulating T_3. In contrast, overfeeding, particularly with carbohydrates, increases T_3 production rates and the serum T_3 concentration, reduces serum rT_3, and increases basal thermogenesis.[187]

During illness, decreases in T_3 and pulsatile TSH release and increases in rT_3 also occur.[183] If illness progresses, the hypothalamic-pituitary-thyroid axis is even further suppressed, with a consequent reduction in the free T_4. Severe decreases in serum T_4 are associated with a high probability of death. This syndrome is associated with a decrease in TRH mRNA in the human PVN.[188] An increase in T_3 production by D2-catalyzed T_4-to-T_3 conversion in the tanycytes lining the third ventricle during illness may contribute to the blunted response of TSH to the reduced serum T_3, particularly during infections.[189] Cytokines, such as interleukin 6 (IL6), also increase during illness and are coincident with the decrease in circulating T_3, although it is not clear whether this is the cause of the hypothalamic changes.[190] An in vitro study indicated that IL6 causes an increase in intracellular and extracellular reactive oxygen species (ROS).[93] As mentioned, the active centers of both D1 and D2 are intracellular and that of D3 has access to extracellular thiols, which do not pass easily through the cell membrane (see Fig. 11.4). The increase in ROS reduces intracellular thiols such as GSH (glutathione) and presumably the GSH/NADPH-dependent thioredoxin and glutaredoxin. Thus T_4-to-T_3 conversion by D1 and D2 is reduced while D3-mediated T_3 and T_4 inactivation continues. In addition to these cofactor changes, the increase in ROS and the activation of MAPK-dependent pathways due to IL6 and ROS induce increases in the transcription of the deiodinases, especially D3.[93] In vitro, all these changes are reversed by addition of *N*-acetylcysteine (NAC) to the media, which rescues intracellular GSH synthesis. Interestingly, follow-up studies in patients with the low T_3 syndrome due to acute myocardial infarction show that the reduction in T_3 and the increase in rT_3 can be prevented by NAC infusions,[191] suggesting these in vitro results are relevant to sick patients. These endogenous changes may be further exaggerated by agents such as dopamine or glucocorticoids, which will also, at least transiently, suppress the TRH-TSH axis.[192] The changes in thyroid function are a continuum, with the abnormalities becoming progressively more severe in parallel with the patient's clinical condition (see Table 11.9). Postmortem studies show that hepatic D1 activity is reduced by about 50%, skeletal muscle D2 is absent, and D3 is present in liver and skeletal muscle.[193] No differences have been found in the T_3 transporter MCT8 in skeletal muscle or liver, and possible abnormalities in other thyroid hormone transporters have not been evaluated. Interestingly, the same global pattern of changes during acute medical illness has been described in patients with primary hypothyroidism receiving levothyroxine.[194] In such patients, serum T_4, T_3, and TSH concentrations all fell about 50% over the first 3 days, presumably due to a disruption of T_4 binding due to a decrease in TBG, TTR, and albumin, as well as a blockade of T_4-protein interactions due to circulating

interfering substances. Contributing to this may be the translational modification of TBG due to a serpin-catalyzed release of a carboxy-terminal fragment of TBG in inflamed tissues discussed earlier (see "Thyroxine-Binding Globulin").

Therapies have been introduced in an attempt to ameliorate certain of the illness-related central abnormalities in the hypothalamic-pituitary axis (including decreases in growth hormone and gonadotropins). One of these, infusions of growth hormone–releasing peptide 2 (GHRP2) combined with TRH, has resulted in increases in TSH, T_3, and T_4, as well as insulin-like growth factor 1 (IGF1), insulin, and the IGF-binding proteins 1, 3, and 5.[195] Although the biochemical improvements were significant, the clinical state did not change, suggesting that the thyroid dysfunction is a marker of the severity of the illness rather than its cause.

Although serum TSH concentrations in severely ill patients are reduced, an increase in TSH above the normal range may appear during recovery, with the elevation in TSH concentration persisting until circulating free T_4 and T_3 levels return to normal.[196] This pattern can be confusing if the elevated TSH concentration is associated with the still-reduced concentrations of free T_4. Such patients meet all laboratory criteria for primary hypothyroidism with the exception of the clinical context. Follow-up generally reveals a normalization of TSH and T_4 within 1 to 2 months (see Table 11.9).

Despite the severity of the abnormalities, particularly in serum T_3, there is still disagreement as to whether therapeutic intervention should be initiated even in the most severely ill patients because most controlled studies have not shown beneficial effects of T_4 or T_3 supplementation in such individuals.[183] The one exception is a possible beneficial effect of T_3 therapy in patients after coronary artery bypass grafting, with one study showing a positive effect but a second study showing none.[197,198] Therefore the cautious use of thyroid hormonal therapy in patients with low T_3 syndrome or a stunned myocardium was proposed.[199]

The Thyroid Axis and Neuropsychiatric Illness

Patients with neuropsychiatric disease can present with any of a number of abnormalities in thyroid function. Patients with bipolar disorders may show slight elevations in serum TSH and reductions in free T_4, whereas patients with severe depression have slight elevation in serum T_4 and reduced serum TSH. Other acutely psychotic patients may have either high or low serum TSH concentrations and tend to have elevations in free T_4.[200] The cause of these minor abnormalities is not clear, but such patients may have thyroid function test results resembling those of patients with primary thyroid disease from whom they must be differentiated.

Effects of Hormones on Thyroid Function

Glucocorticoids

The acute administration of pharmacologic doses of glucocorticoid eliminates pulsatile release of serum TSH concentrations in normal patients, presumably by reducing TRH release. With continued administration, there is an escape from this suppression (Table 11.10). Pharmacologic doses of glucocorticoid decrease the serum T_3 concentration in normal and hyperthyroid patients as well as in hypothyroid patients maintained on levothyroxine. The latter finding and the accompanying increase in rT_3 production suggest that glucocorticoids may increase D3 activity.[120]

Primary adrenal insufficiency may be associated with reduced serum T_4 and elevated serum TSH concentrations, suggesting the coexistence of primary hypothyroidism. However, treatment of the adrenal insufficiency can lead to complete resolution of these abnormalities, suggesting that in some patients they are a consequence of glucocorticoid deficiency rather than primary thyroid disease.[201] Nevertheless, the prevalence of primary hypothyroidism is increased in patients with autoimmune hypoadrenalism, so the two causes must be differentiated (see Chapter 15). Likewise, patients successfully treated for Cushing disease can develop thyroid autoimmunity.

Gonadal Steroids

Estrogen increases TBG by mechanisms already mentioned.[202] Presumably this increases T_4 secretion, in that total T_4 increases and free T_4 is unchanged. Estrogen also increases the levothyroxine requirement in patients with primary hypothyroidism.[66] In contrast, administration of androgens to women decreases TBG and decreases T_4 turnover and levothyroxine requirements in patients with primary hypothyroidism.[203]

Growth Hormone

Growth hormone increases the serum free T_3 and decreases free T_4 in both levothyroxine-treated and normal individuals, suggesting either suppression of D3 activity or increased T_4-to-T_3 conversion.

Physical Evaluation of the Thyroid Gland

Manifestations of thyroid disease are usually due to excessive or insufficient production of thyroid hormone, goitrous enlargement of the thyroid or nodules resulting in local symptoms in the neck through compression of adjacent structures, or, in the case of Graves disease, ophthalmopathy or dermopathy.

TABLE 11.10 Effects of Hormones on Thyroid Function

Glucocorticoids

Excess

Decrease TSH, TBG, TTR (high dose)
Decrease serum T_3/T_4 and increase rT_3/T_4 ratios
Increase rT_3 production (? ↑ D3)
Decrease T_4 and T_3 secretion in Graves disease

Deficiency

Increase TSH

Estrogen

Increase TBG sialylation and half-life in serum
Increase TSH in postmenopausal women
Increase T_4 requirement in hypothyroid patients

Androgen

Decrease TBG
Decrease T_4 turnover in women and reduce T_4 requirements in hypothyroid patients

Growth Hormone

Decrease D3 activity

D3, Type 3 deiodinase; *rT_3*, reverse T_3; *T_3*, triiodothyronine; *T_4*, thyroxine; *TBG*, thyroxine-binding globulin; *TSH*, thyrotropin; *TTR*, transthyretin.

A functional diagnosis of thyroid disease is based on a carefully taken history, a thorough search for the physical signs of hypothyroidism or thyrotoxicosis, and an appraisal of the results of laboratory tests. Although conditioned by the functional diagnosis, the anatomic diagnosis depends largely on the physical examination of the thyroid gland itself. The typical symptoms of an excess or a deficiency of thyroid hormone are discussed in Chapters 12 and 13.

Physical Examination

Examination of the neck is best accomplished with the patient seated in good light with the neck relaxed. The patient should be provided with a cup of water to facilitate swallowing. The physician should first inspect the neck, especially while the patient swallows, with the neck slightly extended. The presence of old surgical scars, distended veins, and redness or fixation of the overlying skin should be noted. The position of the trachea should be noted. If a mass is present, a determination should be made as to whether it moves with swallowing. A midline mass high in the neck, which rises further when the patient extends the tongue, is typical of a thyroglossal duct remnant or cyst. Movement on swallowing is a characteristic of the thyroid gland because it is ensheathed in the pretracheal fascia; this feature distinguishes a goiter from most other neck masses. However, if the thyroid is so large that it occupies all the available space in the neck, movement on swallowing may be lost. The physician should also inspect the posterior dorsum of the tongue, which is the origin of the thyroglossal duct and the location of lingual thyroid tissue.

Except when the thyroid enlargement is extreme, the thyroid examination can be readily performed with the physician facing the seated patient. The thyroid may also be examined with the physician standing behind the seated patient, palpating with the fingertips of both hands. The physician should use gentle thumb pressure to locate the thyroid isthmus just caudal to the cricoid cartilage. This provides a convenient starting point for the palpation of the lobes of the gland, but an increase in the thickness of the isthmus or a firm texture will already suggest the presence of some generalized thyroid enlargement. To examine the right lobe, the right thumb is then moved laterally, without release of gentle pressure, to locate the lobe of the thyroid by pressing it against the trachea as the patient swallows sips of water. This strategy allows the palpating thumb to laterally displace the medial border of the sternocleidomastoid muscle, allowing direct access to the entire thyroid lobe. As the patient swallows with the thumb pressing the lobe against the trachea with sufficient tension to displace it slightly over the midline, it will slide up and down under the ball of the thumb. This permits an appreciation of the size and texture of the gland as well as the presence or absence of nodules. A similar strategy with the left thumb is used for the left lobe.

The examiner should note the shape of the gland, its size in relation to normal, and its consistency, which is usually slightly greater than adipose tissue but less than muscle. The normal thyroid lobe has approximately the same size in frontal projection as the terminal phalanx of the patient's thumb. Whereas a diffuse goiter and the hyperplastic gland of the hyperthyroid patient with Graves disease may be softer than normal, the gland of Hashimoto disease is usually firm. Irregularities of the surface, variations in consistency, and tender areas should be noted. If nodules are palpated, their shape, size, position, translucency, and consistency in relation to the surrounding tissue should be determined. A firm mass may reflect a cyst, more rarely a malignancy. A search should be made for the pyramidal lobe, which is a thin band of tissue extending upward from the isthmus to the thyroid cartilage to the right or left of the midline. A hypertrophied pyramidal lobe may be mistaken for a pretracheal lymph node that sometimes accompanies thyroid carcinoma or thyroiditis. It is usually palpable in patients with generalized thyroid disease, such as Hashimoto or Graves disease. During palpation, a vascular thrill may be felt that, in the absence of cardiac disease, is suggestive of hyperthyroidism. Finally, palpation should always include examination of the regional lymph nodes along the jugular vein, posterior to the sternocleidomastoids and in the supraclavicular region.

Auscultation of the neck may confirm the increased vascularity of an enlarged, hyperactive gland, suggesting Graves disease. A systolic or continuous bruit is sometimes heard over a hyperplastic gland. Care should be taken to distinguish a thyroid bruit from a murmur transmitted from the base of the heart or from a venous hum that can be obliterated by gentle compression of the external jugular vein or by turning the head. A venous hum is generally found in younger patients with high cardiac output, such as occurs in Graves disease or with severe anemia.

An arm-raising test is useful when a retrosternal goiter is suspected. The basis for this maneuver is that if the size of the thoracic inlet is already reduced by such a goiter, raising both arms until they touch the sides of the head further narrows the thoracic inlet and causes congestion and venous engorgement of the face and sometimes respiratory distress *(Pemberton sign)* or even (rarely) syncope.

In addition to examination of the thyroid gland and regional lymph nodes, evidence of compression or displacement of adjacent structures should be sought. Hoarseness may indicate compression or infiltration of the recurrent laryngeal nerve, usually by a malignant thyroid neoplasm, and this should be confirmed by laryngoscopy. Displacement of the trachea may be evident, usually associated with a large nodule(s), and inspiratory stridor may indicate its compression.

Ultrasound is now widely available and has a superior sensitivity for the detection and characterization of thyroid nodules. Qualitative systems and Thyroid Imaging Reporting and Data Systems (TI-RADS) allow categorization of cystic and solid lesions and their relative risk for malignancy. It is also useful in the evaluation of patients with thyroiditis.[204,205] The use of ultrasound should enhance, not replace, the physical examination of the thyroid.

Laboratory Assessment of Thyroid Status

In considering the laboratory assessment of the patient with known or suspected thyroid disease, the physician should seek to arrive at both a functional and, when appropriate, an anatomic diagnosis. Laboratory determinations will confirm whether there is an excess, normal, or insufficient supply of thyroid hormone to verify the inferences from the clinical history and physical examination. Laboratory tests can be divided into five major categories: (1) those that assess the state of the hypothalamic-pituitary-thyroid axis, (2) estimates of the T_4 and T_3 concentrations in the serum, (3) tests that reflect the impact of thyroid hormone on tissues, (4) tests for the presence of autoimmune thyroid disease, and (5) tests that provide information about thyroidal iodine metabolism. The use of iodine and other isotopes for thyroid scanning is discussed in Chapter 14.

Tests of the Hypothalamic-Pituitary-Thyroid Axis

Thyroid-Stimulating Hormone

Although an inherently indirect reflection of thyroid hormone supply, tests that assess the state of the hypothalamic-pituitary-thyroid axis play a critical role in the diagnosis of thyroid disease.

This is because the rate of TSH secretion is exquisitely sensitive to the plasma concentrations of free thyroid hormones, thus providing a precise and specific indicator of the thyroid status of the patient (see Figs. 11.8 and 11.9). The rare exceptions to this rule are discussed later. Immunometric assay technology now makes it possible to define the normal range for serum TSH and hence to ascertain both when thyroid function is inadequate and when the hormone supply is excessive (see Chapter 4). This assay uses the TSH molecule to link a TSH antibody bound to an inert surface (e.g., particles, the side of a test tube) to a second antibody directed against a different TSH epitope that is labeled with a detectable marker (^{125}I, an enzyme, or a chemiluminescent reagent). Thus the signal generated is proportional to the concentration of TSH in the serum. This technique is more specific, sensitive, and rapid than radioimmunoassay.

Many of these assays take advantage of the biotin/streptavidin binding for high affinity for complex formation. This interaction can be affected by circulating biotin (vitamin B_7) in the serum of patients taking large amounts of this substance commonly available over the counter or prescribed for various skin conditions or even for type 2 diabetes. This is especially worrisome since high levels of serum biotin can both result in very low TSH levels and artifactually raise the free T_4 measured in robotic assays based on biotin/streptavidin chemistry (see Chapter 4).[206]

The reference range of the serum TSH concentration by immunometric assay is 0.4 to 4.2 mU/L. The lower limit of 0.4 is too high for pregnancy due to hCG-induced hyperthyroidism as discussed earlier.[162] It should be kept in mind that there is a diurnal variation of TSH secretion with peak values in the late evening and a nadir in the afternoon. A borderline abnormal value should always be repeated within a week or so to be certain that it is representative. A minimally suitable TSH assay should be able to quantitate concentrations of TSH of 0.1 mU/L with a coefficient of variation of less than 20%. Potential artifacts of these assays are discussed in Chapter 4.

The free α-subunit common to TSH, FSH, LH, and hCG is generally detectable in serum with a reference range of 1 to 5 μg/L, but the TSH β-subunit is not. When FSH and LH production are increased, as in postmenopausal women, or when TSH production is increased, as in primary hypothyroidism, the free α-subunit level is also increased. The α-subunit level may also be increased in patients with glycoprotein-producing tumors of the anterior pituitary (see Chapter 9). Its measurement may be useful in the rare patient with hyperthyroidism and a normal or elevated TSH to differentiate between neoplastic and nonneoplastic causes of TSH excess.[207,208]

TSH in Patients with Thyroid Dysfunction

Patients with primary hyperthyroidism (excess thyroid hormone secretion) or thyrotoxicosis (excess thyroid hormone from any cause) will virtually always have a subnormal TSH. The values fall into two general categories: (1) those between the lower limit of normal and 0.1 mU/L and (2) those less than 0.1 mU/L. Individuals in the former category may be asymptomatic *(subclinical hyperthyroidism),* whereas those in the latter group usually have symptomatic thyrotoxicosis and a significant elevation in free T_4. Patients with hypothalamic or pituitary hypothyroidism often have normal or possibly even slightly elevated serum TSH. The circulating TSH generally has reduced biologic activity due to abnormal glycosylation, reflecting the impaired access of TRH to the thyrotroph.[142,143] Patients with primary hypothyroidism have serum TSH concentrations that range from minimally elevated to 1000 mU/L. In general, the degree of TSH elevation correlates with the clinical severity of the hypothyroidism. Patients with serum TSH values in the range of 5 to 15 mU/L have few if any symptoms, and the serum free T_4 or free T_4 index (FT_4I) is typically low-normal, whereas the serum free T_3 concentration is often low-normal. Such individuals with modest TSH elevation are said to have *subclinical hypothyroidism* if the serum free T_4 is in the normal range. These findings may indicate early thyroidal failure with a compensatory increase in TSH secretion. A detailed discussion of the various conditions associated with abnormal serum TSH concentrations follows that describing the quantitation of serum thyroid hormones (see upcoming discussion).

An elevation in both serum TSH and free T_4 is unusual and indicates either autonomous TSH production (due to a TSH-secreting pituitary tumor, resistance to thyroid hormone) or hyperthyroidism with an artifactual elevation in TSH. Differentiating between these diagnoses may require magnetic resonance imaging (MRI) of the hypothalamic-pituitary region, markers of thyroid hormone action (e.g., ferritin or sex hormone–binding globulin), or consultation with the clinical chemistry laboratory to rule out an assay artifact (see Chapter 4).

Quantitation of Serum Thyroid Hormone Concentrations

Total T_4 and T_3

Quantitation of the circulating thyroid hormone concentrations is essential to confirm that the thyroid status abnormality suggested by an abnormal TSH result is accurate and to document its severity. Sensitive and specific radioimmunoassays are available for measuring the total concentrations of T_4 and T_3 and some of their metabolic byproducts (see Chapter 4). Because the thyroid status correlates with the free, rather than with the total, hormone concentration, the physician usually must also obtain an estimate of that (see following discussion). The degree of abnormality in the free T_4 generally correlates with the severity of the hormone excess or deficiency, whereas the serum TSH concentration is an indication of the impact of this abnormality in that specific patient. The reference range for total T_4 in healthy, euthyroid adults with a normal circulating TBG concentration is 64 to 142 nmol/L (5–11 μg/dL). Normal serum T_3 concentrations are 1.1 to 2.9 nmol/L (70–190 ng/dL). At birth (cord serum), T_3 concentrations are about 50% of those in normal adults, but within a few hours T_3 rises abruptly, peaking at about 24 hours at concentrations in the low thyrotoxic range for adults.

Radioimmunoassays for reverse T_3 (rT_3), T_3SO_4, triac, tetrac, and the diiodothyronines are of primary interest in the research setting because these iodothyronines are derived from the circulating T_4 or T_3, both of which can be easily quantitated.

Concentrations of Free T_4 and Free T_3

The most accurate and direct measurements of the concentrations of free T_4 and free T_3 in serum are performed by assay of these hormones in a dialysate or ultrafiltrate of serum. Such assays are not practical for clinical purposes, so alternative strategies have been developed to estimate free thyroid hormone concentrations. The absolute concentration of free hormone is the product of the total hormone concentration and the fraction that is dialyzable or ultrafiltrable. About 0.02% of T_4 and 0.3% of T_3 is free or

unbound (see Table 11.5). The reference range for free T_4 is 9 to 30 pmol/L (0.7–2.5 ng/dL), and for free T_3 the range is 3.5 to 6.5 pmol/L (0.22–0.43 ng/dL).

So-called direct measurements of free T4 are now available by robotic assays in virtually all laboratories. They have largely replaced the free T_4 index discussed in more detail in past editions of this textbook. These automated tests imply that they quantitate free T_4 directly, but they do not, and rarely results in sera with abnormal binding proteins or in very sick patients may not be accurate.[209] In most situations, especially in outpatients, this automated free T_4 estimate is all that is required to ascertain the state of thyroid secretion or supply.

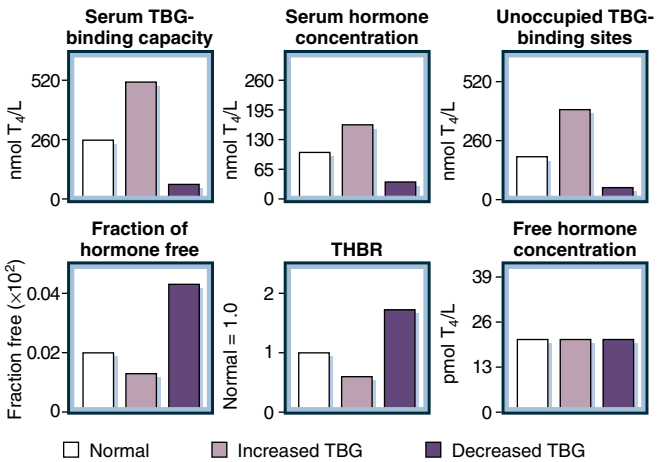
These assays are subject to artifacts from endogenous antibodies to T_4, abnormal binding proteins, or severe illness.[208,210] Thus the clinician must be wary if the free hormone estimate by *any* method does not agree with the clinical state and the TSH. In such situations, the patient should be questioned as to potential excessive biotin (vitamin B_7) ingestion, and another method should be used to estimate the free T_4. A total T_4 and thyroid hormone–binding ratio (THBR) should be measured and the FT_4 I calculated or the result should be ignored. For pregnant or severely ill patients, the automated methods typically give falsely low results. A reasonable alternative for pregnancy is to use the normal range for the serum total T_4 concentration multiplied by 1.5 in lieu of an automated free T_4 assay.[211,212]

The Free T_4 Index

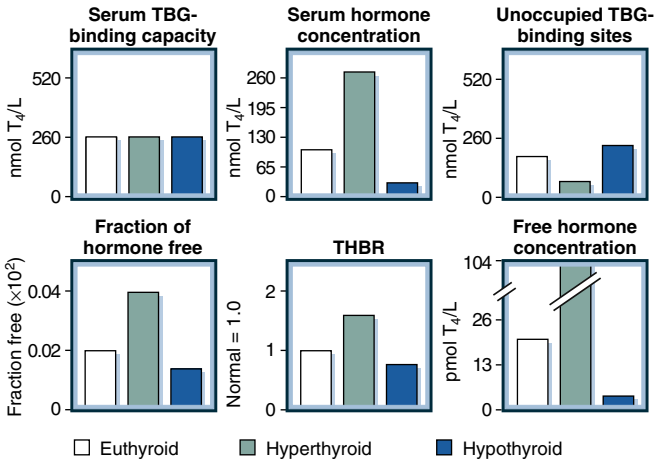
Although rarely necessary with the widespread availability of "direct" free T_4 assays, particularly useful in estimating the free T_4 in severely ill patients is the determination of the THBR, multiplying this result by the total T_4 (or T_3) to obtain a free hormone index (FT_4I or FT_3I). In this test, a tracer quantity of labeled T_4 (or T_3) is added to serum, which is then exposed to a solid phase matrix coated with T_4 or T_3 antibody or to an inert matrix that binds the iodothyronine irreversibly. The proportion of labeled T_4 or T_3 bound by the solid phase is then quantitated. This value varies inversely with the concentration of unoccupied TBG sites in the serum.

The results of such assays are normalized by comparing them with those obtained simultaneously for standard control sera with normal TBG and T_4 concentrations. This step can be performed by dividing the result for the unknown sample by that obtained for control sera in the same assay; in this case, the quotient is called the THBR (thyroid hormone–binding ratio), which typically has a normal range of 0.85 to 1.10. Because the THBR is proportional to the free fraction of the endogenous thyroid hormones in the serum, it can be multiplied by the total T_4 (or T_3) concentration to estimate the free thyroid hormone concentration (i.e., the free T_4 or T_3 index [FT_4I or FT_3I]). Because the midnormal THBR is 1.0, the FT_4I has a normal range in units that are identical to that of the total T_4 (or T_3)—for example, 64 to 142 nmol/L (SI units) and 5 to 11 µg/dL (gravimetric terms). A schematic demonstration of the relationships between total and free T_4, occupied and unoccupied TBG binding sites, and the THBR is shown in Fig. 11.13 for euthyroid individuals with variations in TBG concentrations and in Fig. 11.14 for subjects with a constant TBG and alterations in serum thyroid hormone production rates.

Estrogen, pregnancy, and severe illness are more common causes of changes in total T_4 concentrations than are hyperthyroidism and hypothyroidism (Table 11.11). In the euthyroid person, only about one-third of the available binding sites on TBG are occupied by T_4, and the free T_4 fraction is 2×10^{-4} of the total. During pregnancy, the TBG-binding capacity, the serum T_4, and the number of unoccupied TBG-binding sites approximately double, leading to an approximately 50% reduction of the free T_4 fraction. If the reduced THBR (or free fraction) is multiplied by an increased total T_4, the FT_4I estimate is normal, an accurate reflection of the free T_4 concentration. In patients in whom the serum T_4 concentration is reduced owing to a low TBG, the concentration of unoccupied binding sites is reduced to an even greater extent. This reduction leads to an increase in the free T_4 (and T_3) fractions and the THBR, and both the free T_4 and the FT_4I remain in the normal range. These concepts and expected results in patients with abnormalities in total T_4 or TBG are shown in Figs. 11.13 and 11.14. When concentrations of TBG are altered, the deviation of the total T_4 measurements from normal is in the opposite direction to that of the THBR (see central panels of Fig. 11.13). On the other hand, when the T_4 level is elevated due to increased T_4 secretion or overreplacement, the concentration of



• **Fig. 11.13** Pattern of changes in total serum thyroxine (T_4) concentrations and the thyroid hormone–binding ratio (THBR) in euthyroid patients with alterations in the circulating concentrations of thyroxine-binding globulin (TBG). To convert T_4 from nmol/L to µg/dL (total) or pmol/L (free), divide by 12.87.



• **Fig. 11.14** Pattern of changes in total serum thyroxine (T_4) concentration and thyroid hormone–binding ratio (THBR) in patients with hyperthyroidism or hypothyroidism with normal serum thyroxine-binding globulin (TBG) concentration.

TABLE 11.11 Circumstances Associated with Altered Binding of Thyroxine by Thyroxine-Binding Globulin

Increased Binding	Decreased Binding
Pregnancy	Androgens
Neonatal state	Large doses of glucocorticoids
Estrogens and hyperestrogenemic states	Active acromegaly
	Nephrotic syndrome
Tamoxifen	Major systemic illness
Oral contraceptives	Genetic factors
Acute intermittent porphyria	Asparaginase
Infectious and chronic active hepatitis	
Biliary cirrhosis	
Genetic factors	
Perphenazine	
Human immunodeficiency virus infection	

unoccupied TBG binding sites is reduced, and both the free fraction and the total T_4 are altered in the same direction (see Fig. 11.14).

Several caveats should be kept in mind in the interpretation of these results. The use of labeled T_3 in some assays can produce difficulties in three situations: (1) in cases of *familial dysalbuminemic hyperthyroxinemia* (FDH), (2) in the presence of endogenous antibodies directed against T_3, and (3) in sick patients, as already discussed. In FDH, the abnormal albumin binds T_4, but *not* T_3, with increased avidity. Therefore these patients have an elevated total T_4 and reduced free fraction of T_4 (when measured by the FT_4I) *but not* T_3.[213] It is also possible to measure TBG either by saturation analysis or radioimmunoassay. Normal concentrations of TBG by radioimmunoassay are about 270 nmol/L (1–1.5 mg/dL) and are only slightly higher in women than in men.

Causes of Abnormal TSH or Thyroid Hormone Concentrations

Several causes of an abnormal TSH should be considered by the clinician (Table 11.12). The clinical status and free T_4 results (and sometimes T_3 levels) allow evaluation of the cause for abnormal TSH levels.

Causes of a Suppressed TSH

The most common cause of a reduction in serum TSH is an excess supply of thyroid hormone due to either increased endogenous thyroid hormone production or excessive exogenous thyroid hormone. Because the concentration of TSH is inversely proportional to the degree of thyroid hormone excess, patients with clinical symptoms almost invariably have serum TSH concentrations below 0.1 mU/L. Such patients nearly always have an increase in the free T_4. When thyroid hormone supply is only slightly in excess of the requirement for that patient, serum TSH is suppressed, but clinical manifestations are subtle or absent, and the free T_4 is in the high-normal range. Such minimal changes can sometimes occur with mild Graves disease, autonomous thyroid hormone–producing adenomas, multinodular goiters, subacute or painless thyroiditis, and the ingestion of an amount of exogenous thyroid hormone slightly higher than that required for metabolic needs. This condition is termed *subclinical hyperthyroidism*.

The hypothalamic-pituitary axis may remain suppressed for several months after complete resolution of the thyrotoxic state.[214] The best test for assessing the physiologic state in such patients is the free T_4 (or FT_4I). A common scenario for this pattern is during follow-up of patients receiving antithyroid drugs or ^{131}I for Graves disease. With time, the TSH feedback regulatory loop will normalize, and TSH secretion will return and become appropriate for the circulating free thyroid hormone concentration. As mentioned, patients with few or no symptoms of thyrotoxicosis and subnormal TSH and high free T_4 values should be questioned regarding biotin ingestion.

In severe illnesses, with or without dopamine infusion or excess glucocorticoid, TSH is suppressed, making assessment of thyroid functional status difficult (see earlier discussion). Because the free T_4 may also be reduced in such patients, astute clinical judgment is required to assign the thyroid status.

Because hCG can activate the TSHR, conditions in which hCG is particularly high, such as in the first trimester of pregnancy, with twin pregnancies, and in patients with hydatidiform mole or choriocarcinoma, the TSH concentration is often suppressed.[162] Interestingly, there is a correlation with hCG levels and the occurrence of *hyperemesis gravidarum*. TSH returns to normal in the second and third trimesters in the euthyroid patient. A persistently suppressed TSH (<0.1 mU/L) in the pregnant patient after the first trimester suggests that the hyperthyroidism is due to autonomous thyroid function.

Changes in thyroid test results in patients with psychosis or depression, in the geriatric population, and with the use of long-term glucocorticoids have been discussed earlier. If the serum TSH is suppressed *and* the serum free T_4 is low, one should be suspicious that liothyronine (triiodothyronine) is being ingested before assuming pituitary or hypothalamic dysfunction. Desiccated thyroid also has a high T_3:T_4 ratio and if given in excess may cause a similar abnormality.[215]

Causes of an Elevated TSH

Elevations in TSH nearly always imply a reduction in the supply of T_4 or T_3, which may be permanent or transient. Primary hypothyroidism is far and away the usual explanation. Other causes include acutely ill patients, such as those with renal insufficiency,[216] or the asynchronous return of the hypothalamic-pituitary-thyroid axis to normal as critically ill patients recover.[217] Iodine deficiency is the most common cause of an elevation in TSH worldwide, but this does not occur in North America. The rare patient with RTH due to a mutation in the *THRB* gene (RTHβ) may have a complex phenotype with signs of hypothyroidism in some tissues and hyperthyroidism in others. The most common laboratory pattern is a serum TSH level that is normal or mildly elevated in absolute terms but inappropriately high for the elevated free T_4.[126] They must be differentiated from the patient with a thyrotroph tumor in whom the persistent secretion of TSH causes hyperthyroidism (see Chapters 9 and 12).[207] Patients with mutations in the *THRA* gene (RTHα) have a normal TSH, free T_3, but a low free and total T_4.[127]

Patients with hypothalamic-pituitary dysfunction may have clinical and chemical hypothyroidism but low, normal, or even elevated serum TSH concentrations. The explanation for this

TABLE 11.12 Thyroid Status and Free Thyroid Hormone Levels in Clinical States Associated With Abnormal Serum Thyrotropin (TSH) Concentrations[a]

	Expected TSH (mU/L)	Clinical Thyroid Status	Free T_4	Free T_3
Thyrotropin Reduced[a]				
Hyperthyroidism of any cause	<0.1	↑	↑	↑
Euthyroid Graves disease	0.2–0.5	N, (↑)	N	N, (↑)
Autonomous nodule or multinodular goiter	0.2–0.5	N, (↑)	N	↑
Exogenous thyroid hormone excess	<0.1–0.5	N, ↑	N, ↑	↑
Thyroiditis (subacute or painless)	<0.1–0.5	N, ↑	N, ↑	↑, (N)
Recent thyrotoxicosis due to any cause	<0.1–0.5	↑, N, ↓	N, ↓	N, ↓
Illness with or without dopamine infusion	<0.1–5.0	N	↑, N, ↓	↓
First trimester of pregnancy	0.2–0.5	N, (↑)	N, (↑)	↑
Hyperemesis gravidarum	0.2–0.5	N, (↑)	↑, (N)	↑
Hydatidiform mole	0.1–0.4	↑	↑	↑
Acute psychosis or depression (rare)	0.4–10	N	N, (↑)	N, (↓ or↑)
Elderly (small fraction)	0.2–0.5	N	N	N
Glucocorticoids (acute, high dose)	0.1–0.5	N	N	↓
Biotin	↓	N	N	N
Congenital TSH deficiency				
a. Combined pituitary hormone deficiency (POU1F1/PIT1, PROP1, LHX3, HESX1)	0 - ↓	↓	↓	↓
b. TSH beta gene mutations	0 - ↓	↓	↓	↓
Thyrotropin Elevated				
Primary hypothyroidism	6–500	↓	↓	N, ↓
Recovery from severe illness	5–30	N, (?)	N, ↓	N, ↓
Iodine deficiency	6–150	N, ↓	↓	N
Thyroid hormone resistance	1–20	↑, N, ↓	↑	↑
Thyrotroph tumor	0.5–50	↑	↑	↑
Hypothalamic-pituitary disease	1–20	↓	↓	N, ↓
Psychiatric illnesses	0.4–10	N	N	N, ↓
Adrenal insufficiency	5–30	N	N	N, ↓
Artifact (endogenous antimouse γ-globulin antibodies)	10–500	N	N	N

[a]Arrows indicate the nature of the abnormality in free T_4 or T_3. Parentheses indicate that such a result is unusual but may occur. Excess serum biotin may suppress TSH and elevate free T_4.

N, No change; *T_3*, triiodothyronine; *T_4*, thyroxine.

paradox is that the biologic effectiveness of the circulating TSH is impaired as a result of abnormal glycosylation secondary to reduced TRH stimulation of the thyrotrophs. Nonetheless, the abnormal TSH is a suitable antigen in the immunometric assay. In adrenal insufficiency, TSH may be modestly elevated but returns to normal with glucocorticoid replacement.[201] This may reflect glucocorticoid-mediated amelioration of Hashimoto thyroiditis.

Despite the utility and general efficacy of the serum TSH measurement alone as a screening tool for identifying patients with thyroid dysfunction, a patient should not receive treatment solely on the basis of an abnormal TSH. The TSH assay is an *indirect reflection* of thyroid hormone supply and does not, by itself, permit a conclusive diagnosis of a specific disorder of thyroid hormone production. Accordingly, the TSH abnormality must be confirmed and the expected alteration in thyroid hormone concentrations documented before initiating treatment.

Tests That Assess the Metabolic Impact of Thyroid Hormones

Abnormalities in the supply of thyroid hormone to the peripheral tissues are associated with alterations in a number of metabolic

processes that can be quantitated. Some of these may be useful in the rare patient in whom serum TSH is not an accurate barometer of thyroid status, such as those with RTHβ. These tests may be the sole means of evaluating the metabolic response of the peripheral tissues to thyroid hormones in such patients but are rarely required otherwise.

Basal Metabolic Rate

Thyroid hormones increase energy expenditure and heat production, as manifested by weight loss, increased caloric requirement, and heat intolerance. Because it is impractical to measure heat production directly, the basal metabolic rate (BMR) measures oxygen consumption under specified conditions of fasting, rest, and tranquil surroundings. Under these conditions, the energy equivalent of 1 L of oxygen is 4.83 kcal.

Under basal conditions, approximately 25% of oxygen consumption is due to energy expenditure in visceral organs, including the liver, kidneys, and heart; 10% occurs in the brain, 10% in respiratory activity, and the remainder in skeletal muscle. Because energy expenditure is related to functioning tissue mass, oxygen consumption is related to some index thereof, most often body surface area. Calculated in this way, basal oxygen consumption (resting energy expenditure) is higher in men than in women and declines rapidly from infancy to the third decade and more slowly thereafter. Values in patients, calculated as a percentage of established normal means for sex and age, normally range from −15% to +5%. In severely hypothyroid patients, values may be as low as −40%, and in thyrotoxic patients, these values may reach +25% to +50%. Abnormal, usually elevated, values are seen during recovery in burn patients and in those with systemic disorders, such as febrile illnesses, pheochromocytoma, myeloproliferative disorders, anxiety, and disorders associated with involuntary muscular activity. Resting energy expenditure correlates very well with the free T_4 and TSH in hypothyroid patients given varying doses of exogenous levothyroxine.[218]

Biochemical Markers of Altered Thyroid Status

Occasionally a diagnosis of thyroid dysfunction is first suspected due to an abnormality in a laboratory result performed during an evaluation for an unrelated medical problem. Classic examples are a markedly elevated creatine kinase MM isoenzyme or low-density lipoprotein (LDL) cholesterol reading, leading to the recognition of hypothyroidism.[219] Other similar markers are listed in Table 11.13. These tests are not useful in the diagnosis of thyroid disease, but some, such as sex hormone–binding globulin (SHBG), ferritin, or LDL cholesterol, have been used as end points in clinical studies of the responsivity of the liver to thyroid hormone in patients with thyroid hormone resistance.

Serum Thyroglobulin

The functional sensitivity of most Tg assays is 1 ng/mL or less.[220] The results can be artifactually altered by serum anti-Tg antibodies, and serum should be screened for Tg antibodies with a sensitive Tg-antibody immunoassay. In immunoradiometric assays, interferences lead to underestimations of Tg or false-negative values, whereas values when measured by radioimmunoassay may be falsely elevated.

Tg is normally present in the serum, the concentration ranging up to 50 ng/mL; mean normal values vary with the assay used but are on the order of 20 ng/mL.[221] Concentrations are somewhat higher in women than in men and are elevated several fold in pregnant women and in the newborn. Levels are elevated in three types of thyroid disorders: goiter and thyroid hyperfunction, inflammatory or physical injury to the thyroid, and differentiated follicular cell–derived thyroid tumors and especially in consumptive hypothyroidism.[104] Values are elevated in both endemic and sporadic nontoxic goiter, and the degree of elevation correlates with the thyroid size. Transient elevations occur in patients with subacute thyroiditis and as a result of trauma to the gland during thyroid surgery or after ^{131}I therapy. Subnormal or undetectable concentrations are found in patients with thyrotoxicosis factitia and aid in differentiating this disorder from other causes of thyrotoxicosis with a low thyroid radioiodine uptake (RAIU).

A major clinical value of measuring the level of serum Tg is in the management, but not in the diagnosis, of differentiated thyroid carcinoma.[220,222] Serum Tg concentrations are increased in patients with both benign and differentiated malignant follicular cell–derived tumors of the thyroid and do not serve to distinguish between the two. After total thyroid ablation for papillary or follicular thyroid carcinoma, Tg should not be detectable, and its subsequent appearance typically signifies the presence of persistent or recurrent disease.[222] The serum Tg level is related to the mass of neoplastic tissue and may be undetectable in patients with small lymph node micrometastases (see Chapter 14). Secretion of Tg is TSH dependent. Therefore the serum Tg level may rise when suppressive therapy is withdrawn or after injections of recombinant human TSH (rhTSH), which will increase the sensitivity of the marker for the detection of persistent or recurrent thyroid carcinoma, even when ^{131}I scans are negative (see Chapter 14). Supersensitive assays of Tg with a functional sensitivity less than 0.1 ng/mL improve the sensitivity during thyroid hormone treatment, but at the expense of a decreased specificity.[223,224] This measurement is useful for the follow-up of patients treated with total thyroidectomy with or without radioactive iodine ablation.[225] Unfortunately there is a major potential artifact in some patients due to the presence of autoantibodies binding to Tg. In this situation,

TABLE 11.13 Biochemical Markers of Thyroid Status

Thyrotoxicosis
Increased
Osteocalcin
Urine pyridinium collagen cross-links
Alkaline phosphatase (bone or liver)
Atrial natriuretic hormone
Sex hormone–binding globulin
Ferritin
von Willebrand factor
Decreased
Low-density lipoprotein cholesterol
Lipoprotein(a)
Hypothyroidism
Increased
Creatine kinase (MM isoform)
Low-density lipoprotein cholesterol
Lipoprotein(a)
Plasma norepinephrine
Decreased
Vasopressin

measurement of Tg by radioimmunoassay or liquid chromatography-tandem mass spectrometry (LC-MS/MS) may be more informative.[226]

In the hypothyroid newborn, serum Tg is undetectable in patients with thyroid agenesis and is usually elevated in those with ectopic thyroid tissue or goiter and in consumptive hypothyroidism due to infantile hemangioma.

Tests for Thyroid Autoantibodies

Graves disease and Hashimoto thyroiditis are well characterized and interrelated autoimmune thyroid disorders with a variety of clinical manifestations. The diagnostic hallmark of the autoimmune thyroid disorders is the presence, in most patients, of circulating autoantibodies and reactive T cells against one or more thyroid antigens.[227] Three varieties of thyroid autoantibodies are in common use and widely available in clinical diagnostic laboratories (Table 11.14). In this section, autoantibodies to Tg and TPO are discussed. Antibodies directed against the TSHR, the cause of hyperthyroidism in patients with Graves disease, are detailed in Chapter 12.

Autoantibodies to Thyroid Peroxidase and Thyroglobulin

Current automated assay techniques for thyroid autoantibodies have good precision because they depend on direct measurement of the interaction between autoantibody and autoantigen (i.e., the interaction between thyroid antigen and the patient's serum). In general, the more sensitive an assay, the more precise and antigen specific it is. However, many euthyroid individuals exhibit low levels of autoantibodies, and in this situation, the absolute concentration becomes important. The higher the concentration of autoantibody, the greater the clinical specificity (see Table 11.14).[228]

So that comparisons of thyroid antibody concentrations can be made from one office visit to the next, among different patients and among laboratories, assays for thyroid autoantibodies have been standardized with results expressed as standard units per milliliter. Of course, the actual standard serum preparation cannot be included in every assay. Instead, a serum pool is usually compared and normalized to the original standard. Autoantibodies differ considerably in their affinity and epitope recognition of antigen, and so the slopes of different standard curves may vary. As a result, despite this attempt at standardization, assay results from different commercial assays may still vary considerably. Hence when monitoring antibody titers (e.g., measuring Tg-Ab after the treatment of thyroid cancer), it is always best to consistently use the same autoantibody assay.

TABLE 11.14 Prevalence of Thyroid Autoantibodies

Group	TSHR-Ab (%)	hTg-Ab (%)	hTPO-Ab (%)
General population	0	5–20	8–27
Patients with Graves disease	80–95	50–70	50–80
Patients with autoimmune thyroiditis	10–20	80–90	90–100
Relatives of patients	0	40–50	40–50
Patients with IDDM	0	40	40
Pregnant women	0	14	14

IDDM, Insulin-dependent diabetes mellitus; *hTg-Ab,* human thyroglobulin antibody; *hTPO-Ab,* human thyroid peroxidase antibody; *TSHR-Ab,* thyroid-stimulating hormone receptor antibody.

Do Thyroglobulin and Thyroid Peroxidase Antibodies Have a Pathogenic Role?

Tg-Ab and TPO autoantibodies (TPO-Ab) appear to be a secondary response to thyroid injury and are not thought to cause disease themselves, although they may contribute to its development and chronicity. Both types of antibodies are polyclonal, and although they are of the immunoglobulin G class, they are not restricted to a particular immunoglobulin G subclass. These thyroid antibodies cannot transfer disease from mother to fetus or between animals even though they can pass across the placenta.[229] However, both antibodies may contribute to disease mechanisms. For example, TPO-Ab on the surface of B cells may be involved in antigen presentation, thus activating thyroid-specific T cells.[230] Such autoantibodies may have complement-fixing cytotoxic activity; TPO-Ab, in particular, correlates well with thyroid damage and lymphocytic infiltration.

Thyroid Autoantibodies in Hashimoto Thyroiditis and Graves Disease

The disease most widely associated with Tg-Ab and TPO-Ab is autoimmune thyroiditis, or Hashimoto disease (terms that embrace both goitrous thyroiditis, as first described by Hashimoto, and atrophic thyroid failure, previously referred to as primary myxedema). Both Tg-Ab and TPO-Ab are found in almost 100% of such patients, but TPO-Ab has a higher affinity and occurs in higher concentrations, so testing for TPO-Ab is more helpful.

Tg-Ab and TPO-Ab are also detectable in 50% to 90% of patients with Graves disease, indicative of the associated thyroiditis that is evident histologically as a heterogeneous lymphocytic infiltration. Hence Graves disease tends to develop on a background of autoimmune thyroiditis. Although the presence of such autoantibodies favors an autoimmune cause for hyperthyroidism over other causes, the tests are neither sensitive nor specific in this setting and are interpretable only as part of the clinical scenario. Testing for TSHR antibodies is important in the evaluation of hyperthyroid patients, along with consideration of a radioiodine uptake and scan.

Thyroid Autoantibodies in Nonautoimmune Thyroid Disorders

Tg-Ab and TPO-Ab are more common in patients with sporadic goiter, multinodular goiter, or isolated thyroid nodules and cancer than in the general population. This finding usually represents an associated thyroiditis on histologic examination. Low levels of thyroid autoantibodies may occur transiently in patients with subacute (de Quervain) thyroiditis but correlate poorly with disease course and are probably a nonspecific response to thyroid injury. There is also a higher prevalence of thyroid autoantibodies in many other autoimmune diseases, particularly insulin-dependent diabetes mellitus.

Thyroid Autoantibodies in Pregnancy

Euthyroid women with thyroid autoantibodies have been shown to suffer from increased early pregnancy loss. The miscarriage rate in the presence of TPO-Ab is almost doubled in many studies,[231–233] and the cause is uncertain. It is unclear if this is a reflection of an immune diathesis or due to subtle alterations in thyroid function.

The screening of pregnant women has been advocated by some groups but is controversial.[234]

The Normal Population

Although the prevalence of thyroid autoantibodies depends on the technique used for detection, Tg-Ab and TPO-Ab are common in the general population (see Table 11.14). At all ages, these antibodies are almost five times more common in women than in men. Selected groups at risk include younger women and relatives of patients with an autoimmune thyroid disorder, in whom the incidence is higher. The low levels of TPO-Ab and Tg-Ab found in many individuals are of uncertain significance in the presence of normal thyroid function; however, they remain a significant risk factor in families with autoimmune thyroid disorders.[235]

Radioiodine Uptake

A direct test of thyroid function uses a radioactive isotope of iodine as a marker for the body's stable form of iodine, ^{127}I. Most often the test involves the measurement of the fractional uptake by the thyroid of a tracer (chemically inconsequential) dose of radioiodine. However, several factors make this test less frequently used than in the past. The first is the improvement in indirect methods for assessing thyroid status. The second is the decrease in normal values for thyroid RAIU consequent to the widespread increase in daily dietary iodine intake, reducing the utility of the test in the diagnosis of thyroid disorders. However, the test is still useful in defining the etiology of thyrotoxicosis. It should not be performed in pregnant and breastfeeding women.

^{131}I (half-life 8.1 days) and ^{123}I (half-life 0.55 day) both emit gamma radiation, which permits their external detection and quantitation at sites of accumulation, such as the thyroid. These isotopes (abbreviated I* hereafter) are physiologically indistinguishable, not only from one another but also from the naturally occurring I, which permits their use as valid tracers. The shorter half-life of ^{123}I is preferable because the radiation delivered to the thyroid per amount of administered ^{123}I is only about 1% of that delivered by ^{131}I. ^{131}I also emits beta radiation, hence its utility in the therapy of patients with Graves disease, hyperfunctioning nodules, and well-differentiated thyroid cancer.

Physiologic Basis

When tracer quantities of inorganic radioiodine are administered orally or intravenously, the isotope quickly mixes with the endogenous stable iodide in the extracellular fluid and begins to be removed by the two major sites of clearance, the thyroid and the kidneys. As this process continues, the plasma level of tracer iodide I* decreases exponentially. Low levels are reached by 24 hours, and inorganic I* is virtually undetectable in the plasma 72 hours after its administration. The thyroid content of I* increases rapidly during the early hours and then at a decreasing rate until a plateau is approached. The proportion of administered I* ultimately accumulated by the thyroid is a function of the clearance of iodide by the thyroid and kidneys. The relationship is simply expressed by the following equation:

$$\text{RAIU at plateau} = \frac{C_T}{C_T + C_K}$$

where C_T represents the thyroid iodide clearance rate and C_K the renal iodide clearance rate. The normal thyroid iodide clearance rate is approximately 0.4 L/hour, and the renal iodide clearance rate is 2.0 L/hour, so the uptake of I* normally approximates 20% of the administered dose.

Measurements of RAIU are generally made at 24 hours, both as a matter of convenience and because the value at 24 hours is usually near the plateau, but RAIU can be measured at 6 hours with appropriate determination of a reference range.

Radioactive Iodine Uptake

Little difference will be noted if the uptake is measured at any time during the day following that on which the isotope was administered, and for the calculation of therapeutic radioiodine doses in treating thyrotoxic Graves disease an early uptake at 3 to 6 hours may produce results comparable to those found at 20 to 28 hours.[236] With the use of this modified early RAIU measurement, diagnosis and treatment of thyrotoxic Graves disease can be accomplished on the same day. In general, the reference range in North America is approximately 5% to 25%. Higher values are found in iodine-deficient regions or in patients with thyroid hyperfunction, but as with other procedures, patients with mild hyperthyroidism may display values at or just above the upper limit of the reference range (Table 11.15).

The Perchlorate Discharge Test

In normal individuals, more than 90% of thyroidal radioiodine is organified and present as iodotyrosine and iodothyronine within minutes of its entry into the thyroid. It is then no longer in the intracellular iodide pool. In patients with Pendred syndrome or with other disorders that inhibit the iodination of tyrosine, such as Hashimoto thyroiditis, or those receiving thiourea drugs, this process is delayed, as shown by the exit (discharge) of more than 10% of the thyroidal radioiodine within 2 hours of administration of 500 mg of $KClO_4$.[12] Perchlorate inhibits NIS function by competing with iodide for NIS, eliminating the iodide gradient that is required for maintaining the radioiodide in the gland. This illustrates that both iodide transport by NIS at the basal pole of the thyrocyte and its efflux across the apical membrane by pendrin are required for thyroid hormone synthesis.

States Associated With Increased RAIU

Hyperthyroidism

Hyperthyroidism (sustained excess endogenous thyroid hormone production) as distinct from *thyrotoxicosis*, which refers to the manifestations of excess thyroid hormone levels due to any cause, is accompanied by an elevated RAIU at 24 hours unless body iodide stores are increased. The increased uptake is always evident unless the release of labeled hormone is so rapid that the thyroidal content of I* has decreased to the normal range by the time the measurement is made. This condition is rare, is typically associated with severe thyrotoxicosis, and can be recognized by checking the thyroid uptake at 6 hours.

Aberrant Hormone Synthesis

RAIU can be increased in the absence of hyperthyroidism in disorders in which iodine accumulation is normal but the secretion of hormone is impaired, such as in patients with abnormal Tg synthesis.[237] The magnitude of the increase in uptake and the time at which the plateau is achieved vary with the nature and severity of the disorder. Differentiation of the foregoing states from hyperthyroidism is generally not difficult because in the former, clinical findings and laboratory evidence of hyperthyroidism are lacking, and indeed hypothyroidism may be present.

TABLE 11.15 Factors That Influence 24-Hour Thyroid Iodide Uptake

Factors That Increase Uptake

Increased Hormone Synthesis

- Hyperthyroidism
- Response to glandular hormone depletion
  - Recovery from thyroid suppression
  - Recovery from subacute thyroiditis
  - Antithyroid agents
- Excessive hormone losses
  - Nephrotic syndrome
  - Chronic diarrheal states
  - Soybean ingestion

Normal Hormone Synthesis

- Iodine deficiency
  - Dietary insufficiency
  - Excessive loss (dehalogenase defect, pregnancy)
- Hormone biosynthetic defects

Factors That Decrease Uptake

Decreased Hormone Synthesis

- Primary hypofunction
  - Primary hypothyroidism
  - Antithyroid agents
  - Hormone biosynthetic defects
  - Hashimoto disease
  - Subacute thyroiditis
- Secondary hypofunction
- Exogenous thyroid hormones

Not Reflecting Decreased Hormone Synthesis

- Increased availability of iodine
  - Diet or drugs
  - Cardiac or renal insufficiency
- Increased hormone release
  - Very severe hyperthyroidism (rare)

Iodine Deficiency

RAIU is increased in acute or chronic iodine deficiency, as demonstrated by measurement of urinary iodine excretion, with urinary iodine values lower than 100 μg/day, indicating deficiency. Chronic iodine deficiency is usually the result of an inadequate amount of iodine in food and water (endemic iodine deficiency). Patients with cardiac, renal, or hepatic disease may develop iodine deficiency if given diets severely restricted in salt, especially if diuretic agents are administered.

Response to Thyroid Hormone Depletion

Rebound increases in RAIU are seen after withdrawal of antithyroid therapy, after subsidence of transient or subacute thyroiditis, and after recovery from prolonged suppression of thyroid function by exogenous hormone. A striking increase in uptake occurs in patients with iodide-induced myxedema after cessation of iodide administration. The duration of the rebound depends on the time required to replenish thyroid hormone stores.

Excessive Hormone Losses

In nephrotic syndrome, excessive losses of hormone in the urine occurring in association with urinary loss of binding protein cause a compensatory increase in hormone synthesis and RAIU. A similar sequence may occur when losses of hormone via the gastrointestinal tract are abnormal, as in chronic diarrheal states or during ingestion of agents such as soybean protein and cholestyramine, which bind T_4 in the gut.

States Associated With Decreased RAIU

A general increase in iodine intake has made values of the RAIU in hypothyroidism indistinguishable from those at the lower end of the reference range. Therefore the major indication for measuring the RAIU is to establish whether thyrotoxicosis is due to hyperthyroidism (high RAIU) or thyroiditis (low RAIU).

Exogenous Thyroid Hormone: Thyrotoxicosis Factitia

Except in disorders in which homeostatic control is disrupted or overridden (e.g., Graves disease or autonomously functioning thyroid nodules), administration of exogenous thyroid hormone suppresses TSH secretion and reduces the RAIU, usually to values below 5%.

Low values of the RAIU in a patient who is clinically thyrotoxic may also indicate the presence of thyrotoxicosis factitia, the syndrome produced by the ingestion of excess thyroid hormone. The unmeasurably low level of Tg in serum differentiates thyrotoxicosis factitia from other causes of thyrotoxicosis with decreased RAIU.[238]

Disorders of Hormone Storage

The RAIU is usually low in the early phase of subacute thyroiditis and in chronic thyroiditis with transient hyperthyroidism. Here, inflammatory follicular disruption leads to loss of the normal storage function of the gland and leakage of hormone into the blood. In the early stage of subacute thyroiditis, leakage of hormone is usually sufficient to suppress TSH secretion and the RAIU. Transient hypothyroidism often occurs late in both diseases, when stores of preformed hormone are depleted; the RAIU may return to normal or increased values at that time.

Exposure to Excessive Iodine

Exposure to excessive iodine is a common cause of a subnormal RAIU. Such decreases are spurious in the clinical sense because they do not indicate decreased absolute iodine uptake or decreased hormone production but can be produced by the introduction of excessive iodine in any form: inorganic, organic, or elemental. Common offenders are organic iodinated dyes used as radiograph contrast media and amiodarone (see Table 11.7). The duration of suppression of the uptake varies among individuals and with the compound administered. In general, dyes used for pyelography or computed tomography scanning are cleared within a month, whereas amiodarone may influence the uptake for up to 12 months because of its storage in fat. A single large dose of inorganic iodide can decrease uptake for several days, and chronic ingestion of iodide may depress the uptake for many weeks. Excessive quantities of iodine may also be present in vitamin and mineral preparations, vaginal or rectal suppositories, and iodinated antiseptics such as povidone (see Table 11.7).

The measurement of urinary iodine excretion is an invaluable means of establishing or excluding the existence of excessive body iodide stores; the 24-hour iodine excretion can be roughly extrapolated from the iodide-to-creatinine ratio in a random urine sample. Values in excess of 2 mg/day can account for a low RAIU value, whereas values less than 1 mg/day suggest that a low RAIU is due to one of the other disorders discussed in this section.

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The complete list of references is available online at ExpertConsult.com.

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12

Hyperthyroid Disorders

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CHAPTER OUTLINE

KEY POINTS

- Hyperthyroidism has a prevalence of 1% to 2% in women and 0.1% to 0.2% in men.
- The most common causes of an overactive thyroid are Graves disease and toxic multinodular goiter.
- Graves disease is caused by the development of unique human autoantibodies to the thyroid-stimulating hormone (thyrotropin, TSH) receptor; these autoantibodies act as TSH receptor agonists.
- Graves orbitopathy (GO) remains one of the most difficult endocrine diseases to treat and requires a multidisciplinary approach. It may occur before, during, or even long after resolution of the hyperthyroidism.
- Toxic thyroid nodules are caused by a constitutive activating mutation in the TSH receptor.
- Subacute thyroiditis secondary to infectious agents is usually painful, in marked contrast to the transient autoimmune thyroiditis seen in the postpartum period.
- The treatment of hyperthyroidism is best initiated with the antithyroid drug methimazole; propylthiouracil (PTU) is no longer recommended as first-line therapy because of its rare, but occasionally severe, hepatic toxicity. PTU may be useful in treating severe hyperthyroidism because of its capacity to block conversion of thyroxine (T_4) to triiodothyronine (T_3) by the type 1 deiodinase (D1) in liver, kidney, and Graves thyroid.
- Methimazole embryopathy is also rare. It may be avoided by the use of PTU in the first trimester as well as for women planning a pregnancy while under treatment for hyperthyroidism.
- Overtreatment of the pregnant hyperthyroid patient must be avoided because of the transplacental passage of both PTU and methimazole. Fetal hypothyroidism can impair cognitive development. Typically the TSH should remain suppressed and the free T_4 slightly above normal throughout pregnancy. If possible, the patient should be followed in concert with a high-risk obstetrician.

These days the terms *thyrotoxicosis* and *hyperthyroidism* are used interchangeably and refer to the classic or subtle physiologic manifestations of excessive quantities of the thyroid hormones, which are the characteristics of this condition (Table 12.1). In addition to overstimulation of the thyroid via the TSH receptor and true TSH receptor mutations, other common conditions causing hyperthyroidism include the passive release of thyroid hormones from damaged thyroid follicles; inflammation of the thyroid gland (called *thyroiditis*), which may be autoimmune, postviral, or drug induced; and extrathyroidal sources of thyroid hormone, most often iatrogenic or self-administered. For most patients with thyrotoxicosis, the symptoms and signs caused by an excess of thyroid hormone, whatever the source, lead to medical attention. Others may have surprisingly few symptoms and are referred because of a suppressed TSH. This chapter begins with a brief review of the symptoms and signs of thyrotoxicosis and their

TABLE 12.1 Causes of Hyperthyroidism

I. Excessive TSH-Receptor Stimulation

Graves disease (TRAb)
Pregnancy-associated transient hyperthyroidism (hCG)
Trophoblastic disease (hCG)
Familial gestational hyperthyroidism (mutant TSH receptor)
TSH-producing pituitary adenoma

II. Autonomous Thyroid Hormone Secretion

Multinodular toxic goiter (somatic mutations)
Solitary toxic thyroid adenoma (somatic mutation)
Congenital activating TSH-receptor mutation (genomic mutation)

III. Destruction of Follicles With Release of Hormone

Subacute de Quervain thyroiditis (virus infection)
Painless thyroiditis/postpartum thyroiditis (hashitoxicosis—autoimmune)
Acute thyroiditis (bacterial infection)
Drug-induced thyroiditis (amiodarone, interferon-γ)

IV. Extrathyroidal Sources of Thyroid Hormone

Iatrogenic overreplacement with thyroid hormone
Excessive self-administered thyroid medication
Food and supplements containing excessive thyroid hormone
Functional thyroid cancer metastases
Struma ovarii

hCG, Human chorionic gonadotropin; *TRAb,* thyrotropin receptor antibodies; *TSH,* thyroid-stimulating hormone (thyrotropin).

pathophysiologic basis. The appropriate use of the laboratory tests already described in Chapter 11 is then presented to show how these results can focus the search for a diagnosis.

Clinical Manifestations of Thyrotoxicosis

One very important clinical clue to the cause of the patient's thyrotoxicosis is the duration of symptoms. Patients with hyperthyroidism have generally had manifestations for months before presentation, but because the week-to-week increases in thyroid hormones are small, the effects of the disorder may become rather extreme, while going unnoticed by the patient. In addition, patients will often attribute the symptoms to other causes; for example, they may ascribe their fatigue to family or work responsibilities, heat intolerance to the weather, weight loss to an effective diet, and dyspnea and palpitations to a lack of regular exercise. On the other hand, patients with thyrotoxicosis due to thyroiditis can often date the onset of their symptoms precisely, usually to within a month or so of their seeking medical attention, as might be expected from the effects of the release of the equivalent of 30 to 60 days' supply of thyroid hormone into the circulation over a few days to weeks. Thus ascertaining the chronology as well as the spectrum of symptoms is a critical goal of the interview process.

Another general characteristic is that the symptoms and signs of thyrotoxicosis are more readily recognized in the younger than in the older patient. The term *masked* or *apathetic* thyrotoxicosis is used to describe the syndrome sometimes seen in the elderly, which may present as congestive heart failure with arrhythmia or as unexplained weight loss without the increased appetite and other typical symptoms and signs of the younger patient.

At present, the ready availability of sensitive serum TSH assays, a reliable indicator of excess thyroid hormone in the ambulatory patient (see Chapter 11), has made the more classic and severe manifestations of long-standing thyrotoxicosis less prevalent. In fact, a continued area of some controversy is how aggressively to treat the condition termed *subclinical* hyperthyroidism, a biochemical diagnosis in which a subnormal serum TSH level is accompanied by normal free thyroid hormone concentrations. Nonetheless, the classic presentation is still common, serves to illustrate the pleiotropic physiologic effects of excess thyroid hormones, and, if not recognized, can progress to life-threatening severity despite the fact that hyperthyroidism is a benign condition (accelerated hyperthyroidism). The next sections review the pathophysiology of the most important manifestations of excess thyroid hormone.

Cardiovascular System

Alterations in cardiovascular function in the thyrotoxic patient are in part due to increased circulatory demands that result from the hypermetabolism and the need to dissipate the excess heat produced.[1] At rest, peripheral vascular resistance is decreased, and cardiac output is increased as a result of an increase first in heart rate and, with more severe disease, in stroke volume. Thyroid hormones in excess also have a direct ionotropic effect on cardiac contraction mediated by an increase in the ratio of α-myosin to β-myosin heavy chain expression. Tachycardia is virtually always present and is due to a combination of increased sympathetic and decreased vagal tone.[2] Widening of the pulse pressure is due to the increase in systolic pressure and decrease in diastolic pressure caused by reduced resistance.[3] The decreased resistance is due to increased nitric oxide production via the PI3K/AKT signaling pathway.[4] The increased systolic force is often felt by the patient as a palpitation and is evident on inspection or palpation of the precordium. Because of the diffuse and forceful nature of the apex beat, the heart may seem enlarged, and echocardiography may show an increased ventricular mass. In addition, the preejection period is shortened and the ratio of preejection period to left ventricular ejection time is decreased.[3,5] The heart sounds are enhanced, particularly S_1, and a scratchy systolic sound along the left sternal border, resembling a pleuropericardial friction rub (Means-Lerman scratch), may also be heard. These manifestations abate when a normal metabolic state is restored. Mitral valve prolapse occurs more frequently in Graves or Hashimoto disease than in the normal population[6] and has been suggested as autoimmune in origin.[7] Cardiac arrhythmias are almost invariably supraventricular, especially in younger patients. Between 2% and 20% of patients with thyrotoxicosis have atrial fibrillation, and about 15% of patients with otherwise unexplained atrial fibrillation are thyrotoxic,[1] which may be caused directly by the thyroid hormone excess or by activating autoantibodies to the β_1-adrenergic receptors.[8,9] In the Framingham cohort, individuals over age 60 with a suppressed TSH level had a 2.8-fold increased risk of developing atrial fibrillation compared to those with normal serum TSH values,[10] and such a finding has been widely confirmed.[11]

The increased cardiovascular cost of a standard workload or metabolic challenge is adequately met if the thyrotoxic patient is not, or has not previously been, in heart failure. Thus in most patients without underlying heart disease, cardiac competence is maintained. Mild peripheral edema may occur in the absence of heart failure. Heart failure per se usually, but not always, occurs in patients with preexisting heart disease and therefore is more typically seen in the elderly, but it may not be possible to determine

whether underlying heart disease is present until after thyrotoxicosis is relieved. Atrial fibrillation decreases the efficiency of the cardiac response to any increased circulatory demand and may play a role in causing cardiac failure.[5] Attempts to convert or abate atrial fibrillation to sinus rhythm are not indicated while thyrotoxicosis is present, and about 60% of patients revert spontaneously to sinus rhythm after treatment, most within 4 months. While thromboembolism is not frequent in patients under 50 with thyrotoxicosis, the decision to anticoagulate should be potentially considered in such patients taking into account additional risks for embolic events and risks of therapy.[12] Medical or electrical cardioversion of patients with thyrotoxicosis-induced atrial fibrillation is often successful even after a year has passed.[13]

Protein, Carbohydrate, and Lipid Metabolism

The stimulation of metabolism and heat production is reflected in increased appetite and heat intolerance but only rarely by elevated basal body temperature.[14] Despite an increased food intake, a state of chronic caloric and nutritional inadequacy often ensues, depending on the degree of increased metabolism. Both synthesis and degradation rates of proteins are increased, the latter to a greater extent than the former, with the result that in severe thyrotoxicosis there is a net decrease in tissue protein as indicated by loss of weight, muscle wasting, proximal muscle weakness, and even mild hypoalbuminemia. Preexisting diabetes mellitus may be aggravated, one cause being accelerated turnover of insulin. Both lipogenesis and lipolysis are increased in thyrotoxicosis, but the net effect is lipolysis as reflected by an increase in the plasma concentration of free fatty acids and glycerol and a decrease in serum cholesterol level; triglyceride levels are usually slightly decreased. The enhanced mobilization and oxidation of free fatty acids in response to fasting or catecholamines are due to enhancement of lipolytic pathways by thyroid hormones, including their effects on mitochondrial beta oxidation in the liver.[14,15]

Sympathetic Nervous System and Catecholamines

Many of the manifestations of thyrotoxicosis and of sympathetic nervous system activation are similar. Nonetheless, the plasma concentrations of epinephrine and norepinephrine, as well as their urinary excretion and that of their metabolites, are not increased in patients with thyrotoxicosis, and thyroid hormones exert effects separate from, but similar and additive to, those of the catecholamines. The improvement in cardiac function in patients with hyperthyroidism by β-adrenergic blockade has led to the concept that there is increased sympathetic tone or increased cardiac sensitivity to the sympathetic nervous system.[16] Support for the latter are the results in the transgenic mouse in which overexpression of type 2 deiodinase in the heart increases myocardial T_3 and the cyclic adenosine monophosphate (cAMP) response to norepinephrine in the cardiac myocytes due to alterations in G proteins.[17,18] However, stimulation of the heart by thyroid hormones does not require β-adrenergic receptors in mouse models such that it is likely that β-adrenergic blockade in hyperthyroidism is only influencing part of the disorder. Still intact thyroid hormone signaling pathways are required for adrenergic signaling in white adipocytes leading to lipolysis and for the induction of UCP1 in brown adipocytes by adrenergic signaling pathways.[19,20]

Nervous System

Alterations in the function of the nervous system in thyrotoxicosis are manifested by nervousness, emotional lability, and hyperkinesia. Fatigue may be due both to muscle weakness and to the insomnia that is commonly present. Emotional lability is common, and in rare cases mental disturbance may be severe; manic depressive, schizoid, or paranoid reactions may emerge. The hyperkinesia of the thyrotoxic patient is characteristic and may manifest to such a point that the patient is almost levitating. During the interview the patient shifts positions frequently and movements are quick, jerky, exaggerated, and often purposeless. In children, in whom such manifestations tend to be more severe, inability to focus may lead to deterioration in school performance suggesting attention deficit hyperactivity disorder. There may be a fine tremor of the hands, tongue, or lightly closed eyelids. The electroencephalogram reveals an increase in fast wave activity, and in patients with convulsive disorders the frequency of seizures is increased.

Muscle

Weakness and fatigability are usually not accompanied by objective evidence of muscle disease save for the generalized wasting associated with weight loss. The weakness is most prominent in the proximal muscles of the limbs, causing difficulty in climbing stairs or fatigue from minimal exertion such as using a blow dryer or lifting an infant. Proximal muscle wasting may be out of proportion to the overall loss of weight (often referred to as *thyrotoxic myopathy*). In the most severe forms, the myopathy may involve the more distal muscles of the extremities and the muscles of the trunk and face. Although myopathy of ocular muscles is unusual, the disorder may mimic myasthenia gravis or ophthalmic myasthenia.[21–25] Muscular strength returns to normal when a normal metabolic state is restored, but muscle mass takes longer to recover.

Graves disease occurs in about 3% to 5% of patients with myasthenia gravis, and about 1% of the patients with Graves disease develop myasthenia gravis. Antibodies and T cells specific for the TSH and acetylcholine receptors are involved in the pathogenesis of the two diseases.[26] Unlike thyrotoxic myopathy, the association of myasthenia gravis with Graves disease has a distinct female preponderance. The effect of both thyrotoxicosis and its alleviation on the course of myasthenia gravis is variable, but in the majority of instances, myasthenia is accentuated during the thyrotoxic state and improves when a normal metabolic state is restored. A form of myasthenia affecting mainly the orbital muscles may also occur more commonly in patients with Graves disease and needs to be distinguished from GO by the prominence of bilateral ptosis of a variable degree.[22]

Periodic paralysis of the hypokalemic type may occur together with thyrotoxicosis, and its severity is accentuated by the latter disorder. The coincidence of the two disorders is particularly common in Asian and Latino males.[27,28]

Eyes

Some retraction of the upper or lower eyelids, or both, evident as the presence of a rim of sclera between either lid and the limbus, may be seen in all forms of thyrotoxicosis regardless of the underlying cause and is responsible for the typical stare of the patient. Also common is either lid lag, a phenomenon in which the upper lid lags behind the globe when the patient is asked to shift the gaze

slowly downward, or globe lag, which becomes evident when the eye lags behind the upper lid when the patient looks up. These ocular manifestations appear to be the result of increased adrenergic tone. It is important to differentiate these signs, which may occur in all forms of thyrotoxicosis, from those of infiltrative autoimmune orbitopathy, which are associated with Graves disease and are described later.

Skin and Hair

The most characteristic change in the patient with long-standing thyrotoxicosis is the warm, moist feel of the skin that results from cutaneous vasodilation and excessive sweating. The elbows may be smooth and pink, the complexion is rosy, and the patient blushes readily. Palmar erythema may resemble liver palms (palmar erythema), and telangiectasia may be present. The hair is fine and friable, and hair loss may increase. The nails are often soft and friable. A characteristic but uncommon finding is Plummer nails, onycholysis, typically involving the fourth and fifth fingers. Vitiligo, another autoimmune disease, is more common in patients with autoimmune thyroid disease.

Respiratory System

Dyspnea is common in severe thyrotoxicosis, and several factors may contribute to this condition. Vital capacity is commonly reduced, mainly from weakness of the respiratory muscles. During exercise, ventilation is increased out of proportion to the increase in oxygen uptake, but the diffusing capacity of the lung is normal. Because of the general increase in oxygen consumption associated with thyrotoxicosis, patients with chronic lung diseases may experience a rather severe worsening of the condition if they become thyrotoxic.

Alimentary System

An increase in appetite is common but is usually not seen in patients with mild disease. In more severe disease the increased intake of food is inadequate to meet the increased caloric requirements, and weight is lost at a variable rate. More often, the patient reports a gratifying success with a previously frustrated attempt at weight control. The frequency of bowel movements is increased; diarrhea, although rare, can also be a problem. The increased gastric emptying and intestinal motility in thyrotoxicosis appear to be responsible for slight malabsorption of fat, and these functions return to normal when a normal metabolic state has been restored. Celiac and Graves diseases coexist more commonly than once thought, and there is an increased prevalence of pernicious anemia.

Hepatic dysfunction occurs, particularly when thyrotoxicosis is severe; hypoproteinemia and increases in serum alanine aminotransferase (ALT) and bone or liver alkaline phosphatase levels may be elevated. Progressive hepatomegaly and jaundice was a cause of death prior to the development of successful treatment for Graves patients likely exacerbated by congestive heart failure.

Skeletal System: Calcium and Phosphorus Metabolism

Thyrotoxicosis is generally associated with increased excretion of calcium and phosphorus in urine and stool; with an increase in bone turnover and a net demineralization of bone, as demonstrated by routine bone densitometry; and occasionally with pathologic fractures, especially in elderly women.[21,23,29–32]

In such instances the pathologic changes are variable and may include osteitis fibrosa, osteomalacia, or osteoporosis, most likely varying with vitamin D status. Urinary excretion of collagen breakdown products (teleopeptides) is increased in thyrotoxicosis. Kinetic studies indicate an increase in the exchangeable calcium pool and acceleration of both bone resorption and accretion, particularly the former. Thyroid hormone (T_3) has been shown to accelerate activity of the osteoclasts via its nuclear receptors and helps explain these widespread changes.[21,24,32] Some data indicate that TSH itself may have a local action, which may normally balance thyroid hormone action on osteoclasts and enhance osteoblast activity.[25,33,34] Such an action by TSH would be absent in hyperthyroidism, allowing accentuation of the thyroid hormone effects. However, not all models recapitulate these findings, and taken together it is likely that excess levels of thyroid hormones have a more profound effect on bone mineral density.[35] As the thyrotoxicosis is treated, bone density may normalize in many younger patients but not all.[36] Postmenopausal women, however, may have an accelerated reduction in bone density that requires treatment (see Chapter 30). Much controversy has existed over the induction of decreased bone density by TSH-suppression therapy in patients with thyroid cancer. Suffice it to say that postmenopausal, but not premenopausal, women given a TSH-suppressive dosage of thyroid hormones are at risk of osteopenia and require prophylaxis with calcium and vitamin D or more aggressive approaches.[37,38] The decision to relax TSH suppression in low-risk patients may be influenced by their bone status.

For all the same reasons, hypercalcemia may occur in patients with severe thyrotoxicosis. The total serum calcium concentration is increased in as many as 27% of patients, and the ionized serum calcium is elevated in 47%. The concentrations of heat labile serum alkaline phosphatase and osteocalcin are also frequently elevated. These findings resemble those of primary hyperparathyroidism, but the concentration of parathyroid hormone in serum is low-normal in most. True primary hyperparathyroidism and thyrotoxicosis sometimes coexist. Plasma 25-hydroxycholecalciferol levels are decreased in thyrotoxic patients, and this alteration could contribute to the decreased intestinal absorption of calcium and osteomalacia noted in some.

Renal Function: Water and Electrolyte Metabolism

Thyrotoxicosis produces no symptoms referable to the urinary tract save for mild polyuria, which may lead to nocturia. Nevertheless renal blood flow, glomerular filtration, and tubular reabsorptive and secretory maxima are increased. Total exchangeable potassium is decreased, possibly due to a decrease in lean body mass, but electrolytes are normal except when hypokalemic periodic paralysis occurs.

Hematopoietic System

The red blood cells are usually normal, as judged by the usual indices, but red blood cell mass is increased. The increase in erythropoiesis is due to the direct effect of thyroid hormones on the erythroid marrow, as patients that harbor mutations in the thyroid hormone receptor alpha (TRα) have mild anemia secondary to defects in erythropoiesis.[39] A parallel increase in plasma volume also occurs, with the result that the hematocrit is normal in hyperthyroid patients.

Approximately 3% of patients with Graves disease have pernicious anemia, and a further 3% have antibodies to intrinsic factor but normal absorption of vitamin B_{12}. Autoantibodies against gastric parietal cells may also be present in patients with Graves disease, and the requirements for vitamin B_{12} and folic acid appear to be increased. The total white blood cell count is often low because of a decrease in the number of neutrophils. The absolute lymphocyte count is normal or increased, leading to a relative lymphocytosis. The numbers of monocytes and eosinophils may also be increased. Splenic enlargement occurs in about 10% of the patients, and thymic enlargement is common in Graves disease.[40] The latter may present as a mediastinal mass. Thymic hyperplasia is also due to thyrotoxicosis because it is sometimes seen in patients receiving excess exogenous T_4 for suppression.[41]

Platelet levels and the intrinsic clotting mechanism are normal, but the concentration of factor VIII is often increased and returns to normal when the thyrotoxicosis is treated. Despite this increase, there is an enhanced sensitivity to warfarin because of the accelerated clearance of the vitamin K–dependent clotting factors. Therefore the dosage of warfarin needs to be reduced in thyrotoxic patients.[42] This must be kept in mind if initiating anticoagulant treatment for atrial fibrillation in older patients.[43] Coincidental autoimmune thrombocytopenia may also occur.

Pituitary and Adrenocortical Function

The thyrotoxic state imposes several challenges on pituitary and adrenocortical function. The hepatic inactivation of cortisol is accelerated, including enhanced 5α/5β-reductases and 11β hydroxysteroid dehydrogenase. As a result of these changes, the disposal of cortisol is accelerated, but its rate of secretion is also increased, so the plasma cortisol concentration remains normal. The concentration of corticosteroid-binding globulin in plasma is also normal. The urinary excretion of free cortisol is normal or slightly increased[44] (see Chapter 15). Interestingly, thyroid hormone receptors have now been identified in adrenal cortical cells in the mouse. It is not clear if they are also present similarly in humans.[45]

Reproductive Function

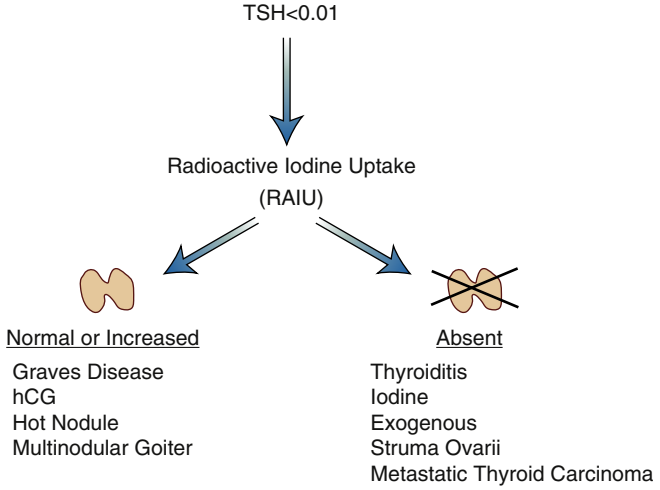
Thyrotoxicosis in early life may cause delayed sexual maturation, although physical development is normal and skeletal growth may be accelerated. Thyrotoxicosis after puberty influences reproductive function, especially in women. The intermenstrual interval may be prolonged or shortened, and menstrual flow is initially diminished and ultimately ceases. Fertility may be reduced, and if conception takes place, there is an increased risk of miscarriage and other complications.[46–48] In some patients, menstrual cycles are predominantly anovulatory with oligomenorrhea, but in most, ovulation occurs as indicated by a secretory endometrium. In the former, a subnormal midcycle surge of luteinizing hormone (LH) may be responsible. In premenopausal women with thyrotoxicosis, basal plasma concentrations of LH and follicle-stimulating hormone (FSH) are reportedly normal but may display enhanced responsiveness to gonadotropin-releasing hormone (GnRH).

Thyrotoxicosis, whether spontaneous or induced by exogenous hormone, is accompanied by an increase in the concentration of sex hormone–binding globulin (SHBG) in plasma.[49] As a result, the plasma concentrations of total testosterone, dihydrotestosterone, and estradiol are increased, but their unbound fractions are normal or transiently decreased. The increased binding in plasma may be responsible for the decreased metabolic clearance rate of testosterone and dihydrotestosterone. In the case of estradiol, however, the metabolic clearance rate is normal, suggesting that tissue metabolism of the hormone is increased. Conversion rates of androstenedione to testosterone, estrone, and estradiol and of testosterone to dihydrotestosterone are increased.[50] The increased rate of conversion of androgens to estrogenic byproducts may be the mechanism for gynecomastia and erectile dysfunction in some 10% of thyrotoxic men and one mechanism for menstrual irregularities in women. Another likely mechanism for menstrual changes is the disruption in amplitude and frequency of LH/FSH pulses due to thyroid hormone influences on GnRH signaling.

Laboratory Diagnosis

The effects of thyrotoxicosis on the major organ systems are the same regardless of the underlying cause. Their frequency and intensity and the other findings with which they are associated are influenced by the cause of the excess thyroid hormone. To a large extent, the same is true of laboratory test results. However, the patient with thyrotoxic symptoms will virtually always have a serum TSH concentration less than 0.1 mU/L and an elevated serum free T_4. In general, serum free T_3 is more elevated than is the free T_4, but free T_4 is relatively high if thyrotoxicosis is caused by thyroiditis or intake of levothyroxine.

If the possibility of exogenous thyroid hormone can be eliminated, the primary differential is between excess thyroid hormone production and excess thyroid hormone release from sick cells, as in thyroiditis. Often this differentiation can be made on the basis of the history and physical. Laboratory tests, including an increased sedimentation rate and a high serum thyroglobulin (Tg), may favor thyroiditis, but the most critical differentiating test is the radioactive iodine uptake (RAIU), which is elevated or inappropriately high-normal given the suppressed serum TSH level with excess thyroid hormone production and very low (<5%) in patients with thyroiditis (Fig. 12.1). However, the RAIU may also be low in a hyperthyroid patient who has recently received an iodine load, usually iodinated contrast for a computed tomography (CT) scan or for angiography. A 24-hour urine iodine measurement can confirm this. Also helpful is measurement of TSH



• **Fig. 12.1** Determination of the cause of hyperthyroidism based on the ^{123}I uptake in the gland. In the setting of a suppressed thyroid-stimulating hormone (TSH) a normal or increased uptake is indicative of something else driving uptake rather than endogenous TSH. In the absence of uptake the gland has either been damaged or an external factor such as exogenous hormone or iodine is playing a role. Rarely, ectopic thyroid hormone production may occur. *hCG*, human chorionic gonadotropin.

receptor antibodies (TSHRAb), which when associated with a suppressed TSH is consistent with Graves disease.

If the physical examination or thyroid ultrasonography indicates the presence of a nodular thyroid, thyroid scanning may confirm which nodules are hyperfunctioning. The association of thyrotoxicosis with an elevated TSH is rare and suggests a TSH-producing pituitary tumor. The possibility of an artifactually elevated TSH in a patient with Graves disease should be ruled out by repeating the assay by a different method in another laboratory (see Chapters 4 and 8). Exceptions to these general guidelines are discussed later within the appropriate subsection.

GRAVES DISEASE

Graves disease is a multisystem autoimmune disorder characterized by TSHRAb. The disease has been described by von Basedow in Germany, Graves in Ireland, and Parry in the United Kingdom, all in the first half of the 19th century and independent of each other.[51] Hallmarks of the disease were palpitations (hyperthyroidism), goiter, and exophthalmos, called the Merseburg triad after the hometown of von Basedow (Merseburg, Germany). The nature of the disease remained unclear for many years, and cardiac, neurologic, and pituitary origins were proposed. The autoimmune nature was not recognized before the late 1950s when Adams reported on the presence of a long-acting thyroid stimulator (LATS) in serum, different from TSH, which turned out to be an antibody capable of stimulating the TSH receptor.[52,53] Self-infusion of sera from patients with Graves disease caused thyroid stimulation and was the first demonstration of the role of TSHRAb in the induction of human hyperthyroidism.[54] Another example of the in vivo effects of TSHRAb is the transplacental passage of maternal TSHRAb, which may stimulate the fetal thyroid gland and cause fetal/neonatal thyrotoxicosis.[55] It is now recognized that TSH receptors are not only expressed on thyrocytes but are also outside the thyroid gland on fibroblasts, fibrocytes, adipocytes, osteoblasts, osteoclasts, and pituitary folliculostellate cells. It is likely that immune responses directed against TSH receptors on orbital fibroblasts and subcutaneous connective tissue cells are involved in other expressions of Graves disease, namely Graves orbitopathy and Graves dermopathy. Besides stimulating TSHRAb there also exist blocking TSHRAb, which are found in a small minority of patients with autoimmune hypothyroidism.[56] The clinical phenotypes of Graves disease are thus manifold (Table 12.2).

TABLE 12.2 Phenotypes of Graves Disease and Their Estimated Incidence Rates

Phenotype	% Affected	Cases/Million/Year
All	100	350
Graves hyperthyroidism	90–95	325
Goiter	50	175
Graves orbitopathy	30	105
Severe orbitopathy	5	17
Hypo + orbitopathy	5	17
Graves dermopathy	0.5	4
Neonatal hyperthyroidism	0.2	1.0
Fetal hyperthyroidism	0.1	0.5
Acropachy	0.1	0.5

Graves Hyperthyroidism

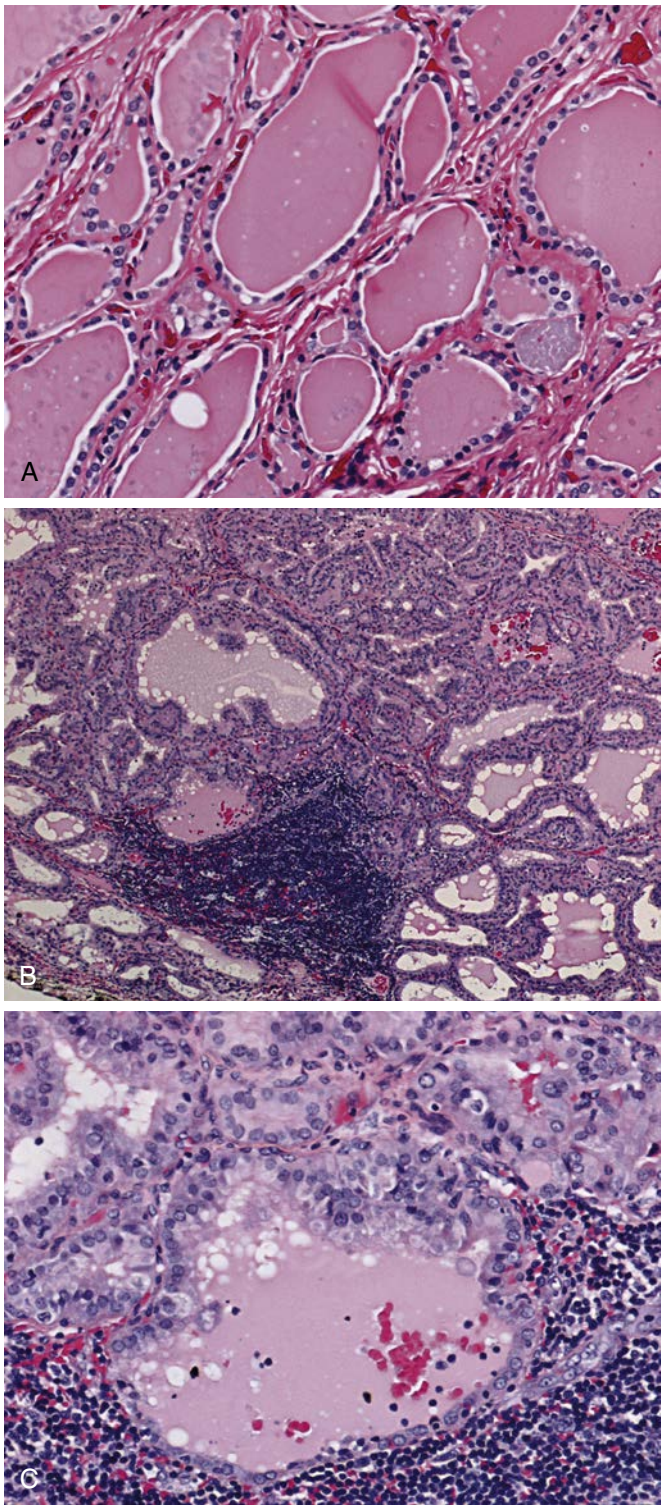
Clinical Presentation

Graves hyperthyroidism (GH) is at least four times more common in women than in men. GH is relatively rare in children. Its incidence starts to increase at the age of 13 years and remains rather stable after the age of 30 years. Average age is 47 years. The typical patient complains about weight loss despite increased appetite, heat intolerance, sweating, palpitations, fine finger tremor, nervousness, and loose stools. Duration of symptoms until diagnosis is less than 6 months in 61% of patients.[57] A positive family history of autoimmune thyroid disease (AITD) is present in about 50%.[57,58] There is some evidence for genetic anticipation, which is a younger age of onset in patients with a family history of AITD.[57–59]

Goiter is present in about half of patients. Typically the goiter is diffuse in nature, with a soft to firm consistency and a smooth surface. In severe cases, a thrill may be felt, usually over the upper or lower poles where the superior and inferior thyroid arteries, respectively, enter the thyroid gland; a thrill is accompanied by an audible bruit in systole. The presence of a nodule in the setting of Graves disease should raise suspicion of thyroid cancer. Advancing age is associated with less severe Graves hyperthyroidism.[58,60] Remarkably symptom-poor hyperthyroidism may occur in elderly people, called apathetic thyrotoxicosis. Graves orbitopathy is present in 26% of patients when Graves hyperthyroidism is diagnosed. Thereafter new cases of GO develop in 9%, rendering a total GO frequency of 35% in Graves hyperthyroidism.[61] Taking into account the severity of the Merseburg triad (as judged from serum free thyroxine [FT_4], thyroid size, and GO signs), 44% of patients have severe Graves disease, 34% moderate disease, and 22% mild disease.[62] The phenotypic appearance of Graves disease is apparently milder than in the past; this may be due to earlier diagnosis and treatment, improved iodine nutrition, and a secular trend to less smoking.

Pathology

The thyroid gland in Graves hyperthyroidism is typically both enlarged and uniformly affected, hence often referred to as diffuse toxic goiter. It is extremely rare that the disease process remains restricted to one lobe.[63] Such unilateral Graves hyperthyroidism is difficult to reconcile with the systemic nature of thyroid-stimulating immunoglobulins. The follicles are small and lined with hyperplastic columnar epithelium and contain scant colloid that displays much marginal scalloping and vacuolization (Fig. 12.2). Papillary projections of the hyperplastic epithelium extend into the lumina of the follicles. Vascularity is increased. There is varying heterogeneous infiltration by lymphocytes and plasma cells that collect in aggregates and may form infrequent B-cell germinal centers (in contrast to their abundance in Hashimoto disease). In such regions, thyroid epithelial cells express human leukocyte antigen (HLA) class II antigens (a phenomenon not seen in normal thyroid glands) and are large (perhaps due to local stimulation by TSHRAb). The intrathyroidal lymphocytic population is mixed, consisting



• **Fig. 12.2** Histopathologic features of the thyroid gland in Graves hyperthyroidism (B and C) as compared to normal tissue (A). (Courtesy Dr. Pamela Unger, Mount Sinai Scholl of Medicine, New York.)

besides B cells mostly of T helper 1 (Th1) and Th2 lymphocytes and less often Th17 and regulatory T cells. When the patient is given iodine or antithyroid drugs (ATDs), the thyroid gland may undergo involution as TSHRAb decrease. Then hyperplasia and vascularity regress, papillary projections recede, and follicles enlarge and become filled with colloid again.

Immunopathogenesis

Autoimmune thyroid diseases can be defined as complex or multifactorial diseases in which autoimmune responses to thyroid antigens develop against a particular genetic background, influenced by exposure to environmental factors. Breakdown of self-tolerance to thyroid antigens results in thyroid autoimmunity. A number of complex regulatory mechanisms serve to protect an immune response directed against self-antigens.[64] During development, immature T cells enter the thymus where they undergo a process of selection.[65] T cells that recognize with high affinity self-peptides expressed on thymic medullary epithelial cells are deleted by apoptosis, and T cells with moderate affinities leave the thymus as mature T cells. The full-length thyroid-stimulating hormone receptor (TSHR) is indeed expressed in the hyperplastic thymus of Graves hyperthyroid patients.[66] Genetic-epigenetic interactions involving a noncoding single-nucleotide polymorphism (SNP) in the *TSHR* gene that regulates thymic *TSHR* gene expression may facilitate escape of TSHR-reactive T cells from central tolerance in the thymus, thereby triggering Graves disease.[67] Central tolerance may not eliminate all self-reactive T cells. Control of autoreactive T cells in the periphery (e.g., by engagement of cytotoxic T-lymphocyte–associated protein 4 [CTLA4] resulting in T-cell anergy) is considered as a secondary or fail-safe mechanism to prevent autoimmune reactions (peripheral tolerance). Self-antigen presentation in the thymus also generates regulatory T cells (T_{reg}) that can inhibit in peripheral tissues those self-reactive T cells that escaped negative selection in the thymus. Besides these natural T_{reg} there are inducible T_{reg} generated in the periphery after antigenic stimulation. T_{reg} are characterized by the expression of CD4, CD25 (the interleukin 2 [IL2] receptor α-chain), and FOXP3 (the transcription factor forkhead box P3 protein); they may act on effector $CD4^+$ and $CD8^+$ lymphocytes by cell-to-cell contact hampering their activation and proliferation or indirectly via secretion of IL10 and transforming growth factor-β (TGFβ). Treg are crucial in maintaining tolerance through the active suppression of self-reactive T-cell activation and expansion. A fourth subset of T helper cells, induced by IL6, are highly proinflammatory and designated Th17 cells. They generate IL17, exacerbating autoimmune responses. Thus a balance between Th17 and T_{reg} is crucial for immune homeostasis. A number of studies now report a decrease in the number of T_{reg} cells and an increase in Th17 cells in peripheral blood of Graves patients, in relationship with stimulating TSHRAb and with improvement upon methimazole treatment.[65,68–70] Low T_{reg} and high IL17 levels are also found in an animal model of Graves disease.[71] Recent studies have identified regulatory beta cells (B_{reg}) that contribute to peripheral tolerance by inhibiting immune reactions to specific self-antigens.[72,73]

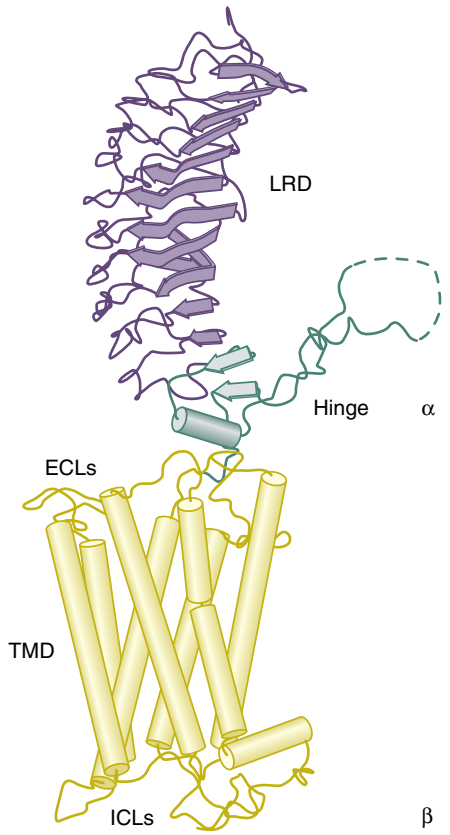
Traditionally Graves disease is viewed as the result of humoral immunity (hallmark TSHR antibodies) and Hashimoto thyroiditis as the result of cell-mediated immunity (hallmark thyroid peroxidase [TPO] antibodies [TPOAb]). However, TPOAb are also present in about 70% of Graves patients, and TSHR antibodies may occur in a minority of Hashimoto patients. Actually, humoral and cellular immune mechanisms are closely connected, and Th1 (interferon gamma [IFNγ]) and Th2 (IL4) subtypes of helper T cell responses are involved in both Graves and Hashimoto disease. IgG1 antibodies arise early in the immune response, whereas IgG4 antibodies (typically Th2 related) arise after prolonged immune stimulation. TPOAb and TgAb may comprise IgG1 as well as IgG4 classes indicating participation of Th1 and Th2 cytokines, respectively. Stimulating TSHRAb are mostly found

in the IgG1 subclass, which is selectively induced by Th1 cells; Graves hyperthyroidism is a predominantly Th1-type cytokine disease.[74] Oligoclonality and light chain restriction of stimulating TSHRAb support their primary role in disease causation.[75,76] Th1 cells may also induce antibody production through secretion of IL10, which in turn activates B cells. B cells may turn into plasma cells secreting antibodies. The thyroidal lymphocytic infiltrate is a major production site of thyroid autoantibodies. Transplantation of Graves thyroid tissue into mice deficient in both T cells and B cells with severe combined immunodeficiency (SCID mice) results in the appearance of human thyroid autoantibodies in serum, including TSHRAb.[77] Thyroid antibodies can also be produced outside the thyroid gland as they may persist after total thyroidectomy.

TSH Receptor, the Major Autoantigen in Graves Disease

A mice animal model of Graves hyperthyroidism is provided by genetic immunization against the TSH receptor, which induces stimulating TSHRAb and hyperthyroidism.[78] The gene encoding the TSH receptor is located on chromosome 14q31. The TSHR belongs to the family of G protein–coupled receptors and has seven transmembrane domains, a large extracellular domain, and a small intracellular domain (Fig. 12.3). The TSH holoreceptor consists of a 100-kDa glycosylated 744–amino acid sequence and a 20–amino acid signal peptide. The holoreceptor is cleaved into two subunits, alpha and beta, which are linked by disulfide bonds. The 50-kDa α-subunit is water soluble and heavily glycosylated. TSH and TSHRAb bind to the leucine-rich repeat regions of the α-subunit. The 30-kDa β-subunit is water insoluble, contains the membrane-spanning domain with its three extracellular loops and three cytoplasmic loops, and is 70% to 75% homologous with the LH/human chorionic gonadotropin (hCG) receptor. The TSHR forms dimers and multimeric complexes on the thyrocyte surface, probably enhancing the stability of the receptor. The holoreceptor undergoes cleavage in the hinge region due to breakage of disulfide bonds. As a result of this post-translational modification, the extracellular α-subunit is shed. The shed TSHR α-subunit, rather than the holoreceptor, is apparently the autoantigen in Graves disease.[79] Enhanced α-subunit shedding from damaged follicular cells may be the cause of the increase of serum TSHRAb concentrations after radioiodine therapy.[80]

Stimulating TSHRAb isolated from patients with Graves hyperthyroidism bind to the TSHR and activate $G\alpha_s$ and G_q signaling pathways, thereby inducing thyroid growth, increased vascularity, and increased production and secretion of thyroid hormones.[81,82] In contrast, some TSHRAb act as TSH antagonists and are referred to as blocking TSHRAb; they may occur in some patients with autoimmune hypothyroidism but also after treatment of Graves hyperthyroidism.[56,81] So-called neutral or cleavage region TSHRAb neither block TSH binding nor block TSH action; they do not induce cAMP but have the potential to induce apoptosis by binding to the hinge region.[81]



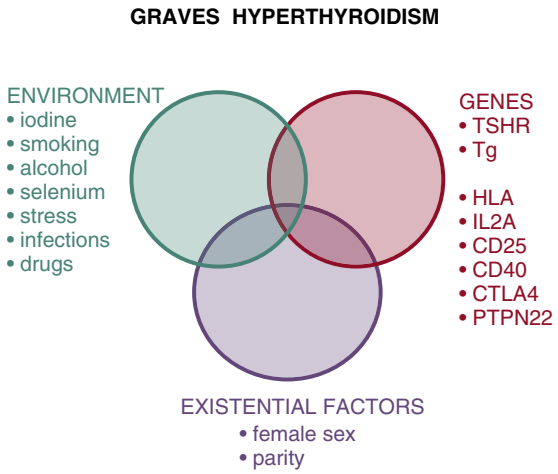
• **Fig. 12.3** Structure of the human thyroid-stimulating hormone receptor (TSHR). *ECLs,* extracellular loops; *ICLs,* small intracellular loops; *LRD,* leucine-rich domain; *TMD,* transmembrane domain. The receptor is cleaved, probably after activation, into α (or A) and β (or B) subunits. The α-subunit is thought to be shed from the cell surface.

Etiology

A complex interaction between existential factors, genetic variants, and environmental insults determines susceptibility of subjects to develop Graves disease[83] (Fig. 12.4).

Existential Factors

The strong female preponderance in Graves patients remains incompletely understood. Among all women with Graves hyperthyroidism, the rate of postpartum onset is 7.2%,[84] and parity as a risk factor has received much attention. Women with children, compared to childless women, have a relative risk for Graves



• **Fig. 12.4** Venn diagram illustrating how the combination of existential factors, genetic variants, and environmental insults may result in susceptibility to Graves hyperthyroidism.

disease of 1.19 (CI 1.14–1.24).[85] Fetal microchimerism (persistence of fetal cells in maternal tissues) may trigger autoimmunity through maternal immune responses against foreign fetal antigens, but its contribution to the female preponderance appears limited.[86] At odds with this hypothesis is a recent report that fetal microchimerism is more frequent in healthy controls than in Graves disease (64% vs. 33%, $p = 0.0004$), suggesting a possible protective role.[87] More relevant might be the epigenetic phenomenon of X-chromosome inactivation (XCI): In female cells, one of the two X chromosomes is inactivated in early embryonic life. Female tissues are thus mosaics of two cell lines, one with the paternal X and the other with the maternal X chromosome, usually in a 50:50 ratio. The consequence of skewed XCI (arbitrarily defined as inactivation of the same X chromosome in ≥80% of cells) might be that self-antigens on one X chromosome are not expressed at sufficiently high levels to induce tolerance.[88] A meta-analysis confirmed significant skewing of XCI in women with Graves disease (odds ratio 2.54, CI 1.58–4.10).[89] *FOXP3* is a key gene in the development of T_{reg}, located on the X chromosome. Polymorphisms in FOXP3 have been linked to Graves disease in some but not all studies.

Genetic Variants

Twin studies suggest that 79% of the liability to develop Graves disease is attributable to genetic factors.[90] Identified susceptibility genes for Graves disease include thyroid-specific genes (*TSHR, Tg*) and genes involved in the regulation of immune responses (*HLA, CD25, CD40, CTLA4,* and *PTPN22*).[91] These genes together probably do not explain more than about 10% of the heritability of Graves disease[92]; it follows there must be many more still undetected genetic loci, each contributing a little. The *TSHR* gene is most tightly associated with Graves disease, but the functional consequences of SNPs in the unusual large intron 1 are not clear.[93] They could give rise to RNA splice variants, increasing the level of autoantigenic TSHR α-subunits. Alternatively, SNP carriers may have fewer thymic *TSHR* transcripts, decreasing central tolerance to TSHR. Multiple SNPs in the thyroglobulin gene are linked to both Graves and Hashimoto disease.[91] Possible interactions between HLA-DRβ1 and thyroglobulin variants have been described in Graves disease, which would result in more effective presentation of disease-associated thyroglobulin-SNP allelles to T cells.[94] The immunoregulatory genes *HLA, CD25, CD40, CTLA4,* and *PTPN22* are all involved in the immunologic synapse, in which antigenic peptides complexed in HLA molecules are presented by antigen-presenting cells (APCs; macrophages, dendritic cells, but also B cells) to T-cell receptors (TCRs) on T cells. Formation of the trimolecular complex (HLA, antigenic peptide, TCR) activates $CD4^+$ T cells via expression of the interleukin 2 receptor (IL2Rα; CD25 is a marker for the IL2R α-chain) and by inducing co-stimulation via induction of CD40 ligand on T cells, which binds to constitutively expressed CD40 on APCs. CTLA4 is finally induced, which should terminate the immune response. Polymorphisms in these genes may functionally hinder the proper development of central and peripheral tolerance and alter T-cell interactions with APCs in the immunologic synapse.[91] They confer susceptibility for AITD but also for other autoimmune diseases, explaining the co-occurrence of various autoimmune diseases.[83] Odds ratios of individual loci for AITD are rather low (<2.0), but slightly higher for *HLA* (2.0–4.0). HLA-C, an HLA class I molecule, is stronger associated with Graves disease than HLA class II molecules (HLA-DRB1, DQA1, and DQB1), at least in Caucasians.[95] This is interesting as HLA class I molecules present endogenous antigens to immune cells, including those derived from viruses, which are possible triggers for AITD. Noteworthy is the lack of an association between Graves disease and mutations in the autoimmune regulator gene (*AIRE*) expressed in thymic medullary epithelial cells, resulting in failure to present self-antigens correctly in the thymus; *AIRE* mutations cause autoimmune polyglandular syndrome type 1.[96] Polymorphisms in the *PTPN22* gene encoding lymphoid protein tyrosine phosphatase is another susceptibility gene, with a gene dose-dependent effect on the age of onset of Graves disease.[97]

Environmental Insults

Iodine

Iodine-induced thyroid autoimmunity is related to TgAb, and the unmasking of a cryptic epitope on Tg contributes to this relationship in humans.[98] Longitudinal epidemiologic studies demonstrate that iodine fortification in iodine-deficient areas initially lead to a transient increase in the incidence of toxic multinodular goiter and Graves hyperthyroidism. In the long term, however, there is a decline in the incidence rate of thyrotoxicosis, mainly due to fewer cases of toxic multinodular goiter but also—although to a lesser extent—to fewer cases of Graves disease.[99,100]

Smoking

Smoking is a well-established risk factor for Graves disease.[101] The odds ratio for Graves hyperthyroidism is 3.30 (CI 2.09–5.22) in current smokers when compared with never smokers. The risk disappears a few years after cessation of smoking.

Alcohol

Moderate alcohol consumption is associated with a considerable reduction in the risk of Graves hyperthyroidism. Odds ratios are 1.73 (CI 1.17–2.56) for 0 units/week, 1.00 for 1 to 2 units/week (reference), 0.56 (0.39–0.79) for 3 to 10 units/week, 0.37 (0.21–0.65) for 11 to 20 units/week, and 0.22 (0.08–0.60) for 21+ units/week.[83,102] No interaction was found with type of alcohol (wine vs beer), smoking habits, gender, or iodine intake.

Selenium

As evident from population-based studies in China, low selenium intake might be a risk factor for autoimmune thyroiditis and hypothyroidism, but not for Graves hyperthyroidism.[103]

Stress

Stressful life events have already been implicated in the early descriptions of Graves hyperthyroidism.[51] Numerous reports on exposure to severe emotional stress prior to the onset of Graves disease support a causal relationship,[104] but prospective studies on this issue are lacking.[83]

Infections

Similarity between different antigens can lead to specificity crossover (molecular mimicry). Antigenic similarity between bacteria/viruses and human proteins is common. The best studied example is infection with *Yersinia enterocolitica* (YE). IgG from Graves patients inhibit TSH binding to outer membranes of YE, and conversely IgG from patients with YE infection inhibit binding of TSH to thyroid membranes. There is cross-reactivity between YE outer membrane proteins and epitopes of TSHR antibodies.[105] Despite this clear evidence of molecular mimicry, epidemiologic studies have not detected an association between YE infection and AITD.[106] A local insult (whether trauma or infection) may cause

an inflammatory infiltrate and production of cytokines in the thyroid gland, which may induce HLA class II expression; this may facilitate presentation of thyroid antigens and activation of local autoreactive thyroid-specific T cells in susceptible individuals.[107] A number of infections (e.g., with enteroviruses, *Helicobacter pylori,* reoviruses) have been implicated in Graves disease, but conclusive evidence for their involvement has not been obtained. The exception is hepatitis C virus (HCV), which seems the only infectious agent that is clearly associated with an increased risk for autoimmune thyroiditis.[108] HCV can infect human thyrocytes resulting in production of proinflammatory cytokines, which may enhance the autoimmune response.[109]

Drugs

Interferon-α has direct cytotoxic effects on thyrocytes but can also provoke destructive bystander immune responses. Treatment of hepatitis C with interferon-α and ribavirin combination therapy is associated with development of a suppressed TSH in 10.4%, due to Graves disease in 0.8%, and to destructive thyroiditis in 9.6%.[110] Exogenous estrogens such as oral contraceptives are associated with a lower risk of Graves hyperthyroidism.[111,112]

Graves hyperthyroidism may occur during periods of lymphocyte recovery after induced lymphopenia, known as the immune reconstitution syndrome. It is observed after bone marrow or hematopoietic stem cell transplantation,[113] during highly active antiretroviral therapy (HAART) for human immunodeficiency virus (HIV) infection in 3%,[114] and during alemtuzumab therapy for multiple sclerosis in 30%.[115] Alemtuzumab is a humanized anti-CD52 monoclonal antibody, inducing a rapid and prolonged depletion of lymphocytes from the circulation, resulting in a profound immunosuppression followed by an immune reconstitution phase. Median onset of Graves hyperthyroidism is 17 months (range 2–107 months) following last dose of alemtuzumab.[116] Graves hyperthyroidism in this context frequently requires definitive or prolonged ATD treatment. Furthermore, fluctuating thyroid status and high frequency of TSHRAb-positive hypothyroidism suggest switches between blocking and stimulating TSHRAb in these patients.

Immune checkpoint inhibitors are emerging as a new class of anticancer drugs associated with frequent thyroidal side effects. Ipilimumab is a monoclonal antibody against CTLA4 that blocks an inhibitory signal to T cells, thereby prolonging a stimulated immune response against tumor cells.[117] Nivolumab and pembrolizumab are antibodies against programmed death protein 1 (PD1), which is upregulated on activated T cells. By interrupting PD1 binding to its ligand PDL1, effector T-cell activity increases in the tumor microenvironment.[117] Treatment with these checkpoint inhibitors may result in subclinical hyperthyroidism due to destructive thyroiditis and, rarely, in Graves hyperthyroidism.[117–119]

Diagnosis and Differential Diagnosis

When the syndromal diagnosis of thyrotoxicosis has been established by the finding of a suppressed serum TSH and an increased serum FT_4 and/or FT_3, a nosologic diagnosis should be reached: Which disease entity causes hyperthyroidism? If Graves orbitopathy is present, it is quite evident Graves hyperthyroidism is present. Otherwise the presence of TSH receptor antibodies (TSHRAb) in serum confirms Graves disease. Most current assays use a competitive binding methodology, measuring TSH-binding inhibitory immunoglobulins (TBII) (Table 12.3). These binding assays do not discriminate between stimulating and blocking TSHRAb. Nevertheless, their sensitivity and specificity for the diagnosis of Graves hyperthyroidism are very high (97% and 98%, respectively).[120] In contrast, cell-based bioassays are able to differentiate between stimulating TSHRAb (TSAb or TSI) and blocking TSHRAb (TBAb).[121,122]

TABLE 12.3 Assays of Thyroid-Stimulating Hormone Receptor Antibodies: Nomenclature and Indications

Nomenclature of TSHRAb Assays	
TBII (TSH binding inhibitory immunoglobulins)	Measurement of inhibition of labelled TSH (or labelled thyroid-stimulating monoclonal antibody) binding to recombinant TSHR by serum antibodies
TSAb or TSI (thyroid-stimulating antibodies)	Measurement of cAMP production by thyroid cell lines transfected with TSHR
TBAb (thyroid-blocking antibodies)	Measurement of inhibition of cAMP production after TSH-mediated stimulation of thyroid cells or TSHR-transfected cells
Indications for Assay of TSHRAb	
Diagnosis	Graves hyperthyroidism Graves orbitopathy and Graves dermopathy Fetal and neonatal thyrotoxicosis
Treatment	Chance of remission of hyperthyroidism at baseline, and during treatment with antithyroid drugs.

cAMP, Cyclic adenosine monophosphate; *TSH*, thyroid-stimulating hormone; *TSHR*, thyroid-stimulating hormone receptor; *TSHRAb*, thyroid-stimulating hormone receptor antibodies.

Large variation exists in the use of diagnostic imaging. A recent study in France among hyperthyroid patients indicated thyroid ultrasonography was done in 94% and isotope scanning in 40%.[123] European Thyroid Association (ETA) guidelines recommend ultrasonography (comprising grey scale analysis and color flow or power Doppler examination) as the preferred imaging procedure for the diagnosis of Graves hyperthyroidism.[124] Graves hyperthyroidism is typically characterized by diffuse thyroid enlargement, hypoechogenicity, and increased vascularity. The latter can be used to differentiate between thyrotoxicosis due to Graves disease and destructive thyroiditis (as in subacute thyroiditis, amiodarone-induced thyrotoxicosis type 2). Typical ultrasound patterns combined with positive TSHRAb may obviate the need for thyroid scintigraphy. ETA guidelines suggest scintigraphy when thyroid nodularity coexists with hyperthyroidism and prior to ^{131}I therapy. The few patients in whom no TSHRAb are detected (TBII <2 U/L) have biochemically less severe Graves hyperthyroidism compared to TSHRAb-positive patients[125]; however, familial nonautoimmune hyperthyroidism due to germline *TSHR* activating mutations is present in 4.5% of such patients.[126]

Natural History and Prognosis

Knowledge about the natural history of Graves hyperthyroidism is limited because nowadays almost no patient escapes therapeutic intervention. Case histories from the older literature and cumulative experience with the outcome of ATDs, however, allow us to hypothesize that patients with Graves hyperthyroidism can be

divided into three groups, each with a different natural history: (1) patients who have a prolonged continuous episode of hyperthyroidism that never goes into remission (~10%), (2) patients who follow a relapsing and remitting course over many years (~50%), and (3) patients who have a single episode of hyperthyroidism followed by permanent remission (~40%). Patients who are in remission after a course of ATDs have a low prevalence of TSHRAb (<30%) and a much higher prevalence of TPOAb (~80%). Long-term follow-up studies of patients in remission indicate development of subclinical hypothyroidism in about 20% and overt hypothyroidism in about 6%, associated with either blocking TSHRAb or TPOAb.[127–129]

A series of population-based studies in Denmark have provided evidence that Graves hyperthyroidism (but also toxic nodular goiter) is associated with increased all-cause mortality (hazard ratio 1.42, CI 1.25–1.60) due to cardiovascular and lung diseases.[130] Excess mortality is related to cumulative periods of low serum TSH.[131] Hyperthyroid patients treated with radioactive iodine remain at a higher risk of cardiovascular diseases compared to patients treated with thyroidectomy, but hypothyroidism during follow-up predicts better cardiovascular outcome.[132]

Treatment

Recent guidelines and reviews give detailed information on the management of Graves hyperthyroidism.[124,133,134] Treatment of hyperthyroidism in the presence of Graves orbitopathy is discussed in the section on Graves orbitopathy. There are three treatment options (ATDs, thyroidectomy, and radioactive iodine) that are effective in restoring euthyroidism. It should be realized, however, that a causal therapy of Graves disease directed against the underlying abnormal immune responses is not yet available.

Antithyroid Drugs: Thionamides

Mechanism of Action

The major agents for treating thyrotoxicosis are drugs of the thionamide class: carbimazole (CBZ), methimazole (MMI), and PTU. CBZ is rapidly decarboxylated in the liver to the active substance MMI. Equivalent doses are 40 mg CBZ, 30 mg MMI, and 400 mg PTU. Each of these agents inhibits the function of thyroid peroxidase, thereby reducing oxidation and organification of thyroid iodide, iodotyrosine coupling, and thyroid hormone biosynthesis. In addition, large doses of PTU, but not methimazole, impair the conversion of T_4 to T_3 by D1 in the thyroid and peripheral tissues.[135] Consequently large doses of PTU may provide more rapid alleviation of very severe thyrotoxicosis.

The plasma half-life of MMI is about 6 hours, whereas that of PTU is about 1.5 hours. Both drugs are accumulated by the thyroid gland. Whereas the daily amount of MMI can be given in one dose, the daily amount of PTU should be divided in three doses with each given every 8 hours. In patients with severe hyperthyroidism, splitting the daily dose of MMI might sometimes be required. Single daily dosing improves compliance and should be used whenever possible. Thionamides cross the placenta and can inhibit fetal thyroid function.

MMI and PTU may also have immunosuppressive effects.[136] In vitro MMI reduces HLA-DR expression on thyroid cells, either directly or indirectly via inhibiting IFNγ. In vivo, MMI decreases the number of intrathyroidal activated T cells and the concentration of serum TSHRAb (but not of nonthyroid antibodies such as parietal cell antibodies). MMI, but not glucocorticoids, given prior to radioiodine therapy blunts the increase in TSHRAb usually seen after ^{131}I.[137] The unresolved question, however, is whether the effects are caused directly by the action of the drug on the immune system or indirectly by restoration of the euthyroid state.

TABLE 12.4 Adverse Events of Antithyroid Drugs

Common (1–5%)	Skin rash Urticaria Arthralgia, polyarthritis Transient mild leukopenia
Rare (0.2–1%)	Gastrointestinal Abnormal smell and taste Agranulocytosis
Very rare (<0.1%)	Aplastic anemia (PTU, CBZ) Thrombocytopenia (PTU, CBZ) Vasculitis, lupus-like, ANCA+ve (PTU) Hepatitis (PTU) Hypoglycemia (anti-insulin antibodies) (PTU) Cholestatic jaundice (CBZ, MMI)

ANCA+ve, Antineutrophil cytoplasmic antibody positive; *CBZ*, carbimazole; *PTU*, propylthiouracil; *MMI*, methimazole.
Adapted from Strieder TG, Prummel MF, Tijssen JG, et al. Risk factors for and prevalence of thyroid disorders in a cross-sectional study among healthy female relatives of patients with autoimmune thyroid disease. *Clin Endocrinol.* 2003;59:396–401.

Adverse Effects (Table 12.4)

Minor cutaneous reactions such as skin rash and urticaria usually can be managed by antihistamine therapy without stopping the drug. The lesions may resolve spontaneously or after switching to another ATD. Prescribing the alternative drug is not recommended in the case of serious allergic reactions.[124,138]

Agranulocytosis (<500 neutrophils/mm^3) is a serious side effect, with an incidence of 0.28% in the first 3 months of therapy.[139] Risk factors are older age, higher doses of ATD, and the presence of particular HLA-B and HLA-DRB1 alleles or rare NOX3 genetic variants.[140–142] The onset of agranulocytosis is rather abrupt, accompanied by fever and sore throat.[138] When therapy with ATD is begun, the patient should be instructed to discontinue the drug and to notify the physician immediately should these symptoms develop. This precaution is more important than the frequent measurement of white blood cell counts because agranulocytosis may develop within 1 to 2 days. Neither the American Thyroid Association (ATA) nor the ETA recommend routine monitoring of white blood cells during ATD therapy. If agranulocytosis occurs, the drug should be discontinued immediately and the patient treated with antibiotics as appropriate. Granulocyte colony-stimulating factor may speed the recovery that invariably takes place. Lymphocytes of patients who have developed agranulocytosis while taking PTU undergo blast transformation when exposed in vitro to PTU or methimazole; consequently, thionamides should not be given again. Granulocytopenia occurs during ATD therapy and is sometimes a forerunner of agranulocytosis, but it can also be a manifestation of thyrotoxicosis itself.

Although rare, PTU has been associated with fulminant hepatic necrosis, and it is the third most common cause of drug-related liver failure, accounting for 10% of all drug-related liver transplants. Children are at a higher risk than adults.[143] Fortunately, stopping PTU results in recovery in most cases. This

PTU-associated liver failure may occur at any time during therapy, so routine monitoring of liver function may not be helpful.[138] Because of this well-known rare but serious PTU side effect of hepatic failure, sometimes requiring liver transplantation, in June 2009 the Food and Drug Administration (FDA) issued an advisory that PTU should not be used as a first-line agent in hyperthyroidism.[144] The use of PTU should be restricted to the first trimester of pregnancy (see later), to thyroid storm, and to patients who experience minor side effects of MMI and are unable or unwilling to undergo ^{131}I therapy or thyroidectomy. MMI is associated in a dose-dependent manner with an increased risk for hepatitis and cholestasis.[145] There are no reported cases of liver transplantation attributed to MMI toxicity.

Practical Use

Guidelines agree MMI (CBZ) should be used in virtually every nonpregnant patient who chooses ATD therapy.[124,134] Guidelines also recommend that patients should be informed of side effects of ATD and the necessity of informing the physician promptly if they should develop jaundice, light-colored stools, dark urine, fever, or pharyngitis. Preferably this information should be in writing. Prior to ATD therapy it is suggested to obtain a baseline complete blood count, including white blood cell count with differential, and a liver profile, including bilirubin and transaminases. Restoration of the euthyroid state by ATD can be done according to the *titration* method or the *block-and-replace* regimen. In the titration method one starts with a relatively high single daily dose of 20 to 30 mg MMI (or 30–40 mg CBZ). Normal FT_4 and T_3 concentrations are usually reached after 4 to 6 weeks, which should be followed by tapering the MMI dose toward a maintenance dose of about 5 to 10 mg MMI, which keeps the patient euthyroid. In the block-and-replace regimen one starts with the same high initial dose of MMI, then adds LT_4 when the patient has become euthyroid under continuation of the high initial MMI dose. In the rare patient given PTU, the high starting PTU dose is 300 to 400 mg daily divided in three to four doses, whereas the maintenance dose is about 50 to 100 mg daily. The advantage of the titration method is the use of lower doses of MMI and thereby slightly fewer side effects; the disadvantage is the need for more frequent blood sampling to adjust the MMI dose and larger fluctuations between hypothyroidism and hyperthyroidism. The advantages of the block-and-replace regimen are fewer blood samples and more stable thyroid function during ATD therapy; the disadvantages are the higher MMI dose and slightly more side effects. The two methods have never been compared in a randomized clinical trial. The recurrence rate of Graves hyperthyroidism is similar between both regimens. In many patients, serum TSH may remain suppressed for a long time despite already normalized serum FT_4 and T_3 concentrations.[146] This phenomenon has been linked to the presence of still high serum concentrations of stimulating TSHRAb, which via binding to TSH receptors in pituitary folliculostellate cells downregulate TSH release.[147]

MMI is administered for 12 to 18 months and then discontinued to see if the disease has gone into remission. Remission

TABLE 12.5 Predictive Scores for the Risk of Recurrent Graves Hyperthyroidism After 1-Year Treatment With Antithyroid Drugs

Items (Assessed Before Starting Therapy)		GREAT Score Range 0–6
Age (yr)	≥40	0
	<40	+1
FT_4 (pmol/L)	<40	0
	≥40	+1
TBII (U/L)	<6	0
	6–19.9	+1
	≥20	+2
Goiter size[a]	gr. 0–I	0
	gr. II–III	+2

GREAT SCORE	
Risk Class	Recurrences
Class I (score 0–1)	16%
Class II (score 2–3)	44%
Class III (score 4–6)	68%

Items Added		GREAT+ Score Range 0–10
PTPN22 C/C wild type		0
	C/T	+1
HLA nr.[b]	0	0
	1–2	+2
	3 (LD)	+3

GREAT+ SCORE	
Risk Class	Recurrences
Class I+ (score 0–2)	4%
Class II+ (score 3–4)	21%
Class III+ (score 5–6)	49%
Class IV+ (score 7–10)	84%

[a]Goiter size: grade 0, thyroid not or distinctly palpable but usually not visible; grade I, thyroid easily palpable and visible with head in normal or raised position; grade II, thyroid easily visible with the head in a normal position; grade III, goiter visible at a distance.

[b]Number of HLA subtypes (*DQB1-02, DQA1-05, DRB1-03)* present.

FT4, Free thyroxine; *HLA*, human leukocyte antigen; *LD*, linkage disequilibrium; *TBII*, thyroid-stimulating hormone–binding inhibitory immunoglobulins.

Modified from Vos XG, Endert E, Zwinderman AH, et al. Predicting the risk of recurrence before the start of antithyroid drug therapy in patients with Graves' hyperthyroidism. *J Clin Endocrinol Metab.* 2016;101:1381–1389.

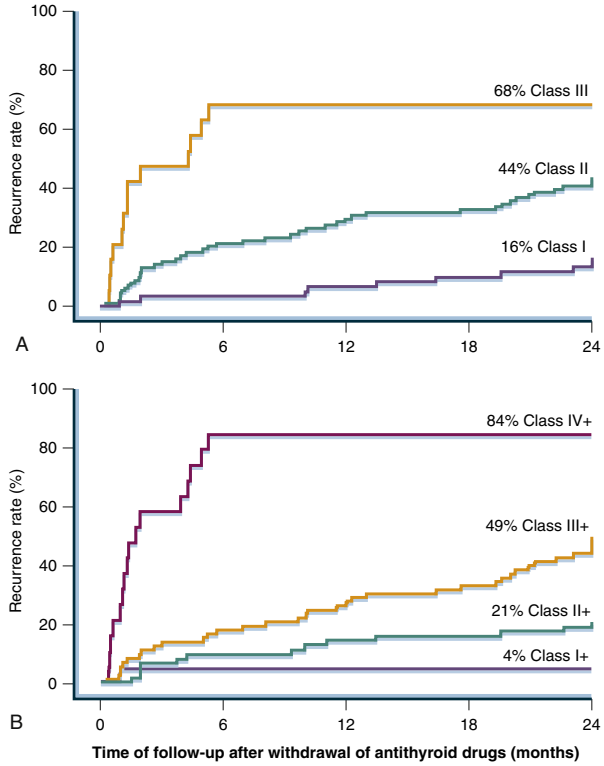
rate is between 40% and 60%; it is not influenced very much by dose or duration of ATD therapy. Most recurrences occur in the first year after stopping ATD. Exposure to stressful life events and daily hassles after withdrawal of ATD increase the risk of recurrent hyperthyroidism.[104,148] It is recommended to check thyroid function annually to detect late recurrences or development of hypothyroidism. Recurrences should be treated with radioiodine or surgery. About one-third of patients experience a lasting remission.

Chances of remission increase if during ATD therapy goiter size and/or TSHRAb decrease and TSH has become normal. It has been recommended to measure TSHRAb just prior to stopping ATD. If TSHRAb have disappeared, chances of remission are high. If TSHRAb can still be detected, one may consider prolonging ATD therapy because chances of remission are low. A major advantage of a more prolonged therapy is that nearly all patients remain euthyroid as long as therapy is given, even if the dose of the drug is low.

Predicting who will experience relapsing hyperthyroidism after stopping ATD has proven to be difficult. A change from stimulating to blocking TSHRAb might play a role, as well as many other factors.[149] A systematic review and meta-analysis of 7595 patients, of whom 48.7% relapsed, identified a significant association with relapse of the following items, all assessed before starting treatment with ATD: smoking, thyroid size (either by sonography or by inspection and palpation), orbitopathy, FT_4, FT_3, and TBII.[150] These risk factors by themselves, however, have not sufficient power for accurate prediction in individual patients. Combining a number of independent risk factors led to a predictive score composed of age, FT_4, TBII, and goiter size (by inspection and palpation), called the GREAT score (Graves Recurrent Events After Therapy) (Table 12.5).[151] The clinical relevance is that the GREAT score provides a reasonable prediction of recurrence risk after a course of ATDs based on just four items, readily available before the start of treatment (Fig. 12.5). It might allow for discussions with the patient of whether the treatment option of ATDs is optimal for them. If the recurrence risk is low, ATD might be a good choice; if the recurrence risk is rather high, surgery or radioiodine could be a better option. The GREAT score has been validated in an independent study.[152] Adding the results of specific genotypes provides the GREAT score with even better prediction. It can be foreseen that such predictive scores will be of great value in personalized medicine.

Other Drugs Used in Hyperthyroidism

Beta-Adrenoceptor Blocking Agents. Thyroid hormone excess increases sensitivity of the sympathetic nervous system to catecholamines. Drugs that block the response to catecholamines at the receptor site (e.g., propranolol) ameliorate some of the manifestations of thyrotoxicosis and are often used as adjuncts in management. Beta blockers rapidly improve complaints of tremulousness, palpitations, excessive sweating, and eyelid retraction. Beta blockers are recommended in all patients with symptomatic thyrotoxicosis, especially in elderly patients and patients with resting heart rates in excess of 90 beats per minute or coexistent cardiovascular



• **Fig. 12.5** Kaplan-Meier curves for recurrent Graves hyperthyroidism after a 1-year course of antithyroid drugs according to risk classes I to III of the GREAT score (A) and risk classes I+ to IV+ of the GREAT+ score (B). (Redrawn from Vos XG, Endert E, Zwinderman AH, et al. Predicting the risk of recurrence before the start of antithyroid drug therapy in patients with Graves' hyperthyroidism. *J Clin Endocrinol Metab.* 2016;101:1381–1389.)

disease.[124,134] Beta blockers are most useful in the interval before the response to thionamides or radioiodine therapy occurs. Propranolol, in addition to its action as a β-adrenoreceptor antagonist, also inhibits 5′-deiodination resulting in a decrease of plasma T_3 and an increase of plasma reverse T_3.[153] Plasma T_3 decreases by 20% at a daily dose of 80 mg propranolol (four 20-mg doses) and by 30% at 160 mg propranolol (four 40-mg doses). Beta blockers without membrane-stabilizing activity do not decrease plasma T_3, but the clinical response to β-blockers is independent of the decrease in T_3. The largest experience is with propranolol, but the drug can be contraindicated (e.g., in asthma and chronic obstructive pulmonary disease). Longer acting drugs with relative β1 selectivity might then be preferred, such as atenolol (25–100 mg once or twice daily) and metoprolol (25–50 mg twice or three times daily).[124]

Perchlorate. Perchlorate inhibits thyroid iodide transport and has been used in the past to treat Graves hyperthyroidism. Although it was effective in restoring euthyroidism and decreasing TSHRAb, it is no longer used in view of its side effects (except in amiodarone-induced thyrotoxicosis type 1).[154,155]

Iodine. Iodine is now rarely used as a sole therapy. The mechanism of action of iodine in relieving thyrotoxicosis differs from that of the thionamides. Although quantities of iodine in excess of several milligrams can acutely inhibit organic binding (acute Wolff-Chaikoff effect), this transient phenomenon probably does not contribute to the therapeutic effect. Instead, the major action of iodine is to inhibit hormone release. Administration of iodine increases glandular stores of organic iodine, but the beneficial effect of iodine is evident more quickly than the effects of even large doses of agents that inhibit hormone synthesis. In patients with Graves disease, iodine acutely retards the rate of secretion of T_4, an effect that is rapidly lost when iodine is withdrawn. These features of iodine action provide both disadvantages and advantages. The enrichment of glandular organic iodine stores that occurs when this agent is given alone may retard the clinical response to subsequently administered thionamides, and the decrease in RAIU produced by iodine prevents the use of radioiodine as treatment for several weeks. Furthermore, if iodine is withdrawn, resumption of accelerated release of hormone from an enriched glandular hormone pool may exacerbate the disorder. Another reason for not using iodine alone is that the therapeutic response on occasion is either incomplete or absent. Even if initially effective, iodine treatment may lose its effect with time. This phenomenon, termed *iodine escape*, should not be confused with the escape from the acute Wolff-Chaikoff effect.[156] Nevertheless, the rapid slowing of hormone release by iodine makes it more effective than the thionamide drugs when prompt relief of thyrotoxicosis is mandatory. Therefore, aside from its use in preparation for thyroid surgery, iodine is useful mainly in patients with actual or impending thyrotoxic crisis, severe thyrocardiac disease, or acute surgical emergencies. If iodine is used in these circumstances, it should be administered with large doses of a thionamide.

Recent studies suggest a potential role for iodine in patients who have adverse reactions to ATD or contraindications for radioiodine or surgery.[157,158] Sometimes iodine is used as a beneficial adjunct to ATD: 38 mg potassium iodide (KI) + 15 mg MMI resulted in better control of hyperthyroidism and fewer adverse reactions than 30 mg MMI alone.[158]

The lowest dose of iodine required for control of thyrotoxicosis is approximately 6 mg daily. Six milligrams of iodine are present in one-eighth of a drop of saturated solution of potassium iodide (SSKI) or approximately 1 drop of Lugol solution; many physicians, however, prescribe 5 to 10 drops of one of these agents three times daily. Although it is advisable to administer amounts larger than the suggested minimal effective dose, huge quantities of iodine are more likely to produce adverse reactions. We recommend the use of a maximum 2 to 3 drops of SSKI twice daily. Adverse reactions to iodine are unusual and in general not serious. They include rash, which may be acneiform; drug fever; sialadenitis; conjunctivitis and rhinitis; vasculitis; and a leukemoid eosinophilic granulocytosis. Sialadenitis may respond to reduction of dosage and the addition of lemon/lime candies to increase salivary flow; in the case of the other reactions, iodine should be stopped.

Lithium. Lithium carbonate inhibits thyroid hormone secretion, but unlike iodine it does not interfere with the accumulation of radioiodine. Lithium, 300 to 450 mg every 8 hours, is used only to provide temporary control of thyrotoxicosis in patients who are allergic to both thionamides and iodide.[159] Another short-term use for lithium has been as an adjunct to radioiodine therapy because the drug slows the release of iodine from the thyroid.[160]

Selenium. Selenium levels were higher in patients in remission and correlated inversely to TSHRAb.[161] Therefore it was hypothesized that selenium supplementation might increase remission rate. Addition of 300 μg sodium selenite daily to methimazole did not, however, increase remission rate in a placebo-controlled trial.[162]

Cholecystographic Agents. The oral iodine-containing cholecystographic contrast agent sodium ipodate causes a prompt decrease in serum T_4 and serum T_3 in Graves hyperthyroidism. After a course of 500 mg sodium ipodate daily for 5 days, plasma T_3 is normalized in all patients, allowing uneventful thyroidectomy on day 5.[163] However, supplies of such agents are generally no longer available.

Cholestyramine. Thyroxine is metabolized in the liver to glucuronides and sulfates that enter the enterohepatic circulation. Cholestyramine interferes with the enterohepatic cycle, thereby acting as an effective and well-tolerated adjunctive therapy in patients with resistant Graves hyperthyroidism.[164,165] It can produce a rapid and complete decline in thyroid hormone levels.

Immunosuppressive Drugs. Because of the autoimmune nature of Graves disease, additional use of immunosuppressive agents might improve outcomes. A systematic review identified seven randomized or controlled trials in which the effect of addition of glucocorticoids or rituximab to standard treatment was compared to standard treatment alone.[166] Relapse rate was much lower in the intervention group with immunosuppressive drugs (24%) than in the control group (59%), with a risk ratio of 0.55 (CI 0.41–0.75, $p < 0.001$). However, the study has several limitations, such as small sample sizes and moderate to high risk of bias.

Future Developments. It is foreseen that novel treatment modalities for Graves hyperthyroidism will become available in the next decade. There may be causal therapies directed against stimulating TSHRAb, the immediate cause of Graves disease. Examples are antigen-specific immunotherapy with tolerogenic peptides of the TSH receptor, monoclonal TSH receptor–blocking antibodies, and low-molecular-weight TSH receptor antagonists.[167,168]

Thyroidectomy. Thyroidectomy has a high cure rate for Graves hyperthyroidism. Total thyroidectomy has a nearly 0% risk of recurrence, whereas subtotal thyroidectomy may have an 8% chance of persistent or recurrent hyperthyroidism at 5 years.[124,134]

Near-total or total thyroidectomy is therefore the procedure of choice. It is recommended to refer the patient to a high-volume thyroid surgeon. Average complication rates, length of hospital stay, and cost are reduced when the operation is performed by a surgeon who conducts many thyroidectomies. Surgeons who perform more than 25 thyroid surgeries per year have superior outcomes compared to surgeons who perform fewer.[169] Complication rates are on average 51% higher when surgery is performed by low-volume surgeons.[134]

Complications. In the hands of high-volume thyroid surgeons the rate of permanent hypoparathyroidism is less than 2% and that of permanent recurrent laryngeal nerve injury is less than 1%. Bleeding into the operative site necessitating reoperation occurs in 0.3% to 0.7%; it is the most serious postoperative complication, which can rapidly produce death by asphyxia and requires immediate evacuation of the blood and ligation of the bleeding vessel. Even with subtotal surgery, the recurrent laryngeal nerve can be damaged. If such damage is unilateral, it causes dysphonia that usually improves in a few weeks but may leave the patient slightly hoarse. Intraoperative recurrent laryngeal nerve monitoring does not necessarily improve long-term outcomes. Hypoparathyroidism can be either transient or permanent. Transient hypoparathyroidism results from inadvertent removal of some parathyroids and/or impairment of blood supply to those that remain. Depending on the severity of these insults, symptoms and signs of hypocalcemia appear, usually within 1 to 7 days after surgery. Severe hypoparathyroidism should be treated with intravenous calcium gluconate. Milder cases can be treated with oral calcium carbonate and cholecalciferol, although the active form of vitamin D (calcitriol) is more effective and preferred in most cases. However, the hypocalcemia that occurs immediately after surgery for thyrotoxicosis may not be due to transient hypoparathyroidism because it occurs more frequently in the Graves patient than after surgery for other thyroid disorders. Instead it may be due to "hungry bones" because of the demineralization of bone that occurs in hyperthyroidism. This begins to be reversed after cure of the hyperthyroid state and may contribute to the modest elevation in alkaline phosphatase during recovery unless the patients have been rendered euthyroid for some time prior to surgery. Many surgeons who fear that they have caused damage to the parathyroid glands at total thyroidectomy may reimplant the apparent parathyroid tissue into local muscles.

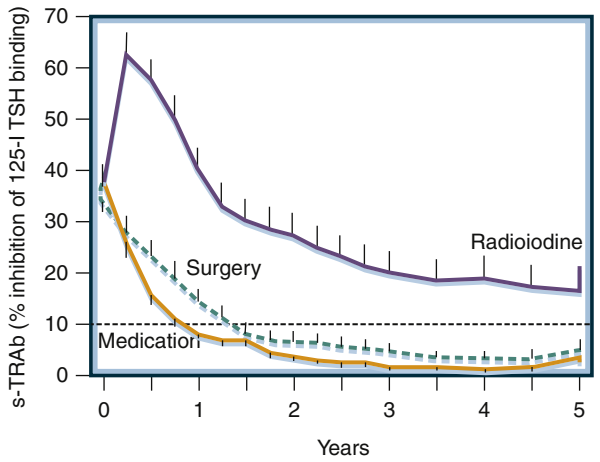
Preparation for Surgery. If surgery is chosen, patients should be rendered euthyroid prior to the procedure with ATD pretreatment, with or without β-adrenergic blockade. These agents do not improve the hyperplasia and hypervascularity of the gland in the short term. Iodine, however, is reported to cause a decrease in height of the follicular cells, enlargement of follicles with retention of colloid, and reduction of hypervascularity. Hence the recommendation to give a KI-containing preparation in the immediate preoperative period should be made.[124,134] SSKI 2 to 3 drops twice daily might be initiated 7 to 10 days before surgery to decrease thyroid blood flow and vascularity and hence intraoperative blood loss during thyroidectomy. During this period a preexisting bruit or thrill may decrease in intensity or disappear entirely, and the gland may become firm. However, little clinical evidence exists that postoperative outcomes are any different following a course of iodine.[170] A recent prospective controlled trial observed that preoperative use of KI decreased gland vascularity but not the overall difficulty of thyroidectomy; however, KI was associated with less transient hypoparathyroidism and transient hoarseness, suggesting KI improves the safety of thyroidectomy.[171] KI or Lugol solution is used by less than 40% of thyroidologists in Europe.[172] In exceptional circumstances, when it is not possible to render a patient euthyroid prior to thyroidectomy, the patient should be adequately treated with KI and β-blockers (eventually also with glucocorticoids and cholestyramine) in the immediate preoperative period.[134] Lastly, checking for calcium and 25-hydroxy vitamin D preoperatively is recommended. Preoperative vitamin D deficiency is a risk factor for postoperative hypocalcemia. Supplementing calcitriol for a brief period preoperatively helps to reduce postoperative hypocalcemia.[124,134]

After thyroidectomy, ATDs are stopped immediately and β-blockers should be weaned. Serum calcium with or without parathyroid hormone (PTH) levels can be measured and oral calcium and calcitriol supplementation be given based on these results; alternatively, prophylactic calcium with or without calcitriol is prescribed empirically. L-Thyroxine should be started at a daily dose appropriate for the patient's weight (1.6 μg/kg) and serum TSH measured 6 weeks postoperatively.[134]

Radioactive Iodine

Radioactive iodine (RAI) has been used since 1941. Prospective trials in this area are few, leaving many questions.[173] The cellular effect of the ionizing radiation leads to cell death and thereby to a decrease in thyroid function and a reduction in thyroid size. One year after ^{131}I therapy, thyroid size is mostly normalized, hyperthyroidism has disappeared in 50% to 90% of patients, and hypothyroidism has developed in up to 50% of patients directly related to the thyroid RAI dose.[173] This is followed by a yearly hypothyroidism rate of 3% to 5%, largely independent of RAI dose.[124] A seminal question thus arises: What is the goal of ^{131}I therapy in Graves hyperthyroidism? Is it to get rid of hyperthyroidism and restore euthyroidism? That has proven to be an elusive goal, as even meticulous calculation of a seemingly appropriate ^{131}I dose does not prevent a high incidence of post-radioiodine hypothyroidism. The ATA therefore recommends the following: "Sufficient activity of RAI should be administered in a single application, typically a mean dose of 10–15 mCi (370–555 MBq), to render the patient with Graves disease hypothyroid."[134] It means that cure of one disease (hyperthyroidism) is exchanged for the creation of another disease (hypothyroidism). ETA guidelines agree: "No dose calculation can secure long-term euthyroidism and it is fully acceptable to offer a fixed dose of RAI."[124] Therefore many have given up meticulous dose calculation and offer fixed activities of, for example, 185, 370, or 555 MBq, depending on thyroid size. An increased mortality rate has been reported in hyperthyroid patients treated with RAI.[174] This was not observed in patients rendered hypothyroid, and these findings support the practice of treating hyperthyroidism with doses of radioiodine sufficient to induce overt hypothyroidism.

There has been concern that RAI therapy might produce cancer. A hypothetical 0.8% lifetime cancer risk attributable to a 15-mCi ^{131}I dose at the age of 20 years has been calculated. This is only a small increase in baseline cancer risk, and most studies have found no significant increase in the prevalence of thyroid cancer or secondary malignancies in adult patients treated with RAI. Also the frequency of genetic damage in the offspring of patients treated earlier with radioiodine does not appear to be increased. In view of the lack of evidence of serious toxicity from RAI in doses generally used for treating adults with hyperthyroidism, the age limit for the use of RAI has been lowered progressively from the initial lower limit of 40 years to age 10 or younger. However, in a 5-year-old child the theoretical lifetime cancer risk



• **Fig. 12.6** Course of TSH receptor antibodies after treatment of Graves hyperthyroidism with antithyroid drugs, thyroidectomy, or radioactive iodine. (Redrawn from Leimberg P, Wallen G, Tallstedt L, et al. TSH-receptor autoimmunity in Graves' disease after therapy with anti-thyroid drugs, surgery, or radioiodine: a 5-year prospective randomized study. *Eur J Endocrinol*. 2008;158:69–75.)

TABLE 12.6 Advantages and Disadvantages of Treatment Options for Graves Hyperthyroidism

Treatment Option	Advantages	Disadvantages
Antithyroid drugs	Chance of permanent remission (~35%)	Side effects of ATDs Long duration (12–18 months) High recurrence risk
Radioactive iodine	Simplicity Low recurrence risk	Risk of orbitopathy Lifelong LT_4 needed Possible small increase in cancer risk
Thyroidectomy	Rapidity Almost no recurrences	Low but unavoidable morbidity Lifelong LT_4 needed

ATDs, Antithyroid drugs; *LT_4*, L-thyroxine.

after 15 mCi ^{131}I is 4%, so in very young children ATDs should be considered. The use of RAI in reproductive-age women also remains unpopular, and RAI is contraindicated during pregnancy. Fetuses exposed to ^{131}I after 10 weeks of gestation may be born athyreotic. In addition, ^{131}I should not be administered for at least 8 weeks after cessation of lactation because it is concentrated in breast milk.

Complications of RAI Therapy. The early induction of euthyroidism and later the development of hypothyroidism are both consequences of radiation-induced destruction of thyroid parenchyma. With large doses of RAI a tender radiation thyroiditis may develop within the first week of treatment, as evidenced by epithelial swelling and necrosis, disruption of follicular architecture, edema, and infiltration with mononuclear cells. Resolution of the acute phase is followed by fibrosis, vascular narrowing, and further lymphocytic infiltration. Radiation thyroiditis may lead to an exacerbation of thyrotoxicosis 10 to 14 days after RAI is administered. The effect of RAI on other tissues that concentrate iodide (e.g., the salivary glands, the gastric glands, and the breasts) is not likely to be a problem with the relatively low doses prescribed for Graves hyperthyroidism.

RAI therapy is associated with development or worsening of Graves orbitopathy in about 15% of cases compared to ATDs or thyroidectomy, as evident from three randomized clinical trials.[175–177] This effect is presumably related to the substantial increase in serum TSHRAb after RAI therapy, whereas in contrast TSHRAb decrease after thyroidectomy or upon treatment with ATD.[80] Five years later the level of TSHRAb is still higher after RAI than after ATD or surgery (Fig. 12.6). Thus the presence of Graves orbitopathy may influence which treatment modality is chosen for hyperthyroidism (see section on Graves orbitopathy).

Preparation for ^{131}I Therapy. Because ^{131}I therapy can cause a transient exacerbation of hyperthyroidism, β-blockers should be considered even in asymptomatic patients who are at increased risk for complications due to worsening of hyperthyroidism (i.e., elderly patients and patients with comorbidities). In addition to β-adrenergic blockade, pretreatment with MMI should also be considered in patients at increased risk for complications. ATD should be paused around 1 week before and after ^{131}I therapy to avoid decreasing RAI efficacy.[124,134] A pregnancy test should be obtained within 48 hours prior to RAI treatment in any woman with childbearing potential. Pregnancy and breastfeeding constitute absolute contraindications to RAI therapy.

The physician administering RAI should provide written advice concerning radiation safety precautions following treatment.[124,134] National differences in radiation regulations have considerable impact on the way RAI therapy is given in different countries (e.g., in the criteria for hospitalization). Attempts have been made to standardize the radiation delivered to the thyroid gland by varying the dose of radioiodine according to the size of the gland, the uptake of ^{131}I, and its subsequent rate of release (*dosimetry*). However, no data support an advantage of dosimetry over a fixed-dose regimen. Most clinics have redefined the goal of RAI therapy from making patients euthyroid to ablating the thyroid with a permanent need for thyroid hormone replacement. A dose of 20 mCi alleviates hyperthyroidism in almost all patients and results in approximately 90% hypothyroidism.

After RAI therapy, patients are seen at intervals of 4 to 6 weeks for 6 months, and FT_4 and TSH levels are monitored. However, TSH is not a reliable guide as to when to begin levothyroxine replacement therapy, as it can remain suppressed for a considerable period of time even after the FT_4 falls below normal. To avoid overt hypothyroidism, levothyroxine replacement therapy may be initiated when the FT_4 reaches the lower portion of the reference range. The starting dose should be relatively low to avoid overtreatment given that the residual thyroid function will not be subject to feedback regulation. When hyperthyroidism persists after 6 months, retreatment with RAI is suggested, generally with about 1.5 times the initial dose of ^{131}I. Conception should be postponed until at least 6 months after RAI therapy, both in males and females.[124]

Choice of Therapy

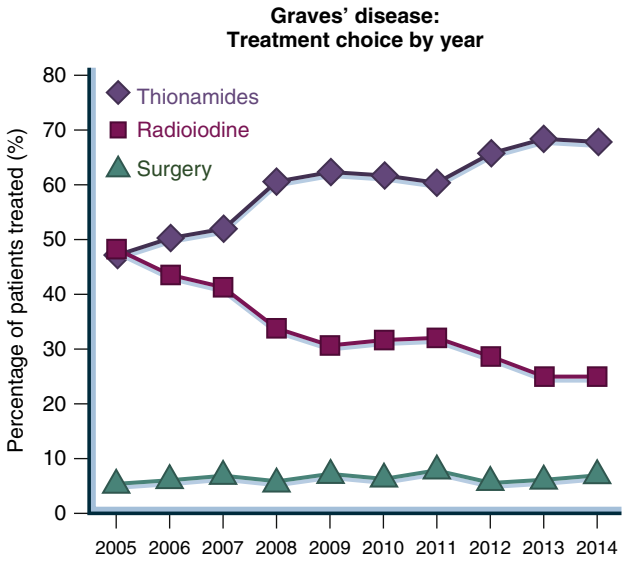
Advantages and disadvantages of each of the three treatment modalities are listed in Table 12.6. Weighing the pros and cons of each treatment by patients varies greatly. Clinical conditions leading to a preferred therapy are summarized in Table 12.7. Patients with high likelihood of remission are ideal candidates for ATD therapy; application of the GREAT score might identify such patients.[151]

TABLE 12.7 Clinical Conditions That Favor a Particular Treatment Modality for Graves Hyperthyroidism

Condition	ATD	RAI	Surgery
High chance of remission	+		
Active Graves orbitopathy	+		
Elderly with comorbidities	+	+	
Increased surgical risk	+	+	
Liver disease		+	
Major adverse reactions ATD		+	
Hypokalemic periodic paralysis		+	
Pulmonary hypertension or congestive heart failure		+	
Previous neck surgery or irradiation		+	
Recurrent hyperthyroidism		+	+
Malignancy suspected			+
Large thyroid nodules			+
Coexistent hyperparathyroidism			+

ATD, Antithyroid drug; *RAI*, radioactive iodine

Modified from Ross DS, Burch HB, Cooper DS, et al. 2016 American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. *Thyroid*. 2016;26:1343–1421.



• **Fig. 12.7** Antithyroid drugs have become the most common treatment modality for Graves hyperthyroidism in the last decade in the United States. (Redrawn from Brito JP, Schilz S, Singh Ospina N, et al. Antithyroid drugs—the most common treatment for Graves' disease in the United States: a nationwide population-based study. *Thyroid*. 2016;26:1144–1145.)

ATD might also be preferred in patients with active Graves orbitopathy. Contraindications are major adverse reactions to ATD. RAI therapy is preferred in patients with contraindications for ATD or surgery, in case of recurrences after ATD, and in patients with hypokalemic periodic paralysis, pulmonary hypertension, or congestive heart failure. Contraindications for RAI are pregnancy or planning pregnancy within 6 months, lactation, and suspicion of thyroid cancer. Thyroidectomy might be preferred in women planning pregnancy within 6 months, in large or compressive goiters (>80 g), when thyroid malignancy is suspected, and in case of coexisting hyperparathyroidism. Thyroidectomy is best avoided in the first and third trimesters of pregnancy and in patients with increased surgical risk and substantial comorbidity. Long-term follow-up of patients randomized to receive one of the three treatment options did not disclose differences in perceived quality-of-life between treatments.[178]

The choice of the most appropriate treatment in a particular patient thus depends on a multitude of factors, including personal ones such as emotional attitudes, economic considerations, and family issues. It is obvious that a satisfactory choice can only be made in close consultation with the patient. One approach is to start with ATD in all patients. This would be useful also in patients who opt later for RAI or surgery. The patient will become euthyroid in 4 to 6 weeks, and this period can be used to discuss with the patient what the optimal treatment option would be. This approach allows the establishment of a workable physician-patient relationship, which is especially important in addressing anxieties about the use of radioiodine.

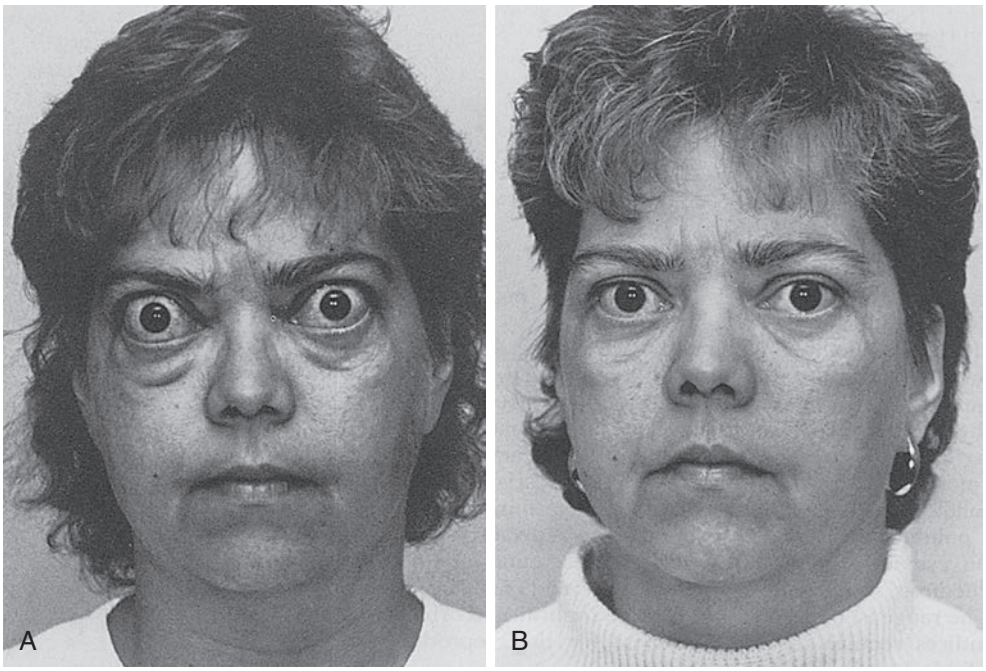
First-line treatment of uncomplicated Graves hyperthyroidism in Europe is with ATD in 84%, with RAI in 14%, and with surgery in 2%.[172] In contrast, the first-line treatment in North America had been RAI in the vast majority of cases in the 20th century. Recently this has changed dramatically; as of 2014, ATD also is the preferred treatment option in the United States, followed by RAI and then surgery[179] (Fig. 12.7).

Graves Orbitopathy

Graves orbitopathy is also known as Graves ophthalmopathy, thyroid-associated ophthalmopathy (TAO), and thyroid eye disease (TED). It is one of the phenotypes of Graves disease. Graves hyperthyroidism is present in about 90% of GO patients, whereas 5% to 10% of GO patients are euthyroid or hypothyroid (Fig. 12.8).[180]

Clinical Presentation

Most patients initially notice a change in appearance. There is redness in the eyes or lids and swelling or feeling of fullness in the upper eyelids and/or bags under the eyes.[181] The most common presenting sign is eyelid swelling, followed by eyelid lagging behind eyeball movement on downgaze (von Graefe sign). Early symptoms are a gritty sensation in the eyes, light sensitivity (photophobia), and excess tearing. With ongoing disease, upper eyelid retraction occurs in most patients. Exophthalmos (also referred to as proptosis) develops in about 60% of patients. These patients are more likely to show incomplete eyelid closure (lagophthalmos); many such patients, especially those with a wide palpebral fissure, will show punctate inferior corneal staining with fluorescein. Patients may complain about retrobulbar pressure and blurred vision. Extraocular muscle involvement may result in aberrant position of the globe or, in extreme cases, fixation of the globe. More common is limitation of eye muscle movements in certain directions of gaze, especially in upward gaze. This may cause diplopia (double vision), occurring in about 50% of patients.



• **Fig. 12.8** Patient with Graves orbitopathy. Note the typical bilateral rather symmetrical eye disease with periorbital swelling, stare, and exophthalmos (A), subsequently corrected by orbital decompression surgery (B). (Courtesy Dr. Jack Rootman, University of British Columbia, Vancouver, BC, Canada.)

Diplopia will not occur if the vision of one eye is very low (such as in amblyopia) or if the impairment of muscle motility is strictly symmetrical. Impairment of elevation and of abduction are most common, related to swollen inferior and medial rectus muscles, respectively, whereas impairment of depression and adduction are less frequent. Corneal ulceration nowadays is rare. It develops only when normal corneal protection is lost.[181] This may happen in patients with lagophthalmos in whom the cornea remains visible when the eyelids are closed. Sight loss due to optic nerve involvement (referred to as dysthyroid optic neuropathy [DON]) occurs in about 5%. Patients with DON may complain about decrease of visual acuity, loss of color vision, visual field defects, and blurred vision.[182] Visual blurring may disappear after blinking (caused by alteration of the tear film on the surface of the cornea due to lacrimation or dry eyes) or after closing one eye (caused by eye muscle imbalance). Visual blurring that persists is of concern as it may indicate DON.

The average age of presentation is 49 years, a few years after the average age of presenting with Graves hyperthyroidism. Female preponderance is obvious, but GO is more severe in males and the elderly. GO is a typical bilateral and rather symmetrical eye disease. Unilateral GO, however, occurs in about 10% of all GO patients. There is good evidence that the GO presentation is changing. Upon referral, GO patients in 2012 had less severe and less active disease than in 2000.[183]

Epidemiology

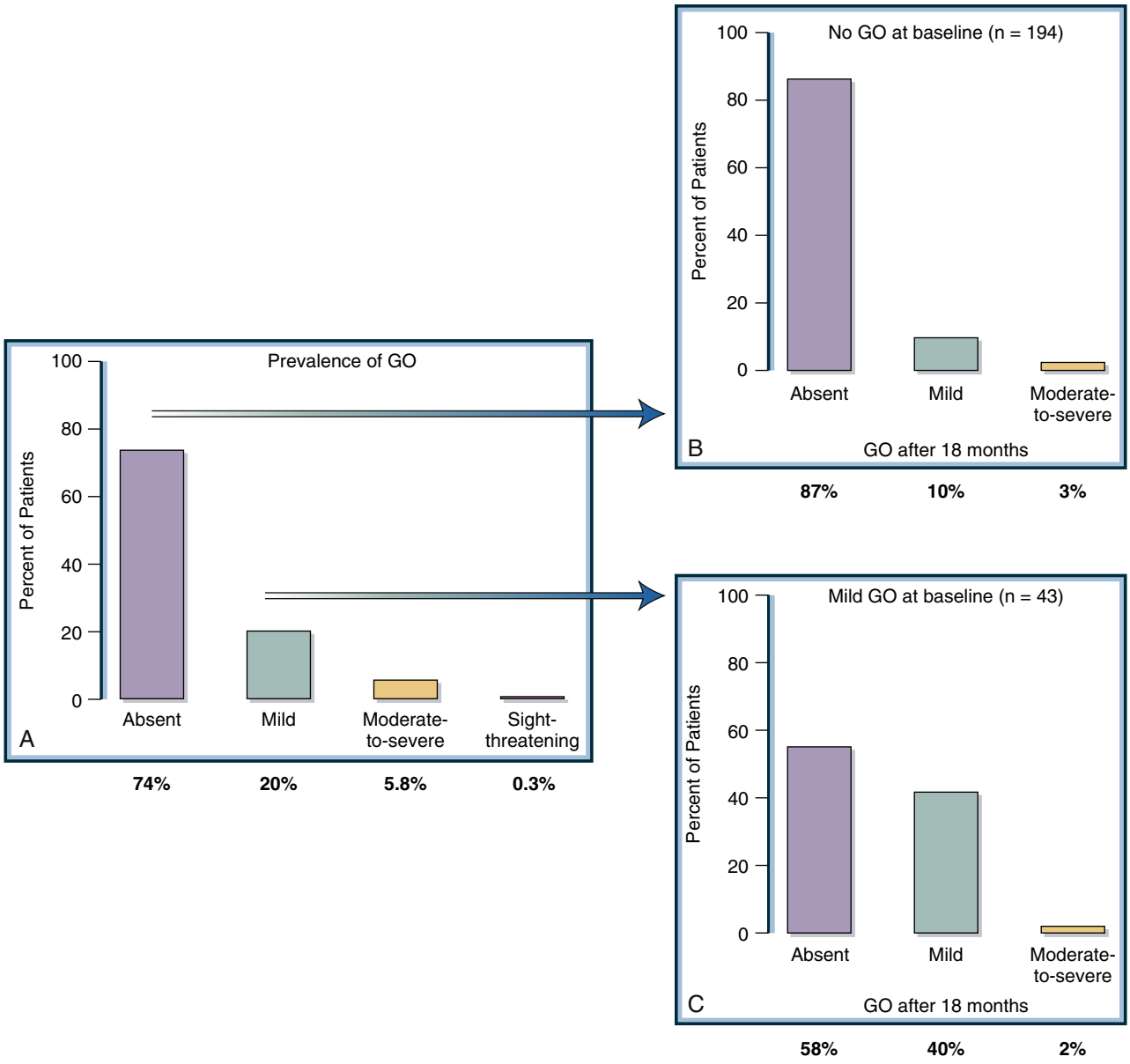
In the general Swedish population the incidence rate of Graves hyperthyroidism is 210 per million per year, and the incidence rate of GO is 42 per million per year.[184] It means 20% of patients with Graves hyperthyroidism also develop GO, which is mild in 15% and more severe in 5%. The data are in good agreement with studies from Denmark and Italy, reporting that moderate-to-severe GO occurs in about 5% of all patients with Graves hyperthyroidism.[61,185] When Graves hyperthyroidism is diagnosed, 74% have no GO, but 13% in this group will develop GO (mild in 10% and moderate to severe in 3%) during subsequent ATD therapy (Fig. 12.9). Mild GO at the time of diagnosing hyperthyroidism disappears spontaneously in 58% of these cases.[61] Thus GO is diagnosed at the same time as Graves hyperthyroidism in about 75% of GO patients, whereas GO is diagnosed later in about 25%.[186] In a few patients GO onset is before that of Graves hyperthyroidism.

There seems to be a secular trend to a lower incidence of GO.[187] Earlier diagnosis and treatment of hyperthyroidism, identification of risks conferred by ^{131}I therapy and postradioiodine hypothyroidism, and focus on the detrimental effects of smoking have likely contributed to this decline.[183,186]

Pathology

The pathologic anatomy of GO orbits is characterized by enlargement of the extraocular muscles and the retrobulbar fat/connective tissue compartment. The increased volumes of muscles and fat are attributed to an increase of the ground substance consisting of collagen and glycosaminoglycans (GAGs). GAGs (mainly hyaluronate) are very hydrophilic and thus attract much water, resulting in edematous swelling. The ground substance accumulates in the endomysial space between muscle fibers. The number of muscle fibers is not increased, and there is no damage to the muscle cells except in very advanced cases. The number of fibroblasts is increased in the endomysial space and in the fat/connective tissue compartment. The orbital fibroblasts (OFs) are responsible for the excessive production of GAGs. A subset of OFs may differentiate into mature adipocytes thereby adding to the volume expansion. OFs have been recognized as the target of the autoimmune attack in GO.

The increased muscle and fat volumes within the confined space of the bony orbit allow a mechanistic explanation of the

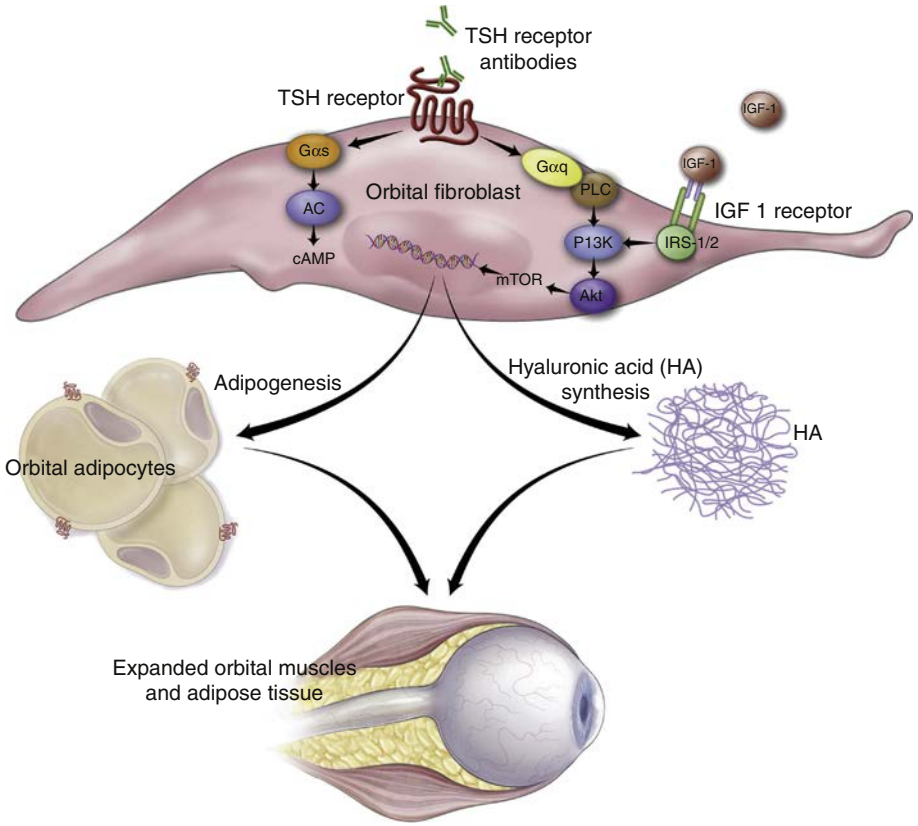


• **Fig. 12.9** (A) Prevalence of Graves orbitopathy (GO) in patients with newly diagnosed Graves hyperthyroidism and during subsequent treatment with antithyroid drugs in those with initially absent GO (B) or mild GO (C). (Modified from Tanda ML, Piantanida E, Liparulo L, et al. Prevalence of natural history of Graves' orbitopathy in a large series of patients with newly diagnosed Graves' hyperthyroidism seen in a single center. *J Clin Endocrinol Metab.* 2013;98:1443–1449.)

eye changes. The swollen retrobulbar tissues will impair venous drainage of eyelids and conjunctiva, resulting in eyelid edema and chemosis. Eyelid swelling can also be caused by herniation of retrobulbar fat through openings in the orbital septum. Increased retrobulbar pressure will push the globe forward, resulting in exophthalmos. Upper eyelid retraction and proptosis contribute to overexposure of the cornea, which may become dry and inflamed. The enlargement of extraocular muscles impairs muscle relaxation, not the ability for muscle contraction. For instance, impairment of elevation is caused by insufficient relaxation of the rectus inferior muscle, which may cause diplopia when gazing up. Marked swelling of rectus muscles in the apex of the orbit (known as apical crowding), close to the entrance of the optic nerve in the optic canal, may compress the optic nerve, resulting in DON.

Immunopathogenesis

Microscopy reveals orbital lymphocytic infiltration, edema, and fibrosis. The lymphocytic infiltration is often focal and consists of T helper cells, cytotoxic T cells, many macrophages, and a few B cells. The infiltrating immunocompetent cells produce cytokines capable of remodeling orbital tissues. The cytokine profile in the early stages of GO is predominantly that of Th1 cells, whereas cytokines are mostly



• **Fig. 12.10** Role of the thyroid-stimulating hormone (TSH) receptor in the immunopathogenesis of Graves orbitopathy. Ligation of the TSH receptor on orbital fibroblasts with stimulating TSH receptor antibodies results in activation of the adenylyl/cyclic adenosine monophosphate (cAMP) pathway and the phosphoinositide 3-kinase (PI3K)/Akt signaling cascade. It induces hyaluronic acid production by the orbital fibroblasts, with a subset exhibiting enhanced adipogenesis. (From Iyer S, Bahn RS. Immunopathogenesis of Graves' ophthalmopathy: the role of the TSH receptor. *Best Pract Res Clin Endocrinol Metab.* 2012;26:281–289.)

derived from Th2 cells in patients with a duration of GO for more than 2 years.[188] The data suggest GO is primarily a T-cell–mediated disease. The cytokines induce expression of immunomodulatory proteins on endothelial cells and fibroblasts, including HLA-DR, heat shock protein 72, and several adhesion molecules. Cytokine-activated OF synthesize IL16 and RANTES (regulated on activation, normal T cell expressed and secreted, also known as CCL5) attracting more T cells to the orbit. Macrophages present antigen to T cells; activated T cells may bind to OF, inducing hyaluronan synthesis, cytokines, COX2, and PGE2. OF are considered the target (and effector) cells of the autoimmune attack in GO. Retrobulbar T cells of GO patients recognize autologous OF (but not eye muscle extract) and proliferate in response to proteins from autologous OF (but not from orbital myoblasts); conversely, OF proliferate in response to autologous T cells dependent on MHC class II and CD40/CD40L signaling.[189]

The TSH receptor is presently viewed as the major autoantigen in GO. OF express full-length functional TSH receptors; the expression is more abundant in active than in inactive GO and is directly related to IL1β levels.[190] Graves immunoglobulins as well as monoclonal TSHR-stimulating antibodies recognize TSHR on OF as evident from increased cAMP and hyaluronan production in cell cultures of OF exposed to these agents. OF not expressing THY1 (present in orbital fat but not in extraocular muscles) may under the influence of IL1 or PPARγ agonists differentiate into mature adipocytes, associated with increased TSHR expression. The role of TSHR is underscored by a direct relationship between TSHRAb and both activity and severity of GO.[191] Genetic immunization against the TSH receptor (but not against the IGF1 receptor) also gives rise to a fair although not perfect animal model of GO.[192,193]

The IGF1 receptor (IGF1R) has been proposed as another major autoantigen in GO. IGF1Rs are indeed overexpressed in OF of GO patients. Graves IgG can induce hyaluronan production in OF, effects that can be attenuated by IGF1R-blocking antibody.[194] Stimulating IGF1R autoantibodies (IGF1RAb) thus have been postulated and have been found in 10% of GO samples and in 10% of control samples; however, the IGF1RAb failed to stimulate IGF1R autophosphorylation but instead inhibited IGF1-induced signaling.[195] These data do not support the hypothesis that IGF1RAb contribute to GO pathogenesis. Immunoglobulins of GO patients stimulate Akt in OF as well as in cells expressing TSHR and IGF1R; knockdown of IGF1R causes a 65% decrease in IGF1-stimulated Akt but has no effect on GO-Ig stimulation of Akt.[196] GO immunoglobulins thus do not activate the IGF1R, and there is no evidence of stimulating IGF1R antibodies (Fig. 12.10).[197] The implication is that TSHRAb stimulate Akt via the PI3K pathway. The therapeutic effect of teprotumumab, an IGF1R-blocking monoclonal antibody, in GO thus could be explained at least in part by decreasing Akt, thereby also impairing the effects of TSHR signaling via the PI3K pathway.[198]

Whereas postreceptor signaling pathways of TSHR and IGF1R partly overlap, it has further been suggested that TSHR and IGF1R form a physical and functional complex in OF[199,200] and

that GO immunoglobulins exert their action via TSHR/IGF1R crosstalk rather than direct binding to IGF1R.[201] A subset of OF are CD34+, and they might be derived from circulating CD34+ fibrocytes, which unexpectedly express relatively high levels of TSH receptors.[202] The relevance of these findings is subject of active research.

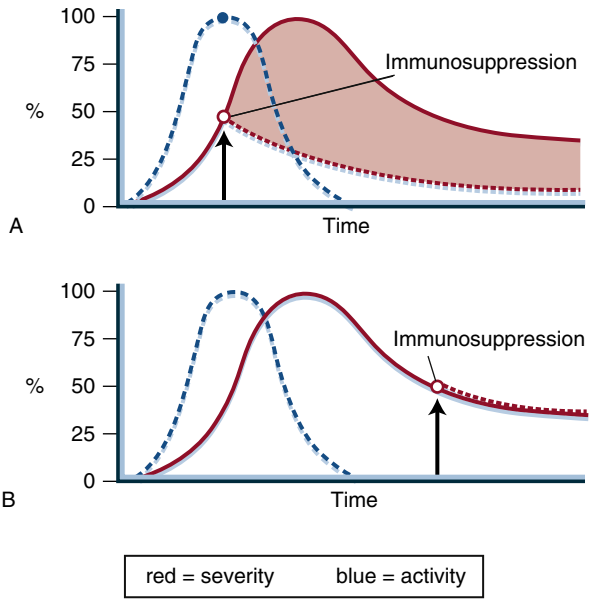
Genetics and Environment

No differences are found in the frequency of known susceptibility genes for Graves disease between patients with Graves hyperthyroidism and Graves orbitopathy. The genetic makeup in both phenotypes seems largely the same.[203] Smoking is a risk factor for GO, as in Graves hyperthyroidism, but the odds ratio is much higher (OR 4.40, CI 2.88–6.73).[101] The risk is dose dependent and decreases in ex-smokers. Exposure of OF in vitro to cigarette smoke extract dose-dependently increases hyaluronan production and adipogenesis.[204]

Exposure to ionizing radiation in the form of RAI therapy is associated with a relative risk for progression of GO of 4.23 (CI 2.04–8.77) when compared to ATD.[205] The immediate and substantial rise of TSHRAb after ^{131}I therapy might be causally connected to the worsening of GO.[80] Risk factors for developing GO in Graves hyperthyroidism were evaluated in a large longitudinal cohort study from Australia.[206] The odds of GO increased by 17% for each decade increase in the age of onset of Graves hyperthyroidism and by 7% for each year increase in the duration of Graves hyperthyroidism. Smoking increased the odds for GO to 2.22 for current smokers and 2.07 for ex-smokers compared with never smokers. The odds for GO were 86% less in patients using ATD than those not (OR 0.14, CI 0.06–0.34). Risk factors for the development of GO in Graves hyperthyroid patients treated for 18 months with ATD were current smoking, mild conjunctival redness or mild eyelid swelling, duration of hyperthyroid symptoms, and TBII.[186] These items, assessed before starting ATD, can be combined in a quantitative manner in a predictive score (called PREDIGO), which is better in predicting those patients who will not develop GO than who will. Another longitudinal study used a large database of a US managed care network.[207] Of Graves hyperthyroid patients, 8.8% developed GO; surgical thyroidectomy alone or in combination with medical therapy was associated with a 74% decreased hazard for GO (HR 0.26, CI 0.12–0.51) compared with RAI therapy alone.[207] Statin use also decreased the risk of GO (HR 0.60, CI 0.37–0.93), an unexpected and hard-to-understand finding.

Natural History and Prognosis

GO has a tendency to spontaneous improvement. Early studies from Rundle in the 1940s and 1950s about the natural history of GO describe the initial development of upper eyelid retraction, exophthalmos, and restricted eye muscle motility, followed by spontaneous but incomplete recovery.[208] Thus in about 60% of patients some eye changes persist. In GO patients who did not require immediate treatment, a 1-year follow-up revealed substantial improvement in 22%, slight improvement in 42%, no change in 22%, and worsening in 14%.[209] The available data suggest there is initially an active stage of the disease characterized by inflammatory edema and lymphocytic infiltration; immunosuppression given in this stage is likely to improve GO (Fig. 12.11).[210] The early active stage is followed by a late inactive



• **Fig. 12.11** Natural history of Graves orbitopathy, as reflected by a curve describing disease severity *(red continuous line)* and a curve describing disease activity *(blue discontinuous line)* over time. Intervention with immunosuppressive agents at peak activity is likely to result in modification of the natural course (gain reflected by the *red stippled area*) (A), whereas late intervention when the disease has become inactive, is unlikely to modify the natural course (B). (Modified from Wiersinga WM. Advances in medical therapy of thyroid-associated ophthalmopathy. *Orbit.* 1996;15:177–186.)

stage of the disease characterized by fibrosis, with immunosuppression less likely to be effective. The time interval between onset of GO and reaching the late inactive stage of the disease varies considerably between patients, from several months to several years. Assessment of GO activity may thus influence the treatment plan. The prognosis of GO has improved substantially in the last few decades, thanks to earlier diagnosis and treatment, more attention to risk factors, and better organization of care. Nowadays it is the rare patient who becomes blind as a result of GO. But the sequelae of GO are substantial: 45% of patients are restricted in their daily activities, 36% are on sick leave, 28% are disabled, 5% went into early retirement, and 3% lost their jobs, all because of GO.[211]

Diagnosis and Differential Diagnosis

Diagnosis of GO can be very easy in the case of a bilateral symmetric ophthalmopathy in a patient with Graves hyperthyroidism. Diagnosis can be difficult in euthyroid or hypothyroid patients presenting with eye changes; in euthyroid or hypothyroid GO patients, GO is milder and more often asymmetric.[180] Unilateral GO occurs in about 10% of all GO patients, whereas GO is the most prevalent cause of unilateral exophthalmos. Schematically, GO is diagnosed along three avenues: ocular symptoms and signs, thyroid autoimmunity, and orbital imaging.

Ocular Symptoms and Signs

It should be realized that none of the symptoms and signs is specific for GO. Guidelines recommend to assess severity and activity of the disease in every patient.[134,212] GO severity is assessed using

TABLE 12.8 Clinical Assessment of the Patient With Graves Orbitopathy

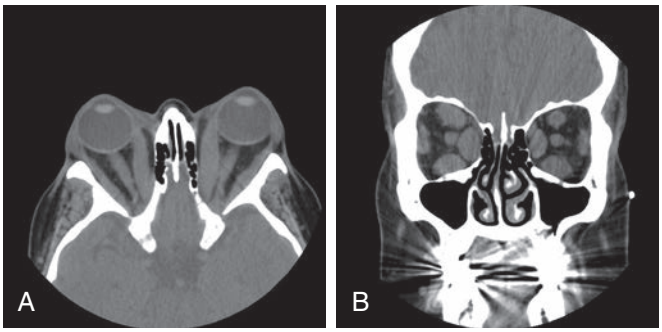
SEVERITY MEASURES (USING THE MNEMONIC NO SPECS)		
NO SPECS Class	**Item**	**Method**
0. **N**o signs or symptoms		
1. **O**nly signs, no symptoms	Lid aperture	With ruler in midline in mm
2. **S**oft tissue involvement	Eyelid and conjunctiva swelling and redness	Inspection, color pictures[a]
3. **P**roptosis	Exophthalmos	Hertel in mm
4. **E**xtraocular muscle involvement	Eye muscle motility Diplopia	Impaired elevation, abduction Subjective grading[b]
5. **C**orneal involvement	Keratitis, ulcer	Fluoresceine
6. **S**ight loss due to optic nerve involvement	Dysthyroid optic neuropathy (DON)	Visual acuity, color vision, visual fields, optic disc

ACTIVITY MEASURES (USING THE CLINICAL ACTIVITY SCORE [CAS])		
Inflammatory Sign	**Item**	**Score**
Pain	Spontaneous retrobulbar pain	1
	Pain on up gaze, side gaze, or down gaze	1
Redness	Redness of the eyelids	1
	Redness of the conjunctiva	1
Swelling	Swelling of the eyelids	1
	Swelling of the caruncle and/or plica	1
	Chemosis	1
Maximum CAS score (assessed momently)		*7*
Impaired function	Increase in proptosis ≥2 mm in 1–3 months	1
	Decrease of ≥8° in eye muscle motility in any direction in 1–3 months	1
	Decrease in visual acuity of more than one line on the Snellen chart (using pinhole) in 1–3 months	1
Maximum CAS score (assessed over time)		*10*

[a]Color atlas in Dickinson AJ, Perros P. Controversies in the clinical evaluation of active thyroid-associated orbitopathy: use of a detailed protocol with comparative photographs for objective assessment. *Clin Endocrinol (Oxf)*. 2001;55:283–303.

[b]Intermittent diplopia = at awakening or when tired; inconstant diplopia = at extremes of gaze; constant diplopia = in primary or reading position.

the mnemonic NOSPECS (Table 12.8). Three degrees of severity are distinguished: mild GO (characterized by lid retraction <2 mm, mild soft tissue involvement, proptosis <3 mm above upper normal limit, absent or intermittent diplopia, absent corneal exposure, and normal optic nerve status), moderate-to-severe GO (lid retraction ≥2 mm, moderate to severe soft tissue involvement, proptosis ≥3 mm above upper normal limit, inconstant or constant diplopia, mild punctuate keratopathy, and normal optic nerve status), and very severe sight-threatening GO (corneal breakdown, or DON). Upper normal limits of proptosis are 23/24 mm in African Americans, 19/21 mm in whites, and 16/17 mm in Asians (female/male values, respectively). Disease activity is best assessed by the clinical activity score (CAS), based on the classic signs of inflammation (see Table 12.8).[213] GO is likely to be inactive at CAS values below 3 and active at CAS values of 3 or greater.



• **Fig. 12.12** Axial (A) and coronal (B) CT scans of a patient with Graves orbitopathy demonstrating generalized enlargement of all extraocular muscles, expansion of the orbital fat, and marked bilateral proptosis.

Thyroid Autoimmunity

Besides assay of TSH and FT_4 to determine thyroid function, assay of TPOAb and TSHRAb are useful to assess whether thyroid autoimmunity is present. This is especially relevant in euthyroid and hypothyroid cases. Knowledge of the level of TSHRAb would anyway be useful in view of their direct relationship with disease activity and severity and their prognostic value for the course of GO.[214]

Orbital Imaging

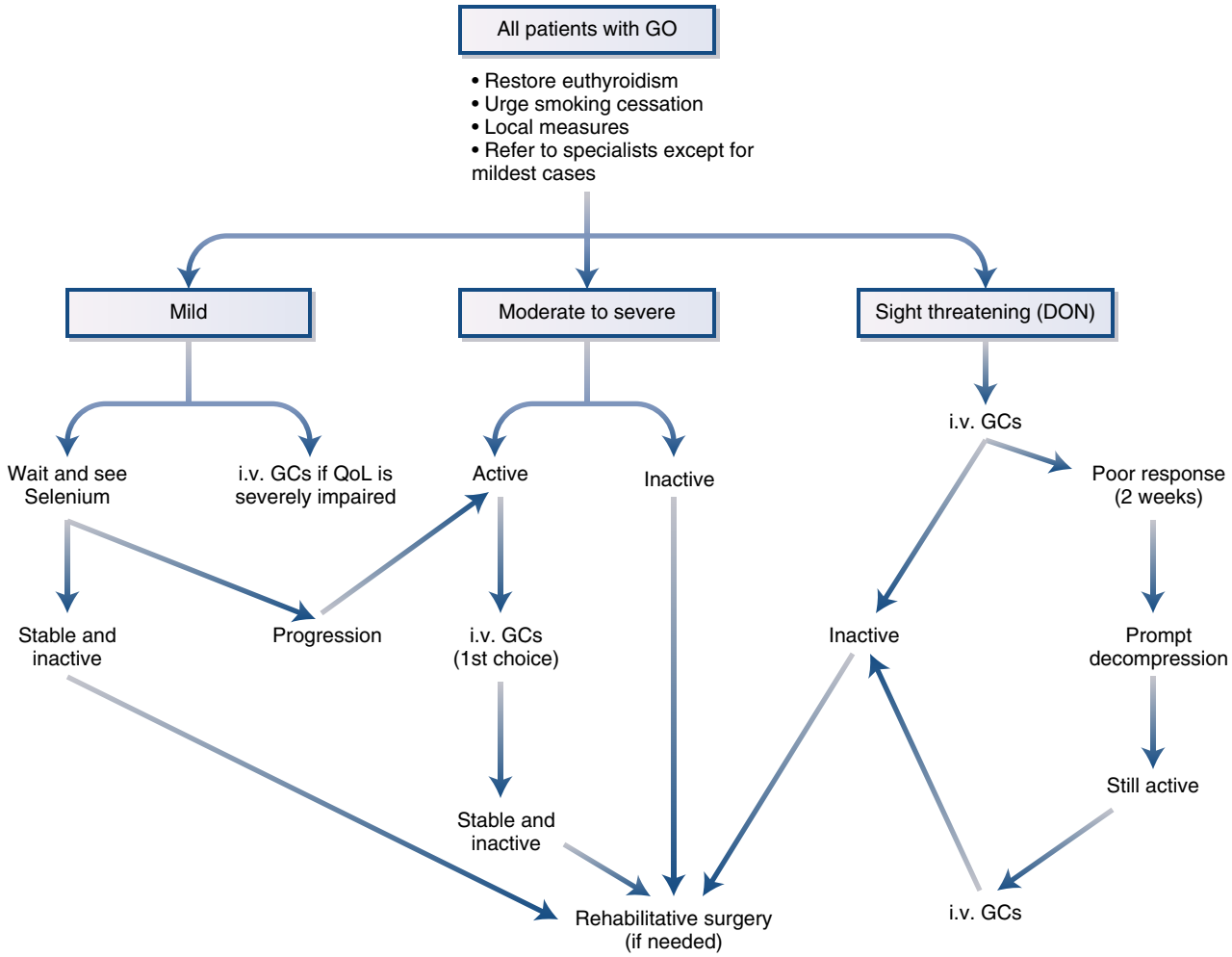
Orbital imaging is not always necessary. It is indicated in patients with suspected DON (to look for apical crowding of enlarged muscles), in euthyroid or hypothyroid GO, and in unilateral GO to exclude alternative diagnoses. Orbital imaging is performed by CT or magnetic resonance imaging (MRI). Orbital CT scanning is indicated before surgical orbital decompression to have a close look at the bony orbit (Fig. 12.12). GO activity might be assessed by MRI provided sophisticated programs are installed measuring T2-weighted relaxation time and/or short tau inversion recovery sequence. The most frequent diseases mimicking GO are orbital myositis, caroticocavernous fistula, non-Hodgkin lymphoma, orbital meningioma, and IgG4-related orbital disease.[215]

Treatment

Specific guidelines for the management of Graves orbitopathy are available.[212] Recommendations are divided into three groups, dealing with general measures, thyroid treatment, and eye treatment.

General Measures

It is recommended that GO patients—except the mildest cases—should be referred to combined thyroid-eye clinics or specialized centers providing endocrinologic and ophthalmologic expertise. A multidisciplinary approach is likely to improve outcomes.[216] A patient-focused approach to the treatment is also advocated, which encompasses the effects of the disease and its treatment on quality of life (QoL) and psychosocial well-being. Use of the GO-QoL, a



• **Fig. 12.13** Algorithm for the management of patients with Graves orbitopathy. (Redrawn from Bartalena L, Baldeschi L, Boboridis K, et al. The 2016 European Thyroid Association/European Group on Graves Orbitopathy guidelines for the management of Graves' orbitopathy. *Eur Thyroid J.* 2016;5:9–26.)

disease-specific QoL questionnaire and well-validated tool, is recommended in routine clinical practice and is available in several languages (www.eugogo.eu).[212,217] It consists of eight questions about visual functioning and eight questions about appearance. It may serve as the primary outcome measure in clinical trials. Treatment should not only *work* from the physician's perspective but also *help* from the patient's perspective. It can also be useful in daily clinical practice to facilitate discussion with the patient on the most disturbing features and to identify patients in need of further counseling. Counseling may reduce anxiety, provide reassurance, and help in developing better coping strategies.

Smokers should be urged to refrain from smoking, and specialized smoking cessation programs or clinics should be offered. The success rate of quitting smoking is rather low, but success rate could be somewhat higher in GO because many patients are very much concerned about their appearance. Patients should be confronted with the evidence that (a) smokers have more severe GO, (b) smokers are more likely to have progression of GO after ^{131}I therapy, and (c) smoking delays or worsens the outcome of immunosuppressive treatment.

Simple measures that may be helpful in any stage of the disease are (1) artificial tears to reduce surface symptoms and protect the epithelium, (2) sunglasses to reduce photophobia but also to comfort patients who are self-conscious of their appearance, (3) lubricant ointments to protect from exposure keratopathy during sleep, (4) prisms to improve diplopia, and (5) botulinum toxin A injections to possibly provide temporary control of eyelid retraction.

Thyroid Treatment

Euthyroidism should be promptly restored and maintained. Antithyroid drugs or thyroidectomy is preferred as neither modifies the natural history of GO, whereas ample evidence indicates ^{131}I therapy carries a risk of developing or worsening of GO. The risk can be reduced by concomitant oral prednisone in a daily dose of 0.3 to 0.5 mg/kg for 3 months. This steroid prophylaxis should be considered in high-risk patients (i.e., in smokers, in active GO, and when TSHRAb are high). Lower doses (0.2 mg/kg prednisone for 6 weeks) can be used in low-risk patients. Patients with inactive GO can safely receive RAI without steroid cover as long as post-radioiodine hypothyroidism is avoided.[218,219] Some experts prefer very long-term ATD treatment until GO has become inactive and no further treatment of GO is required; if a relapse of Graves hyperthyroidism occurs after discontinuation of ATD, it can be safely managed with RAI without flare-up of GO.[218]

Eye Treatment

Management depends on severity and activity of GO (Fig. 12.13).[212] *Mild GO* is best managed by a wait-and-see policy or selenium. Spontaneous improvement is to be expected in about

one-third of cases. Intervention with a 6-month course of selenium (100 µg sodium selenite twice daily) improves QoL and eye manifestations (in 61% vs 36% in placebo) and prevents progression to more severe GO (which occurred in 7% vs 26% in the placebo group).[220] These results were obtained in European countries in which selenium intake is relatively low. It is not known if selenium supplementation is also effective in areas with sufficient selenium intake. Steroids could be considered if the QoL is severely impaired.

Active moderate-to-severe GO qualifies for immunosuppression. Steroids are considered as first-line treatment; they are rather effective in reducing swelling and redness of eyelids and conjunctiva and in improving diplopia, less so in reducing exophthalmos. Intravenous methylprednisolone pulses (IVMP) rather than oral prednisone are recommended because IVMP have greater efficacy than oral prednisone (74% vs 51%) and fewer side effects (56% vs 81%).[212] A dose-finding study indicated a cumulative dose of 4.5 g IVMP is appropriate for most patients, administered as 500 mg IV once weekly for 6 weeks followed by 250 mg IV once weekly for another 6 weeks.[221] Higher doses (750 mg once weekly for 6 weeks followed by 500 mg once weekly for another 6 weeks for a cumulative dose of 7.5 g) are slightly more effective at the expense of more side effects and should be reserved for worst cases. IVMP have been associated with significant cardiovascular or cerebrovascular morbidity and hepatic toxicity if administered in high single doses of 1000 mg or more, in cumulative doses of more than 8 g, and/or as repeat infusions on consecutive days. It is therefore recommended that cumulative doses of IVMP should not exceed 8 g and that IVMP should not be given in patients with recent viral hepatitis, significant hepatic dysfunction, and severe cardiovascular morbidity or psychiatric disorders.[212] Severe hypertension, inadequately managed diabetes, and glaucoma are other contraindications. IVMP therapy should be monitored by regular measurements of blood pressure, blood glucose, and liver function tests. Efficacy of IVMP can be slightly enhanced by coadministration of mycophenolate (one 360-mg tablet twice daily for 24 weeks).[222]

Patients who do not or partially respond to IVMP and patients in whom GO flares up after discontinuation of IVMP may require a second-line therapy. Shared decision making is recommended to select one of the available treatment options: low-dose oral prednisone + either cyclosporine or retrobulbar irradiation (usually 20 Gy fractionated in 10 daily doses of 2 Gy over a 2-week period), repeat IVMP, or rituximab (RTX; 1 g twice at a 2-week interval). RTX is an anti-CD20 monoclonal antibody that effectively causes B-cell depletion. Open studies report a remarkable improvement in CAS and GO severity.[223] Side effects occur in about 30%; notable is the risk of the cytokine-release syndrome with the development of DON. RTX has been tested in two randomized clinical trials. One compared RTX with placebo: CAS decreased similarly in both treatment arms, and RTX was not better than placebo.[224] The other compared RTX with IVMP (cumulative dose 7.5 g). The decrease in CAS was greater in the RTX group than in the placebo group, and RTX was judged to be slightly better than IVMP.[225] Sample size in both trials was very modest, and some differences in patient characteristics and trial execution have been noted.[226] Nevertheless, it remains difficult to reconcile the difference in outcomes. It seems too early to accept RTX as an alternative for IVMP but also too early to dismiss RTX as a disease-modifying drug. The same holds true for tocilizumab, a monoclonal antibody directed against the IL6 receptor. It had good efficacy in GO patients refractory to IVMP in an open study, but the drug has not been tested in a RCT.[227] Teprotumumab is a monoclonal antibody inhibitor of IGF1 receptors. In a placebo-controlled RCT it had great efficacy in reducing CAS, reducing exophthalmos, and improving diplopia and GO-QoL scores.[228] Its potential to reduce exophthalmos is remarkable, and in this respect the drug could be superior to IVMP. However, to displace IVMP as first-line treatment, teprotumumab should be compared to IVMP in a RCT.

Very severe GO, DON requires urgent intervention. It is recommended to start with IVMP: 1 g IV on 3 consecutive days in the first week, followed by 1 g IV on 3 consecutive days in the second week. If visual functions improve by the end of the second week, continue with oral prednisone; if not, then urgent surgical decompression is indicated.[229]

Inactive GO indicates rehabilitative surgery can be done when GO has reached this state. If surgery is performed while the disease is still active, results might be lost because of ongoing disease. Most orbital surgeons require stable eye disease for 6 months before surgery. Rehabilitative surgery includes orbital decompression, eye muscle surgery, and eyelid surgery and should be carried out in this sequence if several procedures are needed.

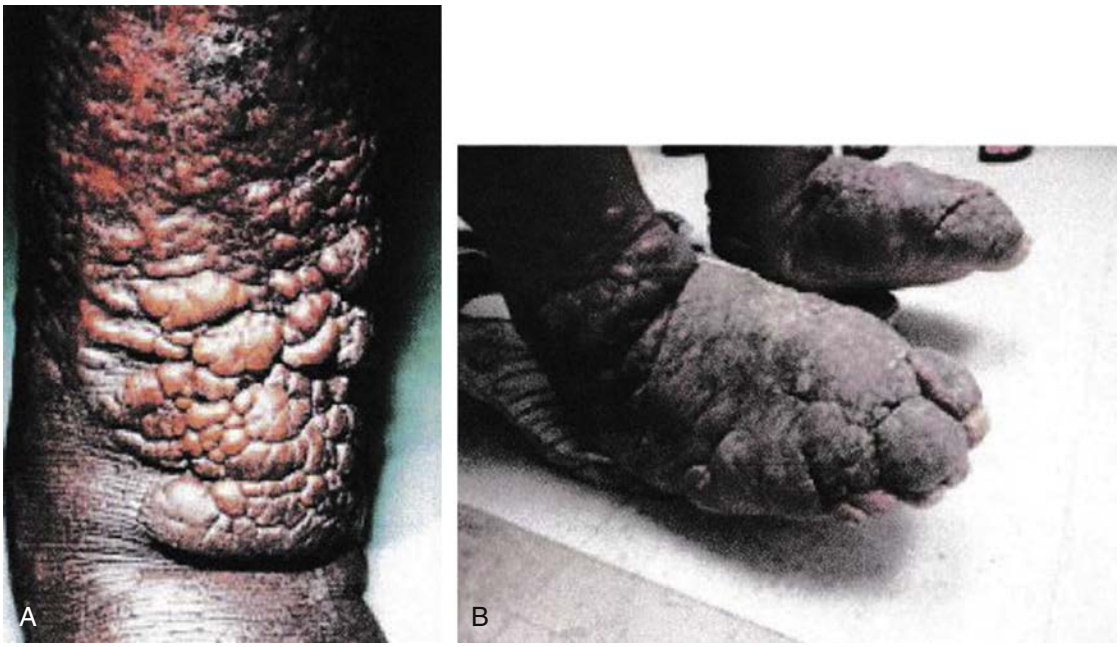
Graves Dermopathy

Graves dermopathy is also known as pretibial myxedema or local myxedema. It is a rather rare phenotype of Graves disease occurring almost always in the presence of Graves orbitopathy and associated with very high levels of TSHRAb. Thus the typical patient with Graves dermopathy has also Graves orbitopathy and Graves hyperthyroidism (sometimes also thyroid acropachy), constituting the most severe expression of Graves disease. The skin lesion consists usually of nonpitting edema with violet discoloration, induration, and prominent hair follicles, giving the appearance and texture of an orange peel *(peau d'orange)*.[230] Other forms are plaques, nodules, and elephantiasis (Fig. 12.14). Its predilection site is the pretibial area (hence the name *pretibial myxedema*), but it has been observed at other sites also exposed to local mechanical pressure.[231] Local trauma may provoke Graves dermopathy as well. Its pathogenesis looks similar to that of Graves orbitopathy, which likewise develops in a confined space with increased local pressure. Dermal fibroblasts seem the target of the autoimmune attack. There is an upregulated expression of TSH receptors on dermal fibroblasts, which also produce cytokine-induced glycosaminoglycans. Treatment is not always required. Spontaneous regression occurs in the long term. If treatment is necessary because of functional or cosmetic complaints, nighttime occlusive dressings with 0.05% to 0.1% triamcinolone acetonide in a cream base might be helpful as well as compressive bandages or stockings. Treatment of GO with glucocorticoids or biologics may also cause regression of Graves dermopathy.

Pregnancy and the Thyroid

Human Chorionic Gonadotropin

hCG is a glycoprotein heterodimer composed of an α-subunit that is common to all glycoprotein hormones (TSH, LH, and FSH) and a specific β-subunit that allows for biologic specificity. Despite this separate structure hCG can bind to and stimulate the hTSHR,[232–234] with an in vitro potency of about 1 U hCG = 0.7 µU of human TSH, depending on its carbohydrate content. In high concentrations as found in hyperemesis gravidarum, multiple gestation, or molar pregnancy hCG will cause hyperthyroidism characterized by a diffuse goiter, elevated free T_4, and a suppressed TSH.



• **Fig. 12.14** (A) Chronic pretibial myxedema in a patient with Graves disease and orbitopathy. The lesions are firm and nonpitting, with a clear edge to feel. (B) Here the chronic myxedema has continued to spread to the foot, causing severe disfiguration and immobility. (A, Courtesy Dr. Andrew Werner, Mount Sinai School of Medicine, New York, NY.)

Transient Gestational Thyrotoxicosis

In the late first trimester of normal pregnancy in humans there is often a physiologic mild transient gestational thyrotoxicosis or hyperthyroidism.[235,236] An exaggeration of this physiologic increase in thyroid stimulation in the first trimester may also be seen in some women and is associated with high levels of hCG (100,000–200,000 U/L), such as those found in twin pregnancies, and is often accompanied by hyperemesis.[237–239] In most patients, the condition is self-limited and the risk of birth defects warrants against the use of antithyroid drugs in early pregnancy. It may be difficult to separate this syndrome from early Graves disease, and a TSHRAb test may be helpful.[240]

Abnormal Responses to Human Chorionic Gonadotropin

A few patients have been reported with an inherited variant of gestational thyrotoxicosis in which a mutation in the *TSHR* gene resulted in a receptor protein with an increase in its responsiveness to hCG.[241] Such patients develop hyperthyroidism with each pregnancy due to even physiologic serum hCG concentrations. Similarly, the use of gonadotropins in in vitro fertilization and indirectly the use of GnRH agonists have been associated with cases of thyroid dysfunction.[242]

Graves Disease During Pregnancy and the Postpartum Period

Although seen more regularly in clinical practice, a truly overactive thyroid gland is uncommon in established pregnancy, affecting approximately 0.2% of women. This low rate is because pregnancy tends to suppress autoimmune responses during pregnancy, and Graves disease, an autoimmune disorder, is the most common cause of thyrotoxicosis in young women. Furthermore, although thyrotoxicosis has a variety of negative influences on fertility itself, it is also associated with increased pregnancy loss and serious medical complications for both the mother and the infant if it should persist.[47,48,243–245] More commonly, a woman under treatment for hyperthyroidism becomes pregnant. Whatever the sequence, pregnancy complicates the diagnosis and treatment of hyperthyroidism in Graves disease and influences its severity and course.

Influence of Pregnancy on the Immune System

The development of pregnancy and the growth of the placenta have profound influences on the immune system, as discussed earlier. The overall suppression of autoimmune responses, which occurs in pregnancy and is mediated by a variety of placental factors, is designed to allow the fetus with its 50% paternal antigens to survive immune assault.[246,247] These changes promote maternal-fetal tolerance, but the increased role of the regulatory T cells and their suppression of maternal responses to the fetus appears to be predominant and long lived.[248,249] It has been shown that a major shift in such T-cell control reduces the effectiveness of all inflammatory T cells.

Thyroid Antibodies in Pregnant Patients With Graves Disease

The hallmark of the immune effects initiated by the placenta is the fall in thyroid autoantibody secretion—TPOAb, TgAb, and TSHRAbs—that is seen in almost all patients as pregnancy progresses.[250,251] This is now considered secondary to enhanced regulatory T-cell activity[252] and precedes a rapid increase in autoantibody levels after the immunosuppression is lost in the postpartum period. Assays for TSHRAbs in the serum of pregnant women with Graves disease may be of clinical value in selected cases because a failure of this immunosuppression may indicate potential fetal problems.[55,253] Because maternal antibodies cross the placenta, there is a correlation between the maternal level of stimulatory TSHRAbs and the development

of fetal thyrotoxicosis. Fortunately fetal and neonatal thyrotoxicosis occurs in only 1% of infants of mothers with Graves disease, and high levels of TSHRAbs, usually greater than three times the upper normal limit, are correlated with fetal thyroid stimulation.[254,255] Pregnant women at risk for failure to suppress thyroid autoantibodies include those with more severe hyperthyroidism and those with significant GO or infiltrative dermopathy. In addition, the prior treatment of the mother, especially with radioiodine, may not always be accompanied by a sufficient reduction in TRAbs. Thus the fetus of a treated patient with Graves disease may still be at risk for development of fetal or neonatal thyrotoxicosis, and the mother may need antithyroid drug treatment and the fetus monitored by umbilic cord blood testing and ultrasonography.[256]

Differential Diagnosis

When mild thyrotoxicosis is present during early pregnancy, it may be due to gestational thyrotoxicosis secondary to hCG stimulation of the thyroid gland (see later).[257,258] When it is more severe it is usually due to Graves disease because toxic multinodular goiters and hot nodules are uncommon in this age group.

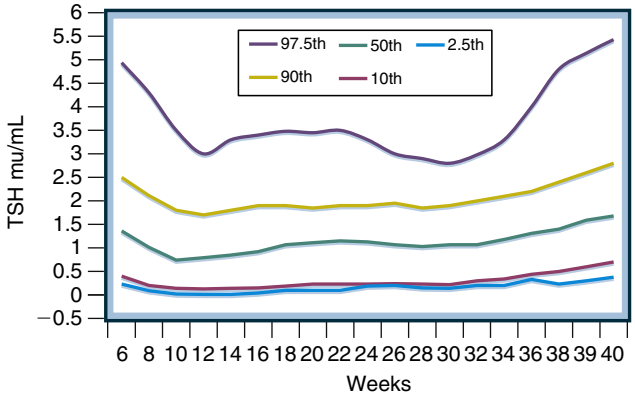
Diagnosis

Pregnancy and hyperthyroidism are both accompanied by thyroid stimulation, a hyperdynamic circulation, and hypermetabolism. In pregnancy, serum TBG levels are increased by estrogen-induced changes in glycosylation, which increases TBG production and lengthens its half-life, and thus in both conditions the total serum T_4 and T_3 levels are elevated so that the upper limit of the normal range during second and third trimesters of gestation is about 1.5 times the upper nonpregnant reference limit.[254] However, serum free T_4 levels, as measured by both analog and equilibrium dialysis methodologies, may actually decrease as pregnancy progresses and the normal third trimester reference range for a given assay is significantly less than its nonpregnant reference range.[236] Serum TSH levels also tend to decrease in early pregnancy and up to 15% of women can have TSH levels below the normal limit.[235] The 95th percentile confidence interval lower limits for serum TSH levels are 0.06 mU/L, 0.3 mU/L, and 0.3 mU/L, respectively, for the first, second, and third trimesters. Nevertheless, each laboratory needs to establish its own normal ranges for thyroid testing in pregnancy.[259,260]

Biochemically, the diagnosis of thyrotoxicosis is confirmed when the serum TSH level is below the trimester-specific lower limit and the total or free T_4 levels are above the normative range for pregnancy (Fig. 12.15). Detection of TSHRAbs can confirm the diagnosis of Graves disease, which may or may not be obvious from the clinical history and examination.

Treatment During Pregnancy

Hyperthyroidism in pregnancy is associated with a variety of complications for mother and child (Table 12.9). Although mild hyperthyroidism in pregnancy does not lead to a significant increase in risk for mother or fetus, severe thyrotoxicosis can lead to many complications and endanger the life of the mother and fetus. Furthermore, the management of hyperthyroidism during pregnancy can be an even greater problem than its diagnosis. Graves disease can worsen in the first trimester, but the subsequent trimesters have an attenuating influence on the hyperthyroid state because of the immunosuppression associated with pregnancy. Pregnancy is also one of the few clinical situations in which an assay of the biologic activity of the TSHRAbs is helpful in predicting its potential effect on the newborn. This assay is especially useful in pregnant women who previously received ablative therapy for Graves hyperthyroidism and who still have high levels of TSHRAb.



• **Fig. 12.15** Gestation age-specific thyroid-stimulating hormone nomogram derived from 13,599 singleton and 132 twin pregnancies. Different percentiles are shown by *colored lines*. (From Dashe JS, Casey BM, Wells CE, et al. Thyroid-stimulating hormone in singleton and twin pregnancy: importance of gestational age-specific reference ranges. *Obstet Gynecol*. 2005;106:753–757.)

TABLE 12.9 Complications of Hyperthyroidism in Pregnancy

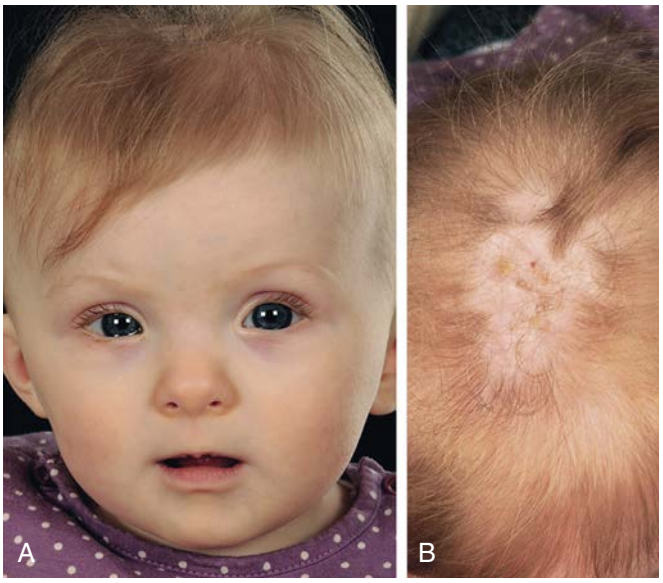
Increased and recurrent pregnancy loss
Preterm delivery
Preeclampsia
Fetal growth restriction
Fetal thyroid hyperfunction or hypofunction caused by TRAb
Fetal goiter from excessive antithyroid drug treatment
Neonatal thyrotoxicosis
Increased perinatal and maternal mortality risk
Potential for decreased IQ of offspring because of excessive use of antithyroid drugs

IQ, Intelligence quotient; *TRAb*, thyrotropin receptor antibodies.

Antithyroid Drugs in Pregnancy

Medical therapy is the usual method of choice in pregnancy. Because of the usual improvement in the disease, the dosage of antithyroid drug required to control the disease in the later phases of pregnancy is generally much less than that required in the same patient were she not pregnant. Overtreatment of the hyperthyroid pregnant woman remains a common but avoidable clinical problem with potentially severe consequences for the fetus. Thus the clinician should prefer mild undertreatment to the risk of hypothyroidism.[256,261–263] Therefore it is imperative for the clinician to understand the therapeutic targets for titrating antithyroid drug dosages during pregnancy.[264]

Certain aspects of placental physiology are relevant to the use of antithyroid drugs. PTU and methimazole readily and rapidly cross the placenta equally well and are concentrated in the fetal thyroid. In excess quantity these agents can cause goitrous hypothyroidism in the fetus.[265] Maternal T_4 crosses the placenta (as evidenced by infants born with significant circulating serum T_4 concentrations despite congenital hypothyroidism) and is the major source of fetal thyroid hormone prior to the complete functional development of



• **Fig. 12.16** Examples of methimazole embryopathy. (A) Dysmorphic astigmatism. (B) Aplasia cutis. (From Bowman P, Osborne NJ, Sturley R, et al. Carbimazole embryopathy: implications for the choice of antithyroid drugs in pregnancy. *QJM*. 2012;105:189–193.)

the hypothalamic-pituitary axis in the fetus at around 20 weeks of gestation. Furthermore, the transplacental passage of maternal TSHRAbs in the latter half of pregnancy can result in fetal thyroid stimulation. Therefore the fetal thyroid is subject to the same factors that influence maternal thyroid hormone production.

Previously, the antithyroid drug of choice throughout pregnancy in the United States was PTU, but because of the rare yet serious side effect of PTU-induced hepatic failure, in June 2009 the FDA issued an advisory that PTU should be reserved for the first trimester of pregnancy while organogenesis is occurring[144] (see later). Subsequently, methimazole could be prescribed. One must keep in mind that the therapeutic antithyroid potency ratio of methimazole to PTU is around 20:1. Thus a patient who requires only 50 mg of PTU in the first trimester may be given 2.5 mg methimazole, and she may not even require a thionamide during the latter part of pregnancy. Of course, the best practice is to ensure definitive treatment prior to pregnancy so that antithyroid drugs are not needed at all.

Danger of Antithyroid Drugs in Pregnancy

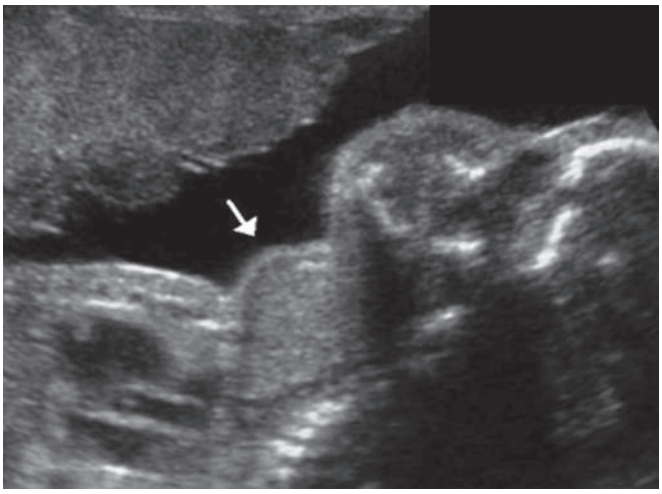
The first report of birth defects after maternal use of antithyroid drugs was a brief letter reporting on congenital scalp skin defects after the use of methimazole.[266,267] Subsequently, several case reports confirmed the association, and other types of defects were described as well.[268] Such defects included a specific combination of facial features, and the condition was termed *methimazole/carbimazole embryopathy*. Because PTU was not at first associated with such birth defects, guidelines have been proposed that recommend the use of PTU in the first trimester and to consider shifting from methimazole to PTU in women planning a pregnancy.[269,270] Recent larger studies have since expanded our knowledge on methimazole-associated birth defects.[271,272] Apart from aplasia cutis, defects are seen in the abdominal wall (gastroschisis, omphalocele), gut (esophageal atresia), upper airways (choanal atresia), urinary system, heart (ventricular septum defect), and eye (Fig. 12.16). Around 1 in 30 women exposed to methimazole or carbimazole in early pregnancy will give birth to children with defects associated with this therapy. This is in addition to the 5% general population risk of giving birth to a child who has a birth defect diagnosed before the age of 2 years.[273] However, PTU also is teratogenic,[274,275] and the use of PTU is also associated with birth defects (estimated to be around 1 in 40 exposed).[272] These abnormalities tend to be milder than with methimazole-associated defects and include preauricular sinuses and cysts and urinary abnormalities.[276]

The period of risk when these drugs may be teratogenic is especially weeks 6 to 10 of pregnancy.[277] Accordingly, to reduce the risk of birth defects, withdrawal of antithyroid medication in patients who are considered in remission of Graves disease or an unavoidable shift from methimazole to PTU has to take place very early in pregnancy. Additionally, the lowest possible dose of an antithyroid drug must be used.[278] The risk of birth defects has to be balanced against the risk of abnormal maternal thyroid function in early pregnancy. It is therefore wise to recommend to young women who receive antithyroid drugs for Graves disease that they test for pregnancy just a few days after a missing menstruation and immediately contact their physician to plan future therapy or withdrawal of medication. No firm international consensus has yet been reached on how to minimize the risk of birth defects from antithyroid drug use in early pregnancy.

Caring for the Pregnant Patient

The therapeutic target for a pregnant Graves patient is a healthy infant. In the second half of pregnancy both the untreated pregnant woman with hyperthyroidism caused by Graves disease and her fetus will be thyrotoxic because TSHRAb passes the placenta and stimulates the fetal thyroid. Antithyroid drugs given to the mother also pass through the placenta and thus treat both the maternal and the fetal hyperthyroidism. However, the drugs may overtreat the fetus compared with the mother, and accordingly the aim of therapy is to keep the mother in a state of subclinical hyperthyroidism analogous to the normal gestational physiology of the first trimester.[264] This is associated with the lowest impact on fetal thyroid function and the highest rate of normal neonatal thyroid hormone levels.[279] The maternal serum free T_4 level should be maintained at or just above the upper normal *nonpregnant* range, and no attempt should be made to normalize the serum TSH concentration. Indeed, a normal TSH during drug therapy is an indication that the dose of drug should be reduced.[280] The clinical status of the patient is an important indication for treatment or increases in dosage. A modest tachycardia is a physiologic response to the increased metabolic demands of pregnancy, and pulse rates of 90 to 100 beats/minute are well tolerated without evidence of myocardial decompensation during delivery. The natural amelioration of Graves disease in the third trimester should be kept in mind, and repeated attempts should be made to reduce or discontinue the thionamide as the delivery date approaches to avoid TSH-induced fetal/newborn goiter, which may cause asphyxia (Fig. 12.17). The serum TSH concentration should be monitored monthly—more to avoid inadvertent overtreatment rather than as the target for normalization. Because thionamides, but not thyroid hormone given to the mother, pass the placenta rather freely, a block-and-replace strategy may induce severe fetal hypothyroidism and goiter, and it is in general not appropriate in the pregnant patient. The rare exception is a pregnant woman who was previously given ablative therapy for Graves disease but who still produces TSHRAb that leads to isolated fetal hyperthyroidism.

All pregnant patients with significant Graves disease should be managed in close cooperation with obstetricians experienced with modern techniques for monitoring the fetus for intrauterine



• **Fig. 12.17** Sagittal view of a fetus at 23.9 weeks showing a large goiter *(arrow)* preventing neck flexion. (From Mayor-Lynn KA, Rohrs JH III, Cruz AC, et al. Antenatal diagnosis and treatment of a dyshormonogenetic fetal goiter. *J Ultrasound Med*. 2009;28:67–71.)

thyroid dysfunction. These techniques normally include fetal heart rate monitoring and ultrasonographic assessment of fetal growth rate. With advanced ultrasonography it is usually possible to examine the fetus for the presence of goiter. Fetal goiter can develop both from the stimulatory effect of maternal TSHRAb passing through the placenta and secondary to antithyroid drugs given to the mother. Occasionally, cordocentesis with fetal thyroid function testing may be appropriate. The amount of fetal monitoring necessary can be judged based on the degree of hyperthyroidism present, which is indicative of the potency of TRAb present and also by the increase in circulating TRAb levels in mothers previously treated for Graves. A recent systematic review demonstrated that values of TRAb of over 3.7 times the upper limit of normal could be associated with fetal thyrotoxicosis.[281] This is consistent with the American Thyroid Association guidelines where fetal monitoring is recommended in mothers who have TSHRAb levels greater than three times the upper limit of normal at any time during pregnancy.[235]

Iodide and Beta Blockers

Obviously therapeutic radioiodine is contraindicated in pregnancy, although no harm has been found after diagnostic doses of ^{123}I.[282] Iodide itself should also not be used as therapy for more than 2 to 3 weeks in the pregnant woman because it readily crosses the placenta and can induce a large goiter that may cause airway obstruction in the newborn. Large amounts of iodide are contraindicated in the last month of pregnancy but can be used at earlier times in emergent situations. Whether propranolol or other beta blockers should be used in the pregnant woman with hyperthyroidism has been a matter of debate. In the experience of some, it can cause intrauterine growth retardation, delayed lung development, and neonatal hypoglycemia or depression,[283] but large studies have suggested that it can be used with safety for short periods or at very low doses.[284,285]

Surgery

Surgery during the first and third trimesters is not desirable because of the possible induction of early pregnancy loss and later premature labor, respectively. Surgery may be successful during the second trimester, but it is best to avoid major surgery during pregnancy if possible. Nevertheless, if antithyroid drug requirements are very high or cannot be used, surgery may be indicated. Iodide can be given for 7 to 10 days to aid in patient preparation with large and highly vascular thyroid glands. Importantly, thyroid surgery may cure the hyperthyroidism of the mother, but TRAb will not disappear immediately. Thus the fetal thyroid may still be stimulated, and withdrawal of antithyroid drugs from the pregnant woman may lead to isolated fetal hyperthyroidism, especially in mothers with high levels of TSHRAb.[286]

Consequences of Overtreatment

The influence of maternal hypothyroidism on fetal brain development and the subsequently reduced IQ of the children of hypothyroid mothers are discussed in Chapter 13. Needless to say, the overuse of antithyroid drugs in pregnancy may lead to the same consequences. There is considerable evidence that many pregnant patients with Graves disease were overtreated in the past as far as the fetus is concerned, as evidenced by transiently elevated serum TSH levels on newborn screening tests.[287] This is another reason why one should accept patients being slightly hyperthyroid rather than slightly hypothyroid.

Graves Disease in the Postpartum Period

Changes in the Immune Response in the Postpartum Period

As discussed earlier, pregnancy induces a variety of immune changes that are responses to placental influences and the paternal foreign antigens and that are designed to prevent rejection of the foreign fetus. These changes include enhanced regulatory T-cell influences and a T-cell shift from Th1 to Th2, resulting in an overall decrease in all autoimmune responses as evidenced by marked decreases in thyroid autoantibodies.[251] Following delivery, these immune changes are slowly lost and a return to normal is observed but only after a period of exacerbated autoimmune reactivity in which large increases in T-cell and autoantibody activity occur. It is at this time—4 to 12 months postpartum—that new-onset or recurrent thyrotoxicosis is seen. Such thyroid dysfunction may be transient or permanent.

Transient Postpartum Thyroiditis

Transient postpartum thyroiditis remains the most common form of hyperthyroidism in the postpartum period and usually precedes a period of hypothyroidism.[288] The transient thyrotoxicosis is due to thyroid cell destruction and may occur in approximately 5% to 10% of patients during the 4 to 12 months postpartum.[289] This can be double in those with other autoimmune diseases. However, the rapid return of true Graves hyperthyroidism is less common but is similarly dependent on the subsequent changes in the immune response.

Presentation of Postpartum Graves Disease

A high percentage of women with Graves disease age 20 to 35 years give a history of pregnancy in the 12 months before the onset of Graves disease.[290,291] Pregnancy and the postpartum state also apparently influence the course of preexisting Graves disease. Patients in clinical remission during pregnancy are prone to postpartum relapse. In 41 pregnancies in 35 patients in remission, 78% were followed by development of thyrotoxicosis during the postpartum period. The patients with Graves disease and postpartum thyrotoxicosis were classified into three categories: (1) Some had persistent recurrent hyperthyroidism with an elevated RAIU (classic Graves disease). (2) Some had a transient disorder associated with a normal or an elevated RAIU (transient Graves

disease). (3) Some patients, especially those with the highest titers of TPOAb, experienced transient thyrotoxicosis with a decreased RAIU (the thyrotoxic phase of postpartum thyroiditis referred to earlier). This phase in turn may be followed by a hypothyroid phase.[292]

Preconception Counseling

A special problem related to hyperthyroidism and pregnancy is presented by the patient who wishes to conceive in the near future and is either in early remission after a course of antithyroid drug treatment or is being treated with antithyroid agents for active Graves disease.[293] For the first scenario, antithyroid drugs can be reluctantly reintroduced if required during pregnancy if symptomatic thyrotoxicosis recurs. In the second situation, definitive therapy (radioiodine therapy or surgery) should be considered to forestall the complexities of managing hyperthyroidism during pregnancy. As with the therapy of Graves disease in general, such decisions must involve education of the patient so that the risks and benefits of the various alternatives are clearly appreciated. Fertile women who receive antithyroid drugs should be educated to perform pregnancy testing already within the first days after a missed menstrual period if pregnancy is possible, and if the test is positive they should immediately contact the physician for further planning of therapy. The 1-year surge in TSHRAb after radioiodine therapy[80,294] may increase the risk of fetal exposure to high TSHRAb levels if the woman is treated with radioiodine shortly before she becomes pregnant. In all cases where radioiodine therapy is used, pregnancy should be delayed for at least 6 months and until normal thyroid function has returned on replacement therapy.

Nursing and Antithyroid Drugs

Older studies suggested that relatively more methimazole than PTU appeared in breast milk of women receiving these drugs during lactation, but more recent evidence shows little difference between them.[265,295,296] It is occasionally recommended that women who take high doses of antithyroid drugs not nurse their infants because of the difficulty in monitoring thyroid function in infants. The drug doses transferred via breast milk are very small, and no drug side effects have been reported in neonates whose mothers were taking antithyroid drugs, including neurologic function.[297]

Inherited Nonimmune Hyperthyroidism

Toxic diffuse thyroid hyperplasia without the pathologic characteristics of autoimmune disease has been reported in families and appears to be inherited as an autosomal dominant condition.[298–300] Polymorphic genomic mutations in the *TSHR* gene have been reported to cause constitutively activated TSHRs differing from family to family.[301] Recessive mutations on both chromosomes have also been described as causing hyperthyroidism while the parents remained euthyroid. These gain of function mutations, mostly in the transmembrane regions of the TSHR, are similar to those somatic mutations seen in toxic adenomas but are in the germline.[302] Treatment is by radioiodine ablation or thyroidectomy, depending on the age of the patient.

Toxic Multinodular Goiter

Toxic multinodular goiter is a disorder in which hyperthyroidism arises in a multinodular goiter, usually of long standing, and is the result of one of several pathogenetic factors.[303] Its incidence is highly dependent on the iodine intake of the population.

Pathogenesis

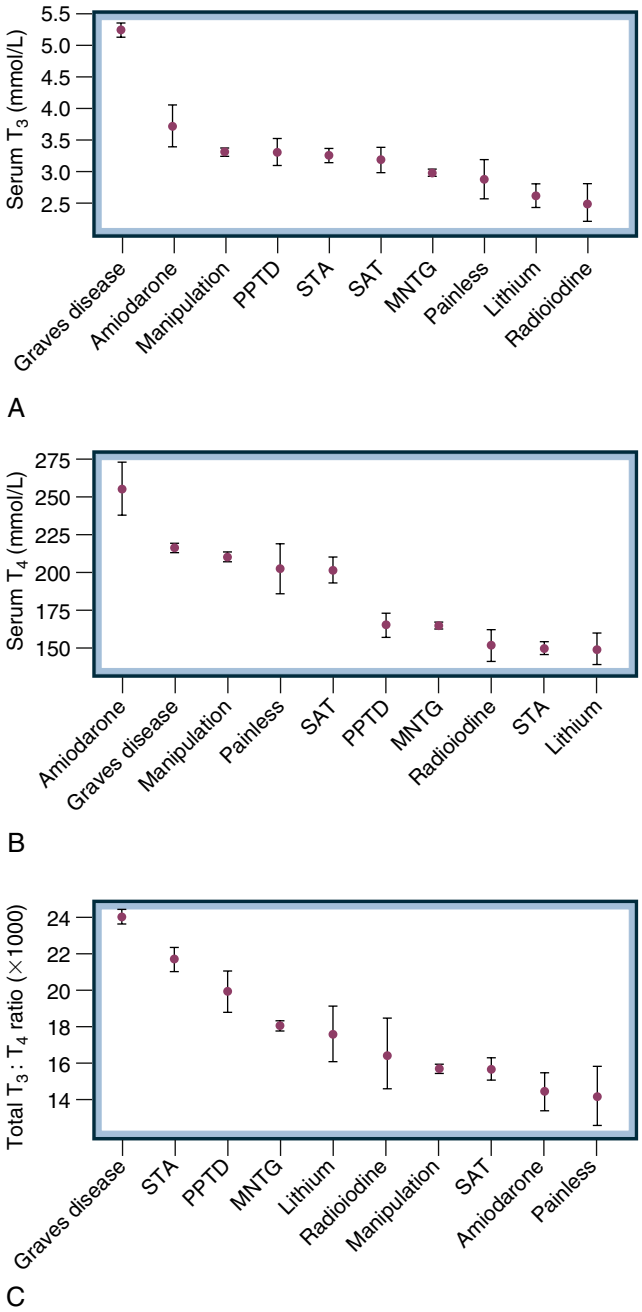
The pathogenesis of toxic multinodular goiter cannot be considered apart from that of its invariable forerunner, nontoxic multinodular goiter, from which it may slowly emerge. Two hallmarks of the disorder, structural and functional heterogeneity and functional autonomy, evolve over time; the increase in the extent of autonomous function causes the disease to move from the nontoxic to the toxic phase. Somatic mutations in the *TSHR* gene, first demonstrated in toxic adenomas,[298] have been demonstrated in toxic multinodular goiter, and the individual mutations appear to differ from nodule to nodule. However, only about 60% of toxic nodules have reported *TSHR* mutations, and only a very few have G protein mutations. Hence there are many nodules with autonomy of undetermined cause[304] and that presumably involve mutations in additional parts of the signaling pathways.

Radioiodine scans show localization of isotope in one or more discrete nodules, whereas iodine accumulation in the remainder of the gland is usually suppressed because TSH is suppressed by the hyperthyroidism. However, the degree of hyperthyroidism can be variable and TSH may not be totally inhibited, so the background uptake of radioisotope may also be variable. Histopathologically the functioning areas may resemble adenomas in being reasonably well demarcated from surrounding tissue. They generally consist of large follicles, sometimes with hyperplastic epithelium, but here, too, architecture correlates poorly with functional state. The remaining tissue appears inactive, and zones of degeneration are present in both functioning and nonfunctioning areas. Hence, from the pathophysiologic standpoint, these thyroids harbor multiple solitary hyperfunctioning and hypofunctioning adenomas interspersed by suppressed normal thyroid tissue.

Clinical Presentation

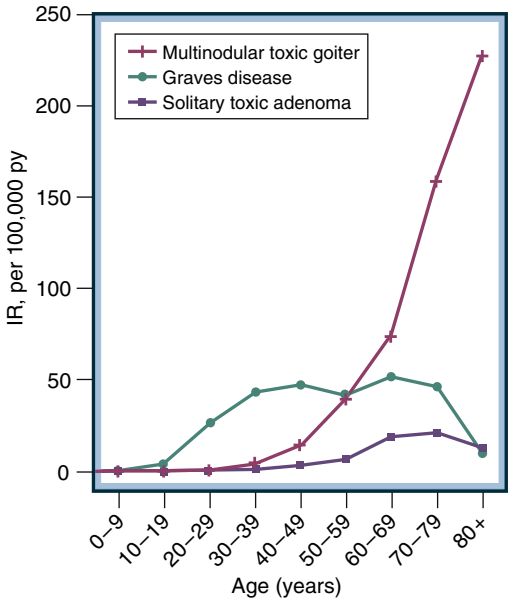
The overproduction of thyroid hormone in toxic multinodular goiter is usually less than that in Graves disease, and the disease presentation is milder (Fig. 12.18); in addition, toxic multinodular goiter usually occurs after the age of 50 in patients who have had nontoxic multinodular goiter for many years (Fig. 12.19). Like its forerunner, toxic multinodular goiter is more common in women than in men (6:1).[305] Sometimes hyperthyroidism develops abruptly, often after exposure to increased quantities of iodine such as the contrast media for CT scanning, which permits autonomous foci to increase hormone secretion to excessive levels and which may simply exacerbate already established mild hyperthyroidism (see "Iodide-Induced Hyperthyroidism"). The serum T_4 and T_3 concentrations may be only marginally increased, and a suppressed TSH may be the major abnormality. The total RAIU is only slightly increased or within the normal range unless following iodine exposure.

A toxic multinodular goiter may also be found as part of Graves disease as confirmed by the presence of TSHRAb of the stimulating variety.[306] Presumably this represents two separate diseases, although TSHRAbs have growth-stimulating activity but this should apply to all cells. Toxic multinodular goiter alone is not accompanied by infiltrative ophthalmopathy, and when the two coexist it represents the emergence of Graves disease.



• **Fig. 12.18** Serum T_3 (A), T_4 (B), and T_3:T_4 ratio (C) in 10 types of hyperthyroidism. Means (±SEM) are shown. *MNTG,* multinodular toxic goiter; *PPTD,* postpartum thyroid disease; *SAT,* subacute thyroiditis; *SEM,* standard error of the mean; *STA,* solitary toxic adenoma; *T_3,* triiodothyronine; *T_4,* thyroxine. (From Carlé A, Knudsen N, Pedersen IB, et al. Determinants of serum T4 and T3 at the time of diagnosis in nosological types of thyrotoxicosis: a population-based study. *Eur J Endocrinol*. 2013;169:537–545.)

Cardiovascular manifestations often predominate, possibly because of patient age, and include atrial fibrillation or tachycardia with or without heart failure. Weakness and wasting of muscles are common, the so-called *apathetic* or *masked thyrotoxicosis*. The nervous manifestations are less prominent than in younger patients with thyrotoxicosis, but emotional lability may be pronounced, and even osteoporosis may be the factor leading to thyroid function testing and diagnosis. Because of the physical characteristics of the thyroid gland and its frequent retrosternal extension, obstructive symptoms are more common than in Graves disease.



• **Fig. 12.19** An example of age-specific incidence rates *(IR)* of the three most common types of hyperthyroidism. *py,* person-years. (From Carlé A, Pedersen IB, Knudsen N, et al. Epidemiology of subtypes of hyperthyroidism in Denmark: a population-based study. *Eur J Endocrinol*. 2011;164:801–809.)

On palpation, the characteristics of the goiter are the same as those of the more common nontoxic multinodular goiter. In as many as 20% of elderly patients with thyrotoxicosis, the thyroid gland is firm and irregular but not distinctly enlarged. A thyroid scan and ultrasound examination will confirm the diagnosis as toxic multinodular goiter rather than a single toxic adenoma or Graves disease.

Laboratory Tests and Differential Diagnosis

All patients with a multinodular goiter should be screened annually with a serum TSH. If suppressed, the free T_4 should be determined. Serum TSH levels intermediate between 0.1 and 0.4 mU/L are not usually associated with significant clinical symptoms. Such patients have thyroid autonomy but are not thyrotoxic (see "Subclinical Hyperthyroidism"). For patients with established thyrotoxicosis, an RAIU with scan will help in gauging the dose of ^{131}I to be administered as well as identify the autonomously functioning nodules. The latter can then be followed by ^{131}I therapy.

Treatment

The treatment of toxic multinodular goiter with overt hyperthyroidism can be approached with surgery, radioactive iodine therapy, or in some cases antithyroid drug therapy. The correct choice depends upon a combination of patient preference with associated risk factors. For example, a gland with significantly high radioactive iodine uptake in select nodules would be ideal for radioiodine therapy. In contrast, a large gland with compressive symptoms could be better addressed with surgery.[134]

Radioiodine Therapy

Radioiodine may be the treatment of choice for patients with toxic multinodular goiter despite disagreement about the size and number of doses required to achieve a therapeutic response.[303,307] In the United States, iodine intake is higher than in many regions

in Europe so that 24-hour RAIU values of 20% to 30% are not unusual. Such patients will require significant doses of radioiodine to restore a euthyroid state and may even require a second treatment.

Because a number of patients with this disorder have underlying or the potential for heart disease, the administration of radioiodine should be preceded by a course of antithyroid therapy with methimazole until a near eumetabolic state is achieved, but still with a suppressed TSH to avoid iodine uptake in normal thyroid tissue. Medication is then discontinued for at least 4 to 7 days before radioiodine is administered. A week later the antithyroid drug may be reinstituted so that the thyrotoxicosis is controlled until radioiodine takes effect, which typically requires 3 to 4 months. A decrease in size of the hyperfunctioning nodules is a positive sign. At that time, the antithyroid drug can be tapered, but if the TSH level remains below 0.1 mU/L after 6 months, a second dose may be required.

Surgery

Surgical therapy is often recommended after adequate preoperative preparation in patients with large goiters or obstructive manifestations. In these patients, a CT scan or MRI is recommended to define the extent of the goiter and the adequacy of the tracheal walls. Respiratory function studies may also be helpful in assessing the need for surgery. Patients with fixed, especially partially retrosternal, goiter should be considered for surgery because of the risk of more complete obstruction should hemorrhage into a nodule occur. However, when surgery is contraindicated, even significant obstructive symptoms can be relieved by adequate radioiodine therapy.[308] In elderly patients who are not candidates for either radioiodine therapy or surgery, lifelong low-dose antithyroid drug therapy remains an option.

Additional Treatment Options

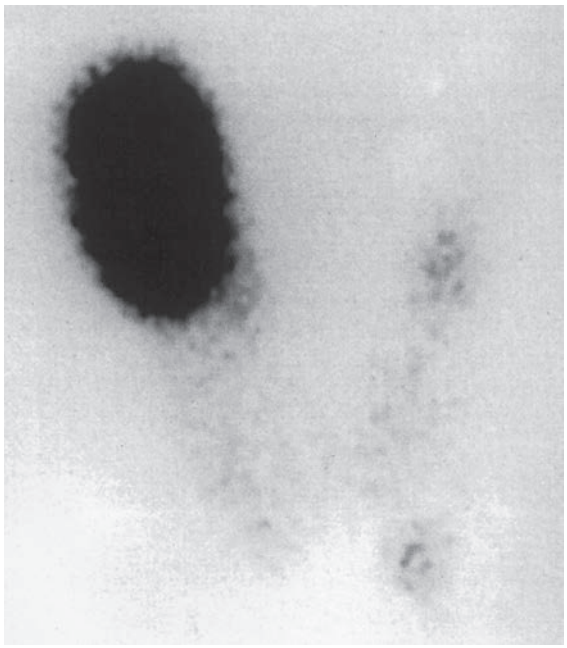
Outside of the United States there is greater experience with newer treatment options, including ethanol ablation and radiofrequency ablation. Success with these techniques in context of diminished nodule size and function is reported. However, their utility has not been broadly studied and they should likely only be used when the more standard modalities of radioactive iodine therapy, surgery, or antithyroid drugs are not possible or contraindicated.[309,310]

Toxic Adenoma

A third, less common form of hyperthyroidism (~5% of cases)[305] is caused by one or more autonomous adenomas of the thyroid gland. As herein used, the term *toxic adenoma* refers to a tumor in a thyroid that is otherwise intrinsically normal. The disorder is usually caused by a single adenoma that is either palpable or seen on ultrasound as a solitary nodule and hence is sometimes referred to as a *hyperfunctioning solitary nodule* or *toxic nodule*. Occasionally two or three adenomas of similar character are present.

Pathogenesis

Toxic adenomas are true follicular adenomas (for histopathologic characteristics, see Chapter 14). The basic pathogenesis of many toxic adenomas (up to 70%) is one of several somatic point mutations in the *TSHR* gene, commonly in the third transmembrane loop.[301,311] These single nucleotide substitutions cause amino acid changes that lead to constitutive activation of the TSHR in the absence of TSH.[312] It appears therefore that the TSHR is allosterically switched from an *off* state to an *on* state. Similarly, loss-of-function rather than gain-of-function mutations may also occur in the *TSHR* gene and cause hypothyroidism (see later). A small number of autonomous adenomas have mutations in the G stimulatory protein genes downstream of the TSH receptor that lead to a similar state of constitutive activation.[304] More recently, mutations have also been identified in the *EZH1* genes in toxic adenomas from patients who possess a TSH receptor activating mutation implying that a second hit may be necessary for full activation of the toxic adenoma phenotype.[313]



• **Fig. 12.20** Radioiodine (^{123}I) thyroid scan shows a hyperfunctioning hot nodule corresponding to physical examination findings with a faint outline of the remaining suppressed gland. In this unusual case, Graves disease developed a few months later after an oral contrast agent load. (From Soule J, Mayfield R. Graves' disease after 131I therapy for toxic nodule. *Thyroid*. 2001;11:91–92.)

Clinical Presentation

The toxic adenoma often presents as a nodule in a patient with a suppressed TSH; on ultrasound it appears as a single hypoechogenic nodule. A radioiodine thyroid scan shows a localized area of increased radioiodine accumulation (Fig. 12.20). This condition may occur at a younger age than toxic multinodular goiter and may be seen in patients in their 30s and 40s.

Frequently there is a history of a long-standing, slowly growing lump in the neck. It is unusual for adenomas to produce thyrotoxicosis until they have achieved a diameter of greater than 3 cm[303]; up to that point, patients have subclinical hyperthyroidism. The adenoma can undergo central necrosis and hemorrhage spontaneously, relieving the thyrotoxicosis, and the remainder of the thyroid may then resume its function. Calcification in the area of hemorrhage may take place and may be evident on sonogram examination. Such calcification is usually macroscopic and irregular and does not resemble the finely stippled calcification suggestive of papillary cancers. The peripheral clinical manifestations of a toxic adenoma are generally milder than those of Graves disease and are notable for the absence of infiltrative orbitopathy and myopathy, although cardiovascular manifestations may occur.

Laboratory Tests

The results of laboratory tests depend on the stage and function of the adenoma. At first, serum thyroid hormone concentrations are normal except for borderline suppression of the serum TSH. This, together with ultrasound examination to exclude multiple nodules, confirms the diagnosis. Later a thyroid scan may show localization of radioisotope in the palpated nodule, but this is not obvious until TSH secretion is suppressed. If the nodule continues to grow, frank hyperthyroidism is accompanied by elevation of serum thyroid hormone levels. Occasionally the serum free T_4 concentration is normal, and only the serum T_3 level is increased (T_3 thyrotoxicosis). Incidental thyroid carcinoma may rarely coexist within a gland exhibiting a hyperfunctioning adenoma. The incidence of carcinoma in these nodules is rare, but there are few clinical indicators of malignancy should it be present. Thus if the nodule is not treated surgically it is reasonable to follow closely if any clinical or radiographic features of malignancy are seen.[314]

Treatment

Although hyperfunctioning adenomas may eventually cause clinical hyperthyroidism, many do so slowly and others not at all.[303] Therefore treatment of asymptomatic patients with functional adenomas is decided on an individual basis. Clinically euthyroid subjects who wish to avoid both surgery and radioiodine can in theory be followed with annual assessments. However, suppression of TSH below normal (particularly to <0.1 mU/L) indicates hyperthyroidism, and therapy may be warranted. Two definitive therapies are available: radioiodine and surgery.

Radioiodine Therapy

In terms of the specificity of treatment, functioning thyroid nodules are candidates for radioiodine therapy. The radiation should in theory be directed almost exclusively to the diseased tissue. This is because TSH is suppressed and the normal thyroid tissue surrounding the nodule does not take up excess radioiodine. However, this suppression may be incomplete, and a significant fraction of patients receiving radioiodine develop thyroid failure. For the patient over age 18 with a nodule 5 cm in diameter or smaller, ^{131}I is an appropriate treatment if the risk of eventual hypothyroidism is acceptable to the patient. For such lesions, doses of radioiodine are given sufficient to result in the presence of 300 to 370 MBq (8–10 mCi) in the nodule at 24 hours based on the uptake.[303] Clearly higher doses of RAI result in enhanced success in treatment of the hyperthyroidism but also lead to an increased incidence of hypothyroidism.[316] Recombinant TSH, used to enhance the RAIU to avoid a large treatment dose in nontoxic multinodular goiters, is not appropriate here because it will direct radioiodine into surrounding normal thyroid tissue. The potential for hypothyroidism also indicates the need for prolonged follow-up.

Surgery

Toxic nodules are readily treated by surgical excision. A hemithyroidectomy may avoid the long-term development of hypothyroidism and with modern surgical procedures can be performed on an outpatient basis or even under local anesthesia. Surgical excision is always preferable in patients younger than 18 years of age to avoid the long-term consequences of irradiation, including effects on perinodular tissue. The toxic adenoma is not diffusely hypervascular, and consequently preoperative preparation with iodine is not required. In the patient with overt thyrotoxicosis, however, a normal metabolic state should be restored with an antithyroid drug before surgery.

Subclinical Hyperthyroidism

Definition

The availability of sensitive assays for TSH allowed the recognition of mild hyperthyroidism, in which there are no signs or symptoms of thyrotoxicosis but the serum TSH is subnormal despite normal serum free thyroid hormone concentrations. The term *subclinical* is somewhat of a misnomer because the condition is defined by biochemical characteristics, and it is preferable to use the term *mild thyroid dysfunction*.[317] Nonetheless, it is still not clear whether a patient is classified as having mild hyperthyroidism simply because our ability to detect physiologic evidence of excess thyroid hormone is less sensitive than our capacity to measure changes in TSH. This is further complicated by the fact that the hypothalamic-pituitary axis is sensitive to both serum free T_4 and T_3, whereas the peripheral tissues such as the heart primarily sense the free T_3 (see Chapter 11).[318,319] It is easy to assume, given the wide normal range for free thyroid hormone concentrations, that an individual with a low-normal free T_4 setpoint for TSH secretion would have a reduced TSH if that concentration were increased by 50% but could still remain within the normal range. In fact, in patients with primary hypothyroidism, small additional quantities of levothyroxine given to patients with normal TSH will decrease TSH below normal without a supranormal free T_4.[318] Thus the true lack of additional sensitive biomarkers in the periphery makes it difficult to determine whether all tissues are in fact hyperthyroid when the TSH is suppressed.

On the other hand, in the now classic studies in the Framingham population over 60 years of age, the cumulative incidence of atrial fibrillation over 10 years was 28% in patients with a serum TSH concentration of 0.1 mU/L or less, whereas it was only 11% in those with serum TSH concentrations falling between 0.1 and 0.4 mU/L. The latter was only slightly higher than that in the normal population.[10,11] Similar results have been obtained in one other prospective study.[11] Furthermore, heart failure is the leading cause of increased cardiovascular mortality rate in both overt and mild hyperthyroidism.[320] Taken together these data support the notion that a suppressed TSH is indicative of hyperthyroidism in the heart.

Bone density is another end point for such studies because it is well known that thyroid hormone causes a net resorption of cortical bone[321] and that lack of TSH may contribute to this phenomenon.[34] Several studies demonstrate lower bone density in patients with mild thyrotoxicosis, although others do not.[38,322] This is a subject of considerable interest because the condition is much more common than overt thyrotoxicosis (0.7% of the population in NHANES III) and has broad implications with respect to the cost of diagnosis, treatment, and follow-up.[323] In general, normalization of thyroid function in postmenopausal women with subclinical hyperthyroidism seems to improve bone density and certain aspects of cardiac function.[324]

Overall these data would generally favor treatment in the older population, but unfortunately there are still no large, long-term randomized studies to allow evidence-based conclusions as to the risk-benefit ratio.[325,326]

Diagnosis

The diagnosis of subclinical hyperthyroidism requires tests revealing several subnormal TSH concentration results spaced months apart in the presence of normal free T_3 and T_4 concentrations. Several studies show that suppressed TSH can normalize spontaneously over several years, particularly in patients without nodular goiter.[327,328] As with overt thyrotoxicosis, there are two sources of excess thyroid hormones, endogenous and exogenous. In a study of over 25,000 individuals attending health fairs in Colorado, 58% of those with a TSH less than 0.3 mU/L were receiving thyroid hormones.[329] When this is not being done intentionally for the treatment of persistent thyroid carcinoma, it is easily treated by more careful monitoring of the levothyroxine dosage using serum TSH concentrations. Endogenous subclinical thyrotoxicosis has the same causes as overt thyrotoxicosis. In the population over 60, multinodular goiter is a more likely cause of hyperthyroidism than it is in younger individuals especially in the United States.

Treatment

There are insufficient data to conclude that individuals with serum TSH concentrations greater than 0.1 mU/L due to hyperthyroidism will benefit from treatment.[134,325] However, treatment should be considered in those over age 65 with cardiac disease or osteoporosis. There is increasing support for treatment of individuals with persistently subnormal TSH concentrations less than 0.1 mU/L (with normal free thyroid hormones) who are over age 65 and for those younger than age 65 with cardiac disease or risk factors for cardiac disease or significant risk for osteoporosis.[134]

Identifying the cause of the hyperthyroidism allows assessment of the potential risks of treatment. At one extreme, the treatment of mild Graves disease with radioiodine usually causes hypothyroidism, whereas this typically does not occur in patients with multinodular toxic goiter. Thus, in an asymptomatic patient with mild Graves disease, watchful waiting for several years, awaiting a possible spontaneous remission, may be the best course of action[330]; if therapy is considered necessary, the chance of prolonged remission after a course of antithyroid drug therapy is good. On the other hand, patients with subclinical hyperthyroidism due to toxic nodular goiter or a solitary hyperfunctioning adenoma can often be treated with a single dose of radioactive iodine with a relatively low risk of subsequent hypothyroidism. Thus the threshold for treatment of such patients is lower. As always, the rationale for treatment, its risks, and its benefits should be carefully discussed with the patient, and one should be guided by common sense and not by the principle of simply treating an abnormal test result.[331]

Induced Hyperthyroidism

The incidence of hyperthyroidism corresponds in general to population iodine nutrition. Rates are lower in iodine-sufficient countries and higher in iodine-deficient countries mostly due to an excess of nodular thyroid disease in the elderly.[332–334] In China, the prevalence of overt and subclinical hyperthyroidism was likewise higher in iodine-deficient areas compared with iodine-sufficient areas.[335] Iodine fortification in previously iodine-deficient regions will result in an adequate iodine intake but is associated with a transient increase in the incidence of hyperthyroidism (mainly due to toxic nodular goiter); in the long run, however, the incidence of hyperthyroidism may be lower than the initial rate due to a decrease in the prevalence of thyroid nodules in the population.[99,333] Exposure to pharmacologic quantities of iodine may cause occasionally iodide-induced thyrotoxicosis (IIT), also called *Jod-Basedow,* more so in iodine-deficient areas than in iodine-sufficient areas.[336,337] Elderly subjects with nodular thyroid disease or latent Graves disease (i.e., with autonomous thyroid function and low TSH) are at risk. Such patients with underlying thyroid disease may have normal or even high thyroidal radioiodine uptake. In contrast, radioiodine uptake is low in IIT patients without preexistent thyroid disease, obviously caused by the cytotoxic effect of iodine excess on thyrocytes resulting in destructive thyroiditis. IIT usually resolves spontaneously in 6 months.

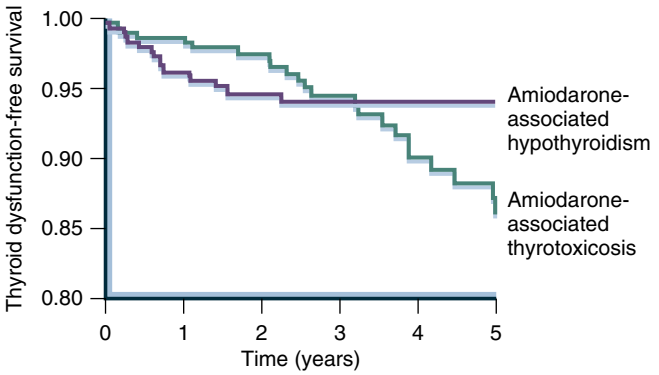
Many drugs contain huge quantities of iodine, such as expectorants, kelp tablets, and iodinated contrast agents. The risk for developing IIT is 0.3% in unselected people after coronary angiography in iodine-deficient areas.[338] Treatment of patients who had thyroid autonomy before coronary angiography (with 20 mg thiamazole and/or 900 mg sodium perchlorate, starting the day before angiography and continued for 2 weeks) was not effective in preventing IIT.[339,340] Close monitoring of high-risk patients rather than prophylaxis is thus recommended, with beta blockers if thyrotoxicosis occurs.[341]

Amiodarone-Induced Thyrotoxicosis

Amiodarone is a potent class III antiarrhythmic drug. It has hypothyroid-like effects such as bradycardia, reduction of myocardial oxygen consumption, lengthening of the cardiac action potential, and hypercholesterolemia[342]; these effects can be reversed by thyroid hormone. Its main metabolite desethylamiodarone acts as a competitive inhibitor of T_3 binding to thyroid hormone receptor α (TRα) and as a noncompetitive inhibitor of T_3 binding to TRβ.[343] One of the mechanisms of action of amiodarone might thus be induction of a hypothyroid-like condition in extrathyroidal tissues. The drug contains 37% iodine by weight and generates iodine excess; one 200-mg tablet/day releases 6 mg of iodide daily. Amiodarone has obligatory and facultative effects on the thyroid. Obligatory effects occur in everyone taking the drug. They consist of a transient rise of serum TSH up to 5 to 10 mU/L, caused by the Wolff-Chaikoff effect induced by the iodine overload, usually followed by an escape of the Wolff-Chaikoff effect and spontaneous normalization of TSH in 3 months. Serum total and free T_4 and reverse T_3 increase, and total and free T_3 decrease due to decreased metabolic clearance rate of T_4 and inhibition of liver deiodinase type 1. Facultative effects of amiodarone develop in a subset of patients. Amiodarone-induced hypothyroidisim (AIH) is caused by a failure to escape from the Wolff-Chaikoff effect. It occurs especially in women with TPO antibodies and is more prevalent in iodine-sufficient regions. Amiodarone-induced thyrotoxicosis (AIT), in contrast, is more prevalent in iodine-deficient regions. AIT type 1 is caused by iodine excess superimposed on preexistent thyroid disease such as Graves disease or nontoxic goiter. AIT type 2 is due to the cytotoxic effect of amiodarone on thyrocytes resulting in transient destructive thyroiditis.[344]

Epidemiology and Screening

In a prospective study with a median follow-up of 3.3 years in an area with an average daily iodine intake of 150 μg, 8% of patients taking amiodarone developed AIT and 6% AIH.[345] Incidence rates of AIT were 1.9 and of AIH 1.6 per 100 person-years (Fig. 12.21). It is striking that no new cases of AIH developed after 2 years on amiodarone therapy, whereas new cases of AIT



• **Fig. 12.21** Kaplan-Meier curve depicting the incidence of amiodarone-associated thyrotoxicosis and hypothyroidism. (Redrawn from Ahmed S, Van Gelder IC, Wiesfeld AC, et al. Determinants of outcome of amiodarone-associated dysfunction. *Clin Endocrinol*. 2011;75:388–394.)

continued to occur with continuation of amiodarone.[345,346] Thus one of every five to six patients taking amiodarone will develop overt thyroid dysfunction. It provides the rationale for the recommendation to measure TSH before starting amiodarone and at regular intervals during amiodarone.[347] Limitations of regular tests are, however, twofold. First, AIT type 2 often has a sudden, unpredictable onset. It may happen shortly after a previous blood test found a perfectly normal TSH, which may thus give rise to a false sense of security. Second, a suppressed TSH might just indicate subclinical hyperthyroidism, which spontaneously reverts to a normal TSH in 30% to 50% of cases despite continuation of amiodarone.[346,348] AIT occurs without preceding subclinical AIT in 64% of patients, and only 25% of patients with subclinical AIT progress to overt AIT.[348] AIT type 1 develops rather shortly after the start of amiodarone (median onset time is 3.5 months with just a single case occurring after 2.5 years), whereas the median onset time of AIT type 2 is 30 months.[349] Nowadays most AIT patients have AIT type 2. An iodine-free analog of amiodarone has been developed, called dronedarone, to get rid of the side effects caused by iodine excess. The pharmacologic properties of dronedarone, however, do not match those of amiodarone, and dronedarone has not displaced amiodarone in clinical practice.

Diagnosis

About half of AIT patients experience complaints, mainly palpitations but also weight loss and agitation.[345] Biochemical diagnosis of AIT is by suppressed TSH and elevated FT_4. FT_3 can be normal, however, due to the drug's inhibition of type I deiodinase, and cases of T_4 toxicosis occur. Although AIT type 1 currently appears to be much less common than type 2, identifying the type may still be of value because the treatments of types 1 and 2 differ. Diagnostic features are listed in Table 12.10. Type 2 patients usually have no goiter and no thyroid antibodies. Most useful is color flow Doppler sonography that provides a noninvasive, real-time assessment of thyroid vascularity.

Management

Specific ETA guidelines recommend treatment with antithyroid drugs in AIT type 1[344] (Fig. 12.22). The iodine-replete thyroid gland of AIT patients is less sensitive to methimazole, requiring rather high daily doses of 40 to 60 mg for longer periods than usual. To increase the response to methimazole, one may opt to coadminister sodium perchlorate (not available in the United States), which acutely inhibits further thyroidal iodine uptake. To restrict its toxic side effects, $NaClO_4$ should be used no longer than 4 to 6 weeks in a daily dose not exceeding 1 g (e.g., 500 mg twice daily). Amiodarone itself should be stopped, if feasible. Management is completely different in AIT type 2. Here the preferred drug is oral prednisone, in a daily dose of 30 mg.[344] Tapering down prednisone can be started when TSH has become normal, which takes about 3 months. In view of the self-limiting character of AIT type 2, it is possible to continue amiodarone in these patients.[350] Time to restoration of euthyroidism is little affected by continuation of amiodarone in type 2 patients.

Occasionally it is difficult to decide whether type 1 or type 2 AIT exists, and mixed forms do occur. One may opt for triple therapy (prednisone + methimazole + $NaClO_4$) under such circumstances, also when the response to instituted treatment is too slow. Some patients do not fare well, and guidelines recommend total thyroidectomy to be performed without delay in AIT patients with deterioration of cardiac function (reduced left ventricular ejection fraction) or severe underlying cardiac disease and in those patients whose thyrotoxicosis is unresponsive to medical therapies.[344]

TABLE 12.10 Differences Between Amiodarone-Induced Thyrotoxicosis Type 1 and Type 2

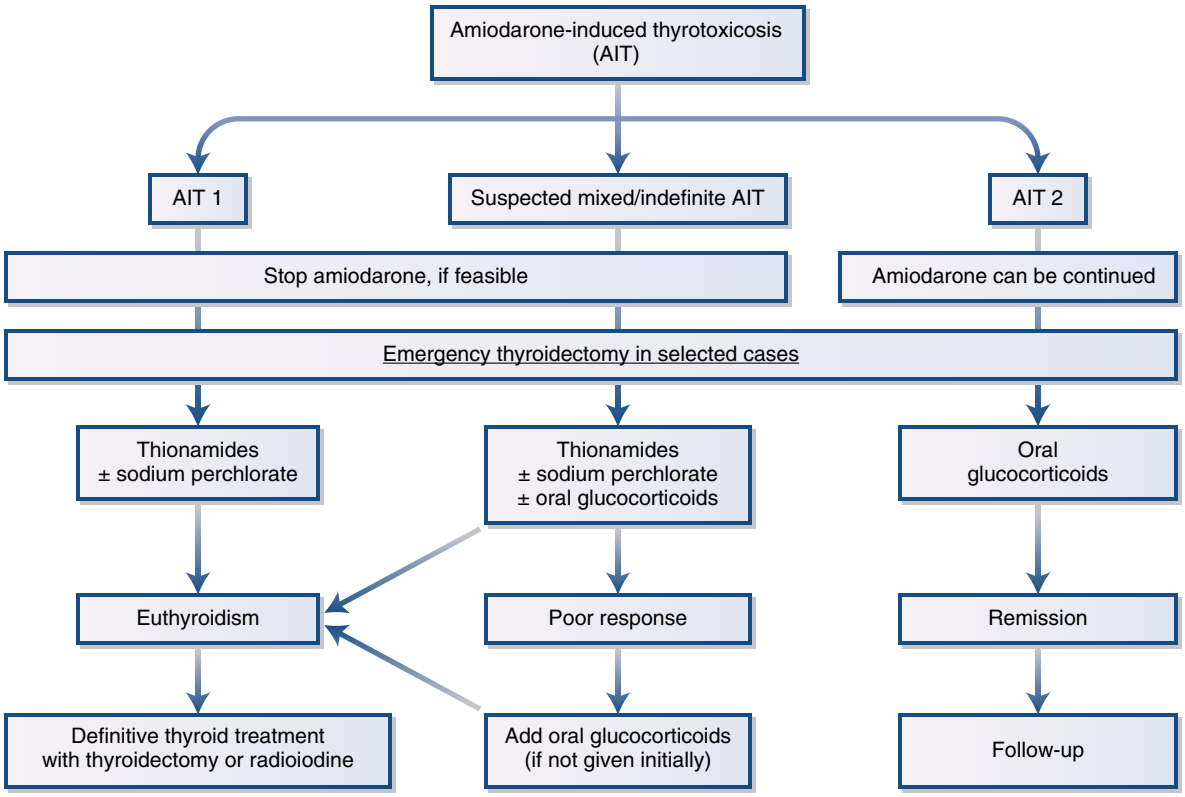
	AIT Type 1	AIT Type 2
Underlying thyroid abnormality	Yes	No
Onset after start amiodarone	Short (3 months)	Long (30 months)
Thyroid antibodies	Present in Graves	Usually absent
Color flow Doppler sonography	High vascularity	Low/absent vascularity
Thyroid radioiodine uptake	Low, normal, high	Suppressed
Preferred treatment	Antithyroid drugs	Oral prednisone
Amiodarone continuation	No	Possible
Spontaneous remission	No	Frequent
Subsequent hypothyroidism	No	Possible (17%)
Subsequent definitive treatment	Generally yes	No

AIT, Amiodarone-induced thyrotoxicosis.

Prognosis

Patients with AIT have more major adverse cardiovascular events than patients who remain euthyroid (31.6% vs. 10.7%, $p < 0.01$).[351] Mortality is also higher in AIT patients with severe left ventricle dysfunction (31% at ejection fraction <50% vs 14% at ejection fraction ≥50%).[352] Total thyroidectomy improves cardiac function and mortality, especially in those with ejection fraction below 40% preoperatively.[353]

Thyroid ablation is preferred before reintroduction of amiodarone after successful treatment of AIT type 1.[354] Continuation of



• **Fig. 12.22** Algorithm for the management of amiodarone-induced thyrotoxicosis. (Redrawn from Bartalena L, Bogazzi F, Chiovato L, et al. 2018 European Thyroid Association (ETA) guidelines for the management of amiodarone-associated thyroid dysfunction. *Eur Thyroid J.* 2018;7:55–66.)

amiodarone after cure of AIT type 2 is feasible; some patients will experience recurrent AIT (6–18%), which apparently is less severe and more easy to handle.[354,355] Permanent hypothyroidism develops in 17% of cured AIT type 2 patients, occurring at 10 months (range 6–24 months) after reaching euthyroidism.[350,356]

Hyperthyroidism Due to Thyrotropin Secretion

Pituitary Tumor

Excess TSH is an exceedingly rare cause of hyperthyroidism. However, pituitary thyrotroph tumors cause this condition and may present as a Graves-like syndrome with diffuse goiter and substantial thyrotoxicosis. Only 1% of pituitary adenomas are TSH producing and 25% may co-secrete growth hormone or prolactin.[357] Guidelines for the management of such patients have recently been released.[358] Laboratory studies demonstrating an inappropriately detectable or somewhat elevated TSH in the presence of elevated thyroid hormone levels must first be confirmed by eliminating assay artifacts. This condition is discussed in depth in Chapter 9 and must be differentiated from the rare patient who has resistance to thyroid hormone (RTH).[359–361]

Thyroid Hormone Resistance

The syndromes of RTH are now known to be caused by inherited mutations in both of the thyroid hormone receptor isoforms. RTHβ (due to mutations in the beta isoform) leads to a syndrome of inappropriate TSH secretion in the presence of elevated circulating levels of thyroid hormones because the beta isoform is responsible for the regulation of TSH. Similarly, TRβ is highly expressed in the liver, which is also functionally hypothyroid in RTHβ patients. However, in tissues that express primarily the TRα isoform, such as the heart and bone, tissue-specific hyperthyroidism may be present because these TRα expressing tissues sense the higher circulating thyroid hormone levels appropriately as high.[359–361] These patients may therefore present with a hyperthyroid appearance with tachycardia, nervousness, and goiter associated with an elevated free T_4 and may require tissue-specific treatment such as with β-adrenergic receptor blocking agents rather than antithyroid drugs (see Chapter 13 for a more extensive discussion of RTH). RTHβ should be suspected in patients who present with hyperthyroidism and inappropriate TSH secretion without an apparent pituitary adenoma. Furthermore, a careful family history should be taken as the disorder is inherited in an autosomal dominant fashion. In contrast to RTHβ, RTHα due to mutations in TRα isoform leads to a syndrome of tissue-specific hypothyroidism because the TRα isoform is not involved in the regulation of the HPT axis and thus thyroid hormone levels are not elevated.[362]

Tumor Chorionic Gonadotropin-Induced Hyperthyroidism

hCG exhibits specificity crossover with the TSHR (see earlier discussion under "Pregnancy and the Thyroid"). Thyroid hyperfunction may therefore accompany hydatidiform mole,

choriocarcinoma, or metastatic embryonal carcinoma of the testis.[258] Such neoplasms, particularly hydatidiform mole, elaborate differentially glycosylated hCG molecules that exhibit crossover specificity for binding to the TSHR and can induce variable degrees of thyroid overactivity.[232,363] Some patients have clinically overt thyrotoxicosis; however, clinical manifestations are usually not prominent, and goiter is absent or minimal. The free T_4 and free T_3 levels are increased, and TSH values are suppressed. The possibility of a molar pregnancy should be considered in a young woman with hyperthyroidism and amenorrhea because the appropriate therapy is evacuation of the uterus.

Transient Thyrotoxicosis

Overview

As mentioned at the outset of this chapter, transient thyrotoxicosis must be differentiated from the sustained hyperthyroidism of Graves disease and other causes of hyperthyroidism. Transient thyrotoxicosis is caused by thyroid cell breakdown, and the hyperthyroid symptoms are of abrupt onset and shorter duration. This process may be followed by recovery of thyroid function or the development of transient or permanent thyroid failure. The discussion in this chapter focuses on thyroiditis as the most common cause of transient thyrotoxicosis, and this disorder is covered more completely in Chapter 13 because Hashimoto disease most commonly causes hypothyroidism after the initial phase of transient hyperthyroidism. Unfortunately transient thyrotoxicosis continues to have a confusing nomenclature, which can be clarified as follows:

Autoimmune thyroiditis: In the autoimmune forms (Hashimoto thyroiditis) there are typically no local symptoms of thyroid inflammation, leading to the terms *silent* or *painless thyroiditis*, also referred to as *lymphocytic thyroiditis* or *hashitoxicosis.* This condition may uncommonly present with thyroid tenderness if the thyroid has expanded rapidly, stretching the capsule.

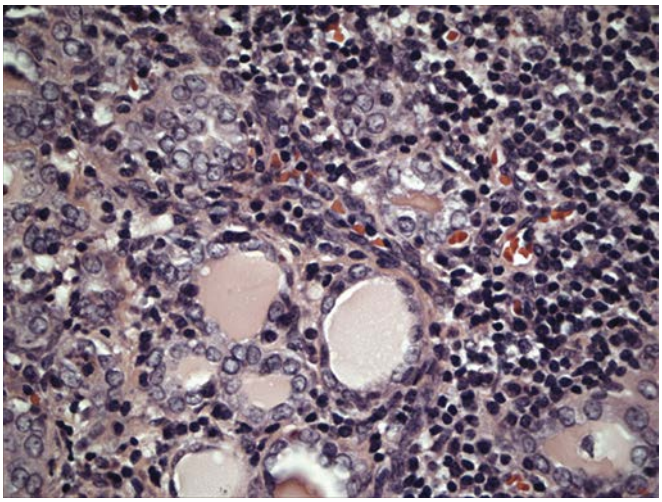
Viral thyroiditis: In what is thought to be postviral thyroiditis (also termed *subacute, de Quervain*, or *granulomatous thyroiditis*), thyroid tenderness may be the most prominent symptom, and thyrotoxicosis is rare and typically self-limited, although this form may rarely also be painless.

Acute thyroiditis: Acute thyroiditis due to bacterial or fungal infections is only rarely accompanied by thyrotoxicosis, and the local symptoms predominate (see Chapter 13).

Drug-induced thyroiditis: Thyroiditis may also be drug induced, the principal offenders being amiodarone and lithium. Some of the new small molecule kinase inhibitors (such as sunitinib) may also cause this form of thyroiditis, resulting eventually in hypothyroidism.[364,365] In addition, the introduction of immunotherapy for cancer treatment, including CTLA4 inhibitors and PD1 inhibitors, has dramatically increased the incidence of several endocrine toxicities, including transient hyperthyroidism.[366]

Transient Thyrotoxicosis Due to Autoimmune (Hashimoto) Thyroiditis

As described earlier, Hashimoto disease causes two different thyrotoxicosis-associated transient syndromes. The most common is the painless form in which the symptoms of thyrotoxicosis are usually mild and predominate; the much more uncommon form has a painful presentation probably secondary to a more acute onset. Histopathologic examination in such patients with thyroiditis shows diffuse or local lymphocytic infiltration, varying degrees of fibrosis, and disruption of the follicular architecture (Fig. 12.23).



• **Fig. 12.23** Lymphocytic thyroiditis in a patient with transient thyrotoxicosis (painless thyroiditis) secondary to autoimmune (Hashimoto) thyroiditis. Notice the diffuse lymphocytic invasion of the tissue, including the follicular epithelium, and the loss of follicles. Multinucleated giant cells may also be seen in the follicular lumen. (Courtesy Dr. Vania Nosé, Brigham and Women's Hospital, Boston, MA.)

Transient Thyrotoxicosis From Painless Autoimmune Thyroiditis

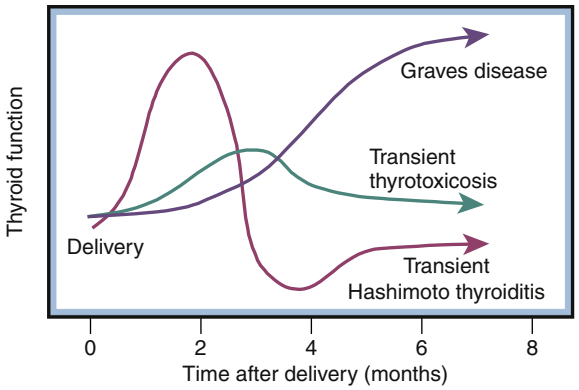
Painless autoimmune thyroiditis may occur postpartum or spontaneously. Postpartum thyroiditis is the most common example; its pathophysiology, postpartum enhancement of thyroid-directed autoimmunity (Hashimoto disease), is analogous to the postpartum exacerbation of Graves disease (see "Graves Disease in the Postpartum Period"). The incidence of postpartum thyroiditis varies but may occur in as many as 10% of women and in more than 30% of those with positive TPOAb and even a larger fraction in patients with type 1 diabetes mellitus.[245,289] In women found to be TPOAb positive prenatally, postpartum assessment of thyroid function is recommended at 3, 6, and 12 months. Thyrotoxicosis from spontaneous autoimmune thyroiditis has all the same characteristics as postpartum thyroiditis and is seen in patients early in their development of classic Hashimoto disease and before the onset of hypothyroidism.

Transient Thyrotoxicosis from Painful Autoimmune Thyroiditis

Although some patients may present with local thyroid tenderness, this occurrence is uncommon. Such tender episodes, which may be unilateral, may recur until the thyroid gland is completely destroyed by the disease process. Only rarely does the pain persist, sometimes requiring surgical intervention.

Clinical Presentation of Transient Autoimmune Thyrotoxicosis

More than 75% of patients are women who present with the acute onset of symptoms of thyrotoxicosis, usually nervousness, palpitations, and irritability; they can often pinpoint the time of recent onset. In the postpartum syndrome, symptoms present 4 to 12 months after delivery but may be mild and overlooked in the myriad of events involved in the care of the newborn.[288] After 1 to



• **Fig. 12.24** The postpartum thyroid syndromes. These potential patterns of thyroid dysfunction may be seen in the postpartum period.

2 months the thyrotoxic symptoms fade but are often replaced by those suggesting hypothyroidism (Fig. 12.24).

In a significant number of postpartum patients the thyrotoxic phase is too mild to be noticed, and the patient presents somewhat later after delivery with hypothyroid symptoms. The physical examination shows mild signs of thyrotoxicosis, tachycardia being the most prominent, without the specific eye signs or dermopathy associated with Graves disease. The thyroid gland is normal in size but may be firm if the Hashimoto disease is chronic.

Diagnosis

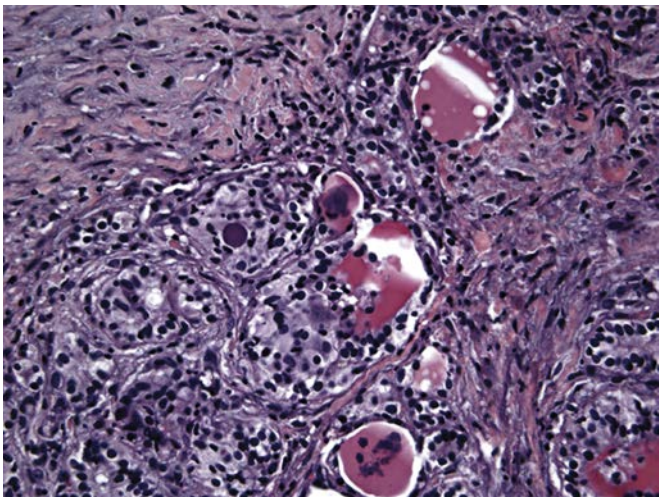
Thyrotoxicosis is usually mild and is reflected in the degree of suppression of the serum TSH level and elevation of the serum free T_4. Significant elevation of the TPOAb is typical. Systemic manifestations of inflammation are lacking, and the erythrocyte sedimentation rate is normal or nearly normal, but the ultrasound may indicate the heterogeneity of an inflamed gland. If true hyperthyroidism cannot be eliminated as a diagnosis on clinical grounds, TRAb should be measured or an RAIU test should be performed unless the patient is nursing. The classic decreased RAIU is due partly to feedback suppression of TSH secretion but also to thyroid follicular cell destruction. The tendency of the disorder to pass through a hypothyroid phase is not surprising in view of the extensive depletion of Tg, which is processed to T_4 and not replaced by the dysfunctional cells.

Natural History

The duration of the thyrotoxic phase, typically not severe enough to require treatment, averages about 1 to 2 months. About one-half of the patients return to a euthyroid phase and remain well in the short term. In the remaining half, a hypothyroid phase may follow and may last from 2 to 9 months. In most, there is eventual restoration of euthyroidism, but some develop permanent hypothyroidism years later.[367,368] About one-third retain a goiter, usually with persistence of thyroid autoantibodies in the serum. The opposite sequela, recurrence of thyrotoxicosis, may also occur months or years after restoration of a euthyroid state or particularly after pregnancy.

Treatment

The thyrotoxic phase may require alleviation of the peripheral manifestations through the use of beta blockers. Prednisone (20–40 mg/day) may decrease the duration of the thyrotoxic phase but is typically not needed except when the painful form of the disease is present. If mild and brief, the hypothyroid phase may also not require treatment. When treatment with levothyroxine is required, it should be withdrawn slowly approximately 6 months later because the hypothyroidism is often not permanent.



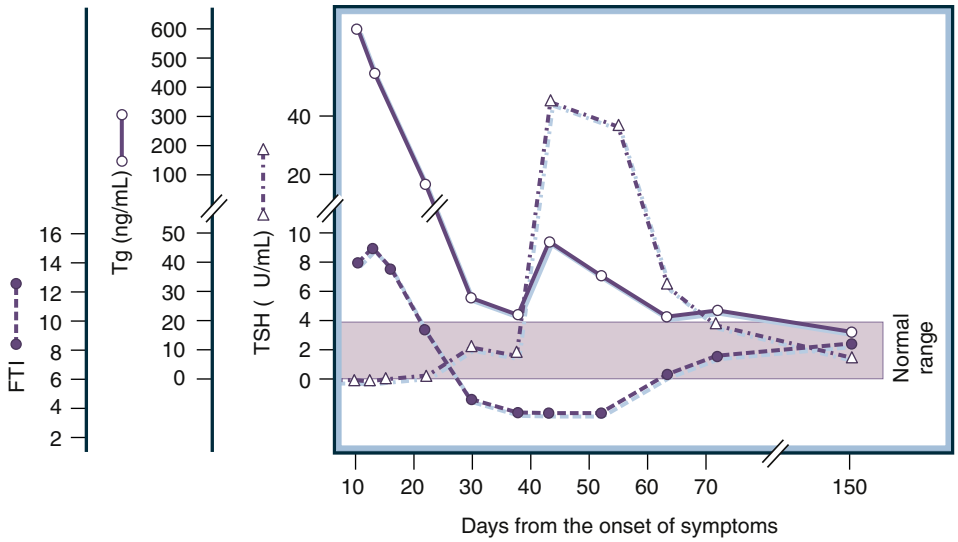
• **Fig. 12.25** Subacute (viral or postviral) thyroiditis. Diffuse neutrophilic invasion with active destruction of follicles and a multinucleated giant cell. Fibrosis and near-complete loss of follicles have occurred. (Courtesy Dr. Vania Nose, Brigham and Women's Hospital, Boston, MA.)

Subacute Thyroiditis

Subacute thyroiditis (also termed *granulomatous, giant cell*, or *de Quervain thyroiditis*) is thought to be caused directly or indirectly by a viral infection of the thyroid gland and often follows an upper respiratory illness. A tendency to appear in the spring in the northern latitudes has been noted, and again it predominates in the female. The mumps virus has been implicated in some cases; coxsackievirus, influenza virus, echovirus, and adenoviruses may also be etiologic agents. Positive TPOAb are present transiently during the active phase of the disease, although some patients may retain evidence of thyroid autoimmunity for many years. A small number of patients eventually develop AITD.[369] Subacute thyroiditis is uncommon, but mild cases may be mistakenly diagnosed as pharyngitis.

Pathology

The histopathologic changes are different from those in Hashimoto disease. The lesions are patchy in distribution and vary in their stage of development from area to area. Affected follicles are infiltrated predominantly with mononuclear cells and show disruption of epithelium, partial or complete loss of colloid, and fragmentation and duplication of the basement membrane (Fig. 12.25). To this extent, the histopathologic appearance may resemble that in Hashimoto disease. A characteristic feature is the well-developed follicular lesion that consists of a central core of colloid surrounded by the multinucleated giant cells (MNGCs), from which stems the designation *giant cell thyroiditis*. Colloid may be found in the interstitium or within the giant cells. The follicular changes progress to form granulomas. Interfollicular fibrosis and an interstitial inflammatory reaction are present to varying degrees. When the disease subsides, an essentially normal histologic appearance is restored.



• **Fig. 12.26** Thyroid function in a patient during the course of subacute (viral or postviral) thyroiditis. During the thyrotoxic phase (days 10–20), the serum thyroglobulin (Tg) concentration was greatly elevated, the free thyroxine index (FTI) was high, and the thyroid-stimulating hormone (TSH) was suppressed; the erythrocyte sedimentation rate was 86 mm/hour, and the thyroidal radioactive iodine uptake (RAIU) was 2%. The Tg level and the FTI declined in parallel. During the phase of hypothyroidism (days 30–63), when the FTI was below normal, a modest transient increase in serum Tg occurred in parallel with the increase in serum TSH. All parameters of thyroid function were normal by day 150, 5 months after the onset of symptoms. (From DeGroot LJ, Larsen PR, Hennemann G. Acute and subacute thyroiditis. In: DeGroot LJ, Larsen PR, Hennemann G, eds. *The Thyroid and Its Diseases*. 6th ed. New York: Churchill Livingstone; 1996:705.)

Pathophysiology

Apoptosis of follicular epithelium and loss of follicular integrity are the primary events in the pathophysiology. Tg, T_4, and iodinated Tg fragments are released into the circulation, often in quantities sufficient to elevate not just the serum Tg level but also the serum free T_4 level, producing clinical thyrotoxicosis and suppressing TSH secretion. As a result, the RAIU decreases to low levels, and hormone synthesis ceases. Later in the disease, when stores of preformed hormone are depleted, serum T_4 and T_3 concentrations decline, sometimes into the hypothyroid range, and the serum TSH level rises, often to elevated values exactly as occurs in silent thyroiditis (Fig. 12.26). As the disease becomes inactive, the RAIU may be greater than normal for a time as hormone stores are repleted. Ultimately, when hormone secretion resumes, serum T_4 and T_3 concentrations rise, and serum TSH concentration decreases to normal values.

Clinical Picture

The characteristic feature is the gradual or sudden appearance of pain in the region of the thyroid gland with or without fever. The pain, which is aggravated by turning the head or swallowing, characteristically radiates to the ear, jaw, or occiput and may mimic disorders arising in these areas. The absence of pain does not exclude the diagnosis, because biopsy-proven painless subacute thyroiditis occurs, but it must be distinguished from acute autoimmune thyroiditis. Hoarseness and dysphagia may be present; patients may complain of palpitation, nervousness, and lassitude. The latter symptoms can be extreme, considering the local nature of the disease, and suggest a systemic component. Although acute manifestations are present in severe cases, in milder disease symptoms may be present for months yet are often overlooked.

On palpation at least part of the thyroid is slightly to moderately enlarged, firm, often nodular, and usually exquisitely tender, one lobe frequently being more severely affected than the other. Indeed the symptoms may be truly unilateral. The overlying skin may be warm and erythematous. Occasionally the locus of maximal involvement migrates over the course of a few weeks to other parts of the gland. The disease usually subsides within a few months, leaving no residual deficiency of thyroid function in 90% of patients. In rare patients the disease smolders, with repeated exacerbations over many months, hypothyroidism sometimes being the final result.

Diagnosis

The laboratory findings vary with the phase of the disease. During the active phase, the erythrocyte sedimentation rate is increased, often to a remarkable extent (>100 mm/hour). Indeed a diagnosis of active subacute thyroiditis is hardly tenable when the sedimentation rate is normal. The white blood cell count is normal or, at most, moderately increased. The serum Tg level is characteristically high, in keeping with the degree of thyroid destruction.

Subacute thyroiditis must be differentiated from acute hemorrhagic degeneration in a preexisting thyroid nodule, Hashimoto disease with painful recurrence (see earlier), acute pyogenic thyroiditis or fungal infection, and rarely thyroid malignancy with painful nodules. Acute painful exacerbations of Hashimoto thyroiditis may be difficult to distinguish from subacute thyroiditis. Lack of elevation of the erythrocyte sedimentation rate and high titers of thyroid autoantibodies strongly suggest the former. Acute pyogenic thyroiditis is distinguished by the presence of a septic focus elsewhere, by a greater inflammatory reaction in the tissues adjacent to the thyroid, and by much greater leukocytic and febrile responses (see Chapter 13). The RAIU and thyroid function are

usually preserved in acute pyogenic thyroiditis. Rarely, widespread infiltrating cancer of the thyroid can present with a clinical and laboratory picture almost indistinguishable from that of subacute thyroiditis. Ultrasound and fine-needle aspiration should be performed if this is a consideration.

Treatment

In mild cases, aspirin or nonsteroidal antiinflammatory drugs or cyclooxygenase 2 (COX2) inhibitors may control the symptoms. With more severe pain, glucocorticoids (e.g., prednisone up to 40 mg/day) are the only solution for the extreme discomfort. This drug may be required for several months and should then be withdrawn gradually. If the TSH is not suppressed, TSH-suppressive therapy with levothyroxine may decrease the size of the gland, relieving the pressure on the thyroid capsule. TSH is needed for thyroid cell regeneration, so such therapy should be decreased as the symptoms subside.

Drug-Associated Thyroiditis

Thyroiditis is an uncommon complication of pharmacotherapy. Amiodarone is an important exception and was discussed earlier. Most of the thyroiditis associated with various therapeutic agents appears to be due to drug-induced exacerbation of underlying autoimmune disease. This effect is understandable with agents that are specifically administered to modify the immune system. They include IL2, interferon alpha, granulocyte/macrophage colony-stimulating factor (GM-CSF), and the newer immune therapy modulators, all of which can precipitate silent thyroiditis.[108,366,370,371] This has also been described with lithium and the GnRH agonist leuprolide, but the pathophysiologic mechanism is obscure.[372–374]

Thyroiditis has been found in association with multitargeting kinase inhibitors such as sunitinib and sorafenib given for a variety of tumors, including gastrointestinal stromal tumors, hepatocellular carcinoma, and renal cell carcinoma.[365,375] This may present as subacute thyroiditis with a suppressed TSH as the major manifestation of the early phase but then progress to destruction of the gland through an unclear mechanism. Although imatinib has been associated with an increase in levothyroxine requirements in hypothyroid patients (analogous to the effects of phenytoin, carbamazepine, and rifampin), those changes are independent of thyroid function.[376]

Other Causes of Thyrotoxicosis With a Low Radioiodine Uptake

In addition to silent and subacute thyroiditis, several other entities should be considered in the patient with thyrotoxicosis in whom the thyroid gland is either not palpable or not enlarged and who has biochemical findings of thyrotoxicosis accompanied by a low RAIU.

Thyrotoxicosis Factitia

Thyrotoxicosis that arises from the ingestion, usually chronic, of excessive quantities of thyroid hormone usually occurs in individuals with a background of underlying psychiatric disease, especially in paramedical personnel who have access to thyroid hormone or in patients for whom thyroid hormone medication has been prescribed in the past. Generally the patient is aware of taking thyroid hormone but may adamantly deny it. In other instances, large doses of thyroid hormone or other thyroactive material may be given without the knowledge of the patient, usually as part of a regimen for weight reduction. Some "natural" products for weight reduction stated not to contain thyroid hormone nonetheless do. Symptoms are typical of thyrotoxicosis and may be severe.

In the absence of preexisting disease of the thyroid, the diagnosis is made from the combination of typical thyrotoxic manifestations, together with thyroid atrophy and hypofunction. Infiltrative ophthalmopathy never occurs, but lid lag, stare, and other thyrotoxic eye signs may be present; TSH levels are suppressed. Serum T_4 concentrations are increased unless the patient is taking T_3, in which case they will be subnormal. Serum T_3 concentrations are increased in either case. Hypofunction of the thyroid gland is evidenced by the subnormal values of RAIU. The presence of low, rather than elevated, values of serum Tg is a clear indication that the thyrotoxicosis results from exogenous hormone rather than thyroid hyperfunction.

This disorder may be confused with other varieties of thyrotoxicosis associated with a subnormal RAIU and absence of goiter, including silent thyroiditis, ectopic thyroid tissue, and hyperfunctioning metastatic follicular carcinoma. Evidence for the two latter disorders can be obtained by demonstration of the ectopic focus or foci by external radioiodine scanning or the presence of normal to elevated serum Tg concentrations. Differentiation from silent thyroiditis may be difficult. The presence of TPOAb points to painless chronic autoimmune thyroiditis, whereas a firm thyroid and brief history suggest the painless variant of subacute thyroiditis. Treatment of thyrotoxicosis factitia consists of withdrawing the offending medication. Psychiatric consultation is often required.

Hamburger Thyrotoxicosis

An unusual form of exogenous thyrotoxicosis occurred in the midwestern portion of the United States in 1984 and 1985. The source was the inclusion of large quantities of bovine thyroid in ground beef preparations.[377] When the slaughtering practices were changed, this condition disappeared. Such a possibility, although remote, should be considered, especially if one is confronted with epidemic exogenous thyrotoxicosis.

Thyrotoxicosis Due to Extrathyroidal Tissue

Struma Ovarii

Thyroid tissue may be present in 5% to 10% of ovarian teratomas, and occasionally such foci are hyperfunctional.[378,379] About 5% to 10% of these tumors are bilateral. Although thyrotoxicosis is unusual, it may occur in as many as 8% to 10% of patients. Rarely, males with germ cell tumors may also develop hCG-induced hyperthyroidism.[380]

Clinical Presentation

Patients present with variable degrees of thyrotoxicosis but without goiter and generally have lower abdominal symptoms such as pain or a mass. Rarely ascites is present. Laboratory studies show reduced TSH and increased free T_4 of a variable degree, but the RAIU is low. The Tg may be elevated, particularly if the teratoma is malignant and has metastasized to the peritoneum. Abdominal CT scan or MRI shows a multilocular ovarian mass or masses.[381] Rarely a struma ovarii is accompanied by Graves disease.[382]

Treatment

The patient should be rendered euthyroid if thyrotoxicosis is significant, followed by removal of the involved ovary or ovaries. Therapeutic radioiodine will be required for metastatic disease after ablation of the normal thyroid gland.

Thyrotoxicosis Due to Metastatic Thyroid Carcinoma

In general, thyroid carcinomas are made up of poorly functioning tissue. On occasion, follicular thyroid carcinomas will have sufficient function when combined with the total mass of the metastases to result in an elevation in serum free T_4 or T_3 and may even be seen in Graves disease with TRAbs activating the tissue.[383] Typically such a course is a complication of a previously diagnosed lesion.[384] The symptoms of thyrotoxicosis will vary and the metastatic disease is usually obvious from radiologic studies. On occasion, the presentation may be confusing if the patient is receiving TSH-suppressive therapy, and diagnosis will require its discontinuation. In spite of that, TSH will remain suppressed and the serum free T_4 is elevated. Treatment of this condition is typical for that of thyroid carcinoma and is described in Chapter 14. In patients with thyrotoxicosis due to metastatic tumor, serum Tg is quite elevated, indicating that the thyrotoxicosis is caused by thyroidal tissue that is not located in the neck. An RAIU during the thyrotoxic phase will show no neck uptake due to TSH suppression even if the thyroid is still present.

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The complete list of references is available online at ExpertConsult.com.

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13

Hypothyroidism and Thyroiditis

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CHAPTER OUTLINE

KEY POINTS

- Autoimmunity is responsible for over 90% of noniatrogenic hypothyroidism in iodine-sufficient areas.
- A variety of genetic factors contribute to susceptibility in autoimmune thyroiditis, but epidemiologic data suggest a strong influence of environmental factors to explain the recent increase in prevalence.
- The risk of progression from *subclinical* to *overt* hypothyroidism is most closely related to the magnitude of serum thyrotropin (thyroid-stimulating hormone [TSH]) elevation and the presence of antithyroid peroxidase (TPO) antibodies.
- In some patients with autoimmune hypothyroidism, a transient exacerbation of thyroiditis or a fluctuation in the balance between TSH receptor-blocking and receptor-stimulating autoantibodies may result in episodes of thyrotoxicosis.
- Hypothyroidism due to direct thyroidal inflammation or activation of autoimmune destruction has been associated with a number of drugs, including tyrosine kinase inhibitors (TKIs).
- The quantity of levothyroxine required to normalize TSH in an athyreotic patient results in a slightly higher serum free thyroxine (T_4) concentration than is present in normal individuals.
- The current approach to thyroid replacement using levothyroxine alone, although not a perfect replication of normal physiology, is satisfactory for virtually all patients.
- Levothyroxine requirements are increased in malabsorption due to bowel diseases and impaired gastric acid secretion or adsorption of levothyroxine to coadministered medications.
- Athyreotic patients who are planning a pregnancy should be advised to increase the dose of levothyroxine by around 30% as soon as the diagnosis is confirmed; the increased dose requirement persists throughout pregnancy, but dosage can return to normal within a few weeks after delivery.

Hypothyroidism

Reduced production of thyroid hormone is the central feature of the clinical state termed *hypothyroidism*.[1,2] Permanent loss or destruction of the thyroid through processes such as autoimmune destruction, referred to as Hashimoto disease,[3] or irradiation injury is described as *primary hypothyroidism* (Table 13.1). Hypothyroidism due to transient or progressive impairment of hormone biosynthesis is typically associated with compensatory thyroid enlargement. Central or secondary hypothyroidism due to insufficient stimulation of a normal gland is the result of hypothalamic or pituitary disease or defects in the TSH molecule.[4] Transient or temporary hypothyroidism can be observed as a phase of subacute thyroiditis.[5] Primary hypothyroidism is the cause in approximately 99% of cases of hypothyroidism, with less than 1% being due to TSH deficiency or other causes. Central hypothyroidism is discussed in Chapter 11.

Reduced action of thyroid hormone at the tissue level, despite normal or increased thyroid hormone production from the thyroid gland, can also be associated with clinical hypothyroidism. Conditions associated with reduced thyroid hormone action are rare and include abnormalities of thyroid hormone metabolism and defects in nuclear signaling.[6] Consumptive hypothyroidism, identified in an increasing number of clinical settings, is the result of accelerated inactivation of thyroid hormone by the type 3 iodothyronine deiodinase (D3).[7] Defects of activation of the prohormone, T_4, to the active form, triiodothyronine (T_3), have also been identified.[8] Polymorphisms in genes regulating thyroid hormone production and activation may influence thyroid hormone action in some tissues.[9,10] Resistance to thyroid hormone (RTH), the result of defects in the thyroid hormone nuclear receptor (TR) or nuclear cofactors, is associated with elevated circulating levels of thyroid hormone. Some tissues, depending on the level of expression of the mutant receptor and other forms of local compensation, have evidence of reduced thyroid hormone action.[11]

Estimates of the incidence of hypothyroidism vary depending on the population studied.[12,13] In the United States, 0.3% have overt hypothyroidism, defined as an elevated serum TSH concentration and reduced free thyroxine concentration (FT_4), and 4.3% have what has been described as subclinical or mild

TABLE 13.1 Causes of Hypothyroidism

Primary Hypothyroidism

Acquired

Hashimoto thyroiditis
Iodine deficiency (endemic goiter)
Drugs blocking synthesis or release of T_4 (e.g., lithium, ethionamide, sulfonamides, iodide)
Drug-induced thyroid destruction (e.g., interferon alpha, interleukin 2, tyrosine kinase inhibitors, blockers of CTLA4 or PD1)
Amiodarone (reversible or permanent)
Goitrogens in foodstuffs or as endemic substances or pollutants
Thyroid infiltration (amyloidosis, hemochromatosis, sarcoidosis, Riedel struma, cystinosis, scleroderma)
Postablative thyroiditis due to ^{131}I, surgery, or therapeutic irradiation for nonthyroidal malignancy
Transient hypothyroidism following painless thyroiditis (including postpartum) or painful subacute thyroiditis

Congenital

Iodide transport or utilization defect (NIS or pendrin mutations)
Iodotyrosine dehalogenase deficiency
Organification disorders (TPO deficiency or dysfunction)
Defects in thyroglobulin synthesis or processing
Thyroid agenesis or dysplasia
TSH receptor defects
Thyroidal G_s protein abnormalities (pseudohypoparathyroidism type 1a)
Idiopathic TSH unresponsiveness

Consumptive Hypothyroidism

Rapid destruction of thyroid hormone due to D3 expression in large hemangiomas or hemangioendotheliomas

Defects of Thyroxine to Triiodothyronine Conversion

Selenocysteine insertion sequence–binding protein 2 (SECISBP2) defect

Central Hypothyroidism

Acquired

Pituitary origin (secondary)
Hypothalamic disorders (tertiary)
Bexarotene (retinoid X receptor agonist)
Dopamine or severe illness

Congenital

TSH deficiency or structural abnormality
TSH receptor defect

Resistance to Thyroid Hormone

Generalized
"Pituitary" dominant

NIS, Sodium-iodide symporter; *TPO,* thyroid peroxidase; *TSH,* thyroid-stimulating hormone (thyrotropin).

hypothyroidism.[13] Although a number of clinical manifestations have been associated with this early or mild phase of hypothyroidism, we will use the term *subclinical* to describe this group, as is used in most clinical studies. Subclinical hypothyroidism is defined as an elevated serum TSH level with a normal serum FT_4 concentration.[14,15] Subclinical hypothyroidism can progress to overt hypothyroidism and be associated with manifestations that, in some patients, may benefit from treatment.[16] The incidence of hypothyroidism is higher among women, in the elderly, and in some racial and ethnic groups.[17] Neonatal screening programs for congenital hypothyroidism identify hypothyroidism (almost all primary) in almost 1 in 3000 newborns.[18]

Clinical Presentation

Hypothyroidism can affect all organ systems, and these manifestations are largely independent of the underlying disorder but are a function of the degree of hormone deficiency. The following sections discuss the pathophysiology of each organ system at various levels of thyroid hormone deficiency, from mild to severe. The term *myxedema,* formerly used as a synonym for hypothyroidism, refers to the appearance of the skin and subcutaneous tissues in the patient in a severely hypothyroid state (Fig. 13.1). Hypothyroidism of this severity is rarely seen today, and the term should be reserved to describe the physical signs.

Skin and Appendages

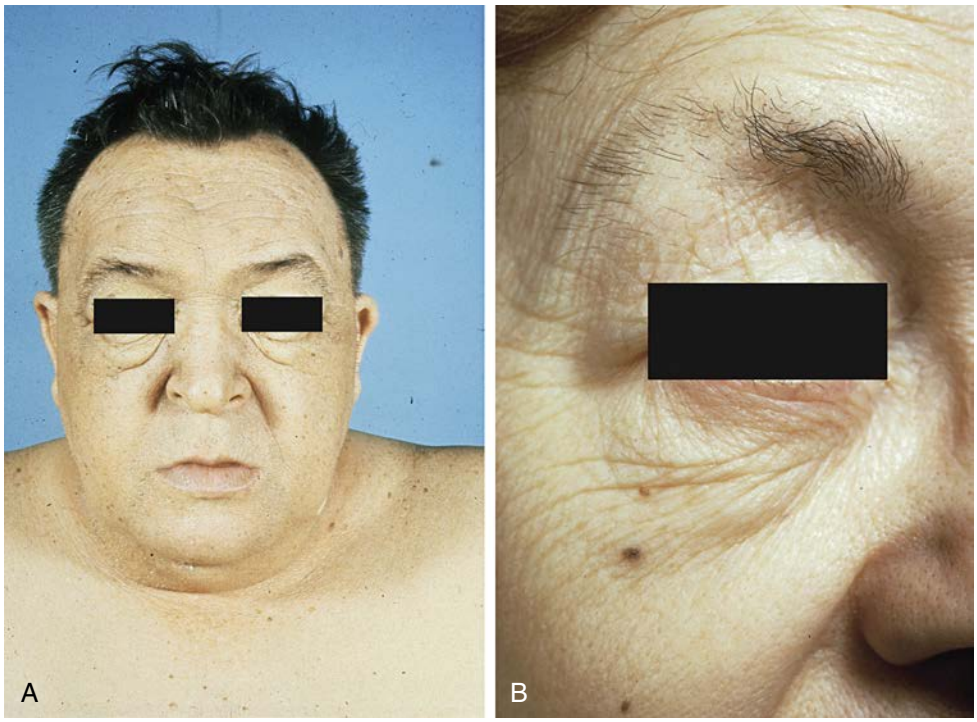
Hypothyroidism causes an accumulation of hyaluronic acid that alters the composition of the ground substance in the dermis and other tissues.[19,20] This material is hygroscopic, producing the mucinous edema that is responsible for the thickened features and puffy appearance (myxedema) with full-blown hypothyroidism. Myxedematous tissue is characteristically boggy and nonpitting and is apparent around the eyes, on the dorsa of the hands and feet, and in the supraclavicular fossae (see Fig. 13.1). It causes enlargement of the tongue and thickening of the pharyngeal and laryngeal mucous membranes.

A clinically similar deposit may occur in patients with Graves disease, usually over the pretibial area (infiltrative dermopathy or pretibial myxedema), but it can be differentiated histologically.[21] In addition to having a puffy appearance, the skin in hypothyroidism is pale and cool as a result of cutaneous vasoconstriction. Anemia may contribute to the pallor; hypercarotenemia gives the skin a yellow tint but does not cause scleral icterus (see Fig. 13.1). The secretions of the sweat glands and sebaceous glands are reduced, leading to dryness and coarseness of the skin, which in extreme cases may resemble that seen in patients with ichthyosis.

Wounds of the skin tend to heal slowly. Easy bruising is due to an increase in capillary fragility. Head and body hair is dry and brittle, lacks luster, and tends to fall out. Hair may be lost from the temporal aspects of the eyebrows, although this is not specific for hypothyroidism (see Fig. 13.1B). Growth of hair is retarded so that haircuts and shaves are required less often. The nails are brittle and grow slowly. Topical T_3 has been shown to accelerate wound healing and stimulate hair growth in a euthyroid mouse model, demonstrating a role for thyroid hormone in these processes.[20]

Histopathologic examination of the skin reveals hyperkeratosis with plugging of hair follicles and sweat glands. The dermis is edematous, and the connective tissue fibers are separated by an increased amount of metachromatically staining, periodic acid–Schiff (PAS)–positive mucinous material. This material consists of protein complexed with two mucopolysaccharides: hyaluronic acid and chondroitin sulfate B. The hygroscopic glycosaminoglycans are mobilized early during treatment with thyroid hormone, leading to an increase in urinary excretion of nitrogen and hexosamine as well as tissue water.[19]

Patients with hypothyroidism due to Hashimoto thyroiditis may also have skin lesions with loss of pigmentation characteristic of the autoimmune skin condition vitiligo. This feature is not a



• **Fig. 13.1** (A and B) Typical appearance with moderately severe primary hypothyroidism or myxedema. Note dry skin and sallow complexion; the absence of scleral pigmentation differentiates the carotenemia from jaundice. Both individuals demonstrate periorbital myxedema. (B) This patient illustrates the loss of the lateral aspect of the eyebrow, sometimes termed *Queen Anne sign.* That finding is not unusual in the age group that is commonly affected by severe hypothyroidism and should not be considered to be a specific sign of the condition.

manifestation of reduced thyroid hormone action but reflects the common association of autoimmune endocrine disease and this skin condition, which is recognized as a component of autoimmune polyendocrine syndromes.[22,23]

Cardiovascular System

The cardiac output at rest is decreased because of reduction in both stroke volume and heart rate, reflecting loss of the inotropic and chronotropic effects of thyroid hormones. Peripheral vascular resistance at rest is increased, and blood volume is reduced. These hemodynamic alterations cause narrowing of pulse pressure, prolongation of circulation time, and decrease in blood flow to the tissues.[24,25] The reduction in cutaneous circulation is responsible for the coolness and pallor of the skin and the sensitivity to cold. In most tissues, the decrease in blood flow is proportional to the decrease in oxygen consumption, so the arteriovenous oxygen difference remains normal. The hemodynamic alterations at rest resemble those of congestive heart failure. However, in hypothyroidism, cardiac output increases and peripheral vascular resistance decreases normally in response to exercise unless the hypothyroid state is severe and of long standing.

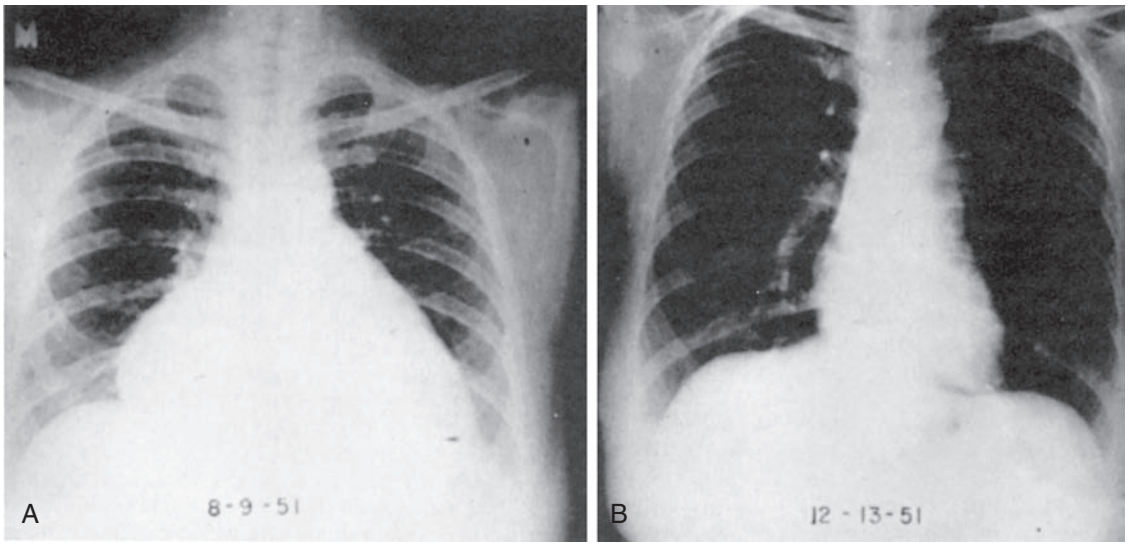
In severe primary hypothyroidism the cardiac silhouette is enlarged (Fig. 13.2), and the heart sounds are diminished in intensity. These findings are the result largely of effusion into the pericardial sac of fluid rich in protein and glycosaminoglycans, but the myocardium may also be dilated. Pericardial effusion is rarely of sufficient magnitude to cause tamponade.

Angina pectoris may first appear or worsen during treatment of the hypothyroid state with thyroid hormone, although most patients with hypothyroidism and coronary artery disease have no change, or improvement, in anginal symptoms with T_4 treatment.[26] Electrocardiographic changes include sinus bradycardia, prolongation of the PR interval, low amplitude of the P wave and QRS complex, alterations of the ST segment, and flattened or inverted T waves. Pericardial effusion is probably responsible for the low amplitude in severe hypothyroidism. Systolic time intervals are altered; the preejection period is prolonged, and the ratio of preejection period to left ventricular ejection time is increased. Echocardiographic studies have revealed resting left ventricular diastolic dysfunction in overt and, in some studies, subclinical hypothyroidism.[27] These findings normalize when the hypothyroidism is treated.

Serum levels of homocysteine, creatine kinase, aspartate aminotransferase, and lactate dehydrogenase may be increased in hypothyroidism.[25,28] Typically the isoenzyme patterns suggest that the source of the increased creatine kinase and lactate dehydrogenase is skeletal, not cardiac, muscle. All levels return to normal with therapy. Sequential cardiac biopsies in a hypothyroid patient with heart failure showed that messenger RNA (mRNA) levels from genes regulated by thyroid hormone and important for the strength of myocardial contraction were normalized after T_4 treatment.[29]

The combination of large heart, hemodynamic and electrocardiographic alterations, and serum enzyme changes has been termed *myxedema heart.* In the absence of coexisting organic heart disease, treatment with thyroid hormone corrects the hemodynamic, electrocardiographic, and serum enzyme alterations of myxedema heart and restores heart size to normal (see Fig. 13.2).

Hypothyroidism is consistently associated with elevations of total and low-density lipoprotein (LDL) cholesterol, which



• **Fig. 13.2** (A and B) Chest roentgenograms in a patient with myxedema heart disease. The patient had signs of severe congestive heart failure and was given thyroid hormone alone. Within 4 months, the heart had returned to normal size (B) and there was no evidence of underlying heart disease.

improve with T_4 replacement.[30] The higher the original serum TSH concentration and elevation of serum LDL, the greater the magnitude of reduction in LDL cholesterol after T_4 therapy. Lipoprotein fractionation has shown that the cholesterol elevation is predominantly due to the less atherogenic large LDL particles. A subset of younger (<50 years) male hypothyroid patients had elevated serum triglycerides and C-reactive protein that improved with T_4 treatment.[31] Most studies have shown that serum high-density lipoprotein (HDL) levels are not influenced by thyroid status.

Hypothyroidism has been shown to be a risk factor for atherosclerosis and cardiovascular disease by several studies, although others have not shown this association.[32] The Whickham Study showed no increase in cardiovascular mortality rate in patients with subclinical hypothyroidism followed for more than 20 years.[12] A prospective study in the United States, following men and women age 65 or older for more than 10 years, showed no influence of hypothyroidism (overt or subclinical) on cardiovascular outcome or mortality rate.[33] Cardiovascular outcome studies suggest that improvement from treatment of hypothyroidism, especially subclinical hypothyroidism, is primarily in those who are middle age and not older individuals (older than 65 years of age).[15,34,35]

Respiratory System

Hypothyroidism affects breathing by actions on the central regulation of respiration as well as the innervation and function of the respiratory muscles, upper airways, and tongue.[36] Pleural effusions usually are evident only on radiologic examination but in rare instances may cause dyspnea. Lung volumes are usually normal, but maximal breathing capacity and diffusing capacity are reduced. In severe hypothyroidism, myxedematous involvement of respiratory muscles and depression of both the hypoxic and the hypercapnic ventilatory drives may cause alveolar hypoventilation and carbon dioxide retention, which in turn can contribute to the development of myxedema coma. An increased prevalence of obstructive sleep apnea is seen in hypothyroid patients, and it is usually reversed with restoration of a euthyroid state.[37]

Alimentary System

Although most patients experience a modest gain in weight, appetite is usually reduced. The weight gain that occurs is caused partly by retention of fluid by the hydrophilic glycoprotein deposits in the tissues but generally does not exceed 10% of body weight. Peristaltic activity is decreased and, together with the decreased food intake, is responsible for the frequent complaint of constipation. The latter may lead to fecal impaction (myxedema megacolon). Gaseous distention of the abdomen (myxedema ileus), if accompanied by colicky pain and vomiting, may mimic mechanical ileus.[38]

Elevations in the serum levels of carcinoembryonic antigen, which may occur on the basis of hypothyroidism alone, add to the impression that an obstruction is present. Ascites in the absence of another cause is unusual in hypothyroidism, but it can occur, usually in association with pleural and pericardial effusions. Like pericardial and pleural effusions, the ascitic fluid is rich in protein and glycosaminoglycans.

Achlorhydria after maximal histamine stimulation may be present in patients with primary hypothyroidism. Circulating antibodies against gastric parietal cells have been found in about one-third of patients with primary hypothyroidism and may be secondary to atrophy of the gastric mucosa. Hypothyroid patients with positive parietal cell antibodies have a higher T_4 requirement compared with antibody-negative patients.[39] Among Swedish celiac disease patients there was a 4.4-fold increased risk for hypothyroidism compared with the general population.[40] Overt pernicious anemia is reported in about 12% of patients with primary hypothyroidism. The coexistence of pernicious anemia and other autoimmune diseases with primary hypothyroidism reflects the fact that autoimmunity plays the central role in the pathogenesis of these diseases (see Chapter 43).[22]

Hypothyroidism has complex effects on intestinal absorption. Although the rates of absorption for many substances are decreased, the total amount absorbed may be normal or even increased because the decreased bowel motility may allow more time for absorption. Malabsorption is occasionally overt.

Liver function test results are usually normal, but levels of aminotransaminases may be elevated, probably because of impaired

clearance.[41] The gallbladder contracts sluggishly and may be distended. In a population study of those without diagnosed thyroid disease, men (but not women) with an elevated TSH had a 3.8-fold increased risk of cholelithiasis.[42] Hypothyroidism is being recognized as a predisposing factor for nonalcoholic fatty liver disease.[43]

Atrophy of the gastric and intestinal mucosa and myxedematous infiltration of the bowel wall may be demonstrated on histologic examination. The colon may be greatly distended, and the volume of fluid in the peritoneal cavity is usually increased. The liver and pancreas are normal.

Central and Peripheral Nervous Systems

Thyroid hormone is essential for the development of the central nervous system.[18,44,45] Deficiency in fetal life or at birth impairs neurologic development, including hypoplasia of cortical neurons with poor development of cellular processes, retarded myelination, and reduced vascularity. If the deficiency is not corrected in early postnatal life, the damage is irreversible. Deficiency of thyroid hormone beginning in adult life causes less severe manifestations that usually respond to treatment with the hormone. Cerebral blood flow is reduced, but cerebral oxygen consumption is usually normal; this finding is in accord with the conclusion that the oxygen consumption of isolated brain tissue in vitro, unlike that of most other tissues, is not stimulated by administration of thyroid hormones. In severe cases, decreased cerebral blood flow may lead to cerebral hypoxia.

All intellectual functions, including speech, are slowed in thyroid hormone deficiency.[46] Loss of initiative is present and memory defects are common, lethargy and somnolence are prominent, and dementia in elderly patients may be mistaken for senile dementia.[47] Positron emission tomography (PET) brain scans of hypothyroid patients before and after T_4 therapy demonstrate reversible reduced glucose uptake in specific brain areas, such as the limbic system, which also correlates with behavioral and psychiatric symptoms.[48] Psychiatric disorders are common and are usually of the paranoid or depressive type and may induce agitation (myxedema madness).[47] Headaches are frequent. Cerebral hypoxia due to circulatory alterations may predispose to confusional attacks and syncope, which may be prolonged and lead to stupor or coma. Other factors predisposing to coma in hypothyroidism include exposure to severe cold, infection, trauma, hypoventilation with carbon dioxide retention, and depressant drugs.

Epileptic seizures have been reported and tend to occur in myxedema coma. Night blindness is due to deficient synthesis of the pigment required for dark adaptation. Hearing loss of the perceptive type is frequent due to myxedema of the eighth cranial nerve and serous otitis media. Perceptive deafness may also occur in association with a defect in the organic binding of thyroidal iodide (Pendred syndrome; see Chapter 11), but in these instances it is not due to hypothyroidism per se.

Thick, slurred speech and hoarseness are due to myxedematous infiltration of the tongue and larynx, respectively.[45] Body movements are slow and clumsy, and cerebellar ataxia may occur. Numbness and tingling of the extremities are frequent; in the fingers these symptoms may be due to compression by glycosaminoglycan deposits in and around the median nerve in the carpal tunnel (carpal tunnel syndrome).[49] The tendon reflexes are slow, especially during the relaxation phase, producing the characteristic "hung-up reflexes"; this phenomenon is due to a decrease in the rate of muscle contraction and relaxation rather than a delay in nerve conduction.

The presence of extensor plantar responses or diminished vibration sense should alert the physician to the possibility of coexisting pernicious anemia with combined system disease. Electroencephalographic changes include slow alpha-wave activity and general loss of amplitude. The concentration of protein in the cerebrospinal fluid is often increased, but cerebrospinal fluid pressure is normal.

Histopathologic examination of the brain in patients with untreated hypothyroidism reveals that the nervous system is edematous with mucinous deposits in and around nerve fibers. In patients with cerebellar ataxia, neural myxedematous infiltrates of glycogen and mucinous material are present in the cerebellum. There may be foci of degeneration and an increase in glial tissue. The cerebral vessels show atherosclerosis, but this finding is much more common if the patient has had coexistent hypertension.

Hypothyroidism has been associated with several neurologic conditions, although a strong etiologic link has not been established. Epidemiologic studies have shown an association between Alzheimer disease and hypothyroidism.[50] It is difficult to convincingly demonstrate this association because the incidence of thyroid disease in the elderly population is high and, like dementia, increases with age. A mechanistic link is suggested by the observation of amyloid deposition in Down syndrome, a condition associated with an increased incidence of Hashimoto disease, and thyroid hormone regulates amyloid gene processing in a number of cellular and animal models. Subclinical hyperthyroidism, however, has also been associated with Alzheimer disease.[51] There is an increase in cerebrospinal fluid reverse T_3 levels in Alzheimer disease patients, all with normal circulating thyroid hormone levels, suggesting the potential for altered thyroid hormone metabolism in the brain.[52] The impact of normalizing T_3 levels in the brain, however, is not known. A corticosteroid-responsive encephalopathy is associated with chronic Hashimoto thyroiditis but may be linked to autoimmunity rather than a process mediated specifically by low thyroid hormone levels or thyroid autoantibodies.[53]

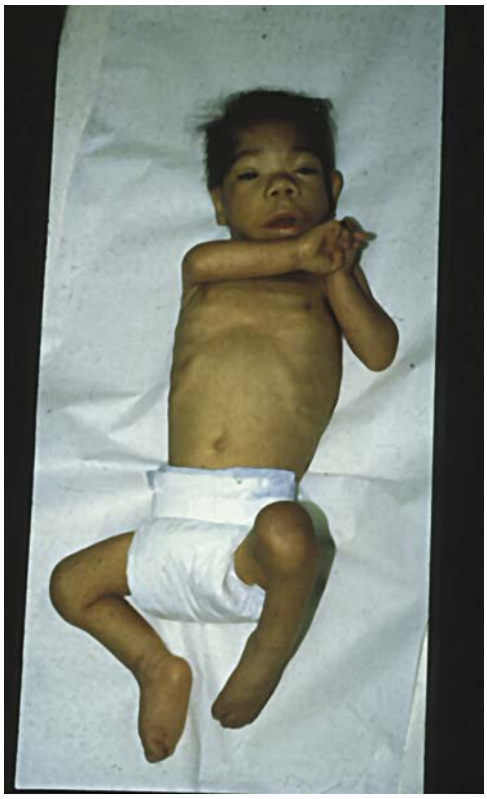
Muscular System

Stiffness and aching of muscles are common in hypothyroidism and are worsened by cold temperatures.[49] Delayed muscle contraction and relaxation cause slowness of movement and delayed tendon jerks.[45] Muscle mass may be reduced or enlarged due to interstitial myxedema. Muscle mass may be slightly increased, and the muscles tend to be firm. Rarely, a profound increase in muscle mass with slowness of muscular activity may be the predominant manifestation (the *Kocher-Debré-Sémélaigne,* or *Hoffmann,* syndrome). Myoclonus may be present. The electromyogram may be normal or may exhibit disordered discharge, hyperirritability, and polyphasic action potentials.

On histopathologic examination, the muscles appear pale and swollen. The muscle fibers may show swelling, loss of normal striations, and separation by mucinous deposits. Type I muscle fibers tend to predominate.

Skeletal System: Calcium and Phosphorus Metabolism

Thyroid hormone is essential for normal growth and maturation of the skeleton, and growth failure is due both to impaired general protein synthesis and to a reduction in growth hormone, but especially of insulin-like growth factor 1 (Fig. 13.3).[54] The thyroid hormone receptor isoforms α and β have specific roles in bone maturation. Before puberty, thyroid hormone plays a major role in the maturation of bone. Deficiency of thyroid hormone in early life leads to both a delay in development and an abnormal, stippled appearance of the epiphyseal centers of ossification



• **Fig. 13.3** The consequences of untreated congenital hypothyroidism are demonstrated in this 17-year-old girl. Her condition had been diagnosed at birth but, through a series of misunderstandings, was not treated with thyroid hormone. Note her size, the poorly developed nasal bridge, the wide-set eyes, and the ears, which are larger than are appropriate for head size. Her tongue is enlarged, and her extremities are inappropriately short in relation to her trunk. (Courtesy Dr. Ronald B. Stein.)

(epiphyseal dysgenesis) (Fig. 13.4). Impairment of linear growth leads to dwarfism, in which the limbs are disproportionately short in relation to the trunk, but cartilage growth is unaffected (see Fig. 13.3). Children with prolonged hypothyroidism, even after adequate treatment, do not reach predicted height based on mid-parental height calculations.[55]

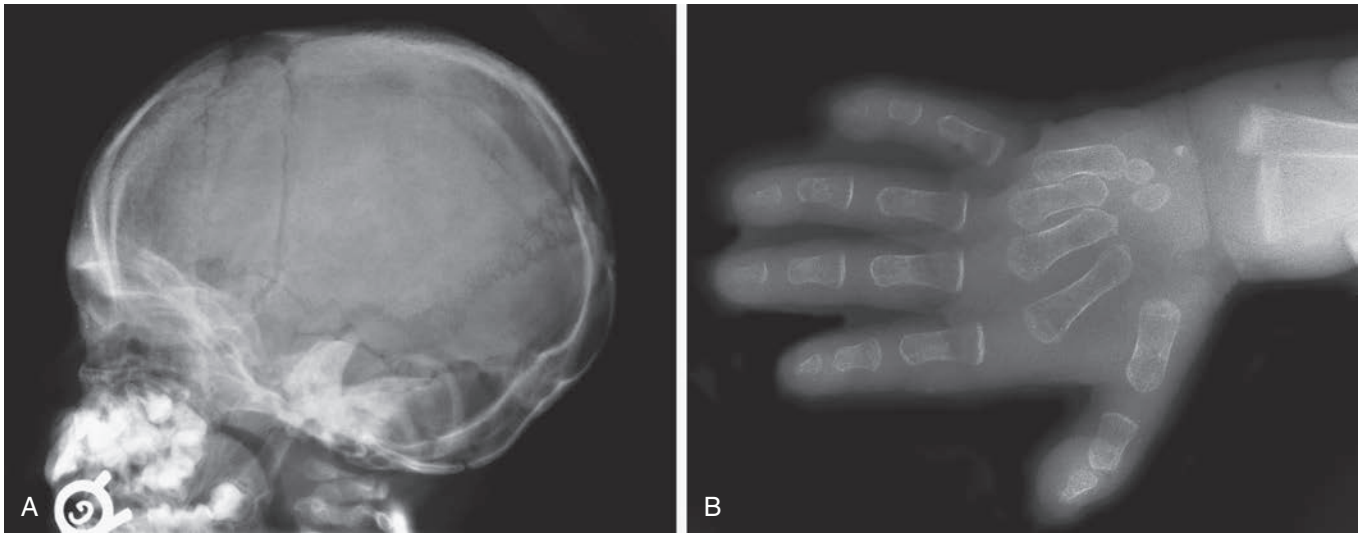
Urinary excretion of calcium is decreased, as is the glomerular filtration rate, whereas fecal excretion of calcium and both urinary and fecal excretion of phosphorus are variable. Calcium balance is also variable, and any changes are slight. The exchangeable pool of calcium and its rate of turnover are reduced, reflecting decreased bone formation and resorption. Because levels of parathyroid hormone are often slightly increased, some degree of resistance to its action may be present; levels of 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) are also increased.

Levels of calcium and phosphorus in serum are usually normal, but calcium may be slightly elevated. The alkaline phosphatase level is usually below normal in infantile and juvenile hypothyroidism. Bone density may be increased. The radiologic appearance of the skeleton in cretinism and juvenile hypothyroidism is discussed subsequently.

Renal Function: Water and Electrolyte Metabolism

Reversible reductions in renal blood flow, glomerular filtration rate, and tubular reabsorptive and secretory maxima are seen in hypothyroidism. Blood urea nitrogen and serum creatinine levels are normal, but uric acid levels may be increased. Urine flow is reduced, and delay in the excretion of a water load may result in reversal of the normal diurnal pattern of urine excretion. The delay in water excretion appears to be due to decreased volume delivery to the distal diluting segment of the nephron as a result of the diminished renal perfusion; evidence supporting inappropriate secretion of vasopressin (syndrome of inappropriate antidiuretic hormone [SIADH] secretion) is less compelling.[56] There is a high prevalence of hypothyroidism in patients with chronic kidney disease, and improvement in renal function has been demonstrated with T_4 treatment.[57]

The impaired renal excretion of water and the retention of water by the hydrophilic deposits in the tissues result in an



• **Fig. 13.4** X-ray films of the skull and hand of the 17-year-old patient illustrated in Fig. 13.3. (A) Skull film shows that the posterior and anterior fontanels are open and that the sutures are not fused. The deciduous and permanent teeth are present. (B) Radiograph of the wrist and hand shows the delayed appearance of the epiphyseal centers of the bones of the hand and the absence of the distal radial epiphysis. The estimated bone age is 9 months. (Courtesy Dr. Ronald B. Stein.)

increase in total body water, even though plasma volume is reduced. This increase accounts for the hyponatremia occasionally noted because the level of exchangeable sodium is increased. The amount of exchangeable potassium is usually normal in relation to lean body mass. Serum magnesium concentration may be increased, but exchangeable magnesium levels and urinary magnesium excretion are decreased.

Hematopoietic System

In response to the diminished oxygen requirements and decreased production of erythropoietin, the red blood cell mass is decreased; this is evident in the mild normocytic, normochromic anemia that often occurs. Less commonly, the anemia is macrocytic, sometimes from deficiency of vitamin B_{12}. Reference has already been made to the high incidence of pernicious anemia (and of achlorhydria and vitamin B_{12} deficiency without overt anemia) in primary hypothyroidism (see Chapter 43). Conversely, overt and subclinical hypothyroidism is present in 12% and 15% of patients, respectively, with pernicious anemia. Folate deficiency from malabsorption or dietary inadequacy may also cause macrocytic anemia. The frequent menorrhagia and the defective absorption of iron resulting from achlorhydria may contribute to a microcytic, hypochromic anemia.

The total and differential white blood cell counts are usually normal, and platelets are adequate, although platelet adhesiveness may be impaired. If pernicious anemia or significant folate deficiency is present, the characteristic changes in peripheral blood and bone marrow will be found. The intrinsic clotting mechanism may be defective because of decreased concentrations in plasma of factors VIII and IX, which, together with an increase in capillary fragility and the decrease in platelet adhesiveness, may account for the bleeding tendency that sometimes occurs.[38,58,59]

Pituitary and Adrenocortical Function

In long-standing primary hypothyroidism, hyperplasia of the thyrotropes may cause the pituitary gland to be enlarged. This feature can be detected radiologically as an increase in the volume of the pituitary gland.[60] Rarely, the pituitary enlargement compromises the function of other pituitary cells and causes pituitary insufficiency or visual field defects. Patients with severe hypothyroidism may have increased serum prolactin levels, stimulated by the elevation in thyrotropin-releasing hormone (TRH) and proportional to the level of serum TSH elevation, and galactorrhea may develop in some patients. Treatment with thyroid hormone normalizes the serum prolactin and TSH levels and causes disappearance of galactorrhea, if present.

In rodents, thyroid hormone directly regulates growth hormone synthesis. Growth hormone is not directly regulated by thyroid hormone in humans, but thyroid status influences the growth hormone axis.[61] Hypothyroid children have delayed growth and the response of growth hormone to provocative stimuli may be subnormal. Individuals affected with RTHα have delayed growth and short stature, indicating the consequences of interference with thyroid hormone signaling through thyroid hormone receptor alpha.[62]

As a result of the decreased rate of turnover of cortisol due to decreased hepatic 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1), the 24-hour urinary excretion of cortisol and 17-hydroxycorticosteroids is decreased, but the plasma cortisol level is usually normal (see Chapter 15). The response of urinary 17-hydroxycorticosteroid to exogenous adrenocorticotropic hormone is usually normal but may be decreased. The response of plasma cortisol to insulin-induced hypoglycemia may be impaired.

In severe, long-standing primary hypothyroidism, pituitary and adrenal function may be secondarily decreased, and adrenal insufficiency may be precipitated by stress or by rapid replacement therapy with thyroid hormone.[61] The rate of turnover of aldosterone is decreased, but the plasma level is normal. Plasma renin activity is decreased, and sensitivity to angiotensin II is increased, which may contribute to the association of hypertension with hypothyroidism (see Chapter 16).[63,64]

Reproductive Function

In both sexes, thyroid hormones influence sexual development and reproductive function.[65] Infantile hypothyroidism, if untreated, leads to sexual immaturity, and juvenile hypothyroidism causes a delay in the onset of puberty followed by anovulatory cycles in girls. Paradoxically, primary hypothyroidism may also rarely cause precocious sexual development and galactorrhea, presumably due to "spillover" of elevated TSH stimulating the luteinizing hormone (LH) receptor and elevated TRH initiating excess prolactin release.

In adult women, severe hypothyroidism may be associated with diminished libido and failure of ovulation. Secretion of progesterone is inadequate, and endometrial proliferation persists, resulting in excessive and irregular breakthrough menstrual bleeding. These changes may be due to deficient secretion of LH and pulse frequency and amplitude. Rarely, in primary hypothyroidism, secondary depression of pituitary function may lead to ovarian atrophy and amenorrhea. Fertility is reduced, and there is an increase in spontaneous abortion and preterm delivery, although many pregnancies are successful.[66] Pregnancy complications are associated with overt and subclinical hypothyroidism, although the impact has varied among different studies.[67] A randomized prospective study of levothyroxine treatment in pregnant women with thyroid peroxidase antibody (TPOAb) positivity but normal range TSH has shown that the increased incidence of preterm delivery and spontaneous abortions is reversed by treatment, although this result remains to be confirmed.[66,68] Primary ovarian failure can also be seen in patients with Hashimoto thyroiditis as part of an autoimmune polyendocrine syndrome.[22,23] Hypothyroidism in men may cause diminished libido, erectile dysfunction, and oligospermia. A significant fraction of men with both hypothyroidism and hyperthyroidism have moderate to severe erectile dysfunction, which improves with treatment of the thyroid disease.[69]

Values for plasma gonadotropins are usually in the normal range in primary hypothyroidism; in postmenopausal women, levels are usually somewhat lower than in euthyroid women of the same age but are nevertheless within the menopausal range. This feature provides a valuable means of differentiating primary from secondary hypothyroidism.

The metabolism of both androgens and estrogens is altered in hypothyroidism. Secretion of androgens is decreased, and the metabolism of testosterone is shifted toward etiocholanolone rather than androsterone. With respect to estradiol and estrone, hypothyroidism favors metabolism of these steroids via 16α-hydroxylation over that via 2-oxygenation, with the result that formation of estriol is increased and that of 2-hydroxyestrone and its derivative, 2-methoxyestrone, is decreased. The sex hormone–binding globulin in plasma is decreased, with the result that the plasma concentrations of both testosterone and estradiol are decreased, but the unbound fractions are increased. The

alterations in steroid metabolism are corrected by restoration of the euthyroid state.[70]

Catecholamines

The plasma cyclic adenosine monophosphate (cAMP) response to epinephrine is decreased in hypothyroidism, suggesting a state of decreased adrenergic responsiveness. The fact that the responses of plasma cAMP to glucagon and parathyroid hormone are also decreased suggests that thyroid hormones have a general modulating influence on cAMP generation.[71] The reduced adrenergic responsiveness associated with hypothyroidism has been linked to all steps of catecholamine signaling, including receptor and postreceptor actions, resulting in an impaired cAMP response. Direct measurement of norepinephrine in abdominal fat of hypothyroid patients shows reduced levels, and there is reduced production of glycerol in response to adrenergic agonist stimulation.[72] Augmentation of α_2-receptor signaling has also been proposed as a factor reducing catecholamine responsiveness.

Energy Metabolism: Protein, Carbohydrate, and Lipid Metabolism

The decrease in energy metabolism and heat production is reflected in a low basal metabolic rate, decreased appetite, cold intolerance, and slightly low basal body temperature.[73–75] Both the synthesis and the degradation of protein are decreased, the latter especially so, with the result that nitrogen balance is usually slightly positive. The decrease in protein synthesis is reflected in retardation of both skeletal and soft tissue growth.

Permeability of capillaries to protein is increased, accounting for the high levels of protein in effusions and in cerebrospinal fluid. In addition, the albumin pool is increased because of the greater decrease in albumin degradation compared to albumin synthesis. A greater than normal fraction of exchangeable albumin is in the extravascular space. The total concentration of serum proteins may be increased.

Hypothyroidism is associated with a reduction in glucose disposal to skeletal muscle and adipose tissue.[76] Thyroid hormone has been shown to stimulate expression of the insulin-sensitive glucose transporter (GLUT4), and the levels of this transporter are reduced in hypothyroidism. Hypothyroidism is also, however, associated with reduced gluconeogenesis. The net effect of these influences is usually a minimal effect of hypothyroidism on serum glucose levels. Thyroid hormone downregulates expression of prohormone processing enzymes, which therefore have increased activity in hypothyroidism. Degradation of insulin therefore is slowed, and the sensitivity to exogenous insulin may be increased. In a patient with preexisting diabetes mellitus who develops hypothyroidism, insulin requirements may be reduced. A further influence on glucose uptake may occur at the tissue level. Polymorphisms in the 5′-deiodinase type 2 (D2) gene, which may affect local T_3 production, have been shown to be associated with impaired glucose disposal.[77]

Both the synthesis and the degradation of lipid are depressed in hypothyroidism. Degradation, however, is reduced to a greater extent, with a net effect of accumulation of LDL and triglycerides.[30,73] The decrease in the lipid degradation rate may reflect the decrease in postheparin lipolytic activity as well as reduced LDL receptors. Plasma free fatty acid levels are decreased, and the mobilization of free fatty acids in response to fasting, catecholamines, and growth hormone is impaired. Impaired lipolysis of white fat in hypothyroid patients at baseline and in response to catecholamine reflects impaired free fatty acid mobilization.[71,72] All of these abnormalities are relieved by treatment.

An elevation in serum LDL cholesterol has been associated, in most studies, with both overt and subclinical hypothyroidism.[30] According to most studies, serum HDL and triglyceride levels are not influenced by hypothyroidism.[73] The reduction in LDL with T_4 therapy is generally related to the original magnitude of LDL and TSH elevation; the higher the initial levels, the greater the reduction in LDL that is observed. A typical reduction in LDL is 5% to 10% of the original level.

The role of adipocytokines, such as leptin, adiponectin, and resistin, in metabolic regulation has been increasingly recognized as well as the potential for interaction with thyroid hormone.[75] Rodent studies have shown that leptin regulates central adaptation between the starved and fed state and that falling leptin levels, associated with starvation, lead to a suppression of the thyroid axis. Hypothyroidism in rodents is associated with reduced leptin and increased resistin levels. Leptin infusion into the cerebral ventricles reverses some of the metabolic changes seen with hypothyroidism, including improved glucose disposal and reduced skeletal muscle fat.[78] Human studies, however, have not shown consistent changes in adipocytokines in hypothyroidism.[79]

Current Clinical Picture

In the adult, the onset of hypothyroidism is usually so insidious that the typical manifestations may take months or years to appear and go unnoticed by family and friends. The gradual development of the hypothyroid state is due to slow progression both of thyroid hypofunction and of the clinical manifestations after thyroid failure is complete. This course is in contrast with the more rapid development of the hypothyroid state when replacement therapy is discontinued in a patient with treated primary hypothyroidism or when the thyroid gland of a normal subject is surgically removed. In such patients, manifestations of frank hypothyroidism are usually present by 6 weeks and myxedema appears by 3 months.

Hypothyroidism continues to be diagnosed at earlier stages.[1,2,80] Based on the most recent data, subclinical or early hypothyroidism is seen approximately 14 times more commonly than overt hypothyroidism. Early symptoms are variable and relatively nonspecific. The reason for the increased prevalence of hypothyroid patients presenting with minimal symptoms is largely the availability of sensitive and specific laboratory tests that allow recognition of the primary form of the disease long before severe symptoms have developed. There should thus be a low threshold to test patients for suspected primary hypothyroidism with a serum TSH determination. Patients with significant biochemical abnormalities of hypothyroidism may not score high on indices of symptoms and signs.[81]

With respect to physical signs of hypothyroidism, the presence of coarse skin, periorbital puffiness that obscures the curve of the malar bone (see Fig. 13.1), cold skin, and delayed ankle reflex relaxation phase are all signs that should lead to appropriate diagnostic tests.

Acute hypothyroidism in the previously hyperthyroid patient seen after radioiodine therapy may also be characterized by painful cramping of large muscle groups, as discussed in Chapter 12 regarding Graves disease.

Hypothyroidism in Infants and Children

Severe hypothyroidism is seldom apparent at birth, perhaps due to the partial protection afforded by transplacental transfer of maternal thyroid hormones, hence the requirement for systematic screening for congenital hypothyroidism.[18] Congenital hypothyroidism can be due to complete thyroid agenesis, ectopic thyroid,

or incomplete thyroid development. Mutations in genes important for thyroid development have been identified in a number of patients and in some cases may explain associated abnormalities in development of other structures, such as the heart, because of their spatial association during development. The age at which symptoms appear depends on the degree of impairment of thyroid function (see Figs. 13.3 and 13.4). Severe hypothyroidism in infancy is termed *cretinism.* As the age at onset increases, the clinical picture of cretinism merges imperceptibly with that of juvenile hypothyroidism. Retardation of mental development and growth, the hallmark of cretinism, becomes manifest only in later infancy, and the former is largely irreversible. Consequently, early recognition is crucial and has been achieved by universal population screening in the developed world by measuring serum T_4 or TSH concentrations routinely in filter paper blood spots from neonates. During the first few months of life, symptoms and signs of hypothyroidism include feeding problems, failure to thrive, constipation, a hoarse cry, somnolence, and jaundice. In succeeding months, especially in severe cases, protuberance of the abdomen, dry skin, poor growth of hair and nails, and delayed eruption of the deciduous teeth become evident. Retardation of mental and physical development is manifested by delay in reaching the normal milestones of development, such as holding up the head, sitting, walking, and talking.

Thyroid hormone plays a major role in bone development, and thyroid hormone receptors are expressed in osteoclasts and osteoblasts.[54] The primary targets of thyroid hormone have been identified in the epiphyseal plates. Impairment of linear growth in congenital hypothyroidism results in dwarfism, with the limbs disproportionately short in relation to the trunk (see Fig. 13.3). Delayed closure of the fontanels causes the head to be large in relation to the body. The naso-orbital configuration remains infantile. Maldevelopment of the femoral epiphyses results in a waddling gait. The teeth are malformed and susceptible to caries. The characteristic appearance includes a broad and flat nose, widely set eyes, periorbital puffiness, large protruding tongue, sparse hair, rough skin, short neck, and protuberant abdomen with an umbilical hernia. Mental deficiency is usually severe.

Radiologic examination of the skeleton is diagnostic. The skull shows a poorly developed base, delayed closure of the fontanels, widely set orbits, and a short and flat nasal bone. The pituitary fossa may be enlarged. Shedding of deciduous teeth and eruption of permanent teeth are delayed (see Fig. 13.4).

The radiologic picture of epiphyseal dysgenesis is virtually pathognomonic of hypothyroidism in infancy and childhood and may involve any center of endochondral ossification, depending on the age at onset of the hypothyroid state; it is usually best seen in the femoral and humeral heads and the navicular bone of the foot. The centers of ossification appear late, so bone age is retarded in relation to chronologic age; when they eventually appear, instead of a single center, multiple small centers are scattered throughout a misshapen epiphysis (see Fig. 13.4). These small centers of ossification eventually coalesce and form a single center with an irregular outline and a stippled appearance (stippled epiphysis). Epiphyseal dysgenesis is evident only in centers that normally ossify at a time after the onset of the hypothyroidism. After a normal metabolic state is restored by treatment, centers destined to ossify at a later age develop normally.

Hypothyroidism that begins in childhood is usually Hashimoto disease and can be transient in this age group. Subclinical hypothyroidism is also seen in children and adolescents, and in one study those affected were more likely to be obese and have a family history of thyroid disease.[82] The clinical manifestations of hypothyroidism in children are intermediate, between those of infantile and those of adult hypothyroidism, in that the developmental retardation is not as severe as that of cretinism, and the manifestations of full-blown adult myxedema are rarely seen. Growth and sexual development are affected predominantly. If left untreated, linear growth is severely retarded and sexual maturation and the onset of puberty are delayed.[55,83] On radiologic examination, epiphyseal dysgenesis may be present, and epiphyseal union is always delayed, resulting in a bone age that is younger relative to chronologic age.

Laboratory Evaluation

Primary and Central Hypothyroidism

A decrease in secretion of the thyroid hormones is common to all varieties of hypothyroidism, except for disorders of thyroid hormone metabolism or action, such as *consumptive hypothyroidism* and *resistance to thyroid hormone* (see later). In patients with primary thyroid disease—the cause of hypothyroidism in more than 99% of the patients—there is a significant increase in basal serum TSH concentration. A strategy for evaluating the patient suspected of hypothyroidism involves a TSH determination (Table 13.2). If the suspicion of hypothyroidism is strong, if a goiter is present, or if central hypothyroidism is part of the differential diagnosis, an FT_4 assay should be included (see Chapter 11). If hypothyroidism is thought to be unlikely but must be excluded, only a TSH determination is required because primary hypothyroidism is almost always the cause. If TSH is elevated, an FT_4 assay can be added to the same determination (Fig. 13.5). As hypothyroidism progresses, the serum TSH increases further, the serum FT_4 falls, and finally at the most severe stage serum T_3 concentrations may become subnormal (see Table 13.2). The persistence of a normal serum T_3 is in part due to preferential synthesis and secretion of T_3 by residual functioning thyroid tissue under the influence of the increased plasma TSH. In addition, the efficiency of conversion of T_4 to T_3 by D2 is increased as the serum T_4 level falls.[84] Consequently, the serum T_3 concentration may remain within the normal range.

The principal differential diagnosis is between primary and central hypothyroidism (see Chapter 9).[85] The serum TSH concentration is the critical laboratory determination that in general allows recognition of the cause of the disease when the serum FT_4 is reduced. An exception is the individual with a recent history of thyrotoxicosis (and suppressed TSH) in whom a low FT_4 level may be associated with a reduced TSH level for several months after treatment of the thyrotoxicosis. In patients with primary hypothyroidism, the absence of TPOAb raises a possible diagnosis of transient hypothyroidism following an undiagnosed episode of painful subacute thyroiditis, also referred to as postviral, de Quervain, granulomatous, or pseudotuberculous thyroiditis.

The differentiation of hypothyroidism due to intrinsic thyroid failure from hypothyroidism due to diminished TSH secretion from hypothalamic or pituitary disease (central or secondary hypothyroidism) is the most critical decision point in this pathway (see Fig. 13.5).[4] A low thyroid hormone level with a normal or low TSH level should lead to an evaluation for the possibility of failure of other endocrine systems that require trophic pituitary hormones for normal function (see Table 13.1) (see Chapters 8 and 9). In some patients with central hypothyroidism, the basal serum TSH concentration (and the response to TRH) may even

TABLE 13.2 Laboratory Evaluation of Patients With Suspected Hypothyroidism or Thyroid Enlargement[a]

TSH, Free T_4	TPOAb	Diagnosis
TSH >10 mU/L		
Low	+	Primary hypothyroidism due to autoimmune thyroid disease
Low-normal	+	Primary "subclinical" hypothyroidism (autoimmune)
Low or low-normal	–	Recovery from systemic illness
		External irradiation, drug-induced, congenital hypothyroidism
		Iodine deficiency
		Seronegative autoimmune thyroid disease
		Rare thyroid disorders (amyloidosis, sarcoidosis, etc.)
		Recovery from subacute granulomatous thyroiditis
Normal	+, –	Consider TSH or T_4 assay artifacts
Elevated	–	Thyroid hormone resistance
		Blockade of T_4 to T_3 conversion (amiodarone) or a congenital 5′-deiodinase deficiency
		Consider assay artifacts
TSH 5–10 mU/L		
Low, low-normal	+	Early primary autoimmune hypothyroidism
Low, low-normal	–	Milder forms of nonautoimmune hypothyroidism (see earlier)
		Central hypothyroidism with impaired TSH bioactivity
Elevated	– (+)	Consider thyroid hormone resistance
		T_4 to T_3 conversion blockade (e.g., amiodarone)
TSH 0.5–5 mU/L		
Low, low-normal	– (+)	Central hypothyroidism
		Salicylate or phenytoin therapy
		Desiccated thyroid or T_3 replacement
TSH <0.5 μU/L		
Low, low-normal	– (+)	"Post-hyperthyroid" hypothyroidism (^{131}I or surgery)
		Central hypothyroidism
		T_3 or desiccated thyroid excess
		Following excess levothyroxine withdrawal

[a]Initial tests: serum TSH, serum free T_4, TPO, or TgAb.

TgAb, Anti-thyroglobulin antibody; *TPOAb,* thyroid peroxidase autoantibody; *TSH,* thyroid-stimulating hormone (thyrotropin); *+,* present; *–,* not present.

be somewhat elevated, but the TSH has reduced biologic potency even though it is immunologically reactive.[4]

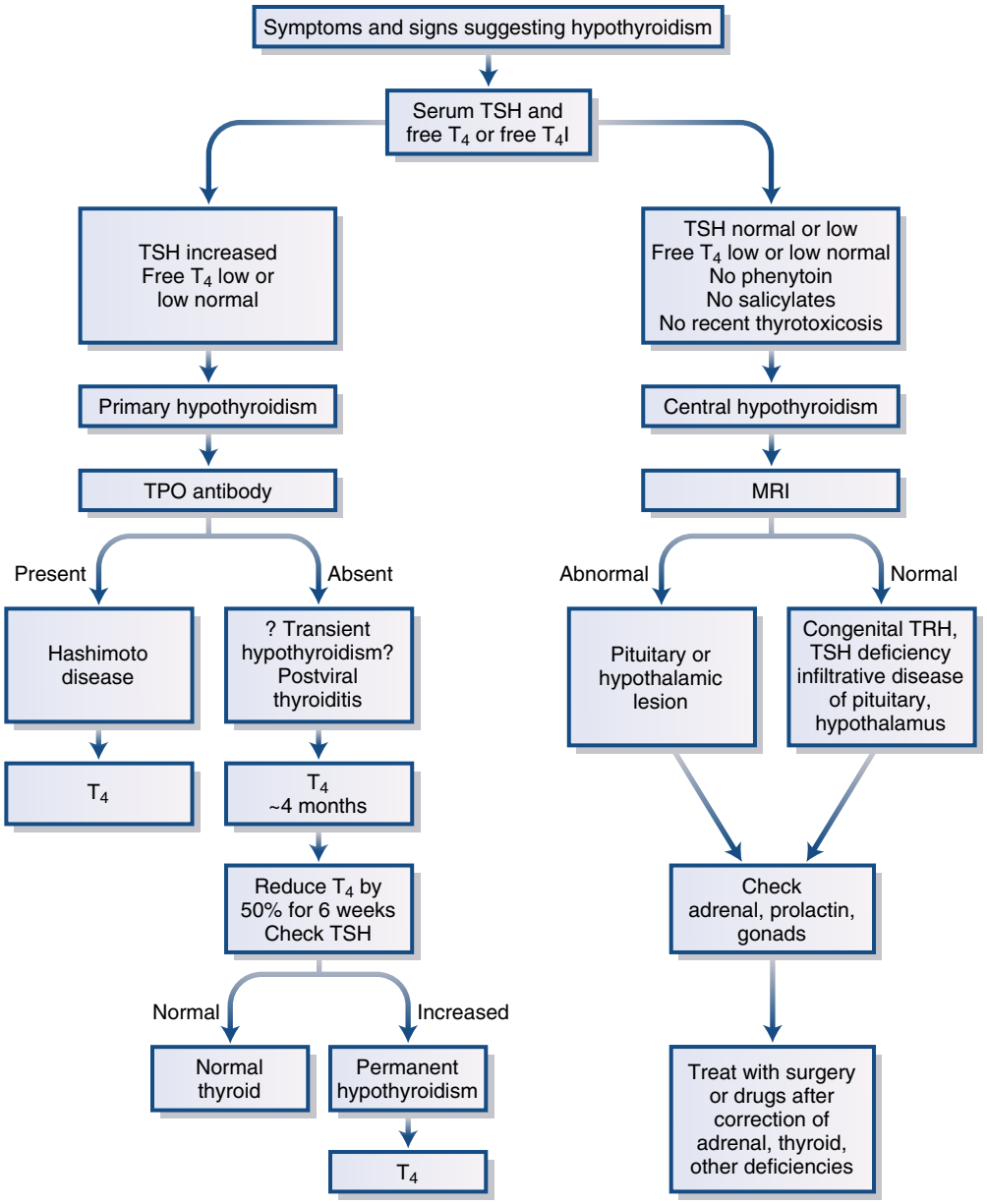
In patients with an elevated TSH level and a reduced FT_4, the presence of TPOAb generally points to autoimmune thyroid disease (Hashimoto disease) as the cause of the hypothyroidism (see Fig. 13.5). On the other hand, the absence of TPOAb raises the possibility of less common causes of hypothyroidism such as transient hypothyroidism, infiltrative thyroid disorders, and external irradiation, as discussed later (see Table 13.1), although rarely patients with Hashimoto disease will not have detectable thyroglobulin or TPOAb.

Measurement of radioactive iodine uptake (RAIU) is rarely required in the evaluation of hypothyroidism. Tests that use radioiodine to assess the function of the thyroid gland display a variable pattern, depending on the underlying thyroid disorder. The diagnostic value of a low RAIU is limited because of the relatively high dietary iodine intake in North America, reducing uptake of the tracer dose of radioiodine, and variation in iodine intake from day to day in the same individual. National surveys of dietary iodine intake had shown a progressive reduction in iodine intake over the past several decades, but the intake has now stabilized.[86] The RAIU may be normal or even increased when hypothyroidism results primarily from a biochemical defect in thyroid hormone synthesis rather than thyroid cell destruction leading to compensatory thyroid enlargement. Specific functional patterns in relation to the causes of hypothyroidism are discussed later. Nonetheless, measurement of RAIU is almost never required in the diagnostic evaluation of the hypothyroid patient.

Differential Diagnosis

The clinical picture of fully developed hypothyroidism is quite characteristic, but the abnormalities can be overlooked, even by experienced clinicians, if the diagnosis is not considered. Despite the availability of inexpensive and specific tests, it is still surprising how often what is retrospectively obvious, severe, primary hypothyroidism is not recognized. A high index of suspicion is required to avoid this oversight.

For the milder forms of hypothyroidism, the clinical presentation overlaps to a significant extent with other conditions. The fact that these disorders often occur in older patients is partly responsible for the diagnostic uncertainty.[2] In some cases, slowing of mental and physical activity, dry skin, and loss of hair may mimic similar findings in hypothyroidism. Furthermore, older people often become hypothermic with cold exposure. In patients with chronic renal insufficiency, anorexia, torpor, periorbital puffiness, sallow complexion, and anemia (e.g., see Fig. 13.1) may suggest hypothyroidism and may call for specific testing. Distinguishing nephrotic states from hypothyroidism by clinical examination alone may be even more difficult. In this disorder, waxy pallor, edema, hypercholesterolemia, and hypometabolism may suggest hypothyroidism. In addition, the total serum T_4 concentration may be decreased if significant thyroid-binding globulin is lost in the urine but the FT_4 and TSH would be normal. In patients with pernicious anemia, psychiatric abnormalities, pallor, and numbness and tingling of the extremities may mimic similar findings in hypothyroidism. Although there is a clinical and immunologic overlap between primary hypothyroidism and pernicious anemia, this association is not invariable (see Chapter 43). The presence of hypothyroidism is often suspected in patients who are severely ill, especially in the elderly.[34,87] In such patients, the total T_4 concentration may be decreased, often markedly so, but the FT_4 is generally normal unless the patient is severely ill (see Chapter 11).



• **Fig. 13.5** Strategy for the laboratory evaluation of patients with suspected hypothyroidism. The principal differential diagnosis is between primary and central hypothyroidism (see Chapter 9). The serum thyrotropin *(TSH)* concentration is the critical laboratory determination that in general allows recognition of the cause of the disease. An exception is the individual with a recent history of thyrotoxicosis (and suppressed TSH) in whom a low free thyroxine *(T_4)* level may be associated with a reduced TSH level for several months after relief of the thyrotoxicosis. In patients with primary hypothyroidism, the absence of thyroid peroxidase *(TPO)* antibodies raises a possible diagnosis of transient hypothyroidism following an undiagnosed episode of subacute or postviral thyroiditis. In such patients, a trial of levothyroxine in reduced dosage after 4 months may reveal recovery of thyroid function, thus avoiding permanent levothyroxine replacement. *MRI,* magnetic resonance imaging; *TRH,* thyrotropin-releasing hormone; *T_4I,* thyroxine index.

These features, together with the absence of an elevation of serum TSH, usually serve to differentiate the ill euthyroid patient from one with primary hypothyroidism. The serum TSH, however, can be transiently increased (up to 20 mU/L) during recovery from severe illness.[88]

Hypothyroidism may develop either because of some extrinsic factor or acquired condition or because of a congenital defect impairing thyroid hormone biosynthesis (see Table 13.1). Inadequate synthesis of hormone leads to hypersecretion of TSH, which in turn produces both goiter and stimulation of all steps in hormone biosynthesis capable of response. In some instances, however, the compensatory TSH response overcomes the impairment in hormone biosynthesis, and the patient is euthyroid with a goiter. The latter condition is discussed in Chapter 14 regarding simple or nontoxic goiter. Less commonly, hypothyroidism is associated with an atrophic gland or, in the case of a congenital abnormality, one that never developed properly. Hypothyroidism occurs in about 20% of patients after surgical lobectomy, with an increased risk in areas of iodine insufficiency or in patients with anti-TPOAb.[89]

Classification

Immune-Mediated

Autoimmune Hypothyroidism

Autoimmunity is responsible for over 90% of noniatrogenic hypothyroidism in countries with iodine sufficiency. The annual incidence of autoimmune hypothyroidism is around 80 per 100,000 men and 350 per 100,000 women.[90] All ages may be affected, although the average age of onset is between 40 and 60 years. The disorder is more frequent in whites and Asians than in African Americans. The initial presentation depends on the stage of disease. Juvenile and adolescent autoimmune thyroiditis may be self-limiting. Hashimoto thyroiditis is the commonest cause of goiter in iodine-sufficient regions; atrophic thyroiditis (primary myxedema) presents as hypothyroidism without a goiter.

Circulating autoantibodies against thyroglobulin and TPO are present in almost all patients with autoimmune hypothyroidism. Up to 20% of patients with autoimmune hypothyroidism have TSH receptor antibodies that block the receptor, rather than stimulating it, as in Graves disease; in rare patients there may be switching from one type of antibody to the other, resulting in alternating hypothyroidism and hyperthyroidism.[91,92] Less commonly, patients produce autoantibodies against the sodium-iodide symporter (NIS), pendrin, and T_4 and T_3, but the functional relevance of these antibodies is not known.

Around 15% of women and 3% of men have positive thyroid autoantibodies but no other clinical features of thyroid disease; most of them, however, will have histologic evidence of focal thyroiditis. Longitudinal studies have shown that euthyroid women with high initial levels of autoantibodies against thyroglobulin or TPO and those whose TSH is within the upper half of the reference interval are the most likely to progress to overt hypothyroidism.[93]

Autoimmune hypothyroidism is commonly found in association with a range of autoimmune disorders, including pernicious anemia, systemic lupus erythematosus, Addison disease, celiac disease, and vitiligo.[23,94] A steroid-responsive encephalopathy (referred to as Hashimoto encephalopathy) has been reported in individuals with positive TPOAb, irrespective of thyroid dysfunction, but it is unclear whether there is a true causal relationship, for instance, through immunologic cross-reactivity with brain tissue.[53,95]

Pathophysiology. The current understanding of autoimmune mechanisms has been discussed in Chapter 12, and the main features associated with autoimmune hypothyroidism are summarized in Fig. 13.6. T-cell–mediated tissue injury is believed to be the most important cause of autoimmune thyroid follicular cell destruction. Perforin-containing cytotoxic $CD8^+$ T cells are abundant in the intrathyroidal lymphocytic infiltrate in Hashimoto thyroiditis. These T cells increase during the evolution of disease and recognize both thyroglobulin and TPO.[96] Apoptosis is an additional pathway for thyroid cell destruction. In Hashimoto thyroiditis, thyroid follicular cells express both Fas (CD95) and Fas ligand (CD95L) and may thus self-destruct when these molecules interact; it is now clear that other decoy death receptors and regulators of apoptosis signaling play an additional role.[97] There is also an increase in the number of intrathyroidal Th17 lymphocytes in Hashimoto thyroiditis, implying a pathogenic role for this proinflammatory T-cell subset.[98] The differentiation of Th17 cells may be enhanced by iodine. Cytokines released by T cells and other inflammatory cells cause Hürthle cell formation and thyroid dysfunction. The thyroid cells also respond to cytokines by expressing a number of proinflammatory molecules, such as chemokines and adhesion molecules, which increase the potential for T-cell binding and cytotoxicity.

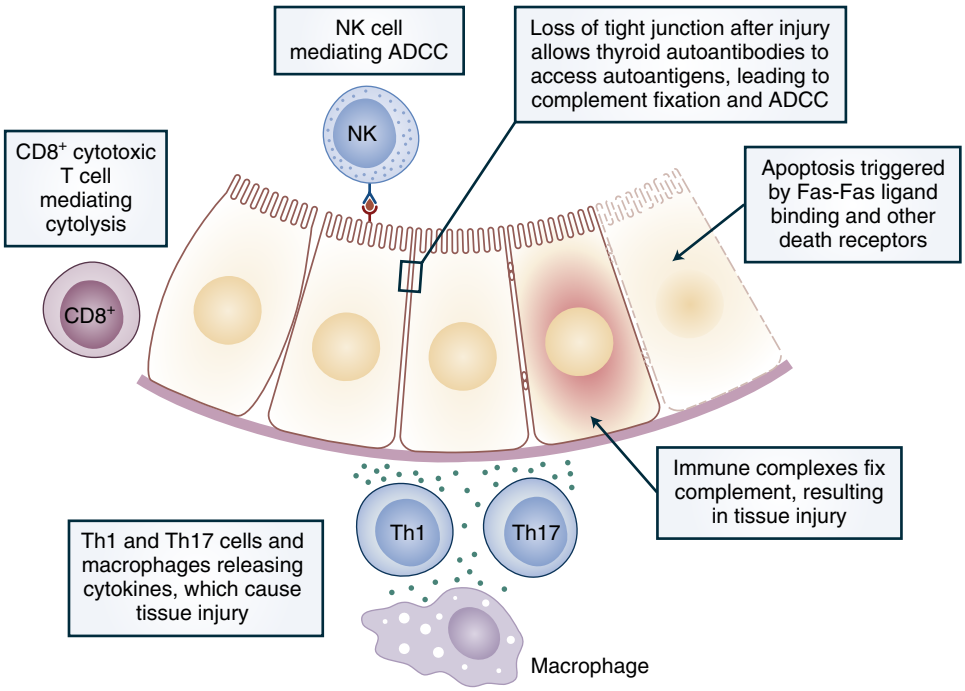
Apart from the striking activity of TSH receptor blocking antibodies, which can induce temporary neonatal hypothyroidism following their transfer across the placenta,[99,100] the pathogenic role of antibodies in autoimmune hypothyroidism is unclear.[101] No neonatal disorders have been associated with the presence of high thyroglobulin or TPO autoantibodies in mothers, indicating that any role in tissue injury is likely to be secondary to an initial phase of T-cell–mediated damage, which allows the autoantibodies to access their target antigens. Such injury may be mediated through antibody-dependent cell-mediated cytotoxicity, involving natural killer (NK) cells, or through complement fixation in the case of TPO antibodies.[102]

Histopathology. The pathologic features of autoimmune hypothyroidism vary from mild focal thyroiditis to extensive lymphocytic infiltration and fibrosis. In classical Hashimoto thyroiditis (originally termed *struma lymphomatosa*), the thyroid gland may be diffusely enlarged or nodular; the tissue is pale and firm and has a rubbery texture (Fig. 13.7A). Typically there is a diffuse lymphocytic infiltration with germinal center formation and obliteration of thyroid follicles, accompanied by a variable degree of fibrosis (see Fig. 13.7B). Destruction of thyroid epithelial cells occurs as disease progresses from euthyroidism to hypothyroidism; in some patients there is follicular cell metaplasia and the formation of Hürthle cells. Rarely, there are concurrent histologic changes of Graves disease, so-called hashitoxicosis. In the other broad type of autoimmune hypothyroidism, termed *atrophic thyroiditis* or *primary myxedema,* the gland is atrophied and consists of extensive fibrotic tissue, moderate lymphocytic infiltration, and widespread loss of thyroid follicles, but the fibrosis is not as extensive as in Riedel thyroiditis (see Fig. 13.7C). The histopathologic changes in painless thyroiditis resemble Hashimoto thyroiditis.

Although it is now generally thought that these variations represent a spectrum of disease arising from a common underlying autoimmune process, a distinct subset of patients with Hashimoto thyroiditis has been delineated recently in whom there are high circulating levels of IgG4 and increased numbers of IgG4-positive plasma cells in the thyroid. Such IgG4-related thyroiditis is characterized pathologically by a greater degree of stromal fibrosis, lymphoplasmacytic infiltration, and hypothyroidism.[103]

Risk Factors

Genetic Susceptibility. The importance of genetic factors in the cause of autoimmune hypothyroidism is indicated by the frequent presence of thyroid autoantibodies, thyroid disease, and other autoimmune disorders in family members and by twin studies, which show a high concordance rate (0.55) in monozygotic but not dizygotic twins.[104] As with all autoimmune endocrinopathies, human leukocyte antigen (HLA)–D region polymorphisms play a role in susceptibility, and Hashimoto thyroiditis is associated with HLA-DR3 and to a lesser extent HLA-DR4.[105] Polymorphisms in the *PD1* gene also confer susceptibility, with lesser contributions from polymorphisms in the *CD40* gene and the gene encoding thyroglobulin, as well as other unconfirmed candidate genes.[106,107] It is clear that new analytic approaches are likely to reveal many other genes, which make small etiologic contributions that account for the diversity of clinical presentation. For example, a combination of novel genetic markers has been described and is associated with an increased risk of progression from TPOAb



• **Fig. 13.6** Summary of the main mechanisms involved in the pathogenesis of autoimmune hypothyroidism. *ADCC,* antibody-dependent cell-mediated cytotoxicity; *NK,* natural killer.

positivity to hypothyroidism, including polymorphism in the *MAGI3* gene.[108]

Shared genetic susceptibility accounts for the frequent occurrence of other autoimmune disorders in patients with autoimmune hypothyroidism. Around half of women with Turner syndrome are positive for thyroid autoantibodies and a third develop hypothyroidism.[109] There is also an increase in autoimmune hypothyroidism in children with Down syndrome, which may evolve into Graves disease in some cases.[110]

Nongenetic Risk Factors. Many of the factors that have been identified as increasing the risk for Graves disease (pregnancy, drugs, age, sex, iodine, and irradiation) apply equally to autoimmune thyroiditis. These factors are detailed in Chapter 12 but briefly considered here. Epidemiologic data suggest a strong influence of environmental factors in Hashimoto thyroiditis, as this was a rare disease before the 1950s but it is now one of the most common autoimmune disorders.[111]

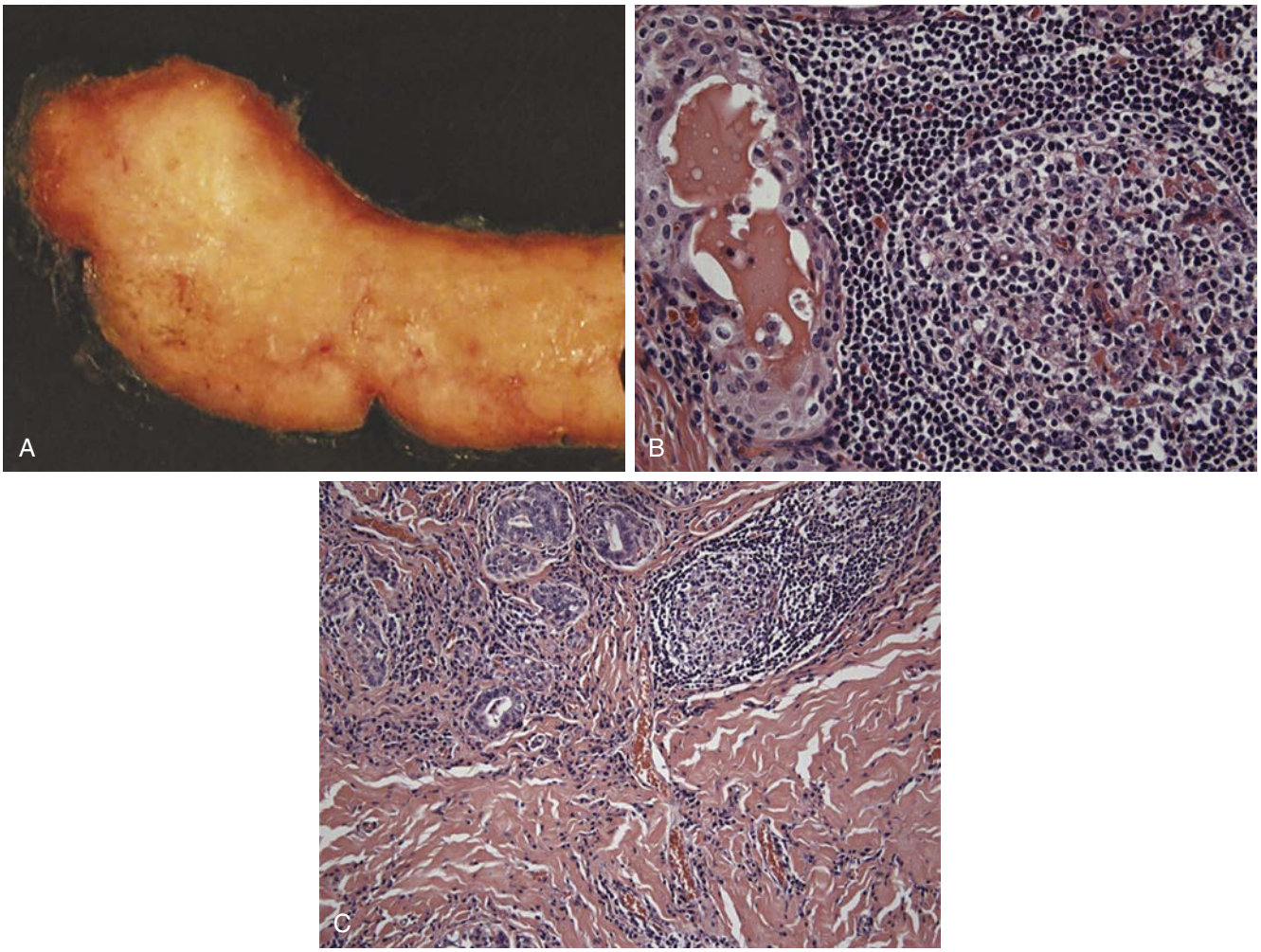
Sex and Pregnancy. The female preponderance of autoimmune hypothyroidism may be due to sex hormones; skewed X-chromosome inactivation has also been proposed as an additional explanation. During pregnancy, fetal tolerance is maintained by changes in immunoregulation that have the coincidental effect of improving thyroid autoimmunity but then lead to postpartum exacerbation of the autoimmune process.[112] This phenomenon results in transient postpartum thyroiditis, a form of painless subacute thyroiditis (see Chapter 12), and in 10% to 50% of these cases permanent hypothyroidism may appear over the next decade.[113] Those women with hypothyroidism and positive TPO antibodies during the phase of postpartum thyroiditis are most at risk of such an outcome.

Iodine and Selenium. An excessive intake of iodine can precipitate autoimmune thyroiditis in susceptible populations.[114] This form of hypothyroidism should be distinguished from direct blockade of a thyroid gland by iodine (the Wolff-Chaikoff effect).[115] Evidence accumulated in animal models suggests that increased iodination of thyroglobulin enhances its immunoreactivity, and iodine may also cause thyroid injury through the generation of reactive oxygen metabolites.[116] There are epidemiologic data to suggest that selenium deficiency exacerbates autoimmune thyroiditis, but trials of selenium supplementation have been inconclusive with regard to clinical benefit.[117,118]

Drugs and Smoking. Treatment of patients with cytokines may precipitate the appearance of autoimmune thyroid disease in the form of Hashimoto thyroiditis or Graves disease (see later).[119] A number of novel anticancer treatments, including TKIs (such as sunitinib), cytotoxic T-lymphocyte–associated protein 4 (CTLA4) blockers (such as ipilimumab), and PD1 blockers (such as pembrolizumab and nivolumab), can also induce autoimmune thyroiditis.[120,121] There is a higher than expected prevalence of autoimmune hypothyroidism in patients treated with lithium. Anthracene derivatives and other chemicals produce autoimmune thyroiditis in animals, but the role of environmental toxins in human disease is poorly studied. Smoking is associated with a decreased risk of autoimmune thyroiditis, but the risk rises temporarily when smoking is stopped.[122] Moderate alcohol consumption is also protective.[123]

Irradiation. Radiation exposure has been shown to induce thyroid autoantibodies and autoimmune thyroid disease in a number of studies. These exposures include radiation from the atomic bomb detonation in Japan[124] and radioactive fallout from the Chernobyl disaster, which was followed by an increase in the prevalence of thyroid autoantibodies in exposed children, with a small overall increase in the prevalence of hypothyroidism 12 to 14 years later.[125] Hodgkin disease survivors have a 17-fold relative risk of developing hypothyroidism, but this may in part be a direct effect of irradiation.[126]

Age. Autoimmune hypothyroidism continues to occur throughout adult life (except in the very elderly whose longevity may be



• **Fig. 13.7** Hashimoto thyroiditis. (A) Gross appearance of a cut section of the thyroid lobe demonstrating the pale color of the tissue due to lymphocytic infiltration, fibrosis, and loss of follicles. (B) Typical histologic appearance illustrating a germinal center, heavy lymphocytic infiltration, and a partially disrupted thyroid follicle. (C) Fibrous variant showing extensive fibrosis and loss of follicles. (A and C, Courtesy Dr. Vania Nosé, Brigham and Women's Hospital, Boston, MA; B, From Nosé V, Asa SL, Erickson LA, et al. *Diagnostic Pathology: Endocrine.* Salt Lake City: Amirsys; 2012.)

associated with superior immunoregulation) so that the prevalence of the disease increases markedly with age.[12,17,87] This feature is similar to other types of autoimmunity and may reflect an increasing loss of tolerance to self.

Infection. There is no direct evidence that infection causes autoimmune thyroiditis in humans, although there is some evidence that the hepatitis C virus may precipitate thyroid disease in susceptible patients.[118] In addition, there is follow-up evidence from patients with painful subacute thyroiditis, following a viral infection (see later), that long-term hypothyroidism occurs in around 15% of patients and some of these cases may have an autoimmune basis.

Clinical Picture. Goiter, the hallmark of classic Hashimoto disease, usually develops gradually and may be found during routine examination or by ultrasonography. On occasion, the thyroid gland enlarges rapidly and, when accompanied by pain and tenderness, may mimic painful subacute thyroiditis (see Chapter 12). Some patients are hypothyroid when first seen. The goiter is generally painless, moderate in size, and firm in consistency and moves freely on swallowing. The surface can be either smooth or nodular. Both lobes are enlarged, but the gland may be asymmetric. The pyramidal lobe may also be enlarged; rarely, adjacent structures, such as the trachea, esophagus, and recurrent laryngeal nerves, may be compressed. Enlargement of regional lymph nodes is unusual.

Other patients with hypothyroidism present without a goiter (atrophic thyroiditis), which is thought to be the end result of autoimmune destruction of the thyroid, although the progression of goitrous Hashimoto thyroiditis to the atrophied state is not commonly seen in the individual patient. The atrophic thyroid is likely the reflection of rapid destruction early in the onset of autoimmune thyroiditis, combined in some patients with TSH receptor antibodies of the blocking variety, although such antibodies may also occur in those with a goiter. Generally the disease tends to progress slowly with an increase in fibrous tissue and loss of thyroid follicular cells. A study of thyroid volume by ultrasound in patients with newly diagnosed autoimmune hypothyroidism found that there was a continuum of thyroid size, with atrophy and goiter representing extremes of the distribution, supporting the idea that these are not distinct entities but part of the same underlying autoimmune process.[127]

Clinically the untreated goiter remains unchanged or enlarges gradually over many years. The manifestations of hypothyroidism vary and often develop over many years in patients who are initially euthyroid. Thyroid lymphoma occurs almost exclusively in patients with underlying Hashimoto thyroiditis and should be suspected if there is rapid and sometimes painful enlargement of the thyroid gland.[128] As mentioned earlier, the presence of coexistent Hashimoto thyroiditis may be a favorable prognostic factor in patients with papillary carcinoma, but the risk of papillary carcinoma is probably not increased in Hashimoto thyroiditis.[129]

Occasionally hyperthyroidism due to Graves disease develops in patients with Hashimoto thyroiditis. In other patients with early autoimmune thyroiditis, transitory thyrotoxicosis (painless thyroiditis with thyrotoxicosis) occurs as the result of thyroid cell destruction. In such cases, evidence of ongoing thyroid hyperfunction is lacking because the thyroid RAIU is depressed. As described earlier, 50% of women with thyroid autoantibodies who are euthyroid in the first trimester of pregnancy develop postpartum thyroiditis, accompanied by transient thyrotoxicosis, hypothyroidism, or fluctuation from one state to the other.[130]

Laboratory Tests. The results of the common tests of thyroid function depend on the stage of the disease (see Table 13.2). Rarely, the tests may suggest thyrotoxicosis with a suppressed TSH and elevated serum T_4 and T_3 levels, due to either release of stored thyroid hormone as a result of rapid tissue destruction or the relative overproduction of autoantibodies, which stimulate rather than block the TSH receptor (sometimes called hashitoxicosis). In the latter case, the RAIU may be increased, whereas it is decreased if there is tissue destruction. Typically, patients with Hashimoto thyroiditis present with a goiter, and blood tests show a normal or slightly raised TSH, with normal serum T_4 and T_3 levels. As tissue destruction continues, the TSH level rises further, but the ability of the thyroid to respond to TSH diminishes, and the RAIU and serum T_4 level decline to subnormal values, resulting in overt hypothyroidism. This is the typical biochemical finding in atrophic thyroiditis, in which there is no goiter to alert the patient or physician to the underlying disorder. The serum T_3 level remains normal until late in the disease process, reflecting maximal stimulation of the failing thyroid by the increased serum TSH. The early phase of the foregoing sequence, when the serum TSH is increased but T_4 and T_3 are still normal, is termed *subclinical hypothyroidism* (see Table 13.2).

The diagnosis of autoimmune hypothyroidism is confirmed by the presence of thyroid autoantibodies in the serum, usually in high levels. TPO and thyroglobulin autoantibodies occur in roughly similar frequencies; the presence of both autoantibodies is twice as frequent as the isolated individual autoantibodies.[131] Thyroid autoantibodies may be absent in rare patients due to assay insensitivity or the occurrence of an entirely intrathyroidal autoimmune process. Sometimes part of a gland with autoimmune thyroiditis may look and feel like a firm thyroid nodule, and ultrasonography or even aspiration biopsy should be performed to confirm the diagnosis.

Differential Diagnosis. Differentiation of autoimmune hypothyroidism from other forms of hypothyroidism is facilitated by the demonstration that high levels of thyroid autoantibodies occur more commonly than in other thyroid disorders. The frequent coexistence of hypothyroidism and Hashimoto thyroiditis serves to distinguish this disease from nontoxic goiter and thyroid neoplasia.

Differentiation of a euthyroid Hashimoto goiter from a multinodular goiter is often difficult without ultrasonography, and diffuse nontoxic goiter tends to be softer than that of Hashimoto thyroiditis. Ultrasound examination typically reveals a diffuse and patchy heterogeneous echotexture or hypoechoic micronodules with echogenic septations in Hashimoto thyroiditis. In adolescents, differentiation of Hashimoto goiter from diffuse nontoxic goiter is even more difficult because in this age group Hashimoto thyroiditis may not be accompanied by such high levels of thyroid autoantibodies. The presence of well-defined nodules usually distinguishes nontoxic multinodular goiter from Hashimoto thyroiditis.

Differentiation between euthyroid Hashimoto thyroiditis and thyroid carcinoma can sometimes be made on clinical grounds, but an ultrasound examination and aspiration biopsy are necessary in any case in which there is uncertainty. Lymphoma must always be excluded if there is a sudden change in a known Hashimoto goiter; core needle biopsy or open surgical biopsy may be required for final diagnosis. Thyroid carcinoma usually occurs as a solitary nodule that is firm or hard, and the gland may be fixed to adjacent structures. Compression of the recurrent laryngeal nerve with hoarseness is virtually pathognomonic of thyroid carcinoma but occurs late in the cancer progression. A history of a recent enlargement of the goiter is more common in thyroid malignancies (either carcinoma or lymphoma) than in Hashimoto thyroiditis. Enlargement of regional lymph nodes also suggests thyroid malignancy but can rarely occur in autoimmune thyroiditis.

Treatment. In euthyroid patients with Hashimoto thyroiditis, no treatment is required because the goiter is usually asymptomatic. Levothyroxine treatment may be indicated in patients when the goiter presses on adjacent structures or is unsightly, and it is most effective in goiters of recent onset. The aim is to keep the TSH level in the lower half of the reference interval. In long-standing goiter, treatment with thyroid hormone is usually ineffective, possibly because of fibrosis. Rarely, the goiter may be painful, and this symptom may respond to levothyroxine therapy. Glucocorticoids, such as prednisolone, are usually ineffective. Surgery may be justified if symptoms or unsightly enlargement persists after a trial of levothyroxine therapy.

Replacement doses of thyroid hormone should be given when hypothyroidism is present, appropriate to the degree of hormone deficiency (see later). There may be a spontaneous return to euthyroidism in up to 10% of patients after starting levothyroxine, associated in some cases with the disappearance of TSH receptor blocking antibodies. However, it has not been established that such remissions are durable and there is no need to routinely stop levothyroxine once it has been started. It has recently been suggested that prednisolone treatment can reverse the hypothyroidism of IgG4-related thyroiditis, but the long-term outcome in such cases is not yet known.[132]

Iodine Deficiency (Endemic Goiter)

The term *endemic goiter* denotes any goiter occurring in a region where goiter is prevalent.[132] As mentioned, endemic goiter almost always occurs in areas of environmental iodine deficiency.[133] Although this condition is estimated to affect more than 200 million people throughout the world and is of major public health significance, it is most common in mountainous areas, such as the Alps, Himalayas, and Andes, or in the Great Lakes and Mississippi Valley regions of the United States, owing to the depletion of iodine consequent to the persistent glacial run-off in these regions.

The causative role of iodine deficiency in the genesis of endemic goiter is supported by the inverse correlation between the iodine content of soil and water and the incidence of goiter, the kinetics

of iodine metabolism in patients with the disorder, and a decrease in incidence after iodine prophylaxis. The latter accounts for its absence in the population residing in the Great Plains region of the United States.

The occurrence of endemic goiter can vary, even within an area of known iodine deficiency; the roles of dietary minerals or naturally occurring goitrogens and of pollution of water supplies have been suggested in instances of this type.[134] For example, in the Cauca Valley of Colombia, waterborne goitrogens have been implicated, and in many areas of endemic iodine deficiency, consumption of cassava meal, which gives rise to thiocyanate, aggravates the iodine-deficient state by inhibiting thyroid iodide transport. Familial clustering of goiters within iodine-insufficient areas, usually with an autosomal dominant inheritance, suggests an important genetic component.[135]

Most abnormalities in iodine metabolism in patients with endemic goiter are consistent with the expected effects of iodine deficiency (see Chapter 11 regarding iodine metabolism). Thyroid iodide clearance rates and RAIU are increased in proportion to the decrease in the urinary excretion of stable iodine. The absolute iodine uptake is normal or low. In areas of moderate iodine deficiency, the serum T_4 concentration is usually in the lower range of normal; in areas of severe deficiency, however, values are decreased. Nevertheless, most patients in these areas do not appear to be in a hypothyroid state because of an increase in the synthesis of T_3 at the expense of T_4 and because of an increase in the activity of thyroidal D1 and D2.[84] TSH levels are typically in the upper range of normal.

The incidence and severity of endemic goiter and the metabolic state of the goitrous patient depend mainly on the degree of iodine deficiency. In the absence of hypothyroidism, the effects of the goiter are mainly cosmetic. When the goiter becomes nodular, however, hemorrhage into a nodule may cause acute pain and swelling, mimicking painful subacute thyroiditis or neoplasia. The goiter may also compress adjacent structures, such as the trachea, esophagus, and recurrent laryngeal nerves. The borderline nature of the iodine supply in many countries of Western Europe is exemplified by the development of compensatory maternal and fetal goiter during pregnancy due to the increased requirement for thyroid hormone during gestation.[133]

The incidence of endemic goiter has been greatly reduced in many areas by the introduction of iodized salt.[133] In the United States, table salt is enriched with potassium iodide to a concentration of 0.01%, which, if the intake of salt is average, would provide an iodine intake of approximately 150 to 300 μg/day, the desired amount in an adult (see Table 11.1). The use of iodine-containing flour in bread products and iodized salt in commercially produced food has been markedly reduced.[136] The iodine content of bread and infant formula is variable within a given product and often does not match the measured content.[136] As mentioned, iodine intake in the United States has been decreasing in recent decades, likely due to reduced iodine in commercial food products, although iodine intake has now stabilized. Pregnant women, however, remain a susceptible population because of their increased iodine requirements.[134] Most prescription prenatal vitamins do not contain iodine.[137] An annual injection of iodized oil is another effective means of administering iodine, and endemic goiter can be treated by the addition of iodine to communal drinking water.

Administration of iodine has little if any effect on a long-standing endemic goiter, but it causes the early endemic hyperplastic goiter of iodine deficiency to regress. Similarly, thyroid hormone usually has no effect on long-standing goiter or on established mental or skeletal changes, but it should be given in full-replacement doses if there is evidence of hypothyroidism. This is of paramount importance in pregnant women. Surgical treatment is indicated if the adjacent structures are compressed or if the goiter is either very large or enlarging rapidly.

Endemic Cretinism

Endemic cretinism is a developmental disorder that occurs in regions of severe endemic goiter.[133] Both parents of an endemic cretin are usually goitrous, and in addition to the features of sporadic cretinism described earlier, endemic cretins often have deafmutism, spasticity, motor dysfunction, and abnormalities in the basal ganglia demonstrable by magnetic resonance imaging.

Three types of cretins can be discerned: (1) hypothyroid cretins, (2) neurologic cretins, and (3) cretins with combined features of the two. The pathogenesis of neurologic cretinism is obscure but may be due to severe thyroid hormone deficiency during a critical early phase of central nervous system development in utero.[44] Some cretins are goitrous, but the thyroid may also be atrophic, possibly as a consequence of exhaustion atrophy from continuous overstimulation or the lack of iodine.

Iodide Excess

Goiter and hypothyroidism, either alone or in combination, are sometimes induced by chronic administration of large doses of iodine in either organic or inorganic form (see Table 11.7).[133,138] Iodide-induced goiter was formerly seen in patients with chronic respiratory disease who were given potassium iodide as an expectorant. The development of iodide goiter has also been reported after a single administration of radiographic contrast medium from which iodide is released slowly over a long period and may also occur during amiodarone administration. Iodide goiter without hypothyroidism may occur endemically, such as on the island of Hokkaido, Japan, where seaweed products are consumed in large quantities.

From an analysis of reported cases and from the fact that only a small percentage of patients who receive iodides chronically develop goiter, it appears that the disorder evolves on a background of underlying thyroid dysfunction.[138] Categories of susceptible individuals include the following: patients with Hashimoto disease, patients with Graves disease especially after treatment with radioiodine, and patients with cystic fibrosis.

Among these groups, many individuals display a positive iodide-perchlorate discharge test, indicating a defect in the thyroidal organic iodine-binding mechanism (see Chapter 11 regarding iodine metabolism). However, intrinsic thyroid disease need not be present because a propensity to develop iodide goiter and hypothyroidism has also been demonstrated in patients who have undergone hemithyroidectomy for a solitary thyroid nodule in whom the remaining lobe was histologically normal.[89] In these patients, as in those with Hashimoto disease or Graves disease studied prospectively, individuals with the highest basal serum TSH concentrations, even within the normal range, were those who developed iodide goiter. Iodinated contrast material, amiodarone, and povidone-iodine are common sources.[138]

Goiter and hypothyroidism commonly occur in newborns of women given large quantities of iodine during pregnancy, and death from neonatal asphyxia has been reported (see Fig. 11.11). In such cases, the mother is usually free from goiter. Pregnant women should not receive large doses of iodine (>1 mg/day) over prolonged periods (>10 days), especially near term. Maternal

amiodarone therapy causes thyroidal dysfunction in up to 20% of newborns.[133] It is not known whether iodide goiter in newborns results from an inherent hypersensitivity of the fetal thyroid or from the fact that the placenta concentrates iodide several-fold, or both.

As discussed earlier (see Chapter 11, "Regulation of Thyroid Function"), large doses of iodine cause an acute inhibition of organic binding that abates in the normal individual, despite continued iodine administration (acute Wolff-Chaikoff effect and escape).[139] Iodide goiter appears to result from a more pronounced inhibition of organic binding and the failure of the escape phenomenon. As a consequence of decreased hormone synthesis and the consequent increase in TSH, iodide transport is enhanced. Because inhibition of organic binding is a function of the intrathyroidal concentration of iodide, a vicious circle, augmented by this increase in serum TSH, is set in motion.

The disorder usually appears as a goiter with or without hypothyroidism, although in rare instances iodine may produce hypothyroidism unaccompanied by goiter. Usually the thyroid gland is firm and diffusely enlarged, often greatly so. Histopathologic examination reveals intense hyperplasia. The FT_4 concentration is low, TSH concentration is increased, and the 24-hour urinary iodine excretion and the serum inorganic iodide concentration are increased. The disorder regresses after iodine is withdrawn. Thyroid hormone may also be given to relieve severe symptoms.

Drugs Blocking Thyroid Hormone Synthesis or Release, Causing Goiter Formation

Ingestion of compounds that block thyroid hormone synthesis or release may cause goiter with or without hypothyroidism. Apart from the agents used in the treatment of hyperthyroidism, antithyroid agents may be encountered either as drugs for the treatment of disorders unrelated to the thyroid gland or as natural agents in foodstuffs.

Goiter with or without hypothyroidism can occur in patients given lithium, usually for bipolar manic-depressive psychosis.[140] Like iodide, lithium inhibits thyroid hormone release, and in high concentrations it can inhibit organic binding reactions. At least acutely, iodide and lithium act synergistically in the latter respect. The mechanisms underlying the several effects of lithium are uncertain; what differentiates patients who develop thyroid disease during lithium therapy from those who do not is also unclear. A promoting effect on underlying autoimmune thyroiditis may be at least one factor because many patients with this combination have autoimmune thyroid disease.

Other drugs that occasionally produce goitrous hypothyroidism include para-aminosalicylic acid, phenylbutazone, aminoglutethimide, and ethionamide. Like the thionamides, these drugs interfere with both the organic binding of iodine and perhaps in later steps in hormone biosynthesis. Although soybean flour is not an antithyroid agent, soybean products in feeding formulas formerly resulted in goiter in infants by enhancing fecal loss of hormone, which, together with the low iodine content of soybean products, produced a state of iodine deficiency. Feeding formulas containing soybean products are now enriched with iodine.

Cigarette smoking reduces the hypothyroidism in patients with underlying autoimmune thyroid disease, although the risk is transiently increased if smoking is stopped.[122] Thiocyanate, hydroxypyridine, and benzopyrene derivatives found in cigarette smoke may also interfere with thyroid hormone action.[141]

Both the goiter and the hypothyroidism usually subside after the antithyroid agent is withdrawn. If continued administration of pharmacologic goitrogens is required, however, replacement therapy with thyroid hormone causes the goiter to regress.

Goitrogens in Foodstuffs or as Endemic Substances or Pollutants

Antithyroid agents also occur naturally in foods. They are widely distributed in the family Cruciferae or Brassicaceae, particularly in the genus *Brassica,* including cabbages, turnips, kale, kohlrabi, rutabaga, mustard, and various plants that are not eaten by humans but that serve as animal fodder.[142] It is likely that some thiocyanate is present in such plants (particularly cabbage).[143] Cassava meal, a dietary staple in many regions of the world, contains linamarin, a cyanogenic glycoside, the preparation of which leads to the formation of thiocyanate. Ingestion of cassava can accentuate goiter formation in areas of endemic iodine deficiency. Except for thiocyanate, dietary goitrogens influence thyroid iodine metabolism in the same manner as do the thionamides, which they resemble chemically; their role in the induction of disease in humans is uncertain. Waterborne, sulfur-containing goitrogens of mineral origin are believed to contribute to the development of endemic goiter in certain areas of Colombia.

A number of synthetic chemical pollutants have been implicated as a cause of goitrous hypothyroidism, including polychlorinated biphenyls and resorcinol derivatives.[144] Perchlorate has also been noted in high concentrations in geographic regions in which explosives and rocket fuel were made. Perchlorate has been detected in water, food, and breast milk, although the amount does not appear to be sufficient to disrupt thyroid function. In an area of Chile with a high level of natural perchlorate contamination in the water, thyroid function in pregnant women was not different from that in a region with no perchlorate, although iodine intake is quite high in this area.[145]

Cytokines

Patients with chronic hepatitis C or various malignancies may be given interferon-α or interleukin 2.[119,146] Such patients may experience hypothyroidism, which is often a transient destructive thyroiditis and associated with an initial thyrotoxic phase but in other cases may persist. Graves disease with hyperthyroidism may also develop, and ablative therapy may be required to treat this condition. Women and those who have positive TPOAb prior to treatment are at higher risk for these complications and should be monitored especially carefully during and after a course of treatment with either of these cytokines.

Congenital Causes of Goiter

Inherited defects in hormone biosynthesis are rare causes of goitrous hypothyroidism and account for only about 10% to 15% of the 1 in 3000 newborns with congenital hypothyroidism.[18,147,148] In most instances, the defect appears to be transmitted as an autosomal recessive trait. Individuals with goitrous hypothyroidism are believed to be homozygous for the abnormal gene, whereas euthyroid relatives with slightly enlarged thyroids are presumably heterozygous. In the latter group, appropriate functional testing may disclose a mild abnormality of the same biosynthetic step that is defective in the homozygous individual. In contrast with nontoxic goiter, which is more common in females than in males, these defects, as a group, affect females only slightly more commonly than males.

Although goiter may be present at birth, it usually does not appear until several years later. Therefore the absence of goiter in a child with functioning thyroid tissue does not exclude

the presence of hypothyroidism. The goiter is initially diffusely hyperplastic, often intensely so, suggesting papillary carcinoma, but eventually it becomes nodular. In general, the more severe the biosynthetic defect, the earlier the goiter appears, the larger it is, and the greater the likelihood of early development of hypothyroidism or even cretinism.[147] Five specific defects in the pathways of hormone synthesis have been identified.

Iodide Transport Defect. An iodide transport defect, a result of impaired iodide transport by the NIS protein mechanism, is rare and is reflected in defective iodide transport in the thyroid, salivary gland, and gastric mucosa.[149,150] Some mutations in such patients produce reduced activity, and others completely inactivate NIS by preventing the protein from being transported and inserted into the membrane. With the milder NIS mutations, administration of iodide raises the plasma and intrathyroidal iodide concentration, permitting the synthesis of normal quantities of hormone.

Defects in Expression or Function of Thyroid Peroxidase. TPO is a protein that is required for normal synthesis of iodothyronines. Quantitative or qualitative abnormalities of TPO have been identified in 1 in 66,000 infants in the Netherlands. The most common of the 16 mutations identified in 35 families was a GGCC insertion in exon 8, leading to a premature stop codon.[151]

Pendred Syndrome. The most common presentation in patients with Pendred syndrome is a defect in iodine organification accompanied by sensory nerve deafness.[152] The abnormality is in the *PDS* gene encoding pendrin, which is involved in the apical secretion of iodide into the follicular lumen (see Fig. 11.1 and the discussion regarding iodine metabolism in Chapter 11). Thyroid function is only mildly impaired in this disorder.

Defects in Thyroglobulin Synthesis. Defects in the synthesis of thyroglobulin due to genetic causes are rare, having been identified only in a small number of families with congenital hypothyroidism.[147] Some defects lead to premature termination of translation, whereas another defect causes deficiency in endoplasmic reticulum processing of the thyroglobulin molecule. The complex regulation and the huge size of this gene make screening for mutations a difficult task, and considerable work is still required to unravel the extent of the defects in this gene.

Iodotyrosine Dehalogenase Defect. The pathogenesis of goiter and hypothyroidism in the iodotyrosine dehalogenase defect is complex. The major abnormality is an impairment of both intrathyroidal and peripheral deiodination of iodotyrosines, presumably because of the dysfunction of the iodotyrosine *DEHAL1B* gene (see discussion regarding iodide metabolism in Chapter 11).[153,154]

As a consequence of intense thyroid stimulation and lack of intrathyroidal recycling of iodide derived from dehalogenation, iodide is rapidly accumulated by the thyroid gland and is rapidly released; monoiodotyrosine (MIT) and diiodotyrosine (DIT) are elevated in plasma and, together with their deaminated derivatives, in the urine. Hypothyroidism is presumed to result from the loss of large quantities of MIT and DIT in the urine and to secondary iodine deficiency. The goiter and hypothyroidism are relieved by administration of high doses of iodine.

Thyroid Infiltration Causing Hypothyroidism and Goiter

A number of infiltrative or fibrosing conditions may cause hypothyroidism. Some are often associated with goiter, such as Riedel struma (see later).[155] Others, such as amyloidosis,[156] hemochromatosis,[157] or scleroderma,[158] may not be. Although the other manifestations of these conditions are usually obvious and hypothyroidism is only a complication, the presence of significant hypothyroidism without evidence of autoimmune thyroiditis should lead to a consideration of these rare causes of this condition.

Postablative Hypothyroidism

Postablative hypothyroidism is a common cause of thyroid failure in adults. One type follows total thyroidectomy usually performed for thyroid carcinoma. Although functioning remnants may be present, as indicated by foci of radioiodine accumulation, hypothyroidism invariably develops. Another etiologic mechanism is subtotal resection of the diffuse goiter of Graves disease or multinodular goiter. Its frequency depends on the amount of tissue remaining, but continued autoimmune destruction of the thyroid remnant in patients with Graves disease may be a factor because some studies suggest a correlation between the presence of circulating thyroid autoantibodies in thyrotoxicosis and the development of hypothyroidism after surgery. Hypothyroidism can be manifested during the first year after surgery, but as with postradioiodine hypothyroidism, the incidence increases with time. In some patients, mild hypothyroidism appears during the early postoperative period and then may occasionally remit, as also occurs after radioiodine treatment.

Hypothyroidism after destruction of thyroid tissue with radioiodine is common and is the one established disadvantage of this form of treatment for hyperthyroidism in adults. Its frequency is determined in large part by the dose of radioiodine and radioiodine uptake, but it is also influenced by other factors, including age, thyroid gland size, magnitude of thyroid hormone elevations, and use of antithyroid drugs.[159] The incidence of postradioiodine hypothyroidism increases with time, approaching 100%. Although the FT_4 is low in patients with postablative hypothyroidism, serum TSH levels may be anomalously low for several months after either surgical or ^{132}I-induced hypothyroidism if TSH synthesis has been suppressed for a long period prior to treatment.

Primary atrophic thyroid failure may also develop in patients with Hodgkin disease after treatment with mantle irradiation or after high-dose neck irradiation for other forms of lymphoma or carcinoma.[126] Surgical, radioiodine, or external beam therapy may also lead to a state of subclinical hypothyroidism (see Table 13.2).

Thyroid Agenesis or Dysplasia

Developmental defects of the thyroid are often responsible for the hypothyroidism that occurs in 1 in 3000 newborns.[18] These defects may take the form of complete absence of thyroid tissue or failure of the thyroid to descend properly during embryologic development. Thyroid tissue may then be found anywhere along its normal route of descent from the foramen cecum at the junction of the anterior two-thirds and posterior third of the tongue (lingual thyroid) to the normal site or below. Absence of thyroid tissue or its ectopic location can be ascertained by scintiscanning.

As indicated, a number of proteins are known to be crucial for normal thyroid gland development.[18] These proteins include the thyroid-specific transcription factor PAX8 as well as thyroid transcription factors 1 and 2 (TTF1 and TTF2, respectively). It might be anticipated that defects in one or more of these proteins may explain abnormalities in thyroidal development. These abnormalities have been identified in several patients with *PAX8* mutations, and a mutation in the human *TTF2* gene was associated with

thyroid agenesis, cleft palate, and choanal atresia. Despite a specific search, no mutations have been found in the *TTF1* gene in infants with congenital hypothyroidism.

Thyroid Aplasia Due to Thyrotropin Receptor Unresponsiveness

Several families exist in which thyroid hypoplasia, high TSH concentrations, and a low FT_4 level are associated with loss-of-function mutations in the TSH receptor.[160] The thyroid gland of these patients was in the normal location but did not trap pertechnetate (TcO_4^-). Somewhat surprisingly, thyroglobulin levels were still detectable. The molecular details of these patients are still under study.

A second type of abnormality that may cause TSH unresponsiveness is a mutation in the G_s protein that occurs in pseudohypoparathyroidism type 1A. These patients have inactivating mutations in the α-subunit of the G_s protein and, consequently, mild hypothyroidism.[161] Other as yet unexplained patients with elevated TSH levels and hypothyroidism in which the molecular nature of the defect has not been defined have been reported.[162]

Transient Hypothyroidism

Transient hypothyroidism is defined as a period of reduced FT_4 with suppressed, normal, or elevated TSH levels that are eventually followed by a euthyroid state. This form of hypothyroidism usually occurs in the clinical context of a patient with painful or painless subacute thyroiditis.[130] These conditions are reviewed in detail in Chapter 12.

The patient reports mild to moderate symptoms of hypothyroidism of short duration, and serum TSH concentrations are typically elevated, although not greatly so. The patient often has a preceding episode of symptoms consistent with mild or moderate thyrotoxicosis. If these symptoms cannot be elucidated from the history, it may be difficult to distinguish such patients from those with a permanent form of hypothyroidism. In the early phases of post-thyroiditis hypothyroidism, TSH concentrations may still be suppressed even though the FT_4 is low because of the delayed recovery of pituitary TSH synthesis, such as in patients with Graves disease or with toxic nodules who have undergone surgery and who have experienced rapid relief of hypothyroidism (see Table 13.2). In that situation, the TSH response to hypothyroidism may be suppressed for many months; in post-thyroiditis hypothyroidism, this period is rarely longer than 3 to 4 weeks.

A significant fraction (50%) of women with autoimmune thyroiditis but normal thyroid function have episodes of hypothyroidism during the postpartum period.[130] In some, the preceding thyrotoxicosis is relatively asymptomatic, which can make an accurate clinical diagnosis difficult. Patients who have had an episode of typical painful subacute thyroiditis, with pain, tenderness, and thyrotoxicosis, are not difficult to recognize.

Diagnostic evaluation should include a determination of TSH, FT_4, and TPOAb. Negative or low antibodies argue strongly for a nonautoimmune cause. This is significant, in that it may be possible for the patient to be treated only temporarily for hypothyroidism. In such patients, a trial of a lower levothyroxine dosage after 3 to 6 months may reveal that thyroid function has recovered (see Fig. 13.5). This may also occur in patients with hypothyroidism that follows painless subacute thyroiditis (e.g., in the postpartum period), but it is somewhat less likely to occur because of the underlying progressive nature of the autoimmune thyroiditis.

In patients with hypothyroidism due to painful subacute thyroiditis, the thyroid gland is usually relatively small and atrophic. In patients with hypothyroidism that follows an episode of painless subacute thyroiditis, the gland is usually slightly enlarged and somewhat firm, reflecting the underlying scarring and infiltration associated with that condition.

Consumptive Hypothyroidism

Consumptive hypothyroidism is the term given to an unusual cause of hypothyroidism that has been identified in infants with visceral hemangiomas or related tumors.[7,163] The first patient reported with this syndrome presented with abdominal distention caused by a large hepatic hemangioma with respiratory compromise secondary to upward displacement of the diaphragm. However, clinical signs suggested hypothyroidism, which was confirmed by finding a markedly elevated TSH level and undetectable T_4 and T_3 levels. The infant's response to an initial intravenous infusion of liothyronine (T_3) was transient, leading to the decision to use parenteral thyroid hormone replacement to relieve the severe hypothyroidism. The accelerated degradation of thyroid hormone was apparent from the fact that 96 μg of liothyronine plus 50 μg of levothyroxine were required to normalize the TSH level. The equivalent dosage of levothyroxine alone is roughly nine times that ordinarily required for treatment of infants with congenital hypothyroidism. The infant succumbed to complications of the hemangioma, and a postmortem tumor biopsy showed D3 activity in the tumor at levels eightfold higher than those normally present in term placenta. The serum reverse T_3 was extremely elevated (400 ng/dL), and the serum thyroglobulin was greater than 1000 ng/mL, indicating the presence of a highly stimulated thyroid gland. Retrospective search revealed two other patients with similar pathophysiology in whom the cause of the hypothyroidism had not been recognized. Significant D3 expression has subsequently been noted in all proliferating cutaneous hemangiomas studied to date. The cutaneous hemangiomas of infancy, although they express D3, are not associated with hypothyroidism owing to their small size. Most infantile hemangiomas involute with propranolol therapy, but such patients must also receive adequate doses of thyroid hormone to prevent the permanent neurologic complications associated with untreated hypothyroidism during this critical phase of neurologic development.[164] Subsequent reports have identified a similar syndrome in adults, including a patient with an epithelioid hemangioendothelioma and an individual with a fibrous tumor as well as extensive gastrointestinal stromal tumors (GISTs).[165] Some of these tumors express D3, or this deiodinase may be induced during treatment with TKIs (see later).[166–169]

Defects in Conversion of Thyroxine to Triiodothyronine

The enzymes that convert the precursor T_4 to the active form, T_3, are 5′-deiodinase 1 (D1) and D2, both of which contain selenocysteine in their active site.[84] A stem loop structure in the 3′-untranslated region of the mRNA, termed a *SECIS element,* directs insertion of selenocysteine at the UGA codon, rather than allowing it to function as a stop codon. Defects in a SECIS-binding protein (selenocysteine insertion sequence–binding protein 2 [SECISBP2]) were found in two families with an elevated FT_4, reduced T_3, and elevated TSH.[170] Affected individuals have growth retardation, compared with unaffected family members.

Polymorphisms in genes associated with thyroid hormone metabolism have been associated with patterns of thyroid function studies as well as obesity. The D2 polymorphism resulting in a change from threonine (Thr) to alanine (Ala) at codon 92 (Thr92Ala) has been associated with obesity, reduced glucose disposal, and lower D2 activity in skeletal muscle.[171] This

polymorphism also has a higher frequency in groups with a high incidence of obesity and type 2 diabetes such as Mexican Americans and Pima Indians.[171] The D2 polymorphism has also been associated with a distinct pattern of gene expression in the cortex, associated with inflammation and chronic disease, linked to prolonged half-life of the D2 protein in the Golgi.[172]

Hypothyroidism Due to Drug-Induced Thyroid Destruction

Thyroid inflammation or activation of autoimmune thyroid destruction has been associated with a number of drugs.[146,173] The TKIs, such as sunitinib, have been associated with a high incidence of hypothyroidism due to thyroid destruction.[166,167] Sunitinib is used to treat renal cell carcinoma and GISTs and inhibits multiple tyrosine protein kinases, including KIT, PDGF, VEGF, and RET. An abnormal TSH was found in 62% of patients receiving sunitinib who were followed for 37 weeks.[168] Patients studied by ultrasound demonstrated no thyroid tissue. Although 40% of hypothyroid patients initially had a suppressed TSH, suggesting thyroiditis, the long-term course was most consistent with sunitinib-induced follicular cell apoptosis. Thus such patients require repeated thyroid function testing. These agents have now been shown to slow disease progression in advanced thyroid cancer unresponsive to radioiodine but may increase thyroid hormone requirements due to increased D3 expression.[174]

Central Hypothyroidism

Central hypothyroidism is due to TSH deficiency caused by either acquired or congenital hypothalamic or pituitary gland disorders (see Chapters 8 and 9). The causes of TSH deficiency may be classified as those of pituitary (*secondary* hypothyroidism) and hypothalamic (*tertiary* hypothyroidism) origins, but this distinction is not necessary in the initial separation of primary from central hypothyroidism.

In many cases, hyposecretion of TSH is accompanied by decreased secretion of other pituitary hormones, with the result that evidence of somatotroph, gonadotroph, and corticotroph failure is also present. Hyposecretion of TSH as the sole demonstrable abnormality (monotropic deficiency) is less common but does occur in both acquired and congenital forms. Hypothyroidism due to pituitary insufficiency varies in severity from instances in which it is mild and overshadowed by features of gonadal and adrenocortical failure to those in which the features of the hypothyroid state are predominant. Because a small but significant fraction of thyroid gland function is independent of TSH (~10–15%), hypothyroidism due to central causes is less severe than primary hypothyroidism.

The causes of central hypothyroidism are both acquired and congenital. The general subject is discussed in Chapters 8 and 9, and those causes with relatively specific thyroid-related deficiencies are mentioned here for completeness. In addition to pituitary tumors, hypothalamic disorders, and the like, an unusual cause of secondary hypothyroidism occurs in individuals given bexarotene (a retinoid X receptor [RXR] agonist) for T-cell lymphoma.[175,176] This drug suppresses the activity of the human TSH β-subunit promoter in vitro. Serum T_4 concentrations are reduced about 50%, and patients experience clinical benefit from thyroid hormone replacement. Dopamine, dobutamine, high-dose glucocorticoids, or severe illness may suppress TSH release transiently, leading to a pattern of thyroid hormone abnormalities suggesting central hypothyroidism.[176] As discussed earlier (see Chapter 11 discussion regarding changes in thyroid function during severe illness), this severe state of hypothalamic-pituitary-thyroid suppression is a manifestation of stage 3 illness (see Table 11.10). Although these agents may be expected to have similar effects when given long term, they do not, nor does somatostatin have a similar effect when given for acromegaly, although it does block the response of TSH to TRH and it has been administered to patients with thyrotropin-secreting pituitary adenomas.[177]

Congenital defects in either the stimulation or the synthesis of TSH or in its structure have been identified as rare causes of congenital hypothyroidism[18] and include the consequences of defects in several of the homeobox genes, including *POU1F1* (formerly termed *Pit1*), *PROP1,* and *HESX1.* The last gene encodes a factor necessary for the development of the hypothalamus, pituitary, and olfactory portions of the brain. Defects in *POU1F1* and *PROP1* cause hereditary hypothyroidism usually accompanied by deficiencies in growth hormone and prolactin.[178] One patient has been identified with a familial defect in the TRH receptor gene.[179] All of these conditions are associated with the typical pattern of central hypothyroidism, a reduced FT_4, and inappropriately low TSH.

Structural defects in TSH have also been described. They include those with a mutation in the CAGYC peptide sequence of the β-subunit, thought to be necessary for its association with the α-subunit,[180] and defects that produce premature termination of the TSH β-subunit gene.[181] As mentioned, some of these abnormalities may be associated with elevations in TSH, suggesting the diagnosis of primary hypothyroidism, but the TSH molecule is immunologically, but not biologically, intact.

Resistance to Thyroid Hormone

The clinical manifestations of resistance to thyroid hormone depend on the nature of the mutation.[6,11,182] The majority of patients with RTH have a mutation in the gene encoding the thyroid hormone receptor β (*TRβ* gene, *THRB*) that interferes with the capacity of that receptor to respond normally to T_3, usually by reducing its T_3-binding affinity (see Chapter 2). A small number of individuals have been identified with *TRα* gene *(THRA)* mutations, referred to as RTHα1,[183] who differ in significant ways from patients with RTH due to *THRB* mutations.[184–186]

RTH is probably produced by the heterodimerization of the mutant *TRβ* with RXR or homodimerization with a normal *TRβ* or *TRα*. These mutant *TRβ*-containing dimers compete with wild-type TR-containing dimers for binding to the thyroid hormone response elements (TREs) of thyroid hormone–dependent genes.[182] Because these complexes bind corepressor molecules that cannot be released in the absence of T_3 binding, genes containing these TREs are more repressed than they would be normally at the prevailing concentrations of circulating thyroid hormones (see Chapter 2).[6] Receptors that contain mutations in the activation domain may have a combination of both decreased affinity for T_3 and impaired activating potential.

Thus the mutant *TRβ* complex can interfere with the function of the wild-type TRs, producing a pattern termed *dominant negative inhibition* with an autosomal dominant pattern of inheritance. At least 400 families have been identified with this condition, and there are probably many more unreported cases. The gene frequency estimate is about 1:50,000 for mutations in *TRβ* and the study of the function of the mutant receptors in this disorder has provided valuable insights into the mechanism of thyroid hormone action.[182] The frequency of *TRα* mutations is probably much lower but undefined.

Patients with RTH usually are recognized because of thyroid enlargement, which is present in about two-thirds of these

individuals. Patients usually report a mixture of symptoms of hyperthyroidism and hypothyroidism. With respect to the heart, palpitations and tachycardia are more common than a reduced heart rate; however, patients may also demonstrate growth retardation and retarded skeletal maturation.[187] This has been attributed to the fact that thyroid hormone effects in the heart and bone appear to be primarily dependent on *TRα* rather than *TRβ*, whereas the hypothalamic-pituitary axis is primarily regulated through *TRβ*.

Abnormalities in neuropsychologic development exist, with an increased prevalence of attention-deficit/hyperactivity disorder, which is found in approximately 10% of such individuals.[187] Other neuropsychologic abnormalities have also been described. Deafness in patients with RTH reflects the important role of *TRβ* and thyroid hormone in the normal development of auditory function. The mixture of symptoms, some suggesting hypothyroidism and others suggesting hyperthyroidism, may even differ in individuals within the same family, despite the identical mutation, thus confusing the clinical picture.

Because patients may present with symptoms suggesting hyperthyroidism, it is important to keep this diagnosis in mind in a patient with tachycardia, goiter, and elevated thyroid hormones. RTH is discussed here because a reduced response to thyroid hormone is the biochemical basis for the condition. However, the laboratory results may be the first clear evidence that a patient, otherwise thought to have hyperthyroidism, has RTH. These tests show the unusual combination of an increased FT_4 accompanied by normal or slightly increased TSH levels (see Table 13.2). Thus the principal differential diagnosis is between a TSH-secreting pituitary tumor causing hyperthyroidism and RTH.[188]

Factors that may assist in the differential diagnosis are as follows: absence of a family history in patients with TSH-producing tumors, normal thyroid hormone levels in family members of individuals with TSH-induced hyperthyroidism due to pituitary tumor, and the presence of an elevated glycoprotein α-subunit in patients with pituitary tumor but not in those with RTH.

A definitive diagnosis requires sequencing of the *TRβ* gene demonstrating the abnormality. Mutations in the *TRβ* gene are found in about 90% of individuals with a clinical diagnosis. In a few individuals this is not the case, suggesting that there may be mutations in coactivator proteins or one of the RXR receptors, which can also present in a similar fashion.[189]

Treatment is difficult because thyroid hormone analogues designed to suppress TSH, thereby relieving the hyperthyroxinemia, may lead to worsening of the cardiovascular manifestations of the condition.[190] Therapy with 3,5,3′-triiodothyroacetic acid (TRIAC) has been used in several patients. The development of analogues of thyroid hormone with *TRβ*, as opposed to mixed or *TRα* preferential effects, as well as analogues that selectively bind mutant TRs, may eventually prove useful in treatment.

Individuals with RTHα1 have more subtle changes in thyroid function studies, compared with RTH due to *TRβ* gene mutations, and a clinical phenotype consistent with hypothyroidism in tissue that predominantly expresses the *TRα* isoform.[184,185] Because regulation of TSH is predominantly mediated by *TRβ*, these individuals have a normal serum TSH but a reduced T_4/T_3 ratio and low concentration of serum reverse T_3, presumably due to reduced activity of the D3 enzyme. Common phenotypic features include growth failure, developmental delays, constipation, and delayed bone maturation, with some improvement associated with T_4 treatment.[186]

Treatment

Hypothyroidism, either primary or central, is gratifying to treat because of the ease and completeness with which it responds to thyroid hormone.[81,191] Treatment is nearly always with levothyroxine, and the proper use of this medication has been reviewed extensively.[80] A primary advantage of levothyroxine therapy is that the peripheral deiodination mechanisms can continue to produce the amount of T_3 required in tissues under the normal physiologic control.[84] If one accepts the principle that replicating the natural state is the goal of hormone replacement, it is logical to provide the "prohormone" and allow the peripheral tissues to activate it by physiologically regulated mechanisms. There is, however, significant interest in combined T_4 and T_3 therapy.[192,193]

Pharmacologic and Physiologic Considerations

Levothyroxine has a 7-day half-life; about 80% of the hormone is absorbed relatively slowly (over hours) and it equilibrates rapidly in its extracellular distribution volume, therefore avoiding large postabsorptive perturbations in FT_4 levels. With its long half-life, omission of a single day's tablet has no significant effect and the patient may safely take an omitted tablet the following day. In fact, the levothyroxine dosage can be calculated almost as satisfactorily on a weekly, as on a daily, basis. Although T_4 is well absorbed and does not require fasting, regular ingestion of levothyroxine on an empty stomach results in the least variation in serum TSH concentration.[194]

The Food and Drug Administration (FDA) has issued standards for single-dose bioequivalence studies in normal volunteers to assess and compare T_4 products in the United States.[195] The area under the curve (AUC) confidence interval must fall within 80% to 125% of the comparison product for a preparation to be considered equivalent. The desirability of a pharmacotherapeutic measurement, such as TSH level as an end point, has been suggested by many professional organizations.[195] The guidelines for measured T_4 content have narrowed from 90% to 110% to 95% to 105% of the stated tablet dose and require that content to be maintained for the entire shelf life.[196] The availability in many countries of a multiplicity of tablet strengths with content ranging from 25 to 300 μg allows precise titration of the daily levothyroxine dosage for most patients with a single daily tablet, improving compliance significantly.

The typical dose of levothyroxine, approximately 1.6 to 1.8 μg/kg ideal body weight per day (0.7 to 0.8 μg/lb), generally results in the prescription of between 75 and 125 μg/day for women and 125 to 200 μg/day for men. Replacement doses need not be adjusted upward in obese patients and should be based on lean body mass.[197] Levothyroxine dose reduction is often required after bariatric surgery, but the dose reduction is proportional to the reduction in lean body mass.[198] This dosage is about 20% greater than the T_4 production rate owing to incomplete absorption of the levothyroxine. In patients with primary hypothyroidism, these amounts usually result in serum TSH concentrations that are within the normal range. Because of the 7-day half-life, approximately 6 weeks are required before there is complete equilibration of the FT_4 and the biologic effects of levothyroxine. Accordingly, assessments of the adequacy of a given dose or the effects of a change in dosage, with rare exceptions such as pregnancy, should not be made until this interval has passed. This long half-life also means that it is safe for a patient to take any missed doses of T_4 for up to 1 week after missing tablets.

By and large, levothyroxine products are clinically equivalent, although problems do occur.[199] However, the variation permitted by the FDA in tablet content can result in slight variations in serum TSH in patients with primary hypothyroidism even when the same brand is used. Using levothyroxine from a single manufacturer reduces variability that may be relevant for patients, such as elderly, pregnant, and thyroid cancer patients, when close titration is required. Although the serum TSH level is an indirect reflection of the levothyroxine effect in patients with primary hypothyroidism, it is far superior to any other readily available method of assessing the adequacy of therapy. Return of the serum TSH level to normal is therefore the goal of levothyroxine therapy in the patient with primary hypothyroidism. Some patients may require slightly higher or lower doses than generally used, owing to individual variations in absorption, and a number of conditions or associated medications may change levothyroxine requirements in patients with established hypothyroidism (see later). Liquid preparations of levothyroxine, including preparations in capsules, may be better absorbed in select patients.[200]

In decades past, desiccated thyroid was successfully used for the treatment of hypothyroidism and still accounts for a small fraction of the prescriptions written for thyroid replacement in the United States. Although this approach was successful, desiccated thyroid preparations contain thyroid hormone derived from animal thyroids that have twofold to threefold higher ratios of T_3 to T_4 than the 1:15 value in normal human thyroglobulin.[201] Accordingly, these preparations may lead to superphysiologic levels of T_3 in the immediate postabsorptive period (2–4 hours) owing to the rapid release of T_3 from thyroglobulin, its immediate and nearly complete absorption, and the 1-day period required for T_3 to equilibrate with its 40-L volume of distribution (see Table 11.5).[202] A prospective double-blind randomized crossover study compared 4 months of levothyroxine monotherapy with desiccated thyroid preparations in the same hypothyroid patients.[203] There were no significant differences in outcomes, although among patients who had a preference, more preferred desiccated thyroid extract, and these patients lost a modest amount of weight.

Mixtures of liothyronine and levothyroxine (liotrix) contain in a 1-grain (64-μg) equivalent tablet (Thyrolar-1 in the United States) the amounts of T_3 (12.5 μg) and T_4 (50 μg) present in the most popular desiccated thyroid tablet.[204] The levothyroxine equivalency of a 1-grain desiccated thyroid tablet or its liotrix equivalent can be estimated as follows: The 12.5 μg of liothyronine (T_3) is completely absorbed from desiccated thyroid or from liotrix tablets.[202] Levothyroxine is approximately 80% absorbed,[205] and about 36% of the 40 μg of levothyroxine absorbed is converted to T_3, with the molecular weight of T_3 (651) being 84% that of T_4 (777). Accordingly, a 1-grain tablet should provide about 25 μg of T_3, which would be approximately equivalent to that obtained from 100 μg of levothyroxine. This equivalency ratio can be used as an initial guide in switching patients from desiccated thyroid or liotrix to levothyroxine. Although levothyroxine is absorbed in the stomach and small intestine, normal gastric acid secretion is required for complete absorption.[206] Patients with impaired acid secretion on levothyroxine therapy require a 22% to 34% higher dose of levothyroxine to maintain the desired serum TSH. In those patients in whom acid secretion was normalized therapeutically, the levothyroxine dose returned to baseline.[206]

As indicated earlier, the use of levothyroxine as thyroid hormone replacement is a compromise with the normal pathway of T_3 production, in which about 80% of T_3 is derived from T_4 5′-monodeiodination and approximately 20% (~6 μg) is secreted directly from the thyroid gland.[84] Studies in thyroidectomized rats, for example, show that it is not possible to normalize T_3 simultaneously in all tissues by an intravenous infusion of T_4.[207] However, it should be recalled from the earlier discussion of T_4 deiodination that the ratio of T_3 to T_4 in the human thyroid gland is about 0.09 but is 0.17 in the rat thyroid gland.[84] Thus about 40% of the rat's daily T_3 production is derived from the thyroid versus about 20% in humans.[84] Accordingly, the demonstration that T_4 alone cannot provide normal levels of T_3 in all tissues in the rat is of interest but is not strictly applicable to thyroid hormone replacement in humans. Nonetheless, the ratio of T_3 to T_4 in the serum of a patient receiving levothyroxine as the only source of T_3 is about 20% lower than that in a normal individual.

Similarly, the quantity of levothyroxine required to normalize TSH in an athyreotic patient results in a slightly higher serum FT_4 concentration than is present in normal individuals. This has been shown in a comparison of thyroid function in the same patient before and after thyroidectomy.[208] Although serum T_3 was the same level in patients before or after thyroidectomy, a higher serum T_4 concentration was necessary when on T_4 replacement to maintain the same serum T_3.[208] T_4 has an independent mechanism for TSH suppression, owing to the intracellular generation of T_3 in the hypothalamic-pituitary-thyroid axis, resulting in a portion of the feedback TSH regulation being independent of the plasma T_3. A retrospective, cross-sectional study compared thyroid function studies in 1800 athyreotic thyroid cancer patients on levothyroxine monotherapy compared to control subjects.[209] In these patients, the serum FT_4 levels were significantly higher, and free T_3 levels significantly lower, than euthyroid control subjects. However, these patients came from a region of variable iodine intake, which makes interpretation of the control data difficult.

Although the concept of combined T_4/T_3 therapy has been recognized for many years, a positive study generated a great deal of interest in this approach.[210] Patients received 12.5 μg of T_3 as a substitution for 50 μg of their levothyroxine preparation and scored, on average, somewhat higher on tests of mood than when they were taking levothyroxine alone. The dosage of thyroid hormone used in these studies was excessive, as judged by the fact that 20% of the group had serum TSH values below normal on either regimen and the test period was only a few months. A large number of subsequent studies using a wide range of replacement strategies and relative T_4/T_3 content were performed in different populations, and none has shown an advantage of combination therapy over T_4 alone.[211] A study that compared T_4 monotherapy to T_4/T_3 combined therapy in hypothyroid patients evaluated the results based on the presence or absence of D2 gene polymorphisms.[212] The hypothyroid patients homozygous for the D2 polymorphism showed greater improvement in measures of well-being while on T_4/T_3 combination therapy compared to T_4 monotherapy. This study requires replication in a separate population but may indicate that T_4/T_3 combination therapy can be targeted to specific hypothyroid patients who will benefit based on a genetic profile of genes important for thyroid hormone metabolism and action.[9,193,213]

On the other hand, the FT_4 index correlated as closely with the resting energy expenditure as TSH levels in a group of patients in whom small adjustments above or below their ideal replacement levothyroxine dosage were made.[214] The correlation with serum T_3 was not statistically significant, suggesting that in humans, perhaps as a result of differences in the peripheral metabolism of T_4 from that in rodents, the FT_4 concentration may be as accurate as the TSH value as an index of satisfactory thyroid hormone

replacement. The practical difficulty with the design of tablets providing combinations of T_3 and T_4 is that the approximate dose of 6 μg of T_3 provided would need to be released in a sustained fashion over 24 hours, as well as replicating the diurnal rhythm of T_3,[215] which is quite different from the rapid absorption of T_3 with a peak at 2 to 4 hours when given in its conventional form. Thus, for the present, it appears that the current approach to thyroid replacement using levothyroxine alone, although not a perfect replication of normal physiology, is satisfactory for virtually all patients. A sustained-release T_3 preparation has been developed and produces more stable levels of serum T_3.[216] The clinical effects of this more "physiologic" replacement are not known.

Institution of Replacement Therapy

The initial dose of levothyroxine prescribed depends on the degree of hypothyroidism and the age and general health of the patient. Patients who are young or middle aged and otherwise healthy with no associated cardiovascular or other abnormalities and mild to moderate hypothyroidism (TSH concentrations of 5–50 mU/L) can be given an initial complete replacement dose of about 1.7 μg/kg of ideal body weight. The resulting increase in serum T_4 concentration to normal requires 5 to 6 weeks, and the biologic effects of T_3 are sufficiently delayed that these patients do not experience adverse effects. At the other extreme, the elderly patient with heart disease, particularly angina pectoris, without reversible coronary lesions, should be given a small initial dose of levothyroxine (25 μg/day), and the dosage should be increased in 12.5-μg increments at intervals of 2 to 3 months with careful clinical and laboratory evaluation.

The goal in the patient with primary hypothyroidism is to return serum TSH concentrations to normal, reflecting normalization of that patient's thyroid hormone supply. This usually results in a mid-normal to high-normal serum FT_4. The serum TSH should be evaluated 6 weeks after a theoretically complete replacement dose has been instituted to allow minor adjustments to optimize the individual dose.[217] In patients with central hypothyroidism, serum TSH is not a reliable index of adequate replacement, and the serum FT_4 should be restored to a concentration in the upper half of the normal range. T_4 dosing based on body weight and a serum FT_4 in the upper reference range improved markers of thyroid hormone action and was superior to replacement with a combination of T_4/T_3.[218] Patients with central hypothyroidism should also be evaluated and treated for glucocorticoid deficiency, if necessary, before institution of thyroid replacement (see Chapter 9).

Although the adverse effects of the rapid institution of therapy are unusual, pseudotumor cerebri has been reported in profoundly hypothyroid juveniles between ages 8 and 12 years who were given even modest initial levothyroxine replacement.[219] This complication appears 1 to 10 months after initiation of treatment and responds to acetazolamide and dexamethasone.

The interval between the initiation of treatment and the first evidence of improvement depends on the strength of dose given and the degree of the deficit. An early clinical response in moderate to severe hypothyroidism is a diuresis of 2 to 4 kg. The serum sodium (Na^+) level increases even sooner if hyponatremia was present initially. Thereafter, pulse rate and pulse pressure increase, appetite improves, and constipation may disappear. Later, psychomotor activity increases and the delay in the deep tendon reflex disappears. Hoarseness abates slowly, and changes in skin and hair do not disappear for several months. In individuals started on a complete replacement dose, the serum FT_4 level should normalize after 6 weeks; a somewhat longer period may be necessary for serum TSH levels to return to normal, perhaps up to 3 months.

In some cases (e.g., myxedema coma [see later]), it is clinically appropriate to alleviate hypothyroidism rapidly. For example, patients with severe hypothyroidism withstand acute infections or other serious illnesses poorly, and myxedema coma may develop as a complication. In such circumstances, rapid near repletion of the peripheral hormone pool in the average adult can be accomplished by a single intravenous dose of 500 μg of levothyroxine. Alternatively, by virtue of its rapid onset of action, liothyronine (25 μg orally every 12 hours) can be administered if the patient can take medication by mouth. With both approaches, an initial biologic effect is achieved within 24 hours. Parenteral therapy with levothyroxine is then continued with a dose that is 80% of the appropriate oral dose but not in excess of 1.4 μg/kg of ideal body weight. Because of the possibility that rapid increases in metabolic rate will overtax the existing pituitary-adrenocortical reserve, supplemental glucocorticoid (intravenous hydrocortisone 5 mg/hour) should also be given to patients with severe hypothyroidism receiving high initial doses of thyroid hormones. Finally, in view of the tendency of hypothyroid patients to retain free water, intravenous fluids containing only dextrose should not be given.

When replacement therapy is withdrawn for short periods (4–6 weeks) for purposes of evaluating therapy for thyroid cancer, rapid reinstitution of levothyroxine using a loading dose of three times the daily replacement dose for 3 days can usually be given unless there are other complicating medical illnesses.

When hypothyroidism results from administration of iodine-containing or antithyroid drugs, withdrawal of the offending agent usually relieves both the hypothyroidism and the accompanying goiter, although it is appropriate to provide interim replacement until the gland recovers its function.[138,220] This is especially true for amiodarone, which may remain in tissues for up to 1 year.

Infants and Children

In infants with congenital hypothyroidism, the determining factor for eventual intellectual attainment is the age at which adequate treatment with thyroid hormone is begun.[18,221] The therapy for infants with congenital hypothyroidism should consist initially of raising the serum T_4 level to more than 130 nmol/L (10 μg/dL) as rapidly as possible and maintaining it at that level for the first 3 to 4 years of life. This is usually accomplished by administering an initial levothyroxine dose of 50 μg/day, which is higher than the adult dose on a weight basis and in keeping with the higher metabolic clearance of the hormone in the infant. The serum TSH concentration may not return to normal even with this high dose because of residual reset of the pituitary feedback mechanism. After 2 years of age, however, a TSH level in the normal range is an index of optimal therapy as it is in adults.[222]

Monitoring Replacement Therapy

Monitoring the adequacy of, and compliance with, thyroid hormone therapy in patients with primary hypothyroidism is easily done by measurement of serum TSH.[9] This value should be within the normal range for an assay sufficiently sensitive to measure, with confidence, the lower limit of the normal range. The normal serum TSH concentration varies between 0.5 and 4 mU/L in most second-generation and third-generation assays, and results within this range are associated with the elimination of all clinical and biochemical manifestations of primary hypothyroidism, except in patients with RTH. Based on analysis of the National Health and Nutrition Examination Survey (NHANES III) reference group,[13]

a reference TSH range with an upper limit of 2.5 mU/L has been suggested. This adjustment, however, would identify a large number of individuals as having abnormal thyroid function, without a clear indication of the clinical significance of TSH levels in this range. A more recent analysis, based on age-specific reference ranges, indicates that older adults, without thyroid autoantibodies, have an increased upper limit TSH (>4.5 mU/L) that is not associated with thyroid disease.[223] In related studies, this progressive shift with age to higher levels of TSH has been associated with extreme longevity in several populations.[224]

After the first 6 months of therapy, the dose should be reassessed because restoration of euthyroidism increases the metabolic clearance of T_4. A dose that was adequate during the early phases of therapy may not be so when the same patient is euthyroid owing to an acceleration in the clearance of thyroid hormone.

Under normal circumstances, the finding of a normal serum TSH level on an annual basis is adequate to ensure that the proper levothyroxine dose is being taken by the patient. If the serum TSH level is above the normal range and noncompliance is not the explanation, small adjustments, usually in 12-μg increments, can be made with reassessment of TSH concentrations after the 6 weeks required for full equilibration have passed. In North America, this strategy is simplified by the availability of multiple tablet strengths, many of which differ by only 12 μg. Most patients can receive the same dose until they reach their seventh or eighth decade, at which point a downward adjustment of 20% to 30% may be indicated because thyroid hormone clearance decreases in the elderly.

Thyroid hormone requirements may be altered in several situations (Table 13.3). Most conditions or medications increase the levothyroxine requirement in patients receiving maintenance therapy. During pregnancy, the levothyroxine requirement is increased by 25% to 50% in most hypothyroid women, and a prospective study demonstrated that the increased requirement occurs early in the first trimester.[225] The required increment is higher in athyreotic patients compared to those with autoimmune hypothyroidism.[226] Athyreotic patients who are planning a pregnancy should be advised to increase the dose by around 30% as soon as the diagnosis is confirmed because the change in requirement appears soon after implantation.[9,113] The increased requirement is probably due to a combination of factors, including increases in T_4-binding globulin and the volume of distribution of T_4, an increase in body mass, and an increase in D3 in the placenta and uterus probably due to estradiol-induced increases in *Dio3* gene transcription.[84] The increased requirement persists throughout pregnancy but returns to normal within a few weeks after delivery. Therefore the dose should be reduced to the original prepregnancy level at the time of delivery. Maternal T_4 is critically important to the athyreotic fetus and in the normal fetus in the first trimester before fetal thyroid function and feedback regulation mature.[227] Maternal hypothyroidism has been associated with fetal loss, preterm delivery, and intellectual deficit in the offspring.[67,113] These findings are not seen in hypothyroid women on T_4 replacement sufficient to normalize their TSH, suggesting that these associations are directly related to maternal thyroid hormone status. A randomized prospective study in pregnant women with anti-TPOAb and normal range TSH demonstrated the benefit of levothyroxine treatment to prevent these complications.[228]

Other conditions in which levothyroxine requirements are increased (see Table 13.3)[9,80,146] include malabsorption due to bowel diseases, impaired gastric acid secretion,[206] and adsorption of levothyroxine to coadministered medications such as sucralfate, aluminum hydroxide, calcium carbonate, ferrous sulfate, lovastatin, or various resins. Certain medications, notably rifampin, carbamazepine, phenytoin, and sertraline, increase the clearance of levothyroxine by inducing CYP3A4 in the liver. Estrogen given to postmenopausal women may act in the same way, although the increases in D3 also play a role.[229] Soy protein and soybean isoflavones have been proposed to interfere directly with thyroid hormone action as well as synthetic T_4 absorption.[230] There is no evidence that soy interferes with thyroid function in euthyroid individuals who are iodine sufficient, and the effect of soy on T_4 absorption in hypothyroid patients is modest.[230] Amiodarone increases levothyroxine requirements by blocking conversion of T_4 to T_3 and perhaps by interfering with T_3–thyroid hormone receptor binding.[231] Selenium deficiency is rare, but because it is rate limiting in the synthesis of D1 (see Fig. 11.2),[84] any significant deficiency, such as may occur in patients receiving diets restricted in protein, may increase levothyroxine requirements.

Occasionally in patients who have been treated with radioactive iodine for Graves disease or toxic nodular goiter, some degree of thyroid hormone secretion persists and, although insufficient to sustain normal thyroid hormone levels, is autonomous. Such patients may have a suppressed TSH on what otherwise would

TABLE 13.3 Conditions That Alter Levothyroxine Requirements

Increased Levothyroxine Requirements

Pregnancy

Gastrointestinal Disorders

Mucosal diseases of the small bowel (e.g., sprue)
After jejunoileal bypass and small bowel resection
Impaired gastric acid secretion (e.g., atrophic gastritis)
Diabetic diarrhea

Therapy With Certain Pharmacologic Agents

Drugs That Interfere With Levothyroxine Absorption

Cholestyramine
Sucralfate
Aluminum hydroxide
Calcium carbonate
Ferrous sulfate

Drugs That Increase the Cytochrome P450 Enzyme (CYP3A4)

Rifampin
Carbamazepine
Estrogen
Phenytoin
Sertraline
? Statins

Drugs That Block T_4 to T_3 Conversion

Amiodarone

Conditions That May Block Deiodinase Synthesis

Selenium deficiency
Cirrhosis

Decreased Levothyroxine Requirements

Aging (≥65 years)
Androgen therapy in women

T_4, Thyroxine; *T_3*, triiodothyronine.

be considered a replacement dose of levothyroxine. The levothyroxine dose in these individuals should be reduced until TSH levels rise to normal, keeping in mind that several months may be required before TSH secretion recovers after its prolonged suppression. Because of either the delayed effects of radioiodine or the natural history of Graves disease per se, this autonomous T_4 secretion may decrease with time, leading to an increase in levothyroxine requirements in subsequent years. Rarely, the opposite occurs; that is, a patient treated with radioiodine develops an increased TSH level, but after several months of therapy the requirement for such replacement is either reduced or eliminated. This response may reflect transient impairment of thyroid function by a combination of preirradiation antithyroid drug therapy and immediate but transient effects of irradiation on the thyroid. In such patients, frequent monitoring of levothyroxine replacement is required to avoid overreplacement.

In North America, based on the recent previously discussed changes in assessment of levothyroxine bioequivalence, the possibility of a difference in tablet levothyroxine content should be considered if a new preparation changes the biologic or biochemical effects of the same dosage. Although the difference in preparation is unlikely to cause a significant difference in most patients, the change in manufacturer introduces another potential source of variability.

Adverse Effects of Levothyroxine Therapy

Although the administration of excessive doses of levothyroxine causes accelerated bone loss in postmenopausal patients, most authorities believe that returning thyroid status to normal does not have adverse effects on bone density.[232] Administration of excessive doses also increases cardiac wall thickness and contractility and, in elderly patients, increases the risk of atrial fibrillation.[16,25]

In some patients, TSH levels remain elevated despite the prescription of adequate replacement doses.[233] This response is most often a consequence of poor adherence. The combination of normal or even elevated serum FT_4 values and elevated TSH levels can occur if the patient does not take levothyroxine regularly but ingests several pills the day before testing. The integrated dose of levothyroxine over prior weeks is best reflected in the serum TSH level, and nonadherent patients require careful education as to the rationale for treatment. Subtle changes in dietary habits, such as increasing the ingestion of bran-containing products, soy, or calcium or proton pump inhibitors, may decrease levothyroxine absorption, and their recognition requires a careful history.[80,146,233] In patients on T_4 replacement with erratic TSH levels and consideration of inconsistent adherence with therapy or malabsorption, a protocol has been developed to evaluate such patients with weekly supervised dosing of LT_4 and monitoring of serum TSH and FT_4 concentrations.[1] Use of weekly dosing of LT_4 should be done very cautiously in older patients or those with known cardiac disease.

Patients With Hypothyroid Symptoms Despite Restitution of Normal Thyroid Function

In patients taking levothyroxine replacement with a normal serum TSH concentration, symptoms consistent with hypothyroidism may persist.[234] A survey of hypothyroid patients on levothyroxine with normal TSH and control patients included questions about symptoms that might be associated with thyroid hormone deficiency.[235] Although a significant fraction of both groups reported such symptoms, a greater fraction of patients on levothyroxine replacement had these symptoms. Patients on thyroxine replacement post-thyroidectomy consistently require a higher serum T_4 than preoperatively to maintain the same serum T_3 concentration.[208] The addition of thyroid replacement containing triiodothyronine continues to be a focus in these patients but has not been shown to confer long-term relief of symptoms.[234] Additionally, such patients should be educated as to the relationship between symptoms of hypothyroidism and the role of thyroid hormone in relieving them, and other causes should be sought for the symptoms.

Special Aspects of Hypothyroidism

Subclinical Hypothyroidism

The term *subclinical hypothyroidism* was originally used to describe the patient with a low-normal FT_4 but a slightly elevated serum TSH level. Other terms for this condition are *mild hypothyroidism, early thyroid failure, preclinical hypothyroidism,* and *decreased thyroid reserve* (see Table 13.2). The TSH elevation in such patients is modest, with values typically between 5 and 15 mU/L, although patients with a TSH above 10 mU/L more often have a reduced FT_4 and may have true hypothyroid symptoms. The definition of this syndrome depends significantly on the reference range for a normal TSH concentration. This syndrome is most often seen in patients with early Hashimoto disease and is a common phenomenon, occurring in 7% to 10% of older women.[14–16]

A number of studies on the effects of thyroid hormone treatment in such patients have used physiologic end points (e.g., measurements of various serum enzymes, systolic time intervals, serum lipids, psychometric testing), and results have been variable.[14–16] In the most carefully controlled studies, one or another of the parameters has returned to normal in about 25% to 50% of patients. A prospective randomized trial of subclinical hypothyroidism in elderly males showed no benefit of therapy.[236] Among those men originally enrolled based on an elevated TSH, 62% had a normal TSH on subsequent measurement, indicating the importance of repeating a TSH measurement in suspected subclinical hypothyroidism. In general, FT_4 and TSH levels normalize, but free T_3, usually normal at the outset, does not change. Modest improvements in cardiac indices and lipid profiles have been noted in most but not all studies, although the benefit on cardiovascular risk is seen in middle-aged patients.[30,34] The association of mild hypothyroidism with an increase in risk for atherosclerotic heart disease has been shown by some studies but not others.[14,16] The impact of treatment to reduce the risk of atherosclerotic heart disease, other than reduction in risk factors such as cholesterol and C-reactive protein, have not yet been studied.

One factor favoring a decision to recommend levothyroxine therapy is the likelihood of developing overt hypothyroidism. The risk of progression from *subclinical* to *overt* hypothyroidism (elevated serum TSH and reduced FT_4) is most closely related to the magnitude of serum TSH elevation and the presence of anti-TPOAb. Prospective studies of women with subclinical hypothyroidism have shown rates of progression from approximately 3% to 8% per year, with the higher rates seen in individuals with initial TSH concentration greater than 10 and those with positive anti-TPOAb.[237] Although most individuals progress slowly to overt hypothyroidism, rapid progression over weeks to months has been reported.[238] Factors that may predispose to rapid progression include increased age, high levels of TPOAb, intercurrent systemic infection or inflammation, iodine contrast agents, and medications such as amiodarone and lithium. The decision to treat with levothyroxine must also take into account the expense

and inconvenience of a daily medication, which is not acceptable to some patients, and the possibility that unintended overdosage may exacerbate osteoporosis or cause cardiac arrhythmias. Ultimately the decision to treat must depend on a careful consideration of the individual clinical situation and patient preference. If a therapeutic trial is performed, the TSH concentration should be monitored carefully and should not be reduced below normal. If no therapy is given, such patients should be monitored at intervals of 6 to 12 months both clinically and biochemically.

Metabolic Insufficiency

Nonspecific symptoms of true hypothyroidism include mild lassitude, fatigue, slight anemia, constipation, apathy, cold intolerance, menstrual irregularities, loss of hair, and weight gain. For this reason, some patients with such complaints but with normal laboratory results for thyroid function have been considered candidates for levothyroxine therapy. The response to thyroid hormone therapy is sometimes gratifying, at least initially, but symptomatic improvement usually disappears after a time unless the dose is increased. Eventually, even larger doses fail to alleviate the symptoms, confirming that they do not arise from a deficiency of thyroid hormone.

Thus thyroid hormone therapy should be avoided in patients with no biochemical documentation of impaired thyroid function. Furthermore, even in patients with subclinical hypothyroidism, symptoms may be out of proportion to abnormalities in the FT_4. It is unwise to raise a patient's expectations that such symptoms will be relieved by correction of mild biochemical abnormalities.

Thyroid Function Testing in Patients Receiving Replacement Therapy for Unclear Reasons

Physicians are frequently confronted with patients receiving levothyroxine in whom the basis for the diagnosis cannot be established. It may be difficult to document previous clinical findings or laboratory data to determine whether thyroid hormone replacement is indicated. If serum TSH is in the normal range and primary hypothyroidism is suspected, a simple way of assessing the need for levothyroxine therapy is to switch levothyroxine to an every-other-day dosage or to reduce the daily dose by 50% and to reevaluate TSH and FT_4 after 4 weeks. If there is no significant increase in TSH concentration and FT_4 remains constant during that period, residual thyroid function is present, although it may still not be completely normal. To answer this question, levothyroxine can then be withdrawn and blood tests repeated 4 to 8 weeks later.

If the initial TSH level is suppressed, indicating overreplacement, the levothyroxine dose should be reduced until TSH becomes detectable before this trial is instituted. If central hypothyroidism is suspected, the FT_4 must be monitored.

Emergent Surgery in the Hypothyroid Patient

The perioperative course of patients with untreated hypothyroidism has been evaluated in several studies. In general, such patients were not recognized to be hypothyroid or did not require surgery despite the presence of significant hypothyroidism. Complications were uncommon. Perioperative hypotension, ileus, and central nervous system disturbances were more common in hypothyroid patients, and patients with major infections had fewer episodes of fever than did euthyroid control subjects.[239] Other complications were delayed recovery from anesthesia and abnormal hemostasis, possibly owing to an acquired form of von Willebrand disease.[59]

From these studies one may conclude that emergent surgery should not be postponed in hypothyroid patients but that such patients should be rigorously monitored for evidence of carbon dioxide retention, bleeding, infection, and hyponatremia. These findings are also relevant to the treatment of hypothyroid individuals with symptomatic coronary artery disease. Considering the lack of significant increase in perioperative complications in the hypothyroid patient, the option of surgery for remediable coronary artery lesions is open to hypothyroid individuals without the risk of a myocardial infarction in association with restitution of the euthyroid state (see later).[240]

Heart Disease and Thyroid Hormone Therapy

Coexisting Coronary Artery Disease and Hypothyroidism

In many patients with coronary artery disease and primary hypothyroidism, cardiac function is improved in response to levothyroxine therapy because of a decrease in peripheral vascular resistance and improvement in myocardial function. However, patients with preexisting angina pectoris should be evaluated for correctable lesions of the coronary arteries and treated appropriately before levothyroxine is administered.[240,241] Retrospective studies indicate that this approach is safer than the institution of replacement therapy prior to angiography and angioplasty or even coronary artery bypass grafting (CABG).[240]

In a few patients, lesions may not be remediable, or small-vessel disease is severe even after stenting or bypass grafting, so that complete replacement cannot be instituted. Such patients must receive optimal antianginal therapy combined with β-adrenergic receptor blockers in judicious quantities, and complete restitution of the euthyroid state may not be possible.

Thyroid Hormone for Compromised Cardiovascular Function

In addition to the issues raised in patients with combined hypothyroidism and coronary artery disease, there is interest in the potential therapeutic use of thyroid hormone in the treatment of patients with cardiomyopathy or those who have undergone CABG or other cardiac procedures.[25] As expected, T_3 levels are reduced in patients with advanced congestive heart failure, as with any illness. In one report, 23 patients with advanced heart failure (mean ejection fraction, 22%) were given up to 2.7 μg/kg of liothyronine over 6 hours with an increase in cardiac output and decrease in systemic vascular resistance but without increase in heart or metabolic rate.[242] Similar effects were seen with a dose of 110 μg liothyronine over 6 hours after CABG.[243]

Liothyronine has also been given postoperatively for congenital heart disease and, again, an improvement in cardiac output and decrease in vascular resistance occurred without adverse side effects.[209] These results suggest that, in certain selected circumstances, liothyronine may be useful as adjunctive therapy in patients with congestive heart failure because of its effect of relaxing vascular smooth muscle.

Although most therapeutic trials of thyroid hormone treatment have used T_3, thyroid hormone analogues have also been used.[25] The most extensively studied is 3,5-diiodothyropropionic acid (DITPA), an analogue that binds both *TRα* and *TRβ* with low affinity. A randomized study of DITPA in heart failure showed some improved cardiac performance,[244] but the study was terminated because of significant metabolic side effects, including weight loss.[245]

Screening for Primary Hypothyroidism

The use of screening for hypothyroidism has been addressed by a number of studies but remains controversial.[191] The conclusions depend, to a great extent, on assumptions regarding the effectiveness and economic value of identifying and treating patients with subclinical hypothyroidism. An evidenced-based medicine review of the literature by an expert panel concluded that there was insufficient evidence to support population-based screening.[246] Aggressive "case finding," based on identification of risk factors such as family history, was advocated for pregnant women, women older than 60 years, and others at high risk. The fraction of patients with hypothyroidism missed when a "case finding" strategy is used, however, is not known. A updated report from the US Preventive Services Task Force concluded that there is still insufficient data to recommend population screening for hypothyroidism in nonpregnant adults.[247] Large, randomized, prospective studies of levothyroxine treatment in patients with subclinical hypothyroidism to establish benefit, however, have not yet been performed. Given the very high incidence of hypothyroidism in older women and the absence of robust clinical symptoms, an assessment of TSH levels at 5-year intervals in women older than age 50 years seems justified until more extensive studies have been performed.

A second complex issue involves whether women planning pregnancy should be screened for the presence of hypothyroidism as a routine part of a prenatal visit.[113] This question is raised because of increasing association of adverse outcomes in pregnancy, even with subclinical hypothyroidism, including impairment of mental development in infants, fetal loss, and preterm delivery.[66,67] The prevalence of overt hypothyroidism during pregnancy is approximately 2%, and screening of all patients has been advocated by several professional organizations. Thyroid testing in high-risk patients, "case finding," has been advocated, although a prospective study showed that approximately a third of pregnant women with underlying thyroid disease are missed by this testing approach.[248]

Isolated maternal hypothyroxinemia in pregnancy, generally defined as a FT_4 in the lowest 2.5 to 5th percentile, with a normal reference range TSH, has been associated with a range of neurologic deficits, including reduced IQ, reduced cortical volume, language delay, and autism.[67] Treatment of hypothyroxinemic women during pregnancy has not been associated with improved cognitive outcome of the offspring,[249,250] and routine treatment is not currently recommended.[113]

A number of questions are raised regarding the appropriate timing of testing, whether thyroid autoantibodies should be measured, the relative importance of TSH and FT_4, the influence of trimester on the normal ranges, and the threshold for intervention. The association of maternal subclinical hypothyroidism and preterm delivery is a much more proximal and defined end point to study compared to intellectual performance in offspring. The morbidity and mortality rates from preterm delivery are significant for the newborn, and these findings are likely to allow for more focused intervention studies to determine the response to T_4 treatment.[66]

For the moment, it appears that any patient with a family history of autoimmune thyroid disease, with symptoms suggesting hypothyroidism, or with thyroid enlargement should be tested for thyroid dysfunction prior to pregnancy or as soon after conception as is feasible. Optimization of levothyroxine therapy for women known to have hypothyroidism prior to conception, when possible, may be the most effective intervention to prevent hypothyroid-related complications of pregnancy. Although the data do not yet reach the threshold to mandate universal screening, the ease of testing, associated adverse outcomes, and demonstrated benefit of intervention make thyroid testing of all pregnant women a reasonable choice.

Myxedema Coma

Myxedema coma is the ultimate stage of severe long-standing hypothyroidism.[251,252] This state, which almost invariably affects older patients, occurs most commonly during the winter months and is associated with a high mortality rate. It is usually accompanied by a subnormal temperature. Values as low as 23°C have been recorded. The external manifestations of severe myxedema, bradycardia, and severe hypotension are invariably present. The characteristic delay in deep tendon reflexes may be lacking if the patient is areflexic. Seizures may accompany the comatose state. Although the pathogenesis of myxedema coma is not clear, factors that predispose to its development include exposure to cold, infection, trauma, and central nervous system depressants or anesthetics. Alveolar hypoventilation, leading to carbon dioxide retention and narcosis, and dilutional hyponatremia resembling that seen with inappropriate ADH secretion may also contribute to the clinical state.

From the foregoing, it appears that myxedema coma should be readily recognized from its clinical signs, but this is not the case. Hypothermia of any cause (e.g., to cold exposure) may cause changes suggestive of myxedema, including delayed relaxation of deep tendon reflexes. The importance of diagnosing myxedema coma is that a delay in therapy worsens the prognosis. Consequently, a rapid serum FT_4 and TSH should be obtained whenever this diagnosis is being considered. Otherwise the diagnosis should be made on clinical grounds; after serum has been sent for thyroid function tests, therapy should be initiated without awaiting the results of delayed confirmatory tests because the mortality rate may be 20% or higher.

Treatment consists of administration of thyroid hormone and correction of the associated physiologic disturbances.[251,252] Because of the sluggish circulation and severe hypometabolism, absorption of therapeutic agents from the gut or from subcutaneous or intramuscular sites is unpredictable, and medications should be administered intravenously if possible. Administration of levothyroxine as a single intravenous dose of 500 to 800 μg repletes the peripheral hormone pool and may cause improvement within hours. Daily doses of intravenous levothyroxine, 100 μg, are given thereafter. Hydrocortisone (5–10 mg/hour) should also be given because of the possibility of relative adrenocortical insufficiency as the metabolic rate increases.

Alternatively, intravenous liothyronine may be given at a dose of 25 μg every 12 hours. Others have used a combination of 200 to 300 μg T_4 and 25 μg T_3 intravenously as a single dose, followed by 25 μg T_3 and 100 μg T_4 24 hours later, and then 50 μg T_4 daily until the patient regains consciousness. *Hypotonic* fluids should not be given because of the danger of water intoxication owing to the reduced free water clearance of the hypothyroid patient. *Hypertonic* saline and glucose may be required to alleviate severe dilutional hyponatremia and the occasional hypoglycemia.

A critical element in therapy is support of respiratory function by means of assisted ventilation and controlled oxygen administration. Internal warming by gastric perfusion may be useful, but external warming should be avoided because it may lead to vascular collapse due to peripheral vasodilatation. Further heat loss can be prevented with blankets. An increase in temperature may be seen within 24 hours in response to levothyroxine. General measures applicable to the comatose patient should be undertaken, such as frequent turning, prevention of aspiration, and attention to fecal impaction and urinary retention.

TABLE 13.4 Causes of Thyroiditis

Autoimmune thyroiditis
Painless subacute thyroiditis, including postpartum thyroiditis (see Chapter 12)
Painful subacute thyroiditis (see Chapter 12)
Acute infectious thyroiditis
Riedel thyroiditis
Postirradiation (^{131}I or external-beam therapy)
Sarcoidosis

Finally, the physician should assess the patient for the presence of coexisting disease, especially infection, cardiac disease, or cerebrovascular disease. The myxedematous patient may be afebrile despite a significant infection. As soon as the patient is able to take medication by mouth, treatment with oral levothyroxine should be instituted.

Thyroiditis

Thyroiditis is a term indicating the presence of thyroid inflammation and thus comprises a large group of diverse inflammatory conditions. These conditions include the following: autoimmune or quasi-autoimmune causes and viral or postviral conditions and infections, including those of bacterial and fungal origins; a chronic sclerosing form of thyroiditis, termed *Riedel thyroiditis* (or struma); and miscellaneous causes of various types, including radiation-induced and granulomatous causes, such as sarcoidosis, as well as lithium.[5]

Not only are the causes of thyroiditis extremely varied, their clinical presentations may also be diverse and are difficult to categorize in a simple fashion (Table 13.4). Thus, as already discussed, autoimmune thyroiditis may present with hypothyroidism but often patients remain euthyroid for long periods after the disease begins. On the other hand, in a euthyroid patient with Hashimoto disease who becomes pregnant, the postpartum period is often complicated by an acute form of thyrotoxicosis due to the transient exacerbation of thyroiditis, often followed by a period of hypothyroidism (see Chapter 12).[66]

A similar syndrome has been observed in nonpregnant patients, called *painless subacute thyroiditis.* It is manifested primarily as thyrotoxicosis of sudden onset without localized pain and often without evidence of autoimmune disease. This condition may be viral in origin in some patients; however, the classic presentation of postviral thyroiditis, a condition referred to as *painful subacute thyroiditis*, is characterized by extreme thyroid tenderness, with pain radiating to the oropharynx and ears, and must be differentiated from acute infectious thyroiditis caused by bacterial or fungal infection.[253]

Thus inflammatory conditions of the thyroid present a didactic dilemma because one must decide whether to discuss these entities as a group with the common denominator of inflammation or to categorize them according to their principal clinical effects, namely, thyrotoxicosis or thyroid hormone deficiency. We have chosen the latter approach and have already discussed autoimmune thyroiditis, the major cause of thyroid gland failure (see Table 13.1). However, patients with acute autoimmune thyroiditis may also develop thyrotoxicosis, such as in postpartum painless thyroiditis (see Chapter 12 discussion regarding autoimmune thyroiditis).[66] These patients must be differentiated from those with Graves disease. In addition, some patients with painful subacute thyroiditis have thyrotoxicosis as a major manifestation with varying degrees of neck discomfort. For that reason, this thyroiditis syndrome is also discussed in Chapter 12, even though the pain associated with the typical form of this condition makes the principal differential diagnosis lie between that and infectious thyroiditis. In that context, subacute thyroiditis is also mentioned later.

Acute Infectious Thyroiditis

Although the thyroid gland is remarkably resistant to infection, congenital abnormalities of the piriform sinus, underlying autoimmune disease, or immunocompromise of the host may lead to the development of an infectious disease of the thyroid gland, acute infectious thyroiditis.[253,254] The cause may be any bacterium, including *Staphylococcus, Pneumococcus, Salmonella,* or *Mycobacterium tuberculosis.* In addition, infections with certain fungi, including *Coccidioides immitis, Candida, Aspergillus,* and *Histoplasma,* have been reported.

The most common cause of repeated childhood infectious thyroiditis, particularly in the left lobe, is a consequence of an internal fistula extending from the piriform sinus to the thyroid.[254] This sinus is the residual connection following the path of migration of the ultimobranchial body from the fifth pharyngeal pouch to the thyroid gland. The predominance of thyroiditis of the left lobe is explained by the fact that the right ultimobranchial body is often atrophic, whereas this is not the case for the left side. Nonetheless, a patient with a completely normal thyroid gland may develop bacterial thyroiditis. This is an extremely rare disease even as a complication of direct puncture of the thyroid gland, such as in fine-needle aspiration. In individuals with midline infections, persistence of the thyroglossal duct should be considered.

Incidence

Infectious thyroiditis is extremely rare, with no more than a few cases being seen in large tertiary care centers.

Clinical Manifestations

The clinical manifestations of infectious thyroiditis are dominated by local pain and tenderness in the affected lobe or entire gland. This is accompanied by painful and difficult swallowing. Because of the tendency for referral of pain to the pharynx or ear, the patient may not recognize the tenderness in the anterior neck. Depending on the virulence of the organism and the presence of septicemia, symptoms such as fever and chills may also accompany the condition.

The major differential diagnosis lies between an infectious form of thyroiditis and painful subacute thyroiditis. It is instructive to compare the principal features of these two diseases to arrive at an accurate diagnosis (Table 13.5). By and large, patients with acute infectious thyroiditis caused by a bacterium are much sicker than patients with painful subacute thyroiditis; they have more severe and localized tenderness and are less likely to have laboratory evidence of thyrotoxicosis, which is present in approximately 60% of patients with painful subacute thyroiditis. Ultrasonographic examination often reveals the abscess in the thyroid gland or evidence of swelling, and needle aspiration may help pinpoint the responsible organism. A gallium scan will be positive as a result of the diffuseness of the inflammation and, particularly in children with infectious thyroiditis of the left lobe, a barium swallow showing a fistula connecting the piriform sinus and left lobe of the thyroid is diagnostic.[254]

Occasionally, pertechnetate scanning is useful in showing normal function of one lobe of the thyroid gland, which is much less common in painful subacute thyroiditis (which more often affects the entire gland). Needle aspiration should be used to drain the affected lobe, although occasionally surgical drainage may be

TABLE 13.5 Features Useful in Differentiating Between Acute Infectious Thyroiditis and Subacute Thyroiditis

Characteristic	Acute Thyroiditis (% with Feature)	Subacute Thyroiditis (% with Feature)
History		
Preceding upper respiratory infection	88	17
Fever	100	54
Symptoms of thyrotoxicosis	Uncommon	47
Sore throat	90	36
Physical Examination of the Thyroid		
Painful thyroid swelling	100	77
Left side affected	85	Not specific
Migrating thyroid tenderness	Possible	27
Erythema of overlying skin	83	Not usually
Laboratory Findings		
Elevated white blood cell count	57	25–50
Elevated ESR (>30 mm/hr)	100	85
Abnormal thyroid hormone levels (elevated or depressed)	5–10	60
Alkaline phosphatase, transaminases increased	Rare	Common
Results of Needle Aspiration		
Purulent, bacteria or fungi present	~100	0
Lymphocytes, macrophages, some polyps, giant cells	0	~100
^{123}I uptake low	Uncommon	~100
Radiologic Findings		
Abnormal thyroid scan	92	—
Thyroid scan or ultrasound helpful in diagnosis	75	—
Gallium scan positive	~100	~100
Barium swallow showing fistula	Common	0
CT scan useful	Rarely	Not indicated
Clinical Course		
Clinical response to glucocorticoid treatment	Transient	100
Incision and drainage required	85	No
Recurrence following operative drainage	16	No
Piriform sinus fistula discovered	96	No

CT, Computed tomography; *ESR,* erythrocyte sedimentation rate.

From DeGroot LJ, Larsen PR, Hennemann G. Acute and subacute thyroiditis. In: *The Thyroid and Its Diseases.* 6th ed. New York: Churchill Livingstone; 1996:700.

required. If a piriform sinus fistula can be demonstrated, it must be removed to prevent recurrence of the problem.

Antibiotics should be administered appropriate to the offending organism. Fungal infections should be treated appropriately, especially because many of these individuals are immunocompromised. Endemic organisms should be kept in mind as a cause, in that both *Echinococcus* and *Trypanosomiasis* infections of the thyroid gland have been reported.

The prognosis is excellent with preservation of thyroid function in general, although post-thyroiditis thyroid function tests should be monitored to ascertain that thyroid failure has not occurred.

Riedel Thyroiditis

Riedel chronic sclerosing thyroiditis is rare and occurs primarily in middle-age women.[155,255,256] The etiologic mechanism is uncertain, and any association with autoimmune thyroid disease is probably coincidental.[257] The morphologic similarities between the fibrosis of Riedel thyroiditis and IgG4-related sclerosing disease suggest that these entities are closely related, with thyroiditis representing an initial manifestation of a more generalized process.[258] Retroperitoneal, orbital, and mediastinal fibrosis, as well as rarer fibrotic syndromes, are associated with Riedel thyroiditis.[259]

Symptoms develop insidiously and are related chiefly to compression of adjacent structures, including the trachea, esophagus, and recurrent laryngeal nerves. Systemic evidence of inflammation is uncommon. The thyroid gland is moderately enlarged, stony hard, and usually asymmetric. The consistency of the gland and the invasion of adjacent structures suggest carcinoma, but there is no enlargement of regional lymph nodes. Temperature, pulse, and leukocyte count are normal. Severe hypothyroidism is unusual but does occur, as does loss of parathyroid function. The RAIU may be normal or low. Elevated circulating thyroid autoantibodies are much less common and are found in lower titers than in Hashimoto disease.

Tamoxifen, 10 to 20 mg/day (with or without corticosteroids), has been successful in many of these patients and is thought to suppress transforming growth factor beta (TGFβ).[155] Surgery may be required to preserve tracheal and esophageal function, although the response to tamoxifen will often preclude the necessity for this. Treatment with thyroid hormone relieves the hypothyroidism but has no effect on the primary process.

Miscellaneous Causes

Only a few causes of generalized inflammation of the thyroid gland have been reported. These include inflammation arising after ^{131}I treatment for Graves disease, a residual thyroid lobe in a patient with thyroid cancer of the contralateral lobe, and thyroiditis arising from external beam therapy for conditions such as Hodgkin or non-Hodgkin lymphoma, breast carcinoma, or other lesions of the oropharynx. Anaplastic thyroid carcinoma may rarely be associated with a diffuse thyroiditis and elevation of thyroid hormone levels.[260] In general, only radioiodine-induced thyroiditis is associated with pain, and glucocorticoid treatment may be useful in symptomatic therapy.

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The complete list of references is available online at ExpertConsult.com.

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14

Nontoxic Diffuse Goiter, Nodular Thyroid Disorders, and Thyroid Malignancies

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CHAPTER OUTLINE

KEY POINTS

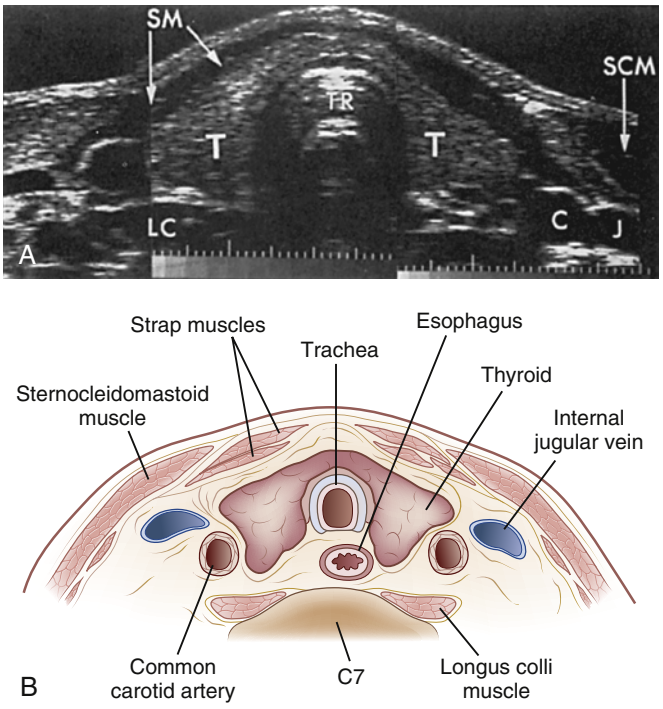
- Neck sonography has become an integral part of the clinical evaluation of thyroid patients and a useful tool in low-risk thyroid cancer patients.
- A more precise risk stratification of thyroid cancer patients has been achieved.
- Surveillance or less invasive surgical strategies can be safely pursued for low-risk thyroid cancer patients.
- Radioiodine treatment for ablation and as adjuvant therapy for intermediate and high-risk patients should be used selectively.
- A more detailed molecular profile of thyroid nodules and thyroid cancer has been achieved. This allows for improved presurgical detection of benign or malignant thyroid nodules, and a more precise profile for high-risk thyroid cancer.
- Targeted therapy has changed the approach to patients with metastatic differentiated and medullary thyroid cancer.

This chapter reviews the imaging techniques available for evaluating thyroid structural abnormalities. Moreover, it deals with the management of nontoxic diffuse goiter as well as benign and malignant thyroid neoplasia. Goiter resulting in thyrotoxicosis and other thyroid conditions arising from autoimmune thyroid disease are considered in Chapters 11–13. The section on management of thyroid nodules and thyroid malignancies is largely based on recently available guidelines from the American Thyroid Association (ATA).[1]

Structural and Functional Imaging of the Thyroid

Ultrasonography

Sonography is a noninvasive technique that has become an integral part of the clinical evaluation of a thyroid patient.[2] High-frequency sound waves are emitted by a transducer and reflected as they pass through the body, whereupon the returning echoes are received by the transducer, which also acts as a receiver. The amplitude of the reflected sound waves is influenced by differences in the acoustic impedance of tissues encountered by the sound; for example, fluid-filled structures reflect few echoes and therefore have no or few internal echoes and well-defined margins, solid structures reflect varying amounts of sound and thus have varying degrees of internal echoes and less well-defined margins, and calcified structures reflect virtually all incoming sound and yield pronounced echoes with an acoustic shadow posteriorly. Thyroid parenchyma, surrounding anatomic structures, and thyroid nodules as small as 2 mm in diameter can be readily detected. Color flow Doppler ultrasonography allows visualization of vessels as well as assessment of nodular vascularity. The thyroid gland must be examined thoroughly in transverse and longitudinal planes. Imaging of patients with thyroid nodules and during follow-up of thyroid cancer should also include evaluation of the regional neck lymph node compartments, with the goal of identifying enlarged and pathologic nodes.[3] The normal thyroid parenchyma has a characteristic homogeneous medium-level echogenicity, with little identifiable internal architecture (Fig. 14.1). The surrounding muscles typically have a hypoechoic appearance. The air-filled



• **Fig. 14.1** Transverse composite sonogram (A) and corresponding anatomic map (B) of the normal thyroid gland. *C,* common carotid artery; *C7,* seventh cervical vertebra; *J,* internal jugular vein; *LC,* longus colli muscle; *SM,* strap muscles; *SCM,* sternocleidomastoid muscle; *T,* thyroid; *TR,* trachea. (From Rifkin MD, Charboneau JW, Laing FC. Special course: ultrasound 1991. In: Reading CC, ed. *Syllabus: Thyroid, Parathyroid, and Cervical Lymph Nodes*. Oak Brook, IL: Radiological Society of North America; 1991:363–377.)

trachea in the midline gives a characteristic curvilinear reflecting surface with an associated reverberation artifact.

A diagrammatic representation of the neck showing the location(s) of any abnormal finding and their characteristics is a useful supplement to the routine film images recorded during an ultrasound examination. Such a cervical map with compartments[4] (Fig. 14.2) can help communicate the anatomic relationships of the findings (and/or pathology) more clearly to the referring clinician and serves as a reference for the sonographer on follow-up examinations.

Neck ultrasonography is clinically useful at each step of thyroid evaluation (Table 14.1). It confirms the presence or absence of a thyroid nodule when the findings on physical examination are equivocal and may reveal the presence of other nonpalpable nodules. Gray-scale and color Doppler ultrasound are used to evaluate the nodule's sonographic features, including size, shape, echogenicity (markedly or mildly hypoechoic, isoechoic, or hyperechoic), margins (irregular or smooth), composition (cystic, solid, or mixed), the presence of coarse or fine (microscopic) calcifications, and internal blood flow. This assessment can be highly useful for estimating the cancer risk in a given nodule. However, descriptions of single ultrasonographic features display wide interobserver variability. In part because of this, the American Thyroid Association[1] and other scientific bodies[5–8] have proposed tiered systems that can be used to estimate a nodule's risk of malignancy (based on its sonographic features) and determine whether it requires fine-needle aspiration biopsy (FNAB) based on size. In 2017, the American College of Radiology (ACR)[6] published a scoring system that promotes systematic assessment of the imaging features of thyroid nodules, the Thyroid Imaging Reporting and Data System (TIRADS), which is inspired by the ACR's recommended approaches to the imaging of the breast (BIRADS) and other organs. Five ultrasound features are assessed and numerically scored, and based on the sum of the five scores, the nodule is assigned to one of five TIRADS classes, each reflecting an estimated risk of cancer and recommendations for management (often including FNAB or surveillance). These classification systems have been shown to improve interobserver agreement.[9] Ultrasound also permits a comprehensive evaluation of regional lymph node compartments.

Elastography assesses tissue stiffness within an isolated, solid thyroid nodule and may prove useful as an indicator of malignancy risk (particularly in nodules that are cytologically indeterminate).[10] Early reports suggested very high specificity and sensitivity, independent of the nodule size. More recent reports suggest that elastographic cancer risk assessment may be inferior to gray-scale ultrasound,[11] with positive predictive values of only 30% to 40%.[12] These contradictory data suggest that elastographic evaluation of thyroid nodules is highly user dependent. Furthermore, it requires special software packages. Elastography cannot be applied to partly or mostly cystic nodules and can prove difficult in a multinodular goiter.

In patients with known thyroid cancer, sonography can be useful in evaluating the extent of disease, both preoperatively and postoperatively.[13] Thus in patients who present with cervical lymphadenopathy caused by papillary thyroid carcinoma (PTC) but in whom the gland is palpably normal, sonography may be used preoperatively to detect an occult primary intrathyroid focus. A preoperative sonogram should be obtained in all patients with differentiated thyroid carcinoma or medullary thyroid carcinoma (MTC) to preoperatively identify the anatomic locations of any sonographically suspicious regional lymph nodes and thereby permit planning of nodal dissection.[1,14] Occasionally impalpable residual cancer that had been identified by preoperative ultrasonography and found cytologically positive by ultrasound-guided FNAB can be identified intraoperatively by the use of a handheld ultrasound probe or by preoperative ultrasound-guided charcoal tattooing.[15]

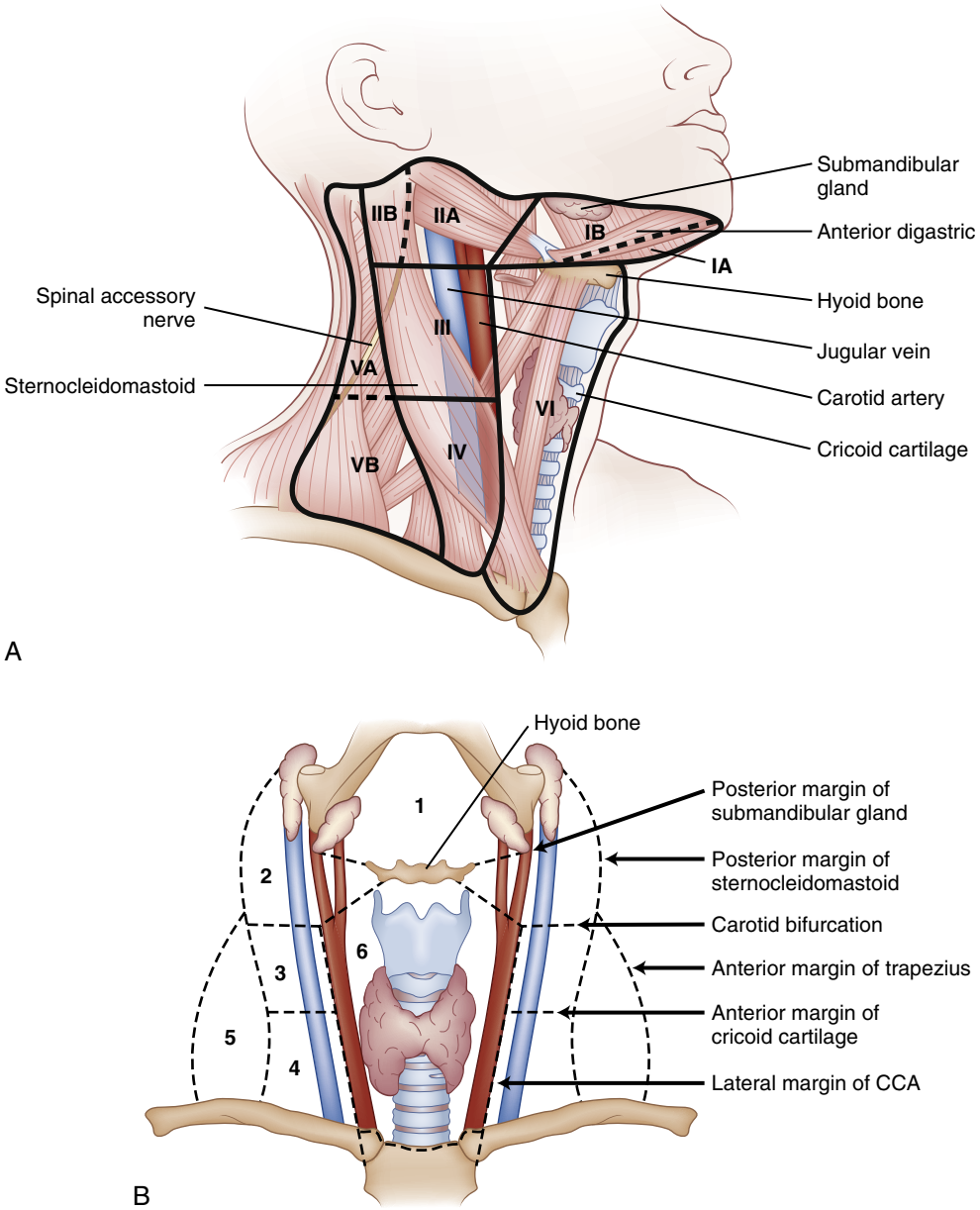
After initial therapy for follicular cell–derived thyroid cancer (FCTC), sonography (together with measurement of serum thyroglobulin [Tg]) represents the most useful method for detecting residual, recurrent, or metastatic disease in the neck.[3,16,17] In patients who have undergone less than a total thyroidectomy, the sonographic appearance of the remaining thyroid tissue may be an important factor in the decision whether to recommend completion thyroidectomy. Also, it is more sensitive than neck palpation in detecting recurrent disease within the thyroid bed and metastatic disease in cervical lymph nodes.

Sonography is also the standard modality for guiding FNAB of most thyroid nodules and cervical lymph nodes, demonstrating improved accuracy and a reduction in nondiagnostic specimens.[1]

External Scintiscanning

Localization of functioning or nonfunctioning thyroid tissue in the area of the thyroid gland or elsewhere is made possible by techniques of external scintiscanning. The underlying principle is that isotopes that are selectively accumulated by thyroid tissue can be detected by a gamma camera and the data transformed into a visual display. Radioactivity in specific areas can be quantified.[18–20] The units of measurement used in evaluation of the radiation dose and radioactivity are defined in Table 14.2.

Several radioisotopes are used in thyroid imaging. Technetium-99m (^{99m}Tc) pertechnetate is a monovalent anion that is actively concentrated by the thyroid gland but undergoes negligible organic binding and diffuses out of the thyroid gland as its concentration in the blood decreases. The short physical half-life of ^{99m}Tc (6 hours), its low fractional uptake, and its transient stay



• **Fig. 14.2** (A) Anatomic scheme of the neck with compartments. (B) Cervical map, derived from sonographic images, helps communicate anatomic relationships of disease to clinicians and serves as a reference for follow-up examinations; standard colors are used to characterize any finding. *CCA,* common carotid artery. (A, from Cooper DS, Doherty GM, Haugen BR, et al. Revised management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid*. 2009;19:1167–1214, used with permission; B, courtesy J.W. Charboneau, Mayo Clinic, used with permission.)

TABLE 14.1 Clinical Utility of Neck Ultrasound

Map of the neck (thyroid and lymph node areas)
Thyroid gland: size, volume, characteristics
Nodules: number and characteristics of each nodule: diameter, shape, echogenicity, composition, limits, presence of calcifications, vascularization
Lymph node compartments
Follow-up: numbers and diameters of nodules
Guidance for fine-needle aspiration biopsy
Follow-up of thyroid cancer: thyroid bed and regional lymph nodes
Guidance for thermal and ethanol ablation

within the thyroid make the radiation delivered to the thyroid gland by standard activity very low. Consequently, the intravenous administration of radioactivities greater than 37 MBq (1 mCi) permits adequate imaging of the thyroid about 30 minutes later.

Two radioactive isotopes of iodine have been used in thyroid imaging. Iodine-131 (^{131}I) was commonly used in the past. Contrary to ^{99m}Tc, iodine isotopes undergo organic binding. However, ^{131}I is a beta emitter, its physical half-life is 8.1 days, and the energy of its main gamma ray is high. Because of this, ^{131}I is poorly adapted for its detection.[20] In many respects, ^{123}I is an ideal isotope for thyroid imaging because of its short half-life (0.55 day) and the absence of beta radiation, but it is unfortunately much more expensive.[21]

TABLE 14.2 Radiation Nomenclature: Traditional and International System (SI) Units

Absorbed Radiation Dose

Units

Gy (gray) and rad (radiation absorbed dose)

Conversions

1 Gy = 100 rad = absorption of 1 J/kg
1 rad = 0.01 Gy = 1 cGy

Dose Equivalent Radiation

Units

Sv (sievert) and rem (roentgen equivalent in man)

Conversion

1 Sv = 100 rem

Radioactivity (or Activity)

Units

Bq (becquerel) and Ci (curie)

Conversions

1 Bq = 1 disintegration per second = 27 pCi
1 mCi = 37 MBq
1 GBq = 10^3 MBq = 10^6 kBq = 10^9 Bq

Notes

Because the becquerel is extremely small, commonly used multiples of the Bq unit are kBq (kilobecquerel), MBq (megabecquerel), and GBq (gigabecquerel).
A curie, however, is extremely large, so commonly used subunits are mCi (millicurie), μCi (microcurie), nCi (nanocurie), and pCi (picocurie).
Gy, Sv, and Bq are SI units; the rad, rem, and Ci are non-SI units.

The most important use of scintigraphic imaging of thyroid tissue is in defining areas of increased or decreased function (hot or cold areas, respectively) relative to the function of the remainder of the gland. Almost all malignant nodules are hypofunctioning, but more than 80% to 85% of benign nodules are also nonfunctioning. Conversely, functioning nodules (hot nodules), particularly if the function of the surrounding tissue is decreased or absent, are rarely malignant.

Scintiscanning with radioactive iodine can also be used to demonstrate that intrathoracic masses represent thyroid tissue and to detect ectopic thyroid tissue in the neck. In FCTC patients, whole-body scanning (WBS) is used to detect functioning metastases.[22,23] This scan is performed after the administration of larger activities of radioiodine, for diagnosis (1–5 mCi ^{131}I or 1–5 mCi ^{123}I), or more commonly for therapy (≥30 mCi ^{131}I). Such dosing must follow intense stimulation with endogenous or exogenous thyroid-stimulating hormone (TSH, thyrotropin) and be performed in the absence of iodine contamination. Superimposition of computed tomography (CT) and gamma camera images (single-photon emission computed tomography [SPECT]/CT) greatly improves both the sensitivity and specificity of the technique and the anatomic localization of any focus of uptake.[24–27]

Computed Tomography

The CT appearance of the anatomic structures depends on the attenuation of the tissue examined. The thyroid gland, because of its high concentration of iodine, has a higher attenuation than the surrounding soft tissues. Recent advances with spiral CT and reconstruction algorithms have improved the performance of this method.[28,29]

CT scanning cannot distinguish benign from malignant nodules or provide information on a nodule's functional status. However, it can define the anatomic extent of large goiters with great clarity. CT scanning can provide useful information regarding the presence and extent of intrathoracic (substernal) goiters. The CT findings of an intrathoracic mass in continuity with the thyroid gland, with high attenuation on non–contrast-enhanced images and marked enhancement after intravenous contrast material injection, all suggest intrathoracic goiter. Radioiodine scanning can also be performed in this clinical setting, but false-negative results can occur when little or no functional tissue is present in the intrathoracic goiter.

In aggressive pathologic processes, such as anaplastic thyroid carcinoma (ATC), CT with infusion of contrast medium is the most frequently recommended first-line technique to identify lymph node metastases and to define the tumor's relationships to surrounding structures, including vessels and the aerodigestive tract.[28,29] CT imaging is less sensitive than neck ultrasonography for the detection of lymph node metastases. However, contrast-enhanced CT can be a useful complement to ultrasound for exploring the mediastinum and for preoperative assessments of vascular invasion and involvement of the retrotracheal space. High-resolution CT without contrast enhancement is the most sensitive method for the detection of micrometastases in the lungs. Because of the necessity of infusing iodine-containing contrast agents for CT scanning of the neck and mediastinum, CT should be performed at least 4 weeks before any administration of radioiodine.[30]

Magnetic Resonance Imaging

Because the hydrogen atoms of different tissues have different relaxation times (termed *T1* and *T2*), a computer-assisted analysis of T1-weighted and T2-weighted signals is used to differentiate the thyroid gland from skeletal muscles, blood vessels, or regional lymph nodes. Normal thyroid tissue tends to be slightly more intense than muscles on a T1-weighted image, and tumors often appear more intense than normal thyroid tissue.

Magnetic resonance imaging (MRI) does not distinguish benign from malignant nodules and does not assess functional status. Recurrent neoplasms in the thyroid bed or regional lymph nodes can be detected with MRI. Recurrence is characterized by a mass with low to medium intensity on T1-weighted images and medium to high signal intensity on T2-weighted images. Conversely, scar tissue or fibrous tissue has low signal intensity on both T1-weighted and T2-weighted images.[28,29] Tumor invasion of adjacent skeletal muscle has high signal intensity on T2-weighted images. Edema or inflammation in the muscle can cause a similar appearance and can be difficult to differentiate from recurrent tumor.

Compared with CT scan, MRI may better delineate any involvement of the aerodigestive axis. It is often used as a second-line imaging technique in patients with demonstrated or suspicious lesions in the upper part of the neck on CT scan to better delineate these lesions from soft tissues. In the lower part of the neck, movements of the aerodigestive axis during the procedure that may last several minutes will decrease the quality of images. Endoscopy of the trachea and/or esophagus, with or without

ultrasonography, looking for evidence of intraluminal extension, can also be helpful in cases of suspected aerodigestive tract invasion.

MRI is superior to CT for detecting brain metastases from solid tumors because it offers higher soft tissue contrast with no bone artifacts and relatively few partial volume effects. The paramagnetic contrast agents used with MRI also produce significantly stronger enhancement than those used with CT. For these reasons, in patients with multiple brain lesions, CT scanning will miss approximately one in five of those seen on MRI.[31] MRI is useful for assessment of the extent of bone involvement in cases of axial bone metastases from follicular cell–derived carcinomas and MTC, which are poorly visualized on bone scintigraphy.[32,33] For MTC patients, contrast-enhanced MRI is more sensitive than three-phase contrast-enhanced CT for the detection of liver metastases.[33]

Positron Emission Tomography (PET)

PET is both quantitative and tomographic. The radionuclide used emits a positron that is converted into a pair of photons after a short path of a few millimeters in the tissue. The coincident detection of the two photons, which travel on a line in opposite directions, permits the localization of the site of the radionuclide decay.

PET is being used with increasing frequency for the assessment of all types of cancers. The tracer used most often in clinical settings is the glucose analog [^{18}F]fluorodeoxyglucose (^{18}FDG). It is taken up by both neoplastic and benign cells and remains metabolically trapped inside tumor cells because of its inability to undergo glycolysis. PET scanners permit in vivo images related to regional glucose metabolism, with high sensitivity and spatial resolution. Superimposition of CT and PET images greatly improves both the sensitivity and specificity of the technique and the anatomic localization of any focus of abnormal uptake. The uptake in any focus can be quantified, and the most frequently used parameter is the maximum standardized uptake value (SUVmax). The sensitivity of ^{18}FDG-PET scanning may be improved with TSH stimulation.[34,35]

PET scanning should be performed in only selected patients with thyroid carcinoma. Low-risk patients are very unlikely to require ^{18}FDG-PET scanning as part of initial staging or follow-up. ^{18}FDG-PET scanning in thyroid cancer[23,34] may be used as follows:

- To localize disease in Tg-positive patients (having serum Tg levels >10 ng/mL) with no other abnormality on diagnostic imaging; it is mostly useful for the detection of lymph node metastases in the posterior neck and mediastinum or distant metastases. In two meta-analyses, the patient-based sensitivity of ^{18}FDG-PET/CT for detecting persistent or recurrent differentiated thyroid cancer (DTC) was found to be appreciably higher than that of PET alone (93–94% vs 83–84%, respectively).[36,37]
- For the initial staging and follow-up of patients with anaplastic, poorly differentiated, or Hürthle cell thyroid cancers to identify sites of disease that may be missed with conventional imaging; in these cancers, FDG uptake is usually high and ^{131}I uptake is either low or absent.
- In patients with known distant metastases in whom high FDG uptake in large metastases indicates a high risk for disease-specific fatality and poor response to ^{131}I therapy.[38,39]
- As a measurement of posttreatment response following local (external beam irradiation, surgical resection, thermal ablation, embolization) or systemic therapy.[40]

Inflammatory lymph nodes, suture granulomas, and increased muscle activity are common causes of false-positive ^{18}FDG-PET findings. Also, asymmetric laryngeal uptake is frequently observed in patients with vocal cord paralysis. Therefore cytologic or histologic confirmation is required before one can be certain that an ^{18}FDG-positive lesion represents metastatic disease. High uptake has also been observed in several thyroid diseases, such as Hashimoto thyroiditis, and PET cannot be used to differentiate benign from malignant thyroid nodules. The discovery of focal thyroid uptake *(thyroid incidentaloma)* on a FDG-PET scan performed for other reasons should lead to a complete workup, including ultrasound and fine-needle aspiration (FNA) cytologic testing, because one-third of these nodules may prove to be malignant.[41,42] Delineation between lymph node metastases or local extension of the tumor and vessels or the aerodigestive axis is often not well visualized on ^{18}FDG-PET/CT in the absence of contrast injection; if necessary, other imaging techniques (CT and MRI with contrast medium) may be performed, especially for a preoperative workup.

PET scans can also be done with iodine-124 (^{124}I) (although this approach is currently used almost exclusively in research settings).[13] Thanks to its tomographic capacity and superior resolution, ^{124}I PET is more sensitive than diagnostic and therapeutic WBS performed with ^{131}I. PET scanning with ^{18}F-labeled dihydroxyphenylalanine (^{18}F-DOPA) can be gainfully used to visualize neoplastic foci of MTC[43,44] because the ^{18}FDG uptake is usually low in MTC patients, and ^{18}FDG-PET scan in that clinical setting is therefore rarely informative.[33,45]

Nontoxic Goiter and Thyroid Nodular Disease

Definitions

Nontoxic goiter is defined as any thyroid enlargement that is characterized by uniform or selective (i.e., restricted to one or more areas) expansion of thyroid tissue separate from a nodule or neoplastic growth. A goiter may or may not be associated with overt hyperthyroidism or hypothyroidism. A thyroid nodule is defined as a discrete lesion within the thyroid gland due to an abnormal focal growth of thyroid cells.

Epidemiology of Goiter

The prevalence of goiter varies widely by global location and can depend on the iodine intake of a given population. Thus goiter may occur endemically, due mainly to iodine deficiency, or sporadically, depending on whether the goiter prevalence in children is more or less than 5%, respectively. In pregnancy, goitrous enlargement is physiologic and usually regresses postpartum. In an adult nonpregnant population, the Framingham survey revealed a 4.6% prevalence of goiter, with a strong female predominance (6.4% in women and 1.5% in men), whereas the Wickham study displayed a 3.2% prevalence (6.6:1 hazard ratio of women to men).[46,47] However, different variables (regional variation in the iodine intake, smoking habits, age and sex distribution, and primarily the methodology [palpation vs. sonography] used to determine thyroid volume) may have influenced these data. Using sonography as the screening method, the prevalence of goiter in an unselected adult population has been reported to be as high as 30% to 50%. This prevalence is even higher in iodine-deficient areas and in older populations. Similarly, a prevalence of thyroid nodules nearing 50% has been described in adult and geriatric

autopsy series[48] and up to 65% in healthy adults screened with sonography. [49]

Etiology and Pathophysiology of Diffuse Goiter

Diffuse goiter has been traditionally regarded as the adaptive response of the thyroid follicular cell to any factor that impairs thyroid hormone synthesis. This classic concept, however, no longer appears to encompass the many aspects of goiters. Indeed, goiter is characterized by a variety of clinical, functional, and morphologic presentations, and whether this heterogeneity represents different entities remains to be clarified. Also, iodine deficiency as the sole factor responsible for goiter appears to be an oversimplification. Thus not all inhabitants in an iodine-deficient region develop goiter; moreover, endemic goiter has been observed in countries with no iodine deficiency, and even in some regions with iodine excess, and has not been observed in some regions with severe iodine deficiency. These findings suggest that other factors (genetic, demographic, and environmental) may play a role in the genesis of diffuse and nodular goiter, and some of these factors may act synergistically. Multiple nodules causing a goiter are often caused by somatic mutations leading to neoplastic growth.

The role of genetic factors in goiter formation is suggested by several lines of evidence,[50] such as (1) the clustering of goiters within families, (2) the higher concordance rate for goiters in monozygotic than in dizygotic twins, (3) the female-to-male ratio (1:1 in endemic vs. 7:1 to 9:1 in sporadic goiters), and (4) the persistence of goiters in areas where a widespread iodine prophylaxis program has been properly implemented. By studying families affected by diffuse goiter, researchers have been able to detect several gene abnormalities involving proteins related to thyroid hormone synthesis, such as mutations in genes encoding TG, sodium/iodide symporter (NIS), thyroid peroxidase (TPO), dual oxidase 2 (DUOX2), pendrin (Pendred syndrome [PDS]), and TSH receptor (TSHR). In addition, three loci for this disorder have been identified that map to chromosomes 14q, Xp22, and 3q26, respectively.[51,52] Although an autosomal dominant inheritance has been demonstrated in several families, multiple genes may be involved in other families, indicating a marked genetic background underlying the cause of the goiter. This complicated genetic pattern may explain why predisposing gene alterations remain unidentified in most patients with nontoxic goiter.

In addition to iodine deficiency and genetic susceptibility, the exposure to a variety of environmental factors has been linked to goiter generation.[53,54] Thus certain endocrine disrupters have been suggested to be involved in goiter development, including phthalates,[55,56] perchlorate, thiocyanate, and nitrate; isoflavones; and organochlorines as well as drugs, smoking, selenium deficiency, insulin resistance, oral contraceptives, parity, and alcohol.[57,58]

TSH has long been considered the major stimulus for thyroid growth in response to any factor impairing thyroid hormone synthesis. Indeed, in the rare clinical setting of a functioning TSH-secreting pituitary adenoma, the increased serum TSH concentrations typically cause enlargement of the thyroid gland.[59] Similarly, goiter is also a typical feature of Graves disease, in which a stimulatory growth effect on thyroid tissue is induced by thyroid-stimulating immunoglobulin (TSI) antibody through TSHR activation. Moreover, thyroid enlargement may appear during the course of Graves disease when increased TSH levels result from overtreatment with antithyroid drugs. In addition, toxic thyroid hyperplasia is usually present in nonautoimmune autosomal dominant hyperthyroidism, a disorder related to germline-activating mutations of the TSHR gene.[60] This clinical condition further emphasizes the role of TSH-TSHR system activation in the genesis of thyroid hyperplasia.[50] Serum TSH concentration is normal in most patients with nontoxic goiter.[53] Experimentally, it has been demonstrated that in rats iodine depletion enhances the promotion of thyroid growth by normal levels of TSH. Hence, any factor that impairs intrathyroidal iodine levels may lead to gradual development of goiter in response to normal concentrations of TSH.

More intriguing is the relationship between TSH levels and iodine supply. Indeed, even small differences in the level of iodine intake are correlated with significant differences in TSH levels; this change has been demonstrated after the 11-year follow-up in the longitudinal population-based Danish program monitoring the nationwide iodine fortification (DanThyr study).[61] A complex network of both TSH-dependent and TSH-independent pathways directs thyroid follicular cell growth and function and plays a role in the goitrogenic process. In particular, a variety of growth factors, derived either from the bloodstream or through autocrine or paracrine secretion, may serve to regulate thyroid cell proliferation and differentiation processes.[50] Typically, early in the course of goiter formation, areas of microheterogeneity of structure and function are intermixed and include areas of functional autonomy and areas of focal hemorrhage. Analysis of hyperplastic nodules by rigid criteria also indicated that morphologically indistinguishable hyperplastic thyroid nodules may be either monoclonal or polyclonal. Monoclonal adenomas within hyperplastic thyroid glands may reflect a stage in progression along the hyperplasia-neoplasia spectrum; accumulation of multiple somatic mutations may subsequently confer a selective growth advantage to this single-cell clone. [62]

Histologically, thyroid nodules contain irregularly enlarged, involuted follicles distended with colloid or clusters of smaller follicles lined by taller epithelium and containing small colloid droplets. The nodules tend to be incompletely encapsulated and are poorly demarcated from and merge with the internodular tissue, which also has an altered architecture. However, the nodules in some glands appear to be localized, with areas of apparently normal architecture elsewhere. Here, the distinction from a follicular adenoma may be difficult, and some pathologists apply terms such as colloid or adenomatous nodules to such lesions. Adenomatoid nodules show distinct gene expression patterns separate from those found in malignant lesions. Recent data have confirmed that distinct somatic mutations in SPOP, *ZNF148*, and *EZH1* are responsible for the formation and growth of most benign nodules.[63]

Natural History of Goiter and Thyroid Nodules

Nontoxic diffuse goiter has a female preponderance. There appears to be no physiologic increase in thyroid volume during normal adolescence. Development of a diffuse goiter during adolescence therefore is a pathologic rather than a physiologic process.[64] A notable exception in women of childbearing age is pregnancy, which causes a diffuse enlargement of the gland as hormone demand and production increase.

Iodine intake influences the natural history of nodular goiter disease. In the DanThyr follow-up study[58] it was demonstrated that 11 years after the iodization program, one-third of solitary thyroid nodules identified at baseline had disappeared; interestingly, one-fifth of previous multinodular goiters turned out to be diffuse. This finding suggested that the iodine intake is the main factor in determining the nodular thyroid disease appearance in

a given area. Furthermore, the survey demonstrated that thyroid nodularity is a dynamic and not necessarily an irreversible process. Therefore dissimilar iodine intake may account for the epidemiologic thyroid nodular disease differences between the United States, which carries an adequate iodine supply, and European as well other countries, which previously displayed a mild to moderate iodine deficiency.

The natural history of benign thyroid nodules, once detected, is to grow slowly if followed long enough, though there is extensive heterogeneity within any population of nodules. While on average nodules will slowly grow,[65,66] many will stay dormant for years or decades.[67] Rarely, benign nodules will shrink. When this happens, it is most often associated with the resorption of cystic fluid in a nodule.[68]

Clinical Presentation of Goiter and Nodules

For most patients, the finding of a palpable abnormality in such a superficial location as the thyroid gland is disconcerting, and the affected patient is likely to seek medical evaluation. At the end of an appropriate investigation, the clinician can usually reassure the patient that the goiter or the nodule is benign. Autonomous nodules or autonomous functional areas in the context of a multinodular goiter may result in an increased thyroid hormone secretion and subsequently a subclinical or overt thyrotoxicosis. This feature is, however, a rare event, especially in the United States, being mainly linked to the iodine deficiency. However, in general, thyroid nodules are usually not associated with abnormal thyroid hormone secretion. Therefore affected patients do not exhibit clinical signs of thyroid dysfunction and are often asymptomatic. The only clinical features of nontoxic goiter may be those of thyroid enlargement. In a health care system in which the extent of cross-sectional imaging has increased, a large proportion of clinically relevant nodules are incidentally detected during carotid ultrasonography or CT and MRI studies of the chest, neck, or head. Such incidentally detected nodules carry the same risk of malignancy as do nodules identified on clinical examination. However, an incidentally discovered thyroid nodule with increased metabolism on ^{18}FDG-PET is different, as discrete uptake of ^{18}FDG in a thyroid nodule is associated with an increased risk of thyroid cancer.[69]

Most thyroid nodules are asymptomatic. However, large nodules, which may displace or compress the trachea, esophagus, and neck vessels, can be rarely associated with symptoms and signs, including neck tightness, dysphagia, and a choking sensation. These obstructive symptoms may be accentuated by the so-called Pemberton maneuver (see Chapter 11). Invasion or compression of the recurrent laryngeal nerve, causing hoarseness, rarely occurs, though when present, it often suggests advanced thyroid carcinoma. More commonly, acute hemorrhage into a cystic nodule may produce acute, painful asymmetric enlargement of the neck and can enhance or induce obstructive symptoms.[53]

The Approach to Thyroid Nodular Disease

Thyroid nodules are generally benign hyperplastic (or colloid) nodules or benign follicular adenomas. However, multiple retrospective studies confirm that about 5% to 15% of clinically relevant nodules prove cancerous.[70,71] The prevalence of thyroid cancer in the United States, as well as in most industrialized countries, has been steadily increasing.[72] There is debate if this increase is mostly due to increased detection (and increased reporting) of small, indolent malignancies or represents a true increase in thyroid cancer incidence.[73–75] An increase in more advanced thyroid cancer has been detected in some studies, raising questions that other factors beyond simply sampling bias may impact this finding. Regardless, the mortality rate attributable to thyroid cancer remains very low.[74]

In general, thyroid nodules larger than 1 to 1.5 cm in diameter are generally considered clinically relevant. Nodules smaller than this size, even if malignant, rarely cause harm and therefore are typically followed conservatively. Recent guidelines of the American Thyroid Association provide guidance for how ultrasound assessment of a nodule can be used to determine the size cutoff at which evaluation is recommended.[1]

In the evaluation of a clinically relevant thyroid nodule (Table 14.3), a thorough history and careful physical examination should be supplemented with laboratory testing, imaging procedures (including neck ultrasonography), and most importantly consideration of fine-needle aspiration for cytologic and/or molecular assessment. With this approach, an individualized assessment of malignancy risk and the specific morbidity and mortality risks attributable to such malignancy can be made. This evaluation allows the health care worker to advise appropriate treatment in relation to the patient's other illnesses and desires. Historic features that suggest malignancy include young age under approximately 30 years,[76] male sex, a history of external neck radiation during childhood or adolescence, total-body radiation for bone marrow transplant, and rapid nodule growth or persistent changes in speaking, breathing, or swallowing. Rarely, a family history of multiple endocrine neoplasia (MEN) type 2, PTEN hamartoma tumor syndrome (Cowden disease), familial adenomatous polyposis, or Carney complex is detected. When identified, this should prompt thyroid evaluation in the family members.[53]

On physical examination, a large, fixed, and firm nodule is worrisome for malignancy, especially when suspicious regional lymphadenopathy is detected.[3,4] It should be noted, however, that most patients are asymptomatic at presentation, and physical examination simply detects a nodule 1 to 3 cm in size that is nontender and mobile with swallowing.

Many studies have shown that nodule size minimally impacts the risk of malignancy[77] and that the incidence of cancer in incidentally identified nodules is the same as in those with palpable nodules. However, in nodules larger than 4 cm in diameter, the incidence of carcinoma may be higher.[78] The presence of multiple nodules does not decrease the likelihood of thyroid cancer. In patients with multiple clinically relevant nodules, the rate of malignancy per nodule decreases, but the decrease is approximately proportional to the number of detected nodules. Therefore

TABLE 14.3 Clinical Findings Associated With Malignant Thyroid Nodules

Historic Features
Young age (<20–30 years old)
Male sex
Neck irradiation during childhood or adolescence
Rapid growth
Recent, persistent changes in speaking, breathing, or swallowing
Family history of multiple endocrine neoplasia type 2
Physical Examination
Firm, fixed, and irregular consistency of nodule
Vocal cord paralysis or hoarseness
Persistent regional lymph adenopathy

the overall cancer rate per patient is the same in those with multiple nodules as in those with a solitary nodule. Importantly, when multiple nodules are present, each must be separately evaluated because the dominant (largest) nodule is not solely representative of thyroid cancer risk.[65]

The Evaluation of Patients With Nodular Disease

In all patients with suspected or known thyroid nodules, measurement of serum TSH is recommended. A low or undetectable serum TSH, even if associated with normal free thyroid hormone levels, should suggest the possibility of toxic, autonomously functioning nodules and prompt thyroid scintigraphy. Higher serum TSH concentrations, even within the normal reference range, may increase the risk that a thyroid nodule is cancerous.[79,80]

Measurement of serum anti-TPO antibody (TPOAb) concentration may assist with the diagnosis of chronic lymphocytic thyroiditis (Hashimoto thyroiditis) when the serum TSH level is elevated. Hashimoto thyroiditis causes a heterogeneous parenchymal appearance on sonography that at times can mimic a pseudonodule. When an elevated TPOAb and heterogeneous sonographic pattern are detected, a thyroid nodule must be sonographically discrete in three separate dimensions to warrant evaluation. Hashimoto disease may also be associated with the presence of bilateral, enlarged but benign-appearing lymphadenopathy. This feature is due to the immune nature of this disease and should not necessarily cause alarm. In some patients, an FNA (described later) will be required to help distinguish benign from suspicious disease.

FCTCs may release increased amounts of Tg into the bloodstream. Unfortunately, there is overlap of serum Tg levels in FCTCs and in most benign conditions. Therefore the measurement of serum Tg levels is not useful in the initial workup of nodular thyroid disease. Some investigators recommend routine measurement of serum calcitonin levels in all patients with nodular thyroid disease to screen for MTC.[81,82] However, because of the rarity of unsuspected MTC, the high frequency of false-positive results that often prompts further workup or thyroidectomy, and the unknown clinical relevance of medullary microcarcinomas (<1 cm), it is neither cost-effective nor necessary to measure serum calcitonin levels in the initial evaluation of patients with nodular thyroid disease. In circumstances of greater suspicion (e.g., the presence of microcalcifications in the nodule), however, the measurement of serum calcitonin may prove useful.[3,53] If the unstimulated serum calcitonin determination is greater than 100 pg/mL, MTC is likely present.[82]

Ultrasonographic evaluation is the optimal means of evaluating the anatomic structure of the thyroid. Ultrasonography allows the health care provider to assess both the morphologic appearance and the size of the gland, while also assessing cancer risk in thyroid nodules.[1,3,5,53] Ultrasonography is capable of detecting even minute thyroid nodules. In fact, of 1000 normal control subjects, 65% had detectable nodularity on high-resolution sonography.[49] Numerous studies demonstrate that ultrasound can effectively stratify malignancy risk in thyroid nodules (Figs. 14.3 and 14.4). Such risk assessment then guides diagnostic and evaluative strategies for any given patient. For example, FNA of higher risk nodules is generally recommended when equal to or larger than 1 cm. In contrast, very low-risk nodules may not require FNA until growth beyond 2 cm is detected.[1] Features with the highest specificity for thyroid cancer include the presence of microcalcifications, hypoechoic parenchyma, and infiltrative or irregular margins.[83–87] Such features are most predictive when present in combination. The presence of abnormal adenopathy, especially when unilateral and in the lower neck, also increases the risk of cancer when a thyroid nodule is confirmed. Macrocalcifications, however, do not predict malignancy, unless seen in combination with microcalcifications.[83,87] A taller-than-wider shape (i.e., the anteroposterior dimension is larger than the transverse dimension on a transverse image) has been associated with increased malignancy risk in some studies, though this remains controversial, especially given the lack of a clear hypothesis suggesting why such a growth pattern would prove more malignant. In contrast, purely cystic nodules, a spongiform parenchyma, and homogeneously hyperechoic lesions carry the lowest risk of malignancy.[5,49,83,84,88]

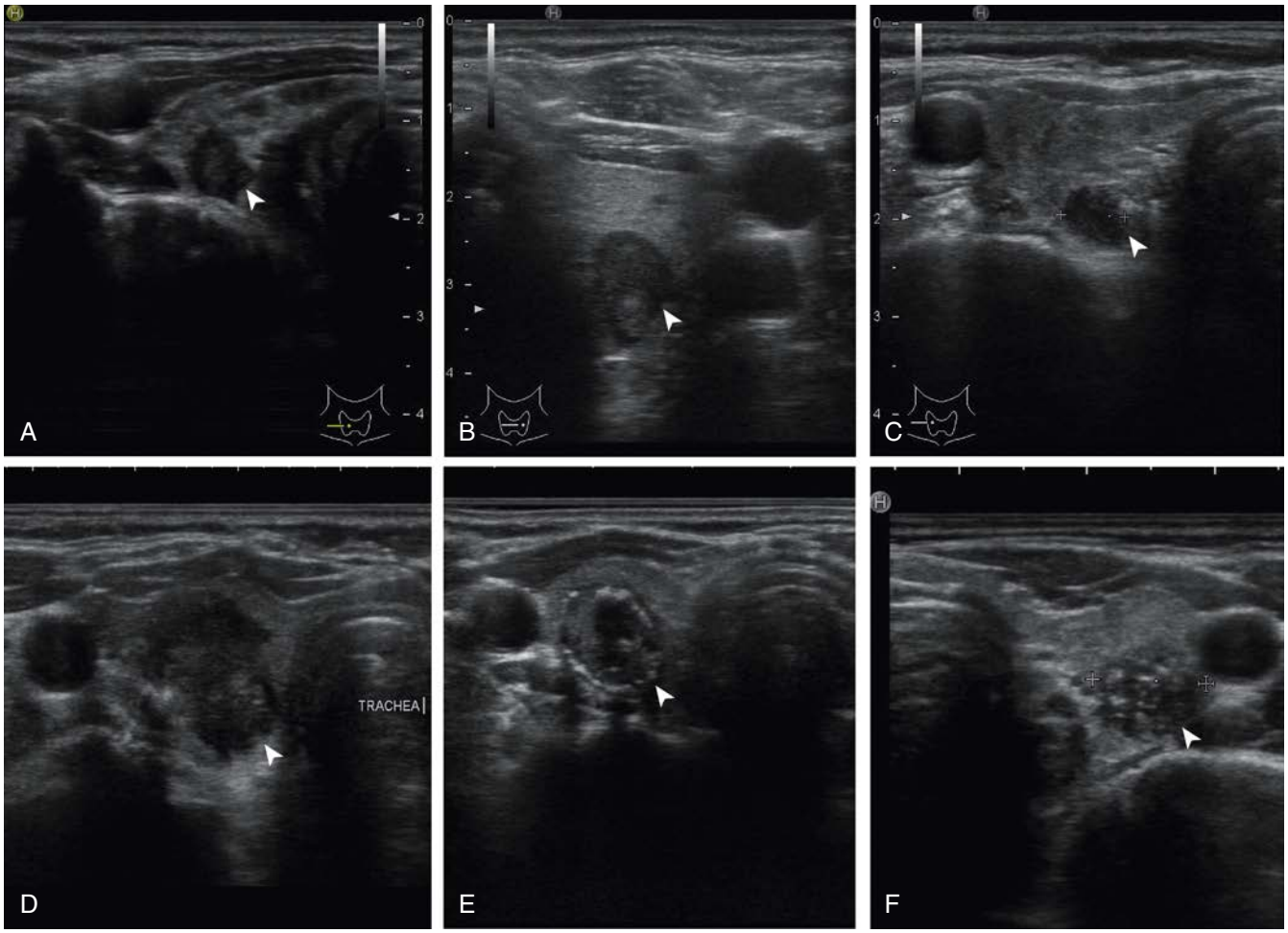
Extensive published research confirming the utility of sonographic risk assessment, combined with substantial advances in ultrasound technology, has led experts to now routinely recommend a sonographic risk classification for all thyroid nodules (ATA guidelines, European Thyroid Association [ETA], American Association of Clinical Endocrinologists [AACE], TIRADS). Nodules should be classified into suspicion categories (high, intermediate, low, and very low), as this allows an evidenced-based strategy to support future intervention such as FNA versus conservative follow-up.

High-risk nodules are solid and hypoechoic with additional findings of microcalcifications or an irregular border. Cancer risk is estimated at 70% to 90% in such lesions. Intermediate-risk and low-risk nodules constitute the majority of nodules seen in clinical practice. Intermediate-risk nodules are solid and hypoechoic but without the additional concerning features listed for high-risk nodules. Low-risk nodules are solid, isoechoic or hyperechoic, or partially cystic, yet they also lack concerning features of microcalcifications, irregular margins, and abnormal adenopathy. Cancer risk in these two groups is approximately 25% and 10%, respectively. Nodules at high or indeterminate risk are generally recommended for FNA if their maximal diameter exceeds 1 cm, whereas low-risk nodules can be followed until growth exceeds 1.5 cm. Very low-risk nodules are mostly cystic or spongiform, and risk of malignancy is very low. For this reason, growing consensus suggests that FNA should not be performed in such nodules unless the maximal nodule diameter exceeds 2 cm.[88–91] Importantly, purely cystic nodules are so rarely malignant that FNA is not indicated for diagnostic purposes.

Such guidelines can provide a roadmap for clinicians to consider, though individual assessment of every patient is nonetheless required. Certain clinical factors, patient or physician concerns, or other findings may appropriately sway a practitioner to biopsy a low-risk nodule even when it is less than 1 cm, or conversely to choose to follow a high-risk nodule even without FNA. These are reasonable decisions, as the overall risk of thyroid cancer is considered in conjunction with the patient's comorbid illnesses, desires, and risks of intervention.

Ultrasound elastography (USE) is a technique that seeks to use pressure and ultrasound as a measurement of tissue stiffness. In general, the stiffer the nodule, the higher the risk of cancer. USE was initially reported as highly predictive of benign or malignant disease.[92] However, more recent trials indicate inferior performance of USE in comparison to ultrasound assessment.

CT and MRI studies of the neck have also been used. Although such tests are highly useful to assess surrounding neck structures in preparation for surgery, their performance is generally inferior to that of thyroid ultrasound. Furthermore, cancer risk characteristics cannot be as readily defined (such as hypoechoic parenchyma or irregular margins) as with ultrasound.

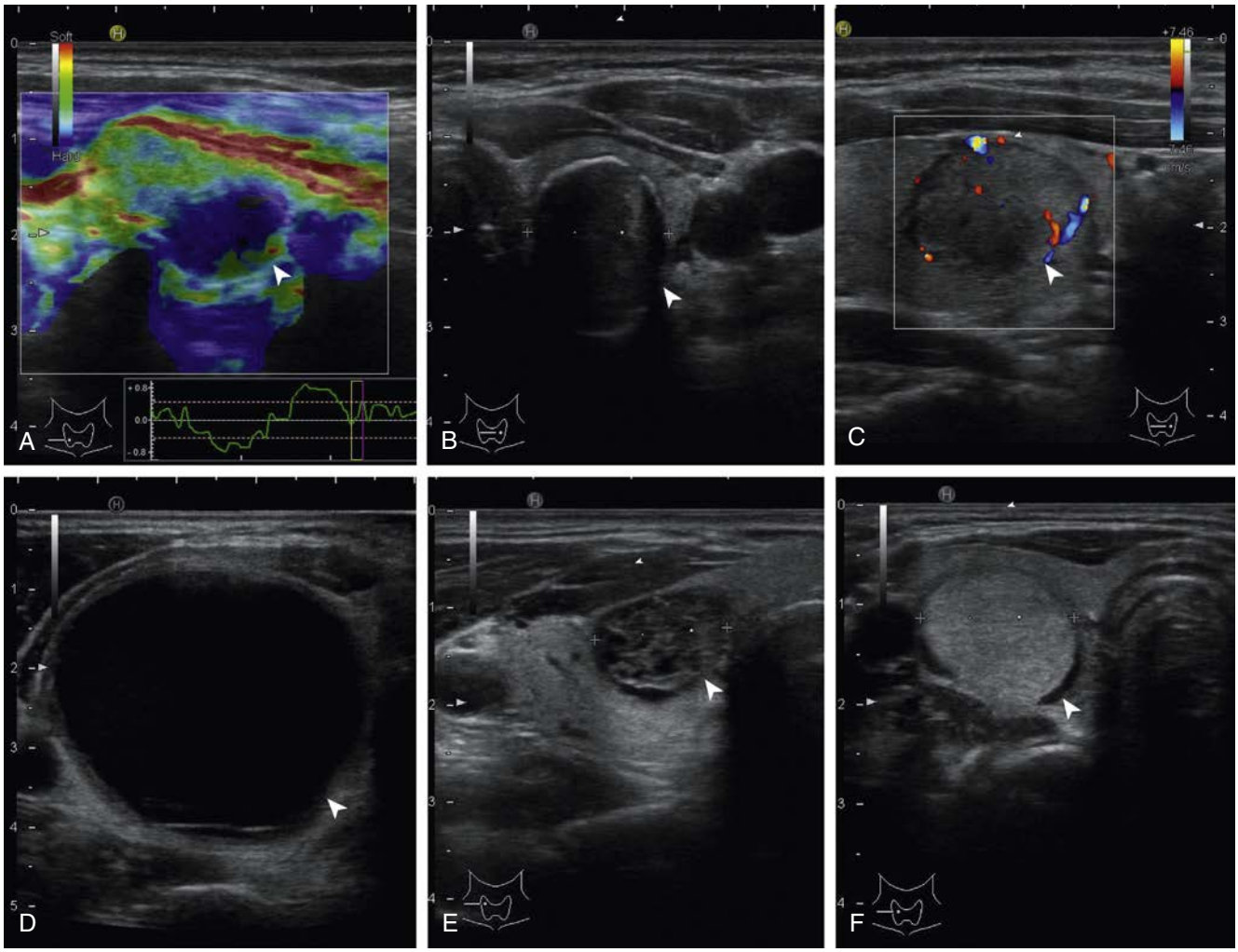


• **Fig. 14.3** Suspicious nodule ultrasound features. (A) Markedly hypoechoic nodule (similar echogenicity as the surrounding strap muscles) with irregular margins. (B) Taller-than-wide hypoechoic nodule. (C) Markedly hypoechoic nodule with regular margins. (D) Hypoechoic nodule with infiltrative margins and suspicious extrathyroidal extension. (E) Multiple interruptions in calcific rim with evidence of extrusive tissue (echogenicity is difficult to interpret because of acoustic shadowing of the calcific rim). (F) Hypoechoic solid nodule with microcalcifications and irregular margins. The *arrows* indicate the thyroid nodule. The gray scale graphically represents the shades of gray that can be provided by the ultrasound equipment. (Modified from Durante C, Grani G, Lamartina L, et al. The diagnosis and management of thyroid nodules: a review. *JAMA*. 2018;319:914–924.)

Before the advent of ultrasound-guided FNA, thyroid scintigraphy with ^{131}I, ^{123}I, or ^{99m}Tc was used to image the gland. Most thyroid carcinomas are inefficient in trapping and organifying iodine and appear on scans as areas of diminished isotope uptake, referred to as a cold nodule. This feature reflects the early decrease of NIS expression during tumorigenesis.[93] Unfortunately, most benign nodules also do not concentrate iodine. Furthermore, not all nodules with normal or slightly increased ^{99m}Tc uptake are benign and some may appear cold on a thyroid scan with radioactive iodine.[15,16] This confirms the limited utility of thyroid scintigraphy. The only situation in which an iodine scan can exclude malignancy with reasonable certainty is in the case of a toxic (hot) adenoma. Such a nodule demonstrates focal ^{123}I uptake though markedly suppressed or absent uptake in the remainder of the gland. These lesions are typically associated with a suppressed serum TSH level. They account for fewer than 5% to 10% of thyroid nodules and are almost invariably benign.[53] Thyroid scintigraphy is used much less often than previously, though it can still prove valuable in the assessment of a patient with multiple thyroid nodules or a borderline low serum TSH concentration. Scintigraphy in such cases allows the practitioner to initially target aspiration of the nonfunctional nodules. Finally, ^{18}FDG-PET is increasingly performed during the evaluation of patients with various illnesses. Although not recommended for the routine evaluation of thyroid nodules, incidental PET-positive nodules have a cancer risk of 30% to 40%.[33,34,69,94–96] In such patients, FNA is warranted. Importantly, diffuse FDG-PET uptake most often is found in the setting of Hashimoto disease and should not be considered pathologic or malignant if ultrasound confirms the absence of any nodularity.

Thyroid Nodule Fine-Needle Aspiration

FNA of thyroid nodules has eclipsed all other techniques for diagnosing thyroid cancer, with reported overall rates of sensitivity and specificity exceeding 90% in iodine-sufficient areas.[3,5,53,87] The technique is easy to perform and safe, with only a handful of complications having been reported in the literature, and causes only mild discomfort. However, care must be taken to obtain an



• **Fig. 14.4** Indeterminate ultrasound features. (A) Elevated stiffness at elastography (*red* indicates soft tissues, *blue* hard tissues, and *green* intermediate values of stiffness). (B) Complete rim calcification. (C) Slightly hypoechoic nodule with intranodular vascularization (the mean flow velocity is converted into a color scale: flow toward the transducer is represented in *red,* while away from the transducer is depicted in *blue*). Low-or very-low suspicion US features: (D) Pure cyst. (E) Mixed composition nodule with isoechoic solid component, without any suspicious features. (F) Solid hyperechoic nodule. The *arrows* indicate the thyroid nodule. The gray scale graphically represents the shades of gray that can be provided by the ultrasound equipment. (Modified from Durante C, Grani G, Lamartina L, et al. The diagnosis and management of thyroid nodules: a review. *JAMA*. 2018;319:914–924.)

adequate specimen; most authors recommend two to four aspirations per nodule. Routine use of ultrasound-guided biopsy even for clinically palpable solid nodules combined with onsite cytologic examination decreases the risk of inadequate sampling.[97,98] A satisfactory specimen must contain at least five groups of 10 to 15 well-preserved cells. FNA samples are routinely evaluated microscopically for cytologic features of benign or malignant disease and classified using the Bethesda system for reporting thyroid cytopathology.[99] Separately, FNA samples can be sent for molecular analysis using RNA expression–based tests or single gene mutation panels.

Not all thyroid nodules require FNA, and many can be safely followed without intervention over time and pose minimal risk. A decision to consider thyroid nodule FNA should initially rest upon an assessment of whether cytologic interpretation would modify the clinical care ahead. If, for example, advancing patient age and comorbid conditions would render further surgical intervention unlikely, FNA may be unnecessary. For those in whom evaluation is warranted, however, recommendation for FNA is then based on nodule size and sonographic features. As mentioned, for those with high-risk or intermediate-risk features, most nodules should generally be considered for FNA when larger than 1 cm. In contrast, low-suspicion and very low-suspicion nodules should be considered for FNA when larger than 1.5 cm and 2 cm, respectively. These guidelines were developed with the hope of identifying clinically relevant thyroid cancer that benefits from therapeutic intervention while avoiding excessive diagnostic intervention, but prospective investigations of this approach are not yet available. Smaller nodules (generally <1 cm) can most often be conservatively followed with repeat sonographic assessment in 1 to 2 years unless unique circumstances or symptoms raise concern.

Thyroid nodule FNA cytologic findings should be reported using diagnostic categories outlined in the Bethesda System for Reporting Thyroid Cytopathology (Table 14.4).[99] The diagnosis

TABLE 14.4 Probability of Malignancy Based on Fine-Needle Aspiration Cytologic Categorization

Cytologic Appearance	% of Results	% of Malignancy, for Each Category
Insufficient/non-diagnostic	~5–10	<5% if cystic nodule; 10–20% in solid nodules
Benign	70 (53–90)	1–5%
Indeterminate	20 (5–23)	
Suspicious for papillary carcinoma		60–70%
Suspicious for a follicular neoplasm (SFN/FN)		15–30%
Atypia (follicular lesion) of undetermined significance (AUS/FLUS)		10–25%
Malignant	5 (1–10)	>97%

of PTC (Bethesda category: malignant) by FNA on the basis of characteristic nuclear changes is both reliable and accurate, with sensitivity and specificity both approaching 100% provided that these changes are evaluated by an experienced cytopathologist. Similarly, a benign result should be viewed as highly accurate, as data confirm a low risk (~1–5%) of false-negative results and negligible mortality risk from false-negative aspirates during an 8.5-year follow-up.[77] However, cytologically indeterminate nodules harbor malignant risk. Bethesda classification allows malignancy risk stratification within this category, ranging from those with highest indeterminate risk (suspicious for papillary carcinoma [SUSP]) to those with lower risk (suspicious for a follicular neoplasm [SFN/FN] or atypia/follicular lesion of undetermined significance [AUS/FLUS]). Regardless, an indeterminate FNA cytologic finding implies concern that such a nodule may be a thyroid malignancy. Cytologic results should be combined with clinical and ultrasound characteristics, allowing further individual assessment. At times, such indeterminate cytologic findings—especially if SUSP, or combined with other clinical factors such as large nodule size, cosmetic concerns, or difficulty swallowing—may prove concerning enough to warrant a recommendation for surgical removal. This approach is reasonable.

Recently, a new diagnostic term was applied to thyroid lesions formerly classified as low-risk malignancy but felt to harbor a very indolent course. Such lesions have been labeled *noninvasive follicular thyroid neoplasm with papillary-like nuclear features* (NIFTP).[100] Such lesions cannot be reliably diagnosed preoperatively and typically harbor *RAS* mutations. FNA cytology of NIFTP lesions often reveals cytology classified as *atypia of undetermined significance*[101] or *suspicious for malignancy*.[102] Increasingly, certain microscopic features can be detected in FNA cytology specimens that allow preoperative concern for an NIFTP diagnosis to be conveyed.[101–103]

Low-risk indeterminate nodules (SFN/FN or AUS/FLUS) often harbor a relatively lower risk of malignancy, and if malignant, often harbor less aggressive variants of cancer.[104] However, the interobserver reproducibility of these diagnoses is poor.[105] Historically, surgical intervention was commonly recommended for nodules with SFN/FN or AUS/FLUS cytologic findings, though the majority of patients would prove to have benign disease. For such patients, surgery was unnecessary yet exposed them to substantial morbidity, time lost for recovery, and excess health care cost. To address these issues, the field has witnessed a rapid expansion in the discovery, development, and validation of thyroid-specific molecular diagnostic tests.

Historically, immunostaining for galectin 3 either alone or combined with TPO was suggested as a valuable adjunct to indeterminate nodules.[106] A series of 17 single-gene oncogenic mutations or translocations in *BRAF, RAS, RET/PTC,* and *PAX8/PPARγ* first demonstrated promise as an effective diagnostic marker for cytologically indeterminate thyroid nodules.[107–111] When detected, such mutations were initially felt to convey a very high positive predictive value and therefore hold promise as a "rule in" test. However, a blinded, multicenter prospective trial confirmed worse test performance for the full 17-gene mutation panel than previously reported.[110] These data raise questions about the overall transferability of initial data into clinical practice, especially when applied to nodules with AUS/FLUS cytologic findings.[110]

Newer versions of this DNA-based mutation panel have been created that also identify genetic rearrangements and copy number derangements.[112,113] With these updated versions, both test sensitivity and specificity have improved, though real-world use of this test has demonstrated lower than expected performance in both blinded and nonblinded analyses.[114–116] Most recently, analytic performance of a third version of this test has been reported.[117] In general, data increasingly suggest that such a test is not robust for ruling in malignancy when applied to low-risk, cytologically indeterminate nodules. However, the test's negative predictive value appears high.[107,111] The most complex output from such DNA-based mutation panels is the finding of a mutation in the *RAS* gene. Many have associated *RAS* mutations with papillary carcinoma.[118] However, *RAS* gene mutations have frequently been identified in benign thyroid nodules that show no evidence of malignant transformation.[119,120]

A separate diagnostic molecular test has investigated the utility of an RNA gene expression classifier (GEC) using microarray technology. Via initial analysis of the expression patterns from 162 genes, a first-generation test was developed for use with nodules with SFN/FN and AUS/FLUS cytologic findings, with the goal of maximizing sensitivity and negative predictive value. A prospective, blinded, multicenter validation trial was performed enrolling nearly 4000 thyroid nodules with SFN/FN or AUS/FLUS cytologic findings.[121] A benign GEC test resulted in negative predictive values of 94% and 95%, respectively, which is similar to the findings of a benign FNA cytologic result itself. The positive predictive values were 37% and 38%, respectively. Follow-up analyses of real-world use confirmed variability from site to site, largely influenced by patient population and variation in the distribution of Bethesda cytologic classification.[122] A recent study compared the long-term follow-up of cytologically indeterminate but GEC benign nodules with the follow-up of cytologically benign nodules using high-resolution ultrasound. Over a mean of about 14 months (duration up to 40 months), no difference between these groups was identified, confirming that GEC benign nodules behave like true

benign lesions.[123] More recently, some data have suggested that GEC performance may be lower when applied to nodules containing abundant Hürthle cells.[124] A newer version of this RNA-based expression classifier has been developed, called the gene sequencing classifier (GSC), demonstrating improved performance.

Increasingly, molecular testing of cytologically indeterminate thyroid nodules is endorsed because of its ability to substantially improve preoperative cancer risk assessment and modify clinical care. Specifically, most molecular tests are demonstrating high sensitivity and thus high negative predicative value. Until recently, use of the GSC is favored because of the strength of its clinical validation. Furthermore, initial cost-effectiveness analyses have demonstrated, in the United States, a cost savings via this approach.[125] The gene mutation panels may prove to have superior specificity and positive predictive values, though the utility of this metric is of limited use as the decision for hemithyroidectomy versus near-total thyroidectomy takes into account variables such as patient demographic and preference, ultrasound findings, and molecular analysis. To date, there has been no head-to-head prospective or blinded investigations comparing the various molecular tests.

MicroRNA testing has been proposed as a separate molecular test for use in cytologically indeterminate nodules. Initial data suggest potential for this approach, though strong validation studies performed in the United States are still pending.[126,127] Thus further prospective validations of these tests are required. The use of large-needle core biopsy in addition to standard FNAB may improve diagnostic accuracy in difficult FNA cases, but the technique is associated with increased morbidity.[128] Particularly for cystic thyroid nodules, sampling from the margin of the nodule under ultrasound guidance, rather than from the cystic fluid and debris in the center, increases accuracy.

Initial nondiagnostic cytologic testing should prompt repeat ultrasound-guided FNA. If available, onsite cytologic evaluation will assist in ensuring an adequate specimen for evaluation.[98,129] When the sonographic pattern of a nondiagnostic nodule is concerning, close observation or consideration for surgical excision should be given.[130] Although most nondiagnostic aspirates are due to cystic content, solid nodules with persistent nondiagnostic aspirates are associated with higher malignant risk. Repeat FNA of an initially nondiagnostic thyroid nodule yields adequate results in 60% to 80% of specimens.[68,131]

Management Options for Patients With Nontoxic Diffuse Goiter and Nodular Thyroid Disease

Patients with small, asymptomatic, nontoxic goiters can be monitored by clinical examination and evaluated periodically with ultrasound measurements. In fact, goiter growth can be variable, and some patients have stable goiters for many years. For more than a century, thyroid hormone supplementation was used to reduce the size of nontoxic goiters. A 1953 report of Greer and Astwood, in which two-thirds of patients' goiters regressed with thyroid therapy, led to widespread acceptance of suppressive therapy[132] despite some doubts about its value.[133] An overview of studies performed from 1960 to 1992 suggested that 60% or more of sporadic nontoxic goiters responded to suppressive therapy. In a prospective placebo-controlled, double-blind randomized clinical trial, 58% of the thyroxine-treated group had a significant response at 9 months, as measured by ultrasonography, in contrast with 5% after placebo.[134]

Patients with nodular thyroid disease appear to be less responsive to suppressive therapy than those with diffuse nontoxic goiters. A meta-analysis failed to demonstrate a significant benefit of thyroxine therapy, which was found to carry a relative risk of nodule shrinkage of only 1.9 (95% confidence interval [CI], 0.95–3.81).[135] Statistical significance emerged from a multicenter, randomized, double-blind, placebo-controlled trial: After 18 months of follow-up the nodule shrinkage was significantly greater in the levothyroxine group than in the placebo group ($p = 0.01$), as well as the proportion of responders ($p = 0.04$).[136] It is likely that a subset of patients respond to thyroxine suppressive therapy, particularly younger patients with small or recently diagnosed nodules.[135] However, thyroid nodules rapidly return to the pretreatment size after discontinuation of therapy. Therefore maintenance of the size reduction may require continuous treatment, which carries long-term risk.

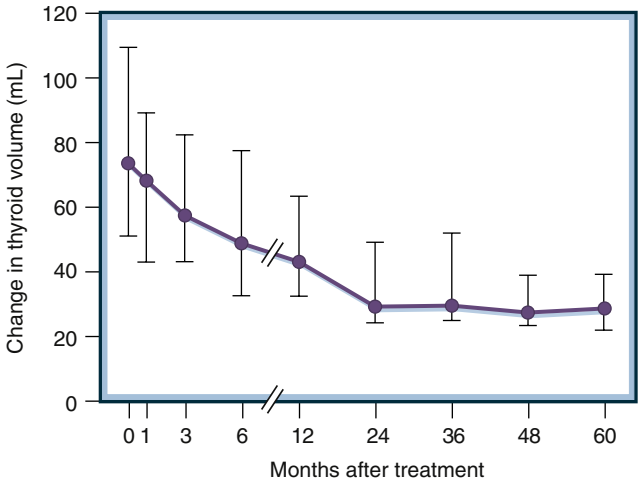
A major concern in relation to long-term thyroxine suppression therapy is the possibility of detrimental effects on the skeleton and heart. TSH suppression therapy is generally associated with variable degrees of bone loss, particularly in postmenopausal women.[137] Furthermore, there is evidence that levothyroxine suppressive therapy is detrimental to the heart, especially in older patients.[137]

Surgery for nontoxic goiter is physiologically unsound because it further restricts the ability of the thyroid to meet hormone requirements. Nevertheless, surgery may become necessary because of persistence of obstructive manifestations despite a trial of levothyroxine. Surgery should consist of a near-total or total thyroidectomy, but recurrence is seen in about 10% to 20% within 10 years.[138] Surgical complications have been reported in 7% to 10% of cases and are more common with large goiters and with reoperation. Prophylactic treatment with levothyroxine after goiter resection probably does not prevent goiter recurrence.[139]

Traditionally, the role of ^{131}I therapy for nontoxic goiter was to reduce the size of a massive goiter in elderly patients who were poor candidates for surgery or to treat goiter that recurs after resection. However, several studies have demonstrated that primary treatment of nontoxic goiter with ^{131}I is followed by a reduction in thyroid volume. In one study, thyroid volume (assessed by ultrasonography) was reduced by 40% after 1 year and 55% after 2 years with no further reduction thereafter, and 60% of the total reduction occurred within the first 3 months.[140]

Considering its effectiveness in reducing the size of the thyroid gland, ^{131}I therapy has also been used for the treatment of nonautonomous thyroid nodules where significant shrinkage has been observed, ranging from 31% to 60%[141] (Fig. 14.5). It was formerly argued that treatment of large goiters or goiters with substernal extension with ^{131}I should be avoided because of the risks of acute swelling of the gland and consequent tracheal compression. Ultrasonographic studies of thyroid volume after ^{131}I have failed to demonstrate significant early volume increase. Moreover, decreased tracheal deviation and increased tracheal lumen size were demonstrable by MRI in patients who had compression by nontoxic goiters with substernal extension.[140]

Therefore it appears that ^{131}I treatment of nontoxic diffuse goiter or multinodular thyroid disease is effective and safe. Hypothyroidism has been reported in 20% to 40%; transient thyrotoxicosis and mild pain can occur.[140] Regular follow-up, preferably by a systematic annual recall scheme, is necessary. The activities used are in the range of those used for ^{131}I treatment of hyperthyroidism, and thus radiation doses are comparable, and long-term thyroid and



• **Fig. 14.5** Median changes in thyroid volume alterations after ^{131}I treatment in 39 patients with nontoxic multinodular thyroid disease who remained euthyroid after a single dose. *Vertical bars* represent quartiles. (From Nygaard B, Hegedus L, Gervil M, et al. Radioiodine treatment of multinodular nontoxic goiter. *BMJ.* 1993;307:828–832.)

TABLE 14.5 Classification of Thyroid Neoplasms

I. Primary Epithelial Tumors
  A. Tumors of Follicular Cells
    1. Benign: follicular adenoma
    2. Borderline follicular tumors
      a. Follicular tumor of uncertain malignancy potential
      b. Well-differentiated tumor of uncertain malignancy potential
      c. Noninvasive follicular neoplasm with papillary-like nuclear features (NIFTP)
    3. Malignant: carcinoma
      a. Differentiated: papillary, follicular, Hürthle cell, poorly differentiated
      b. Undifferentiated (anaplastic)
  B. Tumors of C Cells
    1. Medullary carcinoma
  C. Tumors of Follicular and C Cells
    1. Mixed medullary-follicular carcinomas
II. Primary Nonepithelial Tumors
  A. Malignant Lymphomas
  B. Sarcomas
  C. Others
III. Secondary Tumors

nonthyroidal cancer risk after ^{131}I treatment for hyperthyroidism is reassuring. Stimulation with low doses of recombinant human TSH (rhTSH) (0.01–0.03 mg) increases thyroid ^{131}I uptake and therefore may allow the administration of a lower dosage of ^{131}I, but rhTSH also increases thyroid hormone production, and overproduction of thyroid hormones should be excluded before its use.[142] Long-term randomized studies comparing the effects, side effects, and costs and benefits of surgery and ^{131}I treatment need to be performed.

Percutaneous ethanol injection (PEI) should be used only for recurrent symptomatic cystic nodules.[143] Laser ablation, cryoablation, and radiofrequency ablation are still generally experimental procedures and can be proposed, in experienced centers, for selected patients with symptomatic nodular goiters when surgery is not possible.[144]

Malignant Thyroid Disorders

Thyroid tumors are the most common endocrine neoplasms. The management of a patient with typical well-differentiated, follicular cell–derived thyroid cancer is effective and usually consists of surgical resection, followed by medical therapy and regular surveillance.[1,14,22,145] Some degree of consensus has been achieved regarding the initial management of differentiated thyroid cancer, but many important clinical and biologic questions remain unanswered. In the following discussion, a widely used scheme for classifying and staging tumors of the thyroid gland is presented. The distinguishing features of the principal types of benign and malignant thyroid neoplasms and the controversies in the management of differentiated thyroid carcinoma, based on recent consensus and guidelines, are also reviewed.[1,14,22]

Classification and Staging of Thyroid Cancer

Two monographs have had a major impact on the histologic classification of thyroid tumors. One is from the World Health Organization (WHO); the other was developed by the Armed Forces Institute of Pathology (AFIP).[146] The WHO classification was updated in 2017[147] and is described in Table 14.5.

Lesions of follicular cell origin constitute more than 95% of the cases, and the remainder are largely made up of tumors exhibiting C-cell differentiation. Mixed medullary and follicular carcinomas, made up of cells with both C-cell and follicular differentiation, are rare and of uncertain histogenesis. Nonepithelial thyroid tumors mainly include malignant lymphomas, which may involve the thyroid gland as the only manifestation of the disease or as part of a systemic disease. True sarcomas and malignant hemangioendotheliomas are exceptionally rare. Blood-borne metastases from separate malignancies to the thyroid are not uncommon at autopsy in patients with widespread malignancy, but rarely cause clinically detectable thyroid enlargement.

In addition to the histologic classification of thyroid tumors developed by the WHO and AFIP groups, the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) have agreed on a staging system for thyroid cancer.[148–151] As stated by the AJCC, "The principal purpose served by international agreement on the classification of cancer cases by extent of disease was to provide a method of conveying clinical experience to others without ambiguity."[149]

The main goal of the AJCC-IUCC staging system is to predict disease-specific survival. It is based on the TNM (tumor-node-metastasis) classification system, which focuses on (1) the extent of the primary tumor (T), (2) the absence or presence of regional lymph node metastases (N), and (3) the absence or presence of distant metastases (M). The classification may be either *clinical* (cTNM), based on evidence (often from FNA cytology) acquired before treatment, or *pathologic* (pTNM) when intraoperative and surgical pathologic data are available. The pTNM classification is preferable as this allows a precise size to be assigned to the primary tumor, the type of cancer to be clarified histologically, and the presence of extrathyroid invasion to be demonstrated unequivocally. Whereas head and neck cancers are most often staged entirely on the basis of anatomic extent, for well-differentiated thyroid cancer staging, both the histologic diagnosis and the age of the patient are included because of their importance in predicting the behavior and prognosis ahead. It is noteworthy that in children the risk of recurrence is

high and may be underestimated by the TNM staging system.[152] The most recent (eighth) edition of the AJCC staging system for thyroid cancer was published in 2017 and implemented on January 1, 2018. Table 14.6 summarizes its features and highlights changes introduced since the 2010 edition. The main changes are (1) an increase in the age threshold (>55 years versus >45 years) for defining cases at high risk for thyroid cancer–related death, and (2) attenuation of the previous unfavorable prognostic significance attributed to small cervical lymph node metastasis and microscopic extrathyroidal extension. The age criterion for defining high-risk disease has been raised from 45 years to 55 years. This change increases the proportion of relatively young patients whose mortality risk can be defined solely on the basis of the absence or presence of distant metastases (stages I and II, respectively). As for microscopic extension of the tumor to the perithyroidal soft tissues, this finding is no longer considered an absolute indication for assigning the tumor to the T3 category. The ability to recognize this minimal extrathyroidal invasion can vary widely, even between skilled pathologists. The T3 category now includes tumors whose largest diameter exceeds 4 cm and those that invade the perithyroidal muscles (sternohyoid, sternothyroid, thyroidhyoid, omohyoid). Upper mediastinal lymph node metastases (level VII) are now considered features of N1a disease, which was formerly used solely in the presence of central neck node lesions. The expanded definition reflects the well-known difficulties in distinguishing level VI and VII nodes from an anatomic point of view. Stage I thyroid cancer now refers exclusively to T1 or T2 tumors with no lymph node metastases, whereas stage II includes T3, N0, M0 tumors and any T1-T3 tumor with metastatic lymph node disease (either N1a or N1b).

TABLE 14.6 The Tumor-Node-Metastasis (TNM) Scoring System

	DEFINITION OF TNM	
Category	**2010 Version (AJCC 7th Edition)**	**2017 Version (AJCC 8th Edition)**
Primary Tumor (T)		
T0	No evidence of primary tumor	No evidence of primary tumor
T1	Tumor ≤2 cm limited to the thyroid T1a: ≤1 cm T1b: >1 cm to 2 cm	Tumor ≤2 cm limited to the thyroid T1a: ≤1 cm T1b: >1 cm to 2 cm
T2	Tumor >2 to ≤4 cm limited to the thyroid	Tumor >2 to ≤4 cm limited to the thyroid
T3	Tumor >4 cm limited to the thyroid or any tumor with minimal extrathyroid extension (e.g., extension to sternothyroid muscle or perithyroid soft tissues)	Tumor >4 cm limited to the thyroid or gross extrathyroidal extension invading only strap muscles (sternohyoid, sternothyroid, thyroidhyoid, omohyoid) from a tumor of any size
T4	No evidence of primary tumor	No evidence of primary tumor
T4a	Gross extrathyroidal extension invading subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve from a tumor of any size or intrathyroidal anaplastic thyroid cancer of any size	Gross extrathyroidal extension invading subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve from a tumor of any size
T4b	Gross extrathyroidal extension invading prevertebral fascia or encasing the carotid artery or mediastinal vessels from a tumor of any size or anaplastic thyroid cancer of any size with extrathyroidal extension	Gross extrathyroidal extension invading prevertebral fascia or encasing the carotid artery or mediastinal vessels from a tumor of any size
Regional Lymph Node (N)		
N0	No regional lymph node metastasis	No evidence of locoregional lymph node metastasis
		N0a: one or more cytologically or histologically confirmed benign lymph nodes N0b: no radiologic or clinical evidence of locoregional lymph node metastasis
N1	Regional lymph node metastasis	Regional lymph node metastasis
N1a	Metastases in pretracheal and paratracheal lymph nodes, including prelaryngeal and delphian lymph nodes, unilateral or bilateral	Metastasis in pretracheal, paratracheal, prelaryngeal/delphian, or upper mediastinal lymph nodes, unilateral or bilateral
N1b	Metastases in lateral neck lymph nodes or upper mediastinal lymph nodes, unilateral or bilateral	Metastases in lateral neck lymph nodes, unilateral or bilateral disease

TABLE 14.6 The Tumor-Node-Metastasis (TNM) Scoring System—cont'd

	DEFINITION OF TNM	
Category	**2010 Version (AJCC 7th Edition)**	**2017 Version (AJCC 8th Edition)**
Distant Metastases (M)		
Category	**2010 Version**	**2017 Version**
M0	No distant metastasis	No distant metastasis
M1	Distant metastasis	Distant metastasis
	TNM STAGING FOR PAPILLARY, FOLLICULAR, AND POORLY DIFFERENTIATED THYROID CANCER	
Age Cutoff	**Age <45 Years**	**Age <55 Years**
Stage I	Any T, any N, M0	Any T, any N, M0
Stage II	Any T, any N, M1	
Stage III	None	
Stage IV	None	
Age Cutoff	**Age ≥45 Years**	**Age ≥55 Years**
Stage I	T1, N0, M0	T1-T2, N0, M0
Stage II	T2, N0, M0	T1-T2, N1a-N1b, M0 or T3, any N, M0
Stage III	T3, N0, M0 or any T1-3, N1a, M0	T4a, any N, M0
Stage IV		
Stage IVA	T1-3, N1b, M0 or T4a, any N, M0	T4b, any N, M0
Stage IVB	T4b, any N, M0	Any T, any N, M1
Stage IVC	Any T, any N, M1	-
	TNM STAGING FOR MEDULLARY THYROID CANCER	
Category	**2010 Version**	**2017 Version**
Stage I	T1, N0, M0	T1, N0, M0
Stage II	T2-T3, N0, M0	T2-T3, N0, M0
Stage III	T1-3, N1a, M0	T1-3, N1a, M0
Stage IVA	T1-3, N1b, M0 or T4b, any N, M0	T1-3, N1b, M0 or T4b, any N, M0
Stage IVB	T4b, any N, M0	T4b, any N, M0
Stage IVC	Any T, any N, M1	Any T, any N, M1
	TNM STAGING FOR ANAPLASTIC THYROID CANCER	
Stage IVA	T4a, N0, M0	T1-T3a, N0, M0
Stage IVB	T4b, any N, M0	T1-T3a, N1, M0 or T3b-T4, any N, M0
Stage IVC	Any T, any N, M1	Any T, any N, M1

AJCC, American Joint Committee on Cancer.

To date, prognostic differences and, especially, the differing risk of cause-specific mortality between central lymph node metastases (N1a) and other regional metastases (N1b) have yet to be widely validated. Indeed, the risk of persistent/recurrent disease appears to be more closely related to the number and size of involved lymph nodes as well as the number of lymph nodes with extracapsular extension. These characteristics, however, are not taken into account in the TNM AJCC staging system.[153,154] Stages III and IVa include T4a and T4b tumors, respectively, regardless of their lymph node status. Stage IVb includes any tumor with distant

metastases, and stage IVc has been eliminated entirely in the new eighth edition system.

For MTC, the scheme is similar, in that T1 N0 M0 carcinoma is stage I, but T2-T3 N0 M0 is stage II and a T1-T3 N1a M0 tumor is stage III, underlying the prognostic impact of lymph node involvement. There is no age distinction for MTC, although age is a significant independent prognostic indicator in most multivariate analyses.[155,156]

All anaplastic carcinomas were considered T4 tumors in the 1992, 2002, and 2010 editions of the AJCC/UICC classification. With the eighth edition (2017), the T categories for anaplastic carcinomas are the same as those used for differentiated carcinomas. All anaplastic carcinomas are considered stage IV tumors, with stage IVA referring to intrathyroidal tumors, whereas stage IVB tumors are cases with gross extrathyroidal extension or lymph node metastases. Stage IVC is assigned to anaplastic malignancies with distant metastases.

Although the AJCC staging system has been designed to predict disease mortality risk, it has also been used in practice to predict the risk of persistent/recurrent disease. Other systems, designed specifically to predict postoperative tumor recurrence, are available and are discussed later in this chapter.

Follicular Adenomas and Borderline Thyroid Lesions With Malignant Potential

A follicular adenoma is a benign, encapsulated tumor with evidence of follicular cell differentiation.[146] It is the most common thyroid neoplasm and may be found in 4% to 20% of glands examined at autopsy.[157] The tumor has a well-defined fibrous capsule that is grossly and microscopically complete. There is a sharp demarcation and distinct structural difference from the surrounding parenchyma. These adenomas vary in size, but most have a diameter of 1 to 3 cm at the time of excision. Degenerative changes, including necrosis, hemorrhage, edema, fibrosis, or calcification, are common features, particularly in larger tumors.

Follicular adenomas can be classified into subtypes according to the size or presence of follicles and degree of cellularity. Each adenoma tends to have a consistent architectural pattern. Microfollicular, normofollicular, and macrofollicular adenomas owe their names to the size of their follicles compared with follicles in the neighboring, nonneoplastic areas of the gland. Trabecular adenomas are cellular and consist of columns of cells arranged in compact cords. They show little follicle formation and rarely contain colloid. Hyalinizing trabecular tumor, considered a variant of adenoma, has unusually elongated cells and prominent hyaline changes in the extracellular space.[158] The histologic differences among these subtypes are striking but of no clinical importance. The only practical value of the classification is that the more cellular a follicular nodule is, the more one should search for evidence of malignancy in the form of invasion of blood vessels and capsule, either singly or in combination.[146]

Atypical adenomas are hypercellular or heterogeneous, or both, with gross and histologic appearances that suggest the possibility of malignancy but without invasion. Classification of these tumors is difficult and poorly reproducible among pathologists. They account for fewer than 3% of all follicular adenomas. Follow-up indicates that this lesion behaves in a benign fashion. However, the fact that the tumor does not recur or produce metastases after removal does not prove that it is actually benign. Removal may have interrupted a natural history in such nodules that would have culminated in invasion and metastases. For this reason, they are classified as tumors of uncertain malignancy. Increasingly, molecular analysis of such lesions helps to better define their prognosis and associated risk.

The most important cytologic variant is the oxyphilic or oncocytic (Hürthle cell) adenoma, which is composed predominantly (at least 75%) or entirely of large cells with granular, eosinophilic cytoplasm. Ultrastructurally, the cells are rich in mitochondria and may exhibit nuclear pleomorphism with distinct nucleoli. Although all such neoplasms are thought by some to be potentially malignant, the biologic behavior and clinical course of oncocytic tumors correlate closely with the histologic appearance and the size of the initial lesion. The absence of invasion predicts a benign outcome, but larger tumors may rarely be associated with later recurrence or metastases, even in the absence of obvious microscopic evidence of invasion; fortunately, such an occurrence is rare, and generally a diagnosis of benign Hürthle cell adenoma is reliable.[159]

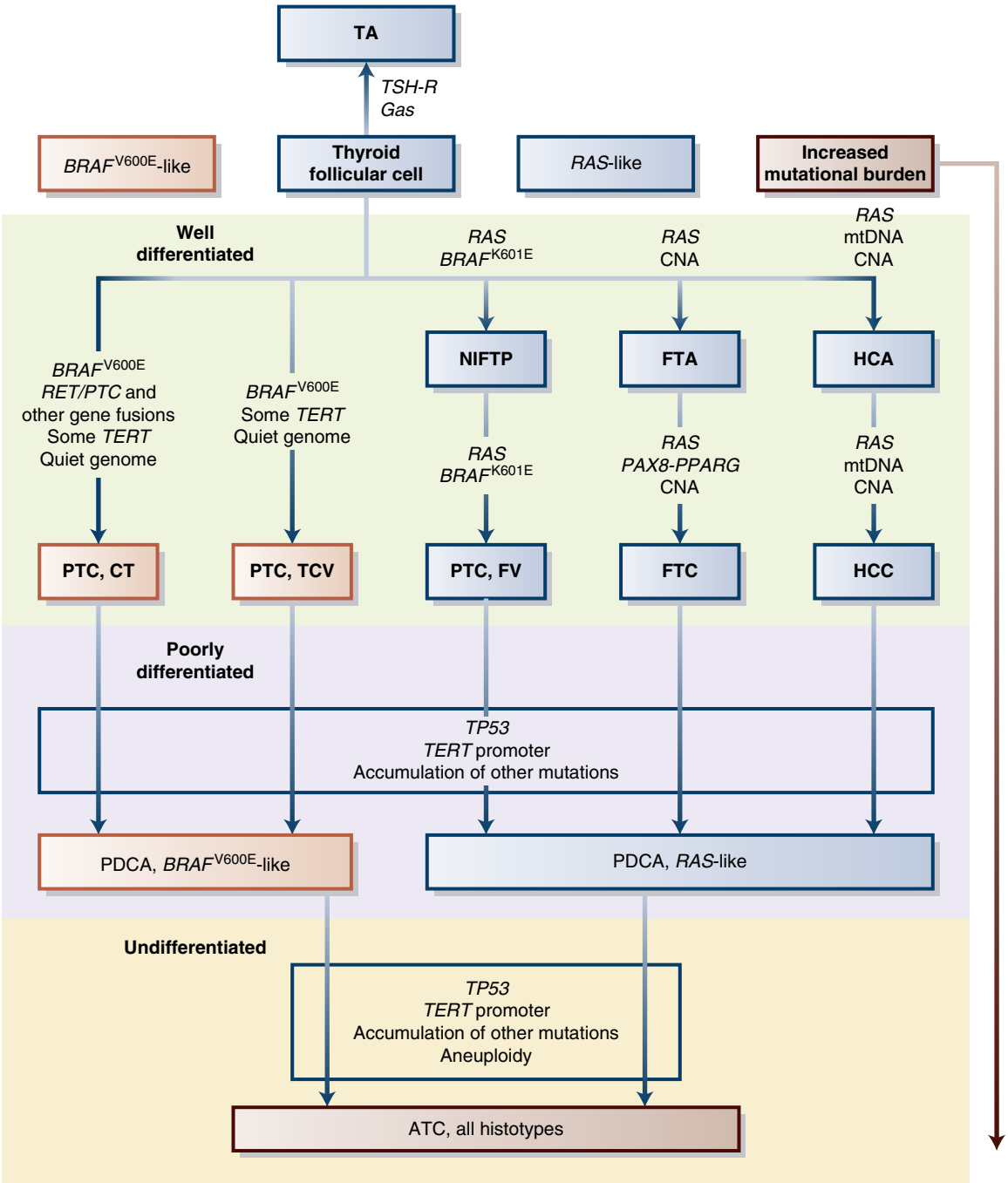
Some follicular adenomas may contain pseudopapillary structures that can be confused with the papillae of papillary carcinoma. These structures are probably an expression of localized hyperactivity and are most common in adenomas that show autonomous function.

In the majority of hyperfunctioning follicular adenomas, activating point mutations have been identified in the *TSHR* or in the α-subunit of the stimulatory guanyl nucleotide protein ($G\alpha_s$) (Fig. 14.6).[60,160] Such mutations result in a constitutive hyperstimulation of the cells. Genetic abnormalities found in hypofunctioning adenomas are detailed later.

In 2017, the WHO introduced a new entity, included in the group of thyroid tumors of follicular origin with borderline histologic features. Such lesions, as mentioned previously, are NIFTP.[100,147,161] These neoplasms are encapsulated follicular variants of papillary thyroid carcinoma (FVPTC) without evidence of either capsular or vascular invasion. Proper pathologic processing is essential for confident exclusion of capsular or vascular spread. NIFTPs are thought to represent up to 18.6% of tumors previously classified as low-risk PTCs. Adverse events, such as cancer-related death, distant or regional metastases, and structural or biochemical recurrence do not occur in patients with NIFTPs that are properly diagnosed. Because of this, the word *cancer* has been eliminated from the definition of these tumors to underscore their outstanding prognosis, thereby discouraging overly aggressive treatment and follow-up. This borderline thyroid entity was first proposed by an international panel of experts and subsequently endorsed by the ATA. However, the presumably excellent outcomes associated with NIFTPs have yet to be independently confirmed with long-term prospective studies. Importantly, the current body of evidence supporting our approach to NIFTPs comes from retrospective studies and is thus of only moderate quality.

Papillary Thyroid Carcinoma

PTC is defined as "a malignant epithelial tumor showing evidence of follicular cell differentiation and a set of distinctive nuclear feature. Papillae, invasion or cytological features of papillary thyroid carcinoma are required" (WHO 2017).[146,162] The most common thyroid malignancy, PTC constitutes 50% to 90% of differentiated FCTCs worldwide.[72,163] The 2017 WHO classification recognizes 15 PTC subtypes (or variants), but the vast majority of PTCs belong to one of the following five subtypes (or variants): classic variant, follicular variant, diffuse sclerosing variant, encapsulated variant, and tall cell variant.[147]



• **Fig. 14.6** Model of thyroid carcinoma (TC) initiation and progression with primary genetic alterations. Benign thyroid tumors (FTA and HCA) and differentiated TCs (PTC, FTC, and HCC) develop from follicular cells and follow two distinct pathways, *BRAF*^{V600E}-like and *RAS*-like, depending on the specific driver mutations present. Some differentiated TCs accumulate additional mutations and progress to more clinically aggressive and less differentiated types (PDCA and ATC). ATC represents the most aggressive form of TC with a high mutational burden, genomic instability, aneuploidy, and high rates of *TP53* and *TERT* promoter mutations. *ATC,* anaplastic thyroid (undifferentiated) carcinoma; *CNA,* copy number alterations; *CT,* classical type; *FTA,* follicular thyroid adenoma; *FTC,* follicular thyroid carcinoma; *FV,* follicular variant; *HCA,* Hürthle cell/oncocytic adenoma; *HCC,* Hürthle cell/oncocytic carcinoma; *mtDNA,* mitochondrial deoxyribonucleic acid; *NIFTP,* noninvasive follicular neoplasm with papillary-like nuclear features; *PDCA,* poorly differentiated carcinoma; *PTC,* papillary thyroid cancer; *TA,* toxic adenoma; *TC,* thyroid carcinoma; *TCV,* tall cell variant; *TSH-R*, thyrotropin receptor. (Modified from Giordano TJ. Genomic hallmarks of thyroid neoplasia. *Annu Rev Pathol*. 2018;13:141–162.)

Papillary thyroid microcarcinomas (PTMs), or pT1a lesions are defined by WHO as PTCs whose largest diameter measures 1 cm or less.[164,165] In the United States, the reported incidence of clinically diagnosed PTMs (approximately 1 per 100,000 persons) is considerably lower than that reported for larger PTCs (approximately 5 per 100,000). It is also lower than the reported incidence of PTMs found at autopsy in various parts of the world (4–36%).[72,146] The increasing detection of small PTCs throughout the industrialized world can be attributed in large part to screening programs.[72–74,166] The incidence of larger tumors has remained essentially stable since the 1980s. Therefore most thyroid cancers being diagnosed today are small tumors with limited extension and excellent prognoses,[167] and protocols for their initial treatment and follow-up must therefore be revised to avoid overzealous management.

Classic PTCs appear as firm, unencapsulated or partially encapsulated tumors. PTCs may be partly necrotic, and some are cystic. The presence of necrosis and a high mitotic rate are associated with aggressive behavior similar to that of poorly differentiated thyroid cancer (PDTC).[39,168] Typically PTC shows a predominance of papillary structures, consisting of a fibrovascular core lined by a single layer of epithelial cells, but the papillae are usually admixed with neoplastic follicles having characteristic nuclear features. The nuclei of PTC cells have a distinctive appearance, which has a diagnostic significance comparable to that of the papillae. Indeed, the preoperative diagnosis of PTC can often be made on the basis of the characteristic nuclear changes seen in FNA material: Nuclei are larger than in normal follicular cells and overlap; they may be fissured like coffee beans; chromatin is hypodense (ground-glass nuclei); limits are irregular; and they frequently contain an inclusion corresponding to a cytoplasmic invagination. Psammoma bodies are often present in the core of papillae or in the tumor stroma; they are microscopic structures of calcified layers.

The term *encapsulated variant of PTC* is used to describe a classic PTC surrounded by a fibrous capsule, regardless of whether the capsule shows signs of tumor infiltration. The tumor is designated a *follicular variant of PTC* when the lining cells of the neoplastic follicles have the same nuclear features as seen in typical PTC and the follicular predominance over the papillae is complete. The encapsulated FVPTC is associated with a favorable outcome, unless extensive vascular invasion is present.[146] The *diffuse sclerosing variant of PTC* is characterized by diffuse involvement of one or both thyroid lobes, widespread lymphatic permeation, prominent fibrosis, and lymphoid infiltration. The *tall cell variant of PTC* is characterized by well-formed papillae that are covered by cells that are twice to three times tall as they are wide. Other rare variants of PTC include the *columnar cell variant,* which differs from other forms of PTC because of the presence of prominent nuclear stratification of elongated cells. The *hobnail variant of PTC* is a recently recognized variant with poor prognostic findings.[169] The tall cell and columnar cell variants are more aggressive, but controversy exists regarding outcome for the diffuse sclerosing variant.

In children, PTCs represent the large majority of newly diagnosed cases, and histologic subtypes include the classic PTC, the presence of the *solid/trabecular variant* features (of unknown prognostic significance), the follicular variant, and the diffuse sclerosing variant. Tumor extension is usually substantial at diagnosis: tumors are large, multifocal, unencapsulated, and invasive. Extension beyond the thyroid capsule, lymph node metastases, and lung metastases are frequently observed.[152,170,171]

Molecular Pathogenesis of Papillary Thyroid Carcinoma

Nearly all PTCs arise as the result of alterations in genes encoding components of the mitogen-activated protein kinase (MAPK) and PI3K/PTEN/AKT/mTOR signaling pathways. The gene most frequently mutated in PTCs is the proto-oncogene *BRAF,* which encodes a serine-threonine protein kinase that plays a central role in regulating signaling through the MAPK pathway. In most cases, *BRAF* is activated as a result of a point mutation that causes a glutamate-for-valine substitution at residue 600 (p.V600E). This alteration is found in up to 40% to 60% of all PTCs,[118,172,173] primarily the classic and tall cell variants.[118] The $BRAF^{V600E}$ mutation occurs early in tumorigenesis,[174] inducing loss of differentiation[175] and promoting tumor invasion and progression.[176]

The second most prevalent mutations found in PTCs involve the Ras family of genes, which encode proteins that act as signal transduction switches to regulate diverse cytoplasmic signaling pathways.[177] Although *RAS* is a classic activator of both the MAPK and PI3K-AKT pathways, the *RAS* mutations associated with thyroid tumorigenesis seem to preferentially activate the PI3K-AKT pathway. Missense mutations at codons 12, 13, and 61 result in constitutive *RAS* signaling, and they are found in 10% to 30% of PTCs[118,178] (FVPTCs and NIFTPs in particular).[100,179]

Of all PTCs, 5% to 10% are characterized by genetic recombination of the *RET* proto-oncogene, which encodes a transmembrane receptor with a tyrosine kinase domain.[118,180] These alterations are characteristic of PTCs linked to radiation exposure (environmental or therapeutic).[180] There are at least 10 different types of *RET/PTC,* all resulting from the fusion of the tyrosine kinase domain of *RET* to the 5′ portion of different genes. *RET/PTC1* and *RET/PTC3* are the most common types, followed by *RET/PTC2*. *RET/PTC1* is formed by an intrachromosomal rearrangement fusing the *RET* tyrosine kinase domain to a gene designated *H4 (CCDC6)*. *RET/PTC2* is formed by an interchromosomal rearrangement fusing the *RET* tyrosine kinase domain to a gene located on chromosome 17 encoding the RIα regulatory subunit of protein kinase A. *RET/PTC3* is formed by an intrachromosomal rearrangement fusing the *RET* tyrosine kinase domain to a gene designated *RFG (NCOA4)*. *RET/PTC3* was more frequently found in aggressive tumors that occurred early after the Chernobyl disaster, and *RET/PTC1* was found in classic variant tumors that are less aggressive and occurred later.

In addition to *RET/PTC* rearrangements, PTCs have been found to harbor fusion genes involving *NRTK1,*[181] *NTRK3,*[182] *BRAF,*[183] *ALK,*[184] *FGFR2, THADA, MET, LTK,*[118] and *ROS1*.[185] PTCs with oncogenic gene fusions are closely associated with radiation exposure, and these tumors usually have distinctive clinicopathologic features.[182] Fusions (especially those involving *NRTK1/3*) are particularly common in PTCs that develop in pediatric patients, where they are associated with more aggressive disease.[186,187] On the whole, adult PTCs with gene fusions are usually associated with an intermediate risk of recurrence.[1] Fusions involving the *PAX8* and *PPARG* genes, which are frequently found in FTCs, have also been reported in a small subset of PTCs, particularly FVPTCs.[118,188]

Telomerase reverse transcriptase *(TERT)* promoter mutations are identified in 8% to 27% of PTCs, particularly those that also harbor mutations affecting the MAPK pathways (e.g., $BRAF^{V600E}$, activating *RAS* mutations).[189–192] They upregulate the expression of TERT, thereby reactivating telomerase to promote cellular immortality. The C228T substitution is the most common (7%), and the C228A and C250T substitutions are less common (0.3%

and 2.1%, respectively).[118] *TERT* mutations are associated with aggressive clinicopathologic features and a high risk of recurrence, particularly when coexisting with *BRAF,* suggesting a synergistic interaction between *BRAF* and *TERT*.[193]

In 2014 there was a major advance in defining the genomic landscape of PTCs: the comprehensive multiplatform analysis of nearly 500 adult tumors carried out as part of The Cancer Genome Atlas (TCGA).[118] Characterization of these tumors in terms of genomic variants, gene expression, microRNA (miR) expression, alterations in methylation, and proteomic profiles shed new light on the genomic hallmarks of PTC, which have greatly enhanced our understanding of its pathogenesis. The overarching conclusion is that PTCs are relatively simple cancers with fairly low mutational burdens (0.41 nonsynonymous mutations per megabase on the average) and with a few copy number variations.

The TCGA study has also identified *EIF1AX* as a novel driver oncogene, whose mutations are nearly mutually exclusive with other MAPK pathway mutations and occur in 1% of PTCs, mostly in FVPTC.[118] An increased prevalence of *EIF1AX* mutations has also been reported in PDTC and ATC (~10% in both), particularly in those that also harbor *RAS* mutations.[194] This intriguing association between *RAS* and *EIF1AX* mutations suggests that the ribosomal protein might also contribute significantly to tumor progression, making it an attractive potential target for further investigation.

Mutations in the DNA repair genes *CHEK2* and *PPMID* have also been found in PTCs, each affecting approximately 1% of all tumors. These mutations occurred concomitantly with MAPK-pathway driver mutations, suggesting that they are late genetic events in PTC tumorigenesis and that the development of particularly aggressive phenotypes may be triggered by an acquired defect in DNA repair.[118]

TCGA data have demonstrated that *BRAF* and *RAS* mutations are almost always mutually exclusive in PTCs. This knowledge has been leveraged to develop the $BRAF^{V600E}$*-RAS* gene expression score (BRS), which has been used to highlight the remarkable biologic differences between *RAS*-driven and $BRAF^{V600E}$-driven PTCs. Using the BRS scheme, tumors with the biologic properties of *RAS*-mutated tumors are designated *RAS*-like, in contrast to tumors with the biologic properties of $BRAF^{V600E}$, which are designated $BRAF^{V600E}$-like. $BRAF^{V600E}$-mutated PTCs are strongly $BRAF^{V600E}$-like with the strongest activation of the MAPK pathway. One tumor harboring a $BRAF^{K601E}$ mutation paradoxically displayed *RAS*-like features consisting of more limited MAPK signaling than that [typically] observed in $BRAF^{V600E}$-driven cancers. Interestingly, PTCs with $BRAF^{K601E}$ (like those bearing *RAS* mutations) are enriched among PTCs with follicular patterns.[195–197] PTCs with *BRAF* indels have also been found to have gene expression patterns that are surprisingly *RAS*-like.

The thyroid differentiation score (TDS) was developed as a measure of the thyroid-specific differentiation of PTCs, based on the expression levels of 16 thyroid-related genes. TDS classification of PTCs characterizes $BRAF^{V600E}$-like PTCs as a whole as less differentiated than *RAS*-like tumors, whose differentiation scores are closer to those of normal thyroid tissue. The distinctive intracellular signaling profiles of $BRAF^{V600E}$-like and *RAS*-like PTCs have also been documented at the transcriptional and protein expression levels.[118]

The pan-genomic differences between $BRAF^{V600E}$-like and *RAS*-like tumors documented by TCGA data are striking, and a revised classification of thyroid carcinomas is needed to reflect these findings. One possible solution would entail the creation of a new entity—separate from PTC—that includes the follicular-patterned tumors (FTC, FVPTC) and highlights the typically *RAS*-like nature of their underlying biology.

TCGA data have also been used to identify subgroups of $BRAF^{V600E}$-like tumors with distinct biologic and pathologic features. This molecular heterogeneity of $BRAF^{V600E}$-like PTCs thus appears to be more substantial than previously believed, and it might at least partially explain the conflicting findings published on the prognostic significance of the $BRAF^{V600E}$ mutation in PTCs.[198] The heterogeneity was particularly evident when the miRNA expression profiles of these PTCs were analyzed: six distinct clusters were identified. Cluster 1 was characterized by relatively high expression of miR-182-5p and miR-183-5p and was enriched for *RAS*-mutated tumors and the follicular variant. *BRAF*-like tumors comprised five miRNA-defined clusters. Three clusters were associated with highly differentiated, relatively nonaggressive PTCs. The other two were associated with less differentiated tumors and a higher likelihood of recurrence. Cluster 5 was characterized by higher levels of miR-146b (3p and 5p isoforms) and miR-375, relatively low levels of miR-204-5p, and overrepresentation of classic PTCs harboring the $BRAF^{V600E}$ mutation. Cluster 6 presented high levels of miR-21-5p, low levels of miR-204-5p, and enrichment for tall cell variant and classic PTCs. This cluster had the highest frequency of $BRAF^{V600E}$ mutation, the lowest differentiation scores, and a very high risk of recurrence.

It remains to be seen whether miRNA profiles can help differentiate low-risk from high-risk *BRAF*-like PTCs, but recent studies have yielded promising findings.[199,200] In addition to clinically informative miRNA expression patterns, several specific miRNAs have roles in thyroid carcinogenesis that have been experimentally demonstrated. MiR-146b-5p, which is currently one of the most thoroughly studied miRNAs in PTC, has been linked to aggressive clinicopathologic features and poor outcomes.[199,201] Both isoforms of this miRNA specifically repress the expression of *PAX8* and *NIS,* which are essential determinants of the differentiated thyroid cancer phenotype.[202] Antagonism of miR-146b in human thyroid cancer cells has been shown to restore *NIS*-mediated iodide uptake.[202] This miRNA is also predicted to repress the expression of other iodide-metabolizing proteins, such as *DEHAL* and *DIO2*.[202] In addition, the transcription of miR-146b is regulated by *PAX8*. As a result, a negative feedback loop is created whereby *PAX8* limits its own activity by inducing the expression of its repressor, miR-146b. This repression also extends to *NIS, DEHAL*, and *DIO2*, which are downstream targets of *PAX8*.[203]

NIS expression is also repressed by miR-21-5p,[202] a miRNA that has been associated with poor differentiation and high recurrence rates in PTC.[118,199] MiR-182, which is predominant in *RAS*-mutated PTCs, is predicted to repress *PAX8* and DEHAL, whereas miR-375, which is more prominent in *BRAF* tumors, is predicted to repress *DEHAL* and *NKX2.1*.[202] Therapies targeting miRNAs in thyroid cancer can thus disrupt regulatory circuits that help maintain tumor cells in a poorly differentiated state, and this approach might be an effective new strategy for inducing redifferentiation and increased iodide uptake in these cells.

A high incidence of PTC has been reported in patients with adenomatous polyposis coli who have a peculiar histologic appearance, with solid areas and elongated cells, and Cowden disease (the multiple hamartoma syndrome), suggesting that the predisposing genes may play a role in the occurrence of papillary carcinoma. The familial risk of thyroid cancer is higher than for other

cancers, and about 3% to 10% of cases of PTC are familial[204]; their behavior is similar to or slightly more aggressive than that of nonfamilial cases.[205] At least five loci of predisposition have been individualized but they do not explain all hereditary cases.[206–210] The gene predisposing to familial thyroid tumors with cellular oxyphilia has been mapped to chromosome 19q13.2, and in a family with PTC and renal carcinoma, a separate gene was mapped to chromosome 1p13.2.q22. Variants of two genes, *FOXE1 (TTF2)* located at chromosomal locus at 9q22, and *NKX2-1 (TTF1)* on 14q, both coding for thyroid-specific transcription factors, confer an increased risk of thyroid cancer.

Presenting Features of Papillary Thyroid Carcinoma

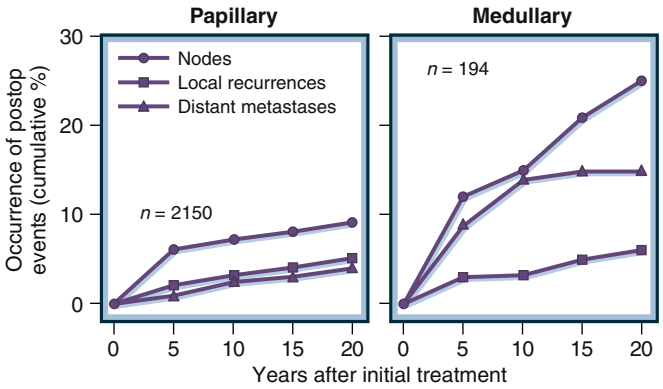
Although PTCs can occur at any age, most occur in patients between 30 and 50 years of age (mean age, 45 years). Women are affected more frequently (female predominance, 60–80%). Most primary tumors are 1 to 3 cm, and in recent years the proportion of small tumors has increased, largely due to detection of small PTCs by screening.[72,73,163,211–213] PTC is frequently multifocal when it occurs in a single lobe and is bilateral in 20% to 80% of cases, depending on whether the thyroid is meticulously examined. Some studies have suggested that contralateral PTCs may have independent clonal origins, but this idea remains controversial.[214] Extrathyroidal invasion of adjacent soft tissues is present in about 15% (range 5–34%) at the time of primary diagnosis.[145,163] About 35% to 50% of excised neck lymph nodes have histologic evidence of involvement (microscopic foci of metastases in most of the cases), though in patients 17 years of age or younger, nodal involvement may be present in up to 90%.[152,170,171,215] Only 1% to 7% of patients with PTC have distant metastases at diagnosis.[163] Spread to superior mediastinal nodes is usually associated with extensive neck nodal involvement.

At presentation, most PTCs were classified as TNM stage I or II tumors (60% and 22%, respectively) using the seventh edition of the AJCC staging system. With the implementation of the eighth edition in 2018, stage I and II tumors account for an even larger proportion of PTCs.[216,217] Fewer than 10% of all PTCs will be classified as stage III or IV using this newer system; these are reserved for cases in which the risk of cancer-related death is substantial.

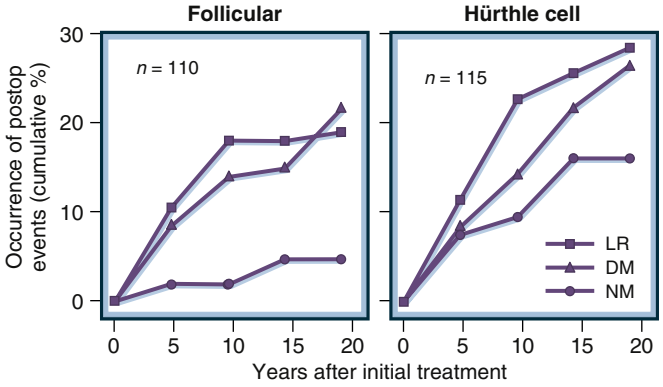
Risk of Papillary Thyroid Carcinoma Recurrence and Mortality

Three types of tumor recurrences may occur following the initial therapy for PTC, including local recurrence (LR), nodal metastases (NM), and distant metastases (DM). Local recurrence may be defined as "histologically confirmed tumor occurring in the resected thyroid bed, thyroid remnant, or other adjacent tissues of the neck (excluding lymph nodes)" after complete surgical removal of the primary tumor.[218] Fig. 14.7 illustrates rates of PTC recurrence at local, nodal, and distant sites in 2150 patients with PTC treated at one institution from 1940 to 1997. After 20 years of follow-up, postoperative NM had been discovered in 9%, and LR and DM occurred in 5% and 4%, respectively. Both local recurrence and distant metastases are less common in PTC than in FTC (Fig. 14.8), though postoperative cases of nodal metastases are more frequent in PTC. Another series of 1020 patients diagnosed between 1990 and 2000 demonstrated a rate of recurrence of only 1.4%, and most such relapses (80%) occurred within 5 years of diagnosis.[219]

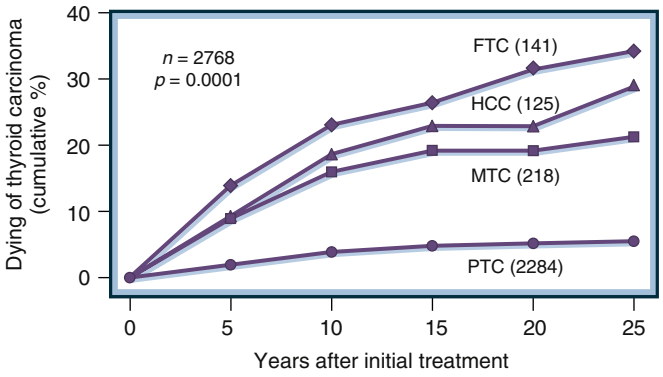
Cause-specific mortality (CSM) rates for differentiated thyroid cancer are shown in Fig. 14.9. CSM rates for PTC were 2% at 5 years, 4% at 10 years, and 5% at 20 years. Among



• **Fig. 14.7** Development of neck nodal metastases, local recurrences, and distant metastases in the first 20 years after definitive surgery for papillary thyroid cancer (PTC) or medullary thyroid cancer (MTC) performed at the Mayo Clinic from 1940 to 1997. Based on 2150 consecutive PTC *(left)* and 194 MTC *(right)* patients who had complete surgical resection (i.e., had no gross residual disease) and were without distant metastases on initial examination. *Postop,* postoperative.



• **Fig. 14.8** Development of neck nodal metastases (NM), local recurrences (LR), and distant metastases (DM) in the first 20 years after definitive surgery for follicular thyroid cancer (FTC) or Hürthle cell cancer (HCC) performed at the Mayo Clinic from 1940 to 1997. Based on 110 consecutive FTC patients *(left)* and 115 HCC patients *(right)* who had complete surgical resection and were without distant metastases on initial examination. *Postop,* postoperative.



• **Fig. 14.9** Cumulative cause-specific mortality rates for patients with differentiated thyroid carcinoma in the first 25 years after treatment with initial surgery performed at the Mayo Clinic from 1940 to 1997. Based on 2768 consecutively treated patients (2284 with papillary thyroid carcinoma [PTC], 141 with follicular thyroid cancer [FTC], 125 with Hürthle cell cancer [HCC], and 218 with medullary thyroid cancer [MTC]).

those with lethal PTC, 20% of deaths occurred in the first year after diagnosis, and 80% of the deaths occurred within 10 years. The 25-year cause-specific survival rate of 95% for PTC was significantly higher than the 79%, 71%, and 66% rates seen with MTC, Hürthle cell cancer (HCC), and FTC, respectively.[163] Survival outcomes according to the 2017 TNM eighth edition staging system were retrospectively evaluated in two large US registry cohorts (Surveillance, Epidemiology, and End Results [SEER] and National Cancer Data Base [NCDB]).[220] Five-year disease-specific survival (DSS) was 99.7% to 96.6% for stage I, 96.7% to 88% for stage II, 85.2% to 74.3% for stage III, and 66.9% to 49.5% for stage IV patients. Fig. 14.10 shows the distribution of SEER and NCDB papillary thyroid cancer populations according to both the TNM seventh and eighth editions. Fig. 14.11 illustrates the rates of DSS according to the TNM eighth edition staging system.

Prediction of Papillary Thyroid Carcinoma Recurrence

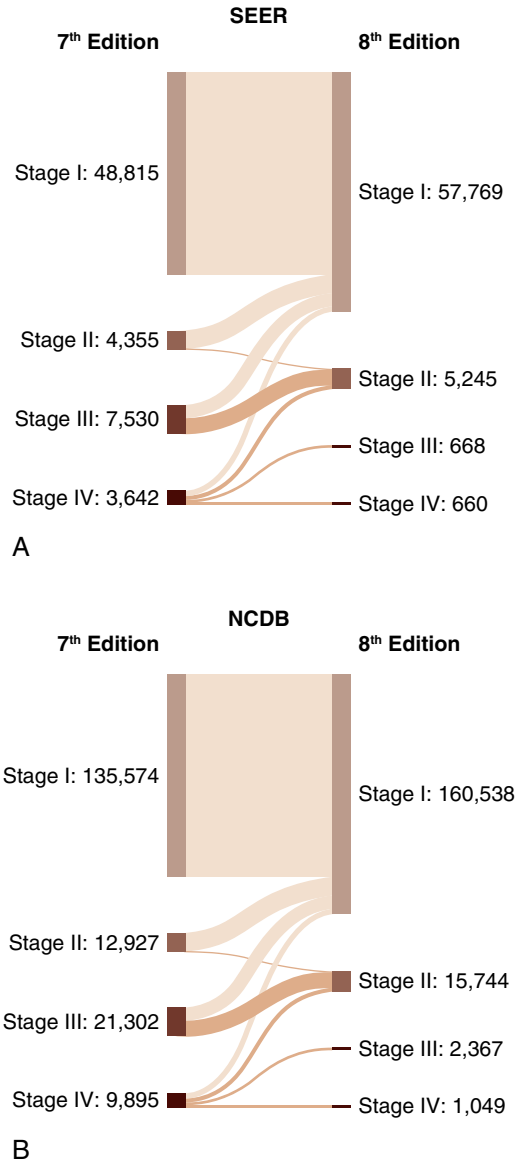
Only a fraction (~15–25%) of patients with PTC are likely to experience relapse of disease, and even fewer (~5%) will prove to have a lethal outcome. Exceptional patients who have an aggressive course tend to experience relapse early, and the rare fatalities usually occur within 5 to 10 years of diagnosis.[163] Multivariate analyses have been used to identify variables predictive of CSM.Multivariate analyses have been used to identify variables predictive of CSM.[221–223] Increasing age of the patient and the presence of extrathyroidal invasion (especially gross extrathyroidal invasion) are independent prognostic factors in all studies.

The presence of initial distant metastases and large size of the primary tumor are also significant variables in most studies,[163,221,223] and some groups[163,221,222,224] have reported that histopathologic grade (degree of differentiation) is an independent variable. The completeness of initial tumor resection (postoperative status) is also a predictor of fatality.[221,223] The presence of initial neck nodal metastases, although relevant to future nodal recurrence, surprisingly does not influence CSM rate (Fig. 14.12).[163,223]

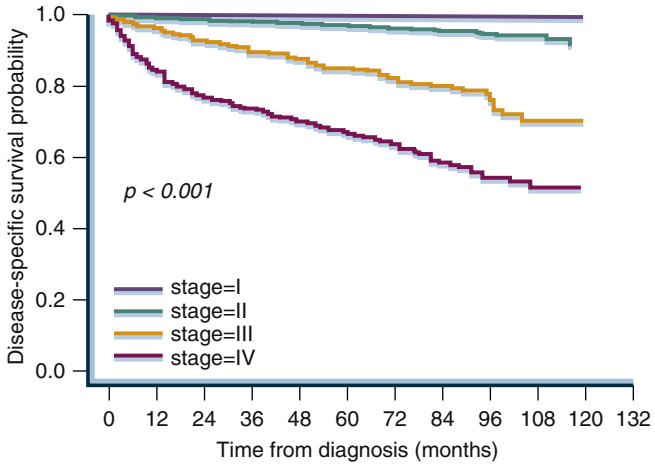
Several scoring systems based on these significant prognostic indicators have been devised. Each system allows one to assign the majority of PTC patients (≥80%) to a low-risk group, in which the CSM rate at 25 years is less than 2%, and the others (a small minority) to a high-risk group, in which almost all cancer-related deaths are observed. In general, these systems provide prediction of postoperative events comparable to that of the internationally accepted TNM staging system.[225]

A scoring index devised to assign PTC patients to prognostic risk groups[226] was named the AGES scheme after the four independent variables: patient's *a*ge, tumor *g*rade, tumor *e*xtent (local invasion, distant metastases), and tumor *s*ize. With the use of such a scoring system, 86% of patients were in the minimal risk group (AGES score <4) and they experienced a 20-year CSM rate of only 1%. By contrast, patients with AGES scores of 4 or above (high risk; 14% of the total) had a 20-year CSM rate of 36%. Such prognostic scoring systems make it possible to counsel patients and to aid in the planning of individualized postoperative management programs in PTC.[223,226]

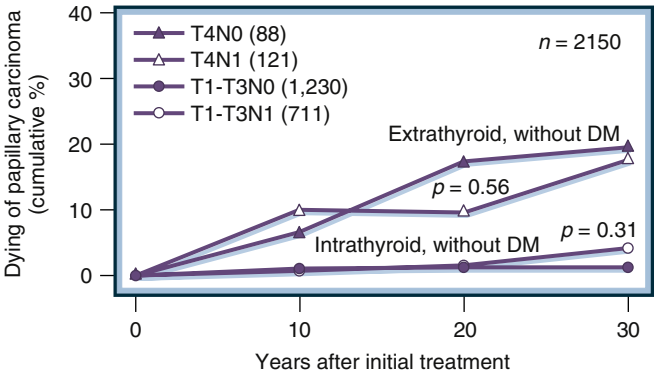
Although the AGES scheme had the potential for universal application, some centers could not include the differentiation (G) variable because their surgical pathologists did not recognize higher-grade PTC tumors.[225] Accordingly, a prognostic scoring system for predicting PTC mortality rates was devised with the use of candidate variables that included completeness of primary tumor resection but excluded histologic grade.[223] Cox model analysis and stepwise variable selection led to a final prognostic model that included five variables: *m*etastasis, *a*ge, *c*ompleteness of resection, *i*nvasion, and *s*ize. This model was termed the MACIS prognostic scoring system. The final score utilized a calculation of (3.1 [age ≤39 years] or 0.08 × age [age ≥40 years]) + (0.3 × tumor size [in centimeters]) + (1 [if tumor not completely resected]) + (1 [if locally invasive]) + (3 [if distant metastases present]).



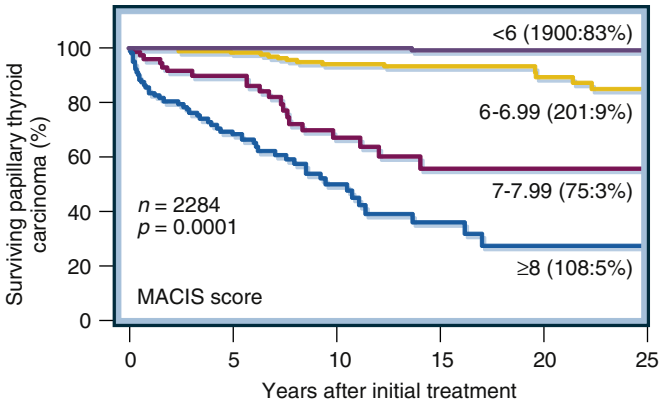
• **Fig. 14.10** Alluvial flow diagram representing the restaging of patient cohorts from the seventh to the eighth edition of the American Joint Commission on Cancer/Union for International Cancer Control (AJCC/UICC) tumor, node, metastasis (TNM) staging system in (A) the Surveillance, Epidemiology, and End Results (SEER) program and (B) the National Cancer Database (NCDB). Numbers represent the absolute number of patients within each stage, with flow line width proportional to the number of patients moving to a new stage classification. (Redrawn from Pontius LN, Oyekunle TO, Thomas SM, et al. Projecting survival in papillary thyroid cancer: a comparison of the seventh and eighth editions of the American Joint Commission on Cancer/Union for International Cancer Control Staging Systems in two contemporary national patient cohorts. *Thyroid*. 2017;27:1408–1416.)



• **Fig. 14.11** Unadjusted disease-specific survival (DSS) curves for patients with papillary thyroid cancer (PTC) in the SEER program using the AJCC/UICC TNM staging eighth edition models. (Redrawn from Pontius LN, Oyekunle TO, Thomas SM, et al. Projecting survival in papillary thyroid cancer: a comparison of the seventh and eighth editions of the American Joint Commission on Cancer/Union for International Cancer Control Staging Systems in two contemporary national patient cohorts. *Thyroid.* 2017;27:1408–1416.)



• **Fig. 14.12** Lack of influence of nodal metastases at initial operation on cumulative mortality from papillary thyroid carcinoma in 1941 patients with pathologic T1 to T3 intrathyroidal tumors (completely confined to the thyroid gland) and 209 pathologic T4 patients with extrathyroidal (locally invasive) tumors. All patients had initial surgical treatment at the Mayo Clinic from 1940 to 1997. *DM,* distant metastases.



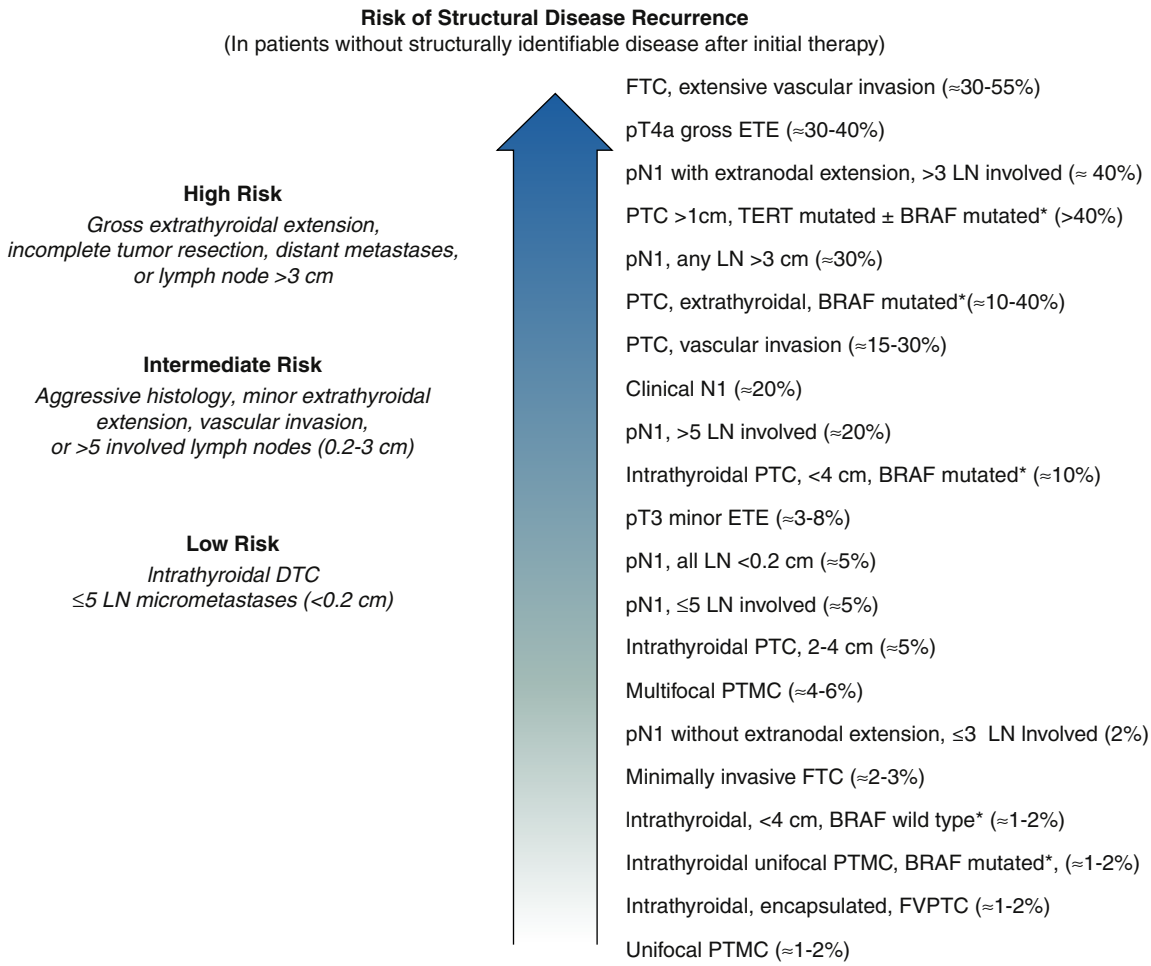
• **Fig. 14.13** Cause-specific survival according to MACIS (*m*etastases, *a*ge, *c*ompleteness of resection, *i*nvasion, and *s*ize) scores of less than 6, 6 to 6.99, 7 to 7.99, and 8+ in a cohort of 2284 consecutive patients with papillary thyroid carcinoma (PTC) undergoing initial treatment at the Mayo Clinic from 1940 to 1997. The numbers in parentheses represent the numbers and percentages of PTC patients in each of the four risk groups.

As illustrated by Fig. 14.13, the MACIS scoring system permits identification of groups of patients with a broad range of risk of death from PTC. Twenty-year cause-specific survival rates for patients with MACIS scores of less than 6, 6 to 6.99, 7 to 7.99, and 8+ were 99%, 89%, 56%, and 27%, respectively (p <0.0001). When the cumulative mortality rate from all causes of death was considered, approximately 85% of PTC patients with AGES scores below 4 or MACIS scores below 6 had no excess mortality rate over rates predicted for control subjects.[223,226]

It should be emphasized that the five variables in MACIS scoring are easy to define after primary operation; consequently, the system can be applied in any clinical setting. The MACIS system can be used for counseling individual PTC patients and can help guide decision making concerning the intensity of the postoperative tumor surveillance and the appropriateness of adjunctive radioiodine therapy. Because the CIS (completeness of resection, invasion, and size) variables require information obtained at surgery, the system should not be used to decide the extent of primary surgery.[227,228]

An important consideration for patients with well-differentiated thyroid cancer is that the risk of thyroid cancer *recurrence* is higher than the risk of thyroid cancer–related *death*. To address this important shortcoming impacting clinical care, the ATA proposed a stratification system predicting the risk of recurrence after initial treatment,[1] which should be taken into account for the indication of postoperative administration of radioiodine and for the subsequent follow-up strategy. The stratification is based on individual factors from various studies and reports a continuous increasing risk of recurrence according to each factor (Fig. 14.14). No multivariate analysis is thus far available. Of note, the age of the patient at the time of initial treatment is not taken into account for the risk of recurrence, but many other prognostic factors for thyroid cancer death are also prognostic for the risk of recurrence, such as some histologic characteristics, the size of the thyroid tumor, the extension of the tumor beyond the thyroid capsule, the presence of lymph node metastases, and the presence of residual disease due to either incomplete surgical resection or presence of distant metastases. The encapsulated follicular variant is associated with a low risk of recurrence. Aggressive histologic subtypes, the presence of necrosis, a high mitotic count, and vascular invasion are, in contrast, associated with higher risk. Indeed, the risk of recurrence is minimal (<2%) for unifocal micropapillary carcinoma, but higher for multifocal micropapillary carcinoma (~4%), and increases with the size of the thyroid tumor but is still low (~5%) in patients with an intrathyroid tumor of 3 to 4 cm without extension beyond the thyroid capsule.[229]

The prognostic impact of extension beyond the thyroid capsule is low in micropapillary carcinomas that are classified as N0. This risk increases with the size of the thyroid tumor and with the extent of the extension.[230–232] Minimal lymph node involvement (i.e., less than three lymph node metastases each <2 mm) is



• **Fig. 14.14** Differentiated thyroid cancer risk of recurrence. *DTC*, differentiated thyroid cancer; *ETE,* extrathyroidal extension; *FTC,* follicular thyroid cancer; *FVPTC,* follicular variants of papillary thyroid carcinoma; *LN,* lymph node; *PTC,* papillary thyroid carcinoma; *PTMC,* papillary thyroid microcarcinoma. (From Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid*. 2016;26:1–133.)

associated with a low risk of recurrence. This risk again increases with the number of lymph node metastases, with the size of the lymph node metastases, and with the presence of extranodal extension.[153,154,233] Therefore minimal lymph node involvement that is usually found at prophylactic lymph node dissection may have a minimal prognostic impact and may not change the indication for postoperative treatment with radioiodine. However, large lymph node metastases (>3 cm) that are usually palpable and easily visualized on neck ultrasonography and are frequently multiple and associated with extracapsular nodal extension have a major prognostic impact on recurrence that may occur in up to 40% of patients.

The risk of PTC recurrence is also associated with the molecular profile and with the presence of *BRAF* mutation associated with a higher risk of recurrence for most tumor stages.[111,234,235] This risk is even higher when both *BRAF* and *TERT* mutations are present in the tumor.[111,193] Each of these characteristics is associated with a risk of tumor recurrence that may range from less than 2% up to more than 40%. For practical purposes, cases can be divided into three discrete groups based on their estimated risk of recurrence (low, <5%; intermediate, 5–20%; high, >20%), and the group to which the case is assigned can be used as a guide for planning subsequent treatment and follow-up (Table 14.7). (See also "Outcome Prediction for Follicular Thyroid Carcinoma.")

Follicular Thyroid Carcinoma

WHO defines follicular thyroid cancer as a malignant epithelial tumor characterized by follicular cell differentiation and absence of the nuclear features typical of PTC.[147] This definition excludes the follicular variant of PTC, and it is also customary to exclude both the poorly differentiated carcinoma[236] and the rare mixed medullary and follicular carcinoma.[237]

Thus FTC is a relatively rare neoplasm whose identification requires invasion of the capsule, blood vessel, or adjacent thyroid. In initial epidemiologic surveys, FTC constituted 5% to 50% of differentiated thyroid cancers and tended to be more common in areas with iodine deficiency.[238] Changing diagnostic criteria and increases in the incidence of PTC associated with dietary iodine supplementation have reduced the frequency of FTC diagnoses. Data from the SEER program from 1980 to 2009 show incidence rates for FTCs (1.19 per 100,000 woman-years, 0.55 per 100,000 man-years) that are markedly lower than those for PTCs (9.21 per 100,000 woman-years, 3.10 per 100,000 man-years).[239]

TABLE 14.7 2015 American Thyroid Association (ATA) Risk Stratification System

ATA Low Risk	Papillary thyroid cancer (with all of the following): • No local or distant metastases • All macroscopic tumor has been resected • No tumor invasion of locoregional tissues or structures • The tumor does not have aggressive histology (e.g., tall cell, hobnail variant, columnar cell carcinoma) • If ^{131}I is given, there are no RAI-avid metastatic foci outside the thyroid bed on the first posttreatment whole-body RAI scan • No vascular invasion • Clinical N0 or ≤5 pathologic N1 micrometastases (<0.2 cm in largest dimension) Intrathyroidal, encapsulated follicular variant of papillary thyroid cancer Intrathyroidal, well-differentiated follicular thyroid cancer with capsular invasion and no or minimal (<4 foci) vascular invasion Intrathyroidal, papillary microcarcinoma, unifocal or multifocal, including *BRAF*^{V600E} mutated (if known)
ATA Intermediate Risk	Microscopic invasion of tumor into the perithyroidal soft tissues RAI-avid metastatic foci in the neck on the first posttreatment whole-body RAI scan Aggressive histology (e.g., tall cell, hobnail variant, columnar cell carcinoma) Papillary thyroid cancer with vascular invasion Clinical N1 or >5 pathologic N1 with all involved lymph nodes <3 cm in largest dimension Multifocal papillary microcarcinoma with extrathyroidal extension and BRAF^{V600E} mutated (if known)
ATA High Risk	Macroscopic invasion of tumor into the perithyroidal soft tissues (gross extrathyroidal extension) Incomplete tumor resection Distant metastases Postoperative serum thyroglobulin suggestive of distant metastases Pathologic N1 with any metastatic lymph node ≥3 cm in largest dimension Follicular thyroid cancer with extensive vascular invasion (>4 foci of vascular invasion)

ATA, American Thyroid Association; *RAI*, radioactive iodine

From Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid.* 2016;26:1–133.

The microscopic appearance of FTC varies from well-formed follicles to a predominantly solid growth pattern.[146] Poorly formed follicles and atypical patterns (e.g., cribriform) may occur, and multiple architectural types may coexist. FTC is best divided into three categories on the basis of degree of invasiveness: (1) minimally invasive or encapsulated, (2) encapsulated angioinvasive, and (3) widely invasive. Minimally invasive FTC is an encapsulated tumor whose growth pattern resembles that of a trabecular or solid microfollicular or atypical adenoma. The diagnosis of malignancy depends on the demonstration of capsular invasion. The criteria for invasion must therefore be strict.[146,147] Interruption of the capsule must involve the full thickness to qualify as capsular invasion. Penetration of only the inner half or the presence of tumor cells embedded in the capsule does not qualify for the diagnosis of FTC. Foci of capsular invasion must be distinguished from the capsular rupture that can result from FNA. The acronym WHAFFT (worrisome histologic alterations following FNA of the thyroid) is applied to such changes.[240] Extensive histologic sampling may be required to distinguish minimally invasive FTCs from follicular adenomas, and the ability to discriminate between the two varies substantially from one pathologist to another.[241] The diagnosis of malignancy of these tumors may be difficult and not reproducible among pathologists, and immunohistochemistry with markers such as TPO, galectin 3, or HMBE1 may help for this purpose,[106] but these techniques did not reliably improve the accuracy in case of suspicious findings. Global gene expression studies with the microarray technology and more recently the gene classifier or a panel of gene mutations may help to differentiate malignant from benign follicular tumors.[242]

Minimally invasive FTC with vascular invasion (encapsulated angioinvasive, defined by some authors as moderately invasive) must also be distinguished from minimally invasive FTCs with capsular invasion alone because vascular invasion increases the risks of recurrence and metastasis. Vascular spread should be diagnosed even when there is only a single focus of angioinvasion. Grossly evident blood vessel invasion is almost never seen.

In contrast, the rare, widely invasive form of FTC can be distinguished easily from benign lesions. Although the tumor may be partially encapsulated, the margins are infiltrative even on gross examination, and vascular invasion is often extensive. The structural features are variable, but a follicular element is always present. When follicular differentiation is poor or absent, or in the presence of trabecular, insular, or solid components, the tumor may be classified as a poorly differentiated carcinoma (see later).[146,236] Focal or extensive clear cell changes can also be seen on histologic analysis. A rare clear cell variant of FTC has been described in which glycogen accumulation or dilatation of the granular endoplasmic reticulum is responsible for the clear cells.[243]

Molecular Pathogenesis of Follicular Thyroid Carcinoma

There is still no accepted paradigm for the pathogenesis of FTC. A multistep adenoma-to-carcinoma pathogenesis, similar to that for colon cancer and other adenocarcinomas, is not universally accepted because pathologists do not recognize follicular carcinoma in situ, and documentation of the evolution of adenoma to carcinoma is rare. Nevertheless, several facts about the pathogenesis of FTC are firmly established.[111,160,244,245] First, most follicular adenomas and all FTCs are probably of monoclonal origin. Second, oncogene activation, particularly by point mutation of the *RAS* oncogene, is common both in follicular adenomas (~20%) and in FTCs (~40%), supporting a role for these mutations in early tumorigenesis.[245,246] This commonality suggests that FTA and FTC are etiologically related or, more specifically, that FTAs are premalignant neoplasms that can (at least in some cases, but likely not all) progress to become FTC.[245] Moreover, the fact that *RAS* mutations are common in FTA, FTC, and FVPTC implies that all these follicular-patterned neoplasms share a common pathogenesis.[247] The *RET* and *BRAF* oncogenes do not appear to be involved in follicular tumors.[111,160,248] Third, cytogenetic abnormalities and evidence of genetic loss are

more common in FTC than in PTC and also occur in follicular adenomas.[244,248]

Of the cytogenetic abnormalities described in FTC, the most common are deletions, partial deletions, and deletion rearrangements involving the p arm of chromosome 3. Loss of heterozygosity (LOH) on chromosome 3p appears to be limited to FTC because no evidence for 3p LOH has been found in follicular adenomas or PTC. A translocation, t(2;3)(q13;p25), resulting in the fusion of the DNA binding domains of the thyroid transcription factor PAX8 to domains of the peroxisome proliferator–activated receptor gamma 1 (PPARγ1) was detected in 30% (range 11–63%) of FTCs, 10% of follicular adenomas, and a smaller percentage of FVPTCs.[249–253] PAX8 encodes a paired-box transcription factor that is highly expressed in thyroid follicular cells, where it plays a key role in their development.[254,255] The fusion gene mentioned earlier therefore results in increased expression of *PPARγ*.[256] *CREB3L2-PPARγ* fusions have also been described in a small number of FTCs,[257] suggesting that *PPARγ* may play a role in the development of these tumors. Compared with classic FTCs, *PAX8-PPARγ* FTCs are more frequently diagnosed in younger patients, and they are more likely to be associated with vascular invasion, although their clinical behavior is relatively indolent.[251] The *PAX8-PPARγ* tumors' gene expression profiles are also distinctive, reflecting as they do the transcriptional output of the PAX8-PPARγ fusion protein.[258]

Genes encoding components of the PI3K/PTEN/AKT signaling pathway are also likely to be genetically altered or epigenetically silenced in FTCs.[259] Somatic mutations involving the tumor suppressor gene *PTEN* are found in up to 1 of 10 sporadic FTCs,[260] and germline *PTEN* mutations are associated with the Cowden syndrome (CS). Up to 10% of all FTCs also harbor activating PIK3CA mutations.[261]

Mutations of the *TERT* promoter are also found in some FTCs, and they are associated with aggressive disease and poor outcomes.[192] Interestingly, *TERT* promoter mutations have also been reportedly found in a small subset of FTAs and atypical adenomas. Some of these tumors ultimately displayed cancer-like behavior, raising the possibility that TERT mutations may be a potential biomarker of FTC for use in cases with nondiagnostic histologic features.[262]

In a recent genomic study of FTA, FTC, and PTC,[263] the transcriptomes of FTA, minimally invasive FTC, and noninvasive FVPTC proved to be indistinguishable, while that of the infiltrative FVPTC was more similar to the classic PTC transcriptome.[263] These findings add support to the view that follicular-patterned tumors are characterized by a common *RAS*-like pathogenesis. The strong biologic relation between FVPTC and FTC has also been confirmed by miRNA expression profiling, which revealed upregulated expression of miR-182-5p and miR-183-5p exclusively in these thyroid carcinoma histotypes.[118,264,265] Nearly 90% of the miRNAs found to be dysregulated in FTC were similarly dysregulated in follicular adenomas (FA), including miR-182-5p, miR-183-5p, and miR-96, which were upregulated, and miR-1247, which was downregulated with respect to levels found in normal thyroid tissues.[264] In contrast, downregulation of miR-150 seemed to be an FTC-specific change[264] and may function as a suppressor gene in tumor cells by inhibiting the RAB11A/WNT/β-catenin pathway.[266] Three well-characterized miRNAs—miR-146b-5p, miR-221-3p, and miR-222-3p—are commonly upregulated in FTCs, PTCs, and ATC. Their dysregulation thus appears to be an early event in the development of follicular cell–derived thyroid cancers that is essential for sustaining the oncogenic process.[118,265,267]

Hürthle Cell Carcinoma

When more than 75% of cells in an FTC exhibit Hürthle cell (or oncocytic) features, the tumor is classified as a Hürthle cell (oncocytic) carcinoma.[146,238] The current version of the WHO classification includes this tumor as a separate entity,[147] although in the past it was considered an oxyphilic variant of FTC.[162] The AFIP monograph already stated that "the tumors made up of this cell type have gross, microscopic, behavioral, cytogenetic features that set them apart from all others and justify discussing them in a separate section."[146] The mutational, transcriptional, and copy number profiles of HCC were distinct from those of PTC and FTC, indicating HCC to be a unique type of thyroid malignancy. Molecular pathways that differentiate Hürthle cell adenoma from widely invasive HCC included the PIK3CA-Akt-mTOR (mammalian target of rapamycin) and Wnt/β-catenin pathways, potentially providing a rationale for new targets for this type of malignancy. Recent data have confirmed that molecular abnormalities are different from those found in follicular cancer.[268]

Presenting Features of Follicular Thyroid Carcinoma

FTC tends to occur in older individuals.[238] The mean age at diagnosis of FTC (49 years) is slightly higher than those for both PTC and follicular variant PTCs (44 and 46 years, respectively).[269] The median age of patients with HCC is about 60 years. As in most thyroid malignancies, women outnumber men (>2:1). Most patients with FTC present with a painless thyroid nodule, with or without background thyroid nodularity, and they rarely (2–8%) have clinically evident lymphadenopathy at presentation.[238] Lymph node metastases to the neck in FTC are so exceptional that "wherever they are observed, the alternative possibilities of follicular variant papillary carcinoma, oncocytic carcinoma, and poorly differentiated carcinoma should be considered."[146] HCC are the exception where up to one-third of these FTCs have cervical lymph node metastases at the time of diagnosis.[238]

In most series in which tumor sizes were reported, the average tumor in FTC and HCC was larger than those seen with PTC.[270] Direct extrathyroidal extension, by definition, does not occur with minimally invasive FTC but is common in the rare patients with widely invasive FTC. Between 15% and 27% of patients may have distant metastases at presentation.[238] The most common sites for distant metastases in FTC are lung and bone.[238] The bones most often involved are long bones (e.g., femur), flat bones (particularly the pelvis, sternum, and skull), and vertebrae. When a distant metastasis is the first manifestation of the disease, definitive proof of its thyroid origin should be obtained, usually by biopsy of a metastasis, before performing any thyroid surgery. It is unusual but possible for patients with FTC to have thyrotoxicosis caused by massive tumor burden producing thyroid hormone.[271]

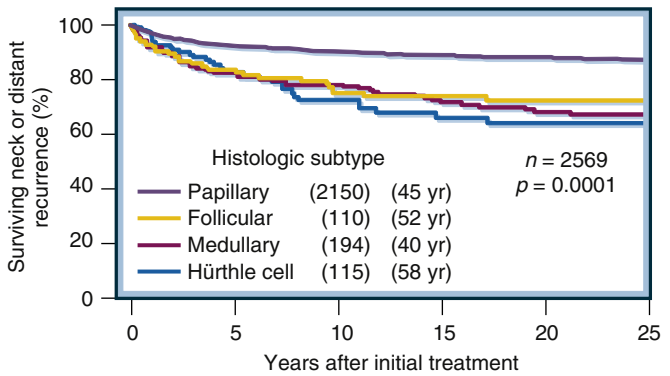
Risk of Follicular Thyroid Carcinoma Recurrence and Mortality

Recurrence rates reported in patients with FTCs vary widely, from 3% to 43.5%, and recurrence is most frequent in widely invasive FTC. Similar recurrence rates are seen in HCC (14–44%). Over half of all FTC recurrences are detected within the 3 years following diagnosis, and 80% are found within the first 6 years. The vast majority of recurrences (up to 85%) occur at distant sites, but lesions can also be found in the thyroid bed and regional lymph nodes.

Nodal metastases are rare in typical FTC, and the nodal recurrence rate at 20 postoperative years is the lowest among differentiated thyroid carcinoma, being around 2% (see Fig. 14.8),

although it is higher (about 17%) in HCC patients.[272] When recurrences at either the neck or distant sites are taken into consideration, patients with HCC (Fig. 14.15) have the highest numbers of tumor recurrences after 10 to 20 years. As illustrated by Fig. 14.8, local recurrences at 20 years have occurred in 20% of FTCs and 30% of HCCs. Comparable distant metastasis rates are 23% and 28%, respectively.

The mortality rate from FTC exceeds that of PTC (Fig. 14.16). CSMs vary with the presenting TNM stage in both FTC and HCC (Fig. 14.17). The death rates tend to parallel the curves for development of distant metastases (see Fig. 14.8). In more than 50 years of experience at the Mayo Clinic, the mortality rate for FTC initially exceeds that of HCC, but by 20 to 30 postoperative years, there are no significant differences in cause-specific survival rates between FTC and HCC, both being around 80% at 20 years and 70% at 30 postoperative years.[270] Curves representing death from all causes differ in FTC and HCC. On average, patients with FTC are about 5 years younger, tend to die within the first 10 postoperative years, and have a high all-cause mortality rate for 10 to 30 postoperative years. Deaths related to HCC occur gradually over the first 15 years. However, by 25 years, the average survivor of HCC is 84 years old, and by that time, almost 50% of the treated cohort would be predicted by the actuarial curve to have died from all causes.

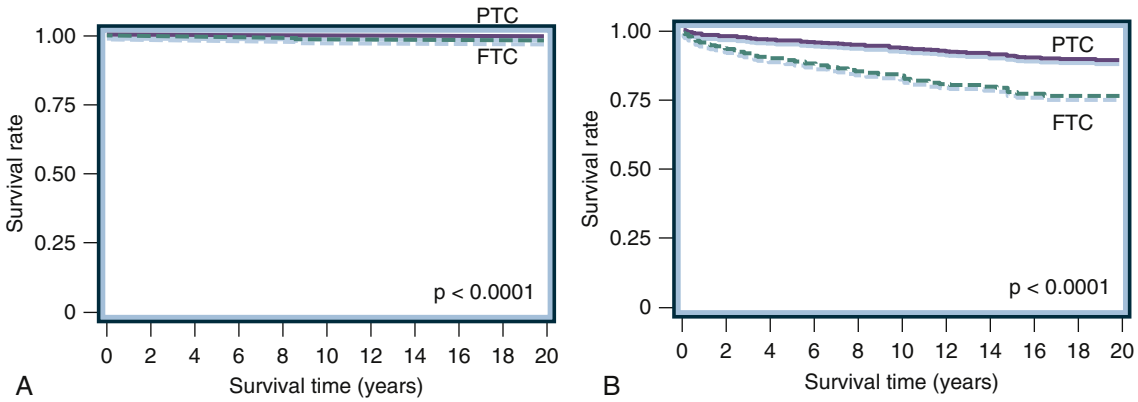


• **Fig. 14.15** Postoperative recurrence (any site) in the first 20 years after definitive surgery for differentiated thyroid carcinoma performed at the Mayo Clinic from 1940 to 1997. Based on 2569 consecutive patients (2150 papillary thyroid carcinoma, 110 follicular thyroid carcinoma, 115 Hürthle cell carcinoma, and 194 medullary thyroid carcinoma) who had complete tumor resection and had no distant metastases at presentation. The ages in parentheses represent the median age at diagnosis for each of the four histologic subtypes.

Outcome Prediction for Follicular Thyroid Carcinoma

The risk factors that predict outcome in FTC are similar to those recognized in PTC[273–276] and include distant metastases at presentation, advanced age at diagnosis (>45 years for most studies), large primary tumors (>4 cm), the presence and extent of local (extrathyroidal) invasion, and the completeness of excision. To a lesser degree, increased mortality risk is associated with male sex. The number of vessels involved (<4 or ≥4) has also prognostic value for both cancer-specific survival and disease-free survival (Fig. 14.18).[238,275]

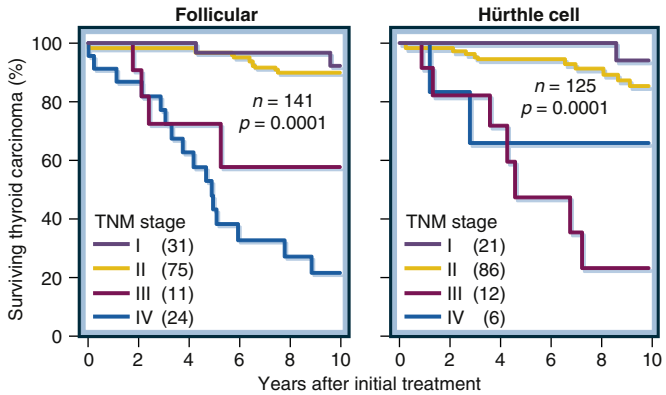
Inconsistencies between the results of different studies in predicting the outcome of FTC patients may reflect the use of different classification systems over long follow-up periods, the quality of the pathologic reviews, and/or the small sample sizes. Several systems have been proposed for staging thyroid cancer. Some were developed solely for use with PTC, others were derived from data on PTC and FTC *(differentiated thyroid cancer),* and still others were developed for use with all histologic types of thyroid cancers (including medullary and anaplastic forms). The AIM (age, invasion, metastases) system is the only one developed specifically for staging FTC. In some of these systems, FTC patients are classified in a higher risk category (GAMES [grade, age, metastases, extent, size], National Thyroid Cancer Treatment Cooperative Study Group [NTCTCSG]). On the contrary, the AMES (age, metastases, extent, size), Clinical Class, MACIS, and AGES prognostic scoring systems and various versions of the UICC/AJCC pTNM system do not include FTC histology as an independent risk factor.[274] The UICC/AJCC pTNM (tumor, nodes, metastasis) system is still the most widely used staging system for thyroid carcinoma and provides fairly accurate prognostic information on the tumors (see Fig. 14.17). One of the most hotly debated staging criteria is the age threshold for defining higher risk disease. In the recently implemented eighth edition of the AJCC/IUCC staging system, the threshold was raised from 45 years to 55 years to avoid encouraging overtreatment. Moreover, a recent reassessment of the NTCTCSG thyroid cancer staging system showed that a new model for FTC, with a threshold of 50 years, outperformed the current system.[277]



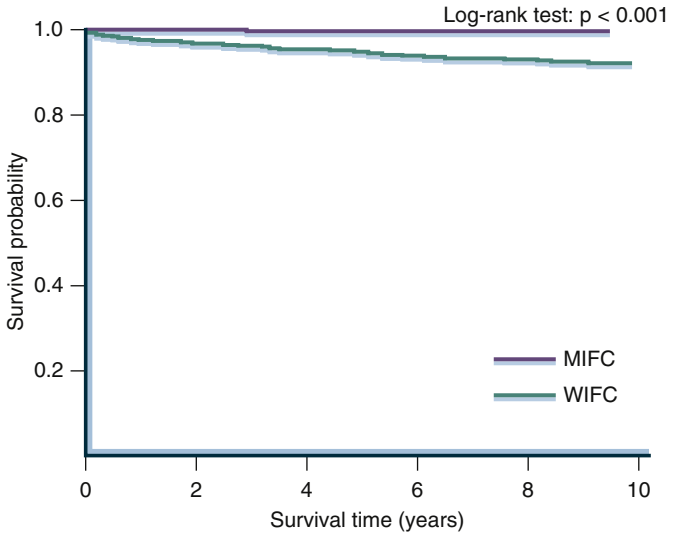
• **Fig. 14.16** Comparison of disease-specific survival by carcinoma type (papillary [PTC] vs follicular thyroid cancer [FTC]) in patients less than 45 years (A) or at least 45 years old (B). (Redrawn from Oyer SL, Fritsch VA, Lentsch EJ. Comparison of survival rates between papillary and follicular thyroid carcinomas among 36,725 patients. *Ann Otol Rhinol Laryngol*. 2014;123:94–100.)

Poorly Differentiated Carcinoma

Poorly differentiated thyroid carcinoma is rare, representing less than 5% of all thyroid cancers. It has been defined as "a tumor of follicular cell origin with morphological and biologic attributes intermediate between differentiated and anaplastic carcinomas of the thyroid."[146] A consensus conference proposed the following diagnostic criteria (the *Turin criteria*) for poorly differentiated carcinoma: (1) solid, trabecular, and insular pattern of growth; (2) absence of the conventional nuclear features of papillary carcinomas; and (3) presence of at least convoluted nuclei, mitotic activity greater than 3 × 10 high-power fields, and tumor necrosis.[236] Similarly, in well-differentiated cancer, necrosis, mitosis, and cellular atypia associated with vascular invasion are considered as features of poor prognosis and aggressiveness.[39,274] Most poorly differentiated tumors are larger than 5 cm in diameter at diagnosis, with extrathyroidal extension and blood vessel invasion.



• **Fig. 14.17** Cause-specific survival according to pathologic tumor-node-metastasis (pTNM) stages in a cohort of 141 patients with follicular thyroid carcinoma *(left panel)* and 125 patients with Hürthle cell carcinoma *(right panel)* treated at the Mayo Clinic from 1940 to 1997. Numbers in parentheses represent the number of patients in each pTNM stage grouping.



• **Fig. 14.18** Disease-specific survival of minimally invasive follicular thyroid cancer (MIFC) versus widely invasive follicular thyroid cancer (WIFC). (From Goffredo P, Cheung K, Roman SA, et al. Can minimally invasive follicular thyroid cancer be approached as a benign lesion? A population-level analysis of survival among 1200 patients. *Ann Surg Oncol*. 2013;20:767–772.)

RAS mutations appear to be a common molecular signature in poorly differentiated tumors, although they were found at a highly variable frequency and wide variation in specific types of *RAS* mutations. The occurrence of β-catenin mutations in PDTC is controversial, ranging from 0% to 32% of tumors. Finally, *TERT* promoter mutations, which have been described in a subset of PDTC, have been proposed as a molecular marker of thyroid tumor dedifferentiation and progression, and they are considered to be a molecular signature of aggressive tumors. *TERT* promoter mutations are found in around 40% of PDTCs, particularly those that also harbor an additional *BRAF* or *RAS* mutation. *TP53* mutations are reported in 10% of the cases, but are more common in anaplastic thyroid cancer (Fig. 14.19).[278] Recently, a prognostic role of specific microRNAs has been demonstrated in PDTC.

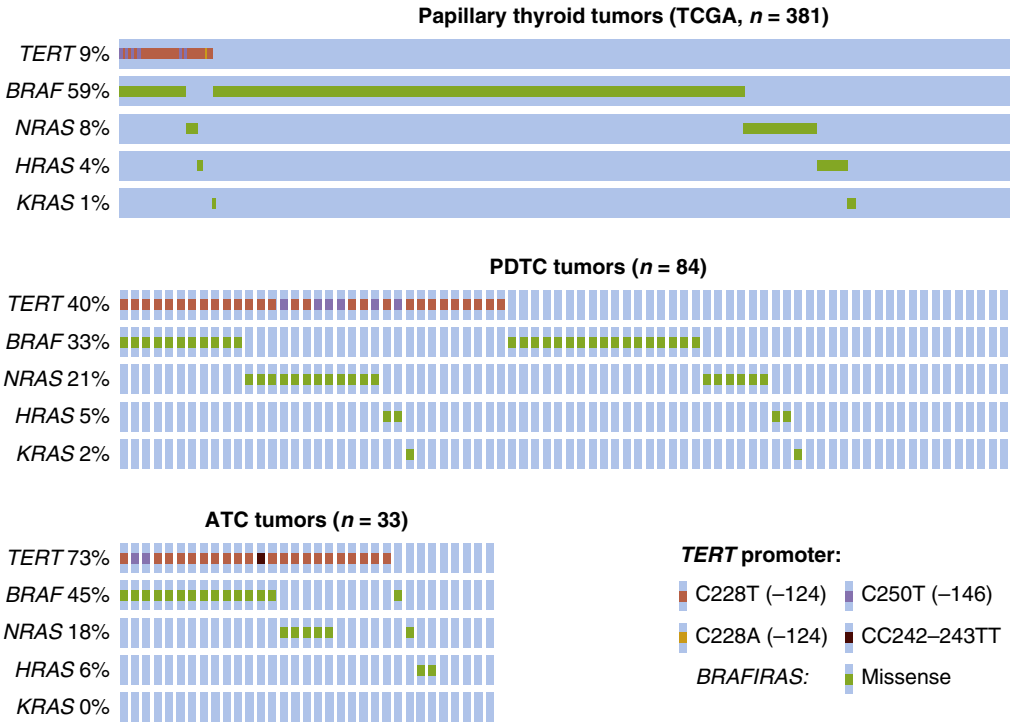
The mean age at diagnosis is about 55 years, and the female-to-male ratio is about 2:1. Poorly differentiated carcinoma is aggressive and can be lethal. Radioiodine uptake is rarely present, and FDG uptake on PET is frequently high; production of Tg in blood may be lower than in differentiated carcinomas. Metastases are common in regional nodes and distant sites (lung, bone, brain). In one series, 56% of patients died from their tumor within 8 years of initial therapy, though not all have replicated these findings.[236,279,280]

Undifferentiated (Anaplastic) Carcinoma

Anaplastic carcinoma constitutes about 1% to 2% of all thyroid carcinomas, usually occurs after the age of 60 years, and is slightly more common in women than men (1.3:1 to 1.5:1).[281] This carcinoma is highly malignant, nonencapsulated, and extends widely. Evidence of invasion of adjacent structures, such as the skin, muscles, nerves, blood vessels, larynx, and esophagus, is common. Distant metastases occur early in the course of the disease in lungs, liver, bones, and brain.

On histopathologic examination, the lesion is composed of atypical cells that exhibit numerous mitoses and form a variety of patterns. Spindled, pleomorphic, squamoid, and even rhabdoid cells have been described. Areas of necrosis and polymorphonuclear infiltration are common, and the presence of PTC or FTC suggests that they may be the precursors of anaplastic carcinoma. Immunohistochemistry revealed that a significant proportion of cells are tumor-associated macrophages.[282,283]

Mutations of the *TP53* gene are present in many (60–80%) undifferentiated carcinomas but may not be found in the residual well-differentiated component, suggesting that these mutations occurred after the development of the original tumor and may have played a key role in tumor progression.[163,284] *BRAF* mutation is found mostly in anaplastic thyroid carcinoma with a papillary component, and *RAS* mutation is found in 20% or more of anaplastic thyroid carcinomas; *PI3KCA* mutation is rare in differentiated thyroid cancer and was found in 23% of anaplastic thyroid carcinoma; finally, *ALK* rearrangement was found in about 10% of anaplastic thyroid carcinomas.[184] *BRAF* and *RAS* mutations are still the principal genetic drivers of thyroid carcinomas, but additional mutations accumulate in ATCs, including those affecting the *TERT* promoter (see Fig. 14.19), mismatch repair genes, and genes encoding components of the PIK3CA-PTEN-AKT-mTOR pathway, the SWI-SNF complex, or histomethyltransferases. Several alterations may serve as adverse prognostic markers in ATC, including *EIF1AX* mutations, loss of chromosome 13q, and gain of chromosome 20q.[278]



• **Fig. 14.19** Oncoprints of *TERT* promoter mutations, *BRAF*, and *RAS* mutations (with reported rate) in PTCs from TCGA (*n* = 381), PDTCs (*n* = 84), and ATCs (*n* = 33). (From Landa I, Ibrahimpasic T, Boucai L, et al. Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. *J Clin Invest*. 2016;126:1052–1066.)

The usual clinical complaint is of rapid, often painful enlargement of a mass that may have been present in the thyroid gland for many years. The tumor invades adjacent structures, causing hoarseness, inspiratory stridor, and difficulty in swallowing. On examination, the overlying skin is often warm and discolored. The mass is tender and is often fixed to adjacent structures. The regional lymph nodes are enlarged, and there may be evidence of distant metastases. Anaplastic carcinomas do not accumulate iodine and do not typically produce Tg; high FDG uptake is usually found on PET, which is the best tool for tumor staging and for control of treatment efficacy.[285,286]

Treatment should be initiated rapidly to avoid death from locally infiltrative disease and possible suffocation. It consists of surgical resection of the tumor tissue present in the neck, when this is feasible, followed by a combination of external irradiation and chemotherapy. The overall prognosis for ATC remains poor despite recent advances (Fig. 14.20). The median overall survival remains around 3 to 5 months with a 1-year survival of approximately 20%. Patient factors associated with poorer prognosis include advanced age (>60–70 years), male gender, presence of leukocytosis (>10,000), and symptoms (such as rapidly growing tumor, pain in the neck, dyspnea, dysphagia, and hoarseness).

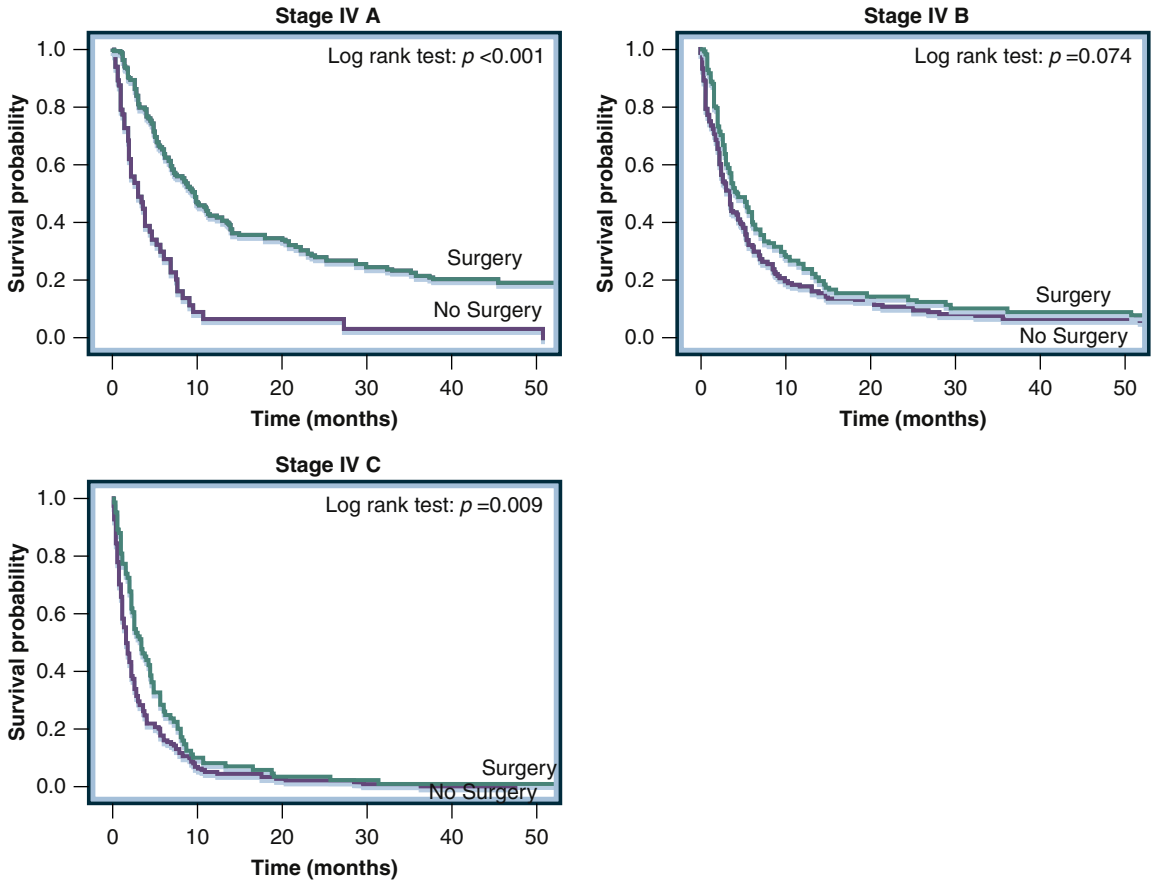
However, treatment paradigms for ATC are evolving rapidly. Patients with *BRAF*-mutant ATCs can now be treated with a combination of selective BRAF and MEK inhibitors. For patients whose disease was initially regarded as unresectable, response to this combination therapy can render them eligible for surgery. For patients interested in taking part in a clinical trial, the tumor should be assessed for a broad range of mutations and fusions. This helps determine whether the patient is suitable for enrollment in trials testing selective inhibitors, such as the selective NTRK or RET inhibitor trials. Other driver mutations that are identified may help identify a clinical trial that is appropriate for the patient, particularly as more targeted therapies become available.[287]

Medullary Thyroid Carcinoma

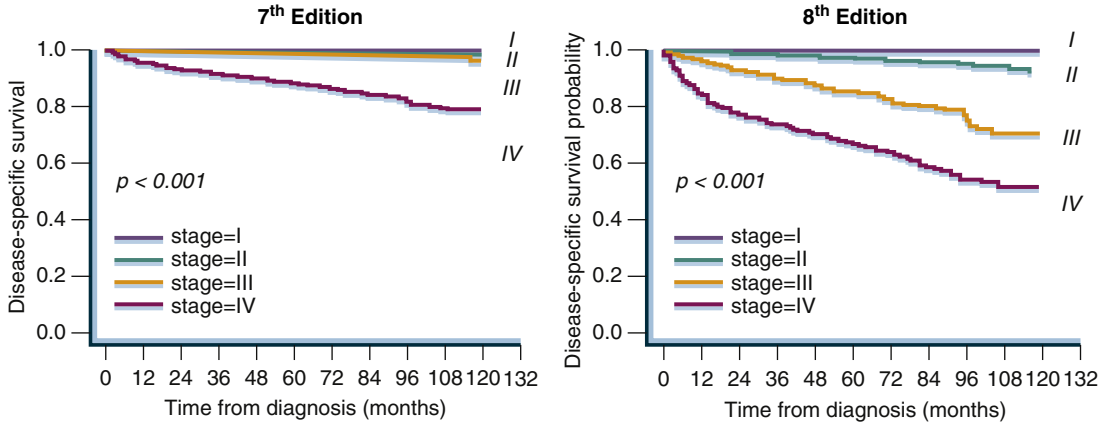
Medullary thyroid carcinoma accounts for approximately 2% of all thyroid cancers. MTCs are rare neuroendocrine cancers that arise from the parafollicular C cells of the thyroid gland. Compared with differentiated thyroid cancers, MTC is associated with less favorable outcomes (Fig. 14.21), including persistent or recurrent disease, need for reoperation, and mortality.[288] However, the prognosis and associated risk are highly variable, in large part determined by the specific molecular mutation detected in the MTC. On histologic assessment, MTC readily invades the intraglandular lymphatics and spreads to other parts of the gland, in addition to the pericapsular and regional lymph nodes. It also regularly spreads through the bloodstream to the lungs, bones, and liver.[14,155,156,289–291]

Histologic Diagnosis of Medullary Thyroid Carcinoma

MTC tumors are firm and usually unencapsulated. On histopathologic examination, the tumor is composed of cells that vary in morphologic features and arrangement. Round, polyhedral, and spindle-shaped cells form a variety of patterns, which may vary from solid and trabecular to endocrine or glandular-like structures. An amyloid stroma is commonly present.[146] Gross or microscopic foci of carcinoma may be present in other parts of the gland, and blood vessels may be invaded. In all cases, the diagnosis can be confirmed by positive immunostaining of tumor tissue for calcitonin and carcinoembryonic antigen (CEA).



• **Fig. 14.20** Overall survival of patients with ATC who did and did not undergo surgery, grouped by AJCC/TNM stage. (From Goffredo P, Thomas SM, Adam MA, et al. Impact of timeliness of resection and thyroidectomy margin status on survival for patients with anaplastic thyroid cancer: an analysis of 335 cases. *Ann Surg Oncol*. 2015;22:4166–4174.)



• **Fig. 14.21** Overall survival according to pathologic tumor-node-metastasis (pTNM) in the National Cancer Database Cohort. (Redrawn from Pontius LN, Oyekunle TO, Thomas SM, et al. Projecting survival in papillary thyroid cancer: a comparison of the seventh and eighth editions of the American Joint Commission on Cancer/Union for International Cancer Control staging systems in two contemporary national patient cohorts. *Thyroid*. 2017;27:1408–1416.)

Clinical Presentation of Medullary Thyroid Carcinoma

MTC first appears either as a hard nodule or mass in the thyroid gland or as an enlargement of the regional lymph nodes. Occasionally a metastatic lesion in a distant site is found first. The neck masses are frequently painful; they are sometimes bilateral and are often localized to the upper two-thirds of each lobe of the gland, which reflects the anatomic location of the parafollicular cells.

Differentiation of sporadic MTC from other types of thyroid nodules on clinical grounds alone may be difficult. FNAB has made it possible to diagnose MTC before surgery. In some

patients, however, cytologic findings may be misleading because the type of carcinoma is difficult to determine and HCC may occasionally be confused with MTC.[146,162] Positive immunocytochemical staining for calcitonin allows confirmation of the diagnosis. Basal plasma calcitonin levels are elevated in virtually all patients with clinical MTC, but it is still controversial whether it should be performed in all patients with thyroid nodules or only in those with suspicious or malignant cytologic findings.[81,82]

When the diagnosis of MTC is made from calcitonin measurements or FNAB, patients should be evaluated for hyperparathyroidism and for pheochromocytoma, unless a hereditary form of the disease has been excluded. If these diagnoses are satisfactorily excluded, total thyroidectomy with removal of regional nodes can safely be performed.[14,292–295]

The tumor occurs in both sporadic and hereditary forms, the latter making up about 20% to 30% of the total. The hereditary variety arises as part of MEN syndrome type 2A or 2B. A germline-activating *RET* point mutation is found in almost all hereditary cases, and *RET* proto-oncogene testing should be performed in all MTC patients. The finding of a germline *RET* mutation indicates a hereditary disease; the mutation should then be sought in all first-degree family members. The hereditary form is typically bilateral and is usually preceded by a premalignant C-cell hyperplasia. Total thyroidectomy at this premalignant stage can cure the disease in more than 95% of cases.[14,81,82,291–293]

As mentioned, there is a strong relationship in MTC between genotype and phenotype: most MEN2B are due to a codon 918 mutation in exon 16; the most frequent mutation found in patients with MEN2A is a codon 634 mutation in exon 11; the other mutations are located in exons 10, 13, 14, and 15 and are usually associated with less aggressive phenotypes. Somatic *RET* mutations are found in 40% of sporadic MTCs, with the codon 918 mutation being the most frequent and associated with a more aggressive course.[296] In up to two-thirds of tumors with no *RET* mutation, a *RAS* point mutation was found in most studies[297–299] but not in all.[300]

Cushing syndrome may occur at an advanced stage of the disease because of secretion of corticotropin by the tumor. Prostaglandins, serotonin, kinins, and vasoactive intestinal peptide may also be secreted and are variously responsible for flushing and for the attacks of watery diarrhea that about one-third of patients experience, usually at an advanced stage of the disease.[155,156,290,291] In MEN2A, hyperparathyroidism occurs late and is usually mild. Pheochromocytomas invariably occur later than MTC; they are often bilateral and may be clinically silent, and patients at risk should be screened with measurements of urinary metanephrine excretion. Familial MTC is a variant of MEN2A. MTC is transmitted as a single entity without any associated abnormality in the family and usually occurs later in life and is less aggressive than MTC occurring in the context of other subgroups of MEN2A; pheochromocytoma should be screened even if its risk is low because it cannot be totally excluded in any hereditary form. In MEN2B, MTC and pheochromocytomas are associated with multiple mucosal neuromas (bumpy lip syndrome), a marfanoid habitus, and typical facies, but such patients do not have hyperparathyroidism.[14,291]

In patients with a family history of thyroid cancer associated with hypertension or hyperparathyroidism, the MEN2A syndrome should be suspected. In patients with MEN2, surgery should be performed for pheochromocytomas before surgery for MTC is performed.

First-degree relatives of patients with MEN should undergo DNA testing for the presence of the mutant *RET* gene (see Chapter 42). Gene carriers should undergo a prophylactic total thyroidectomy at an age that depends on the mutation: within the first year of life for those with MEN2B and before age 5 years for those with the 634 *RET* mutation (the most frequent).[14,292,293] For carriers of other mutations, prophylactic total thyroidectomy may be delayed beyond age 5 years in the setting of a normal annual basal serum calcitonin, normal annual neck ultrasonography, less aggressive MTC family history, and family preference. Surgery is indicated if all of these features are not present and consists of a total thyroidectomy with lymph node dissection; lymph node dissection may be obviated when the thyroid nodule is smaller than 5 mm, when there is no lymph node abnormality on neck ultrasonography, and when the plasma calcitonin level is less than 40 pg/mL.[14]

Prognosis for Medullary Thyroid Carcinoma

Early series of MTC mainly described sporadic cases in which 80% of patients presented with TNM stage II or III. Patients with MEN2A are diagnosed earlier and have curable (stage I) disease.[291,292] Patients with MTC now have outcomes similar to or better than those of patients with nonpapillary FCTC (see Fig. 14.7). In a study of patients undergoing surgery for MTC in California, independent risk factors for disease-specific mortality were older age (HR, 1.36 per decade; 95% CI, 1.17–1.59), tumor size exceeding 2 cm (HR, 2.83; 95% CI, 1.08–7.44 for >2 to 4 cm and HR, 2.89; 95% CI, 1.09–7.71 for >4 cm), and the administration of external beam radiotherapy (HR, 2.14; 95% CI, 1.23–3.71). Disease stage was the strongest predictor of mortality from regional (HR, 4.77; 95% CI, 2.29–9.94) or metastatic (HR, 21.08; 95% CI, 9.90–44.89) disease.[288]

Primary Malignant Lymphoma of the Thyroid

Primary thyroid lymphomas are rare tumors, accounting for less than 2% of all extranodal lymphomas and less than 3% of all malignant thyroid tumors. The peak incidence is during the seventh decade of life and the male-to-female ratio is 1:2–8.[301]

Primary thyroid lymphoma almost always has a B-cell lineage.[301] The majority of thyroid malignant lymphomas are mucosa-associated lymphoid tissue (MALT) lymphomas that usually arise in a background of Hashimoto thyroiditis. These small cell lymphomas are characterized by a low grade of malignancy, a slow growth rate, and a tendency for recurrence at other MALT sites such as the gastrointestinal or respiratory tract, the thymus, or the salivary glands. At diagnosis, diffuse large cell lymphomas account for about 70% to 80% of tumors, and a substantial proportion of clinical cases arise from the transformation of low-grade MALT lymphoma to high-grade B-cell lymphoma. Other histologic findings are rare.

Clinical thyroid lymphomas almost invariably present as a rapidly enlarging, painless neck mass. One-third of the patients have compressive symptoms. The mass is often fixed to surrounding tissues, and half of the patients have unilateral or bilateral cervical lymph node enlargement. Clinically evident distant disease is uncommon. About 20% of patients already have a long-standing goiter, and hypothyroidism is reported in up to 40% of cases. The palpated mass is solid and hypoechoic on ultrasonography, which often depicts a characteristic asymmetric pseudocystic pattern. Most patients have serum antiperoxidase and anti-Tg antibodies.

The diagnosis of lymphoma can often be established by FNA cytologic and flow cytometric tests, particularly in diffuse large B-cell type. The need for core-needle or surgical biopsies has

decreased, but they can still be helpful for distinguishing thyroiditis from low-grade MALT lymphoma and for reliably excluding aggressive histologies because histology, immunostaining, and testing for gene rearrangements may be needed.

Accurate staging is critical for treatment planning. Staging includes a physical examination; complete blood count; serum lactate dehydrogenase; liver function tests; bone marrow biopsy; CT scan or MRI of the neck; CT scan of the thorax, abdomen, and pelvis; and appropriate biopsies at other sites where tumor is suspected. ^{18}FDG-PET scanning may be useful for the initial diagnosis and for monitoring responses to treatment. Involvement of the Waldeyer ring and the gastrointestinal tract has been associated with thyroid lymphomas, and therefore upper gastrointestinal tract radiographs or endoscopy should be performed.

Treatment is guided by the histologic subtype, the extent of the disease, and (in case of diffuse large B-cell lymphoma) the age-adjusted international prognostic index.[302,303] Surgical debulking of thyroid lymphomas is neither feasible nor necessary. Small tumors are often treated initially as primary thyroid carcinomas with surgery, and additional radiotherapy may be necessary in case of indolent lymphoma.

For high-grade B-cell lymphoma, chemotherapy combined with rituximab (chimeric human-mouse anti-CD20 monoclonal antibody) has become the standard treatment.[304] The chemotherapy prescribed should be an anthracycline-based regimen. It usually consists of four to six cycles of the CHOP regimen (cyclophosphamide 750 mg/m^2 on day 1, doxorubicin 50 mg/m^2 on day 1, vincristine 1.4 mg/m^2 on day 1, and prednisone 40 mg/m^2 per day on days 1–5) every 3 weeks. For localized aggressive lymphoma, the combination of chemotherapy and radiotherapy used before the era of rituximab reduced distant recurrence compared with radiotherapy alone, which should be used only for elderly patients who cannot receive medical treatment because a recurrence at distant sites will occur in one third of the patients and generally within the first year of treatment.

For MALT lymphomas, if disease is localized after accurate staging, total thyroidectomy (predicted overall survival rate and disease-free survival, 100% at 5 years) or involved field radiation therapy alone, 2 Gy per fraction for 5 days per week up to a total dose of 30 to 40 Gy (5-year overall survival rate, 90%) may be adequate.[305] For disseminated MALT lymphoma, chemotherapy alone with a single agent such as chlorambucil or combined with local radiation therapy can be proposed.

The Surgical Treatment of Thyroid Malignancy

Surgical resection of the primary tumor, gross disease that has extended beyond the thyroid capsule, and clinically significant lymph node metastases remains the mainstay of initial therapy in thyroid cancer.[1] Effective initial surgery minimizes the risk of disease recurrence, improves disease-specific survival, prevents complications associated with local growth of tumor into important structures in the neck, facilitates postoperative radioactive iodine therapy if indicated, and facilitates accurate staging and risk stratification.

Until recently, a one-size-fits-all approach to the management of thyroid cancer meant that the vast majority of thyroid cancer patients were subjected to high-intensity management approaches (total thyroidectomy with or without prophylactic central neck dissection, radioactive iodine therapy, prolonged TSH suppression, and highly sensitive follow-up evaluations).[23,306] However, the increasing incidence of very low-risk thyroid cancers (which usually display an indolent disease course) coupled with a much more selective use of radioactive iodine (RAI) therapy has resulted in a reevaluation of the need for immediate high-intensity treatment options in low-risk differentiated thyroid cancer patients.[307–310] The current thyroid cancer guidelines now endorse consideration of low-intensity treatment options (observation or thyroid lobectomy without the need for RAI therapy or prolonged TSH suppressive therapy) in properly selected patients.[1,311] Nonetheless, it is important to emphasize that some combination of high-intensity treatment options is still recommended for most patients with intermediate-risk or high-risk thyroid cancers. Because the evidence evaluating the comparative effectiveness of high-intensity versus low-intensity treatment options is largely retrospective, observational and devoid of high-quality prospective randomized clinical trials, the recommendations in these major guidelines are largely based on expert opinion in which the committees are trying to balance the risk and benefits of immediate therapy in low-risk thyroid cancer patients. Reevaluation of the intensity of thyroid cancer therapies is imperative because thyroid cancer mortality has remained stable or slightly increased over the last several decades[75] despite widespread use of highly sensitive disease detection tools (such as high-resolution neck ultrasonography, FNA of small thyroid nodules, and ultrasensitive thyroglobulin assays) and increasing use of aggressive therapies (such as prophylactic and therapeutic neck dissections for small volume disease and repeated administrations of RAI for biochemical evidence of persistent disease).

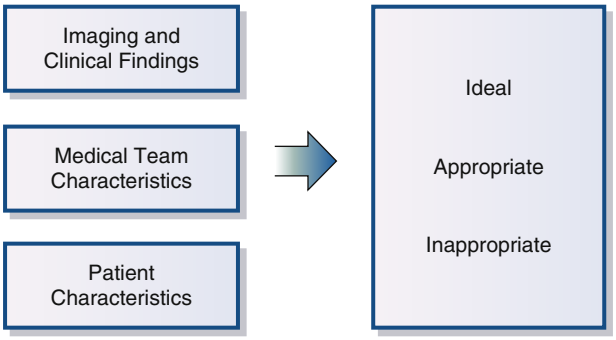
Active Surveillance as an Alternative to Immediate Surgery in Low-Risk Differentiated Thyroid Cancer

When thyroid nodule evaluations are done according to the recommendations of the American Thyroid Association, FNA cytology that is diagnostic for thyroid cancer will almost always lead to thyroid surgery.[1] However, an active surveillance management approach (also known as deferred surgical intervention) can be considered as an alternative to immediate surgery in (a) healthy patients with very low-risk intrathyroidal papillary thyroid cancers, (b) patients with comorbid conditions that would increase the risk of surgery or be associated with relatively short life expectancy, or (c) patients with more pressing concurrent medical or surgical issues that take precedence over treatment of the thyroid cancer.[1]

Building on the experience of our Japanese colleagues, several publications now support the effectiveness of an observational, active surveillance management approach to low-risk papillary thyroid cancers.[1,312–315] In these studies, serial neck ultrasound evaluations of the thyroid and cervical lymph nodes at intervals of 6 to 12 months are offered to properly selected patients as an alternative to immediate surgery. While the majority of patients demonstrate little if any disease progression during observation, salvage therapy at the time of disease progression is very effective when needed.[312]

Obviously the key to safe and effective implementation of an active surveillance management program is proper patient selection. A recent clinical framework describes how consideration of (a) imaging and clinical findings, (b) medical team characteristics, and (c) patient characteristics can be used to classify patients as ideal, appropriate, or inappropriate for low-risk treatment options

(active surveillance or thyroid lobectomy) (Fig. 14.22).[316,317] The definitions associated with the ideal, appropriate, and inappropriate patients with regard to the selection for active surveillance are given in Table 14.8. Although there are multiple patient characteristics that influence decision making, the construct that describes patients as medical maximalists or medical minimalists appears to be particularly relevant.[318] Given the choice, a medical maximalist often chooses high-intensity treatment options while a medical minimalist will often select low-intensity treatment options.[318]



• **Fig. 14.22** A clinical framework to guide proper patient selection for low-intensity therapy.

Patients classified as ideal or appropriate for active surveillance are offered observation as an alternative to immediate surgery. Patients choosing active surveillance are followed with neck ultrasound evaluations every 6 to 12 months for several years (then less frequently over time) unless disease progression occurs or the patient opts for surgery even in the absence of disease progression. Interestingly, it appears that only about 50% of patients eligible for active surveillance will choose an initial observational management program while the remainder elect to proceed with immediate surgery.[312] Patients classified as being inappropriate for active surveillance are recommended to have appropriate surgical resection.[316,317]

Selecting Total Thyroidectomy or Thyroid Lobectomy in Differentiated Thyroid Cancer: A Risk-Adapted Approach

There continues to be uniform agreement that total thyroidectomy is the preferred treatment option for patients with (a) differentiated thyroid cancers with primary tumors larger than 4 cm, (b) gross extrathyroidal extension, (c) clinically apparent disease

TABLE 14.8 Classification System to Aid in Proper Patient Selection for Active Surveillance

Patient Classification	Tumor/Imaging Characteristics	Patient Characteristics	Medical Team Characteristics
Ideal	• Solitary thyroid nodule confined to thyroid • ≤1 cm • Well-defined tumor margins by ultrasound • Surrounded by ≥2 mm normal thyroid parenchyma • Previous US documenting stability • cN0 • cM0	• Medical minimalist • Older patients (>60 yr) • Willing to accept active surveillance • Understands that future surgery may be necessary (deferred intervention) • Understands that lymph node metastases may be identified during follow-up • Compliant with follow-up plans • Supportive significant others (including family and other members of the health care team) • Life-threatening comorbidities or medical conditions requiring therapy	• Experienced team • Expeditious evaluation by multidisciplinary team • High-quality neck US • Prospective data collection • Tracking/reminder program to ensure proper follow-up
Appropriate	• Multifocal papillary microcarcinoma • 1–1.5 cm maximal dimension • Subcapsular location not adjacent to RLN without evidence of extrathyroidal extension • Ill-defined tumor margins • Background US findings that will make follow-up difficult (thyroiditis, reactive lymph nodes, multiple other benign-appearing nodules) • FDG-avid papillary microcarcinomas • Isolated *BRAF*^{V600E} mutation	• Minimalist/maximalist • Patients aged 18–59 yr • Strong family history of PTC • Childbearing potential	• Experienced endocrinologists or thyroid surgeon • Neck US routinely available
Inappropriate	• Aggressive cytologic features (rare) • Location adjacent to RLN/trachea • Evidence of extrathyroidal extension • N1 or M1 disease • High-risk molecular profile • Demonstrated increase of 3 mm diameter or 50% increase in tumor volume over a relatively short period	• Medical maximalist • Young patients (<18 yr) • Unlikely to be compliant with follow-up plans • Not willing to accept an observation approach • Severe anxiety regarding diagnosis	• Reliable neck US not available • Little experience with thyroid cancer management

FDG, Fluorodeoxyglucose; *PTC*, papillary thyroid carcinoma; *RLN*, recurrent laryngeal nerve; *US*, ultrasound.

of the cervical lymph nodes, or (d) known distant metastases.[1,311] Likewise, there is a general consensus that thyroid lobectomy is an adequate oncologic procedure for papillary thyroid cancers smaller than 1 cm without extrathyroidal extension or clinically apparent lymph node metastases.

Thus preoperative evaluations designed to carefully determine the size of the primary tumor, the presence of extrathyroidal extension, and/or metastatic disease assume primary importance in medical decision making. These evaluations include neck ultrasonography (evaluation of the thyroid gland, surrounding structures, and cervical lymph nodes) and voice evaluation in all patients being considered for surgery for differentiated thyroid cancer.[319] Additional functional and imaging studies are usually required for surgical planning and adequate initial staging in patients demonstrating bulky lymph node metastases, evidence of local invasion, or distant metastases. Endoscopic evaluation of the airway or gastrointestinal tract may be necessary to determine the extent of involvement in locally invasive disease.

Decision making in patients with intrathyroidal differentiated thyroid cancers with primary tumors larger than 1 cm and smaller than 4 cm without evidence of extrathyroidal extension or clinically apparent lymph node metastases has become complicated since both the National Comprehensive Cancer Network (NCCN) and the ATA guidelines allow for either thyroid lobectomy or total thyroidectomy in this setting.[1,311] Largely based on the assumption that nearly all of these patients would require RAI therapy and further strengthened by the Bilimoria report demonstrating 10-year survival of 98.4% for total thyroidectomy versus 97.1% for thyroid lobectomy (p <0.05),[320] the ATA guidelines had previously strongly recommended total thyroidectomy for all tumors over 1 cm.[23,306,311] However, multiple publications have failed to convincingly demonstrate a statistically significant survival benefit for total thyroidectomy versus lobectomy in differentiated thyroid cancers less than 4 cm when patients are properly selected and the statistical analysis controls for important confounding variables.[321–327] Decision making must also consider the risk of surgical complications and recognize that the risks of recurrent laryngeal nerve injury, transient and permanent hypoparathyroidism, and hemorrhage/hematoma are increased following total thyroidectomy compared with thyroid lobectomy.[328–330] However, total thyroidectomy is associated with a slightly lower risk of recurrence than a thyroid lobectomy.[327] Nonetheless, experienced centers report recurrence rates of less than 1% to 4% by combining high-quality preoperative ultrasound with appropriate clinical judgment.[325–327,331] These same studies show that salvage therapy is very effective in the few patients who have disease recurrence after low-intensity initial therapy.

Building on the same basic clinical framework described for active surveillance (see Fig. 14.22), patients can be classified as ideal, appropriate, or inappropriate for thyroid lobectomy (or isthmusectomy) (Table 14.9).[317] However, unlike active surveillance, patients selected for lobectomy must understand that information may become available either intraoperatively or postoperatively (primarily the final pathology report) that could result in a recommendation for complete removal of the thyroid gland. Thus the final classification of the patient as ideal, appropriate,

TABLE 14.9 Preoperative Classification System to Aid in Proper Patient Selection for Lobectomy/Isthmusectomy in Differentiated Thyroid Cancer

Patient Classification	Tumor/Imaging Characteristics	Patient Characteristics	Medical Team Characteristics
Ideal	• <1 cm • Intrathyroidal • Thyroid US otherwise normal • Clinical N0 neck	• Medical minimalist • Motivated patient • Willing to accept possibility of small volume disease in contralateral lobe • Desire to preserve normal thyroid function • Desire to minimize surgical complications • Open to intraoperative decision making • Willing to accept a low risk of needing an immediate completion thyroidectomy based on histology findings • TSH <2 mIU/L • Antithyroid antibodies undetectable • Anti-Tg antibodies undetectable	• Experienced MDT • Experienced US • Shared treatment philosophy • Uses RAI very selectively for ablation/adjuvant therapy and follow-up • Frozen section available
Appropriate	• 1–4 cm • Benign-appearing changes on US (thyroiditis, benign nodules) • Clinical N0 neck	• Minimalist/maximalist • Desire to keep normal thyroid (or avoidance of surgical complications) outweighs concern for disease in the contralateral lobe or the desire for RAI • TSH >2 • Antithyroid antibodies present • Anti-Tg antibodies	• Surgeon and endocrinologist agree on postoperative management plan • Unlikely to include need for RAI • Comfortable that follow-up US is adequate for low-risk patient
Inappropriate	• Extrathyroidal extension • Clinical N1 metastases • Distant metastases • High-risk molecular profile	• Medical maximalist • Patient desires total thyroidectomy and/or RAI • Clinical indications for RAI for ablation/adjuvant therapy/staging	• Treatment team desires RAI for ablation/adjuvant therapy/staging/follow-up

MDT, Multidisciplinary team; *RAI,* radioactive iodine; *TSH,* thyroid-stimulating hormone; *US,* ultrasound.

TABLE 14.10 Postoperative Histologic Confirmation of Proper Patient Selection Following Lobectomy/Isthmusectomy in Differentiated Thyroid Cancer

Ideal	• Intrathyroidal unifocal or multifocal papillary microcarcinoma with or without *BRAF*^{V600E} mutation • Intrathyroidal FVPTC with capsular invasion only (no vascular invasion) • NIFTP • Intrathyroidal well-differentiated FTC (invasion of tumor capsule without vascular invasion) • Clinical N0 and pathology N0/Nx neck • Small differentiated thyroid cancers confined to the isthmus
Appropriate	• 1–4 cm intrathyroidal PTC • Minor extrathyroidal extension • Clinical N0 but pathology N1 micrometastases (includes pN1a/b disease with ≤5 microscopic lymph node metastases, all of which are <0.5 cm in maximum diameter) • FVPTC, FTC, or PTC with minor vascular invasion (<4 microscopic foci of vascular invasion) • 1–2 cm, intrathyroidal tumors with potentially aggressive histology (e.g., tall cell, hobnail variant, columnar cell carcinoma)
Inappropriate	• Extensive vascular invasion (FTC or HCC with ≥4 microscopic foci of vascular invasion) • Larger potentially aggressive variants (e.g., poorly differentiated thyroid cancer, tall cell, hobnail variant, diffuse sclerosing, or columnar cell carcinoma with primary tumor >2 cm) • Clinical N1 or pathology N1 disease (includes N1a/b disease involving >5 lymph node metastases or any lymph node metastasis >0.5 cm in maximum diameter) • Gross extrathyroidal extension

FTC, Follicular thyroid cancer; *FVPTC,* follicular variant of papillary thyroid carcinoma; *HCC,* Hürthle cell cancer; *NIFTP,* noninvasive follicular thyroid neoplasm with papillary-like nuclear features; *PTC,* papillary thyroid carcinoma.

or inappropriate requires consideration of additional factors that can only be known postoperatively (Table 14.10). In centers that practice a very risk-adapted management approach and a selective approach to RAI therapy, immediate completion thyroidectomy after lobectomy is only required in 5% to 6% of properly selected patients.[325–327,331] However, other centers report that the findings on the lobectomy final histology report result in recommendations for immediate completion thyroidectomy in up to 20% of the patients selected for thyroid lobectomy.[332–334]

Determining the Extent of Initial Cervical Lymph Node Dissection in Differentiated Thyroid Cancer

Consistent with the goal of primary therapy to remove all visible gross disease, compartment-oriented resection of clinically apparent metastatic cervical lymphadenopathy is routinely recommended.[1,311] This would include removal of all visible metastatic lymph nodes in the central neck (level VI/VII) and/or lateral neck (levels II, III, IV, and V). Rather than "berry picking" abnormal lymph nodes, a compartment-oriented neck dissection is designed to clear all metastatic lymph nodes by systematically removing the fibroadipose tissue in the compartment (which often contains additional subclinical metastatic lymph nodes) with the clinically apparent lymph node metastases. This compartment-oriented approach to the removal of clinically apparent lymph node metastases is associated with decreased rates of recurrent/persistent disease.

Since very small-volume, subclinical lymph node micrometastases are present in up to 70% to 80% of patients with papillary microcarcinomas, it is not surprising that meticulous dissection of central neck lymph nodes would often yield metastatic disease.[335] However, preoperative neck ultrasonography identifies suspicious cervical lymphadenopathy (clinical N1a or N1b disease) in only 20% to 30% of cases.[336–341] It remains controversial whether routine removal of subclinical central neck lymph nodes (prophylactic dissection) has a clinically significant impact on the risk of recurrence or disease-specific survival in papillary thyroid cancer.[342] Prophylactic neck dissections are not routinely recommended for follicular thyroid cancer because they are associated with a much lower rate of subclinical lymph node metastases. While prophylactic neck dissection can be done safely with a low risk of complications in experienced hands, the increased risk of hypoparathyroidism and recurrent laryngeal nerve injury may outweigh the potential benefit when performed by less experienced surgeons.[342]

Surgical Approach to Medullary Thyroid Cancer

Medullary thyroid cancer is usually treated with total thyroidectomy and surgical resection of clinically apparent central or lateral neck lymph nodes in a standard compartment-oriented fashion.[14,311] Depending on the results of preoperative calcitonin and neck ultrasound, prophylactic central or lateral neck dissections may also be recommended.

Surgical Approach to Anaplastic Thyroid Cancer

Ideally, patients with anaplastic carcinoma should be treated with total thyroidectomy and therapeutic lymph node dissection, but these tumors usually present with locally invasive disease, which is seldom amenable to effective surgical resection.[343] In the setting of unresectable disease, patients desiring high-intensity therapies are usually treated with a combination of systemic therapy and external beam irradiation.

Postoperative Management of Thyroid Malignancy

In view of disease-specific uncertainties and the unique needs of individual patients, the postoperative treatment of thyroid carcinoma does not occur with a rigid algorithm.[1] Indeed, physicians must consider the extent of disease at surgery, the histopathology and differentiation of the tumor, the age of the patient and his or her risk group category for tumor-related death and recurrence, and the results of postoperative serum Tg determination with neck ultrasonography.

^{131}I Administration

^{131}I is an effective agent for delivering high radiation doses to the thyroid tissue with low spillover to other portions of the body.

The radiation dose to the thyroid tissue is related to the tissue concentration (the ratio between the total tissue uptake and the volume of functional tissue) and the effective half-life of ^{131}I in the tissue.[93,344] Thyroid tissue is able to concentrate iodine only after TSH stimulation, but even after optimal TSH stimulation, iodine uptake in neoplastic tissue is always lower than in normal thyroid tissue and may not be detectable in about one-third of cases.[93]

^{131}I therapy is given postoperatively for three reasons.[1] First, it destroys normal thyroid remnants *(ablation),* thereby increasing the sensitivity and the specificity of measurements of serum Tg for the detection of persistent or recurrent disease. Ablation is mandatory for interpreting TSH-stimulated Tg determination because Tg may be produced by normal thyroid remnants and by neoplastic foci. During levothyroxine treatment, the production of Tg by normal thyroid cells may be low or suppressed, and serum Tg can be used for the follow-up of these patients.[345] Second, ^{131}I therapy may destroy occult or known microscopic carcinoma, thereby potentially decreasing the long-term recurrence rate. Third, ^{131}I therapy makes it possible to perform a postablative ^{131}I total-body scan (TBS), which is a sensitive tool for detecting persistent carcinoma.

It cannot be emphasized enough that postoperative ^{131}I therapy should be used selectively and that not all patients with a diagnosis of FCTC benefit from routine postoperative radioiodine ablative therapy.[1,22,23,224,346] The classification of the risk of recurrence (low, intermediate, or high) from the 2009 ATA guidelines was redefined in the 2015 guidelines, taking into account (among other criteria) the extent of lymph node involvement and pathologic features.[1,23] In low-risk thyroid cancer, the long-term prognosis after surgery alone is so favorable that ^{131}I ablation is not routinely recommended.[1,22,23,347,348] This is widely applied to patients with microcarcinoma, but unfortunately routine treatment is still given routinely to low-risk patients in some countries. In high-risk patients with a risk of recurrence above 40%, postoperative radioactive iodine administration (see Table 14.7) is routinely performed because such therapy can potentially decrease both recurrence and death rates. Also, radioiodine is administered postoperatively when surgery has not been complete or its success is doubtful. Young children have traditionally been considered as candidates for postoperative radioiodine therapy because they may have extensive neck lymph node involvement and frequently harbor pulmonary metastases that may not be detectable even with CT imaging of the chest.[152,171,215] Finally, in the other patients (intermediate risk of recurrence, and some low risk), there is currently no evidence that radioiodine remnant ablation (RRA) may improve the long-term disease-specific mortality, and prospective randomized trials are needed to validate its current indications.

When the risk of persistent disease is low and postoperative serum Tg is undetectable, ^{131}I administration may not be justified. This is especially true in patients with a low risk of recurrence.[349–353] This recommendation is particularly relevant to patients with N0 disease.[349] On the contrary, the likelihood of identifying ^{131}I avid metastatic disease on the post-therapy ^{131}I TBS increases when postoperative Tg values are greater than 5 to 10 ng/mL, suggesting that ^{131}I should be administered to such patients. Postoperative neck ultrasonography may also provide reassuring data or show abnormalities that should lead to ^{131}I administration.[354]

When considering ^{131}I therapy, levothyroxine treatment is most often administered following surgery, and both the indication and the protocol of stimulation and the ^{131}I activity to be administered are decided according to the ATA risk stratification. Data from postoperative serum Tg determination and neck ultrasonography may also be taken into account because the risk of persistent/recurrent disease in case of undetectable Tg levels (<0.2 ng/mL under thyroid hormones or <1 ng/mL after TSH stimulation) in the absence of Tg antibodies with normal neck ultrasonography is very low (<3%).

To administer ^{131}I therapy, levothyroxine treatment is typically withheld for 3 to 4 weeks. Another approach allows liothyronine to be substituted for 3 to 4 weeks and then discontinued for 1 to 2 weeks before radioiodine studies. At the time of treatment, the serum TSH level should be greater than an empirically determined level of 25 to 30 mU/L. Intramuscular injections of rhTSH (0.9 mg for 2 consecutive days, with ^{131}I administered 1 day after the second injection) given while on levothyroxine treatment may achieve an equally effective stimulation of radioiodine uptake by normal thyroid remnant, with ablation rates similar to those obtained with withdrawal using either a high (100 mCi) or a low (30 mCi) activity.[352,355,356] The use of rhTSH prevents hypothyroidism (as the patient remains on levothyroxine) and induces lower radiation exposure to the body, permitting an earlier discharge from the hospital.[352,355–359] However, in the United States, most ^{131}I therapy is now administered in the outpatient setting, making this latter issue less of a concern. Similar outcomes were reported in intermediate-risk and high-risk patients prepared for ^{131}I therapy using rhTSH versus thyroid hormone withdrawal.[360–362] In addition, short-term recurrence rates have been found to be similar in patients prepared with thyroid hormone withdrawal or rhTSH, even in those with initial lymph node involvement.[363,364] A retrospective study reported a similar outcome at 10 years after ablation with a low activity in patients prepared with either withdrawal or rhTSH.[365] rhTSH is approved for radioactive iodine administration with 100 mCi (or more) or 30 mCi in the United States, Europe, and many other countries around the world. The cost of rhTSH, however, must be factored into care, as thyroid hormone withdrawal is much less costly.

In a patient who has undergone an incomplete thyroidectomy, neck uptake may be measured with a tracer activity of ^{123}I (or rarely, ^{131}I). The activity used should be small enough to avoid stunning (i.e., a decrease of thyroid uptake with the subsequent high activity of therapeutic radioiodine).[366,367] High uptake (>10%) and high risk of persistent disease should lead to completion surgery. Following administration, a total body scan is performed and is highly informative in patients with low uptake (<1%) in the thyroid bed. However, additional metastatic foci have been reported in 10% to 26% of patients scanned following high-dose radioiodine treatment compared with the diagnostic scan.[368] ^{131}I SPECT/CT fusion imaging may provide superior lesion localization.[24–27]

After radioiodine therapy, levothyroxine therapy is then resumed and maintained. Total ablation (defined as no visible uptake) was previously verified by ^{131}I TBS 6 to 12 months following initial therapy, typically with 2 to 5 mCi (74–185 MBq). However, a follow-up ^{131}I TBS is no longer routinely performed when postablation scans have been informative because such repeat scanning does not afford any further information.[369,370] Moreover, total ablation is now currently defined by an undetectable serum Tg level following rhTSH stimulation in the absence of anti-Tg antibody (or a serum Tg level <0.2–0.3 ng/mL on levothyroxine treatment when using a sensitive assay) and unremarkable neck ultrasonography.[1]

Total ablation (eradication of normal thyroid remnants) is achieved after administration of either 100 mCi (3700 MBq) or 30 mCi (1100 MBq) in more than 80% of patients who had at least a near-total thyroidectomy. This is similar whether preparation is with withdrawal or rhTSH.[352,355,356,371,372] After less extensive surgery, ablation is achieved in only two thirds of patients with 30 mCi (1100 MBq). Therefore a total thyroidectomy should be performed in all patients who are to be treated with ^{131}I. Also, in low-risk or intermediate-risk patients, levothyroxine treatment is initiated soon after surgery and 30 mCi (1100 MBq) is administered following injections of rhTSH, with the aim of irradiating normal thyroid remnants. In high-risk patients, higher activity (≥100 mCi) is administered with the aims of both ablating normal thyroid remnants and irradiating residual neoplastic tissue. In patients with known distant metastases, ^{131}I is administered following thyroid hormone withdrawal, but in low-risk or intermediate-risk patients, rhTSH injections represent a valid alternative. High activities should be administered with caution to elderly patients.[373]

^{131}I ablation therapy has no role in the management of patients with anaplastic thyroid cancer, MTC, or thyroid lymphoma.

External Radiotherapy

External radiotherapy to the neck and mediastinum is indicated only for older patients (>55 years) with extensive and aggressive pathology subtypes in whom complete surgical excision is impossible, invasion of critical neck structures is likely, and tumor tissue does not take up ^{131}I. Retrospective studies have shown that in these selected patients, external radiotherapy decreases the risk of neck recurrence.[374,375] The target volume encompasses the thyroid bed, bilateral neck lymph node areas, and the upper part of the mediastinum. Typically, 50 Gy (5000 rad) is delivered in 25 fractions over 5 weeks, with a boost of 5 to 10 Gy on any residual macroscopic focus. The current approach with intensity-modulated radiation therapy permits the delivery of 63 to 66 Gy to gross disease and high-risk areas and 54 to 56 Gy in 30 to 33 fractions to cervical and mediastinal nodal regions. Its use may decrease late morbidity.

In patients with anaplastic thyroid carcinoma, when the extent of disease is limited and surgery is feasible, accelerated external radiotherapy in combination with chemotherapy permits local control of the disease in two-thirds of the patients and long-term survival in about 20%.[281,376,377]

TSH Suppressive Therapy

The growth of thyroid tumor cells is controlled by TSH, and inhibition of TSH secretion with levothyroxine is thought to improve the recurrence and survival rates.[22,23,137] Therefore levothyroxine should be given to all patients with FCTC, whatever the extent of thyroid surgery and other treatment. The initial effective dose is about 1.6 to 2 μg/kg body weight in adults, though children require a higher dose and elderly patients a lower dose. The adequacy of therapy is monitored by measuring serum TSH approximately 3 months after initiation. The initial goal is a serum TSH concentration below about 0.1 mU/L for high-risk thyroid cancer patients and maintenance of the TSH at or slightly below the lower limit of normal (0.1–1.5 mU/L) for low-risk patients. Similar recommendations apply to low-risk patients who have not undergone remnant ablation (i.e., serum TSH 0.1–1.5 mU/L). All TSH targets must also weigh the individual risks associated with TSH suppressive therapy. The dose of levothyroxine is then modified according to the ATA initial response to therapy. In patients with no evidence of disease, the dose of levothyroxine is decreased to maintain the serum TSH level within the normal range. Thus the dosing of levothyroxine (and target TSH concentration) must be continually reevaluated based on disease risk and potential side effects of long-term TSH suppression.

In patients with MTC or thyroid lymphoma, a replacement dose of levothyroxine is given with the aim of obtaining a serum TSH level in the normal range.

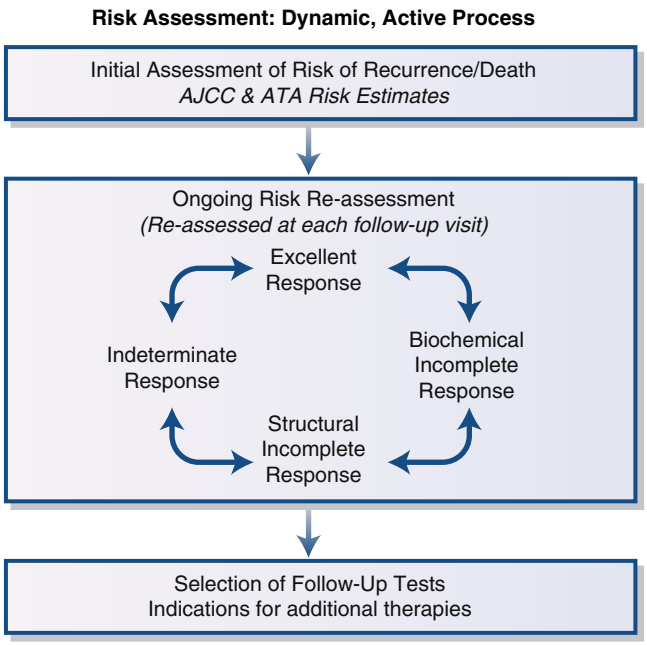
TABLE 14.11 Response to Therapy Definitions

Excellent response	No clinical, biochemical, or structural evidence of disease
Biochemical incomplete response	Persistent abnormal thyroglobulin values or rising antithyroglobulin antibody levels in the absence of localizable disease
Structural incomplete response	Persistent or newly identified locoregional or distant metastases
Indeterminate response	Nonspecific biochemical or structural findings that cannot be confidently classified as either benign or malignant

Regardless of the extent of initial therapy, this provides the general description of each of the responses to therapy categories that can be used to describe the clinical status of a patient with either differentiated or medullary thyroid cancer at any point during follow-up. See text for precise definitions of *excellent, indeterminate,* and *biochemical incomplete responses,* which will vary by tumor type and extent of initial therapy.

Long-Term Follow-Up of Patients With Thyroid Cancer Following Initial Therapy

After completion of initial therapy, the risk-adapted approach to the management of thyroid cancer continues by tailoring the types, extent, and timing of follow-up evaluations to the predicted risks of recurrence and disease-specific survival.[307] Initial treatment and early follow-up recommendations are based on the initial static risk assessments provided by AJCC staging (stage I, II, III, or IV) and ATA risk stratification (low, intermediate, or high).[1,217] These initial risk estimates are then continually modified over time as new data become available so that the management plan can be appropriately altered to become either more or less intensive, depending on the biologic course of the disease and the response to therapy.[1] Over the last several years, a nomenclature has been developed to describe the response to therapy and clinical status of patients during follow-up (Table 14.11).[1,378,379] Unlike the AJCC stage and the ATA risk category, which do not change over the lifetime of the patient, the response to therapy definitions may change during follow-up as new data become available (Fig. 14.23). This approach allows for characterization of the clinical status of each patient based on initial AJCC stage, ATA risk, and response to therapy at each follow-up visit. This information can then be used to guide management recommendations both with regard to the selection of follow-up test and also possible indications for additional therapies or ongoing observational management.



• **Fig. 14.23** Risk-adapted approach to follow-up. Management plans for the first year of follow-up are based on initial assessment of the risk of recurrence and death provided by American Joint Commission on Cancer (AJCC) and American Thyroid Association (ATA) risk staging. These plans are continually modified during follow-up as new data become available, with ongoing risk stratification defined by response to therapy assessments (*excellent, biochemical incomplete, structural incomplete,* or *indeterminate response*). Unlike the AJCC and ATA risk staging, the response to therapy status may change over time, depending on disease behavior and response to therapy.

TABLE 14.12 Overview of Plans for First Year of Follow-Up Following Initial Therapy

Initial Plan Based on ATA Risk for the First Year of Follow-Up	ATA Low Risk	ATA Intermediate Risk	ATA High Risk
Tg, TgAb, TFTs, every 3–6 months	√	√	√
Neck US in 3–6 months	–	√	√
Neck/chest CT with contrast in 6–12 months	–	Consider[a]	√[b]
Cross-sectional imaging of other sites (brain, abdomen, pelvis)	–	–	Consider[c]
Routine surveillance diagnostic RAI scan	–	–	Consider
^{18}FDG-PET scan	–	–	Consider
Dynamic risk assessment at each visit	√	√	√

While most patients will return for physical examination and biochemical testing every 3–6 months for the first year, consideration for additional testing is based on ATA risk and on the dynamic risk assessment done at each follow-up visit.

[a]Considered for intermediate-risk patient status postresection clinical N1a or N1b disease.

[b]Depending on presenting features, CT of the neck/chest may need to be done as early as 2–3 months after initial therapy.

[c]Depending on presenting features, functional imaging results, and serum Tg levels.

ATA, American Thyroid Association; *CT,* computed tomography; ^{18}*FDG-PET,* fluorodeoxyglucose positron emission tomography; *RAI,* radioactive iodine; *Tg,* thyroglobulin; *TgAb,* antithyroglobulin antibodies; *TFTs,* thyroid function tests; *US,* ultrasound;

Initial Follow-Up Recommendations for Differentiated Thyroid Cancer (First Year After Initial Therapy)

Regardless of ATA risk, most patients are seen at intervals of 3 to 6 months with biochemical testing during the first year of follow-up (Table 14.12). ATA low-risk patients are expected to do exceptionally well with very low recurrence rates that probably would not become apparent for at least 3 to 5 years.[167,219] Thus the primary follow-up tools for these patients will be physical examination, serum thyroglobulin, thyroglobulin antibody, and thyroid function test.[1] In these low-risk patients, the primary goal of follow-up is to confirm the absence of disease in early follow-up so that they can rapidly be transitioned to less intensive management approaches. They usually return for follow-up visits every 6 to 12 months for the first year then less frequently after that if they demonstrate the expected excellent response to therapy. Neck ultrasonography is usually done about 1 year after surgery, although the utility of this test is unproven and has the potential to identify more false-positive findings than real disease given the high likelihood of nonspecific findings on surveillance neck ultrasonography coupled with a very low recurrence risk.[380,381] Diagnostic whole-body scans, additional cross-sectional imaging, or FDG-PET scans are not routinely used for follow-up surveillance. Stimulated thyroglobulin values are only done if the available thyroglobulin assay does not reliably read down to less than 0.2 ng/mL.

ATA intermediate-risk patients are initially followed at 6-month intervals for the first year with the same biochemical testing as the low-risk patients but with more intensive ultrasound follow-up, given their higher risk of disease.[1] Intermediate-risk patients usually have follow-up ultrasound in 6 months to establish the baseline and verify the completeness of initial resection. Additional ultrasounds during the first year may be required if this initial evaluation identifies worrisome nonspecific findings or persistent disease. In patients who had extensive bulky metastatic lymphadenopathy, a CT scan of the neck and chest with contrast is usually considered at 6 to 12 months to definitively establish the presence or absence of clinically significant persistent disease. Radioactive iodine scans are not routinely obtained for surveillance in these patients but can be important tools to characterize the functional status of suspicious lesions identified during follow-up and can be considered if thyroglobulin antibodies are rising during follow-up. Additional testing is not routinely planned but may be required, depending on the response to therapy, as described later in the chapter.

ATA high-risk patients require a much more individualized management approach.[1] While still utilizing the same biochemical evaluations as the ATA low-risk and intermediate-risk patients, ATA high-risk patients may also require a wide variety of cross-sectional and imaging studies on the basis of either a structural incomplete response to initial therapy or their high risk of local recurrence and distant metastases. In general, high-risk patients are evaluated every 2 to 3 months with appropriate cross-sectional and functional imaging. Because of the aggressive nature of their disease, their response to therapy can often be characterized within the first 6 to 12 months, leading to early alterations in the initial management plans.

Modifying Initial Follow-Up Recommendations for Differentiated Thyroid Cancer Using Response to Therapy Definitions

While originally conceived to be used as a restaging system to be utilized after 2 years of follow-up, the dynamic risk assessment approach has evolved such that the response to therapy definitions can be used at any point during follow-up to describe the clinical status of a patient.[378,382,383] Thus it is appropriate even within the first year of follow-up to describe the clinical status of a patient using the response to therapy nomenclature (*excellent response, biochemical incomplete response, structural incomplete response, indeterminate*) (see Table 14.11). Even at these early time points, the response to therapy can be used to alter the initial management plans. This is most apparent in patients who have ATA intermediate-risk disease who manifest an excellent response within the first year of initial therapy and can therefore have their management intensity decreased. Or alternatively, ATA low-risk or intermediate-risk patients demonstrating a biochemical or structural incomplete response may require more aggressive follow-up, disease detection studies, or therapy.

In oncologic terms, *excellent response* can be thought of as "remission" as it describes patients who have no biochemical, clinical, or structural evidence of disease (see Table 14.11). Patients with abnormal thyroglobulin values or rising antithyroglobulin antibodies in the absence of localizable disease are described as having a biochemical incomplete response. *Structural incomplete response* is used to describe patients who have persistent or recurrent imaging evidence of disease. The *indeterminate response* category describes patients who have nonspecific either low-level thyroglobulin values or nonspecific findings on imaging that cannot be confidently classified as either benign or malignant. Over time, most patients can be moved out of the indeterminate response category as follow-up studies help to define whether the nonspecific findings are actually benign or malignant.

The precise definitions for each of the response to therapy categories and the management implications are given in Table 14.13.[1] An excellent response to therapy should lead to an early decrease in the intensity and frequency of follow-up and the degree of TSH suppression as these patients do exceptionally well with essentially no mortality and very low recurrence rates. Interestingly, at least 30% of the biochemical incomplete response patients will eventually evolve to having no evidence of disease with observation alone. Unfortunately, patients with a structural incomplete response usually maintained persistent disease (either biochemical or structural incomplete response) despite additional therapies. Fortunately, the majority of patients classified as having an indeterminate response remain disease-free with only 15% to 20% of these patients eventually being classified as having either biochemical or structural incomplete response.

TABLE 14.13 Clinical Implications of Response to Therapy Reclassification in Differentiated Thyroid Cancer Patients Treated With Total Thyroidectomy and RAI Remnant Ablation

Category	Definitions	Clinical Outcomes	Management Implications
Excellent Response	Negative imaging *and either* suppressed Tg <0.2 ng/mL[a] *or* TSH-stimulated Tg <1 ng/mL[a]	1–4% recurrence <1% disease-specific death	An excellent response to therapy should lead to an early decrease in the intensity and frequency of follow-up and the degree of TSH suppression
Biochemical Incomplete Response	Negative imaging *and* suppressed Tg >1 ng/mL[a] *or* stimulated Tg >10 ng/mL[a] *or* rising anti-TgAb levels	At least 30% spontaneously evolve to NED; 20% achieve NED after additional therapy; 20% develop structural disease; <1% disease-specific death	If associated with stable or declining serum Tg values, a biochemical incomplete response should lead to continued observation with ongoing TSH suppression in most patients Rising Tg or Tg antibody values should prompt additional investigations and potentially additional therapies
Structural Incomplete Response	Structural or functional evidence of disease with any Tg level +/- TgAb	50–85% continue to have persistent disease despite additional therapy Disease-specific death rates as high as 11% with locoregional metastases and 50% with structural distant metastases	A structural incomplete response may lead to additional treatments or ongoing observation, depending on multiple clinicopathologic factors, including the size, location, rate of growth, RAI avidity, FDG avidity, and specific pathology of the structural lesions
Indeterminate Response	Nonspecific findings on imaging studies Faint uptake in thyroid bed on RAI scanning Nonstimulated Tg detectable, but <1 ng/mL Stimulated Tg detectable, but <10 ng/mL *or* TgAb stable or declining in the absence of structural or functional disease	15–20% will have structural disease identified during follow-up In the remainder, the nonspecific changes are either stable or resolve <1% disease-specific death	An indeterminate response should lead to continued observation with appropriate serial imaging of the nonspecific lesions and serum Tg monitoring Nonspecific findings that become suspicious over time can be further evaluated with additional imaging or biopsy

[a]In the absence of anti-TgAb.

NED denotes a patient as having no evidence of disease at final follow-up.

FDG, Fluorodeoxyglucose; *NED,* no evidence of disease; *RAI,* radioactive iodine; *Tg,* thyroglobulin; *TgAb,* antithyroglobulin antibodies; *TSH,* thyroid-stimulating hormone.

From Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid.* 2016;26:1–133.

In the past, stimulated thyroglobulin values were a key component of follow-up testing. However, with the development of new ultrasensitive assays, the definition of excellent response to therapy can be established with the suppressed thyroglobulin value of less than 0.2 ng/mL.[1] However, if reliable ultrasensitive thyroglobulin values are not available, then stimulated thyroglobulin values will be needed to establish an excellent response to therapy.

Because the response to therapy definitions rely heavily on serum thyroglobulin measurements, patients with antithyroglobulin antibodies present a challenge. In these cases, the serum thyroglobulin measurement may or may not be reliable and therefore cannot be trusted. Antithyroglobulin antibodies that decline by at least 50% over the first 6 months usually indicate the absence of persistent disease.[384] Conversely, rising thyroglobulin values over time are often an early indicator of subsequent structural incomplete response and would lead to additional structural or functional imaging.

Additionally, the response to therapy definitions were originally developed for patients who had total thyroidectomy and radioactive iodine ablation. Therefore the definitions need to be modified slightly to be able to accommodate patients who were treated with lobectomy alone or total thyroidectomy without radioactive iodine.[385,386] In patients who underwent total thyroidectomy without radioactive iodine, an excellent response to therapy requires a nonstimulated thyroglobulin less than 0.2 ng/mL, an indeterminate response is defined as a serum thyroglobulin of 0.2 to 5 ng/mL, and a biochemical incomplete response requires a serum thyroglobulin greater than 5 ng/mL. In patients treated with lobectomy, any thyroglobulin value less than 30 ng/mL is classified as excellent, whereas a value greater than 30 ng/mL is classified as biochemical incomplete.

Follow-Up Recommendations Based on Response to Therapy Status

ATA low-risk and intermediate-risk patients who demonstrate an excellent response to therapy can transition to low-intensity follow-up. In the long term, patients with an excellent response to therapy are followed primarily with serum thyroglobulin, thyroglobulin antibody, and physical examination every 1 to 2 years. Routine use of neck ultrasonography in the follow-up of these excellent response to therapy patients is likely to yield more false-positive findings than true structural disease and therefore is not routinely recommended.[380,381] While an excellent response to therapy almost always leads to less aggressive follow-up, it is important to note that ATA high-risk patients can still have a risk of recurrence in the 5% to 10% range, despite having been classified as an excellent response to therapy, because they often manifest distant sites of disease that are not apparent on early imaging or they are poor thyroglobulin producers, leading to them being inappropriately classified as excellent response to therapy.[378,382] As a result, we consider cross-sectional imaging in ATA high-risk patients every 3 to 5 years, depending on the specifics of individual cases.

The evaluation of the patient with biochemical incomplete response begins first with an assessment of whether appropriate imaging studies have been done. The level of serum thyroglobulin roughly correlates with the location of disease, with nonstimulated Tg values less than 10 ng/mL usually identifying persistent/recurrent disease in the neck, values in the 10 to 100 range suggesting pulmonary metastasis, and values in the thousands suggesting bone metastases.[387] In addition to meticulous cross-sectional imaging, functional imaging with radioactive iodine or ^{18}FDG-PET may identify the source of the abnormal thyroglobulin. Once appropriate imaging has been done to definitively rule out structurally identifiable disease, then the follow-up of patients with biochemical incomplete response depends on the trend over time in their thyroglobulin and thyroglobulin antibodies. Patients with stable or declining thyroglobulin and thyroglobulin antibodies are usually followed with observation. Patients with rising thyroglobulin or thyroglobulin antibodies are followed with additional cross-sectional imaging with the type, intensity, and frequency of testing dependent on the rate of rise of the biochemical markers. Studies have now verified correlation between thyroglobulin doubling time and clinical outcomes.[388]

Patients with a structural incomplete response are particularly problematic as the majority will maintain persistent biochemical or structural evidence of disease despite additional therapies.[1] Possible treatment options for structural incomplete response include observation, surgical intervention, radioactive iodine therapy, external beam radiation therapy, focal therapies, and systemic therapies. Decision making in this setting is best done in a multidisciplinary team setting. Indications for intervention in patients with a structural incomplete response should be guided by factors such as the size, location, rate of growth, radioactive iodine avidity, FDG avidity, and underlying histology of the metastatic disease.

Patients with an indeterminate response are usually followed with additional observation with serial evaluations of the indeterminate biochemical or structural area of interest.[1] In many cases, the nonspecific biochemical markers or structural findings resolve spontaneously, in which case the patients can be reclassified as having an excellent response to therapy. In other cases, the indeterminate findings become more clearly abnormal and the patient is then reclassified as biochemical or structural incomplete.

While these response to therapy recommendations have been primarily based on patients who underwent total thyroidectomy and radioactive iodine ablation, follow-up recommendations are essentially the same in patients treated either with lobectomy or with total thyroidectomy without radioactive iodine.[385,386] By definition, these patients are low risk and thus are not expected to have early recurrences or distant metastasis. While the significance of a single serum thyroglobulin determination is uncertain in the majority of these patients, the trend and thyroglobulin over time can be informative despite being less sensitive and more nonspecific. Because of the lack of sensitivity in the thyroglobulin measurements or persistent thyroglobulin antibodies, follow-up in these patients relies more heavily on neck ultrasonography done every 3 to 5 years at least for the first decade.

Risk-Adapted Follow-Up Recommendations for Medullary Thyroid Cancer

Recommendations regarding the extent, intensity, and timing of follow-up in medullary thyroid cancer will largely be dependent on postoperative calcitonin and CEA values. As with thyroglobulin values, the calcitonin and CEA doubling-time values provide important clinical insights into both the rate of disease progression and disease-specific survival.[14] Similar to the approach used in differentiated thyroid cancer, ongoing follow-up recommendations are based on response to therapy reevaluations in which the definitions of excellent, biochemical incomplete, and structural

incomplete response are modified using calcitonin and CEA values rather than thyroglobulin values.[389–391] For medullary cancer, an excellent response is defined as an undetectable serum calcitonin and normal range CEA value. Biochemical incomplete response is defined as a measurable postsurgical serum calcitonin or elevated CEA value. Just as with differentiated thyroid cancer, the follow-up plans are adjusted based on response to therapy.

Treatment of Thyroid Cancer Recurrence and Distant Disease

Locoregional Recurrences of PTC and FTC

Locoregional recurrences occur in 5% to 20% of patients with PTC and FTC. However, more than a third of reoperations for persistent or recurrent disease are related to inadequate initial thyroid surgery.[392] Small lymph node metastases may be treated with radioiodine, but their persistence or growth should lead to consideration of surgery.[393] A recurrence larger than 8 to 10 mm in diameter should be excised.[393,394] Compartmental dissection of previously unexplored compartments with clinically significant persistent/recurrent disease while sparing vital structures is performed because microscopic lymph node metastases are commonly more extensive than would appear from imaging studies alone. This dissection provides long-term local control of the disease in the majority of patients.[395] Conversely, compartmental surgical dissections may not be feasible in the setting of compartments that have been previously explored because of extensive scarring, and only a more limited or targeted lymph node resection may be possible.

Total excision can be facilitated by TBS 3 to 5 days after administration of 100 mCi (3700 MBq) of ^{131}I because additional tissue that should be excised may be identified. In rare cases, surgery has been performed 1 day after the total body scan is done, typically using an intraoperative probe. The completeness of resection is verified 1 to 2 days after surgery by another total-body scan, and in one series total excision was achieved in 92% of cases.[394] Other methods may be used to facilitate the excision of small neoplastic foci located in scar tissue or in sites that are difficult to redissect, primarily using intraoperative ultrasound or preoperative ultrasound-guided charcoal tattooing.[15] Considering both normal neck ultrasonography and undetectable Tg levels (<1 ng/mL after TSH stimulation or <0.2 ng/mL under thyroid hormones) to define excellent response after a reoperation for persistent/recurrent disease as defined by the ATA guidelines, the rate of excellent response reaches 63%.[396] In general, external radiotherapy is indicated in FCTC patients with soft tissue recurrences that cannot be completely excised and that do not take up ^{131}I.

In rare cases, patients with DTC who are not eligible for further surgery or ^{131}I therapy have been treated for regional nodal recurrence with ultrasound-guided radiofrequency ablation or percutaneous ethanol injection.[397–403] No studies prospectively and respectively compared the two techniques. Both should be avoided, however, for the treatment of central lesions close to the trachea and/or the laryngeal nerve and/or the esophagus, as well as locations where surgery is also challenging. These procedures are especially safe for nodes not adjacent to critical structures such as lateral neck recurrences. For tumors that invade the upper aerodigestive tract, patient outcome is related to complete resection of all gross disease, with techniques ranging from shaving tumor off the trachea or esophagus for superficial invasion with the preservation of function to more aggressive techniques in case of direct intraluminal invasion, including tracheal resection and anastomosis or laryngopharyngoesophagectomy.[404,405] In these cases, surgery is usually combined with ^{131}I and external beam radiation.

Management of Distant Metastatic Disease

Among patients with differentiated carcinoma (PTC, FTC, and HCC), only 9% develop distant metastases.[406] Mortality rates at 5 and 10 years after the diagnosis of metastasis were 65% and 75% for all patients with distant metastases, and nearly 80% of the deaths were due to thyroid cancer.[406–408] Of note, up to 20% of deaths were due to locoregional disease.[409] Thus the development of distant metastases in FCTC has traditionally foreseen an ominous prognosis. Lung metastases are more frequent in young patients with PTC, and the lung is almost always the only site of distant spread in children. Bone metastases are more common in older patients and in those with FTC. Other less common sites are the brain, liver, and skin.[406–408]

Clinical symptoms of lung involvement are uncommon. By contrast, pain, swelling, or fracture occurs in more than 80% of patients with bone metastases. They are a poor prognostic indicator.[410] The pattern of lung involvement may vary from macronodular to diffuse infiltrates. The latter are usually diagnosed with ^{131}I TBS and may be confirmed by spiral CT; enlarged mediastinal lymph nodes are often present in patients with PTC, especially children. Bone metastases are osteolytic and are better visualized by CT scan, MRI, or ^{18}FDG-PET. ^{18}FDG-PET scanning is useful in these patients for determining the extent of disease and for prognostic assessment.[23,34,38,39] Nearly all patients with distant metastases have high serum Tg concentrations unless the lung metastases are not visible on CT scan, and two-thirds of such patients have ^{131}I uptake in their sites of metastasis.[407]

Focal treatment of bone metastases includes surgery, external beam radiation therapy, or thermal ablation (radiofrequency ablation or cryoablation) and cement injection.[411] Focal treatment is indicated when there are neurologic or orthopedic complications or a high risk of such complications and when bone metastases are visible on CT scan or MRI, even in the presence of ^{131}I uptake, because radioiodine alone will not control the disease. In patients with a single or a few bone metastases, focal treatment may also be performed with curative intent.[412] Surgery and stereotaxic radiation therapy may be indicated in patients with brain metastases. Thermal ablation or stereotaxic radiation therapy may be used in case of a few lung metastases. Completeness of thermal ablation and outcome of the treated lesions can be assessed with ^{18}FDG-PET scanning.[40]

Patients with distant metastases that take up ^{131}I are treated with 100 to 200 mCi (3700–7400 MBq) every 4 to 6 months during the first 2 years and then at longer intervals. Between ^{131}I treatments, suppressive doses of levothyroxine are given to maintain the serum TSH level below 0.1 mUI/L. In one study, the radiation dose to the tumor tissue and outcome of ^{131}I therapy are correlated.[344] A radiation dose higher than 80 Gy (8000 rads) should be delivered to obtain cure; with radiation doses less than 35 Gy (3500 rads), there is little chance for success. This is the rationale for using higher activities of radioiodine either as standard activity or based on individual dosimetry. However, the comparison between repeated administrations of a standard activity of 100 mCi (3.7 GBq) to the administration of higher activities after dosimetric study did not show a benefit of dosimetry over standard activities in terms of overall survival.[413] In patients with functioning metastases, PET scanning with ^{124}I showed that in a given patient uptake may vary between metastases and also within a given metastasis.[414,415] Finally, uptake may be heterogeneous at the cellular level.[93,414] This heterogeneity in the dose distribution in neoplastic foci may explain the ineffectiveness of ^{131}I treatment, despite significant mean uptake on total-body scan. For treatment to be effective in this clinical setting, appropriate

levels of TSH stimulation and absence of iodine contamination are essential. Excess iodine is eliminated 1 month after administration of an iodinated CT scan.[30] The urinary iodine excretion can be obtained to confirm clearance. Similar short-term survival rates were observed in patients with distant metastases after ^{131}I treatment prepared with either withdrawal or rhTSH.[416] However, most patients with ^{131}I uptake in their metastases are alive at 5 years, and there are no available data on long-term outcome after preparation with rhTSH. Prolonged withdrawal usually induces higher uptake in neoplastic foci than rhTSH and should be the preferred method of TSH stimulation in patients with metastatic disease.[417] rhTSH-mediated therapy may be indicated in selected patients with underlying comorbid conditions, making iatrogenic hypothyroidism potentially risky, and in patients with pituitary disease who are unable to raise their serum TSH.[358] Such patients should be given the same or higher activity than would have been given had they been prepared with hypothyroidism or a dosimetrically determined activity. Lower activities (1–2 mCi [37–74 MBq]/kg body weight) are given to children. There is no absolute limit to the cumulative activity of ^{131}I that can be given to patients with distant metastases as long as treatment is efficient. There is, however, an increased risk of leukemia and of solid cancer, especially when the cumulative activity rises above 600 mCi (22,000 MBq)[418–420]; also, above this activity, further ^{131}I therapy may rarely provide a cure.[407]

Disappearance of imaging abnormalities has been obtained overall in about 45% of patients with distant metastases showing avidity for ^{131}I, and responses are more frequent in younger patients, those with small pulmonary metastases, those who had a well-differentiated cancer, and those who have no ^{18}FDG uptake on PET scan; complete responses may be obtained several years after initiation of therapy.[406–408] When response was judged to have been complete after ^{131}I therapy, subsequent relapse rarely (<10%) occurred even though serum Tg levels were persistently detectable in some patients.[407]

Complications of Treatment With ^{131}I

Acute side effects (nausea, sialadenitis, loss of taste) after treatment with ^{131}I are common but are typically mild and resolve rapidly.[421] Radiation thyroiditis is usually trivial, but if the thyroid remnant is large, the patient may have enough pain to warrant corticosteroid therapy for a few days. Tumor in certain locations, such as the brain, spinal cord, and paratracheal region, may swell in response to TSH stimulation or after ^{131}I therapy, causing compressive symptoms, and this problem should be prevented with corticosteroid therapy. Xerostomia and obstruction of lacrimal ducts may occur in 5% to 10% of patients treated with ^{131}I.[422,423] Radiation fibrosis may rarely develop in patients with diffuse lung metastases and can eventually prove fatal if high activities (>150 mCi [5550 MBq]) are administered at short intervals (<3 months).[248]

Particular attention must be paid to avoid administration of ^{131}I to pregnant women. After ^{131}I treatment, spermatogenesis may be transiently depressed,[424] and women may have transient ovarian failure. Genetic damage induced by exposure to ^{131}I before conception has been a major subject of concern. However, no anomaly has been reported to date. Therefore it is recommended that conception be postponed for at least 6 months after treatment with ^{131}I.[425] There is no clear evidence that pregnancy affects tumor growth in women receiving adequate levothyroxine therapy. In case of pregnancy in a patient treated with a replacement dose of levothyroxine, the dose of levothyroxine is increased by 30% as soon as the pregnancy is confirmed and serum TSH, T_3, and T_4 levels are measured every month during the first half of pregnancy.[426] In a patient treated with a suppressive dose of levothyroxine, serum TSH, T_3, and T_4 levels are controlled every month, and the daily dose of levothyroxine is increased when serum TSH is increased or when T_3 and T_4 decrease.

Mild pancytopenia may occur after repeated or high-dose ^{131}I therapy, especially in patients with bone metastases who are also treated with external radiotherapy. The overall relative risk of leukemia and solid tumors was found to be increased in patients treated with radioactive iodine, especially in case of high cumulative activity of ^{131}I (>600 mCi [22,000 MBq]) or in association with external radiotherapy.[418–420]

Refractoriness to Radioactive Iodine

Two-thirds of distant metastases will become refractory to radioiodine, and treatment with ^{131}I will not provide any benefit. This group includes patients with metastatic disease who do not take up ^{131}I at the time of initial treatment or who lose the ability to take up ^{131}I after previous evidence of uptake, patients with ^{131}I uptake retained in some lesions but not in others, and patients with metastatic disease that progresses despite significant uptake of ^{131}I in the metastases.[427] Less clear is the situation for patients with persistent ^{131}I uptake in all lesions who are not cured despite several treatment courses (particularly after receiving >22,000 MBq [600 mCi] of ^{131}I) and whose disease does not progress according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria.[407] The decision to continue ^{131}I treatment in such patients is generally based on their response to previous treatment courses, persistence of a significant level of ^{131}I uptake on the previous post-therapy TBS, low FDG uptake in tumor foci, and absence of side effects.[427] Large distant metastases with high FDG uptake on PET scanning almost never respond to ^{131}I therapy and usually rapidly progress, confirming the clinical prognostic classification.[38,39]

In patients with refractory disease, ^{131}I treatment should be abandoned, and follow-up is performed with imaging every 4 to 6 months on levothyroxine treatment that should maintain serum TSH below 0.1 mUI/L.[427–429] Optimal TSH goals for individual patients must, however, balance the potential benefit of TSH suppression with the possible harm from subclinical thyrotoxicosis, especially in patients with medical conditions that can be exacerbated with aggressive TSH suppression. Focal treatment modalities are used as previously described. In patients with multiple lesions greater than 1 to 2 cm in diameter with documented progression on imaging within 12 months, a systemic treatment may be indicated.[427] Of note, within a given patient, the intensity of FDG uptake cannot be used to determine the lesions at higher risk of progression.[430] Molecular targeted therapies with antiangiogenic effects are used as first-line treatment. [1]

Molecular Targeted Systemic Therapies

An initiating carcinogenic event can be found in most DTC and thus molecular targeted therapy is based upon a sound scientific rationale.[118] The MAP kinase pathway is activated in the majority of papillary thyroid cancers, mainly by either gene rearrangements (RET-PTC and NTRK) or point mutations of the *RAS* and *BRAF* genes. *RAS* point mutations are frequently found in follicular and poorly differentiated carcinomas. Additional genetic abnormalities may be found in poorly differentiated thyroid carcinomas. Angiogenesis is also activated in thyroid cancers,[431] by activation of the VEGFR pathway. Other pathways may also be activated, including the FGFR and PDGFR pathways.

TABLE 14.14 Drugs Used in Refractory Differentiated Thyroid Cancer

Drugs	*N*	Targets(s)	Partial Response (RECIST) (%)	Median Progression-Free Survival (Months)
Axitinib Cohen[432] Locati[433] Capdevilla[434]	45 45 47	*VEGF, RET, PDGFR, KIT*	31 38 28	18.1 16.1 8
Cabozantinib Cabanillas[436] Cabanillas[435]	15 25	*VEGFR, RET, CMET*	53 40	>12.2, not reached 12.7
Lenvatinib Schlumberger[437] (phase III trial)	392	*VEGFR RET, FGFR, PDGFR, C-KIT*	65	18.3 vs 3.6
Pazopanib Bible[439]	37	*VEGFR, PDGFR, KIT*	49	11.7
Motesanib Sherman[438]	93	*VEGFR, PDGFR, KIT, RET*	14	10
Sorafenib Brose[440] (phase III vs placebo)	417	*VEGFR, RET, BRAF, PDGFR*	12	10.8 vs 5.8
Sunitinib Carr[441]	28	*VEGFR, RET, PDGFR, KIT*	11	NA
Vandetanib Leboulleux[443] (phase II vs placebo)	145	*VEGFR, RET, EGFR*	0	11.1 vs 5.9
Vemurafenib Brose[442]	51	*BRAF*	31	18 TKI naïve, 9 prior TKI
Dabrafenib Falchook[444]	13	*BRAF*	29	11.3

NA, Not applicable; *RECIST,* Response Evaluation Criteria in Solid Tumors; *TKI,* tyrosine kinase inhibitor.

Up to now, most drugs used in refractory DTC had an anti-angiogenic action, and some also target from the MAP kinase pathway. With these agents, the rate of partial response observed ranges from 0% to 65% (Table 14.14), with three drugs showing response rates close to or higher than 50% (lenvatinib, cabozantinib, and pazopanib).[432–444] Improvement of progression-free survival (PFS) has also been demonstrated in two phase III trials: sorafenib in the DECISION trial and lenvatinib in the SELECT trial.[437,440,443]

Vandetanib

The ZACTHYF phase II randomized trial with vandetanib produced a significant prolongation of the PFS compared with placebo (HR, 0.63, p = 0.008; median, 11.1 vs 5.9 months, respectively), and an objective partial response rate of 8%.[443] The subsequent VERIFY phase III trial with vandetanib versus placebo has been conducted, and the results are pending (NCT01876784).

Sorafenib

The DECISION phase III trial with sorafenib showed a prolongation of the PFS compared with placebo (HR, 0.587; 95% CI, 0.454–0.758; p <0.0001; median PFS, 10.8 vs 5.8 months, respectively). The improvement in PFS was seen in all clinical subgroups. The partial response rate was 12%, and stable disease for 6 months or longer was achieved in 41.8 % of patients. PFS was improved in all biomarker subgroups, irrespective of *BRAF* and *RAS* mutation status.

Lenvatinib

The SELECT phase III trial with lenvatinib significantly improved the median PFS compared with placebo (HR, 0.21; 99% CI, 0.14–0.31, p <0.001; median PFS, 18.3 vs 3.6 months, respectively) and the objective response rate was 65% with complete responses in 2%. The improvement in PFS was seen in all clinical subgroups, including in the 20% of patients who had received prior vascular endothelial growth factor (VEGF)–targeted therapy and irrespective of *BRAF* and *RAS* mutation status.

None of the studies demonstrated an improvement in overall survival, which might have been related to the crossover design of the studies with treatment in an open phase in case of progression in the placebo arm and the long survival of some patients after their participation in the trial during which other treatment modalities

were used. However, in a subgroup analysis of the SELECT study, a significant benefit in overall survival was observed in patients aged above 65 years, related to the beneficial effects of lenvatinib in patients with more aggressive disease.[445] In a subsequent analysis, a rapid decline in tumor size by 25% was reported at the 8-week evaluation; the duration of PFS was related to the magnitude of the initial tumor size decrease, and a multivariate analysis identified two prognostic indicators, performance Eastern Cooperative Oncology Group (ECOG) status of 0 or 1 and small size of metastases.[446]

The safety profile of sorafenib was as expected, but with a higher incidence of adverse effects than in patients with other cancer types. Most adverse effects were grade 1 and 2, the most common being hand-foot skin reaction (76%), diarrhea (69%), alopecia (67%), and rash/desquamation (50%). Toxicities led to dose reduction in 64% of patients and to drug withdrawal in 19%.

The safety profile of the lenvatinib group includes hypertension (68%), fatigue (64%), diarrhea (59%), and decreased appetite (50%). Proteinuria occurred in 32% and thromboembolic events in 11%. A total of 68% of the patients required dose reduction, 82% required dose interruption, and 14% of patients were taken off the drug.

Prevention and early diagnosis of toxicity are the best ways to decrease the frequency and gravity of the secondary effects. Prior to starting treatment, patients should have normal blood pressure and should be educated for self–blood pressure measurements. They should be educated to use moisturizing lotion and to protect themselves from the sun. They should be checked for normal heart function, normal electrolytes, normal renal function, and the absence of proteinuria. Interactions with concomitant drugs should be checked to avoid drugs interacting with the cytochrome P450 and drugs prolonging QTc.

It is highly recommended after initiation of treatment that clinicians follow up patients at 2-week intervals for the first 2 to 3 months and then once a month, to proactively manage adverse events in accordance with the tolerance of each individual patient.

Tyrosine kinase inhibitors interacting in the MAP kinase pathway have been used to redifferentiate tumor cells and reinduce RAI uptake. A *MEK* inhibitor (selumetinib) and *BRAF* inhibitor (dabrafenib) have been given prior to radioactive iodine administration with partial response in 20% to 25% of the cases.[447,448] The concept is appealing, but these results have to be confirmed in larger prospective studies.

In conclusion, results of DECISION and SELECT led to the approval of sorafenib and lenvatinib for advanced, refractory, and progressive DTC by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). In the SELECT trial and in real life, maximal benefits were achieved in patients with an ECOG performance status of 0 to 1 who were not heavily pretreated and who had a limited tumor burden. There was no unexpected toxicity, but toxicities were significant and led to dose reduction and to drug withdrawal in a significant proportion of patients. This suggests that these treatments should be initiated only in patients with significant tumor burden and with documented progression of the disease, and they should be managed by experienced teams.

Therapy for Recurrence of Medullary Thyroid Carcinoma

Patients with locoregional recurrence of MTC are at high risk of distant metastases, particularly when calcitonin levels exceed 150 pg/mL.[14] Distant metastases frequently involve multiple organs and are usually multifocal in each involved organ. The most frequently invaded organs are liver, lungs, and bones. For a complete workup, the best imaging combination includes a neck ultrasonography, a liver MRI, and a neck-chest and abdominopelvic CT scan, together with a spine MRI or a bone scintigraphy.[33] ^{18}FDG-PET/CT is useful for initial workup, especially when morphologic images are normal or doubtful.

Patients with distant metastases may progress slowly and may be compatible with decades of survival. Calcitonin and CEA doubling time are prognostic of survival, with short doubling time being in favor of a shorter survival, and are correlated to RECIST progression.[449,450] Symptomatic treatments may be necessary, in particular against pain and diarrhea. Cytotoxic chemotherapy is poorly efficient and may be indicated only in cases of rapid tumor progression.[451] Chemoembolization with doxorubicin (Adriamycin) of liver metastases provides a high response rate for both symptoms and tumor masses.[452] Kinase inhibitors directed against tumor cells (RET and other kinases) and endothelial cells (VEGF receptors) provide a high response rate and should be used as first-line treatment.

Vandetanib and Cabozantinib (XL184)

Vandetanib inhibits kinases of VEGFR1, VEGFR2, RET, and EGFR. In a phase II trial, including 30 patients with hereditary MTC, vandetanib given at the dose of 300 mg/day showed a partial RECIST response in 10 patients and stable disease lasting longer than 24 weeks in 16 other patients.[456] In another phase II trial, including 19 patients with hereditary MTC, vandetanib given at the dose of 100 mg/day showed a partial response in 3 patients and prolonged stabilization in 10 cases. There were, however, no differences in the rate of toxicity.[467] Vandetanib also proved its efficacy in children with advanced MTC in the context of multiple endocrine neoplasia type 2B.[468]

The phase II trial with vandetanib randomized the drug at the dose of 300 mg against placebo in patients with metastatic and aggressive MTC.[466] Patients with MTC-related symptoms were included even in the absence of RECIST progression. The PFS under vandetanib was longer (>30.5 months) than under placebo (19.3 months; HR, 0.46; p <0.001). Partial responses were observed in 45% of the cases, with a median length of 22 months. The subgroup analyses showed a benefit of vandetanib in patients regardless of their WHO and RET status. The study did not show an improvement in the overall survival, but a crossover was allowed in the study, and patients under placebo were treated with vandetanib after unblinding of the study. Vandetanib use in real life has demonstrated a response rate in 22% of the patients.[469]

Cabozantinib inhibits kinases of RET, VEGFR2, and c-MET. In a phase I trial of 34 patients with MTC, partial responses were observed in 17 patients and stable disease in 15 other patients.[460] The phase III trial with cabozantinib randomized the drug at the dose of 140 mg against placebo in patients with metastatic and RECIST progressive MTC. The study did not allow crossover.[465] Median PFS was 11.2 months for cabozantinib versus 4 months for placebo (HR, 0.28; p <0.001). The benefits of cabozantinib on PFS were observed in all groups of patients regardless of age, sex, WHO performance status, tumor location, and previous treatment with TKI. The benefits of cabozantinib on PFS were observed in all patients except those without *RET* mutation.[470] The response rate was 28% with a median length of response of 14.7 months. Response rates ranged from 20% to 34% depending on the *RET* and *RAS* status.[470] The median overall survival was 26.6 months in cabozantinib-treated patients versus 21.1 months for placebo (HR, 0.85, p = 0.24). However, in the group of 126 patients with the *RET M918T* mutation, patient overall

TABLE 14.15 Phase II Prospective Trials in Medullary Thyroid Cancer

Drugs	n	Targets(s)	Partial Response (RECIST) (%)	Progression-Free Survival (Months)
Vandetanib Wells[456]	30	*VEGFR, RET, EGFR*	30	27.9
Sorafenib Lam[457]	19	*VEGFR, BRAF*	11	17.9
Motesanib Schlumberger[458]	83	*VEGFR, PDGFR, C-KIT*	2	12.0
Axitinib Cohen[432] Capdevilla[434]	12 3	*VEGFR1,2,3*	22 23	NA 9.4
Sunitinib Carr[441]	6	*VEGFR, RET*	50	NA
Lenvatinib Schlumberger[459]	59	*RET, VEGFR, FGFR, PDGFR, C-KIT*	36	9.0
Cabozantinib Kurzrock[460]	35	*VEGFR, RET, C-MET*	49	NA
Pazopanib Bible[461]	35	*VEGFR, PDGFR, RET, C-KIT*	14	9.4
Gefitinib Pennell[462]	4	*EGFR*	0	NA
Imatinib De Groot[463] Frank-Raue[464]	15 9	*C-KIT, PDGFR*	0 0	NA NA

NA, Not applicable; *RECIST*, Response Evaluation Criteria in Solid Tumors.

survival was 44.3 months in cabozantinib-treated patients compared with 18.9 months for placebo (HR, 0.60, $p = 0.3$) in favor of *RET M918T* being a predictive factor of treatment efficacy of cabozantinib.[471]

The most frequent secondary effects of vandetanib are diarrhea, fatigue, cutaneous manifestations (folliculitis, photosensitivity, rash), hypertension, and QTc segment prolongation on electrocardiogram (EKG). In the phase III trial, 12% of the patients stopped the drug due to toxicity and 35% decreased the doses.[466] The most frequent secondary effects of cabozantinib are diarrhea, abdominal pain, hypertension, hand-foot syndrome, mucositis, loss of weight, nausea, and fatigue. In the phase III trial, 16% of the patients stopped the drug due to toxicity and 79% decreased the doses.[465] In general, prevention and early diagnosis are the best ways to decrease the frequency and gravity of the secondary effects. Prior to starting treatment, patients should have normal blood pressure and should be educated on self–blood pressure measurements. They should be educated to use moisturizing lotion and to protect themselves from the sun. They should be checked for normal heart function, normal electrolytes, normal renal function, and the absence of proteinuria. Diarrhea should be controlled if possible. Interactions with concomitant drugs should be checked to avoid drugs interacting with the cytochrome P450 and drugs prolonging QTc. After starting the drug, patients should be monitored closely to detect prolongation of QTc and to monitor calcium, vitamin D, and thyroid hormone levels.[472] It is highly recommended after initiation of treatment that clinicians follow up with patients at 2-week intervals for the first 2 to 3 months and then once a month to proactively manage adverse events in accordance with the tolerance of each individual patient.

Vandetanib (Caprelsa) and cabozantinib (Cometriq) are both FDA and EMA approved. Vandetanib is approved for the treatment of symptomatic or progressive MTC with unresectable locally advanced or metastatic disease. The use of vandetanib in patients with indolent asymptomatic or slowly progressive disease should be considered carefully because of the treatment-related risks of vandetanib. Cabozantinib is approved for the treatment of progressive, unresectable, locally advanced or metastatic MTC. These drugs should not be used for patients with isolated elevated calcitonin levels with normal morphologic imaging or in patients with a small tumor burden and stable disease. They should be proposed only to MTC patients with large tumor foci and progressive disease on imaging.

Targeted Molecular Therapy

Activating mutations of the proto-oncogene *RET* are involved in the oncogenesis of MTC. They are found in all hereditary MTC and in 50% to 60% of sporadic MTC.[297–299,453] In selected patients with distant and progressive metastases, they are found

TABLE 14.16 Phase III Trials in Metastatic or Locally Advanced MTC

	Vandetanib: ZETA Study Wells[466]	Cabozantinib: EXAM Study Elisei[465]
Patient Characteristics		
Number of patients included	331	330
Frequency of hereditary MTC	10%	6%
RET mutation: present unknown *RETM918T* mutation	38% 41% 31%	45% 39% 35%
Distant metastases	94%	95%
Previous treatment with TKI	Unknown	20%
RECIST progression prior enrollment	Not mandatory	Yes, within 14 months
Results		
Median follow-up	24 months	14 months
Median PFS	>30.5 months (vandetanib) vs 19.3 months (placebo)	11,2 (cabozantinib) vs 4 months (placebo)
Complete response	0%	0%
Partial RECIST response	45%	28%
Overall survival	Not available	26.6 months (cabozantinib) vs 21.1 months (placebo)
Toxicity		
Toxicity any grade (≥ grade 3)	55% (24%)	69% (33%)
Decrease in dose due to toxicity	35%	65%
Stop for toxicity	12%	16%
Deaths	2% (vandetanib) vs 2% (placebo)	5.6% (cabozantinib) vs 2.8% (placebo)

EXAM, Efficacy of XL184 in Advanced Medullary Thyroid Cancer; *MTC,* medullary thyroid carcinoma; *PFS,* progression-free survival; *RECIST*, Response Evaluation Criteria in Solid Tumors; *TKI,* tyrosine kinase inhibitor.

in 91% of the cases.[297–299,453] The most frequent *RET* mutation found is the M918T mutation. In the absence of *RET* mutation, *RAS* mutations are found in 10% to 45% of the cases, with the *HRAS* mutation being more frequent than *KRAS,* which is more frequent than *NRAS.*[297–299,453] ALK rearrangement and *MET* mutation have also been found, but in less than 5% of the specimens.[454] VEGF receptors 1 and 2 also often are overexpressed in MTC, which together with the fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) play a role in tumor angiogenesis.[455] In general, tyrosine kinase inhibitors (TKIs) have shown significant results in patients with advanced MTC.

Many TKIs targeting RET and VEGFR2, among other targets, have been used in phase II trials, with significant tumor response rates ranging from none to 50%[432,434,441,456–464] (Table 14.15). Two drugs outlined next have been evaluated in phase III trials: vandetanib and cabozantinib in prospective randomized double-blind studies.[465,466] Their main objective was to show a benefit in PFS (Table 14.16).

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The complete list of references is available online at ExpertConsult.com.

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15

The Adrenal Cortex

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CHAPTER OUTLINE

KEY POINTS

- This chapter discusses mechanisms and regulation of adrenal steroid production, function of the hypothalamic-pituitary-adrenal axis, and negative regulation.
- The chapter goes on to describe the transactivating and transrepressive actions of glucocorticoids.
- Glucocorticoid excess and Cushing syndrome, adrenal insufficiency and Addison disease, and inherited disorders of the adrenal gland are also discussed.
- Optimizing corticosteroid replacement therapies is addressed.
- The chapter concludes with discussion of adrenal incidentalomas, adenomas, and carcinomas.

The Adrenal Cortex—Historical Milestones

The anatomy of the adrenal glands was described almost 450 years ago by Bartolomeo Eustachius,[1] and the zonation of the gland and its distinction from the medulla were elucidated shortly thereafter. However, a functional role for the adrenal glands was not accurately defined until the pioneering work of Thomas Addison, who described the clinical and autopsy findings in 11 cases of Addison disease in his classic monograph in 1855.[2] Just a year later, Brown-Séquard demonstrated that the adrenal glands were "organs essential for life" by performing adrenalectomies in dogs, cats, and guinea pigs.[3] In 1896, William Osler first administered adrenal extract to a patient with Addison disease, a feat that was repeated by others in animal and human studies over the next 40 years. Between 1937 and 1955, the adrenocorticosteroid hormones were isolated, and their structures were defined and synthesized.[4] Notable breakthroughs included the discovery of cortisone and clinical evaluation of its anti-inflammatory effect in patients with rheumatoid arthritis[5] and the isolation of aldosterone.[6]

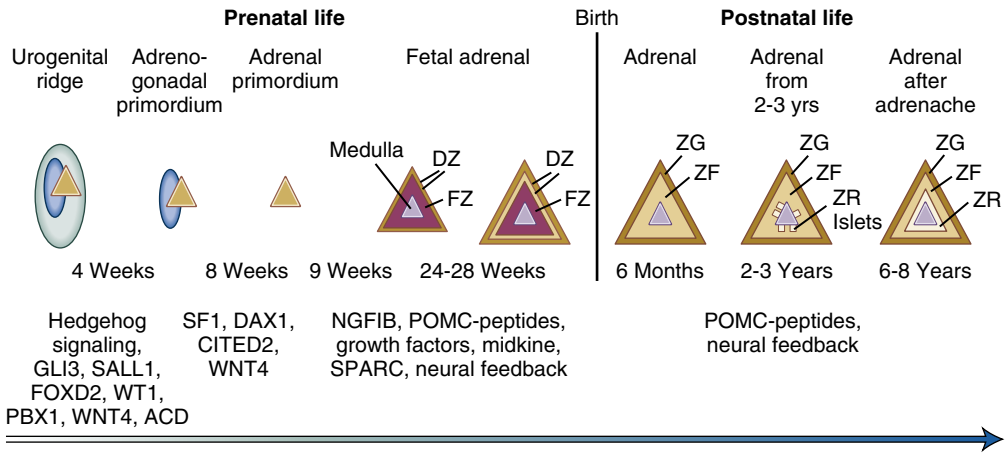
The control of adrenocortical function by a pituitary factor was demonstrated in the 1920s, and this led to the isolation of sheep adrenocorticotropic hormone (ACTH) by Li, Evans, and Simpson in 1943.[7] Such a concept was supported through clinical studies, notably by Harvey Cushing in 1932, who associated his original clinical observations of 1912 (a "polyglandular syndrome" caused by pituitary basophilism) with adrenal hyperactivity.[8] The neural control of pituitary ACTH secretion by corticotropin-releasing factor (later renamed corticotropin-releasing hormone [CRH]) was defined by Harris and other workers in the 1940s, but CRH was not characterized and synthesized until 1981 in the laboratory of Wylie Vale.[9] Jerome Conn described primary aldosteronism in 1955,[10] and the control of adrenal aldosterone secretion by angiotensin II was confirmed shortly afterward. Advances in radioimmunoassay, and particularly molecular biology, have facilitated an exponential increase in the understanding of adrenal physiology and pathophysiology (Table 15.1).

Anatomy and Development

The cells forming the adrenal cortex originate from the intermediate mesoderm. These cells derive from the urogenital ridge and have a common embryologic origin with the gonad and the kidney. Early differentiation of the adrenogonadal primordium from the urogenital ridge requires signaling cascades and transcription factors GLI3, SALL1, FOXD2, WT1, PBX1, and WNT4, and the regulator of telomerase activity, ACD (Fig. 15.1). The adrenogonadal primordium can be seen as the medial part of the urogenital ridge at 4 weeks. Separation of the adrenogonadal primordium and formation of the adrenal primordium seem to depend on the actions of transcription factors SF1 (steroidogenic factor 1),

TABLE 15.1 History of the Adrenal Cortex: Important Milestones

Year	Event
1563	Eustachius describes the adrenals (published by Lancisi in 1714).
1849	Thomas Addison, while searching for the cause of pernicious anemia, stumbles on a bronzed appearance associated with the adrenal glands—*melasma suprarenale.*
1855	Thomas Addison describes the clinical features and autopsy findings in 11 cases of diseases of the suprarenal capsules, at least 6 of which were tuberculous in origin.
1856	In adrenalectomy experiments, Brown-Séquard demonstrates that the adrenal glands are essential for life.
1896	William Osler prepares an oral glycerin extract derived from pig adrenals and demonstrates that it has clinical benefit in patients with Addison disease.
1905	Bulloch and Sequeira describe patients with congenital adrenal hyperplasia.
1929	Liquid extracts of cortical tissue are used to keep adrenalectomized cats alive indefinitely (Swingle and Pfiffner); subsequently, this extract was used successfully to treat a patient with Addison disease (Rowntree and Greene).
1932	Harvey Cushing associates the polyglandular syndrome of pituitary basophilism, which he first described in 1912, with hyperactivity of the pituitary-adrenal glands.
1936	The concept of stress and its effect on pituitary-adrenal function are described by Selye.
1937–1952	Isolation and structural characterization of adrenocortical hormones are reported by Kendall and Reichstein.
1943	Li and colleagues isolate pure adrenocorticotropic hormone from sheep pituitary.
1950	Hench, Kendall, and Reichstein share the Nobel Prize in Medicine for describing the anti-inflammatory effects of cortisone in patients with rheumatoid arthritis.
1953	Isolation and analysis of the structure of aldosterone are reported by Simpson and Tait.
1956	Conn describes primary aldosteronism.
1981	Characterization and synthesis of corticotropin-releasing hormone are reported by Vale.
1980–present	The *molecular era:* cloning and functional characterization of steroid receptors, steroidogenic enzymes, and adrenal transcription factors are reported, and the molecular basis for human adrenal diseases is defined.



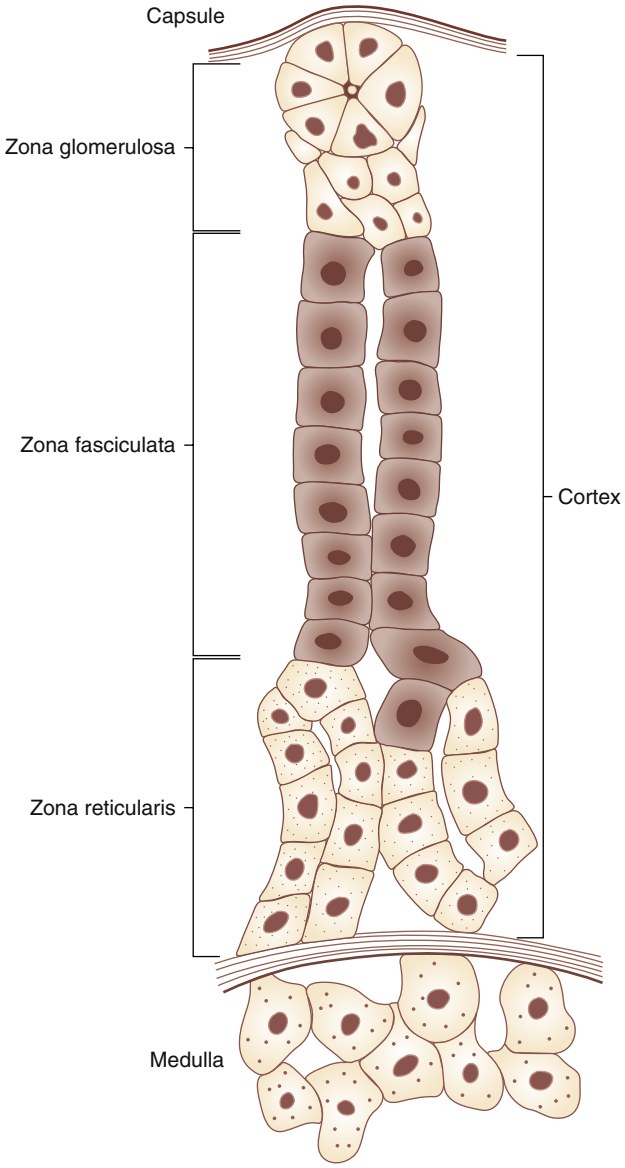
• **Fig. 15.1** Schematic diagram of the development of the human adrenal cortex during prenatal and postnatal life showing transcription factors that are active at each stage (see text for details). *DZ,* definitive zone; *FZ,* fetal zone; *POMC,* pro-opiomelanocortin; *SPARC,* secreted protein, acidic, cysteine-rich (osteonectin); *ZF,* zona fasciculata; *ZG,* zona glomerulosa; *ZR,* zona reticularis.

DAX1, WNT4, and CITED2. The adrenocortical primordium develops at approximately 8 weeks of gestation and can be differentiated into two distinct layers, the inner fetal zone (FZ) and the outer definitive zone (DZ). At approximately 9 weeks, the adrenal blastema encapsulates and the adrenal medulla develops when neural crest cells migrate into the adrenal gland.[11] During the second trimester, the FZ enlarges, becomes larger than the fetal kidney, and secretes abundant amounts of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS). Concentrations of these hormones abruptly decline postnatally, in parallel with the postnatal involution of the FZ. The neocortex develops over the subsequent years into the adult adrenal gland.

In fetal life and up to 12 months of age, two distinct zones are evident, an inner prominent FZ and an outer DZ that differentiates into the adult adrenal gland. After birth, the FZ regresses and the DZ, which contains an inner zona fasciculata (ZF) and an outer zona glomerulosa (ZG), proliferates.[12,13] The innermost zone, the zona reticularis (ZR), is evident after 2 years of life. The differentiation of the adrenal cortex into distinct zones has important functional consequences and is thought to depend on the temporal expression of transcription factors, including Pref1/ZOG, inner zone antigen, and SF1.[14,15] In preadrenarchal children, focal reticular zone islets can be found, but the ZG and ZF are clearly differentiated.[16] The occurrence of these ZR islets is consistent with the observation that DHEA and DHEAS synthesis gradually begins to rise from about 3 years of age.[17] At adrenarche, the inner zone (ZR) thickens, corresponding with increased production of DHEA and DHEAS. Concurrently, changes in zone-specific enzyme expression patterns, such as decreased 3β-hydroxysteroid dehydrogenase type 2 (3βHSD2) and increased cytochrome b_5 and sulfotransferase (SULT2A1) in the ZR, lead to increased flux toward DHEA. Clinically, adrenarche becomes apparent at 6 to 8 years of age. Adrenal androgen production peaks in the third decade and then declines at a variable rate. Mineralocorticoids and glucocorticoids show a less age-specific variation.

The adult adrenal gland is a pyramidal structure, approximately 4 g in weight, 2 cm wide, 5 cm long, and 1 cm thick, that lies immediately above the kidney on its posteromedial surface. Beneath the capsule, the ZG makes up approximately 15% of the cortex (depending on sodium intake) (Fig. 15.2). Cells are clustered in spherical nests and are small, with smaller nuclei in comparison with cells in other zones. The ZF makes up 75% of the cortex; cells are large and lipid laden and form radial cords within the fibrovascular radial network. The innermost ZR is sharply demarcated from both the ZF and the adrenal medulla. Cells there are irregular with little lipid content. The maintenance of normal adrenal size appears to involve a progenitor cell population lying between the ZG and ZF; cell migration and differentiation occur within the ZF, and senescence occurs within the ZR, but the factors regulating this important aspect of adrenal regeneration are unknown. Fetal cells give rise to a subcapsular stem cell population that differentiates in a centripetal direction.[18] ACTH administration results in glomerulosa cells adopting a fasciculata phenotype, and in turn, the innermost fasciculata cells adopt a reticularis phenotype that is reversible on withdrawal of ACTH.

The vasculature of the adrenal cortex is complex. Arterial supply is conveyed by up to 12 small arteries from the aorta and the inferior phrenic, renal, and intercostal arteries. These arteries branch to form a subcapsular arteriolar plexus from which radial capillaries penetrate deeper into the cortex. In the ZR, a dense sinusoidal plexus is created, which empties into a central vein. The right adrenal vein is short, draining directly into the inferior vena cava, whereas the longer left adrenal vein usually drains into the left renal vein.

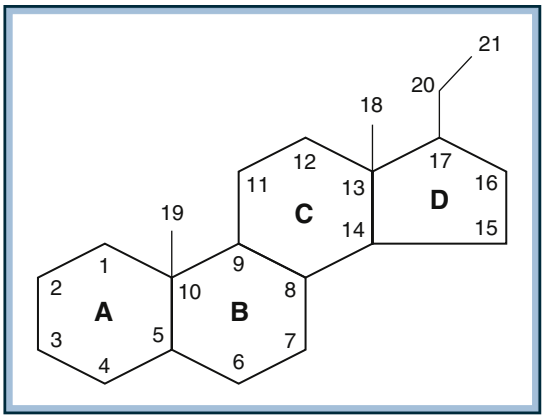


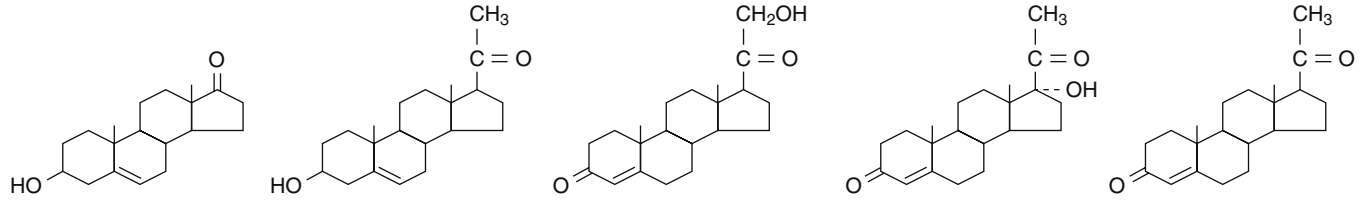
• **Fig. 15.2** Schematic diagram of the structure of the human adrenal cortex, depicting the outer zona glomerulosa and inner zona fasciculata and zona reticularis.

Adrenal Steroids and Steroidogenesis

Three main types of hormones are produced by the adrenal cortex—glucocorticoids (cortisol, corticosterone), mineralocorticoids (aldosterone, deoxycorticosterone [DOC]), androgen precursors (DHEA, DHEAS, androstenedione), and a small amount of androgens (testosterone and 11-oxygenated 19-carbon androgens/precursors). All steroid hormones are derived from the cyclopentanoperhydrophenanthrene structure, that is, three cyclohexane rings and a single cyclopentane ring (Fig. 15.3). Steroid nomenclature is defined in one of two ways: by trivial names (e.g., cortisol, aldosterone) or by the chemical structure as defined by the International Union of Pure and Applied Chemistry (IUPAC).[19] The IUPAC classification is inappropriate for clinical use but does provide an invaluable insight into steroid structure. The basic structure, trivial name, and IUPAC name of some common steroids are given in Fig. 15.3 and Table 15.2. Estrogens have 18 carbon atoms (C18 steroids) and androgens have 19 carbon atoms (C19), whereas glucocorticoids, mineralocorticoids, and progestogens are C21-steroid derivatives.

Cholesterol is the precursor for adrenal steroidogenesis. It is provided principally from the circulation, in the form of low-density lipoprotein (LDL) cholesterol.[20] Uptake is by specific cell-surface LDL receptors present on adrenal tissue[21]; LDL is then internalized via receptor-mediated endocytosis,[22] the resulting vesicles fuse with lysozymes, and free cholesterol is produced after hydrolysis. However, it is clear that this cannot be the sole source





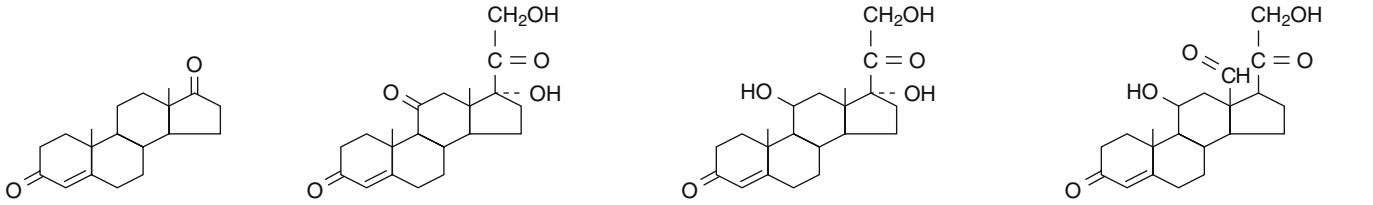
Dehydroepiandrosterone
5-Androsten-3β-ol-17-one

Pregnenolone
5-Pregnen-3β -ol-20-one

Deoxycorticosterone
4-Pregnen-21-ol-3,20-dione

17-OH-Progesterone
4-Pregnen-17α -ol-3,20, dione

Progesterone
4-Pregnen-3,20-dione



Androstenedione
4-Androsten-3,17-dione

Cortisone
4-Pregnen-17a,21-diol-3,11,20-trione

Cortisol
4-Pregnen-11b,17a,21-triol-3,20-dione

Aldosterone
4-Pregnen-11b,21-diol-3,18,20-trione

• **Fig. 15.3** The cyclopentanoperhydrophenanthrene structure of corticosteroid hormones, highlighting the structure of some endogenous steroid hormones together with their nomenclature.

TABLE 15.2 IUPAC and Trivial Names of Natural and Synthetic Steroids

Trivial Name	IUPAC Name
Aldosterone	4-Pregnen-11β,21-diol-3,18,20-trione
Androstenedione	4-Androsten-3,17-dione
Cortisol	4-Pregnen-11β,17α,21-triol-3,20-dione
Cortisone	4-Pregnen-17α,21-diol-3,11,20-trione
Dehydroepiandrosterone	5-Androsten-3β-ol-17-one
Deoxycorticosterone	4-Pregnen-21-ol-3,20-dione
Dexamethasone	1,4-Pregnadien-9α-fluoro-16α-methyl-11β,17α,21-triol-3,20-dione
Dihydrotestosterone	5α-Androstan-17β-ol-3-one
Estradiol	1,3,5(10)-Estratrien-3,17β-diol
Fludrocortisone	4-Pregnen-9α-fluoro-11β,17α,21-triol-3,20-dione
17-Hydroxyprogesterone	4-Pregnen-17α-ol-3,20-dione
Methylprednisolone	1,4-Pregnadien-6α-methyl-11β,17α,21-triol-3,20-dione
Prednisolone	1,4-Pregnadien-11β,17α,21-triol-3,20-dione

Continued

TABLE 15.2 IUPAC and Trivial Names of Natural and Synthetic Steroids—cont'd

Trivial Name	IUPAC Name
Prednisone	1,4-Pregnadien-17α,21-diol-3,11,20-trione
Pregnenolone	5-Pregnen-3β-ol-20-one
Progesterone	4-Pregnen-3,20-dione
Testosterone	4-Androsten-17β-ol-3-one
Triamcinolone	1,4-Pregnadien-9α-fluoro-11β,16α,17α,21-tetrol-3,20-dione

IUPAC, International Union of Pure and Applied Chemistry.

of adrenal cholesterol, because patients with abetalipoproteinemia who have undetectable circulating LDL and patients with defective LDL receptors in the setting of familial hypercholesterolemia still have normal basal adrenal steroidogenesis. Cholesterol can be generated de novo within the adrenal cortex from acetyl coenzyme A (CoA). In addition, there is evidence that the adrenal gland can utilize high-density lipoprotein (HDL) cholesterol after uptake through the putative HDL receptor, SR-B1.[23]

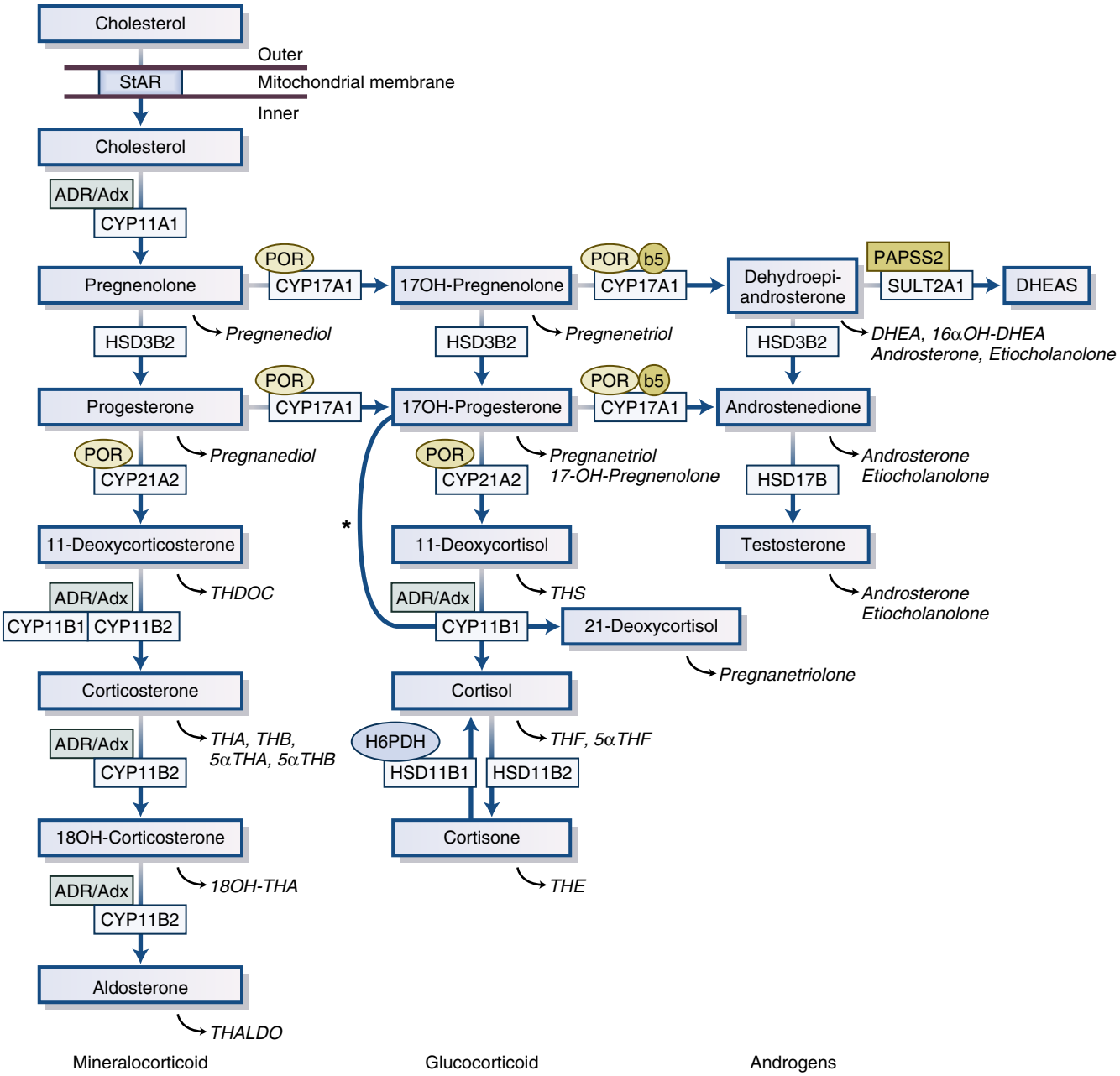
The biochemical pathways involved in adrenal steroidogenesis are shown in Fig. 15.4. The initial hormone-dependent, rate-limiting step is the transport of intracellular cholesterol from the outer to inner mitochondrial membrane for conversion to pregnenolone by cytochrome P450 side-chain cleavage enzyme (P450 11A1). Naturally occurring human mutations have confirmed the importance of a 30-kDa protein, steroidogenic acute regulatory protein (StAR), in mediating this effect. StAR is induced by an increase in intracellular cyclic adenosine monophosphate (cAMP) after binding of ACTH to its cognate receptor, providing the first important rate-limiting step in adrenal steroidogenesis.[24] Other transporters, including the peripheral benzodiazepine-like receptor, may be involved.[25]

Steroidogenesis involves the concerted action of several enzymes, including a series of cytochrome P450 enzymes, all of which have been cloned and characterized (Table 15.3). Cytochrome P450 enzymes are classified into two types, according to their subcellular localization and their specific electron shuttle system. Mitochondrial (type I) cytochrome P450 enzymes such as CYP11A1 (P450 11A1), 11α-hydroxylase (CYP11B1, or P45011B1), and aldosterone synthase (CYP11B2, or P450 11B2) rely on electron transfer facilitated by adrenodoxin and adrenodoxin reductase.[26,27] Micrososomal (type II) cytochrome P450 enzymes localized to the endoplasmic reticulum include the steroidogenic enzymes 17α-hydroxylase (CYP17A1, or P450 17A1), 21-hydroxylase (CYP21A2, or P450 21A2), and P450 aromatase (CYP19A1, or P450 19A1). The functions of cytochrome P450 type II enzymes crucially depend on P450 oxidoreductase (POR), which provides electrons required for monooxygenase reactions catalyzed by the P450 enzyme.[27,28] This category also includes hepatic P450 enzymes involved in drug metabolism and enzymes involved in sterol and bile acid synthesis.[27,28] In addition, the 17,20-lyase activity of P450 17A1 is dependent on a hemoprotein cytochrome b_5, which αβ functions as an allosteric facilitator of P450 17A1 with POR (Fig. 15.5; see also Fig. 15.4).[29]

Mutations in the genes encoding these enzymes result in human disease, so some understanding of the underlying pathways and steroid precursors is required.[30] After uptake of cholesterol to the mitochondrion, cholesterol is cleaved by the P450 11A1 enzyme to form pregnenolone.[31] In the cytoplasm, pregnenolone is converted to progesterone by the type II isozyme 3βHSD through a reaction involving dehydrogenation of the 3-hydroxyl group and isomerization of the double bond at C5.[32] Progesterone is hydroxylated to 17-hydroxyprogesterone (17OHP) through the 17α-hydroxylase activity of P450 17A1. 17α-Hydroxylation is an essential prerequisite for cortisol synthesis, and the ZG does not express 17α-hydroxylase. P450 17A1 also possesses 17,20-lyase activity, which results in production of the C19 adrenal androgens DHEA and androstenedione.[33] In humans, however, 17OHP is not an efficient substrate for P450 17A1, and there is negligible conversion of 17OHP to androstenedione. Adrenal androstenedione secretion is dependent on the conversion of DHEA to androstenedione by 3βHSD. This enzyme also converts 17-hydroxypregnenolone to 17OHP, but the preferred substrate is pregnenolone. The human adrenal gland is capable of synthesis of small but significant amounts of testosterone, which increases in clinical conditions associated with androgen excess. This conversion is facilitated by the enzyme 17βHSD type 5 (17βHSD5), also called aldo-keto reductase 1C3 (AKR1C3).[34] 21-Hydroxylation of either progesterone (in the ZG) or 17OHP (in the ZF) is carried out by the product of the *CYP21A2* gene, 21-hydroxylase, to yield DOC or 11-deoxycortisol, respectively.[35] The final step in cortisol biosynthesis takes place in the mitochondria and involves the conversion of 11-deoxycortisol to cortisol by the enzyme P450 11B1 (11β-hydroxylase).[36] In the ZG, 11β-hydroxylase may also convert DOC to corticosterone. The enzyme P450 11B2 (aldosterone synthase) may also carry out this reaction; in addition, P450 11B2 is required for conversion of corticosterone to aldosterone via the intermediate 18OH corticosterone; CYP11B1 lacks these two enzymatic activities.[37,38] Therefore P450 11B2 can carry out 11β-hydroxylation, 18-hydroxylation, and 18-methyl oxidation to yield the characteristic C11-18 hemiacetyl structure of aldosterone.

Regulation of Adrenal Steroidogenesis: Functional Zonation of the Adrenal Cortex

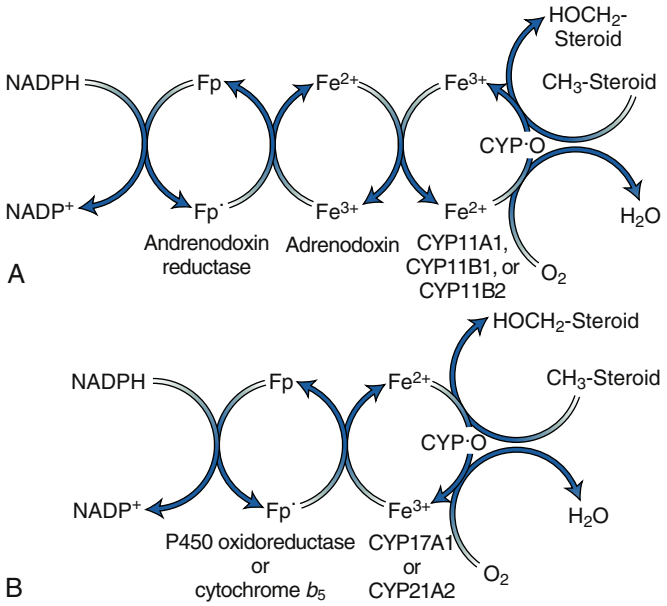
Glucocorticoids are secreted in relatively high amounts (cortisol, 10–20 mg/day) from the ZF under the control of ACTH; mineralocorticoids are secreted in low amounts (aldosterone, 100–150 μg/day) from the ZG under the principal control of angiotensin II. As a class, adrenal androgen precursors (DHEA, DHEAS, androstenedione, 11β-hydroxyandrostenedione) are the most abundant steroids secreted from the adult adrenal gland (>20 mg/day). In each case, secretion is facilitated through the expression of steroidogenic enzymes in a specific zonal manner. The ZG cannot



• **Fig. 15.4** Adrenal steroidogenesis. After the steroidogenic acute regulatory *(StAR)* protein-mediated uptake of cholesterol into mitochondria within adrenocortical cells, aldosterone, cortisol, and adrenal androgens are synthesized through the coordinated action of a series of steroidogenic enzymes in a zone-specific fashion. The mitochondrial cytochrome P450 (CYP) type I enzymes (CYP11A1, CYP11B1, CYP11B2) requiring electron transfer via adrenodoxin reductase (ADR) and adrenodoxin (Adx) are marked with a *box labeled ADR/Adx*. The microsomal CYP type II enzymes *(CYP17A1, CYP21A2)* receive electrons from P450 oxidoreductase *(circle labeled POR)*. The 17,20-lyase reaction catalyzed by CYP17A1 requires, in addition to POR, cytochrome b_5, indicated by a *circle labeled b5*. Urinary steroid hormone metabolites are given in italics below the plasma hormones. The asterisk *(*)* indicates 11-hydroxylation of 17OH-progesterone to 21-deoxycortisol in cases of 21-hydroxylase deficiency. The adrenal conversion of androstenedione to testosterone is catalyzed by the aldo-keto reductase AKR1C3 (HSD17B5). *CYP11A1,* P450 side-chain cleavage enzyme; *CYP11B1,* 11β-hydroxylase; *CYP11B2,* aldosterone synthase; *CYP17A1,* 17α-hydroxylase; *CYP21A2,* 21-hydroxylase; *DHEA,* dehydroepiandrosterone; *DHEAS,* dehydroepiandrosterone sulfate; *H6PDH,* hexose-6-phosphate dehydrogenase; *HSD11B1,* 11β-hydroxysteroid dehydrogenase 1; *HSD11B2,* 11β-hydroxysteroid dehydrogenase 2; *HSD17B,* 17β-hydroxysteroid dehydrogenase; *HSD3B2,* 3β-hydroxysteroid dehydrogenase type 2; *17OH-progesterone,* 17α-hydroxyprogesterone; *PAPSS2,* 3′-phosphoadenosine, 5′-phosphosulfate synthase 2; *SULT2A1,* sulfotransferase 2A1; *THA,* tetrahydro-11-dehydrocorticosterone; *THB,* tetrahydro-corticosterone; *THALDO,* tetrahydro-aldosterone; *THDOC,* tetrahydro-11-deoxycorticosterone; *THF,* tetrahydrocortisol; *THS,* tetrahydro-11-deoxycortisol.

TABLE 15.3 Nomenclature for Adrenal Steroidogenic Enzymes and Their Genes

Enzyme Name	Enzyme Family	Gene	Chromosome
P450 11A1, Cholesterol side-chain cleavage (SCC) (desmolase)	Cytochrome P450 type I	*CYP11A1*	15q23-q24
3β-Hydroxysteroid dehydrogenase (3βHSD) (type II isozyme)	Short-chain alcohol dehydrogenase reductase superfamily	*HSD3B2*	1p13.1
P450 17A1, 17α-Hydroxylase/17,20-lyase	Cytochrome P450 type II	*CYP17A1*	10q24.3
P450 21A2, 21-Hydroxylase	Cytochrome P450 type II	*CYP21A2*	6p21.3
P450 11B1, 11β-Hydroxylase	Cytochrome P450 type I	*CYP11B1*	8q24.3
P450 11B2, Aldosterone synthase	Cytochrome P450 type I	*CYP11B2*	8q24.3



• **Fig. 15.5** (A) Electron shuttle system for the mitochondrial enzymes CYP11A1, CYP11B1, and CYP11B2. Adrenodoxin reductase receives electrons from reduced nicotinamide adenine dinucleotide phosphate *(NADPH)* and reduces adrenodoxin, which transfers reducing equivalents to the cytochrome P450 *(CYP)* enzyme. The enzyme then uses these electrons, plus molecular oxygen, to oxygenate the steroid. (B) Electron shuttle system for the microsomal enzymes CYP17A1 and CYP21A2. P450 oxidoreductase, a flavoprotein, accepts electrons from NADPH and transfers them to the NADPH-P450 enzyme. The enzyme then uses these electrons, plus molecular oxygen, to oxygenate the steroid. A second reducing equivalent may be supplied to CYP17A1 by NADPH-P450 oxidoreductase or cytochrome b_5. *Fp,* flavoprotein; *Fp•,* reduced form of flavoprotein; *$NADP^+$,* nicotinamide adenine dinucleotide phosphate.

synthesize cortisol because it does not express 17α-hydroxylase. In contrast, aldosterone secretion is confined to the outer ZG because of the restricted expression of P450 11B2. Although P450 11B1 and P450 11B2 share 95% homology, the 5′ promoter sequences differ, permitting regulation of the final steps in glucocorticoid and mineralocorticoid biosynthesis by ACTH and angiotensin II, respectively. In the ZR, high levels of cytochrome b_5 confer 17,20-lyase activity on P450 17A1 and androgen precursor production. DHEA is sulfated in the ZR by the DHEA SULT2A1 to form DHEAS. This sulfonation reaction facilitated by SULT2A1 relies on the donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to transfer a sulfonate group to an acceptor molecule. PAPS is synthesized by PAPS synthase, of which two isoenzymes exist (PAPSS1 and PAPSS2).[39]

In the fetal adrenal, steroidogenesis occurs primarily within the inner FZ. The FZ is a characteristic feature of higher primates, but the biologic role of fetal androgen production remains unclear. Because of a relative lack of 3βHSD and high SULT2A1 activity, the principal steroidogenic products are DHEA and DHEAS, which are then aromatized by placental trophoblast to estrogens. Therefore the majority of maternal estrogen across pregnancy is, indirectly, fetally derived.[40]

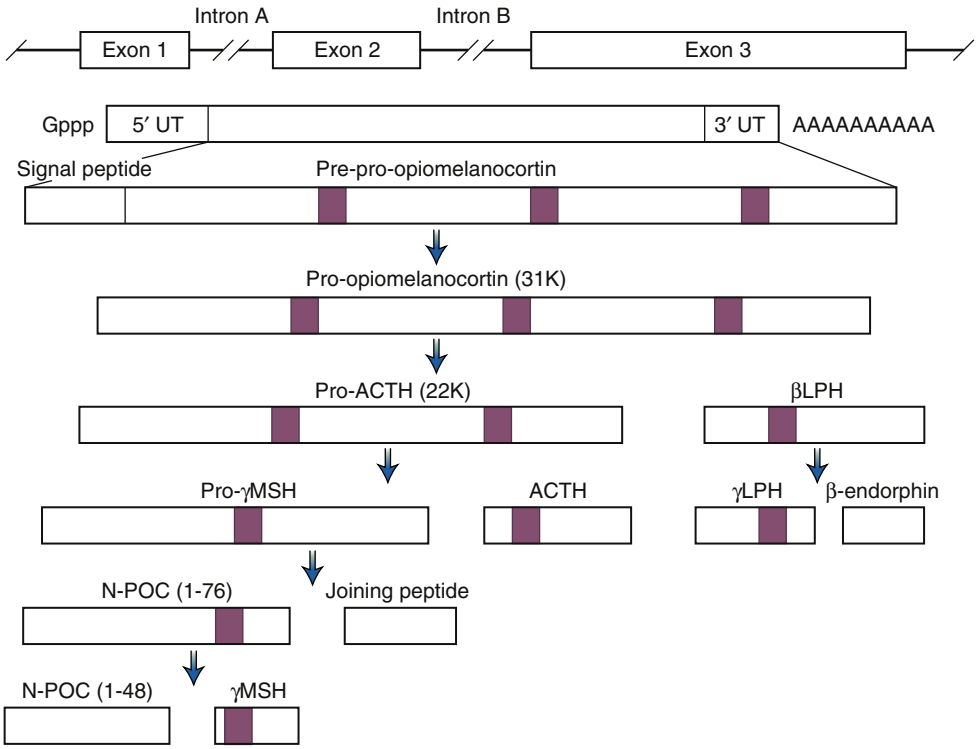
Classic endocrine feedback loops are in place to control the secretion of both hormones. Cortisol inhibits the secretion of CRH from the hypothalamus and ACTH from the pituitary, and aldosterone-induced sodium retention inhibits renal renin secretion.

Glucocorticoid Secretion: The Hypothalamic-Pituitary-Adrenal Axis

Pro-opiomelanocortin and ACTH

ACTH is the principal hormone stimulating adrenal glucocorticoid biosynthesis and secretion. ACTH has 39 amino acids but is synthesized within the anterior pituitary as part of a much larger, 241–amino acid precursor called *pro-opiomelanocortin* (POMC). A transcription factor, TPIT, appears to be essential for differentiation of POMC-expressing cells within the anterior pituitary.[41] POMC is cleaved in a tissue-specific fashion by prohormone convertases to yield smaller peptide hormones. In the anterior pituitary, this results in the secretion of β-lipoprotein (βLPH) and pro-ACTH, the latter being further cleaved to an amino-terminal peptide, joining peptide, and ACTH itself (Fig. 15.6).[42,43] Post-secretion cleavage of the precursor to γ-melanocyte-stimulating hormone (pro-γMSH) by a serine protease (AsP) expressed in the outer adrenal cortex is thought to mediate the trophic action of ACTH on the adrenal cortex.[44] The first 24 amino acids of ACTH are common to all species, and a synthetic ACTH(1-24), tetracosactide or cosyntropin (Synacthen, Cortrosyn), is available commercially for clinical testing of the hypothalamic-pituitary-adrenal (HPA) axis and assessing adrenal glucocorticoid reserve. The hormones αMSH, βMSH, and γMSH are also cleaved products from POMC, but the increased pigmentation characteristic of Addison disease is thought to arise directly from increased ACTH concentrations binding to the melanocortin-1 receptor (MC1R) rather than from αMSH secretion.[45]

POMC is also transcribed in many extrapituitary tissues, notably brain, liver, kidney, gonad, and placenta.[42,46,47] In these



• **Fig. 15.6** Synthesis and cleavage of pro-opiomelanocortin (POMC) within the human anterior pituitary gland. Prohormone convertase enzymes sequentially cleave POMC to adrenocorticotropic hormone *(ACTH)*. Shaded areas represent melanocyte-stimulating hormone *(MSH)* structural units. *βLPH,* β-lipoprotein; *γLPH,* γ-lipoprotein; *N-POC,* amino-terminal pro-opiomelanocortin.

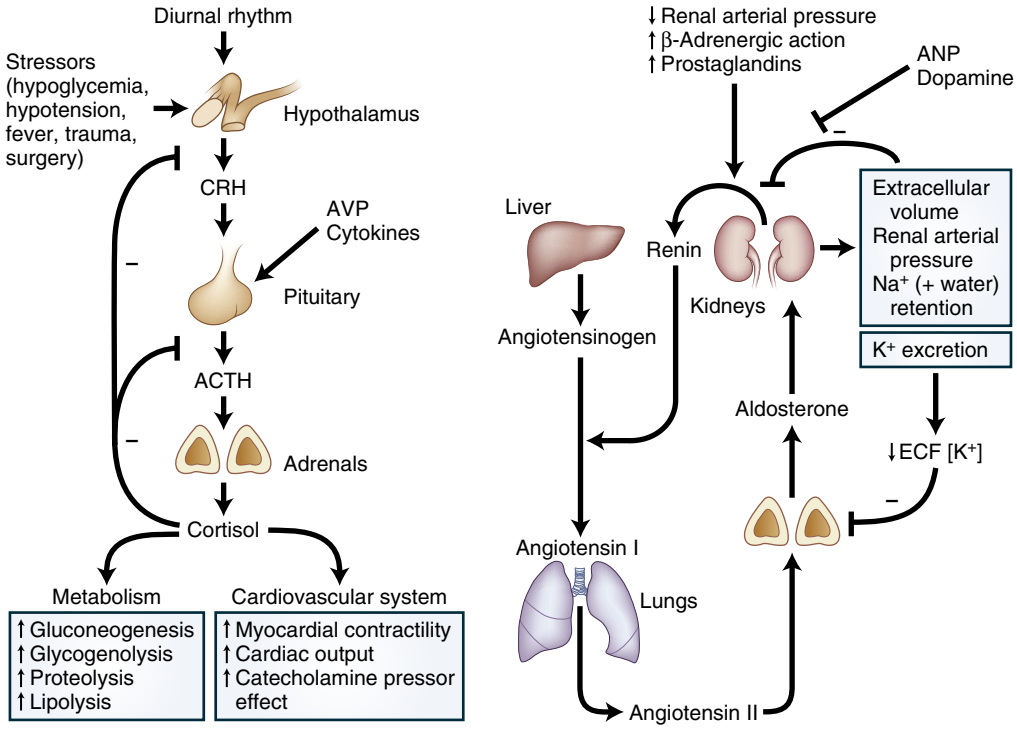
normal tissues, POMC messenger RNA (mRNA) is usually shorter (800nt) than the pituitary 1200nt transcript because of lack of exons 1 and 2 and the 5′ region of exon 3.[48] Because the POMC-like peptide product from this shorter transcript lacks a signal sequence needed to cross the endoplasmic reticulum, it is probable that it is neither secreted nor active in normal circumstances. However, in ectopic ACTH syndrome, additional POMC mRNA species are described that are longer than the normal pituitary POMC species (typically 1450nt) as a result of the use of alternative promoters in the 5′ region of the gene.[49,50] This may, in part, explain the resistance of POMC expression to glucocorticoid feedback in these tumors. Other factors, including interaction with tissue-specific transcription factors[51] and lack of *POMC* promoter methylation,[52] may explain the ectopic expression of ACTH in some malignant tissues. The cleavage of POMC is also tissue specific,[53] and it is possible, at least in some cases of ectopic ACTH syndrome, that circulating ACTH precursors (notably pro-ACTH) may cross-react in current ACTH radioimmunoassays.[54,55] The biologic activity of POMC itself on adrenal function is thought to be negligible.

POMC expression and processing within neurons in the hypothalamus, specifically the generation of αMSH that interacts with melanocortin-4 receptors (MC4R), appears to be of crucial importance in appetite control and energy homeostasis (see later discussion).[56]

Corticotropin-Releasing Hormone and Arginine Vasopressin

POMC secretion is tightly controlled by numerous factors, notably CRH and arginine vasopressin (AVP) (Fig. 15.7).[57,58] Additional control is provided through an endogenous circadian rhythm and by stress and feedback inhibition by cortisol itself. CRH is a 41–amino acid peptide that is synthesized in neurons within the paraventricular nucleus of the hypothalamus.[9,59,60] Human and rat CRH are identical, but ovine CRH differs by seven amino acids[61,62]; ovine-sequence CRH is slightly more potent than human-sequence CRH in stimulating ACTH secretion and has a longer half-life, but both are used diagnostically.

CRH is secreted into the hypophyseal portal blood, where it binds to specific type I CRH receptors on anterior pituitary corticotrophs[63] to stimulate *POMC* transcription through a process that includes activation of adenylyl cyclase. It is unclear whether hypothalamic CRH contributes in any way to circulating levels; CRH is also synthesized in other tissues, and it is likely that circulating CRH reflects synthesis from testis, gastrointestinal tract, adrenal medulla, and particularly the placenta,[64] in which the increased secretion across pregnancy results in a threefold increase in circulating CRH levels.[65] In the circulation, CRH is bound to CRH-binding protein (CRH-BP); levels of CRH-BP also increase during pregnancy so that cortisol secretion is not markedly elevated.[66] CRH is the principal stimulus for ACTH secretion,[67] but AVP is able to potentiate CRH-mediated secretion.[68] In this case, AVP acts through the V_{1b} receptor to activate protein kinase C. The peak response of ACTH to CRH does not differ across the day, but it is affected by endogenous function of the HPA axis in that responsiveness is reduced in subjects treated with corticosteroids but increased in subjects with Cushing disease. Other reported ACTH secretagogues, including angiotensin II, cholecystokinin, atrial natriuretic factor, and vasoactive peptides, probably act to modulate the CRH control of ACTH secretion.[69]



• **Fig. 15.7** Normal negative feedback regulation of cortisol and aldosterone secretion. (A) Hypothalamic-pituitary-adrenal axis. Adrenocorticotropic hormone (ACTH) is secreted from the anterior pituitary under the influence of two principal secretagogues, corticotropin-releasing hormone (CRH) and arginine vasopressin; other factors, including cytokines, also play a role. CRH secretion is regulated by an inbuilt circadian rhythm and by additional stressors operating through the hypothalamus. Secretion of CRH and ACTH is inhibited by cortisol, highlighting the importance of negative feedback control. (B) Renin-angiotensin-aldosterone system (RAAS). Renin is secreted from the juxtaglomerular cells in the kidney dependent on renal arterial blood pressure. Renin converts angiotensinogen to angiotensin I, which is converted in the lungs by angiotensin-converting enzyme (ACE) into angiotensin II. Angiotensin stimulates adrenal aldosterone synthesis. Extracellular fraction (ECF) of potassium has an important direct inhibitory influence on aldosterone secretion. *AVP,* arginine vasopressin (antidiuretic hormone); *ANP,* atrial natriuretic peptide.

The Stress Response and Immune-Endocrine Axis

The proinflammatory cytokines, notably interleukin 1 (IL1), IL6, and tumor necrosis factor-α, also increase ACTH secretion, either directly or by augmenting the effect of CRH.[70,71] Leukemia inhibitory factor (LIF), a cytokine of the IL6 family, is a further activator of the HPA axis.[72] This explains the response of the HPA axis to an inflammatory stimulus and is an important immune-endocrine interaction (see Chapter 7). Physical stresses increase ACTH and cortisol secretion, again through central actions mediated via CRH and AVP. Cortisol secretion rises in response to fever, surgery,[73] burn injury,[74] hypoglycemia,[75] hypotension, and exercise.[76] In all of these cases, this increased secretion can be viewed as a normal counterregulatory response to the insult. Acute psychologic stress raises cortisol levels,[77] but secretion rates appear to be normal in patients with chronic anxiety states and underlying psychotic illness. However, depression is associated with high circulating cortisol concentrations, and this is an important consideration in the differential diagnosis of Cushing syndrome (see later discussion).

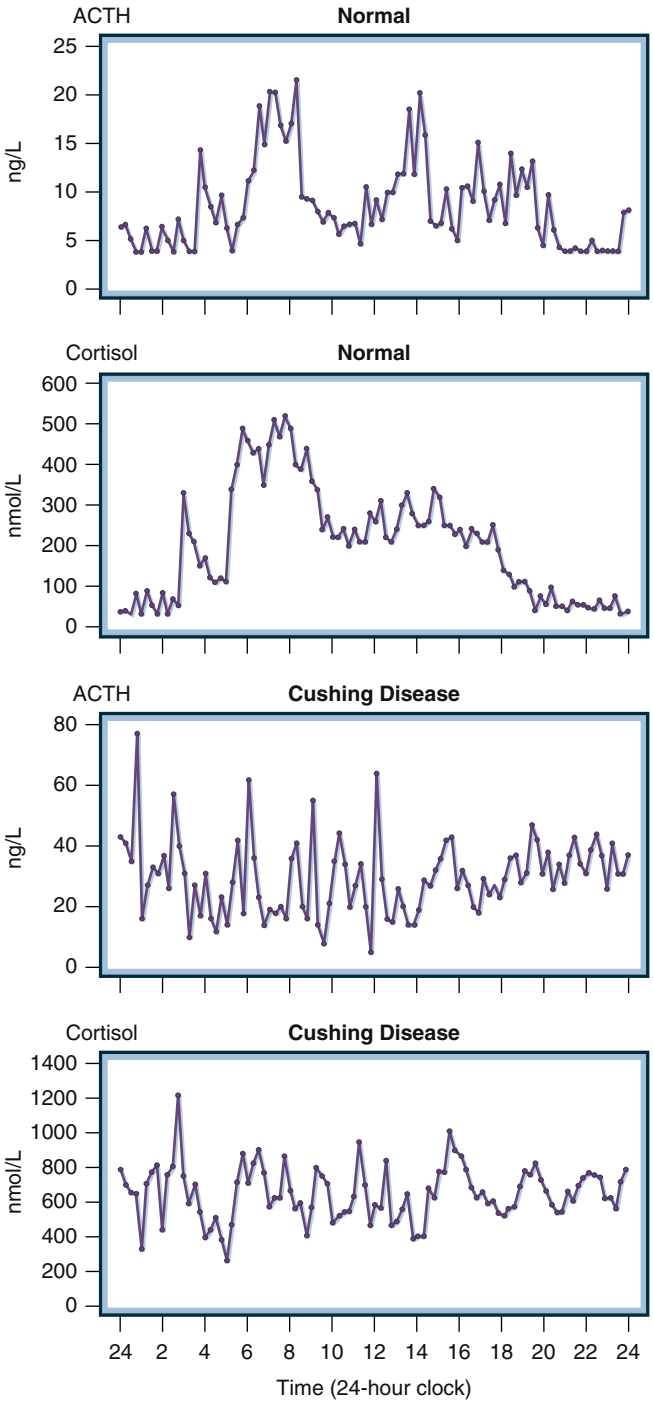
Circadian Rhythm

ACTH, and hence cortisol, is secreted in a pulsatile fashion with a circadian rhythm; levels are highest on awakening and decline throughout the day, reaching nadir values in the evening (Fig. 15.8).[78] The average ACTH pulse frequency is higher in normal adult men compared with women (18 vs. 10 pulses/24 hours, respectively). The circadian ACTH rhythm appears to be mediated principally by an increased ACTH pulse amplitude occurring between 5 and 9 AM but also by a reduction in ACTH pulse frequency occurring between 6 PM and midnight.[79,80] Food ingestion is a further stimulus to ACTH secretion. An ultradian rhythm overlies the circadian and appears to be driven by an oscillator created between the secretion of ACTH, the short delay in response at the adrenal, and the subsequent negative feedback by cortisol at the hypothalamus and pituitary.[81]

Circadian rhythm is dependent on both day-night[82] and sleep-wake[83] patterns and is disrupted by alternating day-night shift work and by long-distance travel across time zones.[84] It can take up to 2 weeks for the circadian rhythm to reset to an altered day-night cycle.

Negative Feedback

An important aspect of CRH and ACTH secretion is the negative feedback control exerted by glucocorticoids themselves. Glucocorticoids inhibit *POMC* transcription in the anterior pituitary[57] and CRH and AVP mRNA synthesis and secretion in the hypothalamus.[85,86] Annexin 1 (formerly called *lipocortin 1*) may also play a critical role in effecting the negative feedback of



• **Fig. 15.8** Circadian and pulsatile secretion of adrenocorticotropic hormone (ACTH) and cortisol in a normal subject *(top two panels)* and in a patient with Cushing disease. In a normal subject, secretion of ACTH and cortisol is highest in early morning and falls to a nadir at midnight. ACTH pulse frequency and pulse amplitude are increased in Cushing disease, and circadian rhythmic secretion is lost.

glucocorticoids on ACTH and CRH release.[87] The negative feedback effect depends on the dose, potency, half-life, and duration of administration of the glucocorticoid and has important physiologic and diagnostic consequences. Suppression of the HPA axis by pharmacologic corticosteroids may persist for many months after cessation of therapy, and adrenocortical insufficiency should be anticipated. Diagnostically, the feedback mechanism explains ACTH hypersecretion in Addison disease, as well as undetectable ACTH levels in patients with a cortisol-secreting adrenal adenoma. Feedback inhibition is principally mediated via the glucocorticoid receptor (GR); patients with glucocorticoid resistance resulting from mutations in the GR[88] and mice lacking the GR gene *(Nr3c1)*[89] have ACTH and cortisol hypersecretion due to perceived lack of negative feedback.

The ACTH Receptor and ACTH Effects on the Adrenal Gland

ACTH binds to a G protein–coupled, melanocortin-2 receptor (MC2R),[90] of which there are approximately 3500 on each adrenocortical cell. Melanocortin-2 receptor accessory protein (MRAP) is required for correct localization and signaling of MC2R.[91] Current data suggest that MRAP might promote three different activities: as a chaperone assisting correct folding of MC2R in the endoplasmic reticulum, as an accessory protein essential for trafficking of MC2R to the plasma membrane, and as a coreceptor enabling MC2R to bind or to signal ACTH response.[92] Downstream signal transduction is mediated principally through the stimulation of adenylyl cyclase and intracellular cAMP,[93] although both extracellular and intracellular Ca^{2+} play a role.[94] Other factors synergize with or inhibit the effects of ACTH on the adrenal cortex, including angiotensin II, activin, inhibin, and cytokines (tumor necrosis factor-α and leptin).[95] Cell-to-cell communication via gap junctions is also important in mediating the effects of ACTH.[96]

ACTH produces both immediate and chronic effects on the adrenal gland; the end result is the stimulation of adrenal steroidogenesis and growth. Acutely, steroidogenesis is stimulated through a StAR-mediated increase in cholesterol delivery to the P450 11A1 enzyme in the inner mitochondrial membrane.[24] Chronically (within 24–26 hours of exposure), ACTH acts to increase the synthesis of all steroidogenic CYP enzymes (P450 11A1, P450 17A1, P450 21A2, P450 11B1) in addition to adrenodoxin,[97,98] the effects of which are mediated at the transcriptional level. ACTH increases synthesis of the LDL and HDL receptors and possibly also synthesis of 3-hydroxy-3-methylglutaryl (HMG)–CoA reductase, the rate-limiting step in cholesterol biosynthesis. ACTH increases adrenal weight by inducing both hyperplasia and hypertrophy. Adrenal atrophy is a feature of ACTH deficiency.

Mineralocorticoid Secretion: The Renin-Angiotensin-Aldosterone Axis

Aldosterone is secreted from the ZG under the control of three principal secretagogues: angiotensin II, potassium, and, to a lesser extent, ACTH (see Fig. 15.7). Other factors, notably somatostatin, heparin, atrial natriuretic factor, and dopamine, can directly inhibit aldosterone synthesis. The secretion of aldosterone and its intermediary 18-hydroxylated metabolites is restricted to the ZG because of the zone-specific expression of P450 11B2 (aldosterone synthase).[99] Corticosterone and DOC, synthesized in both the ZF and ZG, can act as mineralocorticoids, which becomes significant in some clinical diseases, notably some forms of congenital adrenal hyperplasia (CAH) and adrenal tumors. Similarly, it is now established that cortisol can act as a mineralocorticoid in the setting of impaired metabolism of cortisol to cortisone by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2); this is important in patients with hypertension, ectopic ACTH syndrome, or renal disease. The renin-angiotensin system is described in detail in Chapter 16.

Angiotensin II and potassium stimulate aldosterone secretion principally by increasing the transcription of *CYP11B2* through common intracellular signaling pathways. cAMP response elements in the 5′ region of the *CYP11B2* gene are activated after an increase in intracellular Ca^{2+} and activation of calmodulin kinases. The potassium effect is mediated through membrane depolarization and opening of calcium channels and the angiotensin II effect after binding of angiotensin II to the surface AT_1 receptor and activation of phospholipase C.[99]

The effect of ACTH on aldosterone secretion is modest and differs in the acute and chronic situation (see Chapter 16). An acute bolus of ACTH will increase aldosterone secretion, principally by stimulating the early pathways of adrenal steroidogenesis (see earlier discussion), but circulating levels increase by no more than 10% to 20% above baseline values. ACTH has no effect on *CYP11B2* gene transcription or enzyme activity. Chronic continual ACTH stimulation has either no effect or an inhibitory effect on aldosterone production, possibly because of receptor downregulation or suppression of angiotensin II–stimulated secretion because of a mineralocorticoid effect of cortisol, DOC, or corticosterone. Dopamine and atrial natriuretic peptide inhibit aldosterone secretion, as does heparin.

These separate lines of control—through the HPA axis for glucocorticoid biosynthesis and via the renin-angiotensin system for mineralocorticoid synthesis—have important clinical consequences. Patients with primary adrenal failure invariably have both cortisol and aldosterone deficiency, whereas patients with ACTH deficiency due to pituitary disease have glucocorticoid deficiency but normal aldosterone concentrations because the renin-angiotensin system is intact.

Adrenal Androgen Secretion

Adrenal-derived androgens represent an important component (>50%) of circulating androgens in premenopausal women.[100] In men, this contribution is much smaller because of the testicular production of androgens, but adrenal androgen excess even in men may be of clinical significance, notably in patients with CAH, which results in a suppression of the hypothalamic-pituitary-gonadal axis. The adult adrenal secretes approximately 4 mg per day of DHEA, 7 to 15 mg per day of DHEAS, 1.5 mg of androstenedione (AD), and 0.05 mg per day of testosterone. More recently it has been recognized that the 11-oxygenated 19-carbon androgens are important adrenal androgens, with 11β-hydroxyandrostenedione (11OHAD) being the most highly secreted, being derived from AD via the actions of P450 11B1.[101] 11-ketotestosterone (11KT) is derived from 11OHAD following oxidation and reduction, and has equimolar affinity at the androgen receptor as testosterone. These androgens, and 11-ketoandrostendione and 11β-hydroxytestosterone, are significantly increased in patients with CAH due to 21-hydroxylase deficiency and are adrenal-specific biomarkers of androgen excess.[102]

DHEA is a crucial precursor of human sex steroid biosynthesis and exerts androgenic or estrogenic activity after conversion by the activities of 3βHSD, 17βHSD, and aromatase; these enzymes are expressed in peripheral target tissues, a fact that is of clinical importance in many diseases.[103] Some studies have postulated direct effects of DHEA acting as a classic hormone in peripheral tissues. Specific plasma membrane receptors have been identified but await full characterization.[104] Conventionally, desulfated DHEA is thought to be converted downstream to a biologically active hormone. Serum DHEAS was previously thought to represent a circulating storage pool for DHEA regeneration, but it was later suggested that conversion of DHEAS to DHEA by steroid sulfatase plays a minor role in adult physiology and that the equilibrium between serum DHEA and DHEAS is mainly regulated by SULT2A1 activity. This implies that serum DHEAS may not always appropriately reflect the active DHEA pool, particularly if SULT2A1 activity is impaired, as in the inflammatory stress response.[105]

ACTH stimulates androgen secretion; DHEA (but not DHEAS because of its increased plasma half-life) and androstenedione demonstrate a circadian rhythm similar to that of cortisol.[106] However, there are many discrepancies between adrenal androgen and glucocorticoid secretion, leading to the suggestion of an additional cortical androgen-stimulating hormone (CASH). Many putative CASHs have been proposed, including POMC derivatives such as joining peptide, prolactin, and insulin-like growth factor type 1 (IGF1), but conclusive proof is lacking. Efficient adrenal steroidogenesis toward androgen synthesis is crucially dependent on the relative activities of 3βHSD and 17α-hydroxylase and, in particular, on the 17,20-lyase activity of 17α-hydroxylase. Factors that determine whether the 17-hydroxylated substrates will undergo 21-hydroxylation to form glucocorticoid or side-chain cleavage by 17α-hydroxylase to form DHEA and androstenedione are unresolved and seem likely to be important in defining the activity of any putative CASH (Table 15.4).

TABLE 15.4 Dissociation of Adrenal Androgen and Glucocorticoid Secretion: Evidence for an Adrenal-Stimulating Hormone

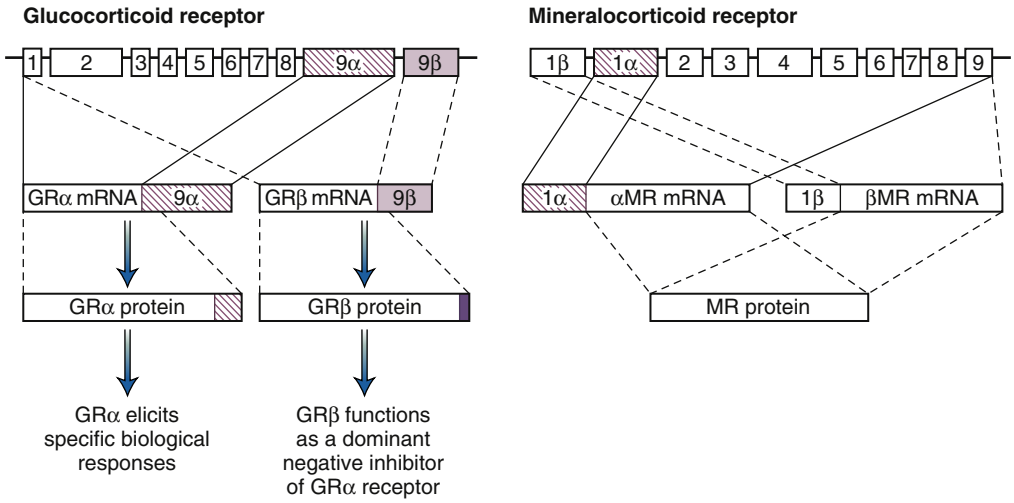
Dexamethasone studies: Complete cortisol suppression with chronic high-dose dexamethasone; DHEA falls by only 20% (lower sensitivity of DHEA to acute low-dose dexamethasone administration causing ACTH suppression).
Adrenarche: Clinically significant rise in circulating DHEA at 6–8 years of age; cortisol production unaltered.
Aging: Reduction in DHEA production; no change in cortisol.
Anorexia nervosa and illness: Fall in DHEA, no change (or increase) in cortisol.

DHEA, Dehydroepiandrosterone.

Corticosteroid Hormone Action

Receptors and Gene Transcription

Both cortisol and aldosterone exert their effects after uptake of free hormone from the circulation and binding to intracellular receptors; these are termed, respectively, the *glucocorticoid receptor* (GR, encoded by *NR3C1*) and the *mineralocorticoid receptor* (MR, encoded by *NR3C2*).[107–109] These are members of the thyroid/steroid hormone receptor superfamily of transcription factors; they consist of a carboxy-terminal ligand-binding domain, a central DNA-binding domain that interacts with specific DNA sequences on target genes, and an amino-terminal hypervariable region. Although only single genes encode the GR and MR, splice variants (i.e., GRα and GRβ) have been described in both receptor types; this, together with tissue-specific post-translational modification (phosphorylation, sumoylation, and ubiquitination), is thought to account for many of the diverse actions of corticosteroids (Fig. 15.9).[110,111]



• **Fig. 15.9** Schematic structure of the human genes encoding the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). In both cases, splice variants have been described. In the case of the GR, there is evidence that the GRβ isoform can act as a dominant negative inhibitor of GRα action. *mRNA,* messenger ribonucleic acid.

Glucocorticoid hormone action has been studied in more depth than mineralocorticoid action. The binding of steroid to the GRα in the cytosol results in activation of the steroid-receptor complex through a process that involves the dissociation of heat shock proteins (HSP90 and HSP70).[112] Following translocation to the nucleus, gene transcription is stimulated or repressed after binding of the dimerized GR-ligand complex to a specific DNA sequence in the promoter regions of target genes.[113] This sequence, known as the *glucocorticoid-response element* (GRE), is invariably a palindromic CGTACAnnnTGTACT sequence that binds with high affinity to two loops of DNA within the DNA-binding domain of the GR (zinc fingers). This stabilizes the RNA polymerase II complex, facilitating gene transcription. The GRβ variant may act as a dominant negative regulator of GRα transactivation.[110]

Naturally occurring mutations in the GR (as seen in patients with glucocorticoid resistance) and GR mutants generated in vitro have highlighted critical regions of the receptor that are responsible for binding and transactivation,[114] but numerous other factors are required (e.g., coactivators, corepressors[115]), and this may make responses tissue specific. This is a rapidly evolving field and beyond the scope of this chapter. However, the interaction between GR and two particular transcription factors are important in mediating the anti-inflammatory effects of glucocorticoids and explain the effect of glucocorticoids on genes that do not contain obvious GREs in their promoter regions.[116] Activator protein 1 (AP1) comprises Fos and Jun subunits and is a proinflammatory transcription factor induced by a series of cytokines and phorbol ester. The GR-ligand complex can bind to c-Jun and prevent interaction with the AP1 site, thereby mediating the so-called transrepressive effects of glucocorticoids.[117] Similarly, functional antagonism exists between the GR and nuclear factor-κB (NF-κB), a ubiquitously expressed transcription factor that activates a series of genes involved in lymphocyte development, inflammatory response, host defense, and apoptosis (Fig. 15.10).[118] In keeping with the diverse array of actions of cortisol, many hundreds of glucocorticoid-responsive genes have been identified. Some glucocorticoid-induced genes and repressed genes are listed in Table 15.5.

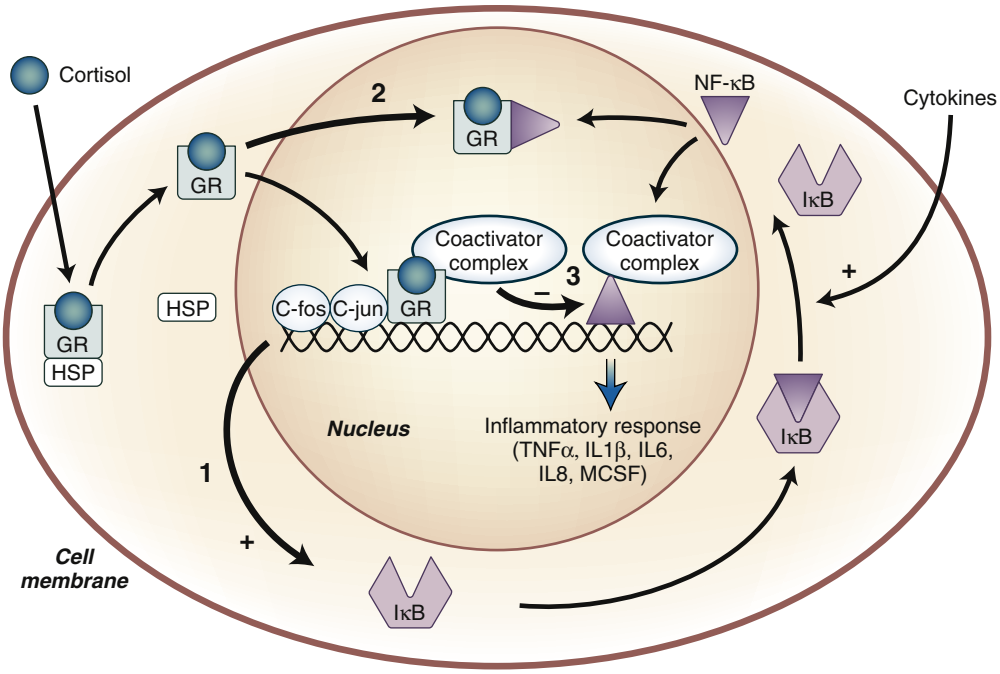
In contrast to the diverse actions of glucocorticoids, mineralocorticoids have a more restricted role, principally stimulation of epithelial sodium transport in the distal nephron, distal colon, and salivary glands.[119] This action is mediated through induction of the apical sodium channel (comprising three subunits—α, β, and γ)[120] and the α_1 and β_1 subunits of the basolateral sodium-potassium adenosine triphosphatase pump (Na^+/K^+-ATPase)[121] through transcriptional regulation of serum-induced and glucocorticoid-induced kinase (SGK).[122] Aldosterone binds to the MR, principally in the cytosol (although there is evidence for expression of the unliganded MR in the nucleus), and the hormone-receptor complex is then translocated to the nucleus (Fig. 15.11).

The MR and GR share considerable homology—57% in the steroid-binding domain and 94% in the DNA-binding domain. It is perhaps not surprising, therefore, that there is promiscuity of ligand binding, with aldosterone (and the synthetic mineralocorticoid, fludrocortisone) binding to the GR and cortisol binding to the MR. For the MR, this is particularly impressive: in vitro, the MR has the same inherent affinity for aldosterone, corticosterone, or cortisol.[108] Specificity on the MR is conferred through the "prereceptor" metabolism of cortisol via the enzyme 11βHSD2, which converts cortisol and corticosterone to inactive 11-keto metabolites, enabling aldosterone to bind to the MR.[123,124] Mineralocorticoid hormone action was extended beyond this classic action in sodium-transporting epithelia with the demonstration that aldosterone can induce cardiac fibrosis and inflammatory changes in renal vasculature. The underlying signaling pathways remain to be fully clarified, but the effects are reversible with MR antagonists.[125]

Finally, for both glucocorticoids and mineralocorticoids, there is accumulating evidence for so-called nongenomic effects involving hormone response obviating the genomic GR or MR. A series of responses have been reported to occur within seconds or minutes after exposure to corticosteroids and are thought to be mediated by as yet uncharacterized membrane-coupled receptors.[126–128]

Corticosteroid-Binding Globulin and Corticosteroid Hormone Metabolism

More than 90% of circulating cortisol is bound predominantly to the α_2-globulin, corticosteroid-binding globulin (CBG).[129] This 383–amino acid protein is synthesized in the liver and binds



• **Fig. 15.10** The anti-inflammatory action of glucocorticoids. Cortisol binds to the cytoplasmic glucocorticoid receptor (GR). Conformational changes in the receptor-ligand complex result in dissociation from heat shock proteins (HSP70 and HSP90) and migration to the nucleus. Binding occurs to specific DNA motifs—glucocorticoid response elements—in association with the activator protein 1 (AP1) comprising C-fos and C-jun. Glucocorticoids mediate their anti-inflammatory effects through several mechanisms: (1) the inhibitory protein IκB, which binds and inactivates nuclear factor-κB (NF-κB), is induced; (2) the GR-cortisol complex is able to bind NF-κB and thereby prevent initiation of an inflammatory process; (3) GR and NF-κB compete for the limited availability of coactivators, which include cyclic adenosine monophosphate response element–binding protein (CREB) and steroid receptor coactivator-1. *IL,* interleukin; *MCSF,* macrophage colony-stimulating factor; *TNFα,* tumor necrosis factor-α.

TABLE 15.5 Some of the Genes Regulated by Glucocorticoids or Glucocorticoid Receptors

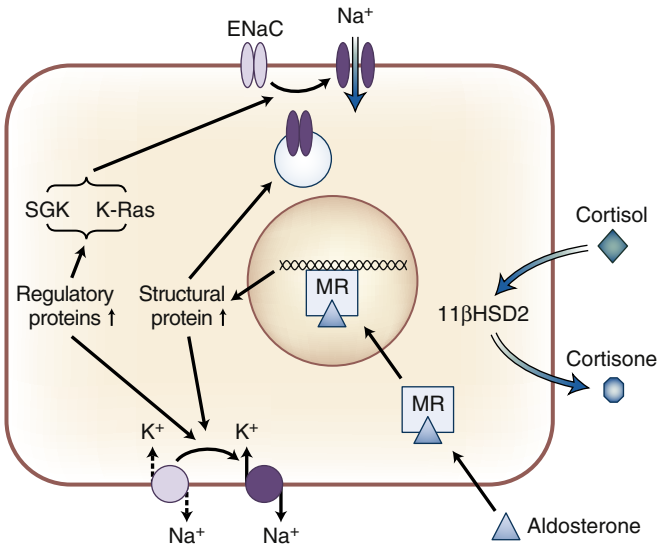
Site of Action	Induced Genes	Repressed Genes
Immune system	IκB (nuclear factor-κB inhibitor) Haptoglobin T-cell receptor (TCR)–ζ p21, p27, and p57 Lipocortin	Interleukins Tumor necrosis factor-α (TNFα) Interferon-γ E-selectin Intercellular adhesion molecule-1 Cyclooxygenase 2 Inducible nitric oxide synthase (iNOS)
Metabolic	PPAR-γ Tyrosine aminotransferase Glutamine synthase Glycogen synthase Glucose-6-phosphatase PEPCK Leptin γ-Fibrinogen Cholesterol 7α-hydroxylase C/EBP/β	Tryptophan hydroxylase Metalloprotease
Bone	Androgen receptor Calcitonin receptor Alkaline phosphatase IGFBP6	Osteocalcin Collagenase
Channels and transporters	ENaCα, ENaCβ, and ENaCγ SGK Aquaporin 1	

TABLE 15.5 Some of the Genes Regulated by Glucocorticoids or Glucocorticoid Receptors—cont'd

Site of Action	Induced Genes	Repressed Genes
Endocrine	Basic fibroblast growth factor (bFGF) Vasoactive intestinal peptide Endothelin Retinoid X receptor GHRH receptor Natriuretic peptide receptors	Glucocorticoid receptor Prolactin POMC/CRH PTHrP Vasopressin
Growth and development	Surfactant proteins A, B, and C	Fibronectin α-Fetoprotein Nerve growth factor Erythropoietin G1 cyclins Cyclin-dependent kinases

CRH, Corticotropin-releasing hormone; *C/EBP/β*, CAAT-enhancer binding protein-β; *ENaC*, epithelial sodium channel; *GHRH*, growth hormone–releasing hormone; *IGFBP6*, insulin-like growth factor–binding protein 6; *PEPCK*, phosphoenolpyruvate carboxykinase; *POMC*, pro-opiomelanocortin; *PPAR*, peroxisome proliferator-activated receptor; *PTHrP*, parathyroid hormone-related protein; *SGK*, serum- and glucocorticoid-induced kinase.

Modified from McKay LI, Cidlowski JA. Molecular control of immune/inflammatory responses: interactions between nuclear factor-κB and steroid receptor-signalling pathways. *Endocr Rev.* 1999;20:435–459.



• **Fig. 15.11** Mineralocorticoid hormone action. An epithelial cell in the distal nephron or distal colon is depicted. The much higher concentrations of cortisol are inactivated by the type 2 isozyme of 11β-hydroxysteroid dehydrogenase (11βHSD2) to cortisone, permitting the endogenous ligand, aldosterone, to bind to the mineralocorticoid receptor (MR). Relatively few mineralocorticoid target genes have been identified, but they include serum-induced and glucocorticoid-induced kinase (SGK), subunits of the epithelial sodium channel (ENaC), and basolateral Na^+/K^+-adenosine triphosphatase.

cortisol with high affinity. Affinity for synthetic corticosteroids is negligible except for prednisolone, which has an affinity for CBG approximately half that of cortisol. Circulating CBG concentrations are approximately 700 nmol/L. Levels are increased by estrogens and in some patients with chronic active hepatitis; they are reduced by glucocorticoids and in patients with cirrhosis, nephrosis, and hyperthyroidism. The estrogen effect can be marked, with levels increasing twofold to threefold across pregnancy, a fact that should be taken into account when measuring plasma total cortisol in pregnancy and in women taking estrogens.

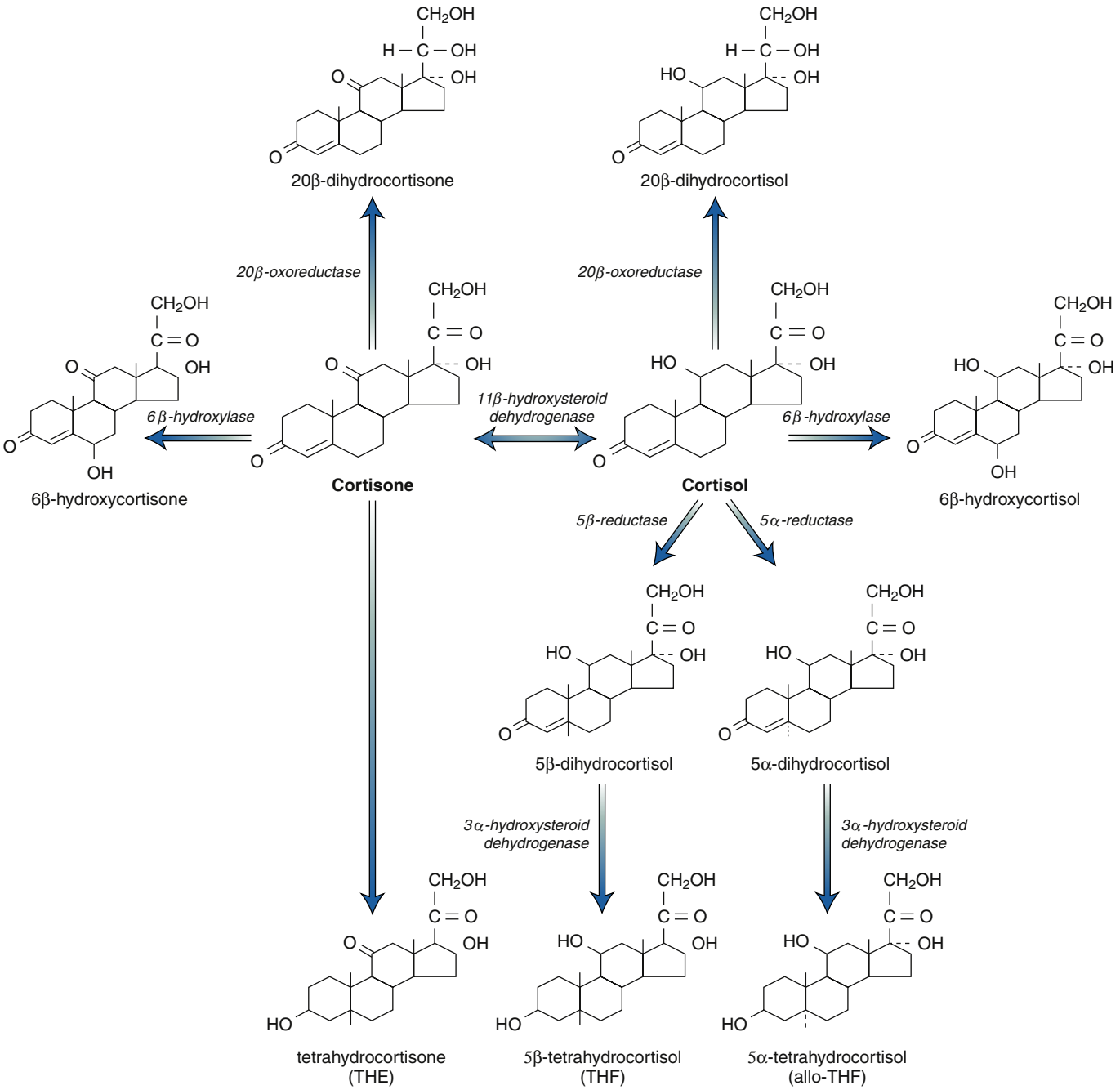
CBG plays a key role in determining circulating cortisol levels.[130] Inherited abnormalities in CBG synthesis are much rarer than those described for thyroxine-binding globulin but include cases of elevated CBG, partial or complete deficiency of CBG, and CBG variants with reduced affinity for cortisol.[131,132] In each case, alterations in CBG concentrations change the total circulating cortisol concentrations accordingly, but free cortisol concentrations are normal. Only this free circulating fraction is available for transport into tissues for biologic activity. The excretion of free cortisol through the kidneys results in *urinary free cortisol*, which represents less than 1% of the total cortisol secretion.

The circulating half-life of cortisol varies between 70 and 120 minutes. The major steps in cortisol metabolism are depicted in Fig. 15.12[133] and can be summarized as follows:

- Interconversion of the 11-hydroxyl group (cortisol, Kendall compound F) to the 11-oxo group (cortisone, compound E) through activity of the 11βHSD system (EC 1.1.1.146).[134,135] The metabolism of cortisol and that of cortisone then follow similar pathways.
- Reduction of the C4-5 double bond to form dihydrocortisol or dihydrocortisone, followed by reduction of the 3-oxo group to form tetrahydrocortisol (THF) or tetrahydrocortisone (THE). The reduction of the C4-5 double bond can be carried out by either 5β-reductase or 5α-reductase, yielding, respectively, 5β-tetrahydrocortisol (THF) and 5α-THF (allo-THF). In normal subjects, the ratio of THF to allo-THF is 2:1. THF, allo-THF, and THE are rapidly conjugated with glucuronic acid and excreted in the urine.
- Further reduction of the 20-oxo group by either 20αHSD or 20βHSD to yield α-cortols and β-cortols and cortolones from cortisol and cortisone, respectively. Reduction of the C20 position may also occur without A-ring reduction, giving rise to 20α-hydroxycortisol and 20β-hydroxycortisol.
- Hydroxylation at C6 primarily by P450 3A4 to form 6β-hydroxycortisol.
- Cleavage of THF and THE to the C19 steroids, 11-hydroxyandrosterone or 11-oxo-androsterone, or etiocholanolone.
- Oxidation of the C21 position or cortols and cortolones to form the extremely polar metabolites, cortolic and cortolonic acids.

Approximately 50% of secreted cortisol appears in the urine as THF, allo-THF, and THE; 25% as cortols/cortolones; 10% as C19 steroids; and 10% as cortolic/cortolonic acids. The remaining metabolites are free unconjugated steroids (cortisol, cortisone, and their 6β-metabolites, and 20α/20β-metabolites).

The principal site of cortisol metabolism has been considered to be the liver, but many of the enzymes listed have been described in mammalian kidney, notably the interconversion of cortisol to cortisone by 11βHSD2. Quantitatively, this is the most important pathway. Furthermore, the bioactivity of glucocorticoids is in part related to the hydroxyl group at C11; because cortisone with a C11-oxo group is an inactive steroid, expression of 11βHSD in peripheral tissues plays a crucial role in regulating corticosteroid hormone action. Two distinct 11βHSD isozymes have been reported: type 1, reduced nicotinamide adenine dinucleotide phosphate (NADPH)–dependent oxo-reductase expressed principally in the liver, which confers bioactivity on orally administered cortisone by converting it to cortisol,[135] and a type 2, nicotinamide adenine dinucleotide (NAD)–dependent dehydrogenase. It is the 11βHSD2, coexpressed with the MR in



• **Fig. 15.12** The principal pathways of cortisol metabolism. Interconversion of hormonally active cortisol to inactive cortisone is catalyzed by two isozymes of 11β-hydroxysteroid dehydrogenase (11βHSD), with 11βHSD1 principally converting cortisone to cortisol and 11βHSD2 doing the reverse. Cortisol can be hydroxylated at the C6 and C20 positions. A ring reduction is undertaken by 5α-reductase or 5β-reductase and 3αHSD.

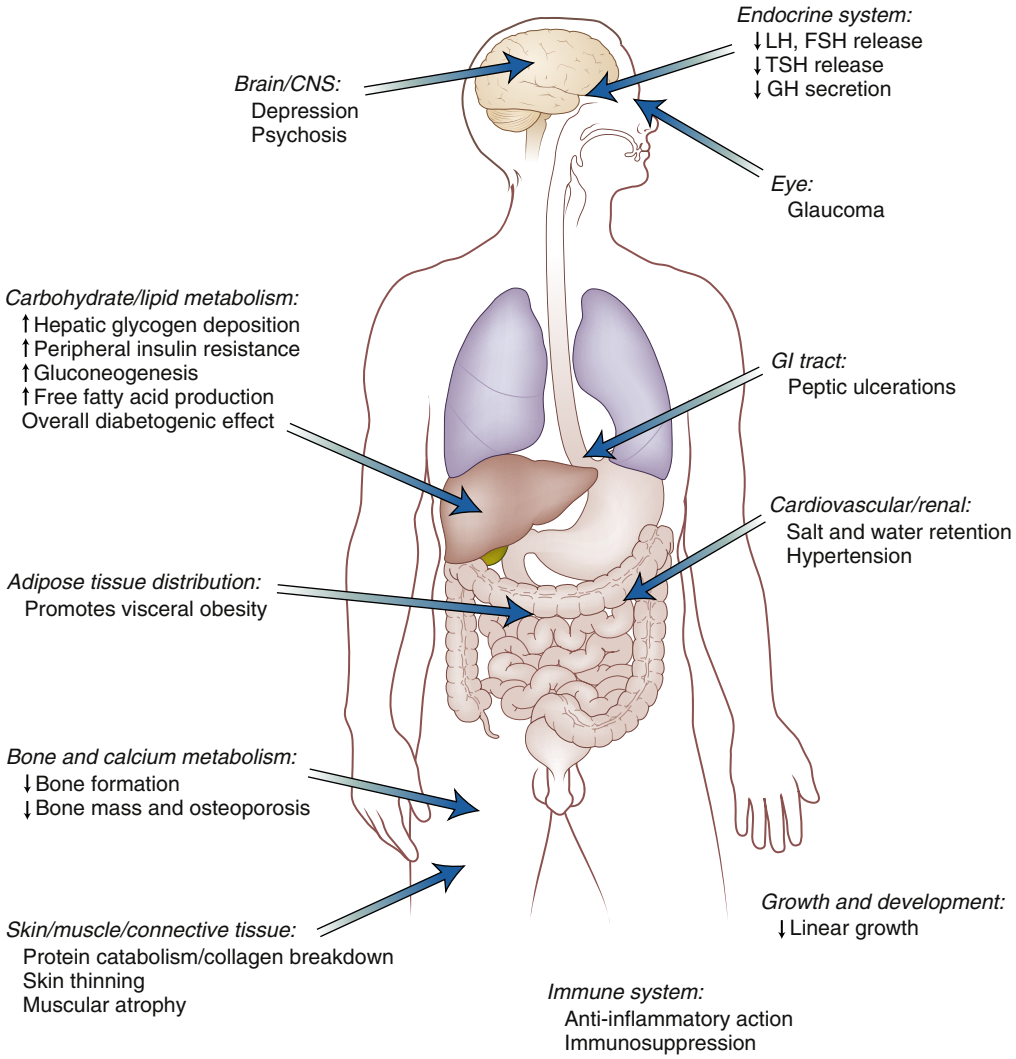
the kidney, colon, and salivary gland, that inactivates cortisol to cortisone and permits aldosterone to bind to the MR in vivo. If this enzyme-protective mechanism is impaired, cortisol is able to act as a mineralocorticoid; this explains some forms of endocrine hypertension (apparent mineralocorticoid excess, licorice ingestion) and the mineralocorticoid excess state that characterizes the ectopic ACTH syndrome.[132,136]

Hyperthyroidism results in increased cortisol metabolism and clearance, and hypothyroidism produces the converse, principally because of an effect of thyroid hormone on hepatic 11βHSD1 and 5α/5β-reductases.[135] IGF1 increases cortisol clearance by inhibiting hepatic 11βHSD1 (conversion of cortisone to cortisol).[137] 6β-Hydroxylation is normally a minor pathway, but cortisol itself induces 6β-hydroxylase activity, so that 6β-hydroxycortisol excretion is markedly increased in patients with Cushing syndrome.[138] Some drugs, notably rifampicin and phenytoin, induce P450 3A4 expression and increase cortisol clearance through this pathway.[139] Patients with renal disease have impaired cortisol clearance because of reduced conversion of renal cortisol to cortisone.[140] These observations have clinical implications for patients with thyroid disease, acromegaly, or renal disease and for patients taking cortisol replacement therapy. Adrenal crisis has been reported in steroid-replaced addisonian patients given rifampicin,[141] and hydrocortisone replacement therapy may need to be increased in treated patients who develop hyperthyroidism or reduced in patients with untreated growth hormone (GH) deficiency.

Aldosterone is also metabolized in the liver and kidneys. In the liver, it undergoes tetrahydro reduction and is excreted in the urine as a tetrahydroaldosterone 3-glucuronide derivative. However, glucuronide conjugation at the 18 position occurs directly in the kidney, as does 3α and 5α/5β metabolism of the free steroid.[142] Because of the aldehyde group at the C18 position, aldosterone is not metabolized by 11βHSD2.[143] Hepatic aldosterone clearance is reduced in patients with cirrhosis, ascites, or severe congestive heart failure.

Effects of Glucocorticoids

The principal sites of action of glucocorticoids and some of the consequences of glucocorticoid excess are shown in Fig. 15.13.



• **Fig. 15.13** The principal sites of action of glucocorticoids in humans, highlighting some of the consequences of glucocorticoid excess. *CNS,* central nervous system; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GI,* gastrointestinal; *LH,* luteinizing hormone; *TSH,* thyroid-stimulating hormone.

Carbohydrate, Protein, and Lipid Metabolism

Glucocorticoids increase blood glucose concentrations through their action on glycogen, protein, and lipid metabolism. In the liver, cortisol stimulates glycogen deposition by increasing glycogen synthase and inhibiting the glycogen-mobilizing enzyme, glycogen phosphorylase.[144] Hepatic glucose output increases through the activation of key enzymes involved in gluconeogenesis, principally glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK).[145,146] In peripheral tissues (e.g., muscle, fat), cortisol inhibits glucose uptake and utilization.[147] In adipose tissue, lipolysis is activated, resulting in the release of free fatty acids into the circulation. An increase in total circulating cholesterol and triglycerides is observed, but HDL cholesterol levels fall. Glucocorticoids also have a permissive effect on other hormones, including catecholamines and glucagon. The result is insulin resistance and an increase in blood glucose concentrations, at the expense of protein and lipid catabolism.

Glucocorticoids stimulate adipocyte differentiation, promoting adipogenesis through the transcriptional activation of key differentiation genes, including lipoprotein lipase, glycerol-3-phosphate dehydrogenase, and leptin.[148] Long-term effects of glucocorticoid excess on adipose tissue are more complex, at least in humans, in whom the deposition of visceral or central adipose tissue is stimulated,[149] providing a useful discriminatory sign for the diagnosis of Cushing syndrome. The predilection for visceral obesity may relate to the increased expression of the GR[150] and 11βHSD1 in omental compared with subcutaneous adipose tissue.[151]

Skin, Muscle, and Connective Tissue

In addition to inducing insulin resistance in muscle tissue, glucocorticoids also cause catabolic changes in muscle, skin, and connective tissue. In the skin and connective tissue, glucocorticoids inhibit epidermal cell division and DNA synthesis and reduce synthesis and production of collagen.[152] In muscle, glucocorticoids cause atrophy (but not necrosis), which seems to be specific for type II (phasic) muscle fibers. Muscle protein synthesis is reduced.

Bone and Calcium Metabolism

Glucocorticoids inhibit osteoblast function, which is thought to account for the osteopenia and osteoporosis that especially affect the axial skeleton and characterize glucocorticoid excess.[153] Up to 1% of Western populations are taking long-term glucocorticoid therapy,[154] and glucocorticoid-induced osteoporosis is becoming a prevalent health concern, affecting 50% of patients treated with corticosteroids for longer than 12 months. However, the complication perhaps most feared by physicians is osteonecrosis. Osteonecrosis (also termed *avascular necrosis*) produces rapid and focal deterioration of bone quality and primarily affects the femoral head, leading to pain and ultimately to collapse of the bone, often necessitating hip replacement. It can affect individuals of any age and may occur with relatively low doses of glucocorticoids (e.g., during corticosteroid replacement therapy for adrenal failure).[155] Importantly, defects may not be detectable on conventional radiographs but are readily seen on magnetic resonance imaging (MRI). Glucocorticoid-induced osteocyte apoptosis has been implicated in the pathogenesis of the condition,[156] and the lack of a direct role for an interrupted blood supply suggests that the term *osteonecrosis* is preferable to *avascular femoral necrosis*. However, there is still no explanation for individual susceptibility.

Glucocorticoids also induce negative calcium balance by inhibiting intestinal calcium absorption and increasing renal calcium excretion. As a consequence, parathyroid secretion is usually increased. In children, glucocorticoids suppress growth, but the increases in body mass index (BMI) are thought to offset a deleterious effect on bone mineral density.[157]

Salt and Water Homeostasis and Blood Pressure Control

Glucocorticoids increase blood pressure by a variety of mechanisms involving actions on the kidney and vasculature.[158] In vascular smooth muscle, they increase sensitivity to pressor agents such as catecholamines and angiotensin II while reducing nitric oxide–mediated endothelial dilatation. Angiotensinogen synthesis is increased by glucocorticoids.[159] In the kidney, depending on the activity of 11βHSD2, cortisol can act on the distal nephron to cause sodium retention and potassium loss (mediated via the MR).[136] Elsewhere across the nephron, glucocorticoids increase the glomerular filtration rate, proximal tubular epithelial sodium transport, and free water clearance.[160] This last effect involves antagonism of the action of vasopressin and explains the dilutional hyponatremia seen in patients with glucocorticoid deficiency.[161]

Anti-inflammatory Actions and the Immune System

Glucocorticoids suppress immunologic responses, and this action has been the stimulus to develop a series of highly potent pharmacologic glucocorticoids to treat a variety of autoimmune and inflammatory conditions. The inhibitory effects are mediated at many levels. In the peripheral blood, glucocorticoids reduce lymphocyte counts acutely (T lymphocytes > B lymphocytes) by redistributing lymphocytes from the intravascular compartment to the spleen, lymph nodes, and bone marrow. Conversely, neutrophil counts increase after glucocorticoid administration. Eosinophil counts rapidly fall, an effect that was historically used as a bioassay for glucocorticoids. The immunologic actions of glucocorticoids involve direct actions on both T and B lymphocytes, including inhibition of immunoglobulin synthesis and stimulation of lymphocyte apoptosis. Inhibition of cytokine production from lymphocytes is mediated through inhibition of the action of NF-κB. NF-κB plays a crucial and generalized role in inducing cytokine gene transcription; glucocorticoids can bind directly to NF-κB to prevent nuclear translocation, and they induce NF-κB inhibitor, which sequesters NF-κB in the cytoplasm, thereby inactivating its effect.[118]

Additional anti-inflammatory effects involve the inhibition of monocyte differentiation into macrophages and macrophage phagocytosis and cytotoxic activity. Glucocorticoids reduce the local inflammatory response by preventing the actions of histamine and plasminogen activators. Prostaglandin synthesis is impaired through the induction of lipocortins, which inhibit phospholipase A2 activity.[162]

Central Nervous System and Mood

Clinical observations of patients with glucocorticoid excess and deficiency reveal that the brain is an important target tissue for glucocorticoids, with depression, euphoria, psychosis, apathy, and lethargy being important manifestations. Both GRs and MRs are expressed in discrete regions of the rodent brain, including hippocampus, hypothalamus, cerebellum, and cortex.[163] Glucocorticoids cause neuronal death, notably in the hippocampus[164]; this effect may underlie the interest in glucocorticoids in relation to cognitive function, memory, and neurodegenerative diseases such as Alzheimer disease.[165] Local blockade of cortisol generation by

11βHSD1 has been shown to improve cognitive function.[166] DHEA has been shown to have neuroprotective effects in the hippocampus region.[167] P450 7B1, an enzyme that metabolizes DHEA to its 7α-hydroxylated metabolite, is highly expressed in brain, but expression was decreased in dentate neurons in the hippocampus.[168]

Eye

In the eye, glucocorticoids act to raise intraocular pressure through an increase in aqueous humor production and deposition of matrix within the trabecular meshwork, which inhibits aqueous drainage. Steroid-induced glaucoma appears to have a genetic predisposition, but the underlying mechanisms are unknown.[169]

Gut

Long-term but not acute administration of glucocorticoids increases the risk of developing peptic ulcer disease.[170] Pancreatitis with fat necrosis is reported in patients with glucocorticoid excess. The GR is expressed throughout the gastrointestinal tract, and the MR is expressed in the distal colon; they mediate the corticosteroid control of epithelial ion transport.

Growth and Development

Although glucocorticoids stimulate transcription of the gene encoding GH in vitro, glucocorticoids in excess inhibit linear skeletal growth,[157,171] probably as a result of catabolic effects on connective tissue, muscle, and bone and through inhibition of the effects of IGF1. The results of experiments on mice lacking the GR gene[89] have emphasized the role of glucocorticoids in normal fetal development. In particular, glucocorticoids stimulate lung maturation through the synthesis of surfactant proteins (SP-A, SP-B, and SP-C),[172] and mice lacking the GR die shortly after birth due to hypoxia from lung atelectasis. Glucocorticoids also stimulate the enzyme phenylethanolamine *N*-methyltransferase (PNMT), which converts norepinephrine to epinephrine in adrenal medulla and chromaffin tissue. Mice lacking the GR do not develop an adrenal medulla.[89] There is also adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency.[173,174]

Endocrine Effects

Glucocorticoids suppress the thyroid axis, probably through a direct action on the secretion of thyroid-stimulating hormone (TSH, thyrotropin). In addition, they inhibit 5′ deiodinase activity that mediates the conversion of thyroxine to active triiodothyronine.

Glucocorticoids also act centrally to inhibit gonadotropin-releasing hormone (GnRH) pulsatility and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).

Therapeutic Corticosteroids

Since the dramatic anti-inflammatory effect of cortisone was first demonstrated in the 1950s, a series of synthetic corticosteroids have been developed for therapeutic purposes. These agents are used to treat a diverse variety of human diseases, principally relying on their anti-inflammatory and immunologic actions (Table 15.6). The main corticosteroids used in clinical practice, together with their relative glucocorticoid and mineralocorticoid potencies, are listed in Table 15.7.

The structures of common synthetic steroids are depicted in Fig. 15.14. The biologic activity of a corticosteroid depends on a delta-4, 3-keto, 11β,17α,21-trihydroxyl configuration.[175] Conversion of the C11 hydroxyl group to a C11 keto group (i.e., cortisol to cortisone) inactivates the steroid. The addition of a 1,2 unsaturated bond to cortisol results in prednisolone, which is four times more potent than cortisol in classic glucocorticoid bioassays such as hepatic glycogen deposition, suppression of eosinophils, and anti-inflammatory actions. Prednisone, widely prescribed in the United States, is the cortisone equivalent of prednisolone and relies on conversion by 11βHSD1 in the liver for bioactivity.[176] Potency is further increased by the addition of a 6α-methyl group to prednisolone (methylprednisolone).

Fludrocortisone is a synthetic mineralocorticoid having 125-fold greater potency than cortisol in stimulating sodium reabsorption. This effect is achieved through the addition of a 9α-fluoro group to cortisol. Fludrocortisone also has glucocorticoid potency (12-fold greater than cortisol). The addition of a 16α-methyl group and 1,2 saturated bond to fludrocortisone results in dexamethasone, a highly potent glucocorticoid (25-fold greater potency than cortisol) that has negligible mineralocorticoid activity.[175,177]

TABLE 15.6 Therapeutic Use of Corticosteroids

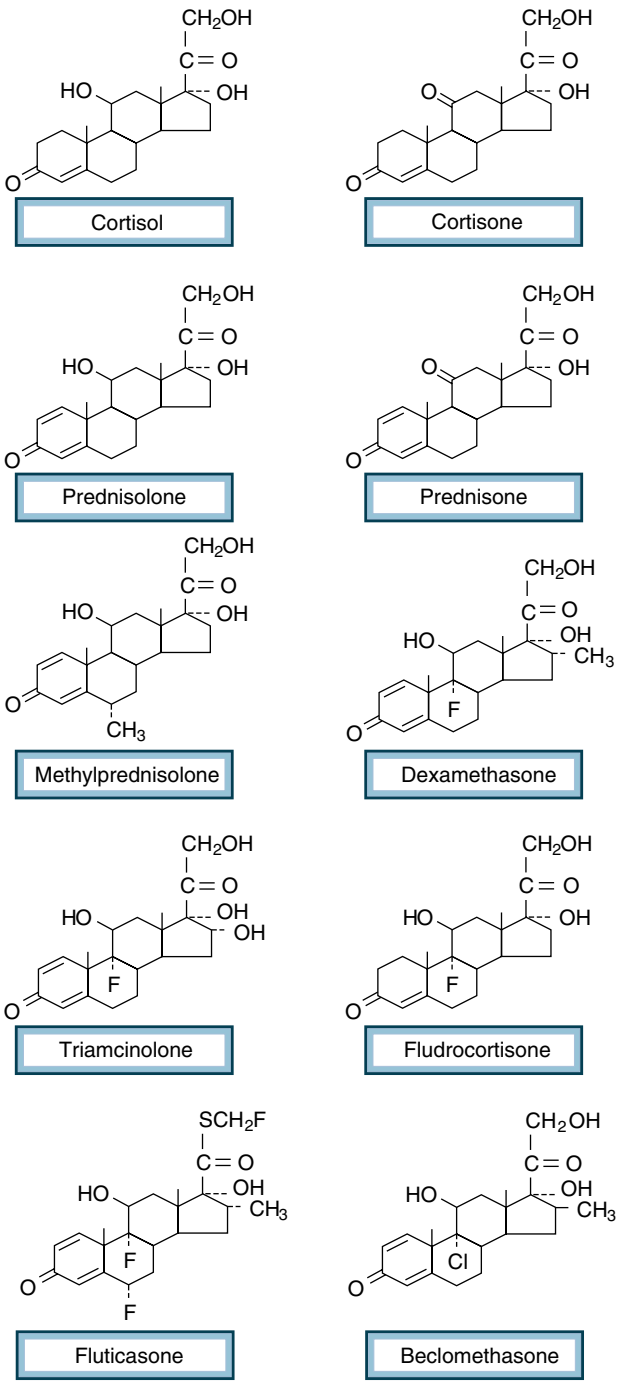
Endocrine: Replacement therapy (Addison disease, pituitary disease, congenital adrenal hyperplasia), Graves orbitopathy
Skin: Dermatitis, pemphigus
Hematology: Leukemia, lymphoma, hemolytic anemia, idiopathic thrombocytopenic purpura
Gastrointestinal: Inflammatory bowel disease (ulcerative colitis, Crohn disease)
Liver: Chronic active hepatitis, transplantation, organ rejection
Renal: Nephrotic syndrome, vasculitides, transplantation, rejection
Central nervous system: Cerebral edema, raised intracranial pressure
Respiratory: Angioedema, anaphylaxis, asthma, sarcoidosis, tuberculosis, obstructive airway disease
Rheumatology: Systemic lupus erythematosus, polyarteritis, temporal arteritis, rheumatoid arthritis
Muscle: Polymyalgia rheumatica, myasthenia gravis

TABLE 15.7 Relative Biologic Potencies of Synthetic Steroids in Bioassay Systems

Steroid	Anti-Inflammatory Action	Hypothalamic-Pituitary-Adrenal Suppression	Salt Retention
Cortisol	1	1	1
Prednisone	3	4	0.75
Prednisolone	3	4	0.75
Methylprednisolone	6.2	4	0.5
Fludrocortisone	12	12	125
Triamcinolone	5	4	0
Dexamethasone	26	17	0

Administration

Widely used synthetic glucocorticoids in respiratory and nasal aerosol sprays are betamethasone, beclomethasone, and fluticasone. Betamethasone has the same structure as dexamethasone but with a 16α-methyl group. Beclomethasone has the same structure as betamethasone apart from the replacement of the 9α-fluoro group with a 9α-chloro group. Fluticasone has the same structure



• **Fig. 15.14** Structures of the natural glucocorticoid cortisol, some of the more commonly prescribed synthetic glucocorticoids, and the mineralocorticoid fludrocortisone. Triamcinolone is identical to dexamethasone except that a 16α-hydroxyl group is substituted for the 16α-methyl group. Betamethasone, another widely used glucocorticoid, has a 16β-methyl group. Beclomethasone is derived from betamethasone by replacement of the 9α-fluoro group with a chloro group. Fluticasone is identical to dexamethasone except that an additional 6α-fluoro group has been added, and the hydroxymethyl group at position 21 has been exchanged by a thiofluoromethyl group.

as dexamethasone with an additional 6α-fluoro group and a 5-fluoromethyl group replacing the hydroxymethyl group.

Corticosteroids are given orally, parenterally, and by numerous topical routes (e.g., eyes, skin, nose, inhalation, rectal suppositories).[177] Unlike hydrocortisone, which has a high affinity for CBG, most synthetic steroids have low affinity for this binding protein and circulate as free steroid (~30%) or bound to albumin (~70%). Circulating half-lives vary depending on individual variability and underlying disease, particularly renal and hepatic impairment. Cortisone acetate should not be used parenterally because it requires metabolism by the liver to active cortisol.

It is beyond the scope of this chapter to describe which steroid should be given and by which route for the nonendocrine conditions listed in Table 15.6. Acute and long-term corticosteroid therapy in patients with hypoadrenalism or CAH is discussed in later sections.

Long-Term Therapy

In addition to the undoubted benefit that corticosteroids provide, there is an increasing incidence of overuse, particularly in patients with respiratory or rheumatologic disease, to such an extent that up to 1% of the population is now prescribed long-term corticosteroid therapy.[154] Because of their established euphoric effect, corticosteroids often make patients feel better but without any objective improvements in underlying disease parameters. In view of the long-term harm of chronic glucocorticoid excess,[178] decisions regarding treatment should be evidence based and subject to regular review based on efficacy and side effects. The endocrinologic consequences of chronic glucocorticoid excess, notably suppression of the HPA axis, are an important aspect of modern clinical practice and are described later (see "Primary and Central Hypoadrenalism"). Endocrinologists need to be aware of the effects of long-term therapy and of steroid withdrawal. Selective glucocorticoid receptor agonists (SEGRAs) are being developed with the aim of dissociating the transrepressive, anti-inflammatory actions of glucocorticoids from the transactivating effects that, by and large, mediate deleterious side effects.[179]

Adrenocortical Diseases

With the exception of common adrenocortical incidentalomas (see upcoming discussion) adrenocortical diseases with a clear clinical phenotype are relatively rare. Their importance lies in their high rates of morbidity and mortality if untreated, coupled with the relative ease of diagnosis and the availability of effective therapy. The diseases are most readily classified on the basis of hormone excess or deficiency (Table 15.8).

Glucocorticoid Excess

Cushing Syndrome

In 1912, Harvey Cushing first described a 23-year-old female with obesity, hirsutism, and amenorrhea, and 20 years later, he postulated that this "polyglandular syndrome" was due to a primary pituitary abnormality causing adrenal hyperplasia.[8] Adrenal tumors were shown to cause the syndrome in some cases,[180] but ectopic ACTH production was not characterized until much later, in 1962.[181] The term *Cushing syndrome* is used to describe all causes, whereas *Cushing disease* is reserved for pituitary-dependent Cushing syndrome.

Cushing syndrome comprises the symptoms and signs associated with prolonged exposure to inappropriately elevated levels of free plasma glucocorticoids. The use of the term *glucocorticoid* in the definition covers excess from both endogenous (cortisol) and exogenous (e.g., prednisolone, dexamethasone) sources. Iatrogenic Cushing syndrome is common,[177,182] occurring to some degree in most patients taking long-term corticosteroid therapy.

TABLE 15.8 Adrenocortical Diseases

Glucocorticoid Excess

Cushing syndrome (pathologic/neoplastic hypercortisolism)
Pseudo-Cushing syndromes (physiologic/nonneoplastic hypercortisolism)

Glucocorticoid Resistance

Glucocorticoid Deficiency

Primary hypoadrenalism
Secondary hypoadrenalism
Postchronic corticosteroid replacement therapy

Congenital Adrenal Hyperplasia

Deficiencies of 21-hydroxylase, 3βHSD, 17α-hydroxylase, 11β-hydroxylase, P450 oxidoreductase, P450 side-chain cleavage, and StAR

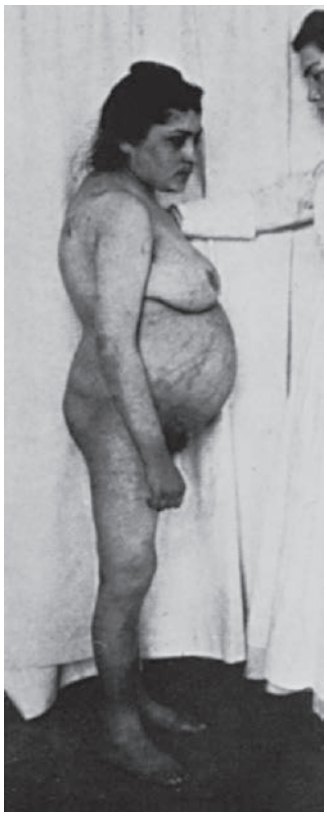
Mineralocorticoid Excess

Mineralocorticoid Deficiency

Defects in aldosterone synthesis
Defects in aldosterone action
Hyporeninemic hypoaldosteronism

Adrenal Incidentalomas, Adenomas, and Carcinomas

HSD, Hydroxysteroid dehydrogenase; *StAR,* steroidogenic acute regulatory (protein).



• **Fig. 15.15** Minnie G., Cushing's index patient, at age 23 years. (From Cushing H. The basophil adenomas of the pituitary body and their clinical manifestations [pituitary basophilism]. *Bull Johns Hopkins Hosp*. 1932;50:137–195.)

Endogenous causes of Cushing syndrome are rare and result in loss of the normal feedback mechanism of the HPA axis and the normal circadian rhythm of cortisol secretion.

The incidence of Cushing disease is estimated to be 2 to 3 cases per 1 million population per year. The incidence of ectopic ACTH syndrome parallels that of bronchogenic carcinoma, and although 0.5% of lung cancer patients have ectopic ACTH syndrome, rapid progression of the underlying disease often precludes an early diagnosis. Cushing disease and adrenal adenomas are four times more common in women, whereas ectopic ACTH syndrome is more common in men. Neuroendocrine tumors have an incidence of 7 to 8 per 100,000 and may be a rare cause of the ectopic ACTH syndrome.

Clinical Features of Cushing Syndrome

The classic features of Cushing syndrome—centripetal obesity, moon face, hirsutism, and plethora—have been well known since Cushing's initial descriptions in 1912 and 1932 (Figs. 15.15, 15.16, and 15.17). However, this gross clinical picture is not always present, and a high index of suspicion is required in many cases. Once the normal physiologic effects of glucocorticoids are appreciated (see Fig. 15.13), the clinical features of glucocorticoid excess are easier to define. They are summarized in Table 15.9, together with the most discriminatory features that will assist in distinguishing Cushing syndrome from simple obesity.[183,184]

Obesity and Weight Gain

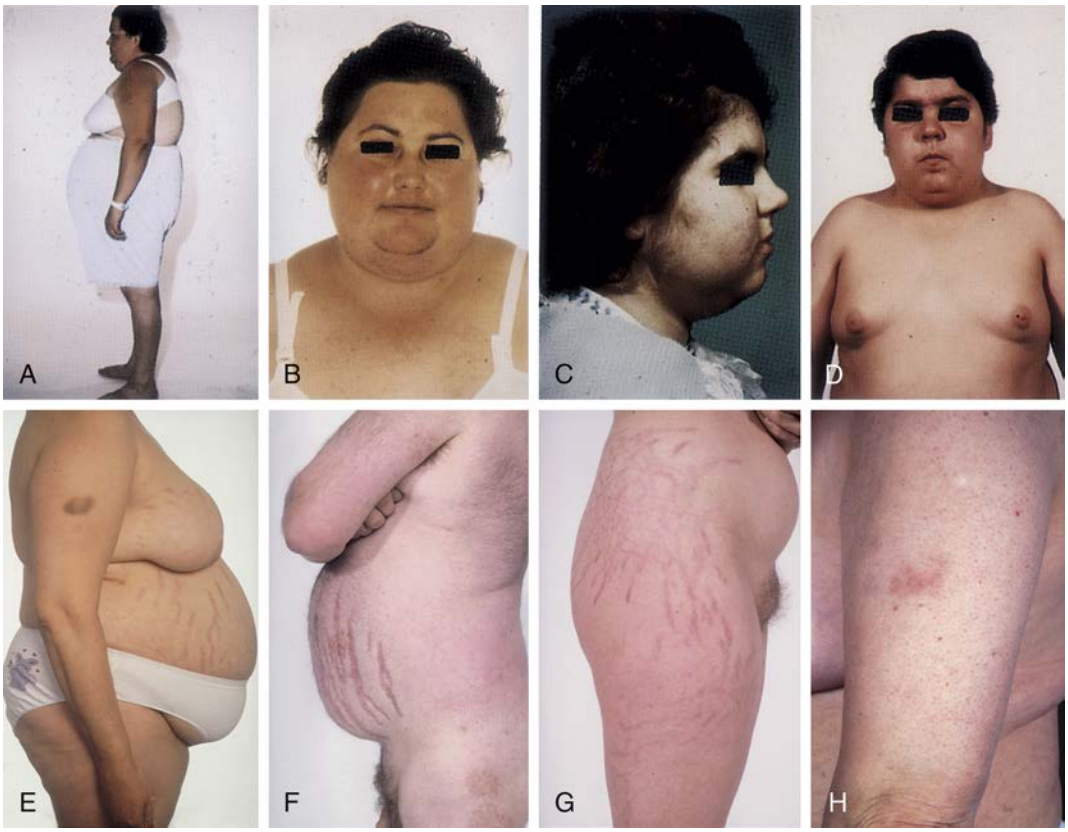
Weight gain and obesity are the most common signs of Cushing syndrome. At least in adults, this weight gain is invariably centripetal in nature.[149,185] In fact, generalized obesity is more common in the general population than it is in patients with Cushing syndrome. One exception is seen in pediatric patients, in whom glucocorticoid excess may result in generalized obesity. In addition to centripetal obesity, patients develop fat depots over the thoracocervical spine (buffalo hump), in the supraclavicular region, and over the cheeks and temporal regions, giving rise to the rounded, moon-like facies. The epidural space, another site of abnormal fat deposition, may lead to neurologic deficits.

Reproductive Organs

Gonadal dysfunction is common, with menstrual irregularity in females and loss of libido in both sexes. Hirsutism is frequently found in female patients, as is acne. The most common form of hirsutism is vellus hypertrichosis on the face; this type should be distinguished from the darker, terminal differentiated hirsutism that may occur because of ACTH-mediated adrenal androgen excess. Hypogonadotropic hypogonadism occurs because of a direct inhibitory effect of cortisol on GnRH pulsatility and LH/FSH secretion, and it is reversible on correction of the hypercortisolism.[186,187]

Psychiatric Features

Psychiatric abnormalities occur in approximately 50% of patients with Cushing syndrome, regardless of cause.[188,189] Agitated depression and lethargy are among the most common problems, but paranoia and overt psychosis are also well recognized. Memory and cognitive function may also be affected, and increased irritability may be an early feature. Insomnia is common, and both rapid eye movement and delta-wave sleep patterns are reduced.[190] Lowering of plasma cortisol by medical or surgical therapy usually results in a rapid improvement in the psychiatric state. Overall quality of life is significantly reduced in patients with Cushing syndrome, particularly affecting physical health and functioning. Quality-of-life scores improve after treatment but do not return to normal.[191]



• **Fig. 15.16** Clinical features of Cushing syndrome. (A) Centripetal and some generalized obesity and dorsal kyphosis in a 30-year-old woman with Cushing disease. (B) Same patient as in (A), showing moon facies, plethora, hirsutism, and enlarged supraclavicular fat pads. (C) Facial rounding, hirsutism, and acne in a 14-year-old girl with Cushing disease. (D) Central and generalized obesity and moon facies in a 14-year-old boy with Cushing disease. (E, F) Typical centripetal obesity with livid abdominal striae seen in a 41-year-old woman (E) and a 40-year-old man (F) with Cushing syndrome. (G) Striae in a 24-year-old patient with congenital adrenal hyperplasia treated with excessive doses of dexamethasone as replacement therapy. (H) Typical bruising and thin skin of a patient with Cushing syndrome. In this case, the bruising occurred without obvious injury.

Bone

In childhood, the most common presentation is poor linear growth and weight gain[155]; as discussed earlier, glucocorticoids have profound effects on growth and development.[171] Many patients with long-standing Cushing syndrome have lost height because of osteoporotic vertebral collapse. This can be assessed by measuring the patient's sitting height or comparing the height with arm span; in normal subjects, height and arm span should be equal. Pathologic fractures, occurring spontaneously or after minor trauma, are not uncommon. Rib fractures, in contrast to those of the vertebrae, are often painless. The radiographic appearance is typical, with exuberant callus formation at the site of the healing fracture. In addition, osteonecrosis of the femoral and humeral heads is a recognized feature of endogenous (although less commonly seen than in exogenous) Cushing syndrome (see Fig. 15.17). Hypercalciuria may lead to renal calculi, but hypercalcemia is not a feature.

Skin

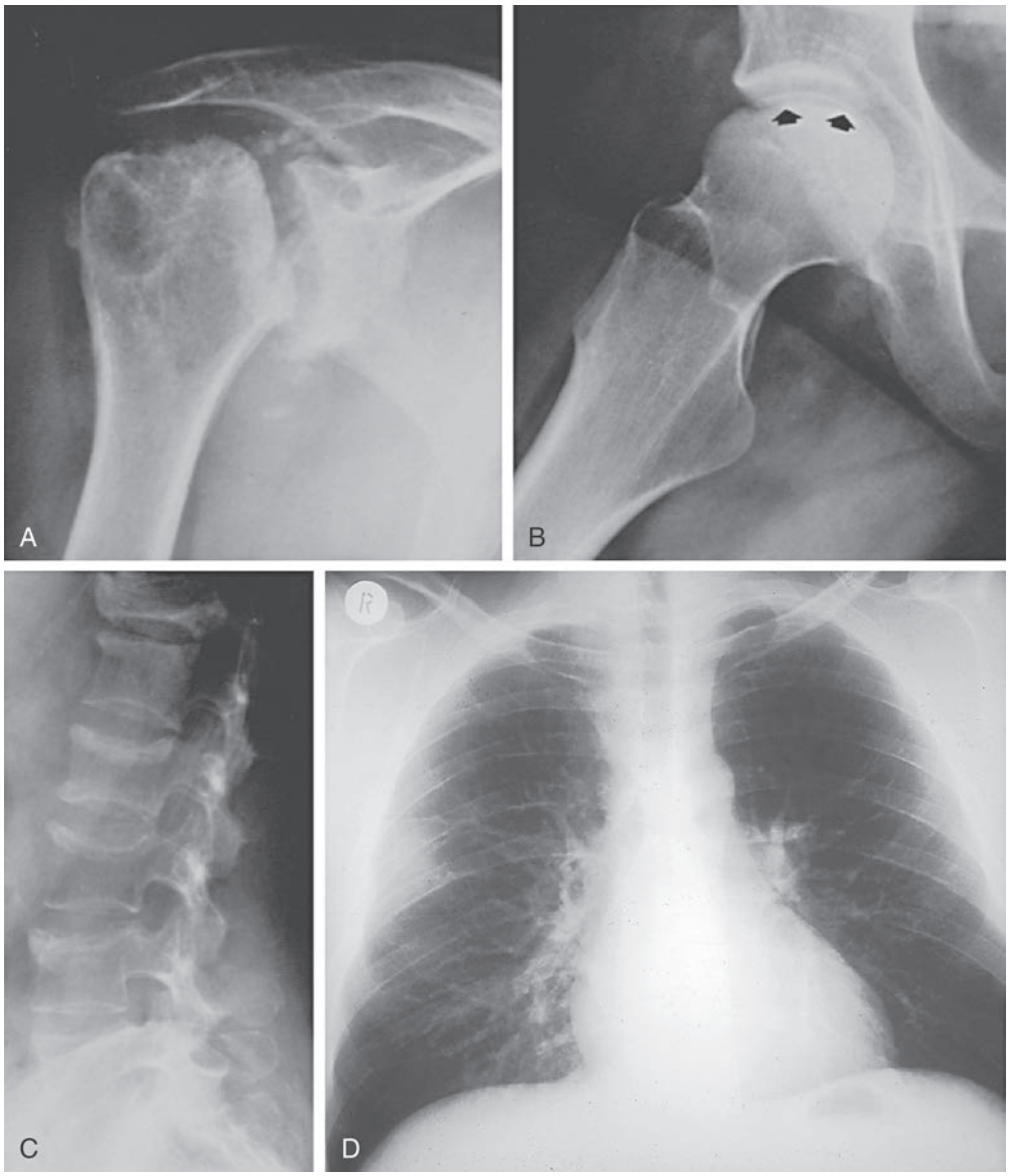
Hypercortisolism results in thinning of the skin and separation and exposure of the subcutaneous vascular tissue. On examination, wrinkling of the skin on the dorsum of the hand may be seen, resulting in a "cigarette paper" appearance (Liddle sign). Minimal trauma may result in bruising, which frequently resembles the appearance of senile purpura. The plethoric appearance of the patient with Cushing syndrome is secondary to the thinning of the skin[192] combined with loss of facial subcutaneous fat and is not caused by true polycythemia. Acne and papular lesions may occur over the face, chest, and back.

The typical, almost pathognomonic, red-purple, nonblanching, livid striae greater than 1 cm in width are most frequently found on the abdomen but may also be present on the upper thighs, breasts, and arms. They are very common in younger patients and less so in those older than 50 years of age. They must be differentiated from the paler, less pigmented striae that occur as a result of pregnancy (striae gravidarum) or in association with rapid weight loss.

Skin pigmentation is rare in Cushing disease but common in the ectopic ACTH syndrome. It arises because of overstimulation of melanocyte receptors by ACTH and possibly POMC-derived peptides.

Muscle

Myopathy and bruising are two of the most discriminatory features of the syndrome.[183] The myopathy of Cushing syndrome involves the proximal muscles of the lower limbs and the shoulder girdle.[193] Complaints of weakness, such as inability to climb stairs or get up from a deep chair, are relatively uncommon, but testing for proximal myopathy by asking the patient to rise from a crouching position often reveals the problem.



• **Fig. 15.17** Bone abnormalities in Cushing disease. (A) Aseptic necrosis of the right humeral head in a 43-year-old woman with Cushing disease of about 8 months of duration. (B) Aseptic necrosis of the right femoral head in a 24-year-old woman with Cushing disease of about 4.5 years of duration. The *arrows* indicate the crescent subchondral radiolucency, best seen in this lateral view. (C) Diffuse osteoporosis, vertebral collapse, and subchondral sclerosis in the same patient shown in (A). (D) Rib fracture in a 38-year-old man with Cushing disease. (A–C, from Phillips KA, Nance Jr EP, Rodriguez RM, et al. Avascular necrosis of bone: a manifestation of Cushing's disease. *South Med J*. 1986;79:825–829.)

Cardiovascular Features

Hypertension is another prominent feature, occurring in up to 75% of cases. Even though epidemiologic data show a strong association between high blood pressure and obesity, hypertension is much more common in patients with Cushing syndrome than in those with simple obesity.[158] This, together with the established metabolic consequences of the disease (diabetes, hyperlipidemia), is thought to explain the increased cardiovascular mortality rate in untreated cases.[194–196] Cardiovascular events are also more common in patients with presumed iatrogenic Cushing syndrome, which results from prescribed corticosteroids.[176] In addition, thromboembolic events may be more common in Cushing patients, but this development appears to be limited to those with ACTH-dependent disease.[197]

Infections

Infections are more common in patients with Cushing syndrome.[198,199] In many instances, infections are asymptomatic and occur because the normal inflammatory response is suppressed. Reactivation of tuberculosis has been reported[200] and has even been the presenting feature in some cases. Fungal infections of the skin (notably tinea versicolor) and nails may occur, as may opportunistic fungal infections. Bowel perforation is more common in patients with extreme hypercortisolism, and the hypercortisolism may mask

TABLE 15.9 Symptoms and Signs for the Diagnosis of Cushing Syndrome

Discriminatory	Less Discriminatory
Signs • Facial plethora • Proximal myopathy • Cutaneous striae (red-purple, >1 cm wide) • Bruising • In children—weight gain with reduced height percentile	Signs • Central obesity • Buffalo hump, supraclavicular fullness • Facial fullness • Acne and hirsutism • Skin thinning • Poor wound healing • Peripheral edema
Symptoms and complications (especially at a young age) • Hypertension • Diabetes mellitus • Osteoporosis and vertebral fractures	Symptoms and complications • Fatigue • Weight gain • Depression, mood and appetite change, impairment of concentration and memory • Back pain • Oligomenorrhea, polycystic ovary syndrome • Recurrent infections • Kidney stones

Data from Nieman LK, Biller BM, Findling JW, et al. Diagnosis of Cushing's syndrome, an Endocrine Society Clinical Guideline. *J Clin Endocrinol Metab.* 2008;93:1526–1540.

TABLE 15.10 Classification of Causes of Cushing Syndrome

ACTH-Dependent Causes

Cushing disease (pituitary dependent)
Ectopic ACTH syndrome
Ectopic CRH syndrome
Macronodular adrenal hyperplasia
Iatrogenic (treatment with [1-24]ACTH)

ACTH-Independent Causes

Adrenal adenoma and carcinoma
Primary pigmented nodular adrenal hyperplasia and Carney syndrome
McCune-Albright syndrome
Aberrant receptor expression (gastric inhibitory polypeptide, interleukin-1β)
Iatrogenic (e.g., pharmacologic doses of prednisolone, dexamethasone)

Other Causes of Hypercortisolism (non-neoplastic)

Alcoholism
Depression
Obesity
Pregnancy

ACTH, Adrenocorticotropic hormone; *CRH,* corticotropin-releasing hormone.

the usual symptoms and signs of the condition. Wound infections are more common and contribute to poor wound healing.

Metabolic and Endocrine Features

Glucose intolerance occurs, and overt diabetes mellitus is present in up to one-third of patients in some series. Hepatic lipoprotein synthesis is stimulated, and increases in circulating cholesterol and triglycerides may be found.[201] Hypokalemic alkalosis is found in 10% to 15% of patients with Cushing disease but in more than 95% of patients with ectopic ACTH syndrome. Several factors may contribute to this mineralocorticoid excess state, including corticosterone and DOC excess, but the principal culprit is thought to be cortisol itself. Depending on the prevailing cortisol production rate, cortisol swamps 11βHSD2 in the kidney and acts as a mineralocorticoid. Hypokalemic alkalosis is more common in ectopic ACTH syndrome because cortisol production rates are higher than in patients with Cushing disease.[136] This can be diagnosed by documenting an increase in the ratio of urinary cortisol to cortisone metabolites. In addition, hepatic 5α-reductase activity is inhibited, resulting in disproportionately greater excretion of 5β-cortisol metabolites.[202]

The functions of the pituitary-thyroid axis and the pituitary-gonadal axis are suppressed in patients with Cushing syndrome because of a direct effect of cortisol on TSH and gonadotropin secretion.[203,204] Cortisol causes a reversible form of hypogonadotropic hypogonadism but also directly inhibits Leydig cell function. GH secretion is reduced, possibly mediated through an increase in somatostatinergic tone.

Eye

Ocular effects include raised intraocular pressure[205] and exophthalmos[206] (in up to one-third of patients in Cushing's original series), the latter occurring because of increased retroorbital fat deposition. Cataracts, a well-recognized complication of corticosteroid therapy, seem to be uncommon,[207] except as a complication of diabetes. In our experience, chemosis is a sensitive and underreported feature of Cushing syndrome.

Classification and Pathophysiology of Cushing Syndrome

Cushing syndrome is most readily classified into ACTH-dependent and ACTH-independent causes (Table 15.10).

ACTH-Dependent Causes

Cushing Disease

When iatrogenic causes are excluded, the most common cause of Cushing syndrome is Cushing disease, accounting for approximately 70% of cases. The adrenal glands in these patients show bilateral adrenocortical hyperplasia with widening of the ZF and ZR.[184]

Cushing himself raised the question as to whether this disease was a primary pituitary condition or secondary to an abnormality in the hypothalamus. The hypothalamus may have an initiating role, but the overwhelming evidence is that, at presentation, the condition is pituitary dependent (Table 15.11). In 85% to 90% of cases, the disease is caused by a pituitary adenoma of monoclonal origin[208,209]; basophil hyperplasia alone is found in 9% to 33% of pathologic series.[210] The majority of tumors are small microadenomas (<1 cm), but larger macroadenomas occur in up to 10% of cases and usually signify a more invasive tumor.[211] Selective surgical removal of a causative corticotroph adenoma results in remission, but on very long-term follow-up, relapse may occur in up to 20% to 30% of microadenoma and macroadenoma patients.[212] Moreover, recent data demonstrate that around 40% of patients with Cushing disease have somatic missense mutations in ubiquitin-specific protease 8 (USP8).[213,214] These mutations cluster in a hotspot region of exon 14 and are not found in other

TABLE 15.11 Etiology of Cushing Disease: Hypothalamic Theory Versus Pituitary Theory

Hypothalamic Theory	Pituitary Theory
Neuroendocrine abnormalities[290,291]	Lack of cure after pituitary stalk section
Loss of circadian rhythm, sleep disturbance, other hypothalamic defects (TSH, LH/FSH secretion)	Circulating and CSF CRH levels are suppressed[292] Reversal of hypothalamic defects on correction of hypercortisolism
Efficacy of centrally acting drugs[293,294] (bromocriptine, cyproheptadine, sodium valproate) Recurrences after pituitary surgery Ectopic CRH-secreting tumors cause Cushing disease,[289] but pathologic examination shows basophil hyperplasia, not adenomas	High surgical cure rate (recurrences resulting from regrowth of inadequately resected tumor rather than real recurrence)[295,300] Secondary hypoadrenalism after successful pituitary surgery (may be prolonged and associated with reduced ACTH expression in surrounding adjacent normal corticotrophs)[514] Pituitary ACTH-secreting adenoma in almost 90% of cases are monoclonal in origin[301,515]

Note: Superscript numbers indicate references listed at the end of the chapter.

ACTH, Adrenocorticotropic hormone; *CRH,* corticotropin-releasing hormone; *CSF,* cerebrospinal fluid; *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone; *TSH,* thyroid-stimulating hormone.

TABLE 15.12 Tumors Associated With the Ectopic Adrenocorticotropic Hormone Syndrome

Tumor Type	Approximate Incidence (%)
Small cell lung carcinoma	50
Non–small cell lung carcinoma	5
Pancreatic neuroendocrine tumors	10
Thymic neuroendocrine tumors	5
Lung neuroendocrine tumors	10
Other neuroendocrine tumors	2
Medullary carcinoma of thyroid	5
Pheochromocytoma and related tumors	3
Rare carcinomas of prostate, breast, ovary, gallbladder, colon	10

pituitary tumors. *USP8* codes for a deubiquitinating enzyme, and the mutations affect the binding of an inhibitory 14-3-3 protein that keeps the deubiquitinating activity in check. Ubiquitination of cell surface receptors usually marks them for degradation by the endosome-lysosome system, downregulating expression. As a result of the *USP8* mutations, deubiquitination is enhanced, and the epidermal growth factor receptor (EGFR) is recycled with enhanced signaling. EGF[213,214] increases POMC mRNA transcription, cell proliferation, and ACTH secretion. Corticotroph[215] adenomas with the USP8 mutant thus express higher levels of EGFR and produce increased amounts of *POMC* mRNA and ACTH compared to tumors with wild-type USP8.[213,214] It is possible that targeting EGFR may have therapeutic potential in Cushing disease.

A key biochemical hallmark of the disease is a relative resistance of ACTH secretion to normal glucocorticoid feedback inhibition.[216] ACTH-secreting pituitary adenomas function at a higher than normal set-point for cortisol feedback. The predominant finding in Cushing disease is an increase in ACTH pulse amplitude with loss of normal circadian rhythm, but ACTH pulse frequency is also increased in some cases (see Fig. 15.8).[217]

Ectopic ACTH Syndrome

In 15% of cases, Cushing syndrome is associated with nonpituitary tumors secreting ACTH—the ectopic ACTH syndrome.[218] On clinical grounds, these tumors can be divided into two entities: highly malignant tumors such as small cell carcinoma of bronchus (Table 15.12) and highly proliferative neuroendocrine carcinoma (e.g., pancreas), and more indolent tumors occurring in patients with underlying neuroendocrine tumors such as bronchial neuroendocrine tumors (carcinoids) with a low proliferative rate. In the former group, the clinical presentation is often that of a wasting syndrome with weakness and pigmentation. Circulating ACTH concentrations and cortisol secretion rates can be extremely high. As a result, duration of symptoms from onset to presentation is short (<3 months); patients are commonly pigmented, and the metabolic manifestations of glucocorticoid excess are often rapid and progressive. Weight loss, myopathy, and glucose intolerance are prominent symptoms and signs. The association of these features with hypokalemic alkalosis and peripheral edema should alert the clinician to the diagnosis.

Depending on local referral practice, approximately 20% of cases of ectopic ACTH syndrome are explained by indolent tumors, such as benign bronchial neuroendocrine tumors, that produce ACTH.[218,219] In these cases, symptoms and signs are commonly present for 18 months before clinical presentation. Such patients present with the typical features of Cushing syndrome and may be biochemically similar to patients with Cushing disease. Therefore, once a diagnosis of Cushing syndrome is established, the principal diagnostic dilemma is in the distinction of pituitary-dependent Cushing disease from these indolent causes of ectopic ACTH syndrome.

Tumors most commonly associated with ectopic ACTH syndrome arise from neuroendocrine tissues, the cells of which possess the ability to uptake and decarboxylate amine precursors (APUD cells). Although *POMC* transcripts of 1200-1450nt are frequently found in small cell lung cancer, only 0.5% to 1% of tumors are associated with ectopic ACTH syndrome, and the explanation for the development of ectopic ACTH secretion remains unclear. In contrast, *POMC* mRNA transcripts of the short 800nt length may be found in tumors not associated with ectopic ACTH syndrome. In addition to aberrant transcriptional regulation of the *POMC*, interaction with tissue-specific transcription factors or the promoter methylation status of *POMC* may be involved. Once translated, POMC is cleaved in the pituitary by specific serine endoproteases to produce ACTH precursors; in ectopic ACTH syndrome, aberrant peripheral processing of POMC may lead to increased concentrations of circulating ACTH precursors (pro-ACTH, N-POC) (see Fig. 15.6). In contrast to ACTH secretion from pituitary adenomas, ectopic POMC/ACTH production is not responsive to normal glucocorticoid feedback[220] because of the defective GR or GR-signaling mechanism.[221] However, this

sensitivity to glucocorticoid feedback is far from absolute, which is one reason why the differential diagnosis of ACTH-dependent Cushing syndrome can be challenging.[218]

Ectopic Corticotropin-Releasing Hormone Syndrome

Ectopic production of CRH is a very rare cause of pituitary-dependent Cushing disease. A number of cases have now been described in which a tumor (usually bronchial carcinoid, medullary thyroid, or prostate carcinoma) has been shown to secrete CRH alone or in combination with ACTH.[222–224] When available, pituitary histologic examination has revealed corticotroph hyperplasia but not adenoma formation. Biochemically, these patients, like those with ectopic ACTH syndrome, lose the normal negative glucocorticoid feedback mechanism—50% have resistance to high-dose dexamethasone therapy. Ectopic CRH production may explain the suppression of cortisol secretion after high-dose dexamethasone that is observed in some patients with the ectopic ACTH syndrome.

Macronodular Adrenal Hyperplasia

In 10% to 40% of patients with Cushing disease, there is bilateral adrenocortical hyperplasia associated with one or more nodules, which may be up to several centimeters in diameter.[225–228] Patients tend to be older and to have had symptoms for a longer time, but they otherwise present with the classic clinical features of Cushing syndrome. Pathologically, the nodules are lobulated and can be markedly enlarged, but internodular hyperplasia is invariably found. Macronodular adrenal hyperplasia (MAH) is thought to result from long-standing adrenal ACTH stimulation, which leads to autonomous adrenal adenoma formation. Therefore, as the adrenals in a patient with Cushing disease become more hyperplastic, they secrete more cortisol for a given ACTH level, which ultimately can lead to autosuppression. Individual clinical cases support this hypothesis, and MAH should be regarded as an ACTH-dependent form of Cushing syndrome, even though ACTH levels may be relatively low and dexamethasone suppressibility less marked than in other cases of Cushing disease.[229] These features can be a trap for the unwary because they may be mistaken for primary adrenal tumors, especially as up to 30% of patients with Cushing disease have asymmetric adrenal hyperplasia.

ACTH-Independent Causes

Cortisol-Secreting Adrenal Adenoma and Carcinoma

Excluding iatrogenic cases, adrenal adenomas are responsible for about 10% to 15% of Cushing syndrome cases, and carcinomas for less than 5%. By contrast, 65% of cases of Cushing syndrome in children have an adrenal cause (15% adenomas, 50% carcinomas).[227–229] Onset of clinical features is gradual in patients with adenomas, but it is often rapid in adrenal carcinoma. Mutations of *PRKACA*, which encodes the catalytic subunit of cAMP-dependent protein kinase A (PKA), at the hotspot L205R have been shown by several independent groups to be the cause of approximately 50% of adrenal adenomas causing overt Cushing syndrome.[230–233]

In addition to the features of hypercortisolism, patients may complain of loin or abdominal pain, and a tumor may be palpable. Adrenal carcinoma may secrete other steroids, such as androgens or mineralocorticoids, but this is very unusual in adenomas. Therefore, in females, there may be features of virilization, with hirsutism, clitoromegaly, breast atrophy, deepening of the voice, temporal recession, and severe acne. In pure cortisol-secreting adenomas, hirsutism is uncommon. Subclinical Cushing syndrome has been reported in up to 10% of patients with adrenal incidentalomas (see later discussion).

TABLE 15.13 Clinical Features of Carney Complex

Feature	Prevalence (%)
Skin lesions	80
Pigmented lesions	
Blue nevi	
Cutaneous myxomas	
Cardiac myxomas	72
Pigmented nodular adrenal hyperplasia	45
Breast lesions	
Bilateral fibroadenomas	45 (females only)
Testicular tumors	56 (males only)
Pituitary lesions, usually growth hormone secreting	10
Neural lesions (gastric schwannomas)	<5
Miscellaneous	
Thyroid cancers	Rare
Acoustic neuromas	Rare
Hepatomas	Rare

Primary Pigmented Nodular Adrenal Hyperplasia and Carney Syndrome

Less than 2% of all Cushing syndrome is caused by ACTH-independent bilateral, small, often pigmented adrenal nodules. Pathologically, these nodules are usually 2 to 4 mm in diameter (although they can be larger) and black or brown on cut section. Adjacent adrenal tissue is atrophic, distinguishing this primary pigmented nodular adrenocortical disease (PPNAD) from MAH. Presentation is with typical features of Cushing syndrome in persons younger than 30 years of age and, in 50% of cases, in persons younger than 15 years of age.[234] Cases of PPNAD have been reported without Cushing syndrome. Bilateral adrenalectomy is curative.

A familial autosomal dominant variant, called *Carney complex* (Table 15.13), comprises mesenchymal tumors (especially atrial myxomas), spotty skin pigmentation, peripheral nerve tumors, and various other tumors, including breast lesions, testicular tumors, and GH-secreting pituitary tumors.[235] Mutations of the gene encoding the PKA regulatory subunit type IA *(PRKAR1A)* lead to abnormal PKA signaling and explain the phenotype in some cases.[236] Other cases have been mapped to chromosome 2p16, but the underlying genetic mutation is unknown.

McCune-Albright Syndrome

In McCune-Albright syndrome, fibrous dysplasia and cutaneous pigmentation may be associated with pituitary, thyroid, adrenal, and gonadal hyperfunction. The most common manifestation is with sexual precocity and GH excess, but Cushing syndrome has been reported.[237] The underlying abnormality is a somatic mutation in the α-subunit of the stimulatory G protein, which is linked to adenylyl cyclase, with a mosaic tissue distribution. The mutation results in constitutive activation of the G protein, mimicking constant ACTH stimulation at the level of the adrenal. ACTH levels are suppressed, and adrenal adenomas may occur.

Macronodular Hyperplasia

Although MAH commonly occurs in patients with ACTH-dependent Cushing syndrome, truly ACTH-independent macronodular adrenal hyperplasia (AIMAH) is also recognized as a distinct entity.[238] The nodules are nonpigmented and greater than 5 mm in diameter; occasionally, the adrenals are massively enlarged. Most cases are explained on the basis of aberrant receptor expression within the adrenal cortex.[239] Food-induced hypercortisolism due to enhanced adrenal responsiveness to gastric inhibitory polypeptide (GIP) was the first cause of AIMAH described, due to expression of GIP receptors within the adrenal cortex, but aberrant expression of the vasopressin V_1, β-adrenergic, LH, serotonin, and angiotensin (AT_1) receptors have also been linked to AIMAH. Protocols have been suggested for the further investigation of AIMAH.[239]

Familial cases suggest a genetic cause for this condition in some patients, and inactivating mutations Armadillo repeat 5 (*ARMC5*) have been demonstrated as a cause.[240]

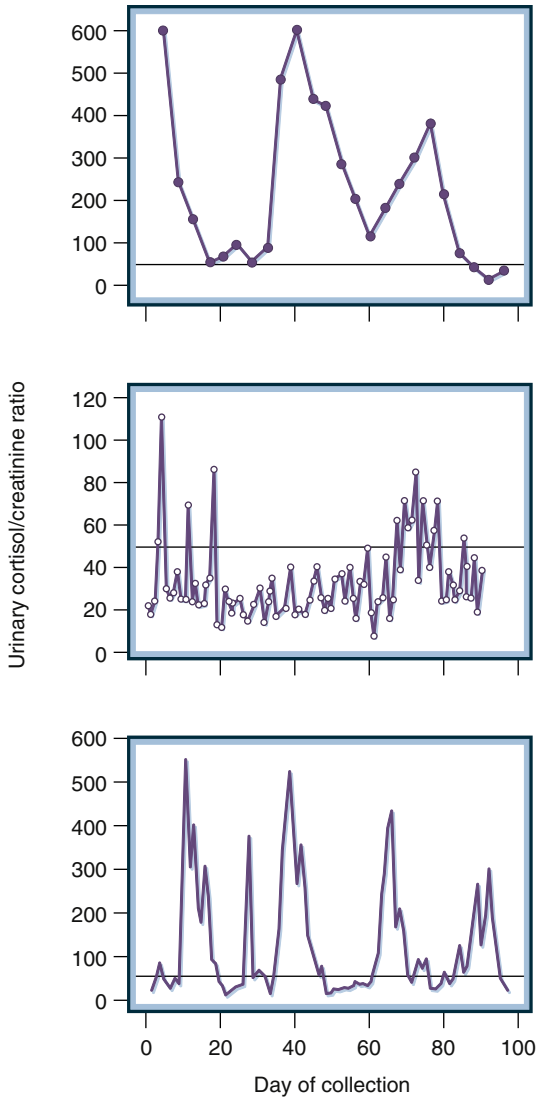
Iatrogenic Cushing Syndrome

A careful drug history is required to exclude iatrogenic Cushing syndrome. Development of the features of Cushing syndrome depends on the dose, duration, and potency of the corticosteroids used in clinical practice. ACTH is rarely prescribed, but long-term administration will also result in cushingoid features. Some features, such as an increase in intraocular pressure, cataracts, benign intracranial hypertension, aseptic necrosis of the femoral head, osteoporosis, and pancreatitis, are more common in iatrogenic than endogenous Cushing syndrome, whereas other features, notably hypertension, hirsutism, and oligomenorrhea/amenorrhea, are less prevalent.

Special Features of Cushing Syndrome

Cyclic Cushing Syndrome

Of particular clinical interest has been a group of patients with cyclic Cushing syndrome, characterized by periods of excess cortisol production interspersed with intervals of normal cortisol production (Fig. 15.18). Some of these patients demonstrate a paradoxic rise in plasma ACTH and cortisol when treated with dexamethasone, and occasionally, a patient is benefited by dopamine agonist (bromocriptine or cabergoline) therapy. Most patients have been thought to have pituitary-dependent disease, and in many of these patients, basophil adenomas have been removed, with long-term cure in some cases. However, cortisol secretion may show some evidence of cyclicity in patients with an ectopic source of ACTH and in PPNAD.[241,242]

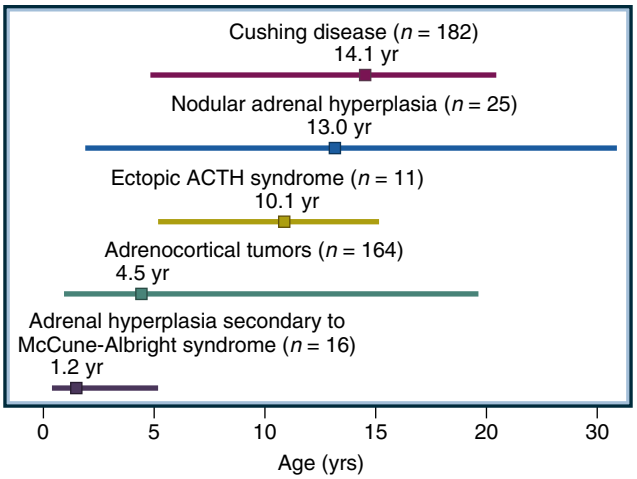


• **Fig. 15.18** Patterns of cortisol secretion in three patients with cyclic Cushing syndrome. In each case, the ratio of early-morning urinary cortisol (in nanomoles per liter) to creatinine (in millimoles per liter) are plotted against time. Variable periodicity in cortisol hypersecretion is shown. (From Atkinson AB, McCance DR, Kennedy L, et al. Cyclical Cushing's syndrome first diagnosed after pituitary surgery: a trap for the unwary. *Clin Endocrinol*. 1992;36:297–299.)

Cushing Syndrome in Children

Cushing syndrome can occur at any age, but the causes differ across age groups (Fig. 15.19). In children, adrenal causes account for 65% of all cases, and in addition to the previously mentioned features, growth arrest is almost invariable.[243] The dissociation between height and weight is obvious, with the height most commonly below the mean, whereas the BMI is almost always above the mean. If the height and weight are increasing along the same percentile line, then the diagnosis of Cushing syndrome is highly unlikely. Obesity in childhood Cushing syndrome tends to be generalized. Most patients have a delayed bone age that is negatively correlated with height standard deviation score (SDS), duration of symptoms, and age at diagnosis. The observed growth failure often precedes other manifestations such as weight gain, pubertal arrest, fatigue, depression, hypertension, and acne. Pubertal development can be advanced in patients with virilizing tumors causing precocious pseudopuberty. However, in patients with true puberty, glucocorticoid-mediated suppression of gonadotropins may occur.[244]

Glucocorticoid excess influences not only the hypothalamic-pituitary-gonadal axis but also the GH/IGF1 axis, leading to both reduction of spontaneous GH secretion and pharmacologic GH response. Furthermore, direct effects of glucocorticoids on epiphyseal chondrocytes, probably together with disturbance of microvascularization of the growth plate, result in a negative effect on growth. Poor catch-up has been reported in children after cure of Cushing disease, and evidence exists for GH inhibition by hypercortisolemia for 1 to 2 years after cure of Cushing disease. GH secretion should be assessed 3 months after treatment. If GH deficiency is demonstrated, GH in a replacement dose of



• **Fig. 15.19** Etiology and age-dependency of pediatric Cushing syndrome. Note how the causes differ across age groups. (From Storr HL, Chan LF, Grossman AB, et al. Paediatric Cushing's syndrome: epidemiology, investigation and therapeutic advances. *Trends Endocrinol Metab*. 2007;18:167–174.)

25 μg/kg per day should be given. Catch-up growth is observed in most patients, and target adult height is achieved. However, many patients remain obese.

Pregnancy

Pregnancy is rare in women with Cushing syndrome because of associated amenorrhea due to androgen excess or hypercortisolism, but when present is due to an adrenal adenoma in 50% of cases.[245] A few cases of true pregnancy-induced Cushing syndrome have been described, with regression after delivery.[246] In these cases, the cause is unknown. Establishing a diagnosis and a cause can be difficult. Clinically, striae, hypertension, and gestational diabetes are common features in normal pregnancies, yet hypertension and diabetes are also the most common signs of Cushing syndrome in a pregnant woman (70% and 30% of all cases, respectively). Furthermore, biochemically normal pregnancy is associated with a threefold increase in plasma cortisol due to increased production of cortisol and CBG. Urinary free cortisol also rises, and dexamethasone does not suppress plasma cortisol to the same degree as the nonpregnant state. Left untreated, the condition is associated with high rates of maternal and fetal morbidity and mortality. Any adrenal or pituitary adenomas should be excised. Metyrapone, which is not teratogenic, has been effective in many cases in controlling the hypercortisolism.

Other Syndromes of Hypercortisolemia

Other states occur when there are clinical and biochemical features of Cushing syndrome, but when hypercortisolemia is secondary to other factors it is often referred to as *pseudo-Cushing syndrome*. Resolution of the underlying cause results in disappearance of the cushingoid state. Several causes are described.

Alcohol

In the original description of alcohol-related pseudo-Cushing syndrome, urinary and plasma cortisol levels were elevated and failed to suppress with dexamethasone. Plasma ACTH has been found to be normal or suppressed. The condition is rare but should be suspected in a patient with an ongoing history of heavy alcohol intake and biochemical or clinical evidence of chronic liver disease.[247] The pathogenesis of this condition remains unknown, but a two-hit hypothesis has been put forward. Chronic liver disease of any cause is associated with impaired cortisol metabolism, but in alcoholic patients there is an increased cortisol secretion rate, rather than concomitant suppression in the face of impaired metabolism.[248] In some studies, alcohol has directly stimulated cortisol secretion; alternatively, AVP levels are elevated in patients with decompensated liver disease and may stimulate the HPA axis. With abstinence from alcohol, the biochemical abnormalities revert to normal within days.

Depression

Although the cause is unknown, it is recognized that patients with depression may exhibit the hormonal abnormalities of patients with Cushing syndrome.[249] These abnormalities are reversible on correction of the psychiatric condition. Conversely, patients with Cushing syndrome are frequently depressed, and a careful clinical and endocrinologic assessment is required.

Obesity

Although one of the most common referrals to a clinical endocrinologist is for exclusion of an underlying endocrine cause in a patient with obesity, the diagnosis of Cushing syndrome in such patients should not cause difficulties. Patients with obesity have mildly increased cortisol secretion rates, and the data suggest that this is due to activation of the HPA axis.[250,251] However, circulating cortisol concentrations are invariably normal, and urinary free cortisol excretion rates are either normal or only slightly elevated. The stimulus for the increased secretion of cortisol appears to be increased peripheral metabolism and clearance of cortisol—principally, reduced hepatic conversion of cortisone to cortisol by 11βHSD1 and increased conversion of cortisol to 5α-reduced derivatives.[251]

Investigation of Patients With Suspected Cushing Syndrome

There are two stages in the investigation of suspected Cushing syndrome: (1) Does this patient have Cushing syndrome? (2) If so, what is the cause? Unfortunately, many investigators fail to make this distinction and ill advisedly use tests that are relevant to the second question in trying to answer the first question. In particular, it is essential that radiologic investigations not be undertaken until Cushing syndrome has been confirmed biochemically. The starting point should be to investigate patients in whom there is a high clinical index of suspicion for the diagnosis of Cushing syndrome, focusing on the features that are most discriminating for the condition (see Table 15.9). Widespread indiscriminate biochemical screening in obese, hypertensive, and diabetic populations is not recommended. Very few laboratories have developed methods for the measurement of free serum cortisol.[252] Because more than 90% of serum cortisol is protein bound, the results of the conventional assay are affected by drugs and by conditions that alter CBG levels. Oral estrogen therapy or pregnancy may elevate CBG and total serum cortisol, and should be stopped for 6 weeks prior to assessment by tests using serum cortisol. The major tests are listed in Table 15.14.[184,249,253,254]

Question 1: Does This Patient Have Cushing Syndrome?

Circadian Rhythm of Plasma Cortisol

In normal subjects, plasma cortisol levels are at their highest early in the morning and reach a nadir (<50 nmol/L [<2 μg/dL] in a nonstressed subject) at about midnight.[255] This circadian rhythm

TABLE 15.14 Tests Used in the Diagnosis and Differential Diagnosis of Cushing Syndrome

Diagnosis—Does the Patient Have Cushing Syndrome?
Late night salivary cortisol/circadian rhythm of plasma cortisol
Urinary free cortisol excretion[a]
Low-dose dexamethasone suppression test[a]
Differential Diagnosis—What Is the Cause of the Cushing Syndrome?
Plasma ACTH
Plasma potassium, bicarbonate
High-dose dexamethasone suppression test
Corticotropin-releasing hormone
Inferior petrosal sinus sampling
CT, MRI scanning of pituitary, adrenals
Scintigraphy
Tumor markers

[a]Valuable outpatient screening tests (see text discussion).

ACTH, Adrenocorticotropic hormone; *CT,* computed tomography; *MRI,* magnetic resonance imaging.

is lost in patients with Cushing syndrome; in the majority, the 9 AM plasma cortisol is normal but nocturnal levels are raised. Random morning plasma cortisol levels are therefore of little value in making the diagnosis, whereas a midnight cortisol level greater than 200 nmol/L (>7.5 μg/dL) indicates Cushing syndrome. However, various factors such as stress of venipuncture, intercurrent illness, and admission to hospital may lead to false-positive results. Conversely, if a serum cortisol value is less than 50 nmol/L at midnight, Cushing syndrome is excluded at that time. Ideally, patients should be hospitalized for 24 to 48 hours before the midnight cortisol level is measured, but some centers have reported discriminant results for midnight levels measured in outpatients. Nevertheless, this is a cumbersome test and has been largely supplanted by measurement of salivary cortisol (see next).

Salivary Cortisol

CBG is absent from saliva, and the use of salivary cortisol measurements offers a sensible alternative in that the test does not require hospitalization. The diagnostic accuracy of a single midnight salivary cortisol level has been established in several studies. The cutoff points that define disease vary depending on the assay used. In one study, a cortisol value greater than 2.0 ng/mL (>5.5 nmol/L) had a 100% sensitivity and a 96% specificity for diagnosis of Cushing syndrome.[253,256,257] It is important to note, however, that late-night salivary cortisol tends to increase with age and cardiovascular comorbid conditions such as hypertension and diabetes, and thus the discriminating power diminishes in the elderly population.[258]

Urinary Free Cortisol Excretion

For many years, the diagnosis of Cushing syndrome was based on the measurement of urinary metabolites of cortisol (24-hour urinary 17-hydroxycorticosteroid or 17-oxogenic steroid excretion, depending on the method used). However, the sensitivity and specificity of these methods are poor, and most centers have replaced these assays with the more sensitive measurement of urinary free cortisol. This is an integrated measure of plasma free cortisol: As cortisol secretion increases, the binding capacity of CBG is exceeded, resulting in a disproportionate rise in urinary free cortisol. Normal values depend on the assay used and tend to be lower when analyzed by liquid chromatography and tandem mass spectrometry (LC-MS/MS). Although in widespread use, urinary free cortisol is less sensitive than salivary cortisol and dexamethasone suppression testing. Patients should make two or three complete consecutive collections to account for patient error in collecting samples and for episodic cortisol secretion, notably from adrenal adenomas. Simultaneous creatinine excretion (which differs by no more than 10% from day to day) may be used to ensure adequacy of collection. Urinary free cortisol is a useful screening test, although it is accepted that the value can be normal in up to 8% to 15% of patients with Cushing syndrome.[253,254,259] Conversely, moderately elevated results should always be verified by further testing before a diagnosis of Cushing syndrome is made. In addition, variations in levels of up to 50% have been observed in patients with proven Cushing disease, reinforcing the need for multiple collections.[260]

Measurement of the cortisol-to-creatinine ratio in the first urine specimen passed on waking obviates the need for a timed collection and has been used as a screening test, particularly when cyclic Cushing syndrome is suspected.[261] Urine aliquots may be sent to the local endocrinology laboratory, with cortisol-to-creatinine ratios greater than 25 nmol/mmol on repeated measurement being indicative of hypercortisolism.

Low-Dose Overnight Dexamethasone Suppression Tests

In normal subjects, the administration of a supraphysiologic dose of glucocorticoid results in suppression of ACTH and cortisol secretion. In Cushing syndrome of whatever cause, there is a failure of this suppression when low doses of the synthetic glucocorticoid dexamethasone are given.[216]

The overnight test is a useful outpatient screening test,[249,253,262] in which 1 mg of dexamethasone is given at 11 PM. A normal response is a plasma cortisol level of less than 50 nmol/L (<1.8 μg/dL) between 8 and 9 AM the following morning. The outpatient overnight test has high sensitivity (95%) but lower specificity, and further investigation is often required.[263,264]

In the 48-hour low-dose dexamethasone test, plasma cortisol is measured at 9 AM on day 0 and again 48 hours later, after administration of dexamethasone 0.5 mg every 6 hours for 48 hours. Using a postdexamethasone plasma cortisol concentration of less than 50 nmol/L (<1.8 μg/dL) as the cutoff point, this test is reported to have a 97% to 100% true-positive rate and a false-positive rate of less than 1%.[249,263]

Certain drugs (e.g., phenytoin, rifampicin) may increase the metabolic clearance rate of dexamethasone, leading to false-positive results. Simultaneous measurement of plasma dexamethasone may be useful in such cases and will also detect whether patients failed to take the drug, and a value of greater than 3.3 nmo/L as measure by LC-MS/MS appears to improve the accuracy of the test.[265]

Other Causes of Hypercortisolemia: Pseudo-Cushing or True Cushing Syndrome?

In patients with depression, urinary free cortisol excretion may be elevated and may overlap with those seen in patients with true Cushing syndrome. Compared with patients with Cushing disease, depressed patients have greater suppressibility after dexamethasone and reduced response to CRH, but neither test is diagnostic.[249,266] The dexamethasone-suppressed CRH test has been proposed as a tool to discriminate between true Cushing syndrome and other states but has been shown to have no advantage over the standard low-dose dexamethasone suppression

test. In normal subjects and in patients with endogenous depression, insulin-induced hypoglycemia results in a rise in ACTH and cortisol levels, a response that usually is not seen in patients with Cushing syndrome, but this test has largely been abandoned for this purpose.[249]

Diagnostic Guidelines

The Endocrine Society, in collaboration with the European Society for Endocrinology, has issued evidence-based guidelines for the diagnosis of Cushing syndrome.[267] Recommendations are to proceed initially with one of four highly sensitive screening tests: urinary free cortisol, late-night salivary cortisol, overnight dexamethasone, or the 2-mg/48-hour dexamethasone suppression test. Abnormality detected by any of these tests in a patient with clinically suspected Cushing syndrome should be confirmed with one of the additional tests; if both test results are abnormal, patients should then undergo testing for the cause of the Cushing syndrome (Fig. 15.20).

Question 2: What Is the Cause of Cushing Syndrome in This Patient?

Having confirmed Cushing syndrome clinically and biochemically, the clinician's next step is to determine the cause (Fig. 15.21).

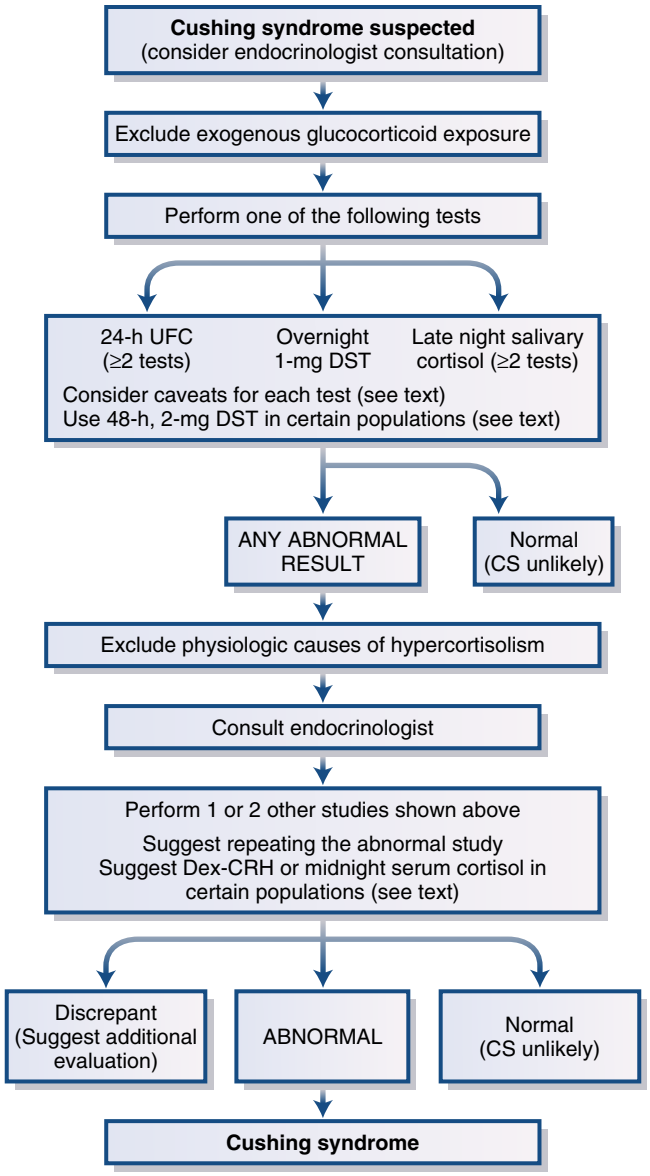
Morning Plasma ACTH

Ideally, ACTH should be measured with the use of a modern, two-site immunoradiometric assay. Such a test differentiates ACTH-dependent from ACTH-independent causes. The samples should be taken in ice cold tubes and immediately separated ahead of storage at −40°C ahead of analysis to prevent inadvertent degradation. In Cushing disease, 50% of patients have a 9 AM ACTH level within the normal reference range (2–11 pmol/L [9–52 pg/mL]); in the remainder, it is modestly elevated. Occasionally, due to episodic secretion, levels may be very low, and thus measurement of at least two values is recommended to avoid misclassification of mild Cushing disease as ACTH independent. ACTH levels in the ectopic ACTH syndrome are high (usually >20 pmol/L [>90 pg/mL]); nevertheless, overlap values are seen in Cushing disease in 30% of cases.[257] Therefore this test cannot be used to differentiate the two conditions (Fig. 15.22). The measurement of ACTH precursors (pro-ACTH, POMC) has been suggested, but it is not routinely available and has not been proven to detect an ectopic source of ACTH.

In patients with adrenal tumors and clear clinical features, plasma ACTH is invariably undetectable (<1 pmol/L) unless there is assay interference. The presence of plasma ACTH levels that are low-normal or intermittently detectable, which may occur in MAH, is problematic. The danger is that in some patients the asymmetry of the nodular hyperplasia may lead to a diagnosis of adrenal adenoma, the plasma ACTH is ignored, and an inappropriate adrenalectomy is performed. Conversely, in some patients with this syndrome, an autonomous adrenal tumor develops, and unilateral adrenalectomy is required despite the detectable ACTH.

Plasma Potassium

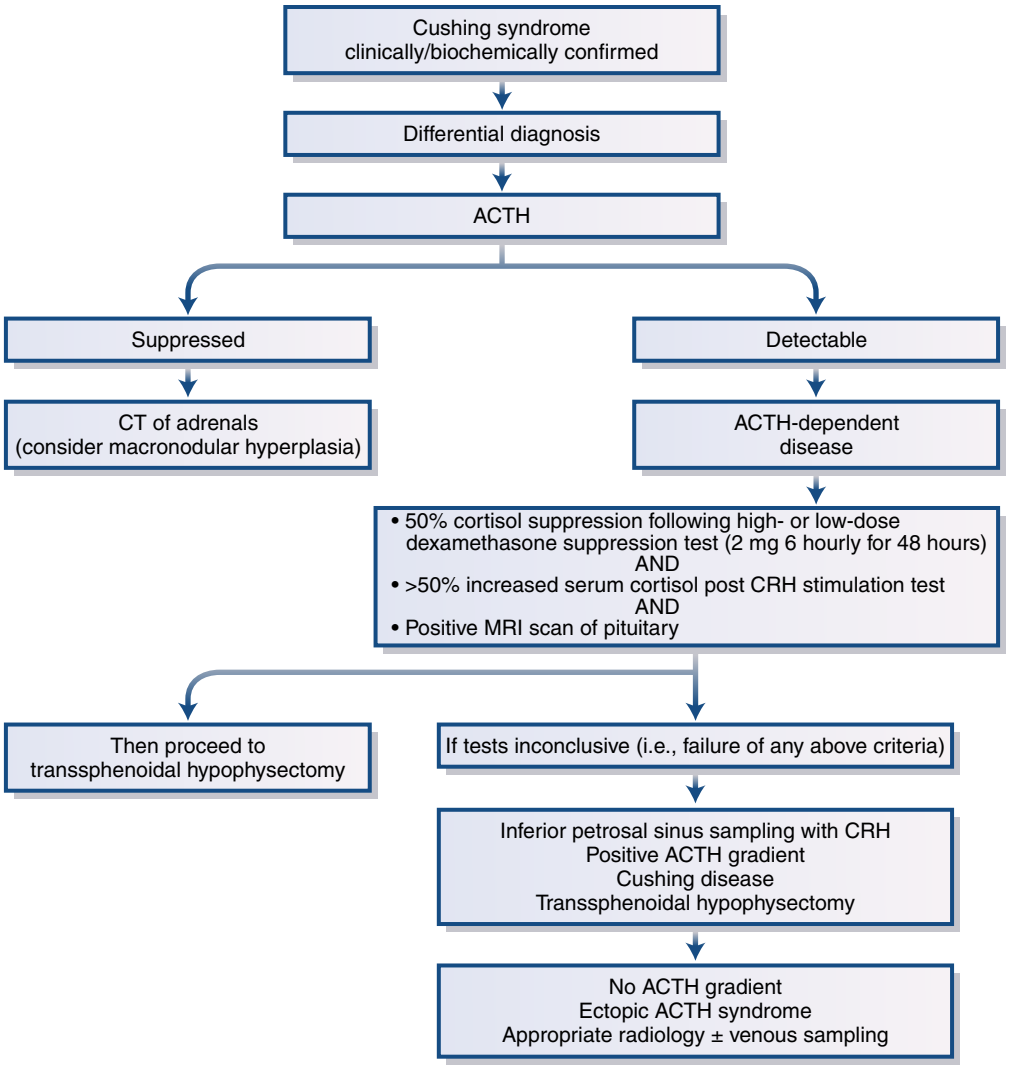
Hypokalemic alkalosis is present in more than 95% of patients with the ectopic ACTH syndrome but in fewer than 10% of those with Cushing disease. The cause of this mineralocorticoid excess state is now established. Patients with the ectopic syndrome usually have higher rates of cortisol secretion. Cortisol saturates the renal-protective 11βHSD2 enzyme, resulting in cortisol-induced mineralocorticoid hypertension (see Chapter 16).[136] In addition, these patients have higher levels of the ACTH-dependent mineralocorticoid, DOC.



• **Fig. 15.20** Algorithm for testing patients with suspected Cushing syndrome *(CS)* according to the 2008 Endocrine Society clinical practice guideline. All statements are recommendations except for those prefaced by the word *Suggest*. Diagnostic criteria that point to Cushing syndrome are a urinary free cortisol *(UFC)* value greater than the normal range for the assay, a serum cortisol level greater than 1.8 μg/dL (>50 nmol/L) after administration of 1 mg dexamethasone *(1-mg DST),* and a late-night salivary cortisol concentration greater than 145 ng/dL (>4 nmol/L). *CRH,* corticotropin-releasing hormone; *Dex,* dexamethasone; *DST,* dexamethasone suppression test. (From Nieman LK, Biller BM, Findling JW, et al. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2008;93:1526–1540.)

High-Dose Dexamethasone Suppression Test

The rationale for the high-dose dexamethasone suppression test is that in Cushing disease the negative feedback control of ACTH is reset to a higher level than normal. Therefore cortisol levels do not suppress with low-dose dexamethasone but do so after high doses.



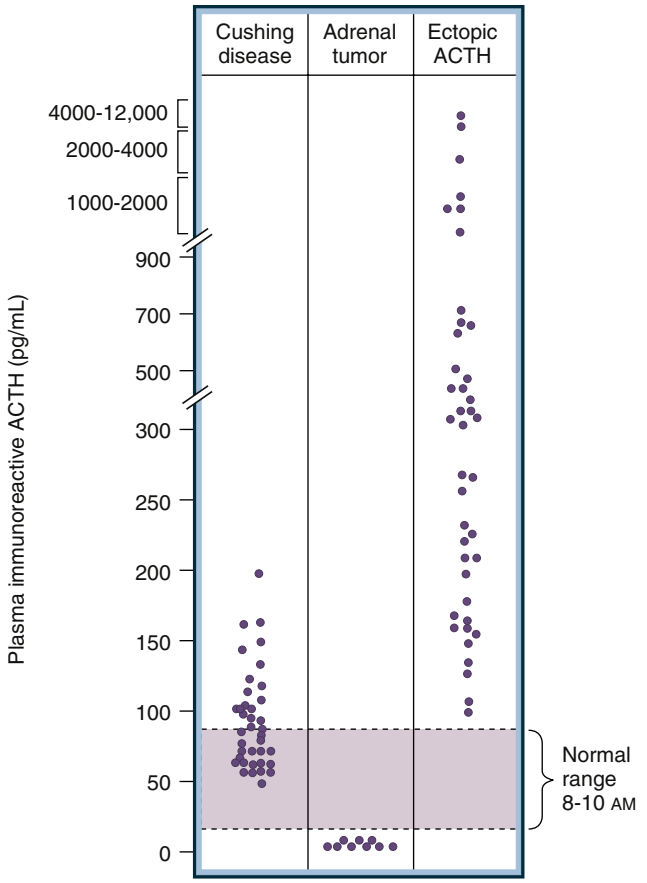
• **Fig. 15.21** The tests to uncover the cause of Cushing syndrome are debatable and differ in any given center depending on many factors, including familiarity and turnaround time of hormone assays and local expertise in techniques such as inferior petrosal sinus sampling. Depicted here is an algorithm in use within many endocrine units based on the reported sensitivity and specificity of each endocrine test. *ACTH,* adrenocorticotropic hormone; *CRH,* corticotropin-releasing hormone; *CT,* computed tomography; *MRI,* magnetic resonance imaging.

The original test introduced by Liddle was based on giving 2 mg dexamethasone every 6 hours for 48 hours and demonstrating a fall of greater than 50% in urinary 17-hydroxycorticosteroids.[216] In the modern test, the plasma or urinary free cortisol (or both) is measured at 0 and +48 hours, and a greater than 50% suppression of plasma cortisol from the basal value has been used to define a positive response. In all cases, the response is graded and is dependent on the original cortisol secretion rate: Greater suppression is often observed in patients with lower basal cortisol values. In women with ACTH-dependent Cushing syndrome, in whom the a priori likelihood of Cushing disease is 90%, the sensitivity of this test for the Cushing disease is 80%, lower than the pretest probability. Because of this, there is little logic to the continued use of this test when inferior petrosal sinus sampling (IPSS) is available (see later). Moreover, if the low-dose dexamethasone suppression test has been used in the diagnosis of Cushing syndrome and a greater than 50% fall in cortisol is observed, there is no added value in the high-dose dexamethasone suppression test.[263]

Corticotropin-Releasing Hormone Test

CRH is a 41–amino acid peptide that was identified by Vale in 1981 from ovine hypothalami. The ovine sequence differs by seven amino acid residues from that of the human hormone but is slightly more effective in stimulating the release of ACTH in humans.[268] The test involves the intravenous injection of either ovine or human sequence CRH in a dose of 1 μg/kg body weight or a single dose of 100 μg (Fig. 15.23). The test can be performed in the morning or afternoon. After basal sampling, CRH is administered, and blood samples for ACTH and cortisol are then taken every 15 minutes for 1 to 2 hours.[261,264,269,270]

In normal subjects, CRH produces a rise in ACTH and cortisol of 15% to 20%. This response is exaggerated in Cushing disease, in which typically an ACTH increase greater than 50% and a cortisol rise greater than 20% over baseline values are seen. Responses are seldom seen in the ectopic ACTH syndrome, but false-positive results have been reported. In distinguishing pituitary-dependent Cushing disease from the ectopic ACTH syndrome, the response



• **Fig. 15.22** Plasma adrenocorticotropic hormone *(ACTH)* concentrations in patients with Cushing disease, Cushing syndrome associated with adrenocortical tumors, or the ectopic ACTH syndrome. To convert values to picomoles per liter, multiply by 0.2202. (From Besser GM, Edwards CRW. Cushing's syndrome. *Clin Endocrinol Metab*. 1972;1:451–490.)

of ACTH and cortisol to CRH has a specificity and a sensitivity of approximately 90%. However, a positive response defined as an ACTH increase of 100% or a cortisol rise of 50% over baseline values effectively eliminates a diagnosis of ectopic ACTH syndrome, which is the real benefit of this test. Up to 10% of patients with Cushing disease do not respond to CRH.

Inferior Petrosal Sinus Sampling and Selective Venous Catheterization

The most robust test to distinguish Cushing disease from the ectopic ACTH syndrome is IPSS.[184] Because blood from each half of the pituitary drains into the ipsilateral inferior petrosal sinus, catheterization and venous sampling of both sinuses simultaneously can distinguish a pituitary from an ectopic source of ACTH (Fig. 15.24).[271,272] In virtually all patients with the ectopic ACTH syndrome, the ratio of the ACTH concentration in the inferior petrosal sinus and that in simultaneously drawn peripheral venous blood is less than 1.4:1. In contrast, the ratio is elevated to greater than 2 in Cushing disease. However, because of the problem of intermittent ACTH secretion, it is useful to take measurements before and at intervals (e.g., 2, 5, and 15 minutes) after intravenous injection of 100 µg synthetic ovine or human CRH.[273,274] Using this approach, an ACTH petrosal sinus/peripheral ratio greater than 3 after CRH administration has a sensitivity of 95% and specificity of nearly 100% for diagnosing Cushing disease.[274] When CRH is not available, some centers use desmopressin as a secretagogue, but central to peripheral gradients have been observed in some patients with ectopic ACTH when using this peptide. When no gradient is found and venograms confirm correct catheter placement, measurement of prolactin in the samples and correction of the ACTH values can reduce the false-negative rate in Cushing disease.[275]

IPSS may also be of value in lateralizing a pituitary tumor in a patient in whom imaging techniques have failed to demonstrate a microadenoma; however, some centers have found this procedure to be of little value in predicting tumor location. Because many tumors are central and drain into both sinuses, current evidence suggests that it is unwise to base the surgical procedure on the results of IPSS studies alone.

IPSS is technically demanding; it has been associated with complications (referred aural pain, thrombosis) and should be performed only in an experienced tertiary referral center. In some centers, a clinical diagnostic algorithm is used (see Fig. 15.21), and IPSS is performed if the differential diagnosis remains in doubt (i.e., lack of adequate suppression after high-dose dexamethasone or CRH response or the absence of a macroadenoma on pituitary MRI scanning).

Imaging

CT/MRI Scanning of Pituitary and Adrenal Glands

High-resolution, thin-section, contrast-enhanced computed tomography (CT) or MRI imaging has revolutionized the investigation of Cushing syndrome.[271,272] However, it is essential that the results of any imaging technique be interpreted alongside the biochemical results if mistakes are to be avoided. When the adrenals are imaged, asymmetric nodular hyperplasia may lead to a false diagnosis of adrenal adenoma. In the general population there is an approximately 10% rate of small abnormalities on pituitary MRI, so-called pituitary incidentalomas, which may mimic a corticotrope adenoma to the unwary, thus emphasizing the need for careful biochemical assessment.[276]

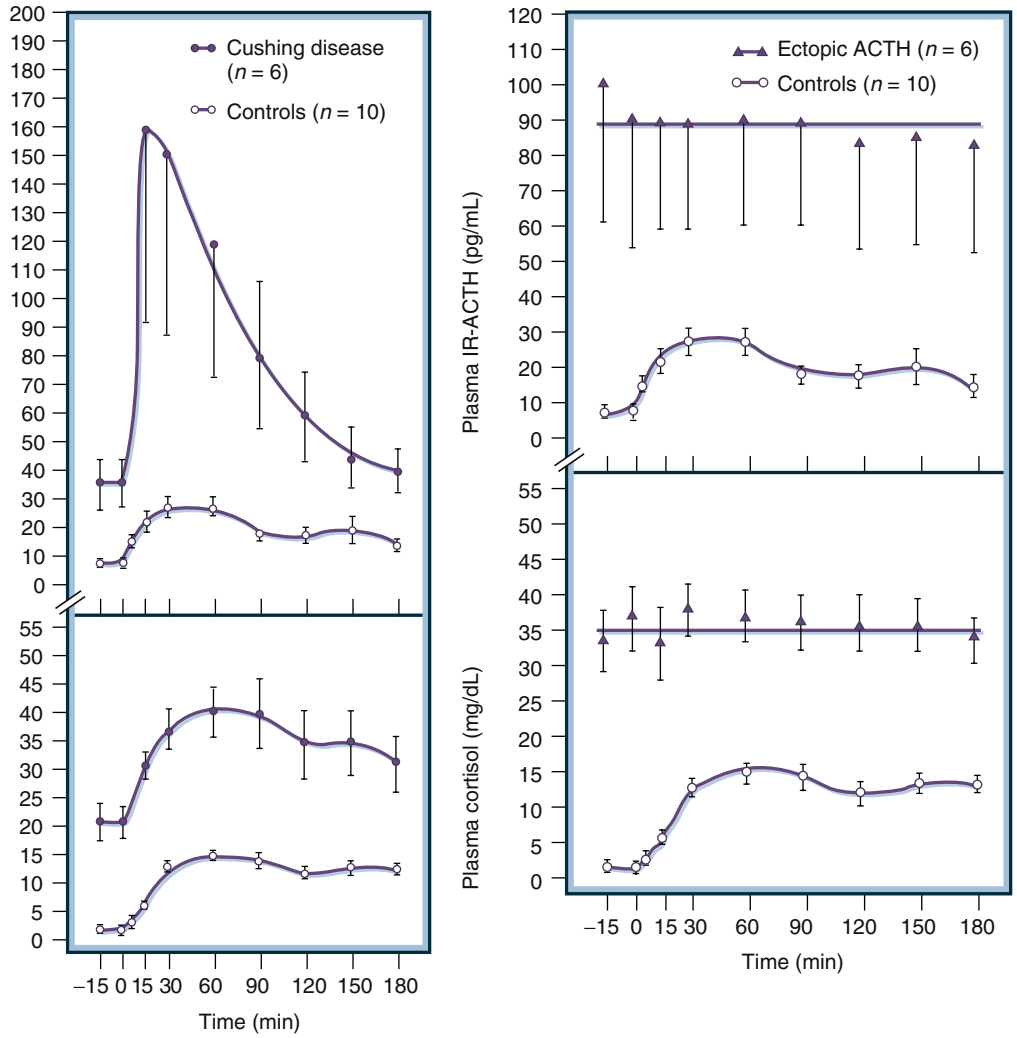
Pituitary MRI is the investigation of choice once the biochemical tests have suggested Cushing disease, with a sensitivity of 60% and specificity of 87%. About 90% of ACTH-secreting pituitary tumors are microadenomas (i.e., <10 mm in diameter). The classic features of a pituitary microadenoma are a hypointense lesion on T1-weighted images after gadolinium contrast enhancement, which may be associated with deviation of the pituitary stalk, and a convex upper surface of the pituitary gland (Fig. 15.25).

For adrenal imaging, CT offers better spatial resolution[277] (Fig. 15.26) and is the procedure of choice, but MRI may provide diagnostic information in patients with suspected adrenal carcinoma. Once again, so-called adrenal incidentalomas are present in up to 5% of normal subjects, so adrenal imaging should not be performed unless biochemical investigation has suggested a primary adrenal cause (i.e., undetectable ACTH concentrations). Adrenal carcinomas are usually large and are often associated with metastatic spread at presentation (Fig. 15.27).

In patients with occult ectopic ACTH syndrome, high-definition CT/MRI scanning of thorax, abdomen, and pelvis with images obtained every 0.1 to 0.5 cm may be required to detect small ACTH-secreting neuroendocrine tumors (Fig. 15.28).

Nuclear Medicine and Molecular Imaging Studies

Scintigraphy is used in some centers for patients with primary adrenal disease. The most commonly used agent is ^{136}I-labeled 6β-iodomethyl-19-norcholesterol,[278] a marker of adrenocortical cholesterol uptake. In patients with adrenal adenomas, the



• **Fig. 15.23** Comparison of cortisol and adrenocorticotropic hormone (ACTH) responses to an intravenous injection of ovine corticotropin-releasing hormone (1 μg/kg) in normal subjects, patients with Cushing disease, and patients with the ectopic ACTH syndrome. *IR,* immunoreactive. (From Chrousos GP, Schulte HM, Oldfield EH, et al. The corticotropin-releasing factor stimulation test: an aid in the evaluation of patients with Cushing's syndrome. *N Engl J Med*. 1984;310:622–626.)

isotope is taken up by the adenoma but not by the contralateral suppressed adrenal gland. Adrenal scintigraphy may be useful in patients with suspected adrenocortical macronodular hyperplasia, although it is not in widespread use; CT scanning may misleadingly suggest unilateral disease, whereas isotope scanning identifies bilateral adrenal involvement.

Many neuroendocrine tumors giving rise to the ectopic ACTH syndrome express somatostatin receptors (sst) and can be imaged by administering radiolabeled analogues of somatostatin, most commonly [^{114}In]-labeled octreotide, or with more sensitive [^{69}Ga]-DOTATATE PET-CT. This latter technique can detect tumors as small as a few millimeters in diameter and should be considered for patients with ACTH-dependent Cushing syndrome in whom pituitary disease has been excluded, although discovery of a lesion that has not been seen on axial imaging is rare.[279] Because the sst2 receptor on the causative tumor to which these analogues bind may be downregulated in Cushing syndrome, medical therapy to either lower or offset the actions of cortisol may allow an initially negative scan to be repeated after some months and detect an occult source.[280]

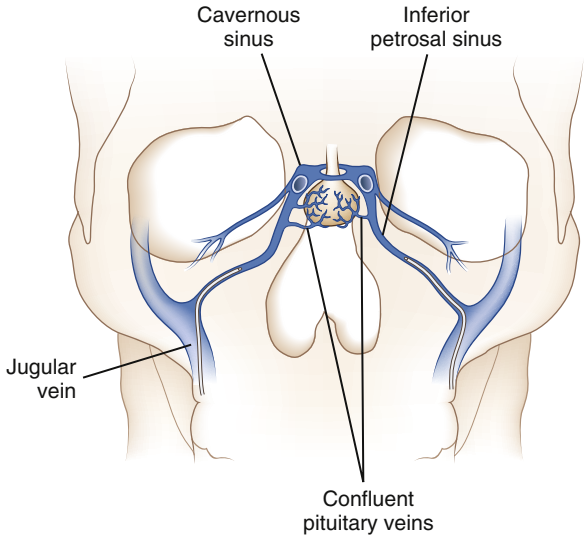
Treatment of Cushing Syndrome

The Endocrine Society has produced evidence-based clinical practice guidelines for the treatment of Cushing syndrome.[281]

Adrenal Causes

Unilateral adrenal adenomas should be removed by adrenalectomy; the cure rate approaches 100%, with success reflected by postoperative serum cortisol values that are less than 50 nmol/L at 9 AM, or even lower in many modern assays.[281,282] Laparoscopic adrenalectomy by an expert surgeon is the standard of care for unilateral tumors, offering reduced surgical morbidity and postoperative hospital stay compared with traditional open approaches.[281,283] After surgery, it may take many months or even years for the contralateral suppressed adrenal to recover. Glucocorticoid replacement regimens vary, but many centers use low doses (15–20 mg) of hydrocortisone, and withdrawal regimens differ. One practical approach is to measure the morning plasma cortisol having omitted the dose of hydrocortisone in the morning at 3-month intervals. Patients with serum

cortisol values less than 200 nmol/L (<7.5 μg/dL) should continue glucocorticoid replacement, whereas in patients with values above 500 nmol/L (>18.3 μg/dL), this replacement can be stopped. In patients with values between 200 and 500 nmol/L, ACTH(1-24) testing can be used to assess whether there is sufficient response to stress, although insulin tolerance testing may be used in some centers. In the interim, all patients should carry a steroid alert card and increase their dose of replacement therapy in the event of an intercurrent illness.



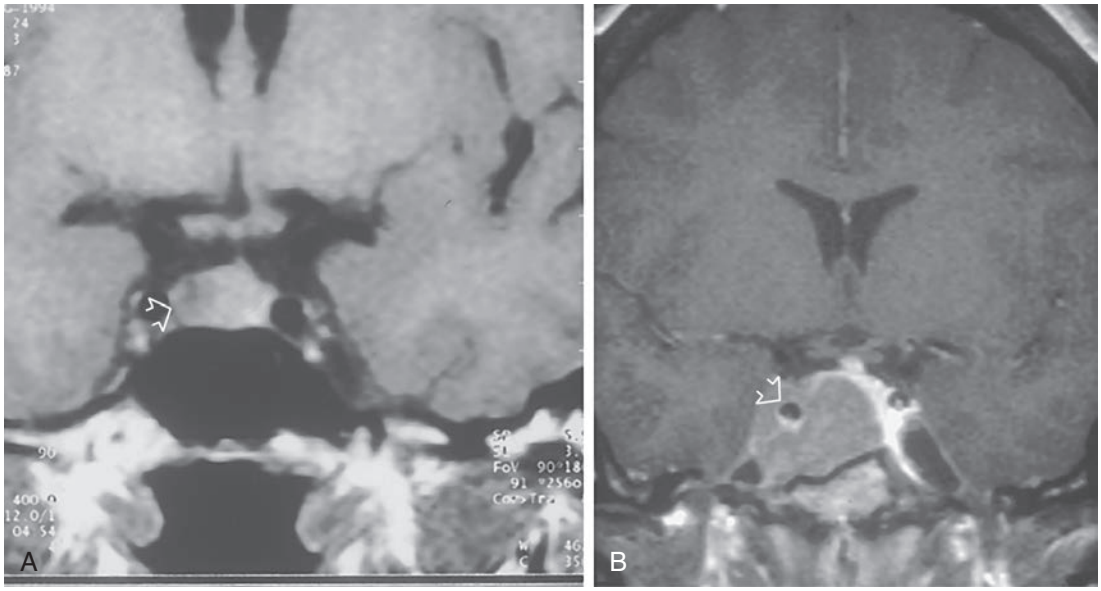
• **Fig. 15.24** Anatomy of the venous drainage of the pituitary gland through the inferior petrosal venous sinuses. (From Oldfield EH, Chrousos GP, Schulte HM, et al. Preoperative lateralization of ACTH-secreting pituitary microadenomas by bilateral and simultaneous inferior petrosal sinus sampling. *N Engl J Med*. 1985;312:100–103.)

Adrenal carcinomas have had a very poor prognosis, and most patients have died within 2 years of diagnosis.[284] It is usual practice to try to remove the primary tumor, even though metastases may be present, so as to enhance the response to the adrenolytic agent *o,p'*-DDD[285] (mitotane). Radiotherapy to the tumor bed and to some metastases, such as those in the spine, may be of limited value. However, in recent years, significant progress has been made by implementing collaborative multicenter studies. Phase III trials of mitotane therapy achieving therapeutic plasma mitotane levels have shown significant benefit[286]; drug combinations include etoposide, doxorubicin, and cisplatin plus mitotane or streptozotocin plus mitotane. Several targeted therapies, including IGF1 inhibitors, sunitinib, and sorafenib, have been tried in cases of mitotane failure, but there are no consistent responses. The 10-year survival rate for patients with T1 N0 M0 disease is about 80% but is significantly impaired with increased tumor mass, positive lymph nodes, and distant metastases, reaching less than 20% for patients with T1-4 N0-1 M1.[287]

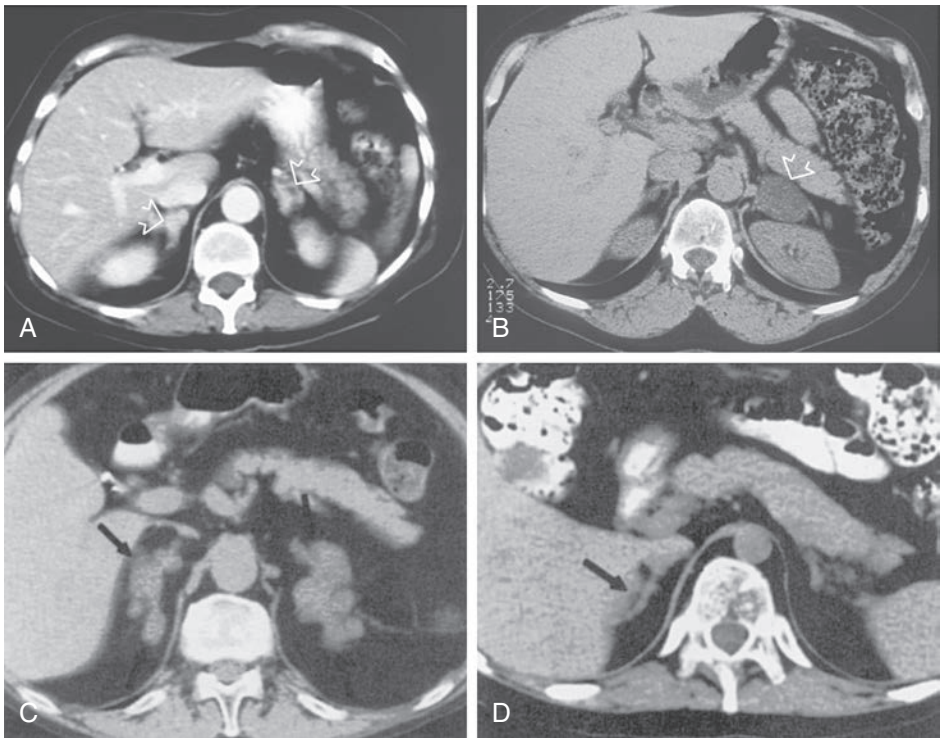
Pituitary-Dependent Cushing Syndrome

The treatment of Cushing disease has been significantly enhanced with transsphenoidal surgery conducted by an experienced surgeon.[281,288]

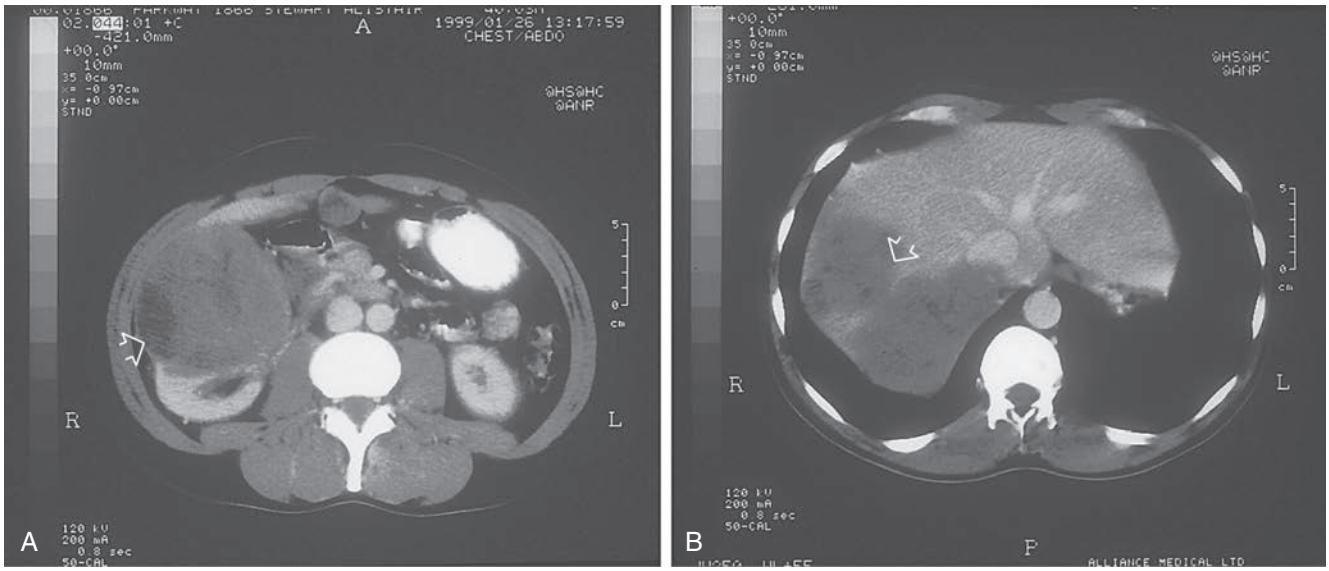
The surgical outcome for transsphenoidal hypophysectomy varies from center to center and with surgical expertise.[289] Because of the hazards of untreated Cushing disease and the potential complications of surgery, the endocrinologist should refer patients only to a recognized surgical specialist at a center in which outcome data have been established. In optimal centers, remission rates are 70% to 90% for microadenomas and 50% for macroadenomas.[290] Rates for postoperative hypopituitarism and permanent diabetes insipidus depend on how aggressive the surgeon was in removing pituitary tissue. The ideal outcome is a cured patient with intact pituitary function, but this result may



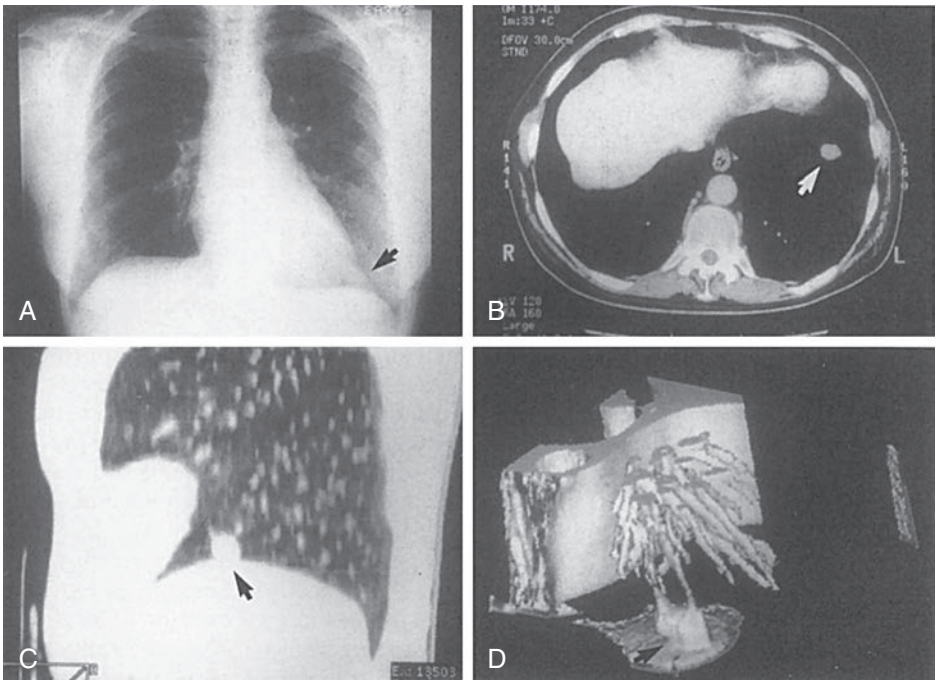
• **Fig. 15.25** (A) Magnetic resonance imaging (MRI) scan of pituitary demonstrates the typical appearance of a pituitary microadenoma. A hypodense lesion is seen in the right side of the gland *(arrow)*, with deviation of the pituitary stalk away from the lesion. After a biochemical diagnosis of Cushing disease, this patient was cured by transsphenoidal hypophysectomy. (B) MRI scan of the pituitary gland demonstrates a large macroadenoma *(arrow)* in a patient with Cushing disease. In contrast to smaller tumors, large macroadenomas are usually invasive and recur after surgery.



• **Fig. 15.26** (A) Adrenal computed tomographic (CT) scan demonstrates bilateral adrenal hyperplasia *(arrows)* in a patient with Cushing disease. (B) CT scan of a typical solitary left adrenal adenoma *(arrow)* causing Cushing syndrome. (C) Cushing syndrome caused by massive macronodular hyperplasia. Adrenal glands are replaced by multiple nodules *(arrows)*. The combined weight of the adrenal glands was more than 100 g. (D) Cushing syndrome caused by surgically proven primary pigmented nodular adrenal disease in a 21-year-old patient. Notice the multiple small nodules with relatively atrophic internodular adrenocortical tissue involving the medial limb of the right adrenal gland *(arrow)*. (C, D, from Findling JW, Doppman JL. Biochemical and radiologic diagnosis of Cushing's syndrome. *Endocrinol Metab Clin North Am.* 1994;23:511–537.)



• **Fig. 15.27** Computed tomographic scan of a patient with rapidly progressing Cushing syndrome caused by an adrenal carcinoma. An irregular right adrenal mass is shown in (A) and a large liver metastasis is seen in (B).

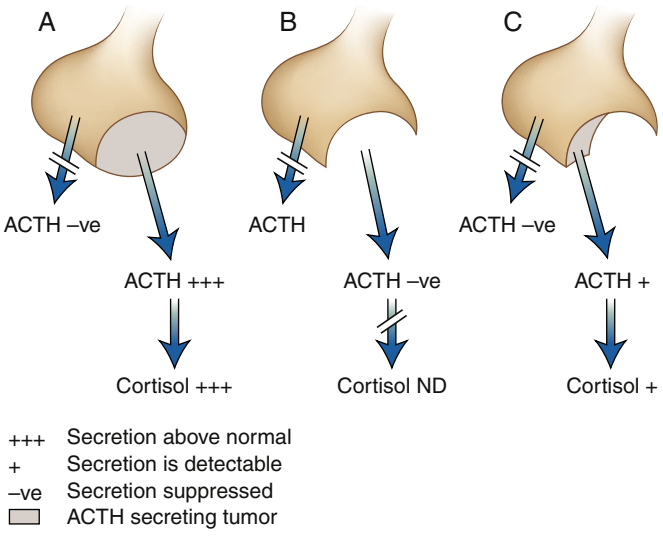


• **Fig. 15.28** Imaging of the thorax in a patient with the ectopic adrenocorticotropic hormone (ACTH) syndrome. (A) Plain chest radiograph demonstrates a suspicious lesion behind the left heart border *(arrow)*. (B, C) Axial and sagittal computed tomographic images demonstrate a bronchial carcinoid tumor *(arrow)* abutting the diaphragm. (D) Three-dimensional reconstruction illustrates adherence of the tumor to the diaphragm *(arrow)*, which was confirmed at surgery. (From Newell-Prince J, Trainer P, Besser M, et al. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. *Endocr Rev*. 1998;19:647–672.)

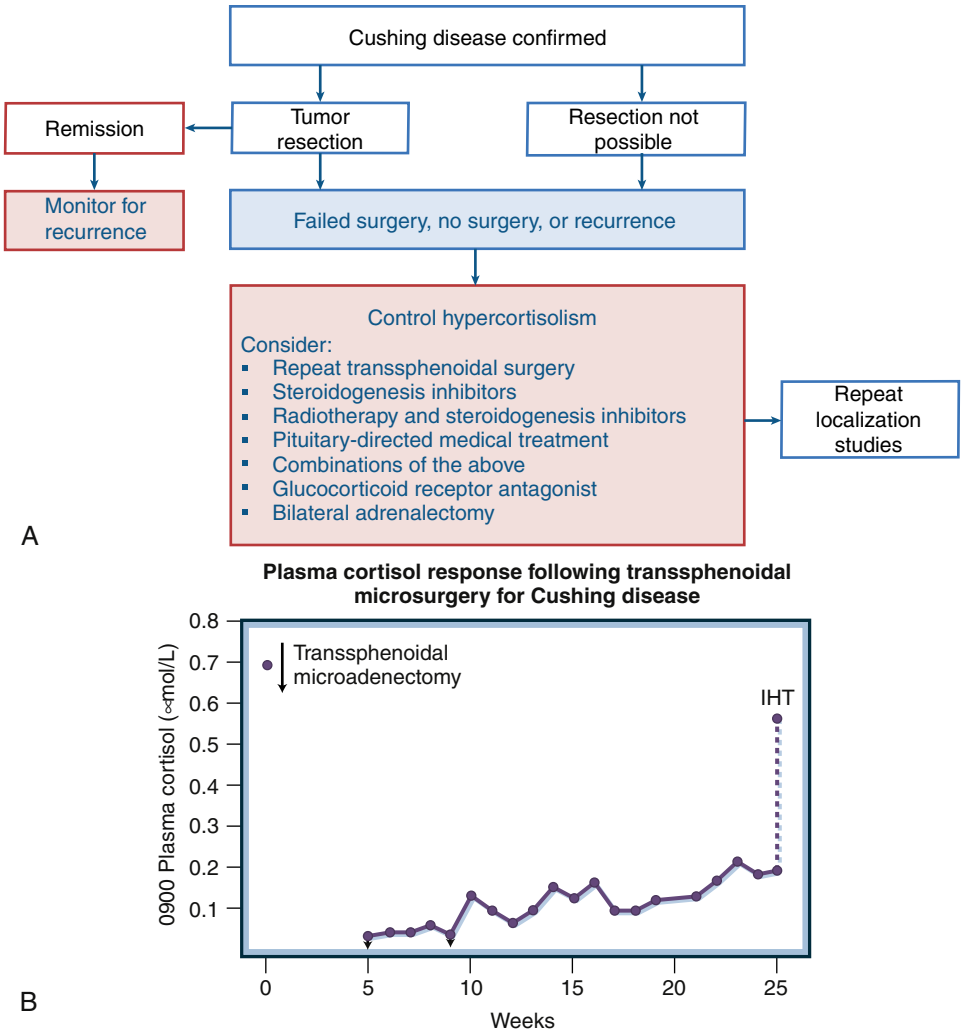
not be possible for a patient with Cushing disease in whom a pituitary adenoma was not identified preoperatively or during the operation itself.

In centers that lack facilities for frequent monitoring of cortisol levels, perioperative and postoperative hydrocortisone cover is advised; this can be reduced to maintenance replacement doses usually within 3 to 7 days. On days 2 to 5, postoperatively, a 9 AM plasma cortisol level should be measured with the patient having omitted hydrocortisone for 24 hours. After selective removal of a microadenoma, the surrounding corticotrophs are usually suppressed (Fig. 15.29). As a result, plasma cortisol levels are less than 30 nmol/L (<1 μg/dL) postoperatively, and ongoing glucocorticoid replacement therapy is required. The HPA axis usually (but not invariably) exhibits gradual recovery, and glucocorticoid therapy is needed until this occurs (Fig. 15.30). A nonsuppressed plasma cortisol postoperatively suggests that the patient is not in remission even though cortisol secretion may have fallen to normal or subnormal values.[291,292] The overall recurrence rate on long-term follow-up even for patients in remission after pituitary surgery is up to 30%, but this value is higher in children (up to 40%).[281,293,294] This emphasizes the need for long-term monitoring. A detailed assessment of residual pituitary function is required in each patient, and close follow-up of such individuals is warranted.

In the past, pituitary irradiation was often used in the treatment of Cushing disease. However, because of the improvements in pituitary surgery, far fewer patients are so treated. In children, pituitary irradiation appears to be more effective.[295] Radiotherapy



• **Fig. 15.29** Selective removal of a microadenoma and its effect on the hypothalamic-pituitary-adrenal axis. (A) Before treatment. (B) After total removal of adenoma. (C) After incomplete excision. Because the surrounding normal pituitary corticotrophs are suppressed in a patient with an adrenocorticotropic hormone (ACTH)-secreting pituitary adenoma, successful removal of the tumor results in ACTH, and hence adrenocortical, deficiency, with an undetectable (<50 nmol/L [2 μg/dL]) plasma cortisol level. A postoperative plasma cortisol level higher than 50 nmol/L (2 μg/dL) implies that the patient is not cured. (Courtesy Professor Peter Trainer.)



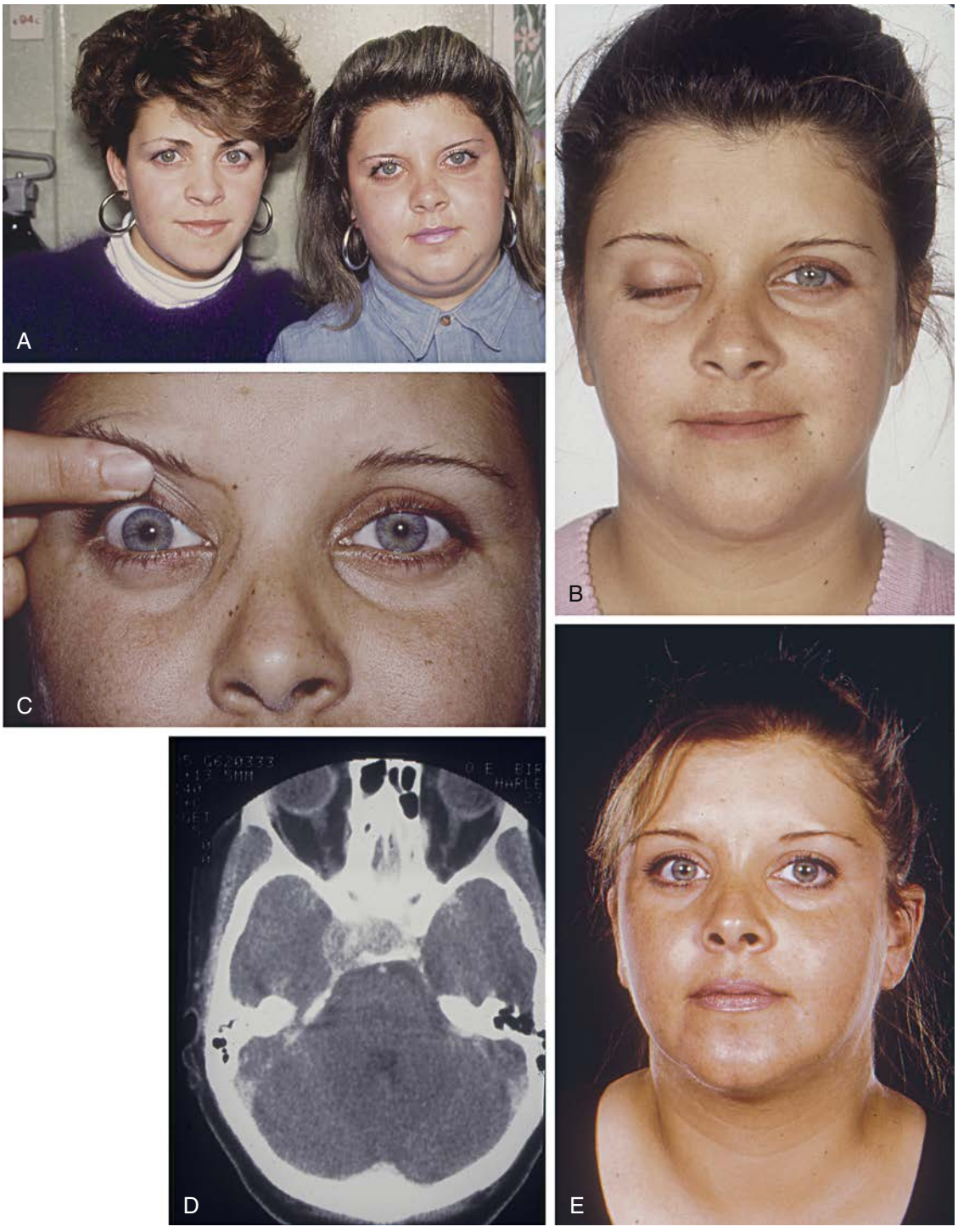
• **Fig. 15.30** (A) Suggested approach for management of Cushing disease. (B) Gradual recovery of function of the hypothalamic-pituitary-adrenal axis in a patient after removal of a pituitary adrenocorticotropic hormone-secreting microadenoma. Morning (9 AM) plasma cortisol levels were measured. The insulin hypoglycemia test (IHT) eventually demonstrated the return of a normal stress response. (A, reproduced from Nieman LK, Biller BM, Findling JW, et al. Treatment of Cushing's syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2015;100:2807–2831.)

is not recommended as a primary treatment but is reserved for patients who are not responding to pituitary microsurgery, patients who have undergone bilateral adrenalectomy, and patients with established Nelson syndrome (see upcoming discussion).

The management of recurrent Cushing disease involves a consideration of repeat pituitary surgery, Gamma Knife radiosurgery, medical therapies, and adrenal surgery.[281,288,296] Bilateral adrenalectomy carries a cure rate approaching 100%. The major risk is the subsequent development of Nelson syndrome (postadrenalectomy hyperpigmentation with a locally aggressive pituitary tumor) (Fig. 15.31), which was attributed to loss of any negative feedback after adrenalectomy but is more likely to be due to an aggressive pituitary tumor from the outset.[297] In an attempt to avoid this complication, pituitary irradiation was often carried out at the time of bilateral adrenalectomy, but this is not widely practiced today.[298] If a pituitary corticotroph tumor is not visible at the time of surgery, the risk of Nelson syndrome is reduced; monitoring is by plasma ACTH and MRI of the pituitary.[297] In addition, these patients require lifelong replacement therapy with hydrocortisone and fludrocortisone. Currently, bilateral adrenalectomy is indicated for patients with Cushing disease if pituitary surgery has failed or if the condition has recurred.

Ectopic ACTH Syndrome

Treatment of the ectopic ACTH syndrome depends on the cause. If the tumor can be found and has not spread, then its removal can lead to cure (e.g., neuroendocrine tumor of the bronchus or thymus). However, the prognosis for small cell lung cancer associated with the ectopic ACTH syndrome is poor. The cortisol excess and associated hypokalemic alkalosis and diabetes mellitus can be ameliorated by medical therapy. Treatment of the small cell tumor itself will also, at least initially, produce improvement. Sometimes, if the ectopic source of ACTH cannot be found, it may be necessary to perform bilateral adrenalectomy and then monitor the patient carefully (sometimes for several years) before the primary tumor becomes apparent.



• **Fig. 15.31** A young woman with Cushing disease, photographed initially beside her identical twin sister (A). In this case, treatment with bilateral adrenalectomy was undertaken. Several years later, the patient presented with Nelson syndrome and a right third cranial nerve palsy (B, C) related to cavernous sinus infiltration from a locally invasive corticotropinoma (D). Hypophysectomy and radiotherapy were performed with reversal of the third cranial nerve palsy (E). Note the advancing skin pigmentation of Nelson syndrome.

Medical Treatment of Cushing Syndrome

Several drugs have been used in the treatment of Cushing syndrome.[288] Metyrapone inhibits 11β-hydroxylase, with a goal of lowering cortisol concentrations, often before definitive therapy or while awaiting benefit from pituitary irradiation. The daily dose must be determined by measurements of plasma or urinary free cortisol. The aim should be to achieve a mean plasma cortisol concentration of about 300 nmol/L (11 μg/dL) during the day or a normal urinary free cortisol level. Since levels of 11-deoxycortisol are increased, use of LC-MS/MS assays are encouraged for monitoring due the potential cross-reactivity with cortisol in immunoassays.[281] The drug is usually given in doses ranging from 250 mg

twice daily to 1.5 g every 6 hours, with lower doses for adrenal adenoma and higher for ectopic ACTH, and overall is effective in around 50% of all patients.[299] Nausea is a side effect that can be helped (if it is not caused by adrenal insufficiency) by giving the drug with milk.[300]

Ketoconazole is an imidazole that has been widely used as an antifungal agent but causes abnormal liver function tests in about 15% of patients. Ketoconazole blocks a variety of steroidogenic cytochrome P450–dependent enzymes and thus lowers plasma cortisol levels. For effective control of Cushing syndrome, 400 to 1600 mg daily has been required, and an acidic stomach is needed for absorption; the drug is effective in around 50% of patients.[301,302] Ketoconazole frequently causes an increase in hepatic enzyme values, and if this level remains less than three times the upper limit of normal, the drug may be continued, but hepatic failure has been described, and the drug has been withdrawn from the market for the treatment of fungal infections.

Mitotane (*o,p′*-DDD) is an adrenolytic drug that is taken up by both normal and malignant adrenal tissue, causing adrenal atrophy and necrosis.[285] Because of its toxicity, it has been used mainly in the management of adrenocortical carcinoma (ACC). Doses of up to 5 g/day are required to control glucocorticoid excess, although evidence that the drug causes tumor shrinkage or improves long-term survival is lacking. This agent also produces mineralocorticoid deficiency, and concomitant high-dose glucocorticoid therapy is required, often with mineralocorticoid replacement if there is no prior glucocorticoid excess due to the ACC. High-dose hydrocortisone replacement is needed in patients with ACC causing Cushing syndrome when mitotane is used in a "block and replace" regime due to the increases in CBG, and induction of CYP3A4 due to the mitotane, which then causes less bioavailability and increased metabolism of hydrocortisone.[303] Side effects are common and include fatigue, skin rashes, neurotoxicity, and gastrointestinal disturbance, and therapeutic drug monitoring is essential during use. It has also been used at lower doses in Cushing disease.[304]

Somatostatin analogues such as octreotide and lanreotide are generally ineffective in Cushing disease. However, the multireceptor somatostatin analogue, pasireotide, which demonstrates high-affinity binding to somatostatin receptor subtypes 1, 2, 3, and 5, normalizes urinary free cortisol in 17% to 40% of patients with Cushing disease depending on the severity of disease, with hyperglycemia being a common side effect.[305,306] The GR antagonist mifepristone has been shown to improve diabetes in patients with Cushing syndrome, but biochemical monitoring is not possible when using this drug.[307]

Prognosis of Cushing Syndrome

Studies performed before the introduction of effective therapy revealed that 50% of patients with untreated Cushing syndrome died within 5 years, principally from vascular disease.[194] Even with modern management, an increased prevalence of cardiovascular risk factors persists for many years after an apparent remission.[195,196] If remission is achieved at the first pituitary surgery the standardized mortality rate approaches the normal population, emphasizing the need for expert surgeons.[308] Paradoxically, on correction of the hypercortisolism, patients often feel worse. Skin desquamation, steroid-withdrawal arthropathy, profound lethargy, and mood changes may occur and can take several weeks or months to resolve.[309] They can usually be ameliorated by a transient increase in glucocorticoid replacement therapy. Patients are invariably GH deficient, and GH replacement therapy may produce clinical benefit.

Features of Cushing syndrome disappear over a period of 2 to 12 months after treatment. Hypertension and diabetes mellitus improve, but as with other secondary causes, they may not resolve completely. The osteopenia of Cushing syndrome improves rapidly during the first 2 years after treatment but resolves more slowly thereafter.[310] Vertebral fractures and osteonecrosis are irreversible, and permanent deformity results. Visceral obesity and myopathy are both reversible features. Reproductive and sexual function usually return to normal within 6 months, provided that anterior pituitary function was not compromised. Long-term health-related quality of life in adults significantly improves after treatment, but quality-of-life scores do not return to normal levels.[191] Similar observations have been made in pediatric patients, in whom significant improvement was noted before and after treatment but residual impairment of health-related quality of life persisted 1 year after cure.[311]

Glucocorticoid Resistance

A small number of patients have been described as having increased cortisol secretion but without the stigmata of Cushing syndrome.[88,312] These patients are resistant to suppression of cortisol with low-dose dexamethasone but respond to high doses. ACTH levels are elevated and lead to increased adrenal production of androgens and DOC. Therefore these patients may present with the features of androgen or mineralocorticoid excess, or both. Treatment with a dose of dexamethasone (usually >3 mg/day) adequate to suppress ACTH results in a fall in adrenal androgens and often returns plasma potassium and blood pressure to normal levels. Many of these patients have been found to have point mutations in the steroid-binding domain of the GR, with consequent reduction of glucocorticoid-binding affinity, but this finding is not invariable. A useful clinical discriminatory test to differentiate this condition from Cushing syndrome is to measure bone mineral density: It is preserved in patients with glucocorticoid resistance or even increased in females because of the androgen excess. In addition, the circadian rhythm for ACTH and cortisol is preserved in patients with glucocorticoid resistance.

Glucocorticoid Deficiency

Primary and Central Hypoadrenalism

Primary hypoadrenalism refers to glucocorticoid deficiency occurring in the setting of adrenal disease, whereas central hypoadrenalism arises because of deficiency of ACTH (Table 15.15). A major distinction between these forms of hypoadrenalism is that mineralocorticoid deficiency invariably accompanies primary hypoadrenalism, but this does not occur in central hypoadrenalism: Here, only ACTH is deficient, and the renin-angiotensin-aldosterone (RAA) axis is intact. A further important cause of adrenal insufficiency in which there may be dissociation of glucocorticoid and mineralocorticoid secretion is CAH.

Primary Hypoadrenalism

Addison Disease

Thomas Addison described the condition now known as *primary hypoadrenalism* in his classic monograph published in 1855.[2]

TABLE 15.15 Etiology of Adrenocortical Insufficiency (Excluding Congenital Adrenal Hyperplasia)

Primary Causes: Addison Disease

- Autoimmune
  - Sporadic
  - Autoimmune polyendocrine syndrome type I (Addison disease, chronic mucocutaneous candidiasis, hypoparathyroidism, dental enamel hypoplasia, alopecia, primary gonadal failure—see Chapter 43)
  - Autoimmune polyendocrine syndrome type II (Schmidt syndrome) (Addison disease, primary hypothyroidism, primary hypogonadism, insulin-dependent diabetes, pernicious anemia, vitiligo—see Chapter 43)
- Infections
  - Tuberculosis
  - Fungal infections
  - Cytomegalovirus
  - HIV
- Metastatic tumor
- Infiltrations
  - Amyloid
  - Hemochromatosis
- Intra-adrenal hemorrhage (Waterhouse-Friderichsen syndrome) after meningococcal septicemia
- Adrenoleukodystrophies
- Congenital adrenal hypoplasia
  - DAX1 *(NR0B1)* mutations
  - *SF1* mutations
- ACTH resistance syndromes
  - *MC2R* gene mutations
  - *MRAP* gene mutations
  - *AAAS* (ALADIN) gene mutations (triple-A syndrome)
- Bilateral adrenalectomy

Secondary Causes: Central Hypoadrenalism

- Exogenous glucocorticoid therapy
- Hypopituitarism
- Selective removal of ACTH-secreting pituitary adenoma
- Pituitary tumors and pituitary surgery, craniopharyngiomas
- Pituitary apoplexy
- Granulomatous disease (tuberculosis, sarcoid, eosinophilic granuloma)
- Secondary tumor deposits (breast, bronchus)
- Postpartum pituitary infarction (Sheehan syndrome)
- Pituitary irradiation (effect usually delayed for several years)
- Isolated ACTH deficiency
  - Idiopathic
  - Lymphocytic hypophysitis
  - TPIT *(TBX19)* gene mutations
  - *PCSK1* gene mutation (POMC processing defect)
  - *POMC* gene mutations
- Multiple pituitary hormone deficiencies
  - *HESX1* gene mutations
  - *LHX4* gene mutations
  - *SOX3* gene mutations
  - *PROP1* gene mutations

ACTH, Adrenocorticotropic hormone; *HIV,* human immunodeficiency virus; *POMC,* pro-opiomelanocortin.

Addison disease is a rare condition with an estimated incidence in the developed world of 0.8 cases per 100,000 and a prevalence of 4 to 11 cases per 100,000 population. Nevertheless, it is associated with significant morbidity and a twofold excess mortality rate,[313] but once the diagnosis is made it can be easily treated.[314,315] Despite treatment, however, patients carry significant burden of metabolic and psychologic comorbidities.[316] Causes of Addison disease are listed in Table 15.15.

TABLE 15.16 Incidence of Other Endocrine and Autoimmune Diseases in Patients With Autoimmune Adrenal Insufficiency

Disease	Incidence (%)
Thyroid disease	
Hypothyroidism	8
Nontoxic goiter	7
Thyrotoxicosis	7
Gonadal failure	
Ovarian	20
Testicular	2
Insulin-dependent diabetes mellitus	11
Hypoparathyroidism	10
Pernicious anemia	5
None	53

Autoimmune Adrenalitis

In the Western world, autoimmune adrenalitis accounts for more than 70% of all cases of primary hypoadrenalism.[317] Pathologically, the adrenal glands are atrophic with loss of most of the cortical cells, but the medulla is usually intact. In 75% of cases, adrenal autoantibodies—specifically anti-21-hydroxylase antibodies—can be detected.[318] Fifty percent of patients with this form of Addison disease have an associated autoimmune disease (Table 15.16), thyroid disease being the most common. Conversely, only 1% to 2% of patients with more common autoimmune diseases such as insulin-dependent diabetes mellitus or thyrotoxicosis have antiadrenal autoantibodies and develop adrenal disease, although the figure is higher in patients with autoimmune hypoparathyroidism (16%). These autoimmune polyglandular syndromes (APSs) have been classified into two distinct variants.[318] APS type I, or autoimmune polyendocrinopathy-candidiasis-ectodermal dysplasia (APECED), is a rare autosomal recessive condition comprising Addison disease, chronic mucocutaneous candidiasis, and hypoparathyroidism. The more common APS type II comprises Addison disease, autoimmune thyroid disease, diabetes mellitus, and hypogonadism. Here, autoantibodies to 21-hydroxylase are usually present and are predictive for the development of adrenal destruction, particularly for APS type I.[318] Polyglandular autoimmune syndromes are discussed in greater detail in Chapter 43.

Infections

Worldwide, infectious diseases are the most common cause of primary adrenal insufficiency. These diseases include tuberculosis, fungal infections (histoplasmosis, cryptococcosis), and cytomegalovirus infection. Adrenal failure may also occur in the acquired immunodeficiency syndrome (AIDS).[319]

Tuberculous Addison disease results from hematogenous spread of the infection from elsewhere in the body; extra-adrenal disease is usually evident. The adrenals are initially enlarged, with extensive epithelioid granulomas and caseation, and both the

cortex and the medulla are affected. Fibrosis ensues, and the adrenals become normal in size or smaller, with calcification evident in 50% of cases.

The adrenals are frequently involved in patients with AIDS[319,320]; adrenalitis may occur after infection with cytomegalovirus or atypical mycobacteria, and Kaposi sarcoma may result in adrenal replacement. Onset is often insidious, but if tested, more than 10% of patients with AIDS will demonstrate a subnormal cortisol response to a short synacthen test (SST). Adrenal insufficiency may be precipitated through the concomitant administration of appropriate anti-infectives such as ketoconazole (which inhibits cortisol synthesis) or rifampicin (which increases cortisol metabolism). Rarely, patients with AIDS and features of adrenal insufficiency are found to have elevated circulating ACTH and cortisol concentrations that fail to suppress normally after low-dose dexamethasone administration. This is thought to reflect an acquired form of glucocorticoid resistance resulting from reduced GR affinity, but the underlying cause remains unknown.[321]

Acquired Primary Adrenal Insufficiency

With the exception of tuberculosis and autoimmune adrenal failure, other causes of Addison disease are rare (see Table 15.15). With the exception of lymphoma, adrenal metastases (most commonly from primary tumors in the lung or breast) uncommonly cause adrenal insufficiency,[322] perhaps because more than 90% of the adrenal cortex must be compromised before symptoms and signs become apparent. Necrosis of the adrenals due to intra-adrenal hemorrhage should be considered in any severely sick patient, particularly a patient with underlying infection, trauma, or coagulopathy.[323] Intra-adrenal bleeding may be found in patients with severe septicemia of any cause, particularly in children, in whom a common cause is infection with *Pseudomonas aeruginosa*. When caused by meningococci, the association with adrenal insufficiency is known as the Waterhouse-Friderichsen syndrome. Adrenal replacement may also occur with amyloidosis and hemochromatosis.

Inherited Primary Adrenal Insufficiency

Adrenal hypoplasia congenita (AHC) is an X-linked disorder comprising congenital adrenal insufficiency and combined primary and central hypogonadotropic hypogonadism. The condition is caused by mutations in the *DAX1 (NR0B1)* gene, a member of a nuclear receptor family that is expressed in the adrenal cortex, gonads, and hypothalamus.[324,325] Depending on the molecular defect, the clinical presentation can be highly variable. Severe cases often manifest with mineralocorticoid deficiency and gradually develop glucocorticoid deficiency. Hypogonadism is combined with primary testicular abnormalities and low gonadotropin levels. However, the so-called minipuberty of infancy can be normal.[326,327] Patients presenting with late-onset adrenal failure have also been described.[328]

Mutations in another transcription factor, SF1, may also result in adrenal insufficiency due to lack of development of a functional adrenal cortex. The transcriptional regulation of many P450 steroidogenic enzymes is dependent on SF1.[15] When it was first described, SF1 mutation was associated with complete sex reversal causing 46,XY disorder of sex development (DSD).[329] However, novel clinical phenotypes in SF1-deficient patients are now emerging; they range from isolated adrenal failure[330] to isolated gonadal failure[331] and ovarian insufficiency.[332] AHC may also occur in association with glycerol kinase deficiency and Duchenne muscular dystrophy caused by a contiguous gene deletion, including the *DAX1* gene.[333]

Adrenoleukodystrophy has a prevalence rate of 1:20,000 and is a cause of adrenal insufficiency in association with demyelination within the nervous system; demyelination results from a failure of β-oxidation of fatty acids within peroxisomes. Increased accumulation of very long-chain fatty acids (VLCFAs) occurs in many tissues, and serum assays can be used diagnostically. Only males have the fully expressed condition, and carrier females are usually normal. Several forms are recognized: a childhood cerebral form (30–40% of cases), adult adrenomyeloneuropathy (40%), and Addison disease (7%). The childhood-onset form manifests at 5 to 10 years of age with progression eventually to a blind, mute, and severely spastic tetraplegic state. Adrenal insufficiency is usually present but does not appear to correlate with the neurologic deficit. Nevertheless, this is the most common form of adrenal insufficiency in a child younger than age 7 years.[334] Adrenomyeloneuropathy, by contrast, manifests later in life with the gradual development of spastic paresis and peripheral neuropathy. Both the childhood and the adult condition result from mutations in the *ABCD1* gene on chromosome Xq28, which encodes for an ABC peroxisomal membrane protein involved in the import of VLCFA into the peroxisome.[335] So far, more than 400 mutations have been reported in the *ABCD1* gene with no relationship between genotype and phenotype.[336,337] Treatment options are few. Monounsaturated fatty acids, which block the synthesis of the saturated VLCFA, have been used; a combination of erucic acid and oleic acid (Lorenzo oil) has led to normal levels of VLCFA. Treatment does not alter the rate of neurologic deterioration but may prevent new neurologic damage in asymptomatic cases.[337] Bone marrow transplantation is a further possibility.

Familial glucocorticoid deficiency (FGD), or inherited unresponsiveness to ACTH, is a rare autosomal recessive cause of hypoadrenalism that usually manifests in childhood. Most patients present with neonatal hypoglycemia or later in life with increasing pigmentation, and they often have enhanced growth velocity. Primary adrenal failure in a child with normal activity of the RAA system is highly suggestive of FGD. The diagnosis can be confirmed by demonstrating low cortisol in combination with increased ACTH concentrations and normal plasma renin and aldosterone measurements.[92] The type 1 variant accounts for approximately 25% of all cases and is explained by inactivating mutations in the ACTH-binding receptor, MC2R.[338–340] The FGD type 2 variant caused by mutations in the *MRAP* gene, which is thought to mediate intracellular trafficking of the *MC2R*, has been reported in some families.[91] However, 50% of patients with FGD do not have mutations in either *MC2R* or *MRAP;* other loci that have been defined include *MCM4, TXNRD2, NNT*, and partial mutations in *STAR* and *CYP11A1*.[341,342]

A variant called the *triple A syndrome* or *Allgrove syndrome* refers to the triad of adrenal insufficiency due to ACTH resistance, achalasia, and alacrima. It is caused by mutations in the *AAAS* gene, which encodes ALADIN, a tryptophan/aspartate WD-repeat–containing protein of the nuclear pore complex.[343,344] The exact function of ALADIN is unknown, but its interaction with other proteins of the nuclear pore complex suggests that it is part of a structural scaffold.

Several syndromic disorders are associated with adrenal insufficiency for which the underlying molecular genetic defect remains to be elucidated.[11]

Secondary Hypoadrenalism

Inherited Central Hypoadrenalism

Central hypoadrenalism may be defined as hypocortisolemia secondary to a deficiency in ACTH. The prevalence of central hypoadrenalism is 125 to 280 per million,[1,345] which is likely to be an underestimate considering the use of therapeutic corticosteroids in the general population. It occurs in up to one-third of patients with pituitary disease.[3,346] ACTH deficiency is an important diagnosis to make; in subjects with tumoral and posttraumatic hypopituitarism, central hypoadrenalism is associated with increased mortality rates.[4–6,347–349] The causes of central hypoadrenalism are outlined in Table 15.15; the most common reason is ACTH suppression by exogenous glucocorticoid treatment.[7,185]

When caused by pituitary disease, other pituitary hormones are often deficient, so the patient presents with partial or complete hypopituitarism. The clinical features of hypopituitarism make this a relatively easy diagnosis. By contrast, isolated ACTH deficiency is rare, and the diagnosis is difficult to make.[350] It may occur in patients with lymphocytic hypophysitis. Mutations in the *TBX19* gene, the product of which (Tpit) regulates *POMC* expression, have been reported in a few cases of isolated ACTH deficiency occurring in neonatal life.[351] A rare but fascinating cause relates to a defect in the normal post-translational processing of POMC to ACTH by the prohormone convertase enzymes (PC1/3 and PC2).[352] Such patients may have more generalized defects in peptide processing (e.g., cleavage of proinsulin to insulin) giving rise to diabetes mellitus.

Some patients have mutations in the *POMC* gene that interrupt the synthesis of ACTH and cause ACTH deficiency. Elucidation of the phenotype of these patients has uncovered a novel role for POMC peptides in regulating appetite and hair color: In addition to adrenal insufficiency, *POMC* mutations result in severe obesity and often red hair pigmentation.[353] A central role for αMSH in regulating food intake via the hypothalamic MC4R has been established,[56] and in recombinant mice lacking the POMC gene, the obese phenotype can be reversed by giving an αMSH agonist peripherally.[354]

Other rare inborn causes of secondary insufficiency are the result of mutations in genes involved in pituitary development, such as *HESX1*,[355] *LHX4*,[356] *SOX3*,[357] and *PROP1*.[358] These defects result in congenital hypopituitarism with multiple pituitary hormone deficiencies: ACTH deficiency may not be present at the time of diagnosis, but it develops progressively over time.

Secondary hypoadrenalism is also observed in patients with Cushing disease after successful and selective removal of the ACTH-secreting pituitary adenoma. The function of adjacent normal pituitary corticotrophs is suppressed and may remain so for many months after curative surgery.[291–293]

ACTH Suppression by Exogenous Glucocorticoids

The ability of exogenously administered corticosteroids to cause adrenal atrophy has been appreciated since their discovery in the 1940s. HPA axis suppression by exogenous glucocorticoid is a serious medical problem and has been described with intra-articular, topical, ocular, rectal, and inhaled, as well as systemic, therapy.[359–361]

There is considerable interindividual variability in response to glucocorticoids; there are no absolute cutoff values for the type of steroid taken, dose, route of administration, duration of treatment, or time since steroid withdrawal that predict adrenal suppression. However, there are some generic issues that can guide diagnosis and therapy. Relative steroid potencies in their affinity/transactivation of the GR have been described based on suppression of corticosterone production, in vitro binding to the glucocorticoid (GR), and functional changes in GC target tissues by individual steroids[362]; these suggest that oral doses of 20 mg of hydrocortisone, 5 mg of prednisolone, and 0.75 mg of dexamethasone are bioequivalent. Dexamethasone has a longer half-life and higher affinity for the GR than hydrocortisone and exerts a more sustained suppressive effect on the HPA axis. Similarly, it is unclear how the potency of oral hydrocortisone compares to that of glucocorticoids taken by other routes. Budesonide has more potent action at the GR than dexamethasone or prednisolone,[363] but its effect on adrenal suppression is dependent on systemic absorption of the inhaled compound. Comparison of inhaled fluticasone with inhaled steroids such as budesonide or beclomethasone indicated that fluticasone was more frequently associated with suppression of the HPA axis and that adults using over 1000 μg of inhaled fluticasone for over a year were at risk of adrenal suppression.[364]

Concomitant therapies can augment potency and adrenal suppression. The coadministration of inhaled fluticasone with one of the many medications that suppress its clearance by inhibition of CYP3A4 is associated with adrenal suppression (e.g., ritonavir).[365] Co-prescriptions of agents that do not affect glucocorticoid clearance but have affinity for the GR are also associated with adrenal suppression, an example being progesterone derivatives such as medroxyprogesterone acetate given in high dose to oncology patients.[179]

In terms of dose and duration, adrenal atrophy and subsequent deficiency should be anticipated in any subject who has taken more than the equivalent of 30 mg hydrocortisone per day orally (>7.5 mg/day prednisolone or >0.75 mg/day dexamethasone) for longer than 3 weeks. In addition to the magnitude of the dose of glucocorticoid, the timing of administration may affect the degree of adrenal suppression. If prednisolone is given as 5 mg at night and 2.5 mg in the morning, there will be more marked suppression of the HPA axis compared with 2.5 mg at night and 5 mg in the morning, because the larger evening dose blocks the early-morning surge of ACTH. LaRochelle and colleagues reported that adrenal function recovered if patients' steroid doses could be tapered to 5 mg of prednisone daily.[366] This report has formed the basis of the practice of assessing HPA axis function in patients who have been on more than 5 mg of prednisolone or equivalent for more than 3 months. Lower doses of glucocorticoid have been shown to suppress cortisol production; in one study, more than 60% of subjects on a glucocorticoid dose equivalent of less than 5 mg of prednisolone per day had a subnormal ACTH or cortisol response to CRH.[367] In patients on long-term tapering of prednisolone who were considered for steroid withdrawal once they had reached a dose of 7 mg/day, 48% had adrenal insufficiency defined by basal cortisol (<100 nmol/L) or response to 250 μg synthetic ACTH (<550 nmol/L); a longer duration of prednisolone therapy (13.7 years) was seen in the hypoadrenal group defined by a low baseline cortisol but not in the group defined by cortisol response to 250 μg synthetic ACTH (6.1 years).[368] Dynamic testing of adrenal function, such as the SST and CRH tests, suggests that cortisol production recovers after withdrawal of long-term glucocorticoids; in a meta-analysis of patients receiving glucocorticoid therapy, 46% to 100% had insufficient cortisol response 1 day after withdrawal, which improved to 26% to 49% in patients at assessment 1 week later.[37] Up to 10% of patients still

TABLE 15.17 Suggested Plan for Steroid Replacement in Patients Withdrawing From Chronic Corticosteroid Therapy

	DURATION OF GLUCOCORTICOID TREATMENT			
Pred Dose (mg/day)	**≤3 wk[a]**		**>3 wk**	
≥7.5	Can stop	↓ rapidly (e.g., 2.5 mg q3-4d)		
		THEN		
5–7.5	Can stop	↓ 1 mg q2-4 wk	OR	Convert 5 mg pred to 20 mg HC, then ↓ 2.5 mg/wk to 10 mg/day
		THEN		THEN
<5	Can stop	↓ 1 mg q2-4 wk		After 2-3 mo HC 10 mg/day, administer SST/ITT: Pass → Withdraw Fail → Continue

[a]Beware of frequent steroid courses (e.g., in asthma).

HC, Hydrocortisone; *ITT,* insulin tolerance test; *pred,* prednisolone; *SST,* short synacthen test. Basal 0900h ACTH can be used to monitor for recovery of HPA axis and levels may be above the normal range ahead of a "pass" on an SST.

have biochemical evidence of hypoadrenalism between 6 and 20 months after withdrawal of glucocorticoids.[369]

All patients receiving long-term therapy with corticosteroids should be treated in a similar fashion to patients with chronic ACTH deficiency; they should carry steroid cards and be offered steroid alert bracelets or necklaces. In the event of an intercurrent stress (e.g., infection, surgery), supplemental steroid cover should be given. If the patient is unable to take drugs orally, parenteral therapy is required.

During recovery from suppression and without replacement therapy, patients may experience symptoms of glucocorticoid deficiency, including anorexia, nausea, weight loss, arthralgia, lethargy, skin desquamation, and postural dizziness (see the later discussions of adrenal insufficiency).[370] To avoid these symptoms, steroids should be cautiously withdrawn over a period of months.[182] Assuming that the underlying disease permits steroid reduction, doses should be reduced from pharmacologic levels to physiologic levels (equivalent to 7.5 mg/day prednisolone) over a few weeks. Thereafter doses should be reduced by 1 mg per day of prednisolone every 2 to 4 weeks depending on patient well-being. An alternative approach is to switch the patient to hydrocortisone 20 mg/day and reduce the daily dose by 2.5 mg/day every week to a level of 10 mg/day. After 2 to 3 months on reduced doses, endogenous function of the HPA axis can be assessed by basal 9:00 AM serum cortisol values and an SST or an insulin-induced hypoglycemia test, as needed. A "pass" response to these tests indicates adequate function of the HPA axis, and corticosteroid therapy can be safely withdrawn. The level of basal 9:00 AM serum cortisol that predicts a pass on an SST varies by the assay used from 336 to 506 nmol/L, indicating the importance of knowledge of assay characteristics.[371] In those patients who are taking physiologic doses of prednisolone (<5–7.5 mg/day) or equivalent corticosteroid, an SST given 12 to 24 hours after omitted steroid therapy will provide an immediate answer as to whether sudden or gradual withdrawal of steroid therapy is indicated (Table 15.17).[361]

Iatrogenic-induced Cushing syndrome occurs in patients who take suppressive doses of corticosteroids for longer than 3 weeks.[182] The rapidity of onset of clinical features depends on the administered dose but can occur within 1 month of therapy.

Hypoadrenalism During Critical Illness

Hypoadrenalism may also complicate critical illness, even in individuals with a previously intact HPA axis.[372] This has been termed *functional adrenal insufficiency* to reflect the notion that hypoadrenalism is transient and is not caused by a structural lesion. Functional adrenal insufficiency has been difficult to define biochemically and is of uncertain cause. Moreover, in patients with hypoproteinemia, with serum albumin less than 2.5 g/dL, total serum cortisol may be low, but free cortisol is normal.[252] Nevertheless, an inability to mount an adequate and appropriate cortisol response to overwhelming stress or sepsis is frequently encountered in intensive care units and substantially increases the risk of death during acute illness.[373] This has stimulated attempts to define functional adrenal insufficiency quantitatively and to treat it with supplemental corticosteroids. Although this diagnosis remains highly contentious, if a suboptimal cortisol response is suspected, the current recommendations suggest (1) treatment with hydrocortisone, 200 mg/day in four divided doses or, preferably, 10 mg/hour as a continuous infusion, for patients with septic shock and (2) treatment with methylprednisolone, 1 mg/kg per day, for patients with severe early acute respiratory distress syndrome. Glucocorticoid treatment should be tapered off rather than stopped abruptly. Treatment of critical illness–related adrenal insufficiency with dexamethasone is not recommended.[374]

Clinical Features of Adrenal Insufficiency

Patients with primary adrenal failure usually have both glucocorticoid and mineralocorticoid deficiencies. In contrast, those with secondary adrenal insufficiency have an intact RAA system. This accounts for differences in salt and water balance in the two groups of patients, which in turn result in different clinical presentations. The most obvious feature that differentiates primary from secondary hypoadrenalism is skin pigmentation (Table 15.18), which is almost always present in cases of primary adrenal insufficiency (unless of short duration) and absent in secondary insufficiency. The pigmentation is seen in sun-exposed areas, recent rather than old scars, axillae, nipples, palmar creases, pressure points, and mucous membranes (buccal, vaginal, vulval, anal). The cause of the pigmentation has long been debated but is

TABLE 15.18 Clinical Features of Primary Adrenal Insufficiency

Feature	Frequency (%)
Symptoms	
Weakness, tiredness, fatigue	100
Anorexia	100
Gastrointestinal symptoms	92
Nausea	86
Vomiting	75
Constipation	33
Abdominal pain	31
Diarrhea	16
Salt craving	16
Postural dizziness	12
Muscle or joint pains	13
Signs	
Weight loss	100
Hyperpigmentation	94
Hypotension (<110 mm Hg systolic)	88-94
Vitiligo	10-20
Auricular calcification	5
Laboratory Findings	
Electrolyte disturbances	92
Hyponatremia	88
Hyperkalemia	64
Hypercalcemia	6
Azotemia	55
Anemia	40
Eosinophilia	17

thought to reflect increased stimulation of the MC1R by ACTH itself. In autoimmune Addison disease, there may be associated vitiligo (Fig. 15.32).

The clinical features relate to the rate of onset and the severity of adrenal deficiency.[314] In many cases, the disease has an insidious onset, and a diagnosis is made only when the patient presents with an acute crisis during an intercurrent illness. Acute adrenal insufficiency, termed an *adrenal crisis* or *addisonian crisis,* is a medical emergency manifesting as hypotension and acute circulatory failure (Table 15.19). Anorexia may be an early feature; it progresses to nausea, vomiting, diarrhea, and sometimes abdominal pain. Fever may be present, and hypoglycemia may occur. Patients presenting acutely with adrenal hemorrhage have hypotension; abdominal, flank, or lower chest pain; anorexia; and vomiting. The condition is difficult to diagnose, but evidence of occult hemorrhage (rapidly falling hemoglobin), progressive hyperkalemia, and shock should alert the clinician to the diagnosis.

Alternatively, the patient may present with vague features of chronic adrenal insufficiency—weakness, tiredness, weight loss, anorexia or nausea, intermittent vomiting, abdominal pain, diarrhea or constipation, general malaise, muscle cramps, arthralgia, and symptoms suggestive of postural hypotension (see Table 15.18). Salt craving may be a feature, and a low-grade fever may be present. Supine blood pressure is usually normal, but almost invariably there is a fall in blood pressure on standing. Adrenal androgen secretion is lost; this is clinically more apparent in women, who may notice loss of axillary and pubic hair and frequently have dry and itchy skin. Psychiatric symptoms may occur in long-standing cases and include memory impairment, depression, and psychosis. Formal quality-of-life measures indicate significant impairment in patients with primary or secondary adrenal insufficiency.[375] Tiredness is often profound, and patients may be inappropriately diagnosed with chronic fatigue syndrome or anorexia nervosa.[376]

In secondary adrenal insufficiency due to hypopituitarism, the presentation may relate to deficiency of hormones other than ACTH, notably LH/FSH (infertility, oligomenorrhea/amenorrhea, poor libido) and TSH (weight gain, cold intolerance). Fasting hypoglycemia occurs because of loss of the gluconeogenic effects of cortisol. It is rare in adults unless there is concomitant alcohol abuse or additional GH deficiency. However, hypoglycemia is a common presenting feature of ACTH/adrenal insufficiency in childhood.[377] In addition, patients with ACTH deficiency present with malaise, weight loss, and other features of chronic adrenal insufficiency. Rarely, the presentation is more acute in patients with pituitary apoplexy.

Investigation of Hypoadrenalism

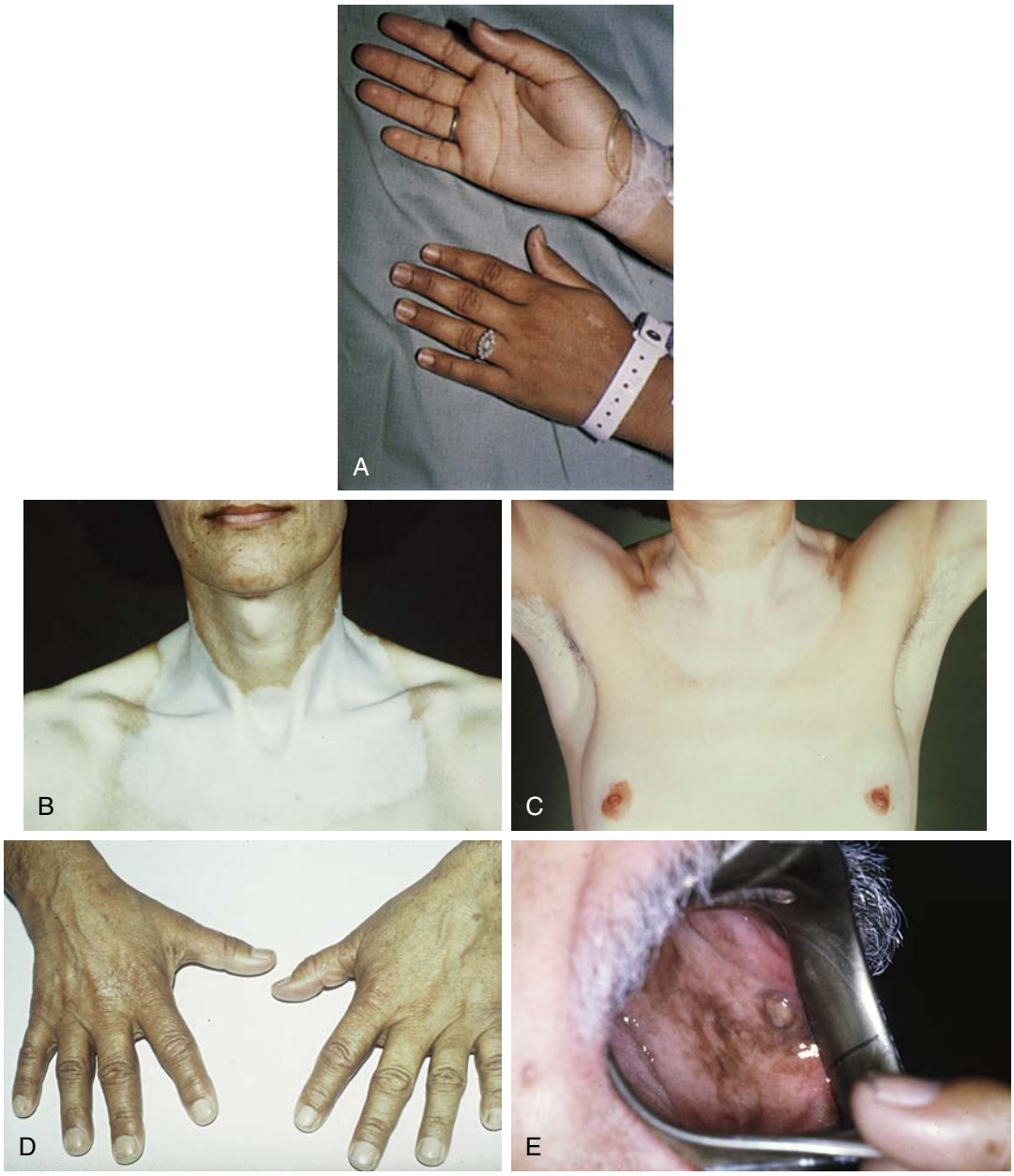
Routine Biochemical Profile

Among patients with established primary adrenal insufficiency, hyponatremia is present in about 90% and hyperkalemia in 65%. The blood urea concentration is usually elevated. Hyperkalemia occurs because of aldosterone deficiency, so it is usually absent in patients with secondary adrenal failure. Hyponatremia may be depletional in an addisonian crisis, but vasopressin levels are elevated, resulting in increased free water retention.[378] Therefore, in secondary adrenal insufficiency, there may be a dilutional hyponatremia with normal or low blood urea.

Reversible abnormalities in liver transaminases frequently occur. Hypercalcemia occurs in 6% of all cases[379] and may be particularly marked in patients with coexisting thyrotoxicosis. Free thyroxine concentrations are usually low or normal, but TSH values are frequently moderately elevated.[380] This is a direct effect of glucocorticoid deficiency and reverses with replacement therapy. Persistent elevation of TSH in association with positive thyroid autoantibodies suggests concomitant autoimmune thyroid disease.

Mineralocorticoid Status

In primary hypoadrenalism, mineralocorticoid deficiency usually occurs, manifested by elevated plasma renin activity and either low or low-normal plasma aldosterone. The investigation of ZG activity is frequently neglected in Addison disease, compared with assessment of ZF function. In secondary adrenal insufficiency, the RAA system is intact.



• **Fig. 15.32** Pigmentation in Addison disease. (A) Hands of an 18-year-old woman with autoimmune polyendocrine syndrome and Addison disease. Pigmentation in a patient with Addison disease before (B) and after (C) treatment with hydrocortisone and fludrocortisone. Notice the additional presence of vitiligo. (D) Similar changes in a 60-year-old man with tuberculous Addison disease before *(left)* and after *(right)* corticosteroid therapy. (E) Buccal pigmentation in the same patient as in (D). (B, C, courtesy Professor C.R.W. Edwards.)

Assessing Adequacy of Function of the HPA Axis

Clinical suspicion of the diagnosis should be confirmed with definitive diagnostic tests. Basal plasma cortisol and urinary free cortisol levels are often in the low-normal range and cannot be used to exclude the diagnosis. However, a basal cortisol value greater than 400 nmol/L (>14.5 μg/dL) indicates an intact HPA axis. In patients with a suspected addisonian crisis, however, treatment should be instigated immediately and prior to the result of biochemical testing being available. The SST involves intramuscular or intravenous administration of 250 μg tetracosactrin, synthetic ACTH(1-24).[381] Plasma cortisol levels are measured at 0 and 30 to 60 minutes after ACTH administration, and a normal response has previously been defined by a peak plasma cortisol level greater than 550 nmol/L (>20 μg/dL).[382] This value equates to the 5th percentile response in normal subjects but is very much assay dependent, with different cortisol radioimmunoassays giving different results. With more recent immunoassays and LC-MS/MS assays that read lower, levels above 430 to 450 nmol/L are usually consistent with normal adrenal function.[383] Incremental responses (i.e., the difference between peak and basal values) are of no value in defining a "pass" response, with the possible exception of diagnosing functional adrenal insufficiency in patients with critical illness. Response is unaffected by the time of day of the test, and the test can be performed in patients who

TABLE 15.19 Clinical and Laboratory Features of an Adrenal Crisis

Dehydration, hypotension, or shock out of proportion to severity of current illness
Nausea and vomiting with a history of weight loss and anorexia
Abdominal pain, so-called acute abdomen
Unexplained hypoglycemia
Unexplained fever
Hyponatremia, hyperkalemia, azotemia, hypercalcemia, or eosinophilia
Hyperpigmentation or vitiligo
Other autoimmune endocrine deficiencies, such as hypothyroidism or gonadal failure

have commenced corticosteroid replacement therapy, as long as this therapy is of short duration and does not include hydrocortisone (which would be detected in the cortisol assay). Depot or prolonged ACTH stimulation tests to discriminate between primary and secondary adrenal insufficiency are no longer indicated if plasma ACTH has been appropriately measured at baseline.

For investigation of secondary adrenal insufficiency the insulin-induced hypoglycemia test or insulin tolerance test (ITT) remains the gold standard test of the integrity of the HPA axis.[384] It should not be performed in patients with ischemic heart disease (always check an electrocardiogram before the test), epilepsy, or severe hypopituitarism (i.e., 9 AM plasma cortisol <180 nmol/L [<6.5 μg/dL]). The test involves the intravenous administration of soluble insulin in a dose of 0.1 to 0.15 U/kg body weight, with measurement of plasma cortisol at 0, 30, 45, 60, 90, and 120 minutes. Adequate hypoglycemia (blood glucose <2.2 mmol/L with signs of neuroglycopenia—sweating and tachycardia) is required for a fail result. In normal subjects, the peak plasma cortisol concentration exceeds 500 nmol/L (18 μg/dL). However, the cortisol response to hypoglycemia can be reliably predicted by the SST—a safer, cheaper, and quicker test.[381,385]

The SST relies on the principle that the cortisol response to an exogenous bolus of ACTH is determined by the endogenous ACTH trophic drive to the adrenal cortex; impaired ACTH secretion from the anterior pituitary results in an impaired cortisol response after synacthen administration. However, the ACTH test should not be used to diagnose central hypoadrenalism in patients with a recent pituitary insult (e.g., surgery, apoplexy). Total hypophysectomy results in a failed cortisol response to ITT immediately thereafter, but it takes 6 weeks for the adrenal cortex to readjust to the reduced level of ACTH secretion; in the interim, a false-positive (normal) cortisol response is seen. The SST should also be avoided in patients with a primary diagnosis of Cushing disease, in whom an exaggerated cortisol response to ACTH may persist following surgical removal of the adenoma.

In clinical practice, if the SST is normal, insulin hypoglycemia testing is not necessary in most cases unless there is also a need to document endogenous GH reserve in a patient with pituitary disease. Some patients have an inadequate SST response but then respond normally to hypoglycemia[385]; they do not require corticosteroid replacement therapy. Conversely, falsely normal results have been reported for the SST.[386] Although these are rare (<2%), the possibility should be noted, particularly in patients with ongoing symptoms and signs indicative of hypoadrenalism.

A low-dose SST giving only 1 μg ACTH(1-24) has been proposed as a screen for adequacy of function of the HPA axis, with the suggestion that it may be more sensitive than the conventional 250-μg test.[387–389] Other researchers dispute this suggestion,[390,391] and although popular with pediatric endocrinologists, wide variations in the dose administered using differing dilutional methods to prepare the 1-μg dose preclude reliance on this test.[392]

Two other tests have been advocated to assess adequacy of function of the HPA axis, but their use in modern clinical practice should be restricted to difficult diagnostic cases. In the overnight metyrapone test, 30 mg/kg (maximum, 3 g) metyrapone is given at midnight, and plasma cortisol and 11-deoxycortisol are measured at 8 AM the following morning. In patients with an intact HPA axis, ACTH levels rise after the blockade of cortisol synthesis by metyrapone (documented with a serum cortisol <5 μg/dL), and a normal result is signified by a peak 11-deoxycortisol value greater than 7 μg/dL.[393] The CRH stimulation test has been used to diagnose adrenal insufficiency; unlike the metyrapone test, it differentiates primary from secondary causes. Patients with primary adrenal failure have high ACTH levels that rise further after CRH stimulation. Patients with secondary adrenal failure have low ACTH levels that fail to respond to CRH. Patients with hypothalamic disease show a steady rise in ACTH levels after CRH administration.[394]

Testing the HPA Axis During Critical Illness

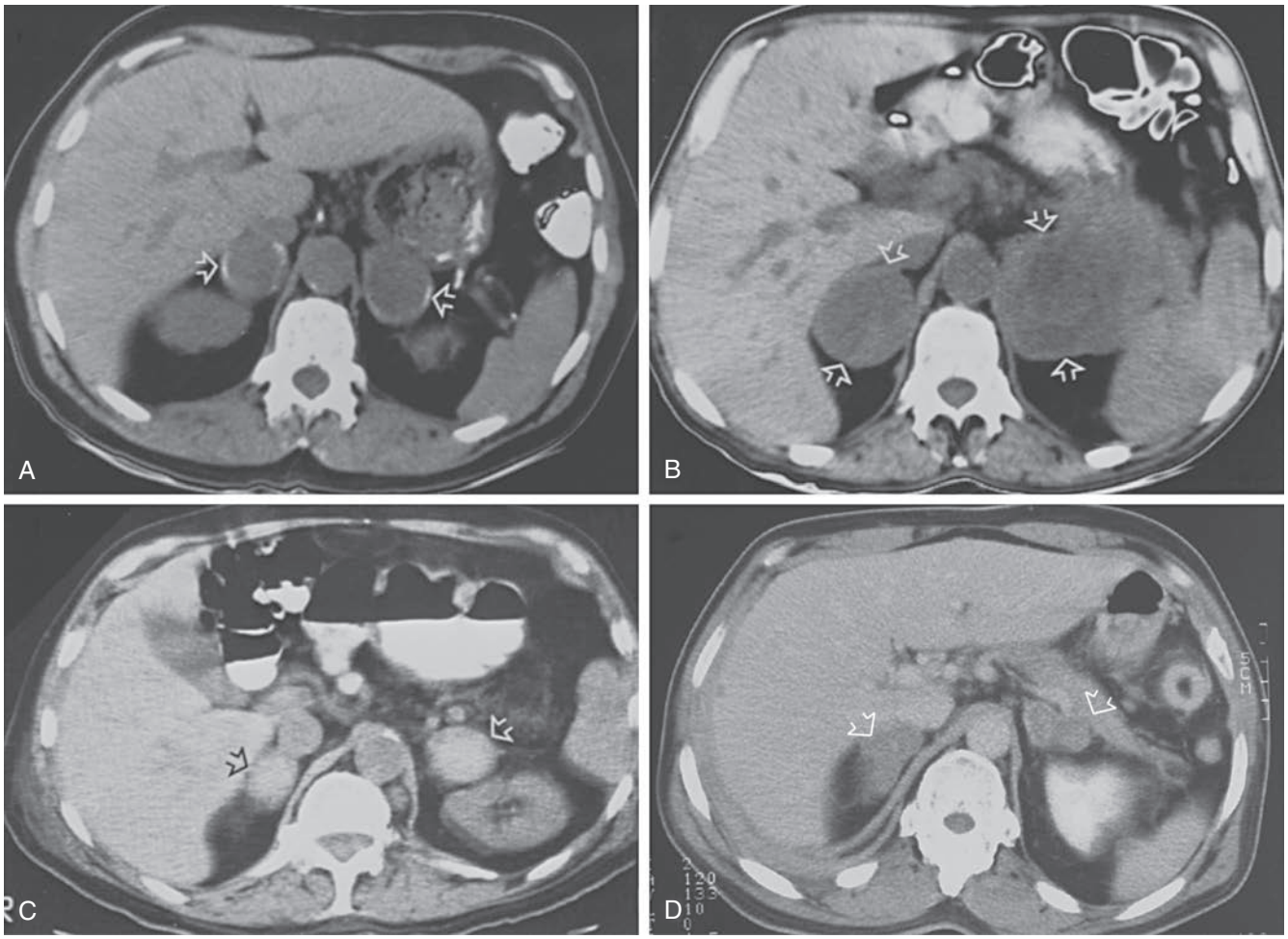
Many factors complicate investigation of the HPA axis during critical illness. Cortisol levels vary broadly with disease severity, making it difficult to define appropriate responses. Additionally, CBG levels decrease substantially, leading to increases in the ratio of free to bound serum cortisol; for this reason, tests that assess the whole axis (e.g., ITT) are not appropriate in the critical care setting. Investigations are therefore limited mainly to basal cortisol levels, with the caveat of low total but preserved free serum cortisol.[252] Methylprednisolone is recommended for patients with severe early acute respiratory distress syndrome, particularly those with poor response to fluid resuscitation and vasopressor agents.[395] The role of glucocorticoids in the management of critically ill patients with other conditions requires further research.

Other Tests

Autoantibodies to 21-hydroxylase should be analyzed in patients with primary adrenal failure. In autoimmune Addison disease, it is also important to look for evidence of other organ-specific autoimmune disease. A CT scan may reveal enlarged or calcified adrenals, suggesting an infective, hemorrhagic, or malignant diagnosis (Fig. 15.33). Chest radiography, tuberculin testing, and early-morning urine samples cultured for *Mycobacterium tuberculosis* should be performed if tuberculosis is suspected. CT-guided adrenal biopsy may reveal an underlying diagnosis in patients with suspected malignant deposits in the adrenal gland. Adrenoleukodystrophy can be diagnosed by measuring circulating levels of VLCFA, which should be obtained in males with primary adrenal insufficiency and negative 21-hydroxylase antibodies. Finally, appropriate investigations, including pituitary MRI scans and an assessment of anterior function, are required for patients with suspected secondary hypoadrenalism who are not taking corticosteroid therapy.

Treatment of Acute Adrenal Insufficiency

Acute adrenal insufficiency is a life-threatening emergency, and treatment should not be delayed while waiting for definitive proof of diagnosis (Table 15.20). However, in addition to measurement



• **Fig. 15.33** Computed tomographic (CT) scans of patients with primary adrenal insufficiency. The affected adrenal glands are indicated by *arrows*. (A) CT scan of a 59-year-old man with histoplasmosis. Notice the subcapsular calcium in both glands. (B) CT scan of a 59-year-old man with metastatic melanoma. (C) CT scan of an 80-year-old man with bilateral adrenal hemorrhage resulting from anticoagulation for pulmonary emboli. (D) Bilateral adrenal tuberculomas in a 79-year-old man with tuberculosis affecting the urogenital tract. (A, B, courtesy Dr. William D. Salmon, Jr.; C, courtesy Dr. Craig R. Sussman.)

of plasma electrolytes and blood glucose, appropriate samples for ACTH and cortisol should be taken before corticosteroid therapy is given. If the patient is not critically ill, an SST can be performed.

In adults, intravenous hydrocortisone should be given in a dose of 100 mg every 6 to 8 hours. If venous access is not possible, then the intramuscular route should be used. In the patient with shock, 1 L of normal saline should be given intravenously over the first hour. Because of possible hypoglycemia, it is normal to give 5% dextrose in saline. Subsequent saline and dextrose therapy will depend on biochemical monitoring and the patient's condition. Clinical improvement, especially in the blood pressure, should be seen within 4 to 6 hours if the diagnosis is correct. It is important to recognize and treat any associated condition (e.g., infection) that may have precipitated the acute adrenal crisis.

After the first 24 hours, the dose of hydrocortisone can be reduced, usually to 50 mg intramuscularly every 6 hours and then to oral hydrocortisone, 40 mg in the morning and 20 mg at 3 to 6 PM. This dose can then be rapidly reduced to a more standard replacement dose of 10 to 20 mg on awakening and 5 to 10 mg at 3 to 6 PM.

Long-Term Replacement Therapy

The aim of long-term therapy is to give replacement doses of hydrocortisone to mimic the normal cortisol secretion rate (Table 15.21). In the past, this rate was thought to be approximately 25 to 30 mg/day, but stable isotope studies have indicated lower normal cortisol production rates of 8 to 15 mg/day.[396] Most patients are adequately treated with less than 30 mg/day (usually 15–25 mg/day in divided doses). Doses are usually given on awakening, with a smaller dose at 3 to 6 PM, but some patients feel better with three-times-a-day dosing. In cases of primary adrenal failure, some clinicians advocate cortisol day curves with simultaneous ACTH measurements to provide some insight into the adequacy of replacement therapy.[397] A simple weight-adjusted regime significantly reduces intraindividual variation in circulating levels.[398] There are no good biomarkers of glucocorticoid adequacy in patients with central hypoadrenalism. Decisions regarding doses of replacement therapy are largely based on crude, yet important, end points such as weight, well-being, and blood pressure.[399] Bone mineral density is moderately reduced in a dose-dependent manner in patients treated with more than

TABLE 15.20 Treatment of Acute Adrenal Insufficiency (Adrenal Crisis) in Adults

Emergency Measures

1. Establish intravenous access with a large-gauge needle.
2. Draw blood for immediate serum electrolytes and glucose and routine measurement of plasma cortisol and ACTH. Do not wait for laboratory results.
3. Infuse 2–3 L of 154 mmol/L NaCl (0.9% saline) solution, or 50 g/L (5%) dextrose in 154 mmol/L NaCl (0.9% saline) solution, as quickly as possible. Monitor for signs of fluid overload by measuring central or peripheral venous pressure and listening for pulmonary rales. Reduce infusion rate if indicated.
4. Inject intravenous hydrocortisone (100 mg immediately and every 6 hours).
5. Use supportive measures as needed.

Subacute Measures After Stabilization of the Patient

1. Continue intravenous 154 mmol/L NaCl (0.9% saline) solution at a slower rate for next 24–48 hours.
2. Search for and treat possible infectious precipitating causes of the adrenal crisis.
3. Perform a short ACTH stimulation test to confirm the diagnosis of adrenal insufficiency (if patient does not have known adrenal insufficiency).
4. Determine the type of adrenal insufficiency and its cause, if not already known.
5. Taper glucocorticoids to maintenance dosage over 1–3 days, if precipitating or complicating illness permits.
6. Begin mineralocorticoid replacement with fludrocortisone (0.1 mg by mouth daily) when saline infusion is stopped.

ACTH, Adrenocorticotropic hormone.

TABLE 15.21 Treatment of Chronic Primary Adrenal Insufficiency in Adults

Maintenance Therapy

Glucocorticoid Replacement

- Hydrocortisone 15–20 mg on awakening and 5–10 mg in early afternoon
- Monitor clinical symptoms and morning plasma ACTH.

Mineralocorticoid Replacement

- Fludrocortisone 0.1 (0.05–0.4) mg orally
- Liberal salt intake
- Monitor lying and standing blood pressure and pulse, edema, serum potassium, and plasma renin activity.
- Educate patient about the disease, how to manage minor illnesses and major stresses, and how to inject steroid intramuscularly.
- Obtain MedicAlert bracelet/necklace, Emergency Medical Information card.

Treatment of Minor Febrile Illness or Stress

- Increase glucocorticoid dose twofold to threefold for the few days of illness; do not change mineralocorticoid dose.
- Contact physician if illness worsens or persists for more than 3 days or if vomiting develops.
- No extra supplementation is needed for most uncomplicated, outpatient dental procedures with local anesthesia. General anesthesia or intravenous sedation should not be used in the office.

Emergency Treatment of Severe Stress or Trauma

- Inject contents of prefilled dexamethasone (4-mg) syringe or contents of hydrocortisone hemisuccinate rapid reconstitution vial (100-mg) intramuscularly.
- Get to physician as quickly as possible.

Steroid Coverage for Illness or Surgery in Hospital

- For moderate illness, give hydrocortisone 50 mg bid PO or IV. Taper rapidly to maintenance dose as patient recovers.
- For severe illness, give hydrocortisone 100 mg IV q8h. Taper to maintenance level by decreasing by half every day. Adjust dose according to course of illness.
- For minor procedures under local anesthesia and most radiologic studies, no extra supplementation is needed.
- For moderately stressful procedures such as barium enema, endoscopy, or arteriography, give a single 100-mg IV dose of hydrocortisone just before the procedure.
- For major surgery, give hydrocortisone 100 mg IV just before induction of anesthesia and continue q8h for first 24 hours. Taper dose rapidly, decreasing by half per day, to maintenance level.

ACTH, Adrenocorticotropic hormone; *bid,* twice a day; *IV,* intravenous; *PO,* orally; *q,* every.

25 mg/day of hydrocortisone,[400] highlighting the need to strive for minimally effective but safe doses.[401,402] Possibly because of the known action of IGF1 to increase cortisol clearance,[137] glucocorticoid requirements are slightly lower in hypopituitary, GH-deficient subjects than in patients with primary adrenal insufficiency.

In primary adrenal failure, mineralocorticoid replacement is usually also required in the form of fludrocortisone (or 9α-fluorinated hydrocortisone), 0.05 to 0.2 mg/day. The mineralocorticoid activity of this drug is about 125 times that of hydrocortisone. After the acute phase has passed, the adequacy of mineralocorticoid replacement should be assessed by measuring electrolytes, supine and erect blood pressures, and plasma renin activity or mass.[403] Too little fludrocortisone may cause postural hypotension with elevated plasma renin activity, whereas too much causes the converse. Mineralocorticoid replacement therapy is all too frequently neglected in patients with adrenal failure.[404]

Patients on glucocorticoid replacement therapy should be advised to double their daily dose in the event of intercurrent febrile illness or accidents. If the patient is vomiting and cannot take medication by mouth, parenteral hydrocortisone must be given urgently. For minor surgery, 50 to 100 mg hydrocortisone hemisuccinate is given with the premedication. For major operations, this pretreatment is followed by the same regimen as for acute adrenal insufficiency (see Table 15.21). Pregnancy proceeds normally in patients taking replacement therapy, but daily doses of hydrocortisone are usually increased modestly (5–10 mg/day) in the last trimester. Progesterone is a mineralocorticoid antagonist, and the rising levels across pregnancy may necessitate an increased dose of fludrocortisone. During labor, patients should be well hydrated with a saline drip and should receive hydrocortisone 50 mg intramuscularly every 6 hours until delivery. Thereafter, doses can be rapidly tapered to prepregnancy levels.

Every patient on glucocorticoid therapy should be advised to register for a medical alert bracelet or necklace and to carry information describing their condition, treatment, and doctors. Patients should receive regular education regarding the requirements of stress-related glucocorticoid dose adjustment, which should involve the patient's partner and family as well. Parenteral preparations of hydrocortisone for self-administration may be required for patients living far from hospitals and those traveling.

For women with both primary and secondary adrenal failure, beneficial effects of adrenal androgen replacement therapy with 25 to 50 mg/day of DHEA have been reported. To date, reported benefit is principally confined to female patients and includes improvement in sexual function and well-being.[405] However, patients with adrenal insufficiency on current steroid replacement regimens have significantly impaired health-related subjective health status irrespective of the origin of disease or concomitant disease.[375] Delayed-release hydrocortisone preparations, such as Plenadren, that more closely replicate normal circadian cortisol concentrations have recently been licensed and approved; early clinical trials show improved quality of life in both primary and central hypoadrenalism compared to conventional twice-daily or thrice-daily hydrocortisone administration.[406]

Congenital Adrenal Hyperplasia

CAH comprises a group of autosomal recessive disorders caused by deficient adrenal corticosteroid biosynthesis.[407,408] It results from defects in one of the steroidogenic enzymes involved in cortisol biosynthesis or in the electron-providing factor, POR. Congenital lipoid adrenal hyperplasia, caused by StAR deficiency affecting mitochondrial cholesterol uptake, is a subform of this disease complex with the unique feature of lipid accumulation leading to cell destruction. In each case, there is reduced negative feedback inhibition of cortisol and, depending on the steroidogenic pathway involved, alteration in adrenal mineralocorticoid and androgen secretion (Table 15.22).

Aldosterone synthase deficiency does not affect glucocorticoid biosynthesis and does not lead to adrenal hyperplasia, but it has been historically grouped into this disease complex. All forms of CAH together represent a disease continuum, ranging from severe forms caused by complete loss-of-function defects to milder forms in which the defective proteins have partial residual activity.

21-Hydroxylase Deficiency

Between 90% and 95% of cases of CAH are caused by 21-hydroxylase deficiency.[407,408] In Western societies, the incidence of classic 21-hydroxylase deficiency (defined by cortisol deficiency) varies from 1 in 10,000 to 1 in 15,000 live births, but in isolated communities the incidence may be much higher (e.g., 1:300 in Alaskan Yupik populations). Nonclassic 21-hydroxylase deficiency is more common, with an incidence of about 1 in 500 to 1000 live births. The condition arises because of defective conversion of 17OHP to 11-deoxycortisol. Reduced cortisol biosynthesis results in reduced negative feedback drive and increased ACTH secretion; as a consequence, adrenal androgens are produced in excess (Fig. 15.34). Seventy-five percent of patients with classic 21-hydroxylase deficiency have clinically manifest mineralocorticoid deficiency because of failure to convert sufficient progesterone to DOC in the ZG. Clinically, several distinct variants of 21-hydroxylase deficiency have been recognized (Table 15.23).

Simple Virilizing Form

In the simple virilizing form of 21-hydroxylase deficiency, the enhanced ACTH drive to adrenal androgen secretion in utero leads to virilization of an affected female fetus. Depending on the severity, clitoral enlargement, labial fusion, and development of a urogenital sinus may occur, leading to sexual ambiguity at birth and even inappropriate sex assignment. Males are phenotypically normal at birth and are at risk of not being diagnosed; this explains the skewed female-to-male ratio of simple virilizing CAH diagnosed in the preneonatal screening era. Such patients may present in early childhood with signs of precocious pseudopuberty such as sexual precocity, pubic hair development, or growth acceleration due to premature androgen excess. If left untreated, this sex steroid production stimulates premature epiphyseal closure, and final adult height is invariably diminished.[409,410]

Salt-Wasting Form

Seventy-five percent of patients of both sexes with classic 21-hydroxylase deficiency have the salt-wasting form and also have concomitant, clinically manifested aldosterone deficiency. In addition to the described features, neonates commonly present after the first 2 weeks of life with a salt-wasting crisis and hypotension if not diagnosed from screening. The clinical signs and symptoms of salt wasting include poor feeding, vomiting, failure to thrive, lethargy, and sepsis-like symptoms. These features may alert the clinician to the diagnosis in a male baby, but the diagnosis is still delayed in many cases, and the condition carries a significant neonatal mortality rate.

Nonclassic or Late-Onset 21-Hydroxylase Deficiency

Patients with nonclassic 21-hydroxylase (NCAH) deficiency present in childhood or early adulthood with premature pubarche or with a phenotype that may masquerade as polycystic ovary syndrome (PCOS).[407,411,412] Indeed, nonclassic 21-hydroxylase deficiency is a recognized secondary cause of PCOS and is far more common than the classic variant. Some evidence suggests that at least 30% of adult patients have an impaired cortisol response to ACTH(1-24)[413] and may be prone to stress-induced adrenal insufficiency, but the rates of adrenal crises in untreated patients appear to be very low. There is overlap in the effects of mutations that cause NCAH and classic 21-hydroxylase deficiency, and if patients fail an SST they are better characterised as occult classic 21-hydroxylase deficiency. In some series from tertiary referral centers, nonclassic 21-hydroxylase deficiency accounts for up to 12% of all PCOS patients, but more realistic prevalence rates are probably 1% to 3%.[414] Females present with hirsutism, primary or secondary amenorrhea, or anovulatory infertility.[411] Androgenic alopecia and acne may be other presenting features.

Heterozygote 21-Hydroxylase Deficiency

Salt wasting, simple virilizing, and late-onset 21-hydroxylase deficiency are all caused by homozygous or compound heterozygote mutations in the human 21-hydroxylase gene *(CYP21A2)*. In the carrier or heterozygote state, only one allele is mutated. The clinical significance of the heterozygote state is uncertain; it does not appear to disadvantage reproductive capability but may cause signs of hyperandrogenism in adult women.[407]

Molecular Genetics

21-Hydroxylase deficiency is inherited as an autosomal recessive trait, and the higher incidence of the condition in some ethnic communities almost certainly relates to consanguinity. The *CYP21A2* gene and its highly homologous pseudogene *(CYP21A1P)* are located on the short arm of chromosome 6 (6p21.3). Because of the genomic localization within the human leukocyte antigen (HLA) locus, a region with a high frequency of genomic recombinations, most of the mutations causing 21-hydroxylase deficiency are generated by gene conversion events from the *CYP21A1P* pseudogene. Complete gene deletions or conversions of the *CYP21A2* gene, eight pseudogene-derived point mutations, and an 8–base

TABLE 15.22 Congenital Adrenal Hyperplasia: Features for Each Enzyme Defect

Deficiency	21-Hydroxylase	11β- Hydroxylase	17α- Hydroxylase	3βHSD Type 2	P450 Oxidoreductase	Lipoid Adrenal Hyperplasia	P450 Side-Chain Cleavage	Aldosterone Synthase	Apparent Cortisone Reductase
OMIM No.	+201910	#202010	#202110	+201810	#201750	[a]600617	+118485	[a]124080	[a]138090
Gene/Protein	CYP21A2	CYP11B1	CYP17A1	HSD3B2	POR	StAR	CYP11A1	CYP11B2	H6PDH
Alias	P450 21A2	P450 11B1	P450 17A1	3β-HSD	CPR, CYPOR		P450 11A1	P450 11B2	
Incidence	*Classic:* 1:10,000 to 1:15,000 *Nonclassic:* 1:500 to 1:1000	1:100,000 to 1:200,000	Rare	Rare	Unknown	Rare	Rare	Rare	Rare
DSD	*Classic:* 46,XX *Nonclassic:* No	46,XX	46,XY	46,XY[a]	46,XX + 46,XY[b]	46,XY	46,XY	No	No
Primary affected organ	Adrenal	Adrenal	Adrenal, gonads	Adrenal, gonads	Adrenal, gonads, liver, all CYP type 2–expressing tissues	Adrenal, gonads	Adrenal, gonads	Adrenal	Liver, adrenal, all H6PDH/11βHSD1-expressing tissues
Glucocorticoids	*Classic:* Reduced *Nonclassic:* Normal	Reduced	Reduced	Reduced	Reduced to normal, impaired stress response	Reduced	Reduced	Normal	Normal, but reduced tissue levels due to increased cortisol clearance
Mineralocorticoids	*Classic:* Reduced in SW *Nonclassic:* Normal	Increased, mainly precursors	Increased	Reduced often	Reduced to increased	Reduced	Reduced	Reduced	Normal
Sex hormones	Increased	Increased	Reduced	Reduced in males, increased in females[c]	Reduced	Reduced	Reduced	Normal	Increased
Increased marker metabolites in plasma	17OHP, 21DOF	DOC, S	Pregnenolone, progesterone, DOC, S	Pregnenolone, 17OH pregnenolone, DHEA	Pregnenolone, progesterone, 17OHP			DOC, B, 18OHB	
Increased marker metabolites in urine	Pregnanetriol, 17OH pregnenolone, pregnanetriolone	THDOC, THS	THDOC, THB, pregnenediol, pregnanediol	Pregnanetriol	Pregnenediol, pregnanediol, pregnanetriol, 17OH pregnanolone				

PRA	*Classic:* Increased *Nonclassic:* Normal to mildly increased	Reduced	Reduced	Increased		Increased	Increased	Increased	Normal
Hypertension	No	Yes	Yes	No	No or mild	No	No	No	No
Plasma sodium	*Classic:* Reduced in SW *Nonclassic:* Normal	Increased	Increased	Reduced in SW	Normal	Reduced	Reduced	Reduced	Normal
Plasma potassium	*Classic:* Increased in SW *Nonclassic:* Normal	Reduced	Reduced	Increased in SW	Normal	Increased	Increased	Increased	Normal
Urinary salt loss	*Classic:* Yes *Nonclassic:* No	No	No	Yes	No	Yes	Yes	Yes	No
Skeletal malformation	No	No	No	No	Yes[d]	No	No	No	No

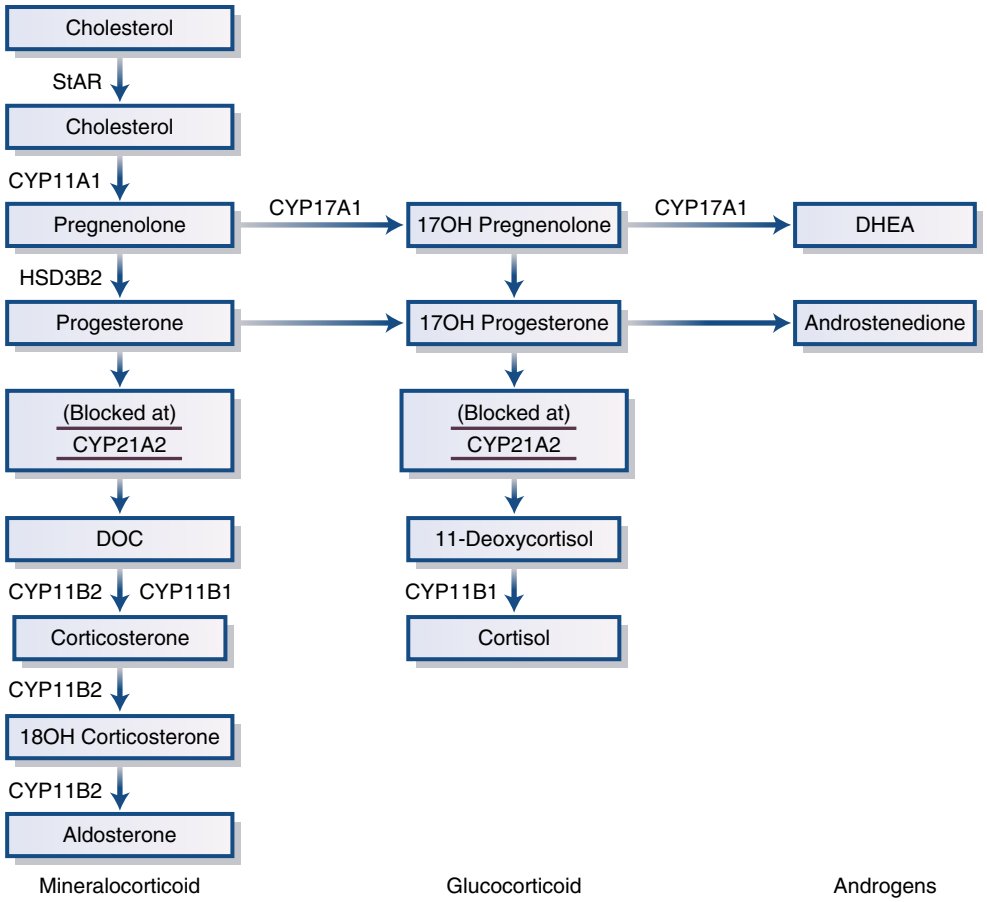
[a]Masculinization of the external genitalia in females at birth is rare and usually mild; signs of increased androgens usually manifest later.

[b]DSD is observed in both sexes, and normal sex-specific sexual development is also reported.

[c]Steroid hormone conversion by 3βHSD1 in peripheral tissues.

[d]In most cases published thus far; however, absence of skeletal malformations does not rule out POR deficiency.

B, Corticosterone; *CYP*, cytochrome P450; *DHEA*, dehydroepiandrosterone; *DOC*, 11-deoxycorticosterone; *21DOF*, 21-deoxycortisol; *DSD*, disorder of sex development; *H6PDH*, hexose-6-phosphate dehydrogenase; *HSD*, hydroxysteroid dehydrogenase; *OMIM*, Online Mendelian Inheritance in Man; *18OHB*, 18-hydroxycorticosterone; *17OHP*, 17-hydroxyprogesterone; *POR*, P450 oxidoreductase; *PRA*, plasma renin activity; *S*, 11-deoxycortisol; *StAR*, steroidogenic acute regulatory protein; *SW*, salt wasting; *THB*, tetrahydrocorticosterone; *THS*, tetrahydro-11-deoxycortisol; *THDOC*, tetrahydro-11-deoxycorticosterone.

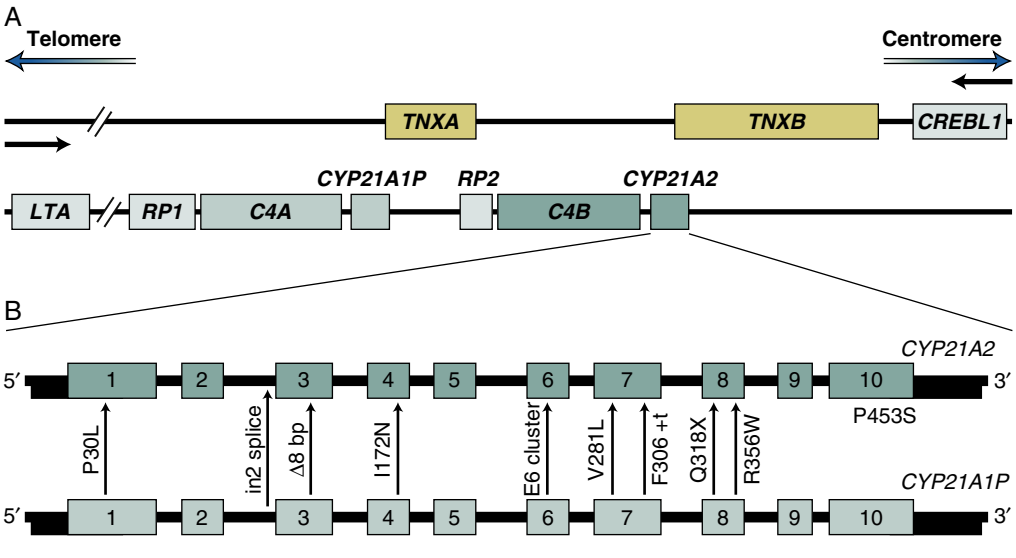


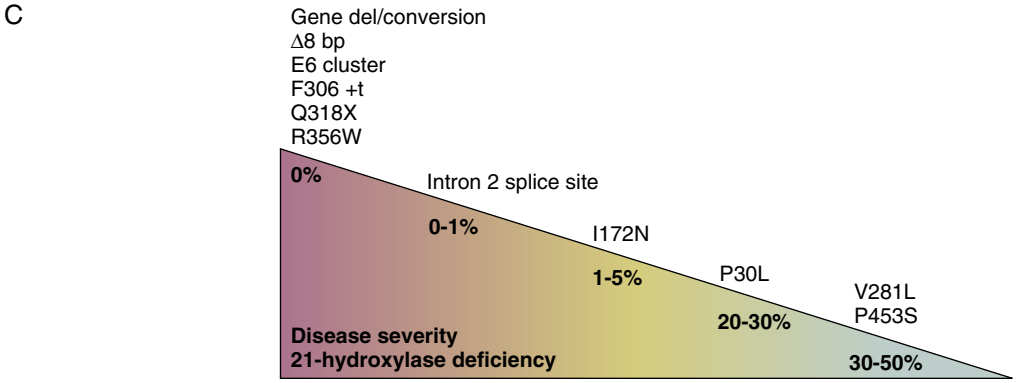
• **Fig. 15.34** Congenital adrenal hyperplasia related to 21-hydroxylase deficiency. The normal synthesis of cortisol is impaired, and adrenocorticotropic hormone (ACTH) levels increase because of loss of normal negative feedback inhibition, resulting in an increase in adrenal steroid precursors proximal to the block. The results are cortisol deficiency, variable mineralocorticoid deficiency, and excessive secretion of adrenal androgens. *CYP,* cytochrome P450; *DHEA,* dehydroepiandrosterone; *DOC,* deoxycorticosterone; *HSD,* hydroxysteroid dehydrogenase; *StAR,* steroidogenic acute regulatory protein.

TABLE 15.23 Forms of 21-Hydroxylase Deficiency

Phenotype	Classic Salt Wasting	Simple Virilizing	Nonclassic
Age at diagnosis	Newborn to 6 mo	*Female:* Newborn to 2 yr *Male:* 2–4 yr	Child to adult
Genitalia	*Female:* Ambiguous *Male:* Normal	*Female:* Ambiguous *Male:* Normal	*Female:* Virilized *Male:* Normal
Incidence	1:20,000	1:60,000	1:1000
Hormones			
Aldosterone	Reduced	Normal	Normal
Renin	Increased	Normal or increased	Normal
Cortisol	Reduced	Reduced	Normal
17OHP	>50,000 ng/dL	10,000-50,000 ng/dL	1000-10,000 ng/dL (ACTH stimulation)
Testosterone	Increased	Increased	Variable, increased
Growth	–2 to –3 SD	–1 to –2 SD	Probably normal
21-Hydroxylase activity (% of wild type)	0	1–5	5–20
Typical *CYP21A2* mutations	Deletions, conversions, nt656g G110Δ8nt, R356W I236N, V237E, M239K, Q318X	I172N Intron 2 splice site (nt656g)	V281L P30L

17OHP, 17-Hydroxyprogesterone; *ACTH,* adrenocorticotropic hormone; *SD,* standard deviation.



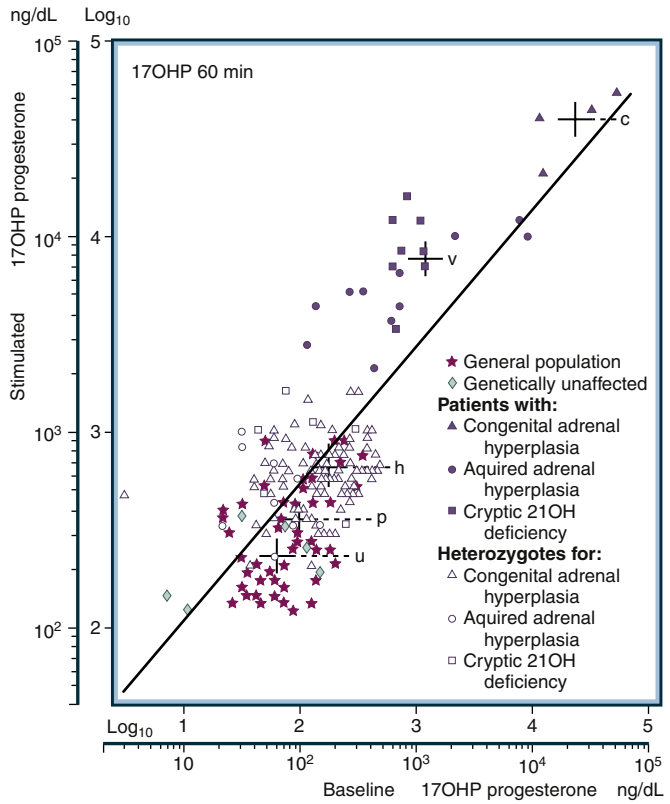


Positive predictive values				
Speiser et al., 1992	96%	85%	73%	63%
Krone et al., 2000	100%	90%	74%	65%
Stikkelbroeck et al., 2003	97%	96%	53%	100%
Prader genital stages				
Speiser et al., 1992	II-IV (IV)	III-V (IV)	I-IV (III)	0-IV (0)
Wedell et al., 1994	III-IV	II-V	I-IV	
Jääskeläinen et al., 1997	II-V	0-V	II-V	0
Krone et al., 2000	III-V (IV)	II-V (IV)	II-V (III)	0-IV (0)

• **Fig. 15.35** Genetics of 21-hydroxylase deficiency. (A) Genomic organization of the functional *CYP21A2* gene and its nonfunctional *CYP21A1P* pseudogene. (B) Nine of 10 common mutations are transferred by microconversions from the *CYP21A1P* pseudogene into the *CYP21A2* gene. (C) Genotype-phenotype correlation in 21-hydroxylase deficiency is well established. Based on the in vitro enzyme activity, the *CYP21A2* gene-inactivating mutations can be categorized into four major mutation groups. Although variation has been reported for the milder mutations, the overall correlation is high regarding expression of the adrenal phenotype. Considerable variability exists for the correlation with genital virilization. *CAH,* congenital adrenal hyperplasia.

pair deletion are found in more than 95% of cases. Other rare pseudogene-independent *CYP21A2*-inactivating mutations have been reported in single families or small populations. Approximately 65% to 75% of CAH patients are compound heterozygous for the disease-causing mutations.[415]

The genotype-phenotype correlation in CAH due to the 21-hydroxylase deficiency is well established. The clinical phenotype correlates with the less severely mutated allele and, consequently, with the residual 21-hydroxylase activity (Fig. 15.35).[416,417] This correlation appears to be high in populations, although divergence between genotype and phenotype has been observed for individuals.[418] The 21-hydroxylase activity measured by in vitro analysis provides a possibility for estimating disease severity, although some phenotypic variability (e.g., salt wasting, age at onset) seems likely to depend on other interacting genes and maturation processes rather than *CYP21A2* itself. One such factor might be the length of the CAG repeats in the androgen receptor modulating androgen action.[419] Potential variations in the degree of recovery from



• **Fig. 15.36** Basal and stimulated plasma 17α-hydroxyprogesterone (17OHP) concentrations in patients with 21-hydroxylase (21OH, CYP21A2) deficiency. To convert values to nmol/L, multiply by 0.0303. The mean for each group is indicated by a large cross and an adjacent letter: *c*, patients with classic CYP21A2 deficiency; *h*, heterozygotes for all forms of CYP21A2 deficiency; *p*, general population; *u*, known unaffected persons (e.g., siblings of patients with CYP21A2 deficiency who carry neither affected parental haplotype as determined by human leukocyte antigen typing); *v*, patients with nonclassic (acquired and cryptic) CYP21A2 deficiency. (From White PC, New MI, Dupont B. Congenital adrenal hyperplasia: part 1. *N Engl J Med*. 1987;316:1519–1524.)

glucocorticoid and mineralocorticoid deficiency during later life might be explained by significant 21-hydroxylase activity of the enzymes cytochrome P450 2C19 and P450 3A4.[420]

Diagnostic Criteria

A diagnosis of 21-hydroxylase deficiency should be considered in any newborn infant with genital ambiguity and salt wasting, hypotension, or hypoglycemia. Hyponatremia and hyperkalemia with raised plasma renin activity are found in salt-wasters. In later life, adrenal androgen excess (DHEAS, androstenedione) is found in patients presenting with sexual precocity or a PCOS-like phenotype. Randomly timed measurements of the plasma 17OHP concentration are significantly increased in classic 21-hydroxylase deficiency. Commonly, 17OHP concentrations in patients with salt-wasting CAH are higher than in non–salt-losing patients.

In nonclassic 21-hydroxylase deficiency, an SST is required to establish normal adrenal glucocorticoid reserve. Clinically useful nomograms have been developed that compare circulating concentrations of 17OHP before and 60 minutes after stimulation to investigate borderline cases and to differentiate between nonclassic 21-hydroxylase deficiency and heterozygous carriers (Fig. 15.36).[421] This test separates patients with classic and nonclassic 21-hydroxylase deficiency from heterozygote carriers and normal subjects, but there is some overlap between values seen in heterozygotes and in normal subjects. 17OHP and cortisol are measured basally and then 60 minutes after administration of 250 μg synacthen. Stimulated values are invariably grossly elevated (>35 nmol/L [>1100 ng/dL]) in patients with classic and nonclassic forms of the disorder. Heterozygote patients usually have stimulated values between 10 and 30 nmol/L (330 and 1000 ng/dL) (see Fig. 15.36). Stimulation tests are not always required to make a diagnosis; for example, a basal 17OHP concentration of less than 5 nmol/L (<150 ng/dL) in the follicular phase of the menstrual cycle effectively excludes nonclassic 21-hydroxylase deficiency.[411] *CYP21A2* genotyping to confirm the clinical and biochemical diagnosis is a useful adjunct to hormonal measurements.[422,423] Androgen excess in 21-hydroxylase deficiency is readily suppressed after glucocorticoid administration.

Prenatal diagnosis of 21-hydroxylase deficiency has been advocated, because treatment of an affected female may prevent masculinization in utero.[424] 17OHP can be assayed in amniotic fluid, but the most robust approach is the rapid genotyping of fetal cells obtained by chorionic villous sampling in early gestation. In patients with known 21-hydroxylase deficiency (male or female) seeking fertility, genotyping the partner before conception will uncover nonclassic or heterozygote cases and provide the endocrinologist/geneticist with some assignment of risk before pregnancy.

Treatment

The objectives for treatment of 21-hydroxylase deficiency differ with age, but at all ages treatment and overall patient management can be fraught with difficulties. In childhood, the overall goal is to replace glucocorticoid and mineralocorticoid, thereby preventing further salt-wasting crises, but also to normalize adrenal androgen secretion so that normal growth and skeletal maturation can proceed. Accurate replacement is essential; in excess, glucocorticoids will suppress growth, whereas inadequate replacement will result initially in accelerated linear growth and ultimately in short stature due to premature epiphyseal closure.[407] Response is best monitored through growth velocity and bone age, with biochemical markers from blood (17OHP, androstenedione, testosterone), urine, and saliva (17OHP, androstenedione, testosterone) being useful adjuncts. In difficult cases, a day curve study, as described for patients with primary adrenal failure but measuring the ACTH and 17OHP response before and after corticosteroid replacement, may confirm overreplacement or underreplacement. The optimal glucocorticoid dose fails to suppress 17OHP and its metabolites and maintains sex hormone concentrations in the middle of the age-specific and sex-specific normal range. Ideally, the biochemical investigations will indicate the need for dose adjustments before physical changes, growth, and skeletal maturation indicate inadequate or excessive glucocorticoid treatment.[424]

Corrective surgery (e.g., clitoral reduction, vaginoplasty) is frequently addressed during childhood. The method of choice should be a one-stage complete repair using the newest techniques of vaginoplasty, clitoral, and labial surgery.[425]

In late childhood and adolescence, appropriate replacement therapy is equally important. Overtreatment may result in obesity and delayed menarche/puberty with sexual infantilism, whereas underreplacement will result in sexual precocity. Compliance with regular medication is often an issue throughout adolescence.

Although much has been written about adequate control in childhood, adults with 21-hydroxylase deficiency often provide an ongoing dilemma for the endocrinologist. The follow-up of such patients should involve multidisciplinary clinics, initially

with transition adolescence clinics to facilitate transfer from pediatric to adult care. Problems in adulthood relate to fertility concerns, hirsutism, and menstrual irregularity in women; obesity, metabolic consequences, and impact of short stature; probable increased cardiovascular risk; sexual dysfunction; and psychologic problems.[407,426,427] Counseling is often required in addition to endocrine support. Males may develop enlargement of the testes due to so-called testicular adrenal rest tumors (TART)—that is, ectopic adrenal tissue, which may regress after glucocorticoid suppression, and if untreated can lead to infertility and affect around one in three male patients. These patients need adequate endocrine therapy rather than urologic referral with ensuing risk of removal of testis mistaken for a tumor.[428]

In the absence of any evidence-based data, there are no prescriptive steroid regimens to treat patients with 21-hydroxylase deficiency at any age, and as a result, many individualized regimens are used in clinical practice. Hydrocortisone is recommended for replacement therapy from the newborn period to adolescence.[425] Usual starting doses of hydrocortisone in childhood are 10 to 15 mg/m^2 per day in three divided doses, with up to 25 mg/m^2 in infancy only seldom required. These doses are higher than those used for replacement of adrenal insufficiency because treatment also aims at normalization of ACTH-driven adrenal androgen excess. The optimal timing for providing the highest dose of hydrocortisone remains an ongoing matter of debate, with no data supporting either circadian replacement (giving the highest dose in the morning) or reverse-phase therapy (giving the largest dose of hydrocortisone at night). Long-acting steroids such as prednisone, prednisolone, and dexamethasone are more effective in this regard but should not be given before the end of puberty to avoid oversuppression and reduction in linear growth.

Fludrocortisone is required for infants with classic 21-hydroxylase deficiency, although this need may spontaneously change with age. Fludrocortisone doses during the first year of life are commonly 150 μg/m^2 per day. Sodium needs to be supplemented, as milk feeds provide only minimal sodium amounts. Adequate mineralocorticoid replacement usually allows hydrocortisone dose reduction. The relative dose in relation to body surface decreases throughout life. Fludrocortisone doses of 100 μg/m^2 per day after the first 2 years of life are commonly sufficient. This requirement drops further with adolescence and adulthood to a daily dose of 100 to 200 μg (50–100 μg/m^2 per day). Mineralocorticoid substitution is monitored by measurements of plasma renin activity (low or suppressed levels indicating overtreatment), serum potassium, and standing blood pressure.[429]

Adrenomedullary dysfunction has been reported in the 21-hydroxylase–deficient adrenal gland, probably because of relative glucocorticoid deficiency, which results in epinephrine deficiency.[173,430] In clinical practice, sufficient supplementation of glucose during exercise and illness should be guaranteed, to prevent hypoglycemic episodes. Bilateral adrenalectomy might appear effective in the short term but should be regarded as a last resort[174]; adrenal rest tumor has appeared after adrenalectomy even in women.[431] In addition, because of the requirement for lifelong corticosteroid replacement therapy, patients could also develop ACTH-secreting pituitary tumors.[432] The adrenalectomy procedure also bears a number of risks, including surgical and anesthetic complications, and the patient is left completely adrenal insufficient.

Prenatal dexamethasone treatment has been proposed to avoid virilization of the external genitalia in the female fetus.[407] Unlike hydrocortisone, which is inactivated by placental 11βHSD2, maternally administered dexamethasone can cross the placenta to suppress the fetal HPA axis. However, due to concerns over the long-term effects of dexamethasone on the mother and fetus, prenatal therapy is not recommended outside research studies.[433,434] Fetal sex and *CYP21A2* genotype can be determined as early as week 6 of gestation with the use of novel molecular diagnostic methods that analyze cell-free fetal DNA from maternal blood using real-time polymerase chain reaction.[435] In this way, the number of unnecessarily treated cases can be substantially reduced. Dexamethasone can lead to maternal cushingoid effects in pregnancy[434,436] and may in turn have long-term, deleterious effects on the fetus, including metabolic, psychologic, and intellectual consequences. Prenatal treatment is controversial and has to be regarded as experimental; patients treated should be included in ongoing multicenter studies.[437]

In adult women with hyperandrogenism and untreated nonclassic 21-hydroxylase deficiency, there is no evidence that final height is affected. In this setting, glucocorticoid therapy in isolation rarely controls hirsutism, and additional antiandrogen therapy is often required (e.g., cyproterone acetate, spironolactone, flutamide together with an oral estrogen-containing contraceptive pill). Most studies have found increased risk of miscarriage in women with nonclassic 21-hydroxylase deficiency, and some retrospective series suggest that the risk is lower in women treated with glucocorticoid replacement during pregnancy.[438,439] Hypogonadotropic hypogonadism in male patients is a consequence of increased aromatization of adrenal androgens, in particular androstenedione to estrone, resulting in suppression of pituitary LH and FSH secretion.[440] The condition is reversible after optimization of glucocorticoid therapy. However, overreplacement in men or women may also lead to hypogonadotropic hypogonadism due to glucocorticoid-mediated suppression of GnRH secretion.

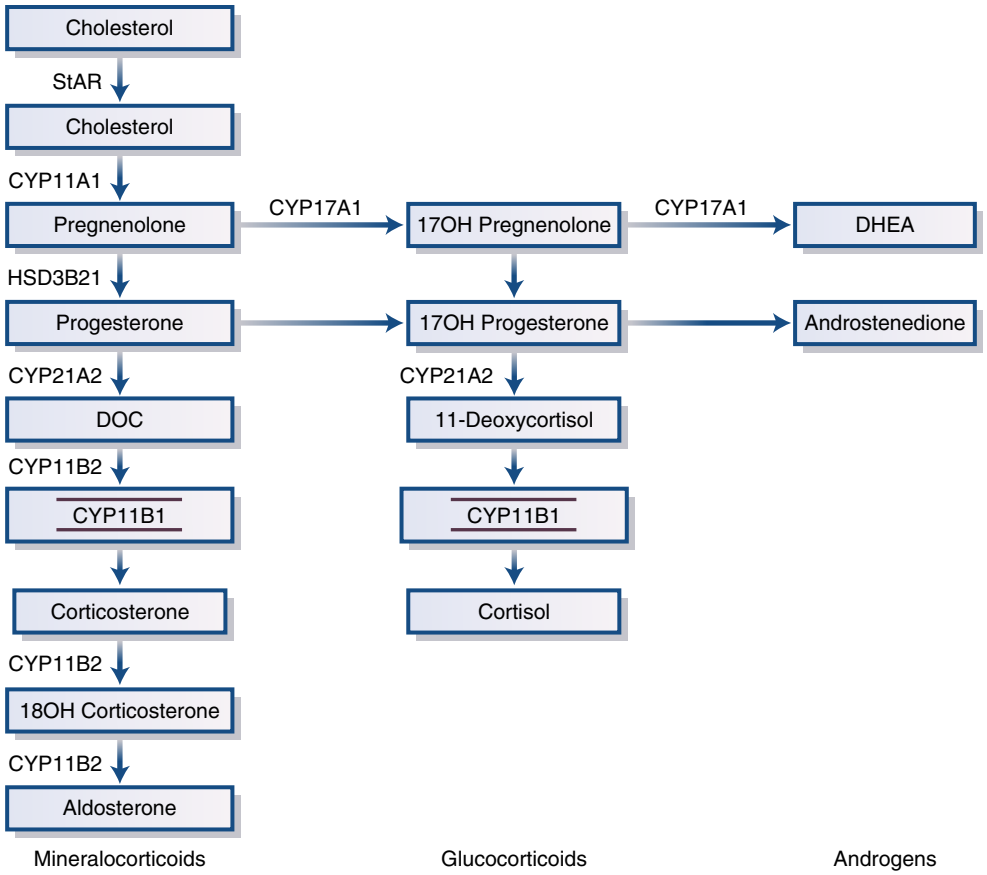
Long-Term Complications and Comorbid Conditions

Outcome assessed by final height is not optimal in many patients treated for 21-hydroxylase deficiency. In a large UK cohort the mean adult height of patients with classic 21-hydroxylase deficiency was 14 cm and 8 cm below the population mean for men and women, respectively.[441] Similar data from the United States shows that final height is -1.1 SDS compared to the control population.[442] The pubertal growth spurt occurs earlier and is less pronounced than normal. An often overlooked problem is glucocorticoid overtreatment during the first 2 years of life; overtreatment suppresses the infant growth spurt, which is characterized by the highest postnatal growth velocity. Therefore the lowest optimal dose of glucocorticoid replacement should be established as early in life as possible.

Increased fat mass and obesity are common among children and adolescents with 21-hydroxylase deficiency.[443–445] Glucocorticoid dose, chronologic age, advanced bone age maturation, and parental obesity all contribute to elevated BMI SDS.[444]

Increased fat mass and higher insulin levels have been described in women older than 30 years of age with 21-hydroxylase deficiency. However, clear evidence of cardiovascular risk factors had not been shown. Women with classic 21-hydroxylase deficiency do have a significantly higher rate of gestational diabetes, a possible forerunner for the development of type 2 diabetes,[446] and women with nonclassic[447] and young adults with classic 21-hydroxylase deficiency[448] have reduced insulin sensitivity. Increased intima media thickness as a marker of atherosclerosis has been detected.[448]

Daytime systolic blood pressure in children and adolescents with 21-hydroxylase deficiency is elevated, and the physiologic



• **Fig. 15.37** Congenital adrenal hyperplasia related to 11β-hydroxylase deficiency. The normal synthesis of cortisol is impaired, and adrenocorticotropic hormone (ACTH) levels increase because of the loss of normal negative feedback inhibition, which results in an increase in adrenal steroid precursors proximal to the block. The results are cortisol deficiency, mineralocorticoid excess related to excessive deoxycorticosterone *(DOC)* secretion, and excessive secretion of adrenal androgens. *CYP,* cytochrome P450; *DHEA,* dehydroepiandrosterone; *HSD,* hydroxysteroid dehydrogenase; *StAR,* steroidogenic acute regulatory protein.

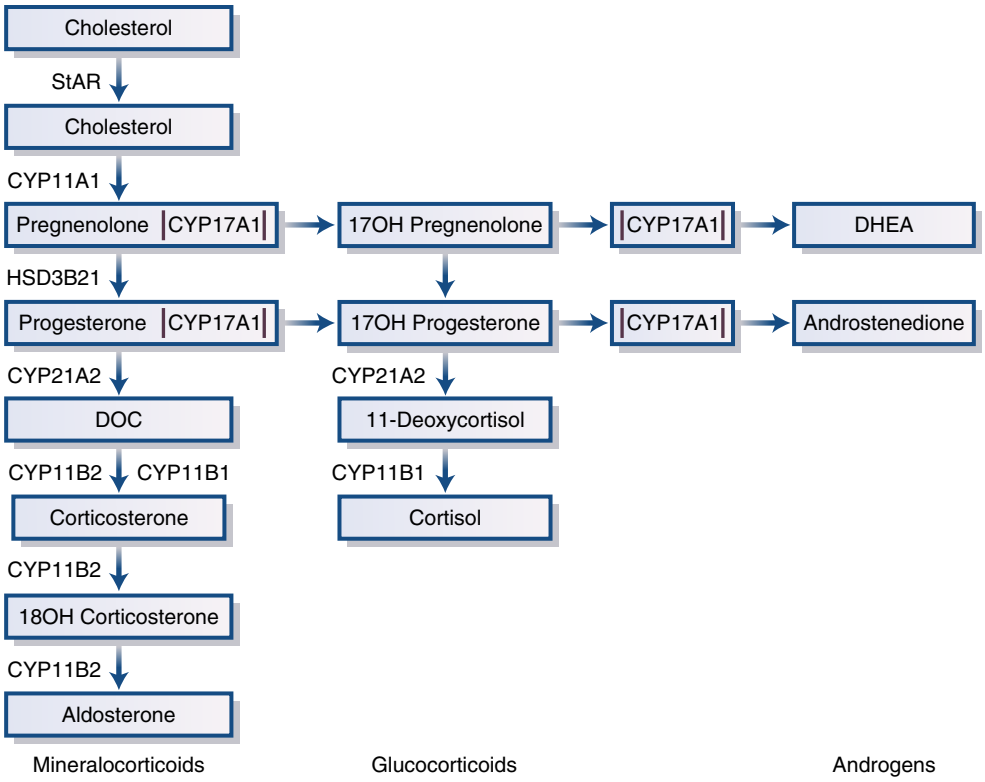
nocturnal dip in blood pressure is absent.[449] Elevated systolic blood pressure correlates with the degree of overweight and obesity.[450] In adults, hypertension was found to be more common in men and in patients with elevated 17OHP,[442] in data from the United States, but in the large UK Congenital adrenal Hyperplasia Adult Study Executive (CaHASE) cohort men with CAH had blood pressure significantly lower than controls. The reasons for these discrepancies are not established.

11β-Hydroxylase Deficiency

11β-Hydroxylase deficiency accounts for up to 7% of all cases of CAH, with an incidence of 1 in 100,000 live births.[451] The condition arises because of mutations in the 11β-hydroxylase *(CYP11B1)* gene that result in loss of enzyme activity and a block in the conversion of 11-deoxycortisol to cortisol. The *CYP11B1* gene is located on chromosome 8q24.3, approximately 40 kilobases from the highly homologous aldosterone synthase gene *(CYP11B2).*[451] *CYP11B1*-inactivating mutations have been shown to be distributed over the entire coding region consisting of nine exons. Although mutation clusters are reported in exons 2, 6, 7, and 8,[415,451] real hot spots, as seen in 21-hydroxylase deficiency, do not exist. Most of the reported mutations lead to absent or almost absent 11β-hydroxylase enzyme activity, with only some cases of mild or nonclassic 11β-hydroxylase deficiency reported.[452–454]

Loss of negative cortisol feedback and enhanced ACTH-mediated adrenal androgen excess occur in 11β-hydroxylase deficiency (Fig. 15.37). Clinical features therefore are very similar to those reported in the simple virilizing form of 21-hydroxylase deficiency (46,XX DSD, including virilization of the external genitalia and sexual ambiguity); again, milder cases can manifest later in childhood or even young adulthood. The principal difference from 21-hydroxylase deficiency is hypertension, which is thought to be secondary to the mineralocorticoid effect of DOC excess. However, there is a poor correlation between DOC secretion and the presence of hypertension, and unexplained salt wasting has been reported in few patients during early life. On this clinical background, the diagnosis can be made by measuring a plasma synacthen–stimulated 11-deoxycortisol value, which will be greater than 52 nmol/L (>1800 ng/dL) and often much higher.[455] Basal concentrations of 17OHP are commonly increased but may be normal even during the first weeks of life.[455]

Treatment is with replacement glucocorticoid therapy; with suppression of DOC secretion, the plasma renin activity, which is suppressed at baseline, increases into the normal range. In general, higher glucocorticoid doses are needed to suppress hyperandrogenism compared with the situation in 21-hydroxylase



• **Fig. 15.38** Congenital adrenal hyperplasia related to 17α-hydroxylase deficiency. The normal synthesis of cortisol is impaired, and adrenocorticotropic hormone (ACTH) levels increase because of loss of normal negative feedback inhibition resulting in an increase in adrenal steroid precursors proximal to the block. The result is cortisol deficiency and mineralocorticoid excess usually related to deoxycorticosterone *(DOC)* excess. Because gonadal 17α-hydroxylase activity is also absent, sex steroid secretion in addition to adrenal androgen secretion is severely impaired, resulting in hypogonadism. *CYP,* cytochrome P450; *DHEA,* dehydroepiandrosterone; *HSD,* hydroxysteroid dehydrogenase; *StAR,* steroidogenic acute regulatory protein.

deficiency, and add-on antihypertensive therapy may be necessary in some cases. Antihypertensive treatment should be commenced at an early stage to avoid excessive glucocorticoid exposure.

17α-Hydroxylase Deficiency

17α-hydroxylase deficiency is less common than 11β-hydroxylase deficiency, except in Brazil.[456–459] Mutations within the *CYP17A1* gene result in failure to synthesize cortisol (17α-hydroxylase activity), adrenal androgens (17,20-lyase activity), and gonadal steroids (Fig. 15.38). Therefore, in contrast to 21-hydroxylase and 11β-hydroxylase deficiencies, 17α-hydroxylase deficiency results in adrenal and gonadal insufficiency and causes 46,XY DSD. A single enzyme is expressed in the adrenal and the gonads and possesses both 17α-hydroxylation and 17,20-lyase activities, but rare patients with isolated deficiency of 17,20-lyase deficiency have been reported.[457,460,461] Loss of negative feedback results in increased secretion of steroids proximal to the block, and mineralocorticoid synthesis is enhanced. Corticosterone has weaker glucocorticoid activity than cortisol, but corticosterone excess generally prevents adrenal crises. Accumulation of corticosterone and DOC results in severe hypokalemic hypertension. Sex steroid deficiency caused by loss of 17,20-lyase activity results in 46,XY DSD; this manifests as undervirilization in male newborns and primary amenorrhea in 46,XX individuals. There is lack of pubertal development due to hypergonadotropic hypogonadism in both sexes.[458]

The 17α-hydroxylase enzyme is a microsomal cytochrome P450 type II enzyme that requires electron transfer from NADPH via POR for catalytic activity.[27] For efficient catalysis of the 17,20-lyase reaction, the P450 17A1-POR complex requires additional allosteric interaction with cytochrome b_5.[460,462] The *CYP17A1* gene consists of eight exons and is located on chromosome 10q24.3. A variety of different mutations have been described, without evidence of a hot spot.[415,463] Relative hydroxylase/lyase activities of P450 17A1 mutants have been shown to vary in in vitro functional assays, but correlations with clinical phenotype are lacking. Patients with clinically pure 17,20-lyase deficiency have P450 17A1 mutations that selectively compromise 17,20-lyase activity.[460,461,464] Mutations underlying isolated 17,20-lyase deficiency are located within the area of the P450 17A1 molecule that is thought to interact with the cofactor cytochrome b_5, thereby disrupting the electron transfer from POR to P450 17A1, specifically for the conversion of 17-hydroxypregnenolone to DHEA.[460,464]

The diagnosis is usually made at the time of puberty when patients present with hypertension, hypokalemia, and hypergonadotropic hypogonadism, the last occurring because of lack of P450 17A1 activities within the gonad and impaired gonadal steroidogenesis. As a result, LH and FSH levels are elevated. Female patients (XX) have primary amenorrhea with absent sexual

characteristics, whereas 46,XY individuals present with 46,XY DSD with female external genitalia but absent uterus and fallopian tubes. The intra-abdominal testes should be removed due to the risk of malignancies, and such patients are usually reared as females.

Glucocorticoid replacement reverses the DOC-induced suppression of the renin-angiotensin system and lowers blood pressure. Additional sex steroid replacement is required from puberty onward.

P450 Oxidoreductase Deficiency: Apparent Combined 17α-Hydroxylase and 21-Hydroxylase Deficiencies

Very few patients have been described with biochemical evidence of apparent combined 17α-hydroxylase and 21-hydroxylase deficiencies.[465] Urinary gas chromatography/mass spectrometry analysis reveals a typical pattern comprising increased pregnenolone and progesterone metabolites, slightly increased corticosterone metabolites, increased pregnanetriolone excretion, and low androgen metabolites (see Fig. 15.4). Mothers pregnant with an affected child present with low serum estriol and a characteristic urinary steroid profile, allowing for prenatal biochemical diagnosis.[466,467] Limited analysis of serum steroids only may lead to misdiagnosis.[468] Cortisol baseline secretion may be normal, but most if not all patients show an insufficient cortisol response to synacthen stimulation and therefore require glucocorticoid replacement. Impaired 17,20-lyase activity results in deficient androgen synthesis, and affected boys are often born undervirilized. Most of the affected girls are born with virilized genitalia. Therefore patients can present with 46,XY or 46,XX DSD or with appropriate development of the external genitalia in both sexes. After birth, virilization does not progress, and circulating androgen concentrations are typically low. Some mothers develop signs of virilization during midpregnancy with an affected child; this commonly resolves soon after birth, further indicating intrauterine androgen excess.[466] In addition to these features of CAH, affected children may also present with bone malformations, including midface hypoplasia, craniosynostosis, and radiohumeral synostosis, in some cases meeting criteria for the Antley-Bixler congenital malformation syndrome.[469,470] The bone phenotype in affected patients with POR deficiency is most likely caused by an impairment of sterol biosynthesis, specifically of POR-dependent 14α-lanosterol demethylase (P450 51A1).

The paradox of fetal virilization but sex hormone deficiency in postnatal life might be mediated by a newly discovered "backdoor" pathway of androgen synthesis in fetal life that relies on neither androstenedione nor testosterone as an intermediate.[470–472] Pubertal development in POR deficiency appears to be dominated by the consequences of sex steroid deficiency,[472] and most patients require sex hormone substitution. The overall incidence of POR deficiency has not been established. However, a relatively large number of patients with POR deficiency were reported within a short period after the initial description of the molecular cause of the disease.[472,473]

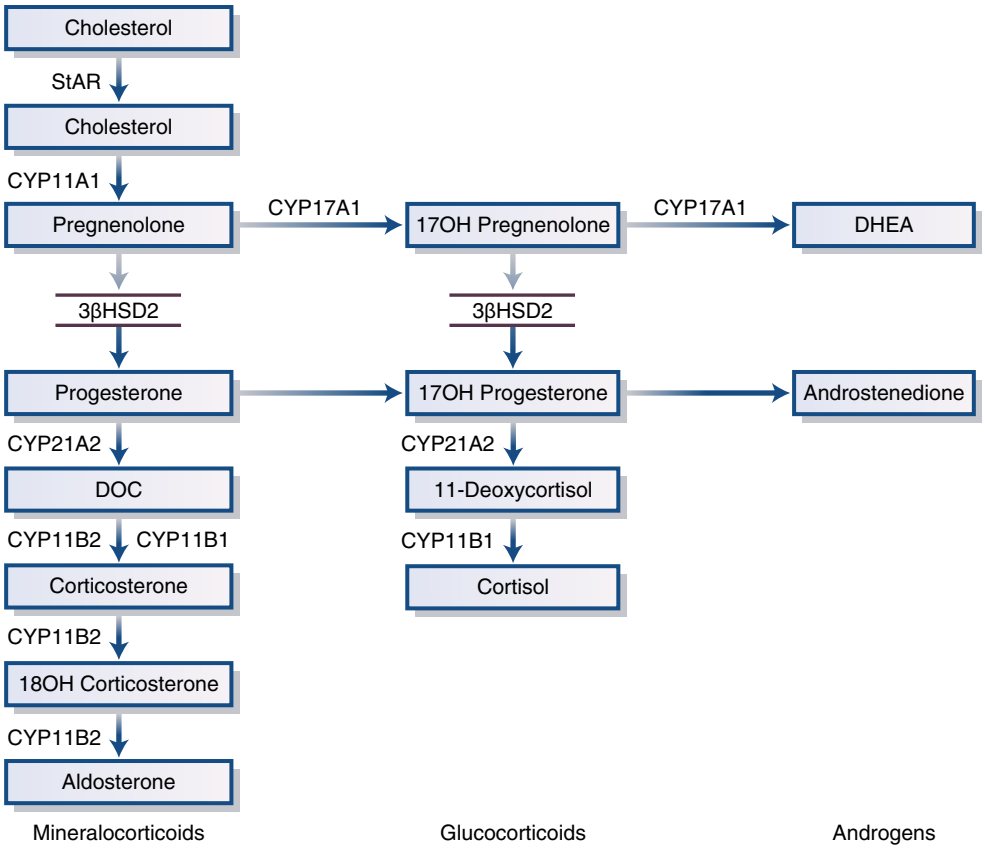
The *POR* gene is located on chromosome 7q11.2 and consists of 15 translated exons spanning 32.9 kilobytes and encoding a protein of 680 amino acids. A variety of *POR*-inactivating mutations have been reported, including missense, frameshift, and splice site mutations. A287P is the most common mutation in Caucasians, whereas R457H is the most frequent founder mutation in the Japanese population. All patients carry *POR* mutations that are either partially inactivating or, in case of major loss-of-function mutations, manifest only in the compound heterozygous state. Homozygous mutations with total loss of function are most likely not viable—a view supported by the nonviability of complete *POR* gene deletion in the murine model.[474]

3β-Hydroxysteroid Dehydrogenase Deficiency

In this rare form of CAH, the secretion of all classes of adrenal and ovarian steroids is impaired due to mutations within the *HSD3B2* gene encoding 3βHSD2.[475,476] There are two isoforms of 3βHSD, encoded by *HSD3B1* and *HSD3B2*, respectively. The *HSD3B2* gene is located on chromosome 1p13.1 and consists of four exons. 3βHSD2 is expressed mainly in the adrenal and the gonad, whereas 3βHSD1 is expressed in the placenta and almost ubiquitously in peripheral target tissues.[28,476] The enzyme 3βHSD2 catalyzes three key reactions in adrenal steroidogenesis: the conversion of the Δ^5 steroids pregnenolone, 17-hydroxypregnenolone, and DHEA to the Δ^4 steroids progesterone, 17OHP, and androstenedione, respectively. 3βHSD2 deficiency affects all three steroid hormone pathways (i.e., mineralocorticoids, glucocorticoids, and sex steroids).

The clinical spectrum shows a wide variety of disease expression. Patients usually present in early infancy with adrenal insufficiency. Loss of mineralocorticoid secretion results in salt wasting, although this is absent in 30% to 40% of cases (Fig. 15.39). As with 21-hydroxylase deficiency, absence of salt wasting may delay the presentation into childhood or puberty, ranging from a severe salt-wasting form with or without ambiguous genitalia in affected male neonates to isolated premature pubarche in infants and children of both sexes and a rare nonclassic variant manifesting with hirsutism and menstrual irregularities. In general, the functional and biochemical data are in close agreement with the expressed phenotype in patients with the non–salt-wasting form of 3βHSD2 deficiency. However, some variability exists, and identical mutations have been found in the *HSD3B2* gene in both salt-wasters and non–salt-wasters.[475,476] The correlation between the impairment in male sexual differentiation and salt wasting is poor. The spectrum of genital development is variable in both sexes. In males, because the 3βHSD2 enzyme is also expressed within the gonad, 46,XY DSD may occur, resulting in female external genitalia. However, most patients present with hypospadias, and even normal male genitalia may be found. In females, genital development can be normal, but usually there is evidence of mild virilization, presumably because of enhanced adrenal DHEA secretion, which is converted peripherally to testosterone. A nonclassic form has been described in patients with premature pubarche and a PCOS-like phenotype (i.e., hirsutism, oligomenorrhea/amenorrhea).[477]

Because activity of the 3βHSD1 enzyme, present in peripheral tissues, is intact, levels of circulating Δ^4 steroids (progesterone, 17OHP, androstenedione) may be normal (or even increased). However, a diagnosis is established by demonstration of an increased ratio of Δ^5 steroids (pregnenolone, 17-hydroxypregnenolone, DHEA) to Δ^4 steroids in plasma or urine. Hormonal criteria have been refined for the diagnosis of 3βHSD2 deficiency based on genotyping of the *HSD3B2* gene. The 17-hydroxypregnenolone concentrations and the ratios of 17-hydroxypregnenolone to cortisol at baseline and after ACTH stimulation are of the highest discriminatory value in differentiating between patients affected by 3βHSD2 deficiency and those with milder biochemical abnormalities, who are commonly negative for *HSD3B2* mutations.[478,479]



• **Fig. 15.39** Congenital adrenal hyperplasia related to 3β-hydroxysteroid dehydrogenase type 2 (3βHSD2) deficiency resulting in cortisol deficiency and variable mineralocorticoid deficiency. Gonadal 3βHSD2 activity is also absent, resulting in 46,XY DSD and hypogonadism or primary amenorrhea in females. Virilization in females can occur due to 3β-hydroxysteroid dehydrogenase type 1 activity. *DOC,* deoxycorticosterone; *DHEA,* dehydroepiandrosterone; *DSD,* disorder of sex development; *StAR,* steroidogenic acute regulatory protein.

StAR Deficiency: Congenital Lipoid Adrenal Hyperplasia

Mutations in the gene encoding StAR result in a failure of transport of cholesterol from the outer to the inner mitochondrial membrane in steroidogenic tissues. StAR-independent cholesterol transport occurs only at a low rate. As a result, there is deficiency of all adrenal and gonadal steroid hormones.[24,480] The adrenal glands are often massively enlarged and full of lipid; before the characterization of StAR, the condition was termed *congenital lipoid adrenal hyperplasia*.[480] StAR deficiency severely but incompletely abolishes pregnenolone synthesis. Cholesterol esters accumulate under the increased tone of ACTH stimulation. Consequently, the lipid accumulation worsens the dysfunction and leads to adrenal cell destruction. Presentation is with acute adrenal insufficiency in the neonatal period, and males exhibit 46,XY DSD due to absent gonadal steroids.

The most severe form of this disorder manifests with 46,XY DSD and combined adrenal insufficiency. Salt wasting typically develops in the neonatal period or after a few weeks of life, but later onset may also occur. Females can show spontaneous pubertal development. A milder form of StAR deficiency has also been described in normally virilized 46,XY individuals who present with adrenal failure during early childhood.[481] Treatment consists of glucocorticoid and mineralocorticoid replacement and substitution of sex hormones in later life.

P450 Side-Chain Cleavage Deficiency

Deficiency of P450scc (P450 11A1) enzyme is a rare inborn error of steroidogenesis. It was previously thought that such mutations would not be viable, because the maintenance of human pregnancy relies on progesterone that is produced by the fetal part of the placenta from the second trimester onward. P450 11A1 deficiency manifests clinically and biochemically with similar signs and symptoms as StAR deficiency, but patients do not have enlarged adrenals.[482–484] Depending on the impairment of P450 11A1 function, a spectrum of clinical presentation ranges from 46,XY DSD with severe adrenal insufficiency in the newborn period to hypospadias and cryptorchidism and later manifestation of adrenal insufficiency during childhood.[485] Concentrations of all steroid hormones are decreased, in keeping with impaired conversion of cholesterol to pregnenolone. Treatment with glucocorticoid, mineralocorticoid, and sex steroid replacement is required.

Cortisone Reductase Deficiency

In cortisone reductase deficiency, adrenal glands become hyperplastic because of ACTH stimulation due to a defect in cortisol metabolism rather than an inherent defect within the gland itself.[135,486,487] Patients with this condition have a defect in the conversion of cortisone to cortisol, suggesting inhibition of 11-oxoreductase activity and, by implication, inhibition of 11βHSD1

TABLE 15.24 Causes of Mineralocorticoid Deficiency

Addison disease
Adrenal hypoplasia
Congenital adrenal hyperplasia (21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies)
Pseudohypoaldosteronism types I and II
Hyporeninemic hypoaldosteronism
Aldosterone biosynthetic defects
Drug induced

(see Fig. 15.12). Cortisol clearance is increased; consequently, ACTH secretion is elevated to maintain normal circulating cortisol concentrations but at the expense of adrenal androgen excess. Female patients present with hirsutism, menstrual irregularity, androgenic alopecia, or some combination of these features. Males may present with premature pubarche. Dexamethasone treatment to suppress ACTH has been used with some success to control the hyperandrogenism in these cases. Urinary tetrahydrometabolites of cortisol and cortisone show almost exclusively THE with little or no detectable THF or allo-THF; the ratio of THF+allo-THF to THE is less than 0.05 (reference range, 0.8–1.3). The molecular bases for cortisone reductase deficiency are inactivating mutations in hexose-6-phosphate dehydrogenase (H6PDH)[487] and dominant negative mutations of 11βHSD1.[488] H6PDH, located in the endoplasmic reticulum, catalyzes the conversion of glucose-6-phosphate to glucose-6-phosphogluconate, thereby generating NADPH, which is crucial in conveying oxo-reductase activity on 11βHSD1.

Patients with PCOS share many of the same clinical characteristics as those with cortisone reductase deficiency. Whereas there is evidence to support increased cortisol secretion rates in PCOS, perhaps indicating a defect in the conversion of cortisone to cortisol, a consensus with respect to THF+allo-THF to THE ratios is still lacking.[489] Association studies using single-nucleotide polymorphic markers in the *HSD11B1* and *H6PDH* genes have largely been negative.

Mineralocorticoid Deficiency

The mineralocorticoid deficiency syndromes are listed in Table 15.24. They can be divided into congenital and acquired syndromes. Mineralocorticoid deficiency may occur in some forms of CAH and with other causes of adrenal insufficiency (e.g., Addison disease, CAH).

Primary Defects in Aldosterone Biosynthesis: Aldosterone Synthase Deficiency

Before the characterization of the *CYP11B2* gene, two diseases were recognized: *corticosterone methyl oxidase type I (CMO I) deficiency* and *corticosterone methyl oxidase type II (CMO II) deficiency.*[490] Subsequently, both variants were shown to be secondary to mutations in aldosterone synthase (P450 11B2), and they are now termed *aldosterone synthase deficiency, types I and II.*[491] Aldosterone synthase catalyzes the three terminal steps of aldosterone biosynthesis, 11β-hydroxylation of DOC to corticosterone, 18-hydroxylation to 18-hydroxycorticosterone, and 18-oxidation to aldosterone. Patients with type I aldosterone synthase deficiency have low to normal levels of 18-hydroxycorticosterone but undetectable levels of aldosterone (or urinary tetrahydroaldosterone), whereas patients with the type II variant have high levels of 18-hydroxycorticosterone and only subnormal or even normal levels of aldosterone. This suggests blockade of only the terminal 18-oxidation step, with some residual aldosterone synthase activity remaining. The explanation for the variable biochemical phenotype is unknown, particularly now that the same mutation in aldosterone synthase has been uncovered in both variants. It is possible that the phenotypic variation may reflect polymorphic variants in the residual and normal product of the *CYP11B1* gene, 11β-hydroxylase.

Both variants are rare and are inherited as autosomal recessive traits.[491] Patients usually present in neonatal life with a salt-wasting crisis involving severe dehydration, vomiting, and failure to grow and thrive. Hyperkalemia, metabolic acidosis, dehydration, and hyponatremia are found. The plasma renin activity is elevated, and plasma aldosterone levels are low. Plasma 18-hydroxycorticosterone levels, the ratio of plasma 18-hydroxycorticosterone to aldosterone, and the levels of their urinary metabolites are used to differentiate the type I and II variants. In most infants, the disorders become less severe as the child ages; indeed, in older children, adolescents, and adults, the abnormal steroid pattern described may be present and may persist throughout life without clinical manifestations.

Patients with P450 11B2 deficiency typically respond well to 9α-fludrocortisone (starting dose, 150 μg/m^2 per day in neonates and infants) and may also benefit from salt supplementation. Patients with failure to grow and thrive usually show a good catch-up growth. Electrolytes often tend to normalize spontaneously between 3 and 4 years of age. However, untreated patients are at significant risk for being growth retarded, although spontaneous normalization of growth can occur. Adults are usually asymptomatic but are more susceptible to salt loss. Rarely, presentation is in adulthood.[492] Mineralocorticoid treatment in later life has to be established on an individual basis.

Postadrenalectomy Hypoaldosteronism

In a patient with a unilateral aldosteronoma (Conn syndrome), the contralateral ZG is frequently suppressed. Without reversal of the chronic volume expansion preoperatively, patients may develop severe hyperkalemia and hypotension lasting several days to several weeks after adrenalectomy. This effect may be exacerbated by the use of spironolactone preoperatively, and is more likely with older age, renal impairment, and proteinuria.[493] Spironolactone has a long half-life and should be discontinued 2 to 3 days before surgery to minimize the risk of postoperative mineralocorticoid deficiency.

Defects in Aldosterone Action: Pseudohypoaldosteronism

Pseudohypoaldosteronism (PHA) is a rare, inherited salt-wasting disorder that was first described by Cheek and Perry in 1958 as a defective renal tubular response to mineralocorticoid in infancy. Patients present in the neonatal period with dehydration, hyponatremia, hyperkalemia, metabolic acidosis, and failure to thrive despite normal glomerular filtration and normal renal and adrenal function.[494] Renin levels and plasma aldosterone are grossly elevated. When patients fail to respond to mineralocorticoid therapy, PHA is suspected as the underlying disorder.

PHA type I can be divided into two distinct disorders based on unique physiologic and genetic characteristics: the renal form of PHA, which is inherited as an autosomal dominant trait, and a generalized autosomal recessive form of PHA. The autosomal dominant form is usually less severe; the patient's condition often improves spontaneously within the first several years of life,

allowing discontinuation of therapy. By contrast, the autosomal recessive form produces a multiorgan disorder, with mineralocorticoid resistance seen in the kidney, sweat and salivary glands, and the colonic mucosa. The condition does not spontaneously improve with age and is generally more severe than the autosomal dominant form.

The underlying basis for the autosomal dominant form of PHA is explained on the basis of inactivating, dominant-negative mutations in the MR (hMR, NR3C2).[494,495] By contrast, inactivating mutations in the α-subunit and, to a lesser extent, in the β-subunit and γ-subunit of the epithelial sodium channel (ENaC) account for the generalized autosomal recessive form of mineralocorticoid resistance.[496,497] (In effect, this represents the opposite of Liddle syndrome—see Chapter 16.) Generalized loss of ENaC activity leads to renal salt wasting (as seen in the renal form) in addition to recurrent respiratory infections and neonatal respiratory distress, cholelithiasis, and polyhydramnios.

PHA type I (PHA-I) is resistant to mineralocorticoid therapy, so standard treatment involves supplementation with salt (2–8 g/day) in the form of sodium chloride and sodium bicarbonate as well as cation exchange resins. This supplementation usually corrects the patient's biochemical imbalance. However, if a patient shows signs of severe hyperkalemia, peritoneal dialysis may be necessary. Hypercalciuria has been reported in some cases of PHA-I. The recommended course of treatment for these patients usually involves indomethacin or hydrochlorothiazide. Indomethacin is thought to act by causing a reduction in the glomerular filtration rate or an inhibition of the effect of prostaglandin E_2 on renal tubules. Indomethacin has been shown to reduce polyuria, sodium loss, and hypercalciuria. Hydrochlorothiazide has been used to diminish hyperkalemia and reduce hypercalciuria in patients with PHA-I.

In patients with the autosomal dominant or renal form of PHA-I, the signs and symptoms of PHA decrease with age; nevertheless, these patients usually require salt supplementation for the first 2 to 3 years of life. In patients with the autosomal recessive or multiorgan type of PHA-I, resistance to therapy with sodium chloride or drugs that decrease serum potassium concentrations often occurs and may even lead to death in infancy from hyperkalemia. PHA-I patients with multiorgan involvement often require very high amounts of salt in their diet (up to 45 g NaCl per day). Carbenoxolone, a derivative of glycyrrhetinic acid in licorice, has been used with moderate success in helping to reduce the high levels of dietary salt needed by renal PHA-I patients. Carbenoxolone acts by inhibiting 11βHSD2 activity and allows unmetabolized cortisol to bind to and activate MRs in a manner similar to that of aldosterone.[498] Carbenoxolone was found to be ineffective in patients with multiorgan PHA-I.

Two other variants of PHA have been described—types II and III. Type II PHA, or Gordon syndrome, is in retrospect a misnomer. Patients with Gordon syndrome share some of the features of patients with PHA-I, notably hyperkalemia and metabolic acidosis, but they exhibit salt retention with mild hypertension and suppressed plasma renin activity rather than salt wasting. The condition is explained by mutations in a serine threonine kinase family (WNK1 and WNK4) that result in increased expression of these proteins with activation of the thiazide-sensitive sodium chloride cotransporter in the cortical and medullary collecting ducts.[499] The condition represents the exact opposite of Gitelman syndrome but is not a true form of PHA.

Type III PHA is an acquired and usually transient form of mineralocorticoid resistance seen in patients with underlying renal diseases, including obstruction and infection and in patients with excessive loss of salt through the gut or skin. Reduced glomerular filtration rate is a hallmark of the condition. The cause is unknown, although increased transforming growth factor-β–mediated aldosterone resistance has been suggested to be an underlying factor.

Hyporeninemic Hypoaldosteronism

Angiotensin II is a key stimulus to aldosterone secretion, and damage or blockade of the renin-angiotensin system may result in mineralocorticoid deficiency. Various renal diseases have been associated with damage to the juxtaglomerular apparatus and subsequent renin deficiency. These include systemic lupus erythematosus, myeloma, amyloid, AIDS, and damage related to use of nonsteroidal anti-inflammatory drugs, but the most common (>75% of cases) is diabetic nephropathy.[500,501]

The usual picture is that of an elderly patient with hyperkalemia, acidosis, and mild to moderate impairment of renal function. Plasma renin activity and aldosterone levels are low and fail to respond to sodium depletion, erect posture, or furosemide administration. In contrast to adrenal insufficiency, patients have normal or elevated blood pressure and no postural hypotension. Muscle weakness and cardiac arrhythmias may also occur. Other factors may contribute to the hyperkalemia, including the use of potassium-sparing diuretics, potassium supplementation, insulin deficiency, and use of β-adrenoceptor blocking drugs or prostaglandin synthetase inhibitors, which inhibit renin release.

Treatment of primary renin deficiency is with fludrocortisone in the first instance together with dietary potassium restriction. However, these patients are not salt depleted and may become hypertensive with fludrocortisone. In such a scenario, the addition of a loop-acting diuretic, such as furosemide, is appropriate. This will increase acid excretion and improve the metabolic acidosis.

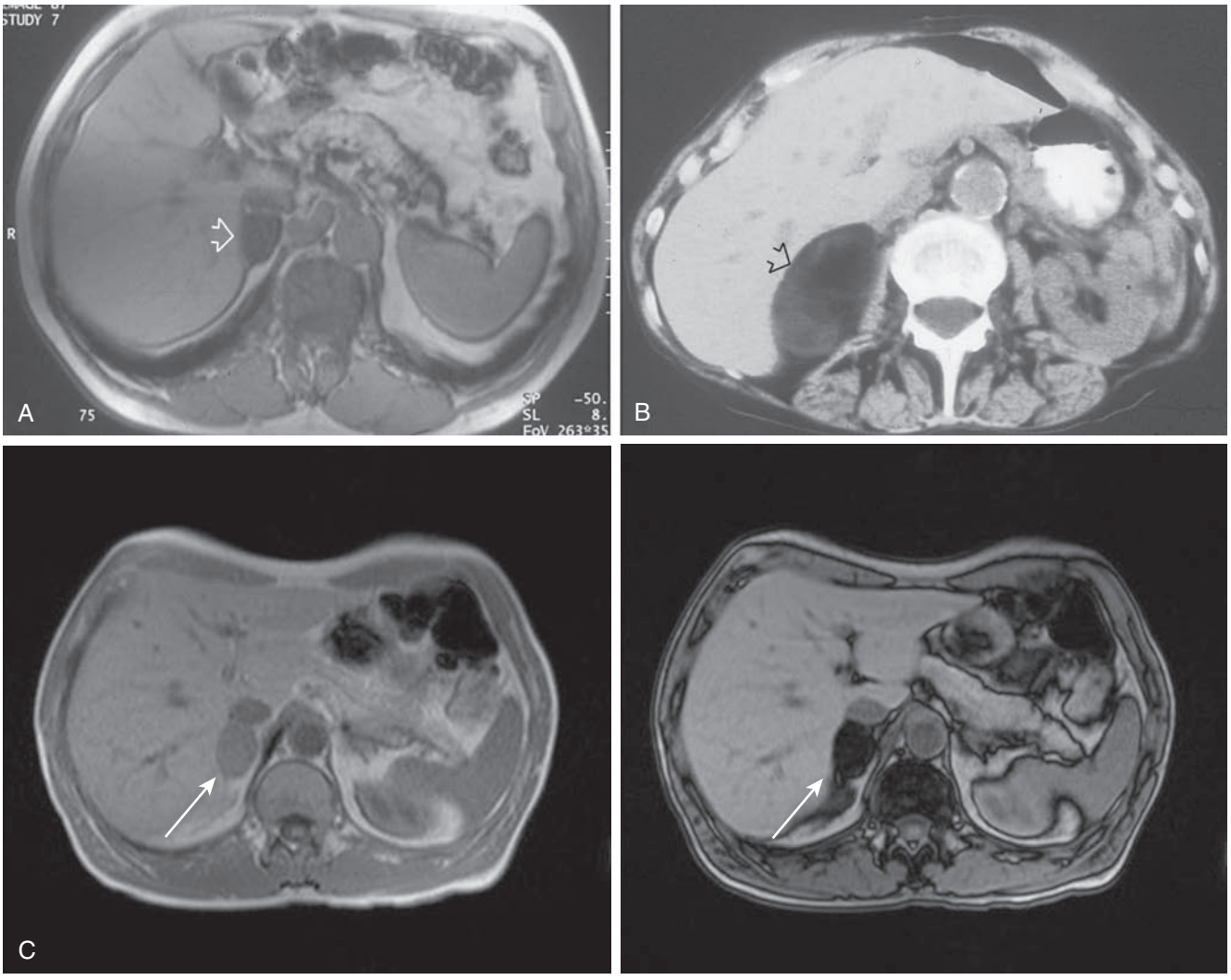
Adrenal Adenomas, Incidentalomas, and Carcinomas

Adenomas

Cortisol-secreting adrenal adenomas were discussed earlier, and aldosterone-secreting adenomas (Conn syndrome) are discussed in Chapter 16. Pure virilizing benign adrenal adenomas are very rare. Most cases occur in women; in males, the disorder is generally recognized in childhood, when presentation is with sexual precocity and accelerated bone age. Such tumors have to be considered in the differential diagnosis of CAH in patients presenting during childhood. In females, most patients present before menopause with marked hirsutism, deepening of the voice, and amenorrhea. Clitoromegaly is found in 80% of cases. Testosterone is usually strikingly elevated, but gonadotropin levels may not be suppressed. By definition, urinary free cortisol is normal. Tumors vary in size and should be treated surgically. Postoperatively, clinical features invariably improve, and normal menses return.[502]

Incidentalomas

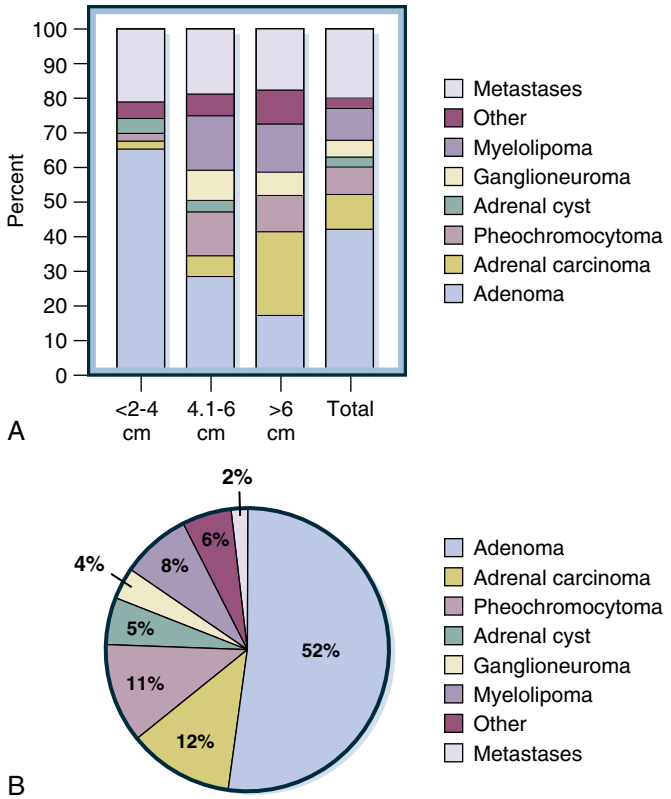
Incidentally discovered adrenal masses have become a common clinical problem. An adrenal mass is uncovered in up to 4% of patients imaged for nonadrenal disease.[503] Incidentalomas are uncommon in patients younger than 30 years of age but increase in frequency with age; they occur more frequent in females, most commonly in the sixth and seventh decades. Clinically, two issues



• **Fig. 15.40** (A) Adrenal incidentaloma *(arrow)* discovered in a woman undergoing investigation for abdominal pain. (B) Incidentally discovered right adrenal myelolipoma *(arrow)*. (C) Chemical shift MRI of adrenal incidentaloma demonstrating signal dropout *(arrow)* for out-of-phase image, consistent with benign disease.

arise: whether the lesion is functional (i.e., secreting hormones) and whether it is malignant. Most incidentalomas are adrenocortical adenomas, but occasionally they represent myelolipomas, hamartomas, or granulomatous infiltrations of the adrenal gland and result in a characteristic CT/MRI appearance (Fig. 15.40). Functioning tumors with a clear clinical phenotype (pheochromocytomas and those secreting cortisol, aldosterone, or sex steroids) and carcinomas account for around 4% of all incidentalomas. In addition, it is established that some incidentalomas cause abnormal hormone secretion without obvious clinical manifestations of a hormone excess state. The best example is so-called subclinical Cushing syndrome, which occurs in up to 20% to 30% of all patients with adrenocortical incidentalomas.[503–505] There is debate as to the best means to biochemically define this phenomenon, but serum cortisol after dexamethasone testing has the widest acceptance, although cutoff values vary. The European Society of Endocrinology and European Network for the Study of Adrenal Tumors (ENSAT) have published guidelines for diagnosis and management.[506] When there is evidence of low-grade excess biochemical hypercortisolism, there is an associated increase in the prevalence of diabetes, obesity, hypertension, new cardiovascular events, osteoporosis, and fatality. However, no prospective study has proven that the adrenal adenoma is the cause of the observed complications, as these are highly prevalent in the population at this age. Intervention by adrenalectomy has shown some benefit, particularly lower blood pressure,[507] but the studies performed have been largely retrospective and highly selected, and hence the approach to each patient needs individualization with most being observed in current clinical practice.

As a result, all patients with incidentally discovered adrenal masses should undergo appropriate endocrine screening tests. This testing should comprise 24-hour urinary metanephrine collection or measurement of plasma metanephrines, an overnight dexamethasone suppression test, and for those with hypertension, measurement of plasma renin and aldosterone. DHEAS should be measured as a marker of adrenal androgen secretion. Low levels may occur in patients with suppressed ACTH concentrations due to autonomous cortisol secretion from the adenoma.[508] Some



• **Fig. 15.41** Distribution of diagnosis of adrenal incidentalomas. (A) Data from eight studies with histologically determined diagnoses (n = 103) relating to tumor size. (B) Distribution of 380 incidental adrenal masses by histologic diagnosis. (From Mansmann G, Lau L, Balk E, et al. The clinically inapparent adrenal mass: update in diagnosis and management. *Endocr Rev.* 2004;25:309–340.)

studies have also documented high levels of 17OHP after ACTH stimulation tests, suggesting partial defects in 21-hydroxylase in some tumors, and this should be measured in cases of bilateral disease.

The possibility of malignancy should be considered in each case. In patients with a known extra-adrenal primary tumor, the incidence of malignancy is obviously much higher; for example, up to 20% of patients with lung cancer have adrenal metastases on CT scanning. In those with no evidence of malignancy, adrenal carcinoma is rare.[509] In true incidentalomas, size appears to be predictive of malignancy: Fewer than 2% of incidentalomas smaller than 4 cm but 25% of those larger than 6 cm in diameter are malignant (Fig. 15.41).[510] Smooth, homogeneous adenomas on an enhanced adrenal scan with a Hounsfield unit (HU) score (a marker of radiodensity) less than 10 HU or signal dropout on out-of-phase MRI sequences (see Fig. 15.40) are invariably benign; malignancy is suspected in irregular, inhomogeneous adenomas with a score greater than 20 HU. On this background, adrenalectomy is considered for functional tumors and for tumors larger than 4 cm in diameter, although if the imaging characteristics are unequivocally benign tumors greater than this size may be left.[506] Repeat CT scanning in patients with smaller tumors can be used to guide management, but development of functional autonomy is very rare, and patients may be discharged if tumors are static. If surgery is indicated, laparoscopic adrenalectomy is the treatment of choice, offering shorter hospital stays and reduced operative complications (e.g., blood loss, morbidity) compared with open adrenalectomy. The exception is the patient with highly suggestive invasive adrenal carcinoma, because breach of the tumor capsule is associated with a poorer outcome. Adequate preparation and close endocrine supervision perioperatively and postoperatively are required for functional tumors.

Carcinomas

Primary adrenal carcinoma is very rare, with an annual incidence of 1 per 1 million population. Women are more commonly affected than men, with a ratio of 2.5:1. The mean age at onset is between 40 and 50 years, although men tend to be older at presentation. Eighty percent of tumors are functional, most commonly secreting glucocorticoids alone (45%), glucocorticoids and androgens (45%), or androgens alone (10%). Fewer than 1% of all tumors secrete aldosterone, and production of hormone precursors is common.[511] Patients present with features of the hormone excess state (glucocorticoid, androgen, or both), but abdominal pain, weight loss, anorexia, and fever occur in 25% of cases. An abdominal mass may be palpable. Current treatment choices for what is often an aggressive tumor are poor. Surgery offers the only chance of cure for patients with local disease, but metastatic spread is evident in 75% of cases at presentation. Radiotherapy is ineffective, as are most chemotherapeutic regimens. Mitotane in high dose, in conjunction with therapeutic drug monitoring, offers benefit in reducing tumor growth[286] and in controlling hormonal hypersecretion in 75% of cases.[285] Overall, the prognosis is poor, with 5-year survival rates of less than 20%. Newer chemotherapies are being evaluated.[287]

Etiology of Adrenal Tumors

Other than the factors discussed under adrenal Cushing syndrome, the underlying basis for adrenal tumorigenesis is unknown. Clonal analysis suggests progression from a normal to an adenomatous to a carcinomatous lesion, but the molecular pathways involved remain obscure. Several factors have been associated with malignant transformation, including the genes encoding p53, p57, cyclin-dependent kinase, menin, IGF2, MC2R, and inhibin-α.[512] Mice lacking the inhibin-α gene develop adrenal tumors through a process that is also gonadotropin dependent.[513]

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The complete list of references is available on line at ExpertConsult.com.

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16

Endocrine Hypertension

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CHAPTER OUTLINE

KEY POINTS

- There are at least 14 endocrine disorders for which hypertension may be the initial clinical presentation. An accurate diagnosis of endocrine hypertension provides the clinician with a unique treatment opportunity: to render a surgical cure or to achieve a dramatic response with pharmacologic therapy.
- Catecholamines affect many cardiovascular and metabolic processes—they increase heart rate, blood pressure, myocardial contractility, and cardiac conduction velocity. The identification of three types of adrenergic receptors (α, β, and dopaminergic receptors) and their subtypes (α_1, α_2, β_1, β_2, β_3, D_1, and D_2) has led to understanding of the physiologic responses to endogenous and exogenous administration of catecholamines.
- Catecholamine-secreting tumors are rare, with an annual incidence of 2 to 8 cases per 1 million people. Nevertheless, it is important to suspect, confirm, localize, and resect these tumors because (1) the associated hypertension is curable with surgical removal of the tumor, (2) a risk of lethal paroxysm exists, (3) at least 10% of the tumors are malignant, and (4) 40% of these tumors are familial and their detection in the proband may result in early diagnosis in other family members.
- Germline mutations are responsible for approximately 40% of all catecholamine-secreting tumors. Mutations contributing to pheochromocytoma and paraganglioma have two general transcription signatures: cluster 1—genes encoding proteins that function in the cellular response to hypoxia, and cluster 2—genes encoding proteins that activate kinase signaling.
- Pheochromocytoma/paraganglioma must be confirmed biochemically by the presence of increased concentrations of fractionated metanephrines and catecholamines in urine or plasma. Then the tumor should be localized with computed tomography (CT) of the abdomen and pelvis. Approximately 85% of these tumors are found in the adrenal glands, and 95% are found in the abdomen and pelvis.
- Some form of preoperative pharmacologic preparation is indicated for all patients with catecholamine-secreting neoplasms, including those who are asymptomatic and normotensive. Surgical resection of a catecholamine-secreting tumor is a high-risk surgical procedure, and an experienced surgeon-anesthesiologist team is required.
- Hypertension, suppressed plasma renin activity, and increased aldosterone excretion characterize the syndrome of primary aldosteronism, which was first described in 1955.
- Aldosterone-producing adenoma and bilateral idiopathic hyperaldosteronism are the most common subtypes of primary aldosteronism. Somatic mutations account for the majority of aldosterone-producing adenomas and include mutations in genes encoding components of the potassium channel *(KCNJ5),* the sodium/potassium and calcium adenosine triphosphatases (ATPases) (*ATP1A1* and *ATP2B3*), a voltage-dependent C-type calcium channel *(CACNA1D),* and β-catenin–activating *CTNNB1* mutations.
- Use of the plasma aldosterone to plasma renin activity ratio as a case-detection test, followed by aldosterone suppression for confirmatory testing, has resulted in prevalence estimates for primary aldosteronism of 5% to 10% of all patients with hypertension.
- The treatment goal in patients with primary aldosteronism is to prevent the morbidity and fatality associated with hypertension, hypokalemia, nephrotoxicity, and cardiovascular damage. Knowing the cause of the primary aldosteronism helps to determine the appropriate treatment. Normalization of blood pressure should not be the only goal—normalization of circulating aldosterone or mineralocorticoid receptor blockade should be part of the management plan for all patients with primary aldosteronism.

An estimated 68 million people in the United States are hypertensive.[1,2] In most, hypertension is *primary* (i.e., *essential* or *idiopathic*), but a subgroup of approximately 15% have *secondary* hypertension. The secondary causes of hypertension can be divided into renal causes, such as renal parenchymal or renovascular disease, and endocrine causes. There are at least 14 endocrine disorders for which hypertension may be the initial clinical presentation (Table 16.1). An accurate diagnosis of endocrine hypertension provides the clinician with a unique treatment opportunity: to render a surgical cure or to achieve a dramatic response with pharmacologic therapy. The diagnostic and therapeutic approaches to endocrine hypertension—ranging from the classic adrenal causes of hypertension (e.g., pheochromocytoma, primary aldosteronism) to pituitary-dependent hypertension (e.g., Cushing syndrome, acromegaly)—are reviewed in this chapter.

Adrenal Medulla and Catecholamines

The adrenal medulla occupies the central portion of the adrenal gland and accounts for 10% of total adrenal gland volume. There is no clear demarcation between the adrenal cortex and the adrenal medulla. The adrenal glands derive blood supply from the superior, middle, and inferior branches of the inferior phrenic artery; from the renal arteries; and directly from the aorta. The adrenal arteries branch and form a plexus under the capsule. This plexus supplies the cortex. Some of the plexus arteries penetrate the cortex and supply the medulla, as do capillaries draining the cortical cells, forming the corticomedullary portal system. The right adrenal vein is short and drains directly into the inferior vena cava (IVC). The left adrenal vein merges with the inferior phrenic vein, and this larger vein (the common phrenic vein) drains into the left renal vein.

TABLE 16.1 Endocrine Causes of Hypertension

Adrenal-Dependent Causes

- Pheochromocytoma
- Primary aldosteronism
- Hyperdeoxycorticosteronism
  - Congenital adrenal hyperplasia
  - 11β-Hydroxylase deficiency
  - 17α-Hydroxylase deficiency
  - Deoxycorticosterone-producing tumor
- Primary cortisol resistance
- Cushing syndrome

AME/11βHSD Deficiency

- Genetic
  - Type 1 AME
  - Type 2 AME
- Acquired
  - Licorice or carbenoxolone ingestion (type 1 AME)
  - Cushing syndrome (type 2 AME)

Thyroid-Dependent Causes

- Hypothyroidism
- Hyperthyroidism

Renin-Secreting Tumor

Pituitary-Dependent Causes

- Acromegaly
- Cushing syndrome

AME, Apparent mineralocorticoid excess; *HSD*, hydroxysteroid dehydrogenase.

Adrenomedullary cells are called *chromaffin cells* (stain brown with chromium salts) or *pheochromocytes*. Cytoplasmic granules turn dark when stained with chromic acid because of the oxidation of epinephrine and norepinephrine to melanin. Chromaffin cells differentiate in the center of the adrenal gland in response to cortisol; some chromaffin cells also migrate to form paraganglia, collections of chromaffin cells located on both sides of the aorta. The largest cluster of chromaffin cells outside the adrenal medulla is located near the level of the inferior mesenteric artery and is referred to as the *organ of Zuckerkandl;* it is quite prominent in the fetus and is a major source of catecholamines during the first year of life. The preganglionic sympathetic neurons receive synaptic input from neurons within the pons, medulla, and hypothalamus, providing regulation of sympathetic activity by the brain. Axons from the lower thoracic and lumbar preganglionic neurons, via splanchnic nerves, directly innervate the cells of the adrenal medulla.

The term *catecholamine* refers to substances that contain catechol (ortho-dihydroxybenzene) and a side chain with an amino group—the catechol nucleus (Fig. 16.1).[3] Epinephrine is synthesized and stored in the adrenal medulla and released into the systemic circulation. Norepinephrine is synthesized and stored not only in the adrenal medulla but also in the peripheral sympathetic nerves. Dopamine, the precursor of norepinephrine, is found in the adrenal medulla and peripheral sympathetic nerves and acts primarily as a neurotransmitter in the central nervous system.

Catecholamines affect many cardiovascular and metabolic processes. They increase the heart rate, blood pressure, myocardial contractility, and cardiac conduction velocity. Activation of G protein–coupled receptors mediates the biologic actions of catecholamines. The identification of three types of adrenergic receptors (α, β, and dopaminergic receptors) and their subtypes (α_1, α_2, β_1, β_2, β_3, D_1, and D_2) has led to understanding of the physiologic responses to endogenous and exogenous administration of catecholamines.[4] The 2012 Nobel Prize in Chemistry was awarded to Brian K. Kobilka and Robert J. Lefkowitz for their studies of G protein–coupled receptors. The α_1 subtype is a postsynaptic receptor that mediates vascular and smooth muscle contraction; stimulation causes vasoconstriction and increases blood pressure. The α_2 receptors are located on presynaptic sympathetic nerve endings; when activated, they inhibit release of norepinephrine. Stimulation causes suppression of central sympathetic outflow and decreased blood pressure.

There are three major β-receptor subtypes. The β_1 receptor mediates cardiac effects and is more responsive to isoproterenol than to epinephrine or norepinephrine. Stimulation causes positive inotropic and chronotropic effects on the heart, increased renin secretion in the kidney, and lipolysis in adipocytes. The β_2 receptor mediates bronchial, vascular, and uterine smooth muscle relaxation. Stimulation causes bronchodilation, vasodilatation in skeletal muscle, glycogenolysis, and increased release of norepinephrine from sympathetic nerve terminals. The β_3 receptor regulates energy expenditure and lipolysis.

D_1 receptors are localized to the cerebral, renal, mesenteric, and coronary vasculatures; stimulation causes vasodilation in these vascular beds. D_2 receptors are presynaptic; they are localized to sympathetic nerve endings, sympathetic ganglia, and the brain.

Stimulation of D_2 receptors in these locations inhibits the release of norepinephrine, inhibits ganglionic transmission, and inhibits prolactin release, respectively.

Most cells in the body have adrenergic receptors. The pharmacologic development of selective α-adrenergic and β-adrenergic agonists and antagonists has advanced pharmacotherapy for various clinical disorders. For example, β_1 antagonists (e.g., atenolol, metoprolol) are now considered standard therapies for angina pectoris, hypertension, and cardiac arrhythmias.[5] Administration of β_2 agonists (e.g., terbutaline, albuterol) causes bronchial smooth muscle relaxation; these agents are commonly prescribed in inhaled formulations for the treatment of asthma.[6]

Catecholamine Synthesis

Catecholamines are synthesized from tyrosine by a process of hydroxylation and decarboxylation (see Fig. 16.1). Tyrosine is derived from ingested food or synthesized from phenylalanine in the liver, and it enters neurons and chromaffin cells by active transport. Tyrosine is converted to 3,4-dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase, the rate-limiting step in catecholamine synthesis. Increased intracellular levels of catechols downregulate the activity of tyrosine hydroxylase; as catecholamines are released from secretory granules in response to a stimulus, cytoplasmic catecholamines are depleted and the feedback inhibition of tyrosine hydroxylase is released. Transcription of tyrosine hydroxylase is stimulated by glucocorticoids, cyclic adenosine monophosphate (cAMP)–dependent protein kinases, calcium/phospholipid-dependent protein kinase, and calcium/calmodulin-dependent protein kinase. α-Methyl-paratyrosine (metyrosine) is a tyrosine hydroxylase inhibitor that may be used therapeutically in patients with catecholamine-secreting neoplasms to decrease tumoral synthesis of catecholamines.[7]

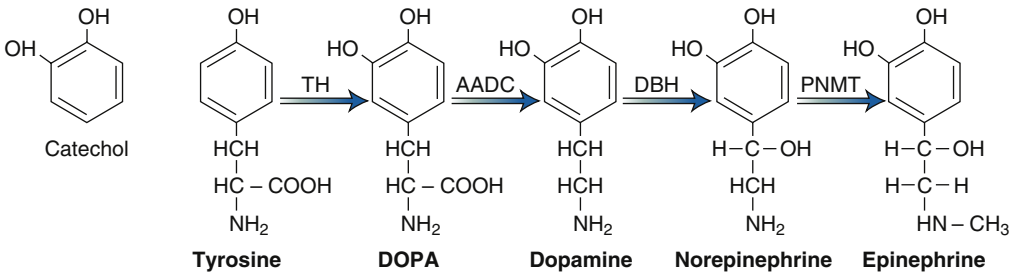
Aromatic L-amino acid decarboxylase catalyzes the decarboxylation of DOPA to dopamine (see Fig. 16.1). Dopamine is actively transported into granulated vesicles to be hydroxylated to norepinephrine by the copper-containing enzyme dopamine β-hydroxylase. Ascorbic acid is a cofactor and electron donor. The enzyme is structurally similar to tyrosine hydroxylase and may share similar transcriptional regulatory elements, and both are stimulated by glucocorticoids and cAMP-dependent kinases. These reactions occur in the synaptic vesicle of adrenergic neurons in the central nervous system, the peripheral nervous system, and the chromaffin cells of the adrenal medulla. The major constituents of the granulated vesicle are dopamine β-hydroxylase, ascorbic acid, chromogranin A, and adenosine triphosphate (ATP).

In the adrenal medulla, norepinephrine is released from the granule into the cytoplasm, where the cytosolic enzyme phenylethanolamine *N*-methyltransferase (PNMT) converts it to epinephrine (see Fig. 16.1). Epinephrine is then transported back into another storage vesicle. The *N*-methylation reaction by PNMT involves *S*-adenosylmethionine as the methyl donor as well as oxygen and magnesium. PNMT expression is regulated by the presence of glucocorticoids, which are in high concentration in the adrenal medulla through the corticomedullary portal system. Therefore, catecholamine-secreting tumors that secrete primarily epinephrine are localized to the adrenal medulla. In normal adrenal medullary tissue, approximately 80% of the catecholamines released are epinephrine.

Catecholamine Storage and Secretion

Catecholamines are found in the adrenal medulla and in sympathetically innervated organs. Catecholamines are stored in electron-dense granules that also contain ATP, neuropeptides (e.g., adrenomedullin, corticotropin [i.e., adrenocorticotropic hormone (ACTH)], vasoactive intestinal polypeptide), calcium, magnesium, and chromogranins. Uptake into the storage vesicles is facilitated by active transport by vesicular monoamine transporters (VMAT).[8] The VMAT ATP-driven pump maintains a steep electrical gradient. For every monoamine transported, ATP is hydrolyzed, and two hydrogen ions are transported from the vesicle into the cytosol. Iodine-123 (^{123}I) and ^{131}I-labeled metaiodobenzylguanidine (MIBG) are imported by VMAT into the storage vesicles in the adrenal medulla, which makes ^{123}I-MIBG useful for imaging localization of catecholamine-secreting tumors and ^{131}I-MIBG potentially useful in treating malignant catecholamine-secreting tumors.[9–11] Catecholamine uptake, as well as that of MIBG, is inhibited by reserpine. The catecholamine stores are dynamic, with constant leakage and reuptake.[8]

Stressful stimuli (e.g., myocardial infarction, anesthesia, hypoglycemia) trigger adrenal medullary catecholamine secretion. Acetylcholine from preganglionic sympathetic fibers stimulates



• **Fig. 16.1** Biosynthetic pathway for catecholamines. The term *catecholamine* comes from the catechol (ortho-dihydroxybenzene) structure and a side chain with an amino group—the catechol nucleus *(shown on left).* Tyrosine is converted to 3,4-dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase (TH); this rate-limiting step provides the clinician with the option to treat pheochromocytoma with a TH inhibitor, α-methyl-paratyrosine (metyrosine). Aromatic L-amino acid decarboxylase (AADC) converts DOPA to dopamine. Dopamine is hydroxylated to norepinephrine by dopamine β-hydroxylase (DBH). Norepinephrine is converted to epinephrine by phenylethanolamine N-methyltransferase (PNMT). Cortisol induces the expression of PNMT, which explains why epinephrine-secreting neoplasms are almost exclusively localized to the adrenal medulla.

nicotinic cholinergic receptors and causes depolarization of adrenomedullary chromaffin cells. Depolarization leads to activation of voltage-gated calcium channels, which results in exocytosis of secretory vesicle contents. A calcium-sensing receptor appears to be involved in the process of exocytosis. During exocytosis, all the granular contents are released into the extracellular space. Norepinephrine modulates its own release by activating the α_2 receptors on the presynaptic membrane. Stimulation of the presynaptic α_2 receptors inhibits norepinephrine release (the mechanism of action of some antihypertensive medications such as clonidine and guanfacine). Catecholamines are among the shortest lived signaling molecules in plasma; the initial biologic half-life of circulating catecholamines is between 10 and 100 seconds. Approximately one-half of the catecholamines circulate in plasma in loose association with albumin. Therefore plasma concentrations of catecholamines fluctuate widely.

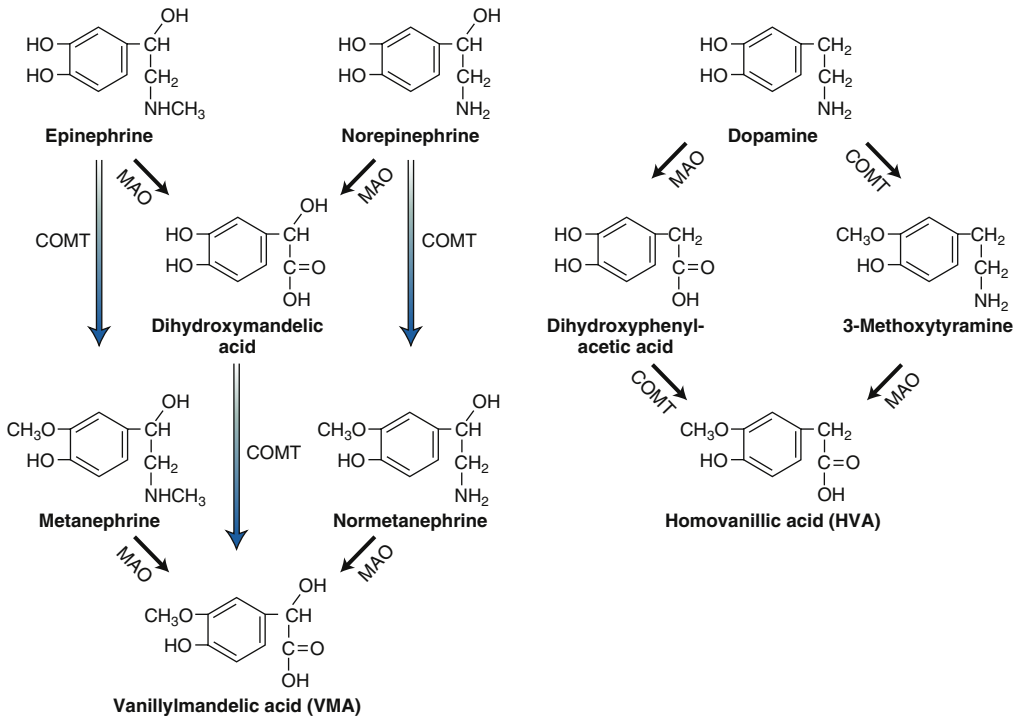
Catecholamine Metabolism and Inactivation

Catecholamines are removed from the circulation either by reuptake in sympathetic nerve terminals or by metabolism through two enzyme pathways (Fig. 16.2), followed by sulfate conjugation and renal excretion. Most of the metabolism of catecholamines occurs in the same cell in which they are synthesized.[8] Almost 90% of catecholamines released at sympathetic synapses are taken up locally by the nerve endings, termed *uptake 1*. Uptake 1 can be blocked by cocaine, tricyclic antidepressants, and phenothiazines. Extraneuronal tissues also take up catecholamines, and this is termed *uptake 2*. Most of these catecholamines are metabolized by catechol-*O*-methyltransferase (COMT).

Although COMT is found primarily outside neural tissue, *O*-methylation in the adrenal medulla is the predominant source of metanephrine (COMT converts epinephrine to metanephrine) and a major source of normetanephrine (COMT converts norepinephrine to normetanephrine) through methylation of the 3-hydroxy group.[8] *S*-Adenosylmethionine is used as the methyl donor, and calcium is required for this enzymatic step. Metanephrine and normetanephrine are oxidized by monoamine oxidase (MAO) to vanillylmandelic acid (VMA) by oxidative deamination. MAO may also oxidize epinephrine and norepinephrine to 3,4-dihydroxymandelic acid, which is then converted by COMT to VMA. MAO is located on the outer membrane of mitochondria. In the storage vesicle, norepinephrine is protected from metabolism by MAO. MAO and COMT metabolize dopamine to homovanillic acid (see Fig. 16.2).

Pheochromocytoma and Paraganglioma

Catecholamine-secreting tumors that arise from chromaffin cells of the adrenal medulla and the sympathetic ganglia are referred to as *pheochromocytomas* and *catecholamine-secreting paragangliomas*, respectively.[12] Because the tumors have similar clinical presentations and are treated with similar approaches, many clinicians use the term *pheochromocytoma* to refer to both adrenal pheochromocytomas and extra-adrenal catecholamine-secreting paragangliomas. However, the distinction between pheochromocytoma and paraganglioma is an important one because of implications for associated neoplasms, risk for malignancy, and genetic testing. Catecholamine-secreting tumors are rare, with an annual incidence of 2 to 8 cases per 1 million people.[13] Based on screening



• **Fig. 16.2** Catecholamine metabolism. Metabolism of catecholamines occurs through two enzymatic pathways. Catechol-*O*-methyltransferase (COMT) converts epinephrine to metanephrine and converts norepinephrine to normetanephrine through meta-*O*-methylation. Metanephrine and normetanephrine are oxidized by monoamine oxidase (MAO) to vanillylmandelic acid (VMA) by oxidative deamination. MAO also may oxidize epinephrine and norepinephrine to dihydroxymandelic acid, which is then converted by COMT to VMA. Dopamine is also metabolized by MAO and COMT to the final metabolite, homovanillic acid (HVA).

studies for secondary causes of hypertension in outpatients, the prevalence of pheochromocytoma has been estimated at 0.1% to 0.6%.[14,15] Nevertheless, it is important to suspect, confirm, localize, and resect these tumors because (1) the associated hypertension is curable with surgical removal of the tumor, (2) a risk of lethal paroxysm exists, (3) at least 10% of the tumors are malignant, and (4) 40% of these tumors are familial, and their detection in the proband may result in early diagnosis in other family members.

History

The association between adrenal medullary tumors and symptoms was first recognized by Fränkel in 1886.[16] He described Fraulein Minna Roll, age 18 years, who had intermittent attacks of palpitation, anxiety, vertigo, headache, chest pain, cold sweats, and vomiting. She had a hard, noncompressible pulse and retinitis. Despite champagne therapy and injections of ether, she died. At autopsy, bilateral adrenal tumors were initially thought to be angiosarcomas, but later a positive chromaffin reaction confirmed pheochromocytoma. A subsequent study published in 2007 documented the presence of a germline *RET* proto-oncogene mutation in four living relatives of Fraulein Roll—proving that the original patient and her family had multiple endocrine neoplasia type 2 (MEN2).[17]

The term *pheochromocytoma*, proposed by Pick in 1912,[18] comes from the Greek words *phaios* (dusky), *chroma* (color), and *cytoma* (tumor)—words that describe the dark staining reaction that is caused by the oxidation of intracellular catecholamines when they are exposed to dichromate salts. In 1926, César Roux in Lausanne, Switzerland, and Charles Mayo in Rochester, Minnesota, successfully surgically removed abdominal catecholamine-secreting tumors.[19,20] In 1929, it was discovered that a pheochromocytoma contained an excess amount of a pressor agent. Subsequently, epinephrine (in 1936) and norepinephrine (in 1949) were isolated from pheochromocytoma tissue. In 1950, it was found that patients with pheochromocytoma excreted increased amounts of epinephrine, norepinephrine, and dopamine in the urine.[21]

Clinical Presentation

Catecholamine-secreting tumors occur with equal frequency in men and women, primarily in the third, fourth, and fifth decades. These tumors are rare in children, and when discovered, they may be multifocal and associated with a hereditary syndrome. The symptoms, listed in Table 16.2, are caused by the pharmacologic effects of excess concentrations of circulating catecholamines. The associated hypertension may be sustained or paroxysmal, and patients whose pheochromocytoma is diagnosed in the presymptomatic stage may have normal blood pressure. The lability in blood pressure can be attributed to episodic release of catecholamines, chronic volume depletion, and impaired sympathetic reflexes. In addition to volume depletion, altered sympathetic vascular regulation may have a role in orthostasis, which may be observed in patients with pheochromocytoma. Symptoms of orthostatic hypotension (e.g., lightheadedness, presyncope, syncope) may dominate the presentation, especially in patients with epinephrine-predominant or dopamine-predominant tumors.

Episodic symptoms may occur in spells, or paroxysms, that can be extremely variable in presentation but typically include forceful heartbeat, pallor, tremor, headache, and diaphoresis.[22] The spell may start with a sensation of a "rush" in the chest and a sense of shortness of breath, followed by a forceful heartbeat and a throbbing headache. Peripheral vasoconstriction associated with a spell results in cool or cold hands and feet and facial pallor. Increased sense of body heat and sweating are common symptoms that occur toward the end of the spell. Spells may be either spontaneous or precipitated by postural change, anxiety, medications (e.g., β-adrenergic antagonists, metoclopramide, anesthetic agents), exercise, or maneuvers that increase intra-abdominal pressure (e.g., change in position, lifting, defecation, exercise, colonoscopy, pregnancy, trauma). Although the types of spells experienced across the patient population are highly variable, spells tend to be stereotypical for each patient. Spells may occur multiple times daily or as infrequently as once monthly. The typical duration of a pheochromocytoma spell is 15 to 20 minutes, but it may be much shorter or last several hours. However, the clinician must recognize that most patients with spells do not have a pheochromocytoma (Table 16.3).[23]

Additional clinical signs of pheochromocytoma include hypertensive retinopathy, orthostatic hypotension, angina, nausea,

TABLE 16.2 Signs and Symptoms Associated With Catecholamine-Secreting Tumors

Spell-Related Signs and Symptoms

Anxiety and fear of impending death
Diaphoresis
Dyspnea
Epigastric and chest pain
Headache
Hypertension
Nausea and vomiting
Pallor
Palpitation (forceful heartbeat)
Tremor

Chronic Signs and Symptoms

Cold hands and feet
Congestive heart failure—dilated or hypertrophic cardiomyopathy
Constipation
Diaphoresis
Dyspnea
Ectopic hormone secretion–dependent symptoms (e.g., CRH/ACTH, GHRH, PTHrP, VIP)
Epigastric and chest pain
Fatigue
Fever
General increase in sweating
Grade II to IV hypertensive retinopathy
Headache
Hyperglycemia
Hypertension
Nausea and vomiting
Orthostatic hypotension
Painless hematuria (associated with urinary bladder paraganglioma)
Pallor
Palpitation (forceful heartbeat)
Tremor
Weight loss

Not Typical of Pheochromocytoma

Flushing

ACTH, Adrenocorticotropic hormone; *CRH,* corticotropin-releasing hormone; *GHRH,* growth hormone–releasing hormone; *PTHrP,* parathyroid hormone–related peptide; *VIP,* vasoactive intestinal polypeptide.

Adapted from Young WF Jr. Pheochromocytoma, 1926-1993. *Trends Endocrinol Metab.* 1993;4:122–127.

constipation (megacolon may be the presenting symptom), hyperglycemia, diabetes mellitus, hypercalcemia, Raynaud phenomenon, livedo reticularis, erythrocytosis, and mass effects from the tumor. Although hypercalcemia may be a sign of primary hyperparathyroidism in patients with MEN2A, in most patients with pheochromocytoma, it is an isolated finding and resolves with resection of the catecholamine-secreting tumor. In addition, calcitonin secretion is in part a catecholamine-dependent process; serum calcitonin concentrations are frequently mildly elevated in patients with pheochromocytoma, usually unrelated to MEN2. Fasting hyperglycemia and diabetes mellitus are caused in part by the α-adrenergic inhibition of insulin release. Painless hematuria and paroxysmal attacks induced by micturition and defecation are associated with urinary bladder paragangliomas.

TABLE 16.3 Differential Diagnosis of Pheochromocytoma-Type Spells

Endocrine Causes
Carbohydrate intolerance
Hyperadrenergic spells
Hypoglycemia
Pancreatic tumors (e.g., insulinoma)
Pheochromocytoma
Primary hypogonadism (menopausal syndrome)
Thyrotoxicosis

Cardiovascular Causes
Angina
Cardiovascular deconditioning
Labile essential hypertension
Orthostatic hypotension
Paroxysmal cardiac arrhythmia
Pulmonary edema
Renovascular disease
Syncope (e.g., vasovagal reaction)

Psychologic Causes
Factitious (e.g., drugs, Valsalva maneuver)
Hyperventilation
Severe anxiety and panic disorders
Somatization disorder

Pharmacologic Causes
Chlorpropamide-alcohol flush
Combination of a monoamine oxidase inhibitor and a decongestant
Illegal drug ingestion (cocaine, phencyclidine, lysergic acid diethylamide)
Sympathomimetic drug ingestion
Vancomycin (red man syndrome)
Withdrawal of adrenergic-inhibitor

Neurologic Causes
Autonomic neuropathy
Cerebrovascular insufficiency
Diencephalic epilepsy (autonomic seizures)
Migraine headache
Postural orthostatic tachycardia syndrome
Stroke

Other Causes
Carcinoid syndrome
Mast cell disease
Recurrent idiopathic anaphylaxis
Unexplained flushing spells

Some of the cosecreted hormones that may dominate the clinical presentation include ACTH (Cushing syndrome), parathyroid hormone–related peptide (hypercalcemia), vasopressin (syndrome of inappropriate antidiuretic hormone secretion), vasoactive intestinal peptide (watery diarrhea), and growth hormone–releasing hormone (acromegaly). Cardiomyopathy and congestive heart failure are the symptomatic presentations caused by pheochromocytoma that are most frequently unrecognized by clinicians.[24] The cardiomyopathy, whether dilated or hypertrophic, may be totally reversible with tumor resection.[24,25] In addition, Takotsubo cardiomyopathy with ventricular apical ballooning can also be a presentation of pheochromocytoma.[26] Myocarditis and myocardial infarction with normal coronary arteries seen on angiography are also cardiac-based presentations of pheochromocytoma.[24] The myocarditis is characterized by infiltration of inflammatory cells and focal contraction-band necrosis.[24,27] Many physical examination findings can be associated with genetic syndromes that predispose to pheochromocytoma; these findings include retinal angiomas, iris hamartomas, marfanoid body habitus, café au lait spots, axillary freckling, subcutaneous neurofibromas, and mucosal neuromas on the eyelids and tongue. Some patients with pheochromocytoma are asymptomatic despite high circulating levels of catecholamines; this type most likely reflects adrenergic receptor desensitization related to chronic stimulation.

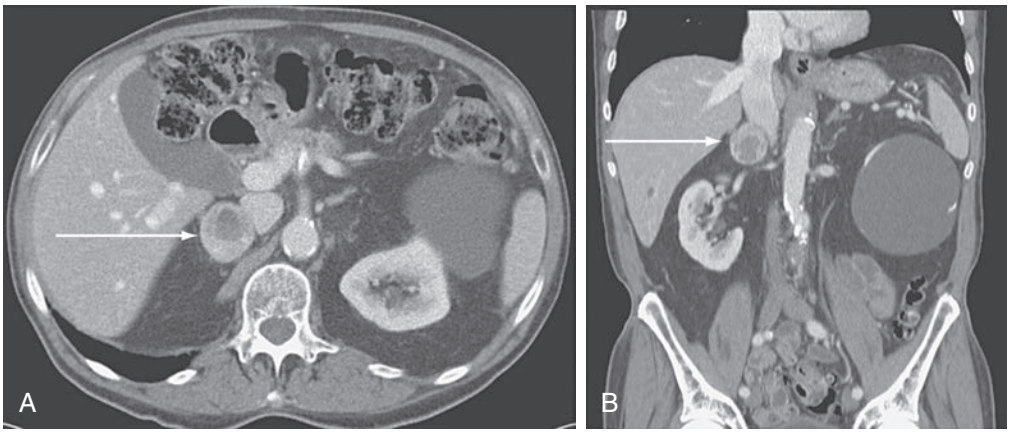
Because of the increased and widespread use of computed tomography (CT) and magnetic resonance imaging (MRI) in patients with abdominal symptoms, pheochromocytoma and abdominal paraganglioma may be detected as an incidental adrenal mass in many patients before any symptoms develop.[28] In the era of widespread use of computed cross-sectional imaging, approximately 60% of adrenal pheochromocytoma patients had their adrenal tumors discovered incidentally on imaging performed for other reasons.[29–31] Although typically these incidentally discovered tumors in asymptomatic patients are small (<3 cm), they may be up to 10 cm in largest diameter.

At the time of symptom-based detection, pheochromocytomas have an average diameter of 4.5 cm (Fig. 16.3).[32] Paragangliomas are found where there is chromaffin tissue: along the para-aortic sympathetic chain, within the organ of Zuckerkandl (at the origin of the inferior mesenteric artery), in the wall of the urinary bladder, and along the sympathetic chain in the neck or mediastinum.[33] During early postnatal life, the extra-adrenal sympathetic paraganglionic tissues are prominent; later they degenerate, leaving residual foci associated with the vagus nerves, carotid vessels, aortic arch, pulmonary vessels, and mesenteric arteries. Odd locations for paragangliomas include the neck, intra-atrial cardiac septum, spermatic cord, vagina, scrotum, and sacrococcygeal region. Paragangliomas in the head and neck region (e.g., carotid body tumors, glomus tumors, chemodectomas) usually arise from parasympathetic tissue and typically do not hypersecrete catecholamines and metanephrines. Paragangliomas in the mediastinum, abdomen, and pelvis usually arise from sympathetic chromaffin tissue and usually hypersecrete catecholamines and metanephrines.

Syndromic Forms of Pheochromocytoma and Paraganglioma

Multiple Endocrine Neoplasia Type 2A

MEN2A (previously known as Sipple syndrome) is an autosomal dominant disorder with age-related penetrance.[34] MEN2A is characterized by medullary thyroid cancer (MTC) in all patients,



• **Fig. 16.3** A computed tomographic (CT) scan of the abdomen with intravenous contrast in a 71-year-old man with an incidentally discovered right adrenal mass. The concentrations of plasma fractionated free metanephrines were abnormal: metanephrine, 0.34 nmol/L (normal, <0.5 nmol/L) and normetanephrine, 8.59 nmol/L (normal, <0.9 nmol/L). The 24-hour urine studies were abnormal: norepinephrine, 455 μg (normal, <170 μg); epinephrine, 7.2 μg (normal, <35 μg); dopamine, 160 μg (normal, <700 μg); metanephrine, 173 μg (normal, <400 μg); and normetanephrine, 3147 μg (normal, <900 μg). (A) The axial CT image shows a typical 3.8-cm heterogeneously enhancing right adrenal mass just lateral to the inferior vena cava and consistent with pheochromocytoma *(arrow)*. (B) Coronal view shows the location *(arrow)* of the mass superior to the right kidney and inferior and medial to the liver. After α-adrenergic and β-adrenergic blockade, a 20-g (2.5 × 1.5 × 1.5 cm) pheochromocytoma was removed laparoscopically.

adrenergic (epinephrine and metanephrines are predominant) pheochromocytoma in 50% (usually bilateral and frequently asynchronous), primary hyperparathyroidism in 20%, cutaneous lichen amyloidosis in 5%, and very rarely Hirschsprung disease. Cutaneous lichen amyloidosis is a pruritic, papular, scaly, and pigmented skin lesion that is typically located in the interscapular region or on the extensor surfaces of the extremities.

MTC is usually detected before the pheochromocytoma is diagnosed. The prevalence of MEN2A is approximately 1 in 200,000 live births. Numerous activating mutations throughout the *RET* (*re*arranged during *t*ransfection) proto-oncogene have been documented in persons with MEN2A. *RET*, located on chromosome 10q11.2, encodes a transmembrane receptor tyrosine kinase that is involved in the regulation of cell proliferation and apoptosis by activation of the PI3K/AKT (phosphoinositide 3-kinase) and MAPK/ERK (mitogen-activated protein kinase/extracellular signal-regulated kinase) signaling pathways. *RET* can be constitutively activated by gain-of-function mutations in seven specific exons of the *RET* gene (exons 8, 10, 11, 13, 14, 15, or 16). Most mutations in MEN2A kindreds (>90%) involve *RET* exons 10 (codons 609, 611, 618, and 620) or 11 (codons 630 or 634). Eighty-five percent of individuals with MEN2A have a mutation in codon 634, particularly p.Cys634Arg (c.1900C>T).

Hirschsprung disease is characterized by the absence of autonomic ganglion cells within the distal colon parasympathetic plexus, which results in constipation, megacolon, or obstipation. Hirschsprung disease may occur in patients with MEN2A who have a Janus mutation—a mutation that acts simultaneously as both a gain-in-function and a loss-of-function mutation—in the *RET* proto-oncogene (exon 10: codons 609, 611, 618, 620).[35] It is important to distinguish the constipation/obstipation due to Hirschsprung disease in patients with MEN2A from that due to ganglioneuromatosis of MEN2B or from the colonic paralytic effects seen in patients with massive elevations in catecholamines (most commonly seen in patients with widespread metastatic paraganglioma or pheochromocytoma).

Multiple Endocrine Neoplasia Type 2B

MEN2B (previously known as Gorlin syndrome) is also an autosomal dominant disorder with age-related penetrance, and it represents approximately 5% of all MEN2 cases.[34] MEN2B is characterized by MTC in all patients, adrenergic (epinephrine and metanephrine predominant) pheochromocytoma in 50%, mucocutaneous neuromas (typically involving the tongue, lips, and eyelids) in most patients, and by skeletal deformities (e.g., kyphoscoliosis, lordosis), joint laxity, myelinated corneal nerves, and intestinal ganglioneuromas.

MEN2B-associated tumors are caused by mutations in the *RET* protein's intracellular domain. A single methionine-to-threonine missense mutation in exon 16 (p.Met918Thr; c.2753T>C) is responsible for more than 95% of MEN2B cases. Another mutation, alanine to phenylalanine at codon 883 in exon 15, has been found in 4% of MEN2B kindreds.

More than 95% of patients with MEN2A and more than 98% of those with MEN2B have an identifiable mutation in the *RET* proto-oncogene. Pheochromocytoma in MEN2 has an adrenergic (epinephrine and metanephrine are predominant) biochemical phenotype, and this knowledge directs genetic testing.[36] Genetic testing for mutations in the *RET* proto-oncogene should be considered for patients with co-phenotype disorders (e.g., MTC) or adrenergic biochemical phenotype pheochromocytoma (unilateral or bilateral). In a family with MEN2, a family member with a clinical diagnosis of MEN2 should be the first to have genetic testing. If a *RET* mutation is found, all family members of unknown status should be offered genotyping. Genetic consultation should be considered before genetic testing is performed. In families with known MEN2, genetic testing shortly after birth facilitates prompt surgical management of the thyroid gland (see Chapter 42 for further discussion of MEN2).

von Hippel-Lindau Disease

von Hippel-Lindau (VHL) syndrome is an autosomal dominant disorder that may manifest with a variety of benign and malignant

neoplasms: noradrenergic (norepinephrine and normetanephrine are predominant) pheochromocytoma or paraganglioma (mediastinal, abdominal, pelvic), hemangioblastoma (involving the cerebellum, spinal cord, or brainstem), retinal angioma, clear cell renal cell carcinoma, pancreatic neuroendocrine tumors, endolymphatic sac tumors of the middle ear, serous cystadenomas of the pancreas, and papillary cystadenomas of the epididymis and broad ligament.[37] The appearance of these VHL-related neoplasms in mutation-positive individuals is close to 100% by age 65 years. The average age of detection of pheochromocytoma is 20 to 29 years of age.[37]

The prevalence of VHL syndrome is between 1 in 35,000 and 1 in 91,000 people.[38,39] The *VHL* tumor suppressor gene, located on chromosome 3p25-26, encodes a protein that regulates ubiquitination and proteosomal degradation of hypoxia-inducible factors (HIFs). Loss-of-function mutations in VHL lead to the inappropriate activation of the hypoxic response—promoting glycolysis, angiogenesis, and proliferation. Genotype-phenotype correlations may be used to divide patients into two groups: type 1 and type 2. Patients from kindreds with the type 1 syndrome have mutations that lead to total loss of biologic activity of the VHL protein and are at very low risk to develop pheochromocytoma; those from kindreds with the type 2 syndrome have missense mutations that allow residual activity of the VHL protein and are at high risk for developing pheochromocytoma.

Genetic testing for VHL syndrome should be considered for patients with bilateral noradrenergic (norepinephrine and normetanephrine predominant) pheochromocytoma, diagnosis of unilateral noradrenergic pheochromocytoma at a young age (e.g., ≤45 years), or pheochromocytoma/paraganglioma patients with co-phenotype disorders (e.g., retinal angioma).

Pheochromocytomas occurring in patients with MEN2 produce predominantly epinephrine and its major metabolite, metanephrine, whereas those occurring in patients with VHL syndrome produce predominantly norepinephrine and its major metabolite, normetanephrine.[36] These biochemical phenotypes result from mutation-specific differential gene expression. PNMT is overexpressed in MEN2-associated tumors (epinephrine and metanephrine profile) and underexpressed in VHL-associated tumors (norepinephrine and normetanephrine profile).[40] In addition, pheochromocytomas occurring in patients with MEN2 have increased tyrosine hydroxylase activity compared with those occurring in patients with VHL; this difference accounts for higher levels of catecholamines and metabolites in patients with MEN2.

Neurofibromatosis Type 1

Neurofibromatosis 1 (NF1), previously known as von Recklinghausen disease, is one of the most common genetic syndromes, with a prevalence of 1 in 2000 to 1 in 5000 persons.[41] NF1 is an autosomal dominant disorder with 100% penetrance and is characterized by neurofibromas, multiple café au lait spots, axillary and inguinal freckling, iris hamartomas (Lisch nodules), bony abnormalities, central nervous system gliomas, pheochromocytoma and paraganglioma, macrocephaly, and cognitive deficits. Although penetrance is 100%, the expression of NF1 features is variable. Approximately 3% of patients with NF1 develop catecholamine-secreting tumors.[42] In these patients, the catecholamine-secreting tumor is usually a solitary, benign adrenal pheochromocytoma, occasionally a bilateral adrenal pheochromocytoma, and rarely an abdominal periadrenal paraganglioma.[42] Frequently the adrenal pheochromocytoma is detected as an incidental adrenal mass on imaging performed for other reasons.[42]

The *NF1* tumor suppressor gene, located on chromosome 17q11.2, encodes neurofibromin, which downregulates RAS proteins and the downstream RAS-RAF-MAPK signaling cascade. Inactivating *NF1* mutations cause the disorder. Unless a patient with pheochromocytoma presents with additional clinical characteristics consistent with an NF1 diagnosis, genetic testing of the *NF1* gene is not recommended.

Congenital Polycythemia

The most common congenital polycythemia-associated somatic mutations that also predispose to pheochromocytoma and paraganglioma occur in HIF2α, which lead to stabilization of HIF2α, resulting in the upregulation of HIF2α-related genes (e.g., erythropoietin gene). Patients with HIF2α mutations are at risk for congenital polycythemia, multiple paragangliomas, and somatostatinomas.[43,44] These HIF2α somatic mutations occur exclusively in females in the postzygotic neural crest precursor cells.[45] Two other congenital polycythemia-associated mutations with potential links to pheochromocytoma and paraganglioma include VHL (Chuvash polycythemia; however, these patients have not been reported to develop pheochromocytoma or paraganglioma) and prolyl hydroxylase domain 2 (PHD2)–containing protein (with only one patient reported to date).[46]

Carney Triad

The Carney triad (gastrointestinal stromal tumor, pulmonary chondroma, and catecholamine-secreting paraganglioma; less frequent neoplasms include esophageal leiomyoma and adrenal adenoma) is another syndrome associated with catecholamine-secreting tumors.[47] This syndrome is a rare (~150 patients have been reported) disorder of unknown cause that primarily affects young women.[48] The gastric stromal tumors are frequently multicentric and associated with early liver metastases; however, most affected patients have a very indolent course.[49] The pulmonary chondromas are benign and, if asymptomatic, require no specific therapy. The paragangliomas secrete catecholamines and should be resected when discovered. Additional features of the Carney triad include esophageal leiomyomas and adrenocortical adenomas. The esophageal leiomyomas are benign and usually asymptomatic. The adrenocortical adenomas may be nonfunctioning or may secrete cortisol autonomously.[50] The molecular pathogenesis of Carney triad is related to the downregulation of the succinate dehydrogenase (SDH) enzyme complex through site-specific hypermethylation of the *SDHC* gene.[51,52]

Congenital Heart Disease

The association between cyanotic congenital heart disease and pheochromocytoma/paraganglioma has been recognized for more than 50 years.[53–56] The underlying pathogenesis appears to be gain-of-function somatic mutations of *EPAS1*, which encodes HIF2α.[57] Mutations in residues 530 and 531 of *EPAS1* result in constitutive HIF2α activation, leading to development of pheochromocytoma and paraganglioma in patients with cyanotic congenital heart disease. *EPAS1* mutations in the setting of chronic hypoxia amplify the oncogenic properties of HIF2α.[57]

Other Genetic Forms of Pheochromocytoma and Paraganglioma

Mutations contributing to pheochromocytoma and paraganglioma have two general transcription signatures: cluster 1—genes encoding proteins that function in the cellular response

to hypoxia, and cluster 2—genes encoding proteins that activate kinase signaling (Table 16.4). Cluster 1 tumors are mostly extra-adrenal paragangliomas (except in VHL, in which most tumors are localized to the adrenal gland), and nearly all have a noradrenergic biochemical phenotype. Cluster 2 tumors are usually adrenal pheochromocytomas with an adrenergic biochemical phenotype (see Table 16.4). Since 1990, 18 pheochromocytoma/paraganglioma susceptibility genes have been reported: *NF1*, *RET*, *VHL*, *SDHD*, *SDHC*, *SDHB*, *EGLN1 (PHD2)*, *EGLN2* (*PDH1*), *KIF1B*, *SDHAF2*, *IDH1*, *TMEM127*, *SDHA*, *MAX*, *HIF2A*, *MDH2*, *FH*, and *DNMT3A*.[58,59]

Succinate Dehydrogenase Gene Mutations

Most cases of familial paraganglioma are caused by mutations in the SDH (succinate:ubiquinone oxidoreductase) subunit genes (*SDHB*, *SDHC*, *SDHD*, *SHDA*, and *SDHAF2*), which make up portions of mitochondrial complex II.[60] The SDHx genes are considered tumor suppressor genes and encode the proteins that form the mitochondrial complex II, a crucial link between the Krebs cycle and mitochondrial electron transport chain. SDH is a heterotetramer protein complex consisting of four subunits encoded by nuclear genes. *SDHA* and *SDHB* form the catalytic domain, and *SDHC* and *SDHD* anchor the complex to the inner mitochondrial membrane. The assembly factors, *SDHAF1* and *SDHAF2*, are needed for functional and structural integrity of the complex. Defects in the *SDH* genes result in succinate accumulation, which is a competitive inhibitor of the 2-oxoglutarate-dependent dioxygenases (e.g., HIF prolyl hydroxylases and histone or DNA demethylases), which leads to the stabilization of HIF isoforms and the activation of hypoxic signaling and to epigenetic modifications.

In patients with *SDHD* or *SDHAF2* mutations, penetrance depends on the mutation's parent of origin. With rare exceptions,[61,62] the disease is not manifested when the mutation is inherited from the mother but is highly penetrant when inherited from the father.[63] This phenomenon is known as *maternal imprinting*.[64]

TABLE 16.4 Germline Mutations Associated With Pheochromocytoma and Paraganglioma

Syndrome/Name	Gene	Typical Tumor Location and Other Associations
Hypoxic Pathway: Cluster 1[a]		
SDHD mutation (familial paraganglioma type 1)[b]	*SDHD*	Primarily skull base and neck; occasionally adrenal medulla, mediastinum, abdomen, pelvis; GIST; possible pituitary adenoma
SDHAF2 mutation (familial paraganglioma type 2)[b]	*SDHAF2*	Primarily skull base and neck; occasionally abdomen and pelvis
SDHC mutation (familial paraganglioma type 3)	*SDHC*	Primarily skull base and neck; occasionally abdomen, pelvis, or chest; GIST; possible pituitary adenoma
SDHB mutation (familial paraganglioma type 4)	*SDHB*	Abdomen, pelvis, and mediastinum; rarely adrenal medulla, skull base, and neck; GIST; renal cell carcinoma; possible pituitary adenoma
SDHA mutation	*SDHA*	Primarily skull base and neck; occasionally abdomen and pelvis; GIST; possible pituitary adenoma
von Hippel-Lindau (VHL) disease	*VHL*	Adrenal medulla, frequently bilateral; occasionally paraganglioma that may be localized from skull base to pelvis; see text for VHL-associated findings
Hereditary leiomyomatosis and renal cell carcinoma (Reed syndrome)—fumarate hydratase mutation	*FH*	Multifocal and metastatic; associated with hereditary leiomyomatosis, uterine fibroids, and renal cell cancer
Hypoxia-inducible factor (HIF) 2α	*HIF2A*	Paraganglioma, polycythemia, and rarely somatostatinoma
Familial erythrocytosis associated with mutation in prolyl hydroxylase isoform 1 (PDH1)	*EGLN2*	Polycythemia associated with pheochromocytoma and paraganglioma
Familial erythrocytosis associated with mutation in prolyl hydroxylase isoform 2 (PDH2)	*EGLN1*	Polycythemia associated with pheochromocytoma and paraganglioma
KIF1B	*KIF1B*	Neuroblastoma
Kinase Signaling Pathway: Cluster 2[c]		
MEN2A and MEN2B	*RET*	Adrenal medulla, frequently bilateral; see text for MEN2A and MEN2B associated findings
Neurofibromatosis type 1 (NF1)	*NF1*	Adrenal or periadrenal; see text for NF1 associated findings
MAX[b]	*MAX*	Adrenal medulla
Familial pheochromocytoma	*TMEM127*	Adrenal medulla; possible renal cell carcinoma

[a]Cluster 1 tumors are mostly extra-adrenal paragangliomas (except in VHL where most tumors are localized to the adrenal) and nearly all have a noradrenergic biochemical phenotype.

[b]Associated with maternal imprinting—see text.

[c]Cluster 2 tumors are usually adrenal pheochromocytomas with an adrenergic biochemical phenotype.

GIST, Gastrointestinal stromal tumor; *MEN,* multiple endocrine neoplasia; *SDH,* succinate dehydrogenase.

TMEM127 *Mutations*

TMEM127 is a negative regulator of mammalian target of rapamycin (mTOR) effector proteins. In a study of 990 individuals with pheochromocytoma or paraganglioma, germline mutations in *TMEM127* were identified in 20 individuals with adrenal tumors (7 bilateral), 5 of whom had a family history of pheochromocytoma.[65] Among 547 patients who presented with sporadic pheochromocytoma (unilateral adrenal tumor with negative family history), 11 (2%) had *TMEM127* mutations.

MAX *Mutations*

Loss-of-function mutations in the MYC-associated factor X *(MAX)* gene are associated with familial pheochromocytoma.[66] In an initial study of three individuals with familial pheochromocytoma (who did not have mutations in any of the nine previously described susceptibility genes), *MAX* germline mutations were found. *MAX* is a component of the MYC-MAX-MXD1 transcription factors that regulate cell proliferation, differentiation, and apoptosis. In an extension of this study, *MAX* mutations were found in 5 of 59 patients (8.5%) with suspected familial pheochromocytoma (based on age of onset <30 years, bilateral pheochromocytoma, or positive family history).[66]

FH *Mutations*

Germline mutations in the *FH* gene encoding fumarate hydratase were identified in 5 of 598 patients (1%) with pheochromocytoma/paraganglioma without mutations in known susceptibility gene mutations.[67] Clinically, a metastatic phenotype and multiple tumors were significantly more frequent in patients with *FH* mutations than those without such mutations.

Genetic Testing

Genetic testing should be considered if a patient has one or more of the following: (1) paraganglioma, (2) bilateral adrenal pheochromocytoma, (3) unilateral adrenal pheochromocytoma and a family history of pheochromocytoma/paraganglioma, (4) unilateral adrenal pheochromocytoma with onset at a young age (<45 years), or (5) other clinical findings suggestive of one of the previously discussed syndromic disorders. An asymptomatic person at risk for disease on the basis of family history of pheochromocytoma/paraganglioma should have genetic testing only if an affected family member has a known mutation. Genetic testing can be complex, and testing of one family member has implications for related individuals. Genetic counseling is recommended to help families understand the implications of genetic test results, to coordinate testing of at-risk individuals, and to help families work through the psychosocial issues that may arise before, during, or after the testing process.

A sequential genetic testing algorithm based on biochemical phenotype, age, and tumor has been proposed.[22] However, the field of genetic testing is rapidly evolving, and at many clinical laboratories, sequential genetic testing is no longer done as it is less expensive to utilize next-generation sequencing technology for all clinically available mutations as a package.[68]

Evaluation and Monitoring of Carriers of Succinate Dehydrogenase Mutations

If an SDHx mutation is identified in a relative of a proband, annual clinical assessment (including blood pressure check and biochemical testing) is indicated for early detection of pheochromocytoma/paraganglioma. Prospective studies to guide the clinician in the frequency, age to start, and type of testing are lacking. Biochemical testing for fractionated metanephrines in plasma or in a 24-hour urine collection for fractionated metanephrines and catecholamines should be performed annually in all carriers of an SDHx mutation starting around age 10 years. Because paragangliomas may be nonfunctioning or may be detected before catecholamine-secretory autonomy is evident, periodic imaging studies are advised. For example, at-risk SDHx (e.g., paternally inherited for *SDHD* and *SDHAF2*) mutation carriers should have MRI of the abdomen, pelvis, chest, and neck every 2 to 3 years; total body imaging with either ^{123}I-MIBG scintigraphy or Gallium 68 (68-Ga) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-octreotate (DOTATATE) positron emission tomography (PET) CT should be performed every 5 years. Patients with SDHx mutations are at risk (albeit low) to develop renal cell carcinoma, and the periodic abdominal cross-section imaging also screens for this possibility.[69] In addition, as new tumor associations are identified, additional surveillance testing will be indicated. For example, several reports have identified an association between SDHx mutations and pituitary tumor risk.[70,71] If this risk proves to be real and clinically important, then pituitary-directed MRI may become part of the surveillance program.

Diagnostic Investigation

Differential Diagnosis

Numerous disorders can cause signs and symptoms that may prompt the clinician to test for pheochromocytoma (see Table 16.3). The disorders span much of medicine and include endocrine disorders (e.g., primary hypogonadism), cardiovascular disorders (e.g., idiopathic orthostatic hypotension), psychologic disorders (e.g., panic disorder), pharmacologic causes (e.g., withdrawal from an adrenergic inhibitor), neurologic disorders (e.g., postural orthostatic tachycardia syndrome), and miscellaneous disorders (e.g., mast cell disease). Indeed, most patients tested for pheochromocytoma do not have it. In addition, levels of fractionated catecholamines and metanephrines may be elevated in several clinical scenarios, including withdrawal from medications or drugs (e.g., clonidine, alcohol), any acute illness (e.g., subarachnoid hemorrhage, migraine headache, preeclampsia), and administration of many drugs and medications (e.g., tricyclic antidepressants, levodopa, buspirone, antipsychotic agents, cocaine, phencyclidine, amphetamines, ephedrine, pseudoephedrine, phenylpropanolamine, isoproterenol) (Table 16.5).[22]

Case Detection

Pheochromocytoma should be suspected in patients who have one or more of the following:

- Hyperadrenergic spells (e.g., self-limited episodes of nonexertional forceful palpitations, diaphoresis, headache, tremor, or pallor)

TABLE 16.5 Medications that May Increase Measured Levels of Fractionated Catecholamines and Metanephrines

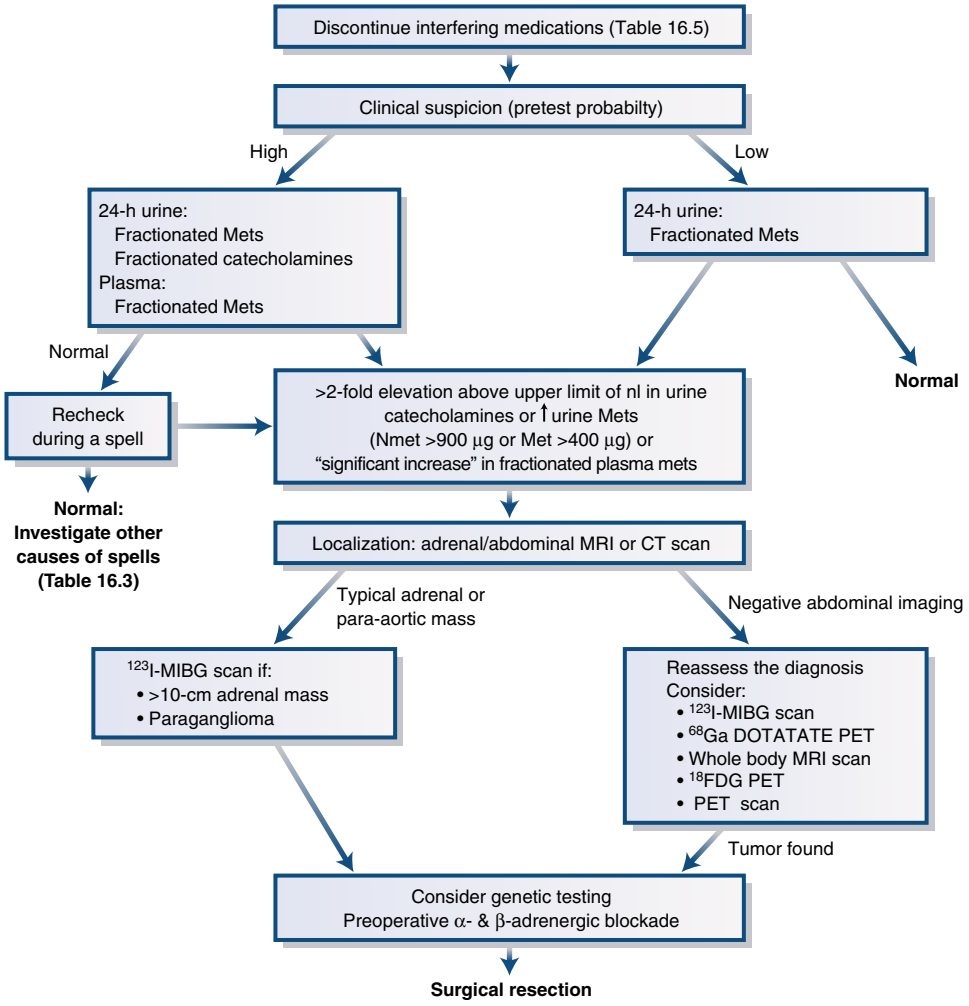
Tricyclic antidepressants (including cyclobenzaprine)
Levodopa
Drugs containing adrenergic receptor agonists (e.g., decongestants)
Amphetamines
Buspirone and antipsychotic agents
Prochlorperazine
Reserpine
Withdrawal from clonidine and other drugs (e.g., illicit drugs)
Illicit drugs (e.g., cocaine, heroin)
Ethanol

- Resistant hypertension
- A familial syndrome that predisposes to catecholamine-secreting tumors (e.g., MEN2, NF1, VHL)
- A family history of pheochromocytoma
- An incidentally discovered adrenal mass with imaging characteristics consistent with pheochromocytoma
- Pressor response during anesthesia, surgery, or angiography
- Onset of hypertension at a young age (<20 years)
- Idiopathic dilated cardiomyopathy
- Cyanotic congenital heart disease

Measurement of Fractionated Metanephrines and Catecholamines in Urine and Plasma

The diagnosis must be confirmed biochemically by the presence of increased concentrations of fractionated metanephrines and catecholamines in urine or plasma (Fig. 16.4).[22,72,73] The metabolism of catecholamines is primarily intratumoral, with formation of metanephrine from epinephrine and normetanephrine from norepinephrine.[8] Most laboratories now measure fractionated catecholamines (dopamine, norepinephrine, and epinephrine) and fractionated metanephrines (metanephrine and normetanephrine) by high-performance liquid chromatography with electrochemical detection or tandem mass spectrometry. These techniques have overcome the problems with fluorometric analysis, which include false-positive results caused by α-methyldopa, labetalol, sotalol, and imaging contrast agents.

At Mayo Clinic, the most reliable case-detection strategy is measurement of fractionated metanephrines and catecholamines in a 24-hour urine collection (sensitivity, 98%; specificity, 98%).[72,74] Because of the higher false-positive rate with plasma fractionated metanephrines, they should be reserved for high clinical suspicion cases. The index of suspicion for pheochromocytoma should be high in the following scenarios: resistant hypertension, spells with associated pallor, a family history of pheochromocytoma, a genetic syndrome that predisposes to pheochromocytoma (e.g., MEN2), a past history of resected pheochromocytoma and present history of recurrent hypertension or spells, and an incidentally discovered adrenal mass that has imaging characteristics consistent with pheochromocytoma (Table 16.6).[28] In addition, measurement of plasma fractionated metanephrines is a good first-line



• **Fig. 16.4** Evaluation and treatment of catecholamine-secreting tumors. Clinical suspicion is triggered by paroxysmal symptoms (especially hypertension); hypertension that is intermittent, unusually labile, or resistant to treatment; a family history of pheochromocytoma or associated conditions; or an incidentally discovered adrenal mass (see text for details). *CT,* computed tomography; *^{18}FDG,* ^{18}F-fluorodeoxyglucose; *^{68}Ga-DOTATATE,* Gallium-68 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-octreotate; *^{123}I-MIBG,* iodine-123–labeled metaiodobenzylguanidine; *Mets,* metanephrines; *MRI,* magnetic resonance imaging; *nl,* normal; *Nmet,* normetanephrine; *PET,* positron emission tomography. (Modified from Young Jr WF. Pheochromocytoma, 1926-1993. *Trends Endocrinol Metab.* 1993;4:122–127, used with permission.)

test for children because obtaining a complete 24-hour urine collection is difficult in pediatric patients. Measurement of urinary dopamine or plasma methoxytyramine can be very useful in detecting the rare tumor with selective dopamine hypersecretion because plasma metanephrine fractions are not direct metabolites of dopamine and may be normal in the setting of a dopamine-secreting tumor.[74–76]

The 24-hour urine collection for fractionated metanephrines and catecholamines should include measurement of urinary creatinine to verify an adequate collection. The diagnostic cutoffs for most 24-hour urinary fractionated metanephrine assays are based on normal ranges derived from normotensive volunteer reference groups, and this can result in excessive false-positive test results. For example, in normotensive laboratory volunteers, the 95th percentiles are 428 μg for normetanephrine and 200 μg for metanephrine, whereas the corresponding values in individuals who are being tested for pheochromocytoma as part of routine clinical practice but who do not have the neoplasm are, respectively, 71% and 51% higher than those of the normal volunteers (<900 μg for normetanephrine and <400 μg for metanephrines).[73]

Although it is preferred that patients not receive any medication during the diagnostic evaluation, treatment with most medications may be continued. Tricyclic antidepressants are the drugs that interfere most frequently with the interpretation of 24-hour urinary catecholamines and metabolites. To effectively screen for catecholamine-secreting tumors, treatment with tricyclic antidepressants and other psychoactive agents listed in Table 16.5 should be tapered and discontinued at least 2 weeks before any hormonal assessments. In some clinical situations it is contraindicated to discontinue certain medications (e.g., antipsychotics), and if case-detection testing is positive, then CT or MRI of the abdomen and pelvis would be needed to exclude a catecholamine-secreting tumor. Furthermore, catecholamine secretion may be appropriately increased in situations of physical stress or illness (e.g., stroke, myocardial infarction, congestive heart failure, obstructive sleep apnea). There are no reliable reference ranges for fractionated metanephrines or catecholamines in patients requiring intensive care unit hospitalization. Therefore the clinical circumstances under which catecholamines and metanephrines are measured must be assessed in each case.

Other Tests That Have Been Used to Assess for Pheochromocytoma

Because of poor overall accuracy in testing for pheochromocytoma, measurement of plasma catecholamines no longer has a role except to detect dopamine-secreting paragangliomas. Chromogranin A is stored and released from dense-core secretory granules of neuroendocrine cells and is increased in 80% of patients with pheochromocytoma. Chromogranin A is not specific for pheochromocytoma, and elevations may be seen with other neuroendocrine tumors. The 24-hour urinary VMA excretion has poor diagnostic sensitivity and specificity compared with fractionated 24-hour urinary metanephrines.

The high false-positive rate for plasma fractionated catecholamines and fractionated metanephrines triggered the development of a confirmatory test, the clonidine suppression test.[77] This test is intended to distinguish between pheochromocytoma and false-positive increases in plasma fractionated catecholamines and metanephrines. Clonidine is a centrally acting α_2-adrenergic receptor agonist that normally suppresses the release of catecholamines from neurons but does not affect the catecholamine secretion from a pheochromocytoma.[78]

TABLE 16.6 Typical Imaging Phenotypes of Adrenal Masses

Tumor Type	Size (cm)	Shape	Texture	Laterality	Contrast Enhancement	CT[a]	MRI[b]	Necrosis, Hemorrhage, or Calcifications	Growth
Cortical adenoma	≤3	Round to oval with smooth margins	Homogeneous	Usually unilateral	Limited	<10 HU; >50% washout	Isointense	Rare	Slow
Cortical carcinoma	>4	Irregular with unclear margins	Inhomogeneous	Usually unilateral	Marked	>10 HU; <50% washout	Hyperintense	Common	Rapid
Pheochromocytoma	>3	Round to oval with smooth margins	Inhomogeneous with areas of cystic degeneration	Usually solitary and unilateral	Marked	>10 HU; <50% washout	Hyperintense	Common	0.5–1 cm/yr
Metastasis	Variable	Oval to irregular with unclear margins	Inhomogeneous	Often bilateral	Marked	>10 HU; <50% washout	Hyperintense	Common	Variable

[a]Precontrast CT attenuation (measured in HU) and percentage of contrast medium washout at 10 min.

[b]Relative intensity compared with liver on T2-weighted images.

CT, Computed tomography; *HU,* Hounsfield unit; *MRI,* magnetic resonance imaging.

Because of advances in the methodology for measuring catecholamines and metanephrines, phentolamine, glucagon, histamine, metoclopramide, and tyramine tests are rarely needed. From 1975 to 1994 at Mayo Clinic, we performed histamine and glucagon stimulation testing in 542 patients in whom pheochromocytoma was highly suspected despite normal 24-hour urinary excretion of total metanephrines or catecholamines; not one patient had a positive stimulation test in this setting.[79]

Renal Failure

Measurements of urinary catecholamines and metabolites may be invalid if the patient has advanced renal insufficiency.[80] Serum chromogranin A levels have poor diagnostic specificity in these patients.[81] In patients without pheochromocytoma who are receiving hemodialysis, plasma norepinephrine and dopamine concentrations are increased, respectively, threefold and twofold above the upper limit of normal.[82] However, standard normal ranges can be used for interpreting plasma epinephrine concentrations.[83] Therefore when patients with renal failure have plasma norepinephrine concentrations more than three times above the upper normal limit or epinephrine concentrations greater than the upper normal limit, pheochromocytoma should be suspected. The findings of one study suggested that plasma concentrations of fractionated metanephrines are increased approximately twofold in patients with renal failure and may be useful in the biochemical evaluation of patients with marked renal insufficiency or renal failure.[84] However, the results from other studies suggested that concentrations of plasma fractionated metanephrines cannot distinguish between patients with pheochromocytoma and end-stage renal disease.[85,86]

Factitious Pheochromocytoma

As with other similar disorders, factitious pheochromocytoma can be very difficult to confirm.[87] The patient usually has a medical background. The patient may "spike" the 24-hour urine container, or the catecholamines may be administered systemically.[88] Other patients take drugs that mimic the symptoms of pheochromocytoma and can cause false-positive biochemical testing.

Localization

Localization studies should not be initiated until biochemical studies have confirmed the diagnosis of a catecholamine-secreting tumor (see Fig. 16.4). CT or MRI of the abdomen and pelvis should be the first localization test (sensitivity, >95%; specificity, >65%).[22] Approximately 85% of these tumors are found in the adrenal glands, and 95% are found in the abdomen and pelvis. The most common locations of catecholamine-secreting paragangliomas include superior abdominal para-aortic region, 46%; inferior abdominal para-aortic region, 29%; urinary bladder, 10%; mediastinum, 10%; head and neck, 3%; and pelvis, 2%.[33]

Imaging Phenotype

The term *imaging phenotype* refers to the characteristics of the mass on CT or MRI (see Table 16.6).[28] The lipid-rich nature of cortical adenomas is helpful in distinguishing these benign neoplasms from pheochromocytoma. On CT scans, the density of the image (with darker tissues being less dense) is attributed to x-ray attenuation. At the extremes of the CT density spectrum are air (black) and bone (white). The Hounsfield scale is a semiquantitative method of measuring x-ray attenuation. Typical Hounsfield unit (HU) values are -20 to -150 HU for adipose tissue and 20 to 50 HU for kidney. If an adrenal mass is less than 10 HU on unenhanced CT, it cannot be a pheochromocytoma.[89] Adrenal cortical adenomas show a much earlier washout of contrast enhancement than do nonadenomas.[90] For example, Korobkin and colleagues[90] found that the mean percentage washout for adenomas was 51% at 5 minutes and 70% at 15 minutes, compared with 8% and 20%, respectively, for nonadenomas.

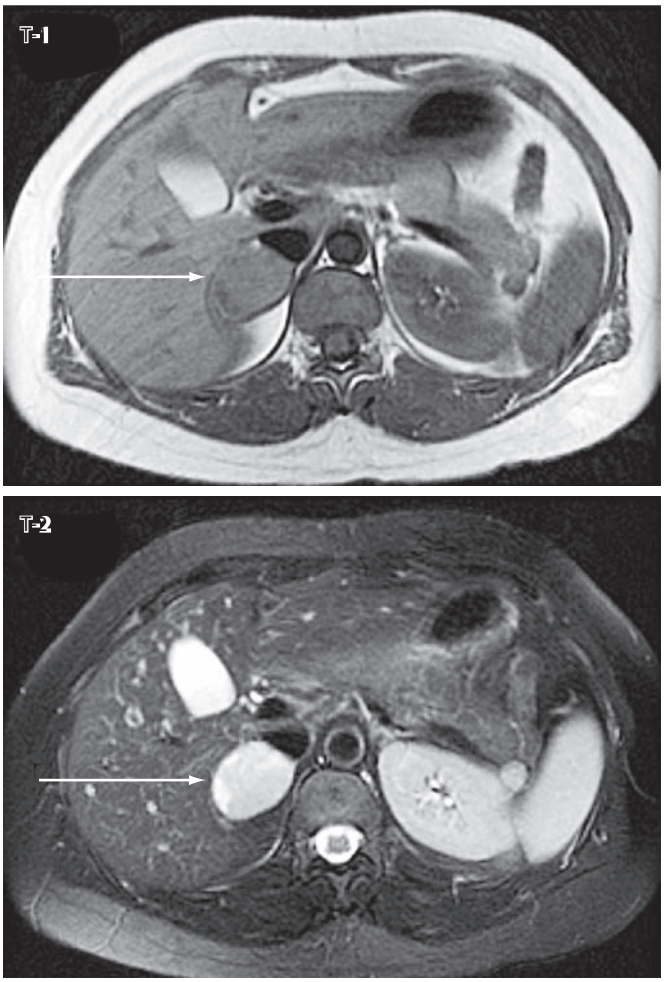
Although CT is still the primary adrenal imaging modality, MRI has advantages in certain clinical situations.[91] Several different MRI techniques have been used to characterize adrenal masses. Conventional spin-echo MRI was the first and is still the most frequently used technique. Early in the history of abdominal MRI, it became clear that with low-strength or midfield-strength magnets, T1-weighted and T2-weighted imaging could be used to differentiate pheochromocytoma and malignancies from benign adenomas. On gadolinium-diethylenetriamine penta-acetic acid (DPTA)–enhanced MRI, pheochromocytomas and malignant lesions show rapid and marked enhancement and a slower washout pattern, whereas adenomas demonstrate mild enhancement and a rapid washout of contrast agent.[90] Similar findings are made with CT.

Chemical shift MRI is a form of lipid-sensitive imaging. Chemical shift MRI is based on the principle that the hydrogen protons in water and lipid molecules resonate at different frequencies. Benign cortical adenomas contain approximately equal amounts of lipid and water, whereas the lipid content of pheochromocytomas is usually low. When the protons of water and lipid are aligned, they are said to be in phase, and when opposite each other, they are out of phase. When fat and water are in phase on MRI, the signal intensity is maximized; when they are out of phase, the signal intensity is reduced. This in-phase and out-of-phase process is the chemical shift technique. Benign adrenal cortical adenomas lose signal on out-of-phase images due to high lipid content but appear relatively bright on in-phase images. A modification of the chemical shift MRI technique uses gradient echo pulse sequences to produce a similar effect.

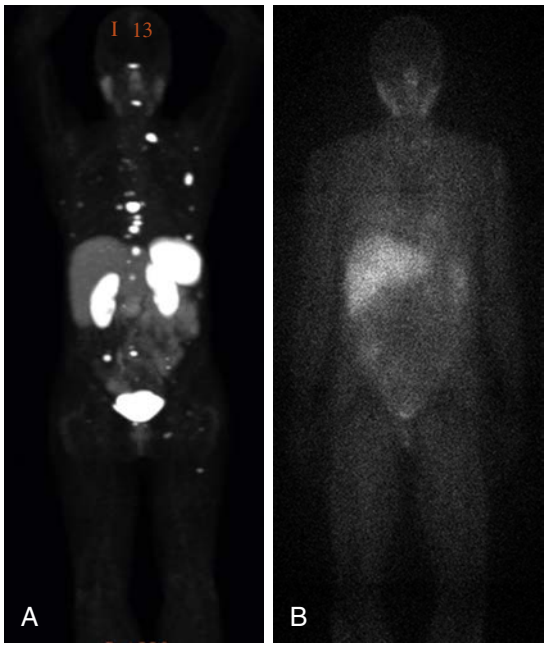
Imaging characteristics consistent with a benign cortical adenoma include round and homogeneous density, smooth contour with sharp margination, diameter usually less than 3 cm, unilateral location, low unenhanced CT attenuation values (e.g., <10 HU) with rapid contrast medium washout at 10 minutes after administration of contrast medium,[90,92] isointensity with liver on both T1-weighted and T2-weighted MRI sequences, and chemical shift evidence of lipid on MRI (see Table 16.6). The imaging phenotype consistent with pheochromocytoma includes noncontrast CT attenuation above 20 HU, marked enhancement with intravenous contrast medium on CT (see Fig. 16.3), high signal intensity on T2-weighted MRI and no signal loss on out-of-phase images (Fig. 16.5), cystic and hemorrhagic changes, and variable size with potential to be bilateral. Although it has been suggested that patients with apparent simple adrenal cysts do not require hormonal evaluation, pheochromocytoma can mimic an adrenal cyst.[93]

^{68}Ga-DOTATATE PET/CT and ^{123}I-MIBG Scintigraphy

In patients with a biochemically confirmed catecholamine-secreting tumor where the results of abdominal and pelvic imaging are negative, additional localization studies are indicated with either ^{68}Ga-DOTATATE PET/CT or scintigraphy with ^{123}I-MIBG (Fig. 16.6). These agents accumulate preferentially in catecholamine-producing tumors. ^{123}I-MIBG scintigraphy is not as sensitive as was initially hoped (sensitivity, 80%; specificity, 99%).[10] In a study of 282 patients with catecholamine-secreting tumors that were surgically confirmed, the overall sensitivity was 89% for CT,



• **Fig. 16.5** Magnetic resonance images of the abdomen of a 34-year-old woman with a recent onset of palpitations and hypertension. She presented with acute left ventricular failure after a single dose of a β-adrenergic blocker. The 24-hour urine test for total metanephrines and catecholamines showed the following: total metanephrines, 3800 μg (normal, <1000 μg); norepinephrine, 37 μg (normal, <170 μg); epinephrine, 7.7 μg (normal, <35 μg); and dopamine, 147 μg (normal, <700 μg). The images show a slightly heterogeneous, right adrenal mass (3.3 × 3.5 × 4.5 cm) consistent with pheochromocytoma *(arrows)* that has increased signal intensity on T2-weighted images *(lower panel)*. After α-adrenergic blockade and restoration of normal left ventricular function, the patient had a laparoscopic adrenalectomy to remove a 5 cm × 4 cm × 3 cm, 33-g pheochromocytoma. Postoperatively, the 24-hour urinary excretion of total metanephrines normalized.



• **Fig. 16.6** Gallium-68 (^{68}Ga) 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-octreotate (DOTATATE) positron emission tomography (PET) and ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) imaging from a 57-year-old woman who had an 8.7-cm right adrenal pheochromocytoma resected 7 years previously. The results from a 24-hour urine for fractionated metanephrines and catecholamines suggested recurrent disease. Contemporaneous ^{123}I-MIBG and ^{68}Ga-DOTATATE PET scans demonstrate the superiority of ^{68}Ga-DOTATATE PET. (A) ^{68}Ga-DOTATATE PET scan shows widespread metastatic lesions that were seen throughout the axial and appendicular skeleton and retroperitoneal lymph node involvement. (B) ^{123}I-MIBG scintigraphy shows very faint increased radiotracer uptake in three vertebral bodies (T3, T8, and T10) and medial right iliac bone.

98% for MRI, and 81% for ^{131}I-MIBG.[10] If a typical (<10 cm) unilateral adrenal pheochromocytoma is found on CT or MRI, ^{123}I-MIBG scintigraphy is superfluous, and the results may even confuse the clinician.[94,95] On the other hand, if the adrenal pheochromocytoma is more than 10 cm in diameter or if a paraganglioma is identified on CT or MRI, then ^{68}Ga-DOTATATE PET/CT or ^{123}I-MIBG scintigraphy is indicated because the patient has increased risk of metastatic disease and additional paragangliomas. It is important for the clinician to recognize the medications that may interfere with ^{123}I-MIBG uptake and have the patient discontinue them before imaging is performed (Table 16.7).[96]

^{68}Ga-DOTATATE PET/CT is proving to be more sensitive in some patients than ^{123}I-MIBG, CT/MRI, or ^{18}F-fluorodeoxyglucose (FDG) PET/CT for detection of metastatic disease (see Fig. 16.6).[97] In addition, ^{68}Ga-DOTATATE PET/CT offers higher spatial resolution than conventional ^{111}In-pentetreotide scintigraphy. ^{68}Ga-DOTATATE injection as a radioactive diagnostic agent for PET imaging was approved by the US Food and Drug Administration (FDA) in June 2016.

Other Localizing Procedures

Other localizing procedures that may be used include computer-assisted cross-sectional imaging of the chest, neck, and skull base. Because of activation of aerobic glycolysis in patients with pheochromocytoma or paraganglioma associated with SDHx mutations, ^{18}FDG-PET/CT is an ideal imaging technique for localization of primary and metastatic tumors.[98] Selective adrenal venous sampling (AVS) for catecholamines is usually misleading and should be avoided.[99]

Treatment

The treatment of choice for pheochromocytoma is complete surgical resection. Surgical survival rates are 98% to 100% and are highly dependent on the skill of the endocrinologist, endocrine surgeon, and anesthesiologist team.[22,32] The most common complications are intraoperative blood pressure lability and postoperative hypotension. Careful preoperative pharmacologic preparation is crucial for successful treatment.[22,100] Most catecholamine-secreting tumors are benign and can be totally excised. Tumor excision usually cures hypertension.

TABLE 16.7 Drugs That May Interfere With Metaiodobenzylguanidine (MIBG) Uptake

Uptake-1 Inhibition[a]

Antiemetics (e.g., prochlorperazine)
Antipsychotics (e.g., chlorpromazine, haloperidol)
Cocaine
Labetalol
Phenylpropanolamine
Tricyclic antidepressants (e.g., amitriptyline, amoxapine, desipramine, doxepin, imipramine, nortriptyline)

Depletion of Storage Vesicle Contents[b]

Amphetamines (e.g., dextroamphetamine, fenfluramine, phentermine)
Dopamine
Labetalol
Reserpine
Sympathomimetics (e.g., ephedrine, phenylephrine, pseudoephedrine, salbutamol, terbutaline)

Inhibition of Vesicular Monoamine Transporters[b]

Reserpine

Unknown Mechanism[a]

Calcium channel blockers (e.g., diltiazem, nicardipine, nifedipine, nimodipine, verapamil)

[a]Should be stopped at least 48 hours before MIBG administration.
[b]Should be stopped at least 72 hours before MIBG administration.

TABLE 16.8 Orally Administered Drugs Used to Treat Pheochromocytoma

Drug	Initial Dosage, mg/day[a] (Maximum)	Side Effects
α-Adrenergic Blocking Agents		
Phenoxybenzamine	10[b] (100)[b]	Postural hypotension, tachycardia, miosis, nasal congestion, diarrhea, retrograde ejaculation, fatigue
Prazosin	1 (20)[c]	First-dose effect, dizziness, drowsiness, headache, fatigue, palpitations, nausea
Terazosin	1 (20)[b]	First-dose effect, asthenia, blurred vision, dizziness, nasal congestion, nausea, peripheral edema, palpitations, somnolence
Doxazosin	1 (20)	First-dose effect, orthostasis, peripheral edema, fatigue, somnolence
Combined α-Adrenergic and β-Adrenergic Blocking Agent		
Labetalol	200[b] (1200)[b]	Dizziness, fatigue, nausea, nasal congestion, impotence
Calcium Channel Blocker		
Nicardipine sustained-release	30[b] (120)[b]	Edema, dizziness, headache, flushing, nausea, dyspepsia
Catecholamine Synthesis Inhibitor		
α-Methyl-p-L-tyrosine (metyrosine)	1000[c] (4000)[c]	Sedation, diarrhea, anxiety, nightmares, crystalluria, galactorrhea, extrapyramidal symptoms

[a]Given once daily unless otherwise indicated.
[b]Given in two doses daily.
[c]Given in three or four doses daily.

Preoperative Management

Some form of preoperative pharmacologic preparation is indicated for all patients with catecholamine-secreting neoplasms, including those who are asymptomatic and normotensive.[22,100] However, no randomized controlled trials have compared the different approaches.[101] Combined α-adrenergic and β-adrenergic blockade is one approach to control blood pressure and prevent intraoperative hypertensive crises.[100] α-Adrenergic blockade should be started 7 to 10 days preoperatively to normalize blood pressure and expand the contracted blood volume. A longer duration of preoperative α-adrenergic blockade is indicated for patients with recent myocardial infarction, catecholamine cardiomyopathy, or catecholamine-induced vasculitis. Blood pressure should be monitored with the patient in the seated and standing positions twice daily. Target blood pressure is low-normal blood pressure for age (e.g., <120/80 mm Hg in the seated position), with systolic blood pressure greater than 90 mm Hg (standing); both targets should be modified on the basis of the patient's age and comorbid disease. Orthostasis is not a goal of treatment but rather a side effect. Therefore, on the second or third day of α-adrenergic blockade, patients are encouraged to start a diet high in sodium content (≥5000 mg/day) because of the catecholamine-induced volume contraction and the orthostasis associated with α-adrenergic blockade. This degree of volume expansion may be contraindicated in patients with congestive heart failure or renal insufficiency. After adequate α-adrenergic blockade has been achieved, β-adrenergic blockade is initiated, typically 2 to 3 days preoperatively.

α-Adrenergic Blockade

Phenoxybenzamine is the preferred drug for preoperative preparation to control blood pressure and arrhythmia. It is an irreversible, long-acting, nonspecific α-adrenergic blocking agent. The initial dosage is 10 mg once or twice daily, and the dose is increased by 10 to 20 mg in divided doses every 2 to 3 days as needed to control blood pressure and spells (Table 16.8). The final dosage of phenoxybenzamine is typically between 20 and 100 mg daily. The patient should be warned about the orthostasis, nasal congestion, retrograde ejaculation in men, and marked fatigue that occur in almost all patients. With their more favorable side effect profiles, selective α_1-adrenergic blocking agents (e.g., prazosin, terazosin, doxazosin) are preferable to phenoxybenzamine when long-term pharmacologic treatment is indicated (e.g., for metastatic pheochromocytoma). However, due to the high cost of phenoxybenzamine, selective α_1-adrenergic blocking agents are being used more commonly for preoperative preparation.

β-Adrenergic Blockade

The β-adrenergic antagonist should be administered only after α-adrenergic blockade is effective because with β-adrenergic blockade alone, severe hypertension or cardiopulmonary decompensation may occur as a result of the unopposed α-adrenergic

stimulation. Preoperative β-adrenergic blockade is indicated to control the tachycardia associated with both the high concentrations of circulating catecholamines and the α-adrenergic blockade. The clinician should exercise caution if the patient is asthmatic or has congestive heart failure. Chronic catecholamine excess can produce a myocardiopathy that may become evident with the initiation of β-adrenergic blockade, resulting in acute pulmonary edema. Therefore, when the β-adrenergic blocker is administered, it should be used cautiously and at a low dose. For example, a patient is usually given 10 mg of propranolol every 6 hours to start. On the second day of treatment, the β-adrenergic blockade (assuming the patient tolerates the drug) is converted to a single long-acting dose. The dose is then increased as necessary to control the tachycardia (goal heart rate is 60–80 beats/minute).

Catecholamine Synthesis Inhibitor

Metyrosine should be used with caution and only after other agents have been ineffective or in patients in whom tumor manipulation or destruction (e.g., radiofrequency ablation of metastatic sites) will be marked.[102] Most centers reserve metyrosine for patients who cannot be treated with the typical combined α-adrenergic and β-adrenergic blockade protocol for cardiopulmonary reasons.[7] Metyrosine inhibits catecholamine synthesis by blocking the enzyme tyrosine hydroxylase.[103] The side effects of metyrosine can be disabling; with long-term therapy, they include sedation, depression, diarrhea, anxiety, nightmares, crystalluria and urolithiasis, galactorrhea, and extrapyramidal signs. Metyrosine may be added to α-adrenergic and β-adrenergic blockade if the resection will be difficult (e.g., malignant paraganglioma) or if destructive therapy is planned (e.g., radiofrequency ablation of hepatic metastases).[7] Our typical protocol with short-term preprocedure preparation is to start with metyrosine 250 mg every 6 hours on day 1, 500 mg every 6 hours on day 2, 750 mg every 6 hours on day 3, and 1000 mg every 6 hours on the day before the procedure, with the last dose (1000 mg) given on the morning of the procedure.[7] With this short-course therapy, the main side effect is hypersomnolence.

Calcium Channel Blockers

Calcium channel blockers, which block norepinephrine-mediated calcium transport into vascular smooth muscle, have been used successfully at several medical centers to preoperatively prepare patients with pheochromocytoma.[104] Nicardipine is the most commonly used calcium channel blocker in this setting; the starting dose is 30 mg twice daily of the sustained-release preparation (see Table 16.8).[105,106] Nicardipine is given orally to control blood pressure preoperatively and if needed is given as an intravenous infusion intraoperatively (Table 16.9). Although there is less collective experience with calcium channel blockers than with α-adrenergic and β-adrenergic blockers, when calcium channel blockers are used as the primary mode of antihypertensive therapy, they may be just as effective.[106,107] Clearly, the exclusive use of calcium channel blockers for the perioperative management of patients with catecholamine-secreting tumors does not prevent all hemodynamic changes; however, its use has been associated with low morbidity and mortality rates.[107] The main role for this class of drugs may be either to supplement the combined α-adrenergic and β-adrenergic blockade protocol when blood pressure control is inadequate or to replace the adrenergic blockade protocol in patients with intolerable side effects.

Acute Hypertensive Crises

Acute hypertensive crises may occur before or during an operation, and they should be treated with intravenously administered sodium nitroprusside, phentolamine, or nicardipine (see Table 16.9). Sodium nitroprusside is an ideal vasodilator for intraoperative management of hypertensive episodes because of its rapid onset of action and short duration of effect. It is administered as an intravenous infusion at 0.5 to 5 μg/kg of body weight per minute and adjusted every few minutes for target blood pressure response; to keep the steady-state thiocyanate concentration below 1 mmol/L, the rate of a prolonged infusion should be no more than 3 μg/kg per minute. Phentolamine is a short-acting, nonselective α-adrenergic blocker that is available in lyophilized form in 5-mg vials. An initial test dose of 1 mg is administered and is followed, if necessary, by repeat 5-mg boluses or continuous infusion. The response to phentolamine is maximal 2 to 3 minutes after a bolus injection and lasts 10 to 15 minutes. Nicardipine can be started at an infusion rate of 5 mg per hour and titrated for blood pressure control (the infusion rate may be increased by 2.5 mg/hour every 15 minutes up to a maximum of 15 mg/hour) (see Table 16.9).

TABLE 16.9 Intravenously Administered Drugs Used to Treat Pheochromocytoma

Agent	Dosage Range
For Hypertension	
Phentolamine	Administer a 1-mg IV test dose, then 2-mg to 5-mg IV boluses as needed or continuous infusion.
Nitroprusside	IV infusion rates of 2 μg/kg of body weight per minute are suggested as safe. Rates >4 μg/kg per minute may lead to cyanide toxicity within 3 hours. Doses >10 μg/kg per minute are rarely required, and the maximal dose should not exceed 800 μg/min.
Nicardipine	Initiate therapy at 5 mg/hr; the IV infusion rate may be increased by 2.5 mg/hr q15 min up to a maximum of 15 mg/hr.
For Cardiac Arrhythmia	
Lidocaine	Initiate therapy with an IV bolus of 1–1.5 mg/kg (75–100 mg); additional boluses of 0.5–0.75 mg/kg (25–50 mg) can be given q5–10 min if needed up to a maximum of 3 mg/kg. Loading is followed by maintenance IV infusion of 2–4 mg/min (30–50 μg/kg per minute) adjusted for effect and settings of altered metabolism (e.g., heart failure, liver congestion) and as guided by blood level monitoring.
Esmolol	An initial IV loading dose of 0.5 mg/kg is infused over 1 minute, followed by a maintenance infusion of 0.05 mg/kg per minute for the next 4 minutes. Depending on the desired ventricular response, the maintenance infusion may then be continued at 0.05 mg/kg per minute or increased stepwise (e.g., by 0.1 mg/kg per minute increments to a maximum of 0.2 mg/kg per minute), with each step being maintained for ≥4 minutes.

IV, Intravenous; *q,* every.

Anesthesia and Surgery

Surgical resection of a catecholamine-secreting tumor is a high-risk surgical procedure, and an experienced surgeon-anesthesiologist team is required. The last oral doses of α-adrenergic and β-adrenergic blockers can be administered early in the morning on the day of the operation. Fentanyl, ketamine, and morphine should be avoided because they potentially can stimulate catecholamine

release from a pheochromocytoma.[108] Also, parasympathetic nervous system blockade with atropine should be avoided because of the associated tachycardia. Anesthesia may be induced with intravenous injection of propofol, etomidate, or barbiturates in combination with synthetic opioids.[108] Most anesthetic gases can be used, but halothane and desflurane should be avoided. Cardiovascular and hemodynamic variables must be monitored closely. Continuous measurement of intra-arterial pressure and heart rhythm is required. If the patient has congestive heart failure or decreased cardiac reserve, monitoring of pulmonary capillary wedge pressure is indicated. The preoperative and perioperative treatment approach outlined here is the same for adults and children.[109,110]

The laparoscopic approach to the adrenal gland is currently the procedure of choice for patients with solitary adrenal pheochromocytomas smaller than 8 cm in diameter.[111–113] If the pheochromocytoma is in the adrenal gland, the entire gland should be removed. Laparoscopic adrenalectomy for pheochromocytoma should be converted to open adrenalectomy in cases of difficult dissection, invasion, adhesions, or surgeon inexperience. Great care should be taken to avoid tumor capsule rupture—this can create incurable diffuse peritoneal disease implants from an apparent benign pheochromocytoma.[114] If the tumor is malignant, as much of the tumor should be removed as possible. If a bilateral adrenalectomy is planned preoperatively, the patient should receive glucocorticoid stress coverage while awaiting transfer to the operating room. In addition, glucocorticoid coverage should be initiated in the operating room if unexpected bilateral adrenalectomy is necessary. Cortical-sparing bilateral adrenalectomies have been used to treat patients with VHL disease.[115,116]

An anterior midline abdominal surgical approach is indicated for abdominal paragangliomas. The midline abdomen should be inspected carefully. Paragangliomas of the neck, chest, and urinary bladder require specialized approaches.[117] Unresectable cardiac pheochromocytomas may require cardiac transplantation.[118]

Hypotension may occur during and after surgical resection of the pheochromocytoma, and it should be treated with fluids and colloids and then intravenous pressor agents if necessary. Postoperative hypotension occurs less frequently in patients who have had adequate preoperative α-adrenergic blockade and volume expansion. If both adrenal glands were manipulated during surgery, adrenocortical insufficiency should be considered as a potential cause of postoperative hypotension. Because hypoglycemia can occur in the immediate postoperative period, blood glucose levels should be monitored, and fluid given intravenously should contain 5% dextrose. Blood pressure is usually normal by the time of hospital discharge. Long-standing, persistent hypertension does occur and may be due to resection-related renal injury, resetting of baroreceptors, hemodynamic changes, structural changes of the blood vessels, altered sensitivity of the vessels to pressor substances, functional or structural renal changes, or coincident primary hypertension.

Long-Term Postoperative Follow-Up

Approximately 1 to 2 weeks after surgery, 24-hour urinary fractionated catecholamines and metanephrines should be measured. If the levels are normal, the resection of the pheochromocytoma should be considered complete. The survival rate after removal of a benign pheochromocytoma is almost equal to that of age-matched and sex-matched normal control subjects. Increased levels of fractionated catecholamines and metanephrines detected postoperatively are consistent with residual tumor (i.e., a second primary lesion or occult metastases). If bilateral adrenalectomy was performed, lifelong glucocorticoid and mineralocorticoid replacement therapy is prescribed.

The risk for recurrent disease (usually metastatic) in patients with apparent benign pheochromocytoma or paraganglioma is approximately 15% on long-term follow-up.[119] The 24-hour urinary excretion of fractionated catecholamines and metanephrines or plasma fractionated metanephrines should be checked annually for life.[22] Annual biochemical testing assesses for metastatic disease (which can occur even 50 years later),[120] tumor recurrence in the adrenal bed, and delayed appearance of multiple primary tumors. Recurrence rates are highest for patients with familial disease, large tumor size (>5 cm), or paraganglioma.[121] Follow-up CT or MRI is not needed unless metanephrine or catecholamine levels become elevated or the original tumor was associated with minimal catecholamine excess.

Genetic testing should be considered for patients younger than 45 years of age or those with one or more of the following: a family history of pheochromocytoma; paraganglioma; and any sign that suggests a genetic cause, such as retinal angiomas, axillary freckling, café au lait spots, cerebellar tumor, MTC, or hyperparathyroidism. In addition, all first-degree relatives of a patient with pheochromocytoma or paraganglioma should have biochemical testing (e.g., 24-hour urine collection for fractionated metanephrines and catecholamines). If mutation testing in a patient is positive, the patient's first-degree relatives should be offered genetic testing.

Metastatic Pheochromocytoma and Paraganglioma

Distinguishing between benign and malignant catecholamine-secreting tumors is difficult on the basis of clinical, biochemical, or histopathologic characteristics.[12] Malignancy is rare in patients with MEN2 or VHL syndrome, but it is common in those with familial paraganglioma caused by mutations in *SDHB*.[122,123] In a series of 272 patients with metastatic pheochromocytoma or paraganglioma, the median age at initial tumor diagnosis was 39 years (range, 7–83 years), and in 65% of patients the metastases developed at a median of 5.5 years (range, 0.3–53.4 years) from the initial diagnosis.[120] The median overall and disease-specific survivals were 24.6 and 33.7 years, respectively.[120] The clinician should first assess the pace of the malignant disease and then target the level of therapy to the aggressiveness of tumor behavior. A multimodality, multidisciplinary, individualized approach is indicated to control catecholamine-dependent symptoms, local mass effect symptoms from the tumor, and overall tumor burden. Long-term pharmacologic therapy for the patient with metastatic pheochromocytoma is similar to that outlined for preoperative preparation in a patient with a catecholamine-secreting tumor.

Metastatic sites include local tissue invasion, bone, liver, lung, omentum, and lymph nodes. Metastatic lesions should be resected, if possible, to decrease tumor burden.[124] Skeletal metastatic lesions that are painful or threaten structural function can be treated with external radiotherapy or thermalablation or approached surgically.[102,125] Thrombotic therapy for large, unresectable liver metastases and radiofrequency ablation for small (<3 cm) liver metastases are options to be considered.[102] In selected cases, long-acting octreotide has been beneficial.[126] Because of the risk of massive catecholamine release, ablative therapy should be performed with great caution and only at centers with experience with these techniques; in addition to α-adrenergic and β-adrenergic blockade, these patients are usually treated with metyrosine before the procedure.[102] External radiotherapy can also be used to treat unresectable soft tissue lesions.[125]

Local tumor irradiation with therapeutic doses of ^{131}I-MIBG has produced partial and temporary responses in approximately one-third of patients.[9,11,127] If the tumor is considered aggressive and the patient's quality of life is affected, combination chemotherapy can provide disease stabilization.[128,129] In a nonrandomized, single-arm trial, the efficacy of chemotherapy with a combination CVD protocol (cyclophosphamide 750 mg/m^2 body surface area on day 1, vincristine 1.4 mg/m^2 on day 1, and dacarbazine 600 mg/m^2 on days 1 and 2; repeated every 21 days) was studied in 14 patients with malignant pheochromocytoma.[130,131] This protocol produced a complete and partial response rate of 57% (median duration, 21 months; range, 7 to >34 months). Complete and partial biochemical responses were seen in 79% of patients (median duration, >22 months; range, 6 to >35 months). All responding patients had objective improvement in performance status and blood pressure.[130,131] CVD chemotherapy can be continued until the patient develops new lesions or there is a significant (e.g., >25%) increase in the size of known tumor sites. Because CVD chemotherapy may induce massive catecholamine release, it is important that the patient be optimally α and β blocked, just as for surgery. In addition, the first cycle of CVD should be completed in the hospital and with close medical observation. Management of metastatic pheochromocytoma can be frustrating because there are no curative options. Tyrosine kinase inhibitors (e.g., sunitinib) may have a role in the treatment of metastatic pheochromocytoma; however, they are not curative.[132]

Pheochromocytoma in Pregnancy

Pheochromocytoma in pregnancy can cause the death of both the fetus and the mother.[133,134] The approach to the biochemical diagnosis is the same as that for the nonpregnant patient. MRI (without gadolinium enhancement) is the preferred imaging modality, and ^{123}I-MIBG scintigraphy and ^{68}Ga-DOTATATE PET/CT are contraindicated. The treatment of hypertensive crises is the same as for nonpregnant patients except that use of nitroprusside should be avoided. Although the most appropriate management is debated, adrenal pheochromocytomas should be removed in the second trimester if diagnosed during the first or second trimester of pregnancy. The preoperative preparation is the same as for a nonpregnant patient. If the pregnancy is already in the third trimester, medical management is indicated and delivery completed by cesarean section. Spontaneous labor and delivery should be avoided. The pheochromocytoma can be resected postpartum. The management of catecholamine-secreting paragangliomas in pregnancy may require modification of these guidelines depending on tumor location.

Renin-Angiotensin-Aldosterone System

The components of the renin-angiotensin-aldosterone (RAA) system are shown in Fig. 16.7.[135] Aldosterone is secreted from the zona glomerulosa under the control of three primary factors: angiotensin II, potassium, and ACTH. The secretion of aldosterone is restricted to the zona glomerulosa because of zone-specific expression of aldosterone synthase (P450 11B2) (see Chapter 15). Dopamine, atrial natriuretic peptide, and heparin inhibit aldosterone secretion.

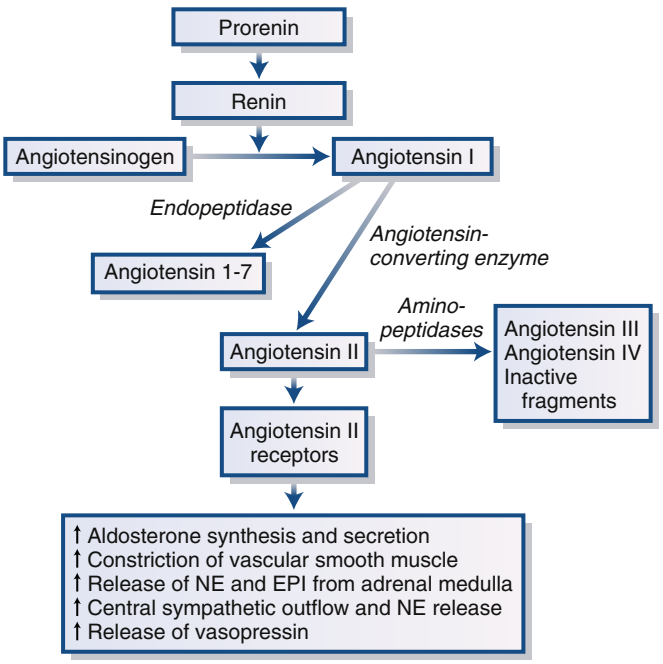
Renin and Angiotensin

Renin is an enzyme that is produced primarily in the juxtaglomerular apparatus of the kidney; it is stored in granules and released in response to specific secretagogues. The protein consists of 340 amino acids, of which the first 43 are a prosegment that is cleaved to produce the active enzyme. The release of renin into the circulation is the rate-limiting step in the RAA system. Renal renin release is controlled by four factors:

1. The macula densa, a specialized group of convoluted distal tubular cells that function as chemoreceptors for monitoring the sodium and chloride loads present in the distal tubule
2. Juxtaglomerular cells acting as pressure transducers that sense stretch of the afferent arteriolar wall and thus renal perfusion pressure
3. The sympathetic nervous system, which modifies the release of renin, particularly in response to upright posture
4. Humoral factors, including potassium, angiotensin II, and atrial natriuretic peptides

Renin release is maximized in conditions of low renal perfusion pressure or low tubular sodium content (e.g., renal artery stenosis, hemorrhage, volume depletion). Renin release is suppressed by elevated perfusion pressure at the kidney (e.g., hypertension) and a high-sodium diet. Renin release is increased directly by hypokalemia and decreased by hyperkalemia.

Angiotensinogen, an α_2-globulin synthesized in the liver, is the only known substrate for renin and is broken down into the angiotensin peptides. The protein consists of 485 amino acids, 33 of which constitute a presegment that is cleaved after secretion. The action of renin on angiotensinogen produces angiotensin I. Angiotensin I comprises the first 10–amino acid sequence after the presegment and does not appear to have biologic activity. Angiotensin II, the main biologically active angiotensin, is created by cleavage of the two carboxy-terminal peptides of angiotensin I by angiotensin-converting enzyme (ACE) (see Fig. 16.7). ACE is localized to cell membranes in the lung and intracellular granules in certain tissues that produce angiotensin II. Amino peptidase A can remove the amino-terminal aspartic acid to produce the



• **Fig. 16.7** Components of the renin-angiotensin system. *EPI,* epinephrine; *NE,* norepinephrine. (Adapted and redrawn from Williams GH, Chao J, Chao L. Kidney hormones. In: Conn PM, Melmed S, eds. *Endocrinology: Basic and Clinical Principles.* Totowa, NJ: Humana Press; 1997:393–404.)

heptapeptide, angiotensin III. Angiotensin II and angiotensin III have equivalent efficacy in promoting aldosterone secretion and modifying renal blood flow. The half-life in the circulation of angiotensin II is short (<60 seconds). Elements of the RAA system are present in the adrenals, kidneys, heart, and brain. For example, the adrenal glomerulosa cells contain the proteins needed to produce and secrete angiotensin II. Other tissues contain one or more components of the system but require other cells or circulating components, or both, to generate angiotensin II.

Angiotensin II functions through the angiotensin receptor to maintain normal extracellular volume and blood pressure by (1) increasing aldosterone secretion from the zona glomerulosa via increased transcription of *CYP11B2*; (2) constricting vascular smooth muscle, thereby increasing blood pressure and reducing renal blood flow; (3) releasing norepinephrine and epinephrine from the adrenal medulla; (4) enhancing the activity of the sympathetic nervous system by increasing central sympathetic outflow, thereby increasing norepinephrine discharge from sympathetic nerve terminals; and (5) promoting the release of vasopressin.

Aldosterone

Approximately 50% to 70% of aldosterone circulates bound to albumin or weakly bound to corticosteroid-binding globulin; 30% to 50% of total plasma aldosterone is free. The half-life is relatively short at 15 to 20 minutes. In the liver, aldosterone is rapidly inactivated to tetrahydroaldosterone. The classic functions of aldosterone are regulation of extracellular volume and control of potassium homeostasis. These effects are mediated by the binding of free aldosterone to the mineralocorticoid receptor in the cytosol of epithelial cells, principally in the kidney.

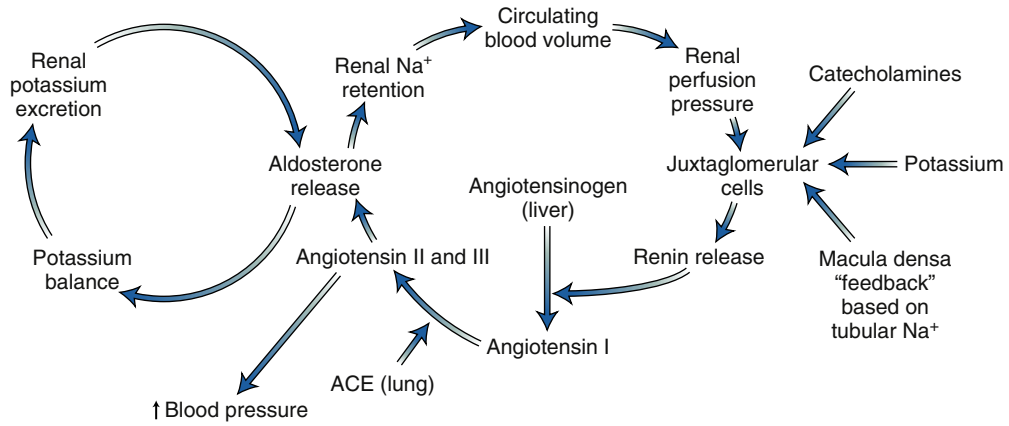
Mineralocorticoid receptors have tissue-specific expression. For example, the tissues with the highest concentrations of these receptors are the distal nephron, colon, and hippocampus. Lower levels of mineralocorticoid receptors are found in the rest of the gastrointestinal tract and heart. Transport to the nucleus and binding to specific binding domains on targeted genes leads to their increased expression. The aldosterone-regulated kinases, primarily serum-inducible and glucocorticoid-inducible kinase-1 (SGK1) appear to be key intermediaries, and increased SGK1 expression leads to modification of the apical sodium channel, resulting in increased sodium ion transport across the cell membrane (see Chapter 15). The increased luminal negativity augments tubular secretion of potassium by the tubular cells and of hydrogen ion by the interstitial cells.

Endogenous glucocorticoids and mineralocorticoids bind equally to the mineralocorticoid receptor. Specificity of action is provided in many tissues by the presence of a glucocorticoid-inactivating enzyme, 11β-hydroxysteroid dehydrogenase 2, which prevents cortisol and corticosterone from interacting with the receptor (see Chapter 15). Mineralocorticoid escape refers to the counterregulatory mechanisms that are manifested after 3 to 5 days of excessive mineralocorticoid administration. Several mechanisms contribute to this escape, including renal hemodynamic factors and increased levels of atrial natriuretic peptide.

In addition to the classic genomic actions mediated by aldosterone binding to cytosolic receptors, mineralocorticoids have acute, nongenomic actions resulting from activation of an unidentified cell surface receptor. This action involves a G protein–signaling pathway and probably a modification of the sodium-hydrogen exchange activity. This effect has been demonstrated in both epithelial and nonepithelial cells.[136]

Aldosterone has additional, nonclassic effects primarily on nonepithelial cells.[137] These actions, although probably genomic and therefore mediated by activation of the cytosolic mineralocorticoid receptor, do not include modification of sodium-potassium balance. Aldosterone-mediated actions include the expression of several collagen genes, genes controlling tissue growth factors (e.g., transforming growth factor-β, plasminogen activator inhibitor type 1) and genes mediating inflammation.[138] The resultant actions lead to microangiopathy, necrosis (acutely), and fibrosis in various tissues, such as the heart, the vasculature, and the kidney.[137] Increased levels of aldosterone are not necessary to cause this damage; an imbalance between the volume or sodium balance state and the level of aldosterone appears to be the critical factors.[137]

The action of angiotensin II on aldosterone involves a negative feedback loop that also includes extracellular fluid volume (Fig. 16.8).[139] The major function of this feedback loop is to modify sodium homeostasis and, secondarily, to regulate blood pressure. Sodium restriction activates the RAA axis. The effects of angiotensin II on both the adrenal cortex and the renal



• **Fig. 16.8** Renin-angiotensin-aldosterone and potassium-aldosterone negative feedback loops. Aldosterone production is determined by input from each loop. *ACE,* angiotensin-converting enzyme; *Na+,* sodium. (Adapted and redrawn from Williams GH, Dluhy RG. Diseases of the adrenal cortex. In: Braunwald E, Fauci AD, Kasper D, et al, eds. *Harrison's Principles of Internal Medicine.* 15th ed. New York, NY: McGraw-Hill; 2001:2087.)

vasculature promote renal sodium conservation. On the other hand, with suppression of renin release and suppression of the level of circulating angiotensin, aldosterone secretion is reduced and renal blood flow is increased, promoting sodium loss. The RAA loop is very sensitive to dietary sodium intake. Sodium excess enhances the responsiveness of the renal and peripheral vasculature and reduces the adrenal responsiveness to angiotensin II. Sodium restriction has the opposite effect. Therefore, sodium intake modifies target tissue responsiveness to angiotensin II, a fine-tuning mechanism that appears to be critical to maintaining normal sodium homeostasis without a chronic effect on blood pressure. Excess aldosterone secretion causes hypertension through two main mechanisms: mineralocorticoid-induced expansion of plasma and extracellular fluid volume and increased total peripheral vascular resistance.

Primary Aldosteronism

Hypertension, suppressed plasma renin activity (PRA), and increased aldosterone excretion characterize the syndrome of primary aldosteronism, which was first fully described in 1955.[140] Aldosterone-producing adenoma (APA) and bilateral idiopathic hyperaldosteronism (IHA) are the most common subtypes of primary aldosteronism (Table 16.10). Somatic mutations account for nearly all of APAs and include mutations in genes encoding components of the Kir 3.4 (*GIRK4*) potassium channel (*KCNJ5*), the sodium/potassium and calcium ATPases (*ATP1A1* and *ATP2B3*), a voltage-dependent C-type calcium channel (*CACNA1D*), and β-catenin–activating mutations (*CTNNB1*).[141,142] A much less common form, unilateral hyperplasia or primary adrenal hyperplasia (PAH), is caused by micronodular or macronodular hyperplasia of the zona glomerulosa of predominantly one adrenal gland. Familial hyperaldosteronism (FH) is also rare, and four types have been described (see later discussion).[141]

History

In his presidential address at the annual meeting of the Central Society for Clinical Research, Chicago, Illinois, October 29, 1954, Dr. Jerome W. Conn stated[140]: "I have prepared no comprehensive review of my personal philosophy of clinical investigation. Instead, I plan to make a scientific report to you about a clinical syndrome, the investigation of which has been most exciting to me since I initiated it in April of this year." Conn, a professor of medicine at the University of Michigan, had been active in government-funded research on the mechanisms of human acclimatization to humid heat. He established that the body's acclimatization response was to rapidly diminish renal salt and water loss and to abruptly curtail the salt content of body sweat and saliva. He suggested that these responses were the result of increased adrenocortical function with elaboration of salt-retaining steroids. He also showed that intramuscular administration of deoxycorticosterone acetate (DOCA) produced similar changes in the electrolyte composition of urine, sweat, and saliva.

In April 1954, Professor Conn was asked to see M.W., a 34-year-old woman with a 7-year history of muscle spasms, temporary paralysis, tetany, and weakness and a 4-year history of hypertension. She was found to have a blood pressure of 176/104 mm Hg, severe hypokalemia (1.6–2.5 mEq/L), mild hypernatremia (146–151 mEq/L), and alkalosis (serum pH 7.62). Because there were no signs or symptoms of glucocorticoid or androgen excess, Conn suspected, based on his past research, that M.W.'s clinical presentation could result from excess secretion of the adrenal salt-retaining corticoid. Conn studied M.W. in the Metabolism Research Unit for 227 days. The Streeten bioassay technique developed to measure sodium retention in adrenalectomized rats after intraperitoneal injection of human urine was used, and M.W. averaged 1333 μg DOCA equivalent per day, compared with normotensive control subjects at 61.4 μg/day. In his presidential address, Conn stated: "It is believed that these studies delineate a new clinical syndrome, which is designated temporarily as primary aldosteronism."[140] (Note: The word *temporarily* was used because aldosterone was yet to be measured in any human bodily fluid.)

Conn planned for a bilateral adrenalectomy for his patient on December 10, 1954. In 1995, Gittler and Fajans described the surgical scene: "To the immense delight of Conn and those in the operating room, the surgeon, Dr. William Baum, encountered a right 13-g adrenal tumor, which was removed while leaving the contralateral gland intact. The patient's postoperative studies showed an almost total reversal of the preoperative metabolic and

TABLE 16.10 Adrenocortical Causes of Hypertension

Low Renin and High Aldosterone

Primary Aldosteronism

- Aldosterone-producing adenoma (APA)—30% of cases
- Bilateral idiopathic hyperplasia (IHA)—60% of cases
- Primary (unilateral) adrenal hyperplasia—2% of cases
- Aldosterone-producing adrenocortical carcinoma—<1% of cases
- Familial hyperaldosteronism (FH)
  - FH type I (*CYP11B1/CYP11B2* germline chimeric gene)—<1% of cases
  - FH type II (APA or IHA; germline *CLCN2* mutations—<6% of cases
  - FH type III (germline *KCNJ5* mutations)—<1% of cases
  - FH type IV (germline *CACNA1H* mutations)—<0.1% of cases
- Ectopic aldosterone-producing adenoma or carcinoma—<0.1% of cases

Low Renin and Low Aldosterone

- Congenital adrenal hyperplasia
  - 11β-Hydroxylase deficiency
  - 17α-Hydroxylase deficiency
- Deoxycorticosterone-producing tumor
- Primary cortisol resistance
- Apparent mineralocorticoid excess (AME)/11β-HSD 2 deficiency
  - Genetic
    - Type 1 AME
    - Type 2 AME
  - Acquired
    - Licorice or carbenoxolone ingestion (type 1 AME)
    - Cushing syndrome (type 2 AME)

Cushing Syndrome

- Exogenous glucocorticoid administration—most common cause
- Endogenous
  - ACTH-dependent—85% of cases
    - Pituitary
    - Ectopic
  - ACTH-independent—15% of cases
    - Unilateral adrenal disease (adenoma or carcinoma)
    - Bilateral adrenal disease
      - Bilateral adenoma
      - Macronodular hyperplasia
      - Primary pigmented nodular adrenal disease (rare)

ACTH, Corticotropin; *HSD,* hydroxysteroid dehydrogenase.

clinical abnormalities. Conn had achieved irrefutable proof of the validity of his investigative conclusions and established for the first time the relationship among an adrenal aldosterone-producing tumor, hypertension, and hypokalemia. A new era had arrived in the study of hypertension and adrenal mineralocorticoids."[143]

By 1964, Conn had collected 145 cases,[144] and he suggested that up to 20% of patients with essential hypertension might have primary aldosteronism.[145] This suggestion was downplayed by most as a gross overestimate.[146,147] Later, Conn decreased his predicted prevalence of primary aldosteronism to 10% of hypertensives,[148] a prediction that was substantiated nearly 40 years later.

Prevalence

In the past, clinicians would not consider the diagnosis of primary aldosteronism unless the patient presented with spontaneous hypokalemia, and then the diagnostic evaluation would require discontinuation of antihypertensive medications for at least 2 weeks. This diagnostic approach resulted in predicted prevalence rates of less than 0.5% of hypertensive patients.[14,146,147,149–152] However, it is now recognized that most patients with primary aldosteronism are not hypokalemic[153,154] and that screening can be completed while the patient is taking antihypertensive drugs.[153] Measurement of plasma aldosterone concentration (PAC) and renin activity or concentration (PRA or PRC, respectively) as a case-detection test, followed by aldosterone suppression for confirmatory testing, has resulted in much higher prevalence estimates for primary aldosteronism—5% to 10% of all patients with hypertension.[153–158]

Clinical Presentation

The diagnosis of primary aldosteronism is usually made in patients who are in the third to sixth decades of life. Few symptoms are specific to the syndrome. Patients with marked hypokalemia may have muscle weakness and cramping, headaches, palpitations, polydipsia, polyuria, nocturia, or a combination of these.[159] Periodic paralysis is a very rare presentation in Caucasians, but it is not an infrequent presentation in patients of Asian descent.[160] The polyuria and nocturia are a result of hypokalemia-induced renal concentrating defect, and the presentation is frequently mistaken for prostatism in men. There are no specific physical findings. Edema is not a common finding because of the phenomenon of mineralocorticoid escape, described earlier. The degree of hypertension is typically moderate to severe and may be resistant to the usual pharmacologic treatments.[159,161] In the first 262 cases of primary aldosteronism diagnosed at Mayo Clinic (1957–1986), the highest blood pressure was 260/155 mm Hg; the mean (± SD [standard deviation]) was 184/112 ± 28/16 mm Hg.[161] Patients with APA tend to have higher blood pressures than those with IHA.

Hypokalemia is frequently absent, so all patients with hypertension are candidates for this disorder. In other patients, the hypokalemia becomes evident only with the addition of a potassium-wasting diuretic (e.g., hydrochlorothiazide, furosemide). Deep-seated renal cysts are found in up to 60% of patients with chronic hypokalemia.[162] Because of a reset osmostat, the serum sodium concentration tends to be high-normal or slightly above the upper limit of normal. This clinical clue is very useful in the initial assessment for potential primary aldosteronism.

Several studies have shown that patients with primary aldosteronism are at higher risk than other patients with hypertension for target-organ damage of the heart and kidney.[163,164] Chronic kidney disease is common in patients with long-standing primary aldosteronism.[165] When matched for age, blood pressure, and duration of hypertension, patients with primary aldosteronism have greater left ventricular mass measurements than patients with other types of hypertension (e.g., pheochromocytoma, Cushing syndrome, essential hypertension).[166] In patients with APA, the left ventricular wall thickness and mass were markedly decreased 1 year after adrenalectomy.[167] A case control study of 124 patients with primary aldosteronism and 465 patients with essential hypertension (matched for age, sex, and systolic and diastolic blood pressure) found that patients presenting with either APA or IHA had a significantly higher rate of cardiovascular events (e.g., stroke, atrial fibrillation, myocardial infarction) than the matched patients with essential hypertension.[163] A negative effect of circulating aldosterone on cardiac function was found in young nonhypertensive subjects with glucocorticoid-remediable aldosteronism (GRA) who had increased left ventricular wall thickness and reduced diastolic function compared with age-matched and sex-matched control subjects.[168]

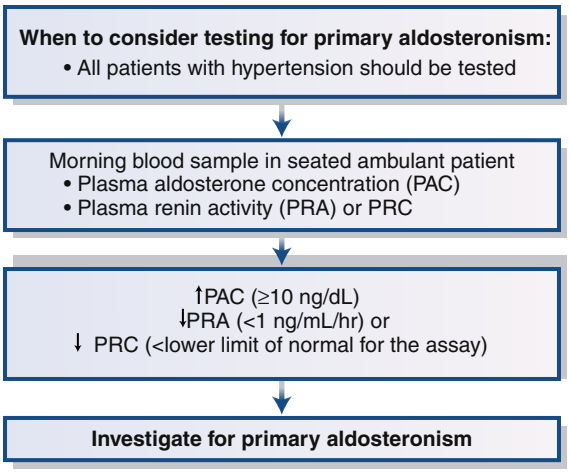
The risk to develop new onset diabetes mellitus was documented in a study of 2367 patients with primary aldosteronism who had no prior diagnosis of diabetes mellitus, where 754 surgically treated patients with APA were matched with 3016 essential hypertension controls.[169] The patients with primary aldosteronism who underwent adrenalectomy had statistically significant reduced risk for incident diabetes and all-cause of mortality, compared with matched hypertensive controls.[169]

Several studies have documented the negative impact of primary aldosteronism on quality of life.[170] Untreated patients with primary aldosteronism have an impaired physical and mental quality of life compared to the general population. Symptoms of anxiety, demoralization, stress, depression, and nervousness were more frequently reported in untreated patients with primary aldosteronism than in the general population and in patients with hypertension.[170] A prospective quality-of-life study compared patients with primary aldosteronism who were managed surgically to those managed medically.[171] After 1 year, almost all quality-of-life measures had normalized for surgically managed patients; whereas for patients on medical treatment, most of the measures had improved but not to the level of the general population.[171]

Diagnosis

There is no typical clinical phenotype to guide the clinician for when to suspect primary aldosteronism—72% of patients with primary aldosteronism are normokalemic.[155,157] Most clinical practice guidelines recommend case-detection testing in high-risk groups for primary aldosteronism (e.g., sustained blood pressure >150/100 mm Hg, hypertension resistant to three conventional antihypertensive drugs, controlled blood pressure on four or more antihypertensive drugs, hypertension and spontaneous or diuretic-induced hypokalemia, hypertension and adrenal incidentaloma, hypertension and sleep apnea, hypertension and a family history of early onset hypertension or cerebrovascular accident at a young age).[153] However, most primary care physicians do not test patients for primary aldosteronism—most patients go undiagnosed and untreated.[172–174] Due to the excess cardiovascular morbidity and nephrotoxicity associated with primary aldosteronism, a case can be made that all patients with hypertension should be tested for this disorder at least once (Fig. 16.9).

The diagnostic approach to primary aldosteronism can be considered in three phases: case-detection tests, confirmatory tests, and subtype evaluation tests.



• **Fig. 16.9** This algorithm provides guidance on when to consider testing for primary aldosteronism and use of plasma aldosterone concentration (PAC) and plasma renin activity (PRA) as a case-detection tool. *PRC,* plasma renin concentration.

Case-Detection Tests

Case-detection testing can be accomplished by paired measurements of PAC and PRA in a random morning ambulatory blood sample (preferably obtained between 8 and 10 AM) (see Fig. 16.9). This test may be performed while the patient is taking antihypertensive medications (with some exceptions, discussed later) and without posture stimulation.[159] Hypokalemia reduces the secretion of aldosterone, and it is optimal to restore the serum level of potassium to normal before performing diagnostic studies.

It may be difficult to interpret data obtained from patients treated with a mineralocorticoid receptor antagonist (MRA) (spironolactone and eplerenone). These drugs prevent aldosterone from activating the receptor, resulting sequentially in sodium loss, a decrease in plasma volume, and an elevation in renin, which can lead to false-negative case-detection testing. For this reason, spironolactone and eplerenone should not be initiated until the evaluation is completed and the final decisions about treatment are made. However, there are exceptions to this rule. For example, if the patient is hypokalemic despite treatment with spironolactone or eplerenone, then the mineralocorticoid receptors are not fully blocked, and PRA or PRC should be suppressed in such a patient with primary aldosteronism. Thus if renin is suppressed, the evaluation for primary aldosteronism (case-detection testing, confirmatory testing, and AVS) can proceed despite treatment with MRAs. Whereas if renin is not suppressed in a patient treated with a mineralocorticoid antagonist, therapy should be discontinued for at least 6 weeks before retesting. Other potassium-sparing diuretics, such as amiloride and triamterene, usually do not interfere with testing unless the patient is on high doses.

ACE inhibitors and angiotensin receptor blockers (ARBs) have the potential to elevate renin in patients with primary aldosteronism. Therefore the finding of detectable renin in a patient taking one of these drugs does not exclude the diagnosis of primary aldosteronism. However, a PRA level less than 1 ng/mL per hour or PRC below the reference range in a patient taking an ACE inhibitor or ARB makes primary aldosteronism likely.

The PAC:PRA ratio, first proposed as a case-detection test for primary aldosteronism in 1981,[175] is based on the concept of paired hormone measurements. The PAC is measured in nanograms per deciliter; the PRA is measured in nanograms per milliliter per hour. In a hypertensive hypokalemic patient, secondary hyperaldosteronism should be considered if both PRA and PAC are increased and the PAC:PRA ratio is less than 10 (e.g., renovascular disease). An alternative source of mineralocorticoid receptor agonism should be considered if both PRA and PAC are suppressed (e.g., hypercortisolism). Primary aldosteronism should be suspected if the PRA is suppressed (e.g., <1.0 ng/mL per hour) and the PAC is increased (e.g., >10 ng/dL) (see Fig. 16.9). It is critical for the clinician to recognize that the measurement of PAC and renin is only a case-detection tool, and most positive results should be followed by a confirmatory aldosterone suppression test to verify autonomous aldosterone production before treatment is initiated.[153] The exception to the requirement for confirmatory testing involves patients with hypertension, spontaneous hypokalemia, and marked primary aldosteronism (e.g., PAC >20 ng/dL and PRA <1.0 ng/mL per hour) (see Fig. 16.9).[153]

Confirmatory Tests

A positive case detection test with PAC above 10 ng/dL and PRA below 1 ng/mL per hour is not diagnostic by itself, and (in the absence of spontaneous hypokalemia—see earlier discussion) primary aldosteronism must be confirmed by demonstration of inappropriate aldosterone secretion.[154] The list of drugs and hormones capable of affecting the RAA axis is extensive, and a medication-contaminated evaluation is frequently unavoidable in patients with poorly controlled hypertension despite a three-drug program. Calcium channel blockers and α_1-adrenergic receptor blockers do not affect the diagnostic accuracy in most cases.[153] However, as long as renin is suppressed and PAC is above 10 ng/dL, the clinician can proceed with confirmatory testing regardless of medications. No medication causes false-positive testing for primary aldosteronism when PAC is above 10 ng/dL.

Aldosterone suppression testing can be performed with orally administered sodium chloride and measurement of urinary aldosterone or with intravenous sodium chloride loading and measurement of PAC.[153,159]

Oral Sodium Loading Test

After hypertension and hypokalemia have been controlled, patients should receive a high-sodium diet (supplemented with sodium chloride tablets if needed) for 3 days, with a goal sodium intake of 5000 mg (equivalent to 218 mEq sodium or 12.8 g sodium chloride).[161] The risk of increasing dietary sodium in patients with severe hypertension must be assessed in each case.[176] Because the high-sodium diet can increase kaliuresis and hypokalemia, vigorous replacement of potassium chloride may be needed, and the serum level of potassium should be monitored daily. On the third day of the high-sodium diet, a 24-hour urine specimen is collected for measurement of aldosterone, sodium, and creatinine. To document adequate sodium repletion, the 24-hour urinary sodium excretion should exceed 200 mEq. Urinary aldosterone excretion of more than 12 µg/24 hours in this setting is consistent with autonomous aldosterone secretion.[161] The sensitivity and specificity of the oral sodium loading test are 96% and 93%, respectively.[177]

Intravenous Saline Infusion Test

The intravenous saline infusion test has also been used widely for the diagnosis of primary aldosteronism.[153,154] Normal subjects show suppression of PAC after volume expansion with isotonic saline; subjects with primary aldosteronism do not show

this suppression. The test is done in the seated position after an overnight fast.[178] Two liters of 0.9% sodium chloride solution is infused intravenously with an infusion pump over 4 hours. Blood pressure and heart rate are monitored during the infusion. At the completion of the infusion, blood is drawn for measurement of PAC. PAC levels in normal subjects decrease to less than 5 ng/dL, whereas most patients with primary aldosteronism do not suppress to less than 10 ng/dL. Postinfusion PAC values between 5 and 10 ng/dL are indeterminate and may be seen in patients with IHA.

Fludrocortisone Suppression Test

In the fludrocortisone suppression test, fludrocortisone acetate is administered for 4 days (0.1 mg every 6 hours) in combination with sodium chloride tablets (2 g three times daily with food). Blood pressure and serum potassium levels must be monitored daily. In the setting of low PRA, failure to suppress the upright 10 AM PAC to less than 6 ng/dL on day 4 is diagnostic of primary aldosteronism.[179] Increased QT interval dispersion and deterioration of left ventricular function have been reported during fludrocortisone suppression tests.[176] Most centers no longer use this test.

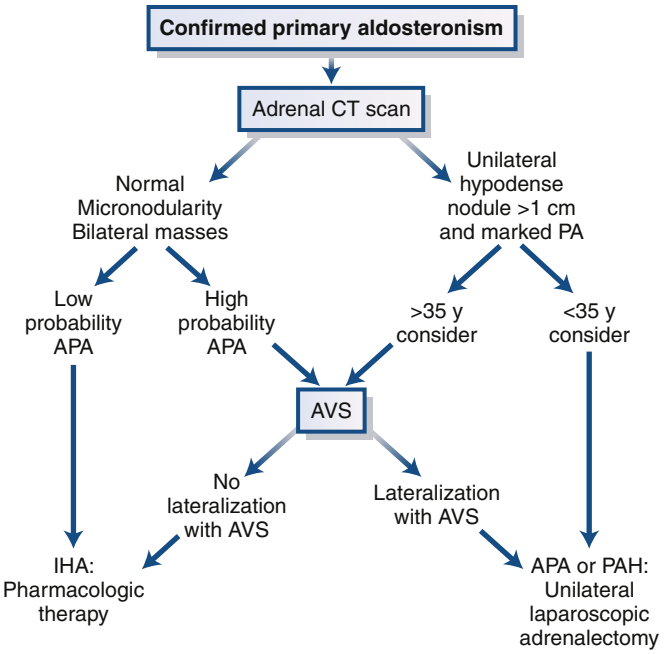
Subtype Studies

After case-detection and confirmatory testing, the third management issue guides the therapeutic approach by distinguishing APA and PAH from IHA and GRA. Unilateral adrenalectomy in patients with APA or PAH results in normalization of hypokalemia in all cases; hypertension is improved in all cases and is cured in 30% to 60%.[88,180–182] In IHA unilateral or bilateral adrenalectomy seldom corrects the hypertension.[161] IHA and GRA should be treated medically. APA is found in approximately 35% of cases and bilateral IHA in approximately 60% (see Table 16.10). APAs are usually small hypodense adrenal nodules (<2 cm in diameter) on CT and are golden yellow when resected. IHA adrenal glands may be normal on CT or may show nodular changes. Aldosterone-producing adrenal carcinomas are almost always larger than 4 cm in diameter and have an inhomogeneous imaging phenotype on CT (see Table 16.6).

Computed Tomography of the Adrenal Glands

Primary aldosteronism subtype evaluation may require one or more tests, the first of which is imaging of the adrenal glands with CT (Fig. 16.10). If a solitary unilateral low noncontrast CT attenuation (<10 HU) macroadenoma (>1 cm) and normal contralateral adrenal morphologic appearance are found on CT in a young patient (<35 years) with severe primary aldosteronism, unilateral adrenalectomy is a reasonable therapeutic option (see Fig. 16.10).[153,183,184] However, most patients with primary aldosteronism are over age 35 years, and in many cases, CT shows normal-appearing adrenals, minimal unilateral adrenal limb thickening, unilateral microadenomas (≤1 cm), or bilateral macroadenomas (Fig. 16.11). In these cases, additional testing is required to determine the source of excess aldosterone secretion.

Small APAs may be labeled incorrectly as IHA on the basis of CT findings of bilateral nodularity or normal-appearing adrenals. Also, apparent adrenal microadenomas may actually represent areas of hyperplasia, and unilateral adrenalectomy would be inappropriate. In addition, nonfunctioning unilateral adrenal macroadenomas are not uncommon, especially in older patients (>40 years).[185] Unilateral PAH may be visible on CT, or the PAH may appear normal on CT. In general, patients with APAs have more severe hypertension, more frequent hypokalemia, higher levels

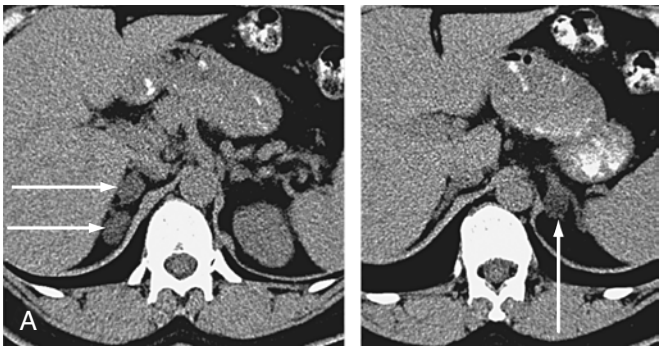


• **Fig. 16.10** Subtype evaluation of primary aldosteronism. For patients who want to pursue a surgical treatment for their hypertension, adrenal venous sampling is frequently a key diagnostic step (see text for details). *APA,* aldosterone-producing adenoma; *AVS,* adrenal venous sampling; *CT,* computed tomography; *IHA,* idiopathic hyperaldosteronism; *PA,* primary aldosteronism; *PAH,* primary adrenal hyperplasia. (Modified from Young Jr WF, Hogan MJ. Renin-independent hypermineralocorticoidism. *Trends Endocrinol Metab.* 1994;5:97–106.)

of plasma aldosterone (>30 ng/dL) and urinary aldosterone (>30 µg/24 hours), and are younger (<50 years), compared with those who have IHA.[161] Patients fitting these descriptors are considered to have a high probability of APA regardless of the CT findings (see Fig. 16.10), and 41% of patients with a high probability of APA and a normal adrenal CT scan prove to have unilateral aldosterone hypersecretion.[186]

Adrenal CT is not accurate in distinguishing between APA and IHA.[183,186–188] In a study of 203 patients with primary aldosteronism who were evaluated with both CT and AVS, CT was accurate in only 53% of patients; based on the CT findings, 42 patients (22%) would have been incorrectly excluded as candidates for adrenalectomy and 48 patients (25%) might have had unnecessary or inappropriate surgery.[186] In another study of 208 patients with primary aldosteronism, based on CT findings, 19 patients (12%) would have been bypassed for curative surgery and 48 patients (30%) would have had noncurative surgery.[188] In a systematic review of 38 studies involving 950 patients with primary aldosteronism, adrenal CT/MRI results did not agree with the findings from AVS in 359 patients (38%); based on CT/MRI, 19% of the 950 patients would have undergone noncurative surgery, and 19% would have been offered medical therapy instead of curative adrenalectomy.[187]

A study published in 2016 caused some confusion regarding the well-documented superiority of AVS compared to CT.[189] The reported success rate of CT and failure rate of AVS were much higher than any other series. In addition, the interpretation of the data was complicated by the use of MRAs postoperatively—leading to blood pressure control in the patients with uncured primary aldosteronism from both groups. The authors used an inappropriately low cutoff for the adrenal-to-IVC cortisol gradient of greater



Results of bilateral adrenal venous sampling

Vein	Aldosterone (A)(ng/dL)	Cortisol (C)(∞g/dL)	A/C Ratio	Aldosterone Ratio*
R adrenal vein	29,338	668	43.9	62.7
L adrenal vein	363	540	0.7	
Inferior vena cava	259	31	8.4	

*R adrenal vein A/C ratio divided by L adrenal vein A/C ratio.

B

• **Fig. 16.11** A 43-year-old woman had a 2-year history of hypertension and hypokalemia. The screening test for primary aldosteronism was positive, with a plasma aldosterone concentration (PAC) of 37 ng/dL and low plasma renin activity (PRA) at less than 0.6 ng/mL per hour (PAC/PRA ratio >61). The confirmatory test for primary aldosteronism was also positive, with the 24-hour urinary excretion of aldosterone measured at 53 μg on a high-sodium diet (urinary sodium, 196 mEq/24 hours). (A) Adrenal computed tomography shows a 12-mm, low-density mass *(arrow, right panel)* in the medial limb of the left adrenal and two low-density, 10-mm nodules *(arrows, left panel)* within the right adrenal gland. (B) Adrenal venous sampling lateralized aldosterone secretion to the right, and two cortical adenomas (1.8 × 1.2 × 0.8 cm and 2.5 × 1.5 × 1.2 cm) were found at laparoscopic right adrenalectomy. The postoperative plasma aldosterone concentration was less than 1 ng/dL. Hypokalemia was cured, and blood pressure was normal without the aid of antihypertensive medications.

than 3:1.[189] When cosyntropin is used during AVS, the minimal cortisol gradient cutoff to determine successful catheterizations is greater than 5:1 (see AVS discussion to come). The persistent postoperative primary aldosteronism in 11% of the AVS-directed treatment group simply reflected the lack of reliable AVS data—likely related to multiple factors.[189] The key message from this study was not that CT and AVS were equivalent in subtype testing; rather, centers that have a suboptimal AVS program will have poor AVS-directed outcomes. Thus centers that have low-volume AVS programs or enlist multiple interventional radiologists (and dilute the expertise) or simply lack the effort to ensure accurate AVS,[190] should refer their patients with primary aldosteronism to centers that champion expertise in AVS.

Therefore AVS is essential to direct appropriate therapy in patients with primary aldosteronism who have a high probability of APA and are seeking a potential surgical cure.

Adrenal Venous Sampling

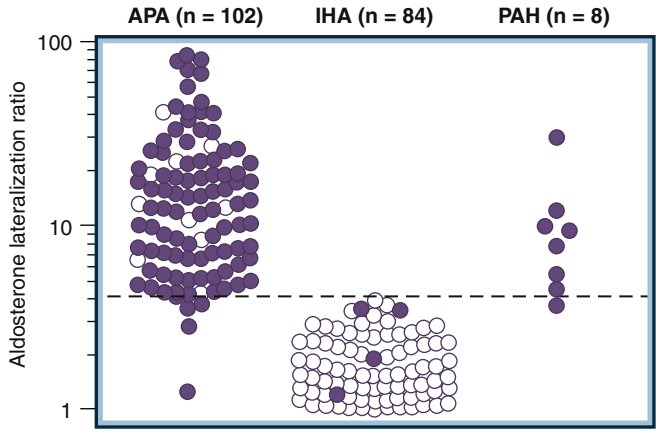
AVS is the criterion standard test to distinguish between unilateral and bilateral disease in patients with primary aldosteronism.[153,183,187,188] AVS is an intricate procedure because the right adrenal vein is small and may be difficult to locate and cannulate; the success rate depends on the proficiency of the angiographer.[191] A review of 47 reports found that the success rate for cannulation of the right adrenal vein in 384 patients was 74%.[161] With experience and focusing the expertise to one or two radiologists at a referral center, the AVS success rate can be as high as 96%.[186,192–194]

The five keys to a successful AVS program are (1) appropriate patient selection, (2) careful patient preparation, (3) focused technical expertise, (4) defined protocol, and (5) accurate data interpretation.[191] A center-specific written protocol is mandatory. The protocol should be developed by an interested group of endocrinologists, hypertension specialists, internists, radiologists, and laboratory personnel. Safeguards should be in place to prevent mislabeling of the blood tubes in the radiology suite and to prevent sample mixup in the laboratory.[191]

At Mayo Clinic, we use continuous cosyntropin infusion during AVS (50 μg/hour starting 30 minutes before sampling and continuing throughout the procedure) for the following reasons: (1) to minimize stress-induced fluctuations in aldosterone secretion during nonsimultaneous AVS, (2) to maximize the gradient in cortisol from adrenal vein to IVC and thus confirm successful sampling of the adrenal veins,[188,195] and (3) to maximize the secretion of aldosterone from an APA.[186,191,195] The adrenal veins are catheterized through the percutaneous femoral vein approach, and the position of the catheter tip is verified by gentle injection of a small amount of nonionic contrast medium and radiographic documentation. Blood is obtained from both adrenal veins and from the IVC below the renal veins and assayed for aldosterone and cortisol concentrations. To be sure that there is no cross-contamination, the IVC sample should be obtained from the external iliac vein. The venous sample from the left side typically is obtained from the common phrenic vein immediately adjacent to the entrance of the adrenal vein. Whereas the blood sample from the right adrenal vein is obtained just at the orifice of the vein—to stabilize the catheter in proper position and avoid the error of advancing the catheter tip too deep into the adrenal vein—a guide wire may be used.[196] The cortisol concentrations from the adrenal veins and IVC are used to confirm successful catheterization—a minimal adrenal vein-to-IVC cortisol gradient is 5:1 or more. At Mayo Clinic, the mean adrenal-to-IVC cortisol gradient was 33.9:1 on the right side and 23.8:1 on the left.[186]

Dividing the right and left adrenal vein PAC values by their respective cortisol concentrations corrects for the dilutional effect of the inferior phrenic vein flow into the left adrenal vein; these are termed *cortisol-corrected ratios* (Fig. 16.12). In patients with APA, the mean cortisol-corrected aldosterone ratio (i.e., the ratio of PAC/cortisol from the APA side to that from the normal side) is 18:1.[186] A cutoff point of more than 4:1 for this ratio is used to indicate unilateral aldosterone excess (see Fig. 16.12). In patients with IHA, the mean cortisol-corrected aldosterone ratio is 1.8:1 (high side to low side), and a ratio of less than 3:1 suggests bilateral aldosterone hypersecretion (see Fig. 16.12).[186] Therefore most patients with a unilateral source of aldosterone have cortisol-corrected aldosterone lateralization ratios greater than 4, and ratios greater than 3 but less than 4 represent a zone of overlap. Ratios no higher than 3 are consistent with bilateral aldosterone secretion.

In addition to the AVS aldosterone lateralization ratio, a contralateral suppression index can be calculated: The contralateral (nondominant adrenal) aldosterone-cortisol (A/C) ratio is divided by the IVC A/C ratio. In the early Mayo Clinic series, the contralateral suppression index was less than 1 in 93.4% of patients with surgically confirmed APA.[186] Although a supportive finding, if the aldosterone lateralization ratio is 4 or above, a contralateral



• **Fig. 16.12** Adrenal vein aldosterone lateralization ratios for patients with unilateral aldosterone-producing adenomas (APA), bilateral idiopathic hyperplasia (IHA), and unilateral primary adrenal hyperplasia (PAH). Shaded symbols indicate that the diagnosis was confirmed surgically. The sensitivity and specificity of a cortisol-corrected plasma aldosterone concentration lateralization ratio greater than 4 for unilateral disease are 95.2% and 100%, respectively. (From Young Jr WF, Stanson AW, Thompson GB, et al. Role for adrenal venous sampling in primary aldosteronism. *Surgery.* 2004;136:1227–1235. Used with permission.)

suppression index less than 1 is not associated with better postoperative blood pressure outcomes and should not be a requirement to send patients to surgery.[197,198] However, there are several situations where the contralateral suppression index can be very helpful. For example, when the aldosterone lateralization ratio is in the gray zone (3–<4), a contralateral suppression index of less than 1 is predictive of good operative outcomes.[199] In addition, if AVS is not bilaterally successful, a contralateral suppression index of less than 0.5 is highly predictive of contralateral disease.[200] Also, patients with a contralateral suppression index of less than 0.47 are at increased risk for postoperative hyperkalemia (see treatment section).[201]

The test characteristics of AVS using an aldosterone lateralization ratio of 4 or above for detection of unilateral aldosterone hypersecretion (APA or PAH) are 95% sensitivity and 98.6% specificity.[186,202] The use of AVS should be based on patient preference, patient age, clinical comorbid conditions, and the clinical probability of finding an APA. A practical approach is the selective use of AVS as outlined in Fig. 16.10.[203]

At centers with experience with AVS, the complication rate is 2.5% or less.[186,192] Complications can include symptomatic groin hematoma, adrenal hemorrhage, and dissection of an adrenal vein.

Noninvasive Alternatives to Adrenal Vein Sampling

Noninvasive alternatives to AVS are being explored. For example, ^{11}C-metomidate can be used as a PET radiotracer. In an initial study of 25 patients with PA, dexamethasone-suppressed ^{11}C-metomidate PET/CT had a specificity of 87% and sensitivity of 76% for unilateral adrenal disease.[204] It was recently reported that there is a high expression of CXC chemokine receptor type 4 (CXCR4) in adrenal glands and APAs, which correlates with the expression of P450 11B2 (aldosterone synthase).[205] The specific CXCR4 ligand ^{68}Ga-pentixafor was used as a PET imaging molecule in localizing APAs in nine patients with primary aldosteronism.[205] Finally, in vitro and animal studies have shown a high selectivity of a ^{18}F PET imaging molecule (CDP2230) for P450 11B2 over P450 11B1 with a favorable biodistribution for imaging P450 11B2.[206] Clinical studies will be needed to see if this ^{18}F-based PET imaging molecule or those similar to it will prove to be clinically useful.

Familial Hyperaldosteronism

Familial hyperaldosteronism should be considered when primary aldosteronism is diagnosed before age 20 years or when it is diagnosed in more than one family member. To date, four forms of FH have been described.[141] However, it is very likely that many more disease-causing germline mutations will be described in the coming years, and labeling each mutation with FH type V, VI, VII, VIII, IX, etc., will become impractical. A more practical approach may be to simply indicate FH due to the specific mutation.

Glucocorticoid-Remediable Aldosteronism: Familial Hyperaldosteronism Type I—*CYP11B1/CYP11B2* Germline Chimeric Gene

GRA (FH type I; Online Mendelian Inheritance in Man [OMIM] 103900) was first described in a single family in 1966.[207] Twenty-six years later the causative *CYP11B1/CYP11B2* chimeric gene was discovered.[208] GRA is a form of hyperaldosteronism in which the hypersecretion of aldosterone can be reversed with physiologic doses of glucocorticoid.[209] It is rare, as illustrated by a study of 300 consecutive patients with primary aldosteronism; only two patients were diagnosed with GRA (prevalence = 0.66%) (see Table 16.10).[210] GRA is characterized by early-onset hypertension that is usually severe and refractory to conventional antihypertensive therapies, aldosterone excess, suppressed PRA, and excess production of 18-hydroxycortisol and 18-oxocortisol. Mineralocorticoid production is regulated by ACTH instead of by the normal secretagogue, angiotensin II. Therefore aldosterone secretion can be suppressed by glucocorticoid therapy. In the absence of glucocorticoid therapy, this mutation results in overproduction of aldosterone and the hybrid steroids 18-hydroxycortisol and 18-oxocortisol, which can be measured in the urine or plasma to make the diagnosis.

Genetic testing is a sensitive and specific means of diagnosing GRA and obviates the need to measure the urinary levels of 18-oxocortisol and 18-hydroxycortisol or to perform dexamethasone suppression testing. Genetic testing for GRA should be considered for patients with primary aldosteronism who have a family history of primary aldosteronism, onset of primary aldosteronism at a young age (<20 years), or family history of strokes at a young age.

Familial Hyperaldosteronism Type II—*CLCN2* Chloride Channel Germline Mutations

FH type II is autosomal dominant in inheritance.[211–213] The hyperaldosteronism in FH type II does not suppress with dexamethasone, and GRA mutation testing is negative. FH type II is more common than FH type I, but it still accounts for fewer than 6% of all patients with primary aldosteronism.[210] In a recent report of a family with FH type II and 80 additional probands with unsolved early-onset primary aldosteronism, a germline *CLCN2* chloride channel mutation was found in 8 of the probands.[214] All relatives with early-onset primary aldosteronism carried the *CLCN2* variant found in the proband. *CLCN2* encodes a voltage-gated chloride channel expressed in adrenal glomerulosa cells. Mutations in the *CLCN2* chloride channel may be responsible for all or some of the individuals who have been classified at FH type II.[215] Over time we may learn of a series of mutations that contribute to what has been classified as FH type II.

Familial Hyperaldosteronism Type III—Germline *KCNJ5* Mutations

FH type III (OMIM 613677) was first described in a 5-year-old boy in 1959.[216] However, the familial nature of the disorder did not become apparent until the detection of primary aldosteronism in the index patient's two daughters in 2008; all presented with refractory hypertension before age 7 years and were treated with bilateral adrenalectomy.[217] The adrenal glands showed massive hyperplasia. Three years later the causative germline mutation in this family was discovered: a point mutation in and near the selectivity filter of the potassium channel *KCNJ5*.[218] This *KCNJ5* mutation produces increased sodium conductance and cell depolarization, triggering calcium entry into glomerulosa cells, the signal for aldosterone production and cell proliferation. Thus far, 12 kindreds carrying 6 different *KCNJ5* mutations have been identified: p.Thr158Ala, p.Gly151Glu, p.Gly151Arg, p. Ile157Ser, p.Tyr152Cys, and p.Glu145Gln.[218–223] The estimated prevalence of germline *KCNJ5* mutations is 0.3% in patients with primary aldosteronism and 8% among patients with familial primary aldosteronism.[220] Most patients with germline *KCNJ5* mutations present with polyuria, polydipsia, and refractory hypertension in childhood—investigations show marked hypokalemia and marked primary aldosteronism. In most cases the degree of aldosterone hypersecretion is so marked that bilateral adrenalectomy is required. However, there is some heterogeneity in age at presentation (as old as 48 years),[221] and the hypertension and hypokalemia in some patients can be controlled with MRAs.

Familial Hyperaldosteronism Type IV—Germline *CACNA1H* Gene Mutations

FH type IV (OMIM 617027) is inherited in an autosomal dominant fashion and with incomplete penetrance and is caused by mutations in the *CACNA1H* gene, which encodes the α-subunit of an L-type voltage-gated calcium channel (Cav3.2).[224,225] A novel germline *CACNA1H* mutation (p.Met1549Val) was identified in 5 of 40 unrelated patients affected by hypertension and primary aldosteronism in childhood.[224] Four additional germline *CACNA1H* mutations have been reported in patients with primary aldosteronism.[225]

Primary Aldosteronism With Seizures and Neurologic Abnormalities (PASNA)—Germline *CACNA1D* Mutations

PASNA is caused by de novo germline mutations in *CACNA1D* (OMIM 615474). *CACNA1D* encodes for the α1D-subunit of a L-type voltage-gated calcium channel (Cav 1.3). PASNA has been reported in two children with primary aldosteronism, seizures, and neurologic abnormalities.[226] The severe neurologic abnormalities do not allow these individuals to reproduce; thus, although due to a germline mutation, it is not technically a familial form of primary aldosteronism. In addition, a missense *CACNA1D* germline mutation was reported in a patient affected by autism and epilepsy.[227]

Primary Aldosteronism and *ARMC5* Mutations

Heterozygous germline mutations of the armadillo repeat containing 5 (*ARMC5*) gene are most commonly linked to subclinical glucocorticoid secretory autonomy or Cushing syndrome in patients with bilateral macronodular adrenal hyperplasia (BMAH).[228] However, there are reports of *ARMC5* mutations in patients with both primary aldosteronism and Cushing syndrome due to BMAH, a co-occurrence seen more commonly in African-American patients.[229]

Somatic Mutations in *KCNJ5, ATP1A1, ATP2B3, CACNA1D,* and *CTNNB1* Genes

Somatic mutations account for the majority of APAs and include mutations in genes encoding components of the Kir 3.4 (GIRK4) potassium channel *(KCNJ5),* the sodium/potassium and calcium ATPases (*ATP1A1* and *ATP2B3*), and a voltage-dependent C-type calcium channel *(CACNA1D).* In a multicenter study of 351 APAs from patients with primary aldosteronism and 130 other adrenocortical lesions, two somatic mutations in *KCNJ5* (G151R or L168R) were identified in 47% of APAs.[230] Somatic *KCNJ5* mutations were absent in adrenal tissue from patients with primary aldosteronism due to unilateral hyperplasia and in 130 non–aldosterone-secreting adrenal lesions. *KCNJ5* mutations were overrepresented in APAs from women compared with men (63% vs 24%), and APAs with *KCNJ5* mutations had larger lesional diameters than those without the mutation (2.7 cm vs 1.7 cm, respectively).[230] The larger size of *KCNJ5* APAs is explained in part by the zona fasciculata–like cells and lower levels of aldosterone synthase expression compared to APAs caused by somatic mutations in *ATP1A1*, *ATP2B3,* or *CACNA1D*.[231] In a meta-analysis including 13 studies with 1636 patients, the overall prevalence of somatic *KCNJ5* mutations in patients with APAs was 43%.[232]

Somatic APA mutations have been identified in three other genes: *ATP1A1* encoding Na^+/K^+-ATPase 1, *ATP2B3* encoding Ca^{2+}-ATPase 3, and *CACNA1D* encoding a voltage-gated calcium channel. In a study of 112 APAs, somatic APA mutations in *KCNJ5, ATP1A1,* and *ATP2B3* were present in 39.2%, 6.3%, and 0.9%, respectively.[233] To date, 13 different *ATP1A1* and 9 *ATP2B3* somatic mutations have been reported.[234] In addition, 31 different *CACNA1D* mutations have been described and account for approximately 9% of sporadic APAs.[234] *CACNA1D* APAs are composed of zona glomerulosa–like cells and are smaller compared with those with either no somatic mutation or *KCNJ5* APAs.[231] Somatic *CACNA1D* mutations are the most frequent somatic mutation in patients with APAs with negative or minimal nodularity (<1 cm) on preoperative cross-sectional imaging.[235]

The *CTNNB1* gene encodes β-catenin, and activating *CTNNB1* mutations have been found in 3% of sporadic APAs.[236] Similar to *KCNJ5* APAs, *CTNNB1* APAs are associated with female sex and larger adenomas. It is likely that nearly 100% of APAs will eventually be associated with somatic driver mutations.

Aldosterone-Producing Cell Clusters

Immunohistochemical staining studies for P450 11B2 and P450 11B1 have detected focal subcapsular nests of adrenocortical cells that extend into the zona fasciculata and stain strongly for P450 11B2 and are termed aldosterone-producing cell clusters (APCCs).[237] APCCs have been identified in normal adrenals and in pathology specimens from patients with primary aldosteronism, where APCCs may occur adjacent to APAs.[237] APCCs have been shown to harbor somatic mutations in *CACNA1D* and *ATP1A1* and raise the possibility that APCCs may secrete aldosterone autonomously and could be precursors to APAs.[238] Interestingly, APCCs increase with age and may in part simply reflect the aging adrenal gland.[239,240] The clinical relevance of APCCs is yet to be determined.

Cortisol Cosecretion

Patients with primary aldosteronism have been shown to be at increased risk for insulin resistance and metabolic syndrome,[241–243] depression,[244] and osteoporotic fractures.[245] These associations are more in keeping with glucocorticoid secretory autonomy or excess. A steroid metabolome study found that (a) patients with primary

aldosteronism (APA and IHA) had significantly increased cortisol and total glucocorticoid metabolite excretion compared to controls and patients with subclinical Cushing syndrome (p <0.001); (b) surrogate parameters of metabolic risk correlated with glucocorticoid, but not mineralocorticoid excretion; and (c) unilateral adrenalectomy in patients with APA resolved both mineralocorticoid and glucocorticoid excess.[246] These findings suggest that some degree of cortisol cosecretion is common in patients with primary aldosteronism and is linked to metabolic risk—a finding that may indicate that treatment with mineralocorticoid receptor antagonists in patients with IHA and APA may not prevent glucocorticoid-dependent metabolic risks.[246] However, these data should be interpreted with caution because this study did not simply identify cortisol cosecretion from APAs but rather an increased corticosteroid metabolome in patients with APA and IHA. The hypothalamic-pituitary-adrenal axis is not suppressed in these patients, and there is no adrenal crisis or steroid withdrawal in patients with APAs who are treated surgically. Thus the etiology of the increased glucocorticoid metabolites in patients with primary aldosteronism is not clear and may be due to a yet to be determined mechanism.

A separate and less common issue is APAs that cosecrete cortisol that may be clinically important for perioperative management (e.g., perioperative corticosteroid coverage and postoperative replacement and taper).[247] When should clinicians test for cortisol cosecretion in patients with APAs? In general, clinically important cortisol secretion from an adrenal adenoma is correlated with tumor size. Unlike aldosterone secretion from an adenoma, clinically important cortisol secretion requires a "large factory"—typically with cortical adenoma diameters greater than 2 cm.[247] Thus it is reasonable to test patients with primary aldosteronism for cortisol cosecretion when the adrenal adenoma is greater than 1.5 cm in diameter; testing should include baseline dehydroepiandrosterone-sulfate and an overnight 1-mg dexamethasone suppression test.[248] When glucocorticoid secretory autonomy is documented in a patient with primary aldosteronism who has a single cortical adenoma greater than 1.5 cm in diameter, AVS is not needed, assuming that the contralateral adrenal appears normal on CT.

Principles of Treatment

The treatment goal is to prevent the morbidity and fatality associated with hypertension, hypokalemia, cardiovascular damage, and nephrotoxicity. Knowing the cause of the primary aldosteronism helps to determine the appropriate treatment. Normalization of blood pressure should not be the only goal. In addition to the kidney and colon, mineralocorticoid receptors are present in the heart, brain, and blood vessels. Excessive secretion of aldosterone is associated with increased risk of cardiovascular disease and morbidity. Therefore normalization of circulating aldosterone or mineralocorticoid receptor blockade should be part of the management plan for all patients with primary aldosteronism. However, clinicians must understand that most patients with long-standing primary aldosteronism have some degree of renal insufficiency that is masked by the glomerular hyperfiltration associated with aldosterone excess.[249,250] The true degree of underlying chronic kidney disease may become evident only after effective pharmacologic or surgical therapy.[249,250]

Surgical Treatment of Aldosterone-Producing Adenoma and Unilateral Hyperplasia

Unilateral laparoscopic adrenalectomy is an excellent treatment option for patients with APA or unilateral hyperplasia.[111] As outlined earlier, in terms of quality-of-life parameters, the beneficial effects of surgery were superior to chronic medical treatment with mineralocorticoid receptor antagonists.[171] Unilateral adrenalectomy is the optimal treatment for patients with unilateral adrenal disease. Surgical treatment with bilateral adrenalectomy is not a good treatment option for patients with IHA; instead lifelong treatment with mineralocorticoid antagonists is the treatment of choice.

Laparoscopic adrenalectomy with an expert adrenal surgeon is the preferred surgical approach. Because APAs are small and may be multiple, the entire adrenal gland should be removed.[188,251] To decrease the surgical risk, hypokalemia should be corrected with potassium supplements or a mineralocorticoid receptor antagonist, or both, preoperatively (but discontinued immediately postoperatively). PAC should be measured 1 to 2 days after surgery to confirm a biochemical cure.[183] In general, the number and dosages of antihypertensive medications can be decreased by 50% postoperatively—discontinuing any that may predispose to hyperkalemia (e.g., ACE-I, ARB). The full impact of surgery on hypertension may take up to 3 months. Because of the risk of temporary hypoaldosteronism due to chronic suppression of the RAA axis, serum potassium levels should be monitored weekly for 4 weeks after surgery, and a generous sodium diet should be followed.[201,252,253] In a multicenter study of 142 surgically treated patients, the prevalence of postoperative hyperkalemia was 9.9%; the hyperkalemic patients were older and had worse renal function than the nonhyperkalemic patient group.[254] In a study of 192 patients with primary aldosteronism who were treated surgically, 12 (6.3%) developed postoperative hyperkalemia (median serum potassium 5.5 mmol/L, range 5.2–6.2 mmol/L); median time to onset was 13.5 days (range, 7–55 days).[201]

Although blood pressure control improves in almost 100% of patients postoperatively, average long-term cure rates of hypertension after unilateral adrenalectomy for APA range from 30% to 60%.[88,182,183] Persistent hypertension after adrenalectomy is directly correlated with having more than one first-degree relative with hypertension, use of more than two antihypertensive agents preoperatively, older age, increased serum creatinine level, and duration of hypertension and is most likely caused by coexistent primary hypertension.[88]

Pharmacologic Treatment

IHA and GRA should be treated medically. In addition, although not optimal, APA may be treated medically if therapy includes mineralocorticoid receptor blockade. A sodium-restricted diet (<100 mEq sodium per day), maintenance of ideal body weight, tobacco avoidance, and regular aerobic exercise contribute significantly to the success of pharmacologic treatment. No placebo-controlled, randomized trials have evaluated the relative efficacy of drugs in the treatment of primary aldosteronism.[255]

In a longitudinal study assessing 602 patients with primary aldosteronism treated with mineralocorticoid receptor antagonists, the incidence of cardiovascular events was higher in patients with primary aldosteronism compared to patients with essential hypertension.[256] The excess risk for cardiovascular events and mortality was limited to patients with primary aldosteronism who had suppressed PRA on medical treatment—suggesting inadequate dosing of mineralocorticoid receptor antagonists.[256] Due to concomitant low-renin essential hypertension in some patients with primary aldosteronism, measurement of PRA may not be optimal to guide management; rather, a more practical treatment target to document effective mineralocorticoid blockade is a high-normal serum potassium without the aid of oral potassium supplements.

Spironolactone has been the drug of choice to treat primary aldosteronism for more than 50 years. It is available as 25-mg, 50-mg, and 100-mg tablets. The dosage is 12.5 to 25 mg per day initially and can be increased to 400 mg per day if necessary to achieve a high-normal serum potassium concentration without the aid of oral potassium chloride supplementation. Hypokalemia responds promptly, but hypertension can take as long as 4 to 8 weeks to be corrected. After several months of therapy, the dosage of spironolactone often can be decreased to as little as 25 to 50 mg per day; dosage titration is based on a goal serum potassium level in the high-normal range. Serum potassium and creatinine should be monitored frequently during the first 4 to 6 weeks of therapy (especially in patients with renal insufficiency or diabetes mellitus). Spironolactone increases the half-life of digoxin, and the digoxin dosage may need to be adjusted when treatment with spironolactone is started. Concomitant therapy with salicylates should be avoided because they interfere with the tubular secretion of an active metabolite and decrease the effectiveness of spironolactone. However, spironolactone is not selective for the mineralocorticoid receptor. For example, antagonism at the androgen receptor may result in painful gynecomastia, erectile dysfunction, and decreased libido in men, and agonist activity at the progesterone receptor results in menstrual irregularity in women.

Eplerenone is a steroid-based antimineralocorticoid that acts as a competitive and selective mineralocorticoid receptor antagonist and was approved by the US Food and Drug Administration for the treatment of uncomplicated essential hypertension in 2003. The 9,11-epoxide group in eplerenone results in a marked reduction of the molecule's progestational and antiandrogenic actions; compared with spironolactone, eplerenone has 0.1% of the binding affinity to androgen receptors and less than 1% of the binding affinity to progesterone receptors. In a randomized, double-blind trial comparing the efficacy, safety, and tolerability of eplerenone to that of spironolactone (100–300 mg vs 75–225 mg, respectively) in patients with primary aldosteronism, researchers found spironolactone to be superior in terms of lowering blood pressure but associated with higher rates of male gynecomastia (21% vs 5% for eplerenone) and female mastodynia (21% vs 0%).[257] Eplerenone is available as 25-mg and 50-mg tablets. For primary aldosteronism, it is reasonable to start with a dose of 25 mg twice daily (twice daily because of the shorter half-life of eplerenone compared with spironolactone) and titrated upward; the target is a high-normal serum potassium concentration without the aid of potassium supplements. The maximum dose approved by the FDA for hypertension is 100 mg per day. However, potency studies with eplerenone show 25% to 50% less milligram-per-milligram potency compared with spironolactone. Thus it is common to treat patients with primary aldosteronism with 200 to 300 mg of eplerenone per day to reach the target serum potassium concentration. As with spironolactone, it is important to monitor blood pressure, serum potassium, and serum creatinine levels closely. Side effects include dizziness, headache, fatigue, diarrhea, hypertriglyceridemia, and elevated liver enzymes.

Patients with IHA frequently require a second antihypertensive agent to achieve good blood pressure control. Hypervolemia is a major reason for resistance to drug therapy, and low doses of a thiazide (e.g., 12.5–50 mg of hydrochlorothiazide daily) or a related sulfonamide diuretic are effective in combination with the mineralocorticoid receptor antagonist. Because these agents often lead to further hypokalemia, serum potassium levels should be monitored.

Before treatment for GRA is initiated, the diagnosis of GRA should be confirmed with genetic testing. In the GRA patient, chronic treatment with physiologic doses of a glucocorticoid normalizes blood pressure and corrects hypokalemia. The clinician should be cautious about iatrogenic Cushing syndrome with excessive doses of glucocorticoids, especially when dexamethasone is used in children. Shorter-acting agents such as prednisone or hydrocortisone should be prescribed, using the smallest effective dose in relation to body surface area (e.g., hydrocortisone, 10–12 mg/m^2/day). Target blood pressure in children should be guided by age-specific blood pressure percentiles. Children should be monitored by pediatricians with expertise in glucocorticoid therapy, with careful attention paid to preventing retardation of linear growth due to overtreatment. Treatment with mineralocorticoid receptor antagonists in these patients may be just as effective as glucocorticoids and avoids the potential disruption of the hypothalamic-pituitary-adrenal axis and risk of iatrogenic side effects. In addition, glucocorticoid therapy or mineralocorticoid receptor blockade may even have a role in normotensive patients with GRA.[168]

Primary Aldosteronism in the Setting of Pregnancy

Primary aldosteronism is uncommon in pregnancy, and fewer than 50 patients are reported in the medical literature.[258–263] Primary aldosteronism in pregnancy can lead to preterm delivery, intrauterine growth retardation, placental abruption, and intrauterine fetal demise.[263–265] In some women, the high blood concentrations of pregnancy-related progesterone are antagonistic at the mineralocorticoid receptor and partially block the action of aldosterone; these patients actually have an improvement in the degree of hypertension and hypokalemia during pregnancy.[266,267] Whereas in other pregnant women, increased expression of the luteinizing hormone/chorionic gonadotropin receptor in APAs harboring β-catenin mutations has been documented, and the degree of hypertension and hypokalemia can be aggravated by the increased pregnancy-related blood levels of human chorionic gonadotropin.[268,269]

Case detection testing is identical to that in nonpregnant women; a morning blood sample should be obtained for the measurement of PAC and renin (PRA or PRC). If spontaneous hypokalemia is present in the pregnant woman with suppressed renin and the PAC is greater than 20 ng/dL, additional confirmatory testing is not needed. However, if the patient is normokalemic, confirmatory testing should be pursued. Confirmatory testing in the setting of pregnancy can be challenging because the captopril challenge test is contraindicated in pregnancy, and the saline infusion test may not be well tolerated because of edema. The optimal confirmatory test in the setting of pregnancy is measurement of aldosterone excretion in a 24-hour urine collection on an ambient sodium diet. To avoid exposure to radiation or contrast material, subtype testing in the setting of pregnancy should start with abdominal MRI without gadolinium. Unilateral APA can be diagnosed in the unique clinical setting of a pregnant woman with marked primary aldosteronism (spontaneous hypokalemia and PAC >30 ng/dL) and a clear-cut unilateral adrenal adenoma on MRI.[202]

The severity of hypertension and hypokalemia dictates the optimal treatment of primary aldosteronism in pregnancy. For example, if the patient is in the subset of patients who have a

remission in the degree of primary aldosteronism, then surgery or treatment with a mineralocorticoid receptor antagonist can be avoided until after delivery. However, if hypertension and hypokalemia are marked, then surgical and/or medical intervention is indicated. Unilateral laparoscopic adrenalectomy during the second trimester can be considered in those women with severe primary aldosteronism and documented unilateral APA. The US FDA lists spironolactone as a pregnancy category C drug because feminization of newborn male rats has been documented. However, only one human case has been reported where treatment with spironolactone in pregnancy led to ambiguous genitalia in a male infant due to spironolactone treatment for polycystic ovary syndrome pre-pregnancy and through the fifth week of gestation.[270] Eplerenone is a FDA pregnancy category B drug. When primary aldosteronism is managed medically in pregnant women, the hypertension should be treated with standard antihypertensive drugs that are approved for use during pregnancy. Hypokalemia should be treated with oral potassium supplements. In those cases of severe primary aldosteronism in pregnancy where surgery is not an option, low-dose eplerenone may be cautiously considered.[258,271,272]

Other Forms of Mineralocorticoid Excess or Effect

The medical disorders associated with excess mineralocorticoid effect from 11-deoxycorticosterone (DOC) and cortisol are listed in Table 16.10. These diagnoses should be considered if PAC and PRA are low in a patient with hypertension and hypokalemia.

Hyperdeoxycorticosteronism

Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders caused by enzymatic defects in adrenal steroidogenesis that result in deficient secretion of cortisol (see Chapter 15).[273] Approximately 90% of CAH cases are caused by 21-hydroxylase deficiency, which does not result in hypertension.[274] Deficiencies of 11β-hydroxylase (CYP11B1, P450 11B1) or 17α-hydroxylase (CYP17A1, P450 17A1) cause hypertension and hypokalemia because of hypersecretion of the mineralocorticoid DOC. The mineralocorticoid effect of increased circulating levels of DOC also decreases renin and aldosterone secretion. These mutations are autosomal recessive in inheritance and typically are diagnosed in childhood. However, partial enzymatic defects have been shown to cause hypertension in adults.

11β-Hydroxylase Deficiency

Approximately 5% of all cases of CAH are caused by 11β-hydroxylase deficiency; the prevalence in Caucasians is 1 in 100,000.[275] More than 40 mutations have been described in *CYP11B1*, the gene encoding 11β-hydroxylase.[276] There is an increased prevalence among Sephardic Jews from Morocco due to a founder effect. The impaired conversion of DOC to corticosterone results in high levels of DOC and 11-deoxycortisol; the substrate mass effect results in increased levels of adrenal androgens. Girls present in infancy or childhood with hypertension, hypokalemia, acne, hirsutism, and virilization. Boys with CAH due to 11β-hydroxylase deficiency present with hypertension, hypokalemia, and precocious pseudopuberty. Approximately two-thirds of patients have mild to moderate hypertension. The initial screening tests include measurement of blood levels of DOC, 11-deoxycortisol, androstenedione, testosterone, and dehydroepiandrosterone sulfate (DHEAS)—all of which should be increased above the upper limit of the respective reference ranges. Confirmatory testing includes germline mutation testing.

17α-Hydroxylase Deficiency

17α-Hydroxylase deficiency is a very rare cause of CAH; good prevalence data are not available, but the prevalence is likely less than 1 in 1 million live births.[277] 17α-Hydroxylase is essential for the synthesis of cortisol and gonadal hormones, and deficiency results in decreased production of cortisol and sex steroids. Genetic 46,XY males present with either genital ambiguity or as phenotypic females, and 46,XX females present with primary amenorrhea. Therefore a person with this form of CAH may not come to medical attention until puberty. Children, adolescents, and young adults present with hypertension and spontaneous hypokalemia and low levels of aldosterone and renin. Although very rare, there is an increased prevalence of 17α-hydroxylase deficiency among Dutch Mennonites. The initial screening tests include measurement of blood levels of androstenedione, testosterone, DHEAS, 17-hydroxyprogesterone, aldosterone, and cortisol—all of which should be either low or at the lower quartile of the respective reference ranges. The plasma concentrations of DOC and corticosterone should be above the upper limit of the respective reference ranges. Confirmatory testing includes germline mutation testing.

Deoxycorticosterone-Producing Tumor

Pure DOC-producing adrenal tumors are very rare and usually large and malignant. Some patients have been documented to have benign DOC-producing adrenocortical adenomas. Some of these adrenal neoplasms cosecrete androgens and estrogens in addition to DOC, which may cause virilization in women or feminization in men. The typical clinical presentation would be that of relatively rapid onset of marked hypertension associated with hypokalemia and low blood levels of aldosterone and renin. A high level of plasma DOC or urinary tetrahydrodeoxycorticosterone and a large adrenal tumor seen on CT confirm the diagnosis. Aldosterone secretion in these patients is typically suppressed.

Primary Cortisol Resistance

Increased cortisol secretion and plasma cortisol concentrations without evidence of Cushing syndrome are found in patients with primary cortisol resistance (or glucocorticoid resistance), a rare familial syndrome.[278,279] Primary cortisol resistance is caused by genetic defects in the glucocorticoid receptor and the steroid-receptor complex. The syndrome is characterized by hypokalemic alkalosis, hypertension, increased plasma concentrations of DOC, and increased adrenal androgen secretion. The hypertension and hypokalemia result from the combined effects of excess DOC and increased cortisol access to the mineralocorticoid receptor, resulting from high rates of cortisol production that overwhelm 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) activity. Most affected individuals present in childhood with hypertension and spontaneous hypokalemia and low levels of aldosterone and renin. The initial screening tests include measurement of blood levels of cortisol, DOC, 11-deoxycortisol, androstenedione, testosterone, and DHEAS—all of which should be increased above the upper limit of the respective reference ranges. In addition,

24-hour urinary cortisol excretion is above the upper limit of the reference range, and serum ACTH is not suppressed. Confirmatory testing includes germline mutation testing.

Apparent Mineralocorticoid Excess Syndrome

Apparent mineralocorticoid excess is the result of impaired activity of the microsomal enzyme 11βHSD2, which normally inactivates cortisol in the kidney by converting it to the inactive 11-keto compound, cortisone.[280] Cortisol can be a potent mineralocorticoid, and when 11βHSD2 is genetically deficient or its activity blocked, high levels of cortisol accumulate in the kidney. Decreased 11βHSD2 activity may be hereditary or it may be secondary to pharmacologic inhibition of enzyme activity by metabolites of glycyrrhizic acid, the active principle of licorice root *(Glycyrrhiza glabra)*.[281] The congenital forms are rare autosomal recessive disorders; fewer than 50 patients have been identified worldwide.[282] Congenital apparent mineralocorticoid excess typically presents in childhood with hypertension, hypokalemia, low birth weight, failure to thrive, hypertension, polyuria and polydipsia, and poor growth.[276] Acquired apparent mineralocorticoid excess due to licorice root ingestion presents with hypertension and hypokalemia—the cause becomes evident when a good medical history is obtained. In addition, when 11βHSD2 is overwhelmed by massive cortisol hypersecretion associated with Cushing syndrome due to ectopic ACTH syndrome, hypokalemic hypertension may be one of the outcomes. The clinical phenotype of patients with apparent mineralocorticoid excess due to congenital deficiency of or inhibition of 11βHSD2 includes hypertension, hypokalemia, metabolic alkalosis, low renin, low aldosterone, and normal plasma cortisol levels. The diagnosis of apparent mineralocorticoid excess is confirmed by demonstration of an abnormal (high) ratio of cortisol to cortisone in a 24-hour urine collection. The characteristic abnormal urinary cortisol-cortisone metabolite profile reflects decreased 11βHSD2 activity; the ratio of cortisol to cortisone is typically increased 10-fold above the normal value.[280] DOC levels may also be increased in severe ACTH-dependent Cushing syndrome and contribute to the hypertension and hypokalemia in this disorder.

Liddle Syndrome: Abnormal Renal Tubular Ionic Transport

In 1963, Grant Liddle described an autosomal dominant renal disorder with a presentation similar to primary aldosteronism with hypertension, hypokalemia, and inappropriate kaliuresis.[283] However, blood levels of aldosterone and renin were very low, so the disorder was termed *pseudoaldosteronism*. Liddle syndrome is caused by autosomal dominant mutations in the β-unit or γ-subunit of the amiloride-sensitive epithelial sodium channel.[276] It is extremely rare, with fewer than 30 families reported worldwide.[284] This mutation results in enhanced activity of the epithelial sodium channel, and patients present with increased renal sodium reabsorption, potassium wasting, hypertension, and hypokalemia. However, as mentioned earlier, blood levels of aldosterone and renin are low. Affected individuals usually present as children or young adults with hypertension and spontaneous hypokalemia and low levels of aldosterone and renin. A family history of hypertension associated with hypokalemia makes Liddle syndrome more likely. The finding of low aldosterone and renin levels in the hypokalemic hypertensive patient should raise the possibility of Liddle syndrome. When the other causes of this presentation have been excluded, then a treatment trial with amiloride or triamterene should be considered. Liddle syndrome can easily be distinguished from apparent mineralocorticoid excess based on a good clinical response to amiloride or triamterene combined with a sodium-restricted diet, lack of efficacy of spironolactone and dexamethasone, and normal 24-hour urine cortisone/cortisol ratio. Clinical genetic testing is available.

Other Endocrine Disorders Associated With Hypertension

Cushing Syndrome

Iatrogenic Cushing syndrome is relatively common. However, endogenous Cushing disease is rare, with an incidence of less than 1 case per 1 million people per year.[285] Hypertension occurs in 75% to 80% of patients with Cushing syndrome (see Chapter 15). The mechanisms of hypertension include increased production of DOC (in ACTH-dependent Cushing syndrome), enhanced pressor sensitivity to endogenous vasoconstrictors (e.g., epinephrine, angiotensin II), increased cardiac output, activation of the RAA system by increased hepatic production of angiotensinogen, and cortisol activation of the mineralocorticoid receptor.

Thyroid Dysfunction

Hyperthyroidism

When excessive amounts of circulating thyroid hormones interact with thyroid hormone receptors on peripheral tissues, both metabolic activity and sensitivity to circulating catecholamines increase. Thyrotoxic patients usually have tachycardia, high cardiac output, increased stroke volume, decreased peripheral vascular resistance, and increased systolic blood pressure. The initial management in patients with hypertension and hyperthyroidism includes use of a β-adrenergic blocker to treat hypertension, tachycardia, and tremor. The definitive treatment of hyperthyroidism is cause specific (see Chapter 12).

Hypothyroidism

The frequency of hypertension (usually diastolic) is increased threefold in hypothyroid patients and may account for as many as 1% of cases of diastolic hypertension in the general population.[286,287] The mechanisms for the elevation in blood pressure include increased systemic vascular resistance and extracellular volume expansion. Treatment of thyroid hormone deficiency decreases blood pressure in most patients with hypertension and normalizes blood pressure in one-third of them. Synthetic levothyroxine is the treatment of choice for hypothyroidism (see Chapter 13).

Renin-Secreting Tumor

Renin-secreting tumors are rare and usually benign mesenchymal tumors localized to the kidney. These juxtaglomerular cell tumors typically present with secondary hyperaldosteronism (hypertension, hypokalemia, high PAC, and elevated renin) in young adults.[288] The renal neoplasm can usually be localized with contrast-enhanced renal CT or MRI. Occasionally renal vein renins may be needed to confirm localization. Surgical resection cures the secondary hyperaldosteronism.

Acromegaly

Hypertension occurs in 20% to 40% of the patients with acromegaly and is associated with sodium retention and extracellular volume expansion (see Chapter 9).[289,290] The hypertension of acromegaly is treated most effectively by curing the excess of growth hormone.[290] If a surgical cure is not possible, the hypertension usually responds well to medications that either block growth hormone effect (e.g., pegvisomant) or suppress growth hormone secretion (e.g., somatostatin analogs). Residual hypertension can be treated with diuretic therapy.

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The complete list of references is available online at ExpertConsult.com.

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17

Physiology and Pathology of the Female Reproductive Axis

SERDAR E. BULUN

CHAPTER OUTLINE

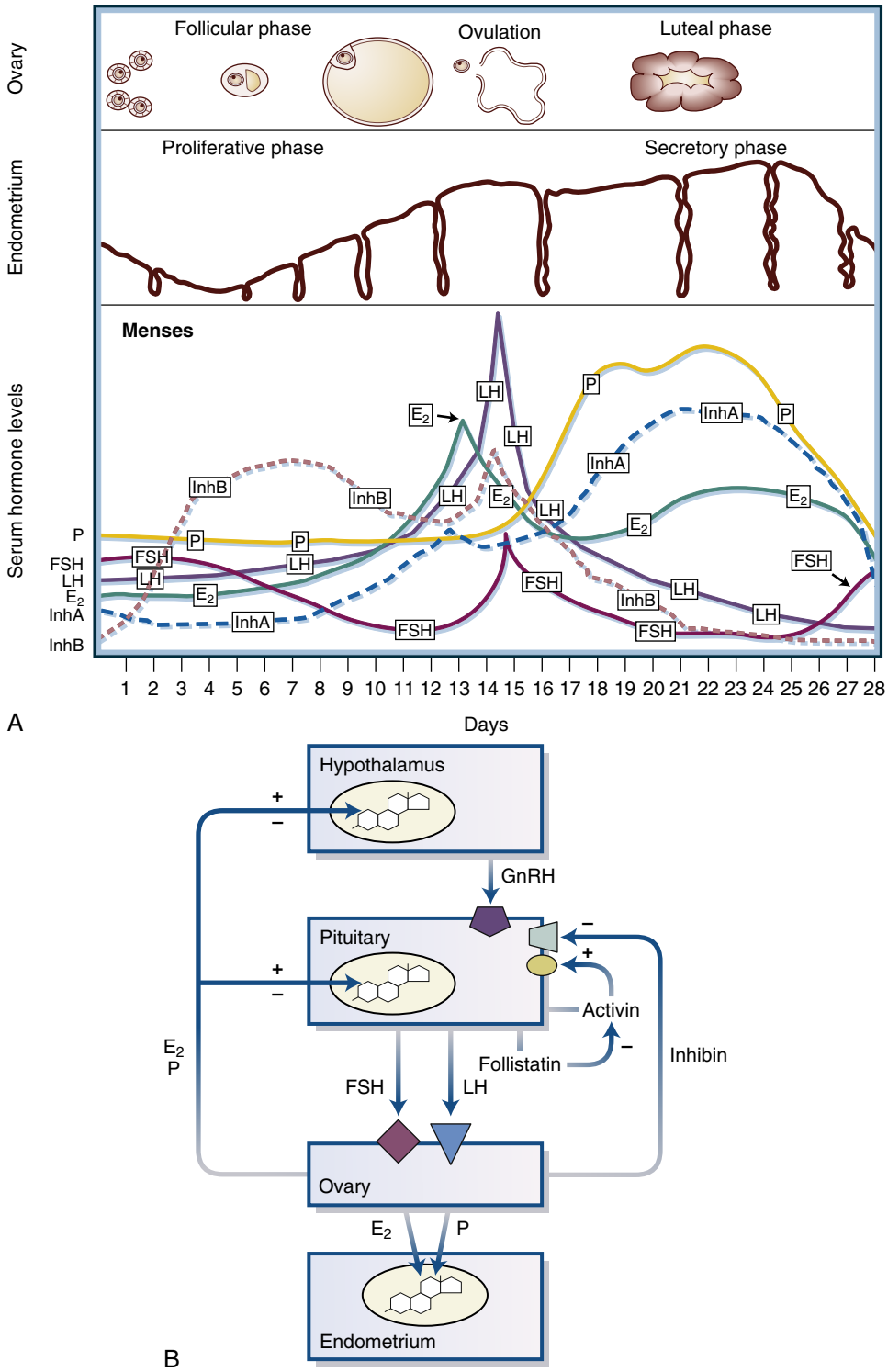
KEY POINTS

- Ovulation and the preparation of the uterus for pregnancy are extremely delicate and parallel physiologic processes that are tightly regulated by a number of hormones released primarily by the hypothalamus, pituitary, and ovary.
- In women, the biologically active steroids—estradiol, testosterone, and dihydrotestosterone (DHT)—are formed in the ovary, peripheral tissues, and locally in androgen or estrogen target tissues. Additionally, the adrenal gland and ovary secrete androgen and estrogen precursors that are converted to biologically active steroids in the peripheral tissues.
- A premenopausal woman often seeks medical help because of disorders that disrupt or complicate ovulation, normal menses, or fertility; the most common disorders include hypothalamic anovulation, hyperprolactinemia, polycystic ovary syndrome, ovarian insufficiency, endometriosis, and uterine fibroids.
- Combination oral contraceptives are commonly prescribed to suppress ovarian activity for the management of various benign causes of anovulatory uterine bleeding or androgen excess, such as polycystic ovary syndrome, and for the management of cyclic or chronic pelvic pain associated with endometriosis.
- Menopause is the depletion of all ovarian follicles; it effectively stops secretion of estradiol and progesterone. The management of postmenopausal ovarian deficiency, characterized thus by vasomotor symptoms, bone loss, and vulvovaginal atrophy, is challenging and still highly debated in regard to the effectiveness and the side effects of existing treatment regimens.

Reproductive Physiology

Tightly coordinated functions of the hypothalamus, pituitary, ovaries, and endometrium give rise to cyclic, predictable menses that indicate regular ovulation. Regular ovulation requires normal functioning of other endocrine glands, such as the thyroid and adrenals, and patients with hypothyroidism, hyperthyroidism, Cushing syndrome, or glucocorticoid resistance may present with anovulation. Clinicians need a thorough knowledge of the functions and interactions of the hypothalamus, pituitary, ovaries, and uterus with other systems to correctly diagnose reproductive disorders and design treatment strategies.

A prominent reproductive function of the hypothalamus is pulsatile secretion of gonadotropin-releasing hormone (GnRH). Negative feedback effects of several factors, including ovarian steroids, regulate hypothalamic GnRH secretion into the portal vessels. Dopamine, norepinephrine, serotonin, and opioids produced in the brain may mediate the regulation of GnRH secretion by ovarian hormones or other stimuli. In response to GnRH, the anterior pituitary cells secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Steroids (e.g., estradiol, progesterone) and peptides (e.g., inhibin) of ovarian origin and activin and follistatin of pituitary origin modify secretion of FSH and LH. LH stimulates androstenedione production in theca cells of the ovary; FSH regulates estradiol and inhibin B production in the granulosa cells and follicular growth. Release of an egg from the mature follicle depends on a sudden rise in LH levels in midcycle. After ovulation, the follicle transforms into a corpus luteum that secretes estradiol and progesterone under the control of FSH and LH. LH also stimulates granulosa-lutein cells of the corpus luteum to secrete inhibin A (Fig. 17.1A).



• **Fig. 17.1** (A) Changes in the ovarian follicle, endometrial thickness, and serum hormone levels during a 28-day menstrual cycle. Menses occur during the first few days of the cycle. (B) Endocrine interactions in the female reproductive axis. Some of the well-characterized endocrine interactions among the hypothalamus, pituitary, ovary, and endometrium for regulation of the menstrual cycle are depicted. *E_2,* estradiol; *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *Inh,* inhibin; *LH,* luteinizing hormone; *P,* progesterone.

The endocrine effects of FSH, LH, estradiol, progesterone, inhibin A, and inhibin B have been deduced from changes in their serum levels throughout the menstrual cycle (see Fig. 17.1A). The postulated endocrine effects were then demonstrated in cell-based and in vivo studies (see Fig. 17.1B). Activin and follistatin are produced in the ovary and the pituitary. They appear to regulate FSH release from the pituitary via autocrine or paracrine but not endocrine pathways. Activin stimulates FSH production, whereas follistatin suppresses this action of activin.

Endometrium, the mucosal lining of the uterine cavity, has extremely high concentrations of nuclear receptors for estrogen and progesterone and is highly sensitive to these hormones. The biologically active estrogen, estradiol, induces the growth of endometrium; progesterone limits this estrogenic effect and enhances differentiation. Sloughing off of the functional layer (stratum functionalis) of the endometrium follows withdrawal of estrogen or progesterone. The remaining basal layer (stratum basalis) is capable of full regeneration in response to estrogen.

Ovaries remain quiescent until puberty because the GnRH-releasing system in the hypothalamus is immature in prepubertal children associated with very low circulating FSH and LH. The entire reproductive function and most of the endocrine function of the ovaries cease after menopause because ovaries are depleted of all oocytes and surrounding steroidogenic cells by this time. These prepubertal and postmenopausal states, characterized by the absence of ovarian function, are associated with the lack of menses.

In summary, the female reproductive function from puberty to menopause can be viewed as an extremely delicate ticking clock. The normal function of this apparatus depends on coordinated actions of the hypothalamus, pituitary, ovaries, and endometrium. The result is regular menses every 24 to 35 days. Any disorder of these tissues or dysfunction of other systems that affect these reproductive units secondarily may result in anovulation and consequent irregular uterine bleeding.

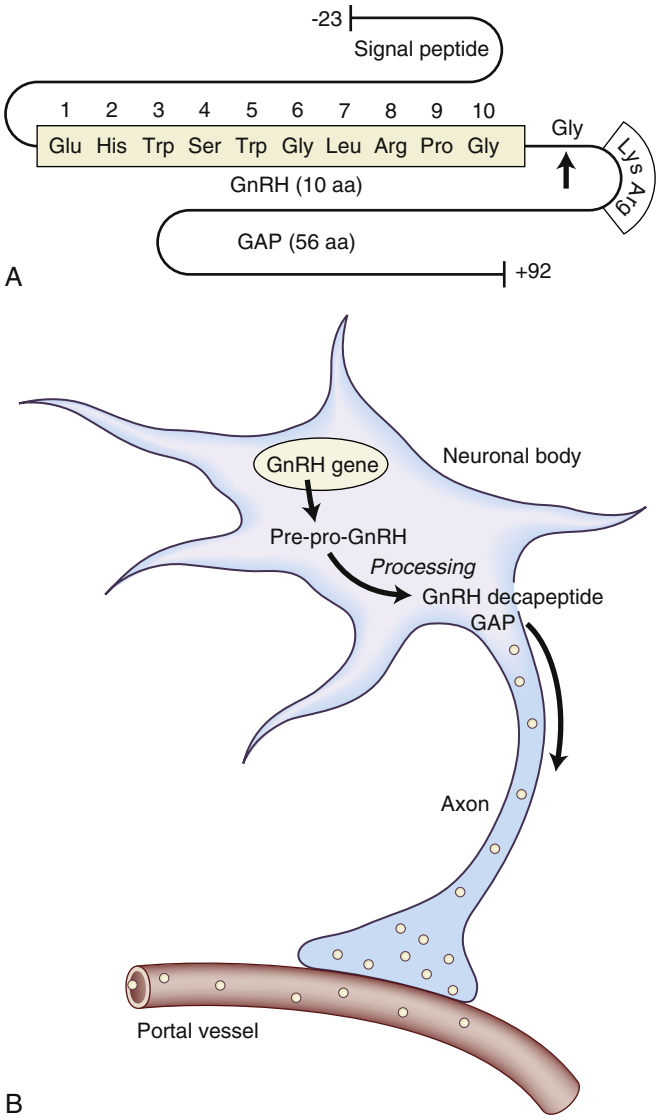
Reproductive Functions of the Hypothalamus

Gonadotropin-Releasing Hormone

GnRH and its analogues are used for the treatment of hormone-dependent disorders and assisted reproductive technologies such as in vitro fertilization (IVF).[1,2] In a number of vertebrates, three GnRHs and three cognate receptors with distinct distributions and functions have been identified. In humans, the hypothalamic GnRH is primarily encoded by the GnRH type I (GnRH-I) gene *(GNRH1)* and regulates gonadotropin secretion through the pituitary GnRH type I receptor, which functions as a G protein–coupled receptor. Binding of GnRH-I to its type I receptor leads primarily to activation of G_q. A second form of GnRH, called *GnRH-II*, is conserved in all higher vertebrates, including humans.[3] In contrast to GnRH-I, GnRH-II is expressed at the highest levels outside the brain. A cognate receptor for GnRH-II has been cloned from various vertebrate species, including primates.[3] The human gene homologue of this receptor has a frameshift and stop codon, and it appears that GnRH-II signaling occurs through the type I GnRH receptor. There seems to be considerable plasticity in the use of different GnRHs, receptors, and signaling pathways for diverse functions.[3] For practical purposes, GnRH-I is referred to as *GnRH* in this chapter.

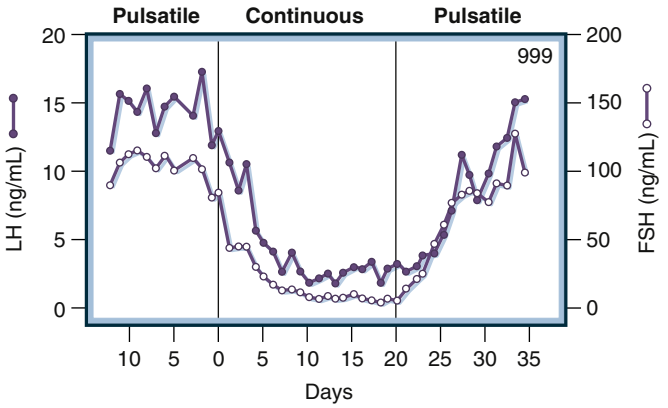
GnRH is a 10–amino acid peptide that is synthesized primarily in specialized neuronal bodies of the arcuate nucleus of the medial basal hypothalamus.[3] Axons from GnRH neurons project to the median eminence and terminate in the capillaries that drain into the portal vessels.

The portal vein is a low-flow transport system that descends along the pituitary stalk and connects the hypothalamus to the anterior pituitary. The direction of the blood flow in this hypophyseal portal circulation is from the hypothalamus to the pituitary. GnRH originating in the neurons of the arcuate nucleus is secreted at the median eminence into the portal circulation, which delivers this hormone to the anterior pituitary (Fig. 17.2).

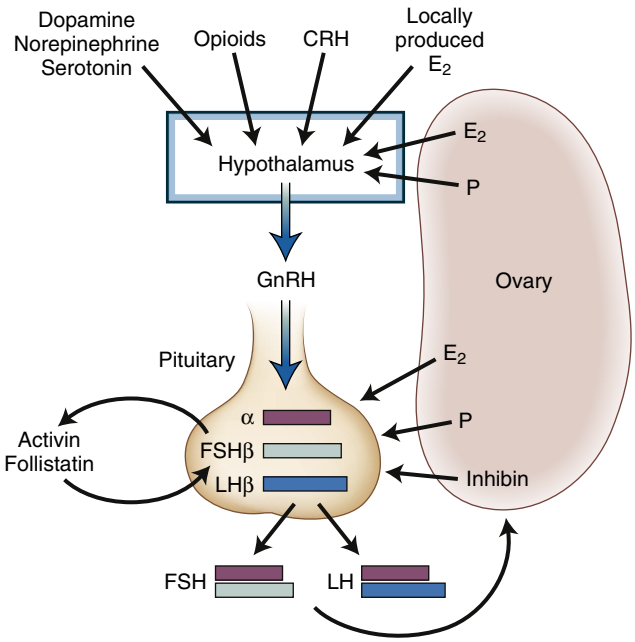


• **Fig. 17.2** Gonadotropin-releasing hormone (GnRH) production. (A) The GnRH gene encodes a precursor protein, pre-pro-GnRH, in the neuronal body. GnRH is released from this protein by proteolytic processing, which gives rise to GnRH and GnRH-associated protein (GAP) within the neuronal body. Both GnRH and GAP are transported in an axon to the nerve terminal and secreted into the portal circulation. (B) Pre-pro-GnRH is a 92–amino acid (aa) protein. The biologically active decapeptide (amino acids 1–10) is sandwiched between the 23–amino acid signal peptide and the Gly-Lys-Arg sequence. The *arrow* indicates the site of proteolytic processing. The C-terminal 56–amino acid peptide is cleaved to produce GAP. *LHRH,* luteinizing hormone–releasing hormone (gonadotropin-releasing hormone 1). (From Yen SSC. Endocrine regulation of the reproductive system. In: Yen SSC, Jaffe RB, Barbieri RL, eds. *Reproductive Endocrinology*. 4th ed. Philadelphia, PA: WB Saunders; 1999:44.)

The mature decapeptide GnRH is derived from the post-translational processing of a large precursor molecule, pre-pro-GnRH (see Fig. 17.2).[4] This precursor peptide is the product of the *GNRH* gene.[3] The pre-pro-GnRH consists of 92 amino acids and contains four parts (from amino-terminal to carboxyl-terminal): a 23–amino acid signal domain, the GnRH decapeptide, a 3–amino acid proteolytic processing site, and a 56–amino acid domain called *GnRH-associated peptide*.[5] The cleavage products of this precursor, GnRH and GnRH-associated peptide (GAP), are transported to the nerve terminals and secreted into the portal



• **Fig. 17.3** Effect of pulsatile or continuous administration of gonadotropin-releasing hormone (GnRH) to ovariectomized monkeys previously rendered GnRH deficient by placement of a lesion in the hypothalamus. Release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) was restored by hourly GnRH infusion, inhibited during a continuous infusion, and again restored after reinstitution of pulsatile GnRH administration. (Adapted from Belchetz PE, Plant TM, Nakai Y, et al. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin releasing hormone. *Science*. 1978;202:631–633. Copyright © 1978 by American Association for the Advancement of Science.)



• **Fig. 17.4** Regulation of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) secretion. Locally synthesized and systemic hormones regulate the pulsatile secretion of GnRH from the hypothalamus into the portal circulation. GnRH and a number of steroid and peptide hormones regulate the synthesis of gonadotropin subunits, including the common α-subunit and specific β-subunits for LH and FSH, and the formation and secretion of FSH and LH. *CRH,* corticotropin-releasing hormone; E_2, estradiol; *P,* progesterone.

circulation (see Fig. 17.2).[4,6] A physiologic role for GAP has not been established.[6]

In humans, GnRH neurons are located primarily in the arcuate nucleus of the medial basal hypothalamus and the preoptic area of the anterior hypothalamus.[7] The population of GnRH-producing neurons is relatively limited and is in the range of 1000 to 2000. The neurons that produce GnRH originate from the olfactory area during embryogenesis.[7] GnRH and olfactory neurons migrate together along cranial nerves connecting the nose and forebrain to the hypothalamus during embryologic development, and disruption of this process causes idiopathic hypogonadotropic hypogonadism with anosmia, or Kallmann syndrome.[7] Individuals with Kallmann syndrome usually have lack of pubertal development and subsequent infertility due to deficient GnRH and pituitary gonadotropins. The neuronal proteins anosmin 1 (encoded by the *KAL1* gene) and fibroblast growth factor receptor type 1 (encoded by the *FGFR1* gene) affect olfactory and GnRH neuron migration. Mutations in these genes cause Kallmann syndrome.[7] Data suggest that mutations of the genes for nasal embryonic LH-releasing hormone factor *(NELF)* and chromodomain helicase DNA-binding protein 7 *(CHD7)* may cause Kallmann syndrome, but this correlation is not as conclusively established as it is for *KAL1* and *FGFR1*.[7] Selective clinical phenotypes in men and women are highly associated with genetic causes of Kallman syndrome. Synkinesia *(KAL1)*, dental agenesis *(FGF8/FGFR1)*, digital bony abnormalities *(FGF8/FGFR1)*, and hearing loss *(CHD7)* may be useful for prioritizing genetic testing.[8,9]

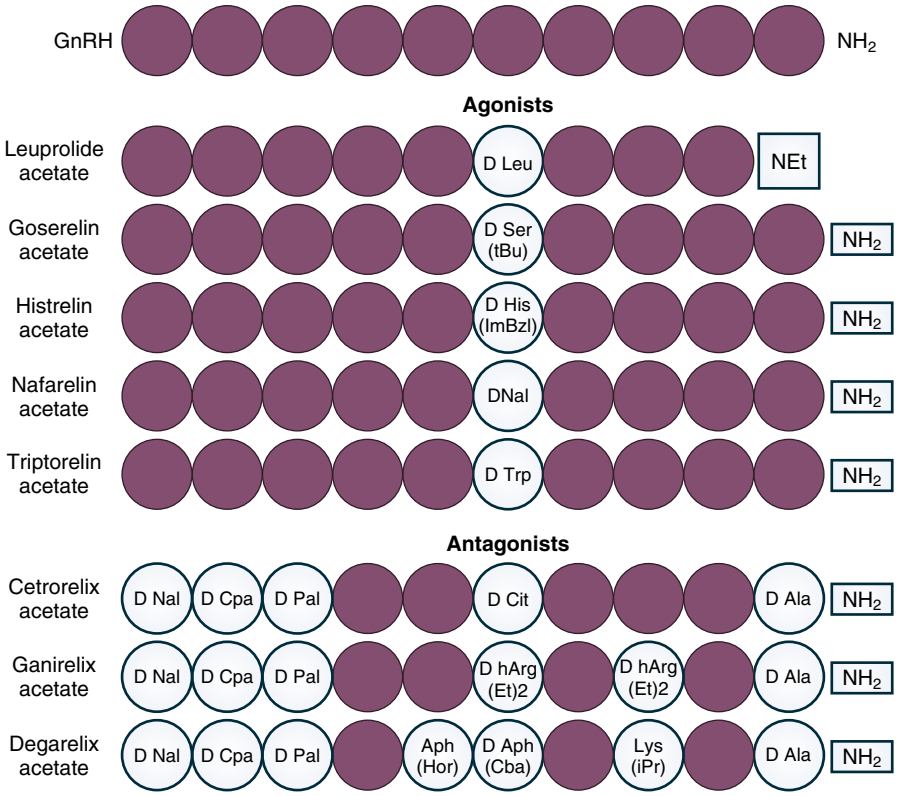
Knobil demonstrated in a pioneering series of experiments that normal gonadotropin secretion requires pulsatile GnRH discharge within a critical frequency and amplitude.[10] The periodicity and amplitude of the pulsatile rhythm of GnRH and gonadotropin secretion are crucial in regulating gonadal activity and therefore the entire reproductive axis (Fig. 17.3). The self-priming effect of GnRH in upregulating its receptors on pituitary gonadotropin-producing cells manifests only at the physiologic periodicity of 60 to 90 minutes.[11,12] Slower frequency causes anovulation and amenorrhea because of inadequate stimulation. Higher frequency or constant exposure to GnRH also gives rise to anovulation by downregulating expression of GnRH receptors, thereby abolishing gonadotropin responses.

The activation of gene expression for gonadotropin subunits, including the common α-subunit and specific β-subunits for LH and FSH, dimerization of αβ subunits, and glycosylation appear to be governed by intermittency of GnRH inputs to pituitary gonadotrophs.[13] In humans, measurement of LH pulses is commonly used as an indication of GnRH pulsatile secretion.[14] The LH pulse frequency is approximately 90 minutes during the early follicular phase, 60 to 70 minutes during the late follicular phase, 100 minutes during the early luteal phase, and 200 minutes during the late luteal phase.[15] This variation is accompanied by predictable changes in FSH and LH levels and ovarian steroid release during these phases of the menstrual cycle. More rapid pulse frequencies favor LH secretion, whereas slower pulse frequencies favor FSH. It appears that variations in GnRH pulse frequency markedly influence both the absolute levels and the ratio of LH and FSH release.

Regulation of Gonadotropin-Releasing Hormone Secretion

Cyclic, predictable menses require the pulsatile release of GnRH within a critical range of frequencies. Pulsatile, rhythmic activity is an intrinsic property of GnRH neurons, and various hormones and neurotransmitters modulate this rhythm (Fig. 17.4).

The variations in GnRH pulse frequency are achieved, in part, by gonadal steroid feedback. Estradiol increases GnRH pulse frequency, and elevated progesterone levels decrease GnRH pulsatility.[13] Increased progesterone levels may decrease GnRH pulse



• **Fig. 17.5** Gonadotropin-releasing hormone (GnRH) agonist and antagonist analogues in clinical practice. *Purple circles* indicate amino acids in the wild-type GnRH decapeptide, and *white circles* are labeled with the changes made to the analogues. (Modified from Millar RP, Lu Z, Pawson AJ, et al. Gonadotropin-releasing hormone receptors. *Endocr Rev*. 2004;25:235–275. Copyright © 2004 by the Endocrine Society.)

frequency and thereby lead to preferential biosynthesis and secretion of FSH, as observed in the late luteal phase.[13]

GnRH pulsatility is also modulated by the actions of locally released neurotransmitters. Norepinephrine stimulates GnRH release, whereas dopamine exerts an inhibitory effect (see Fig. 17.4).[16] β-Endorphin and other opioids may suppress the hypothalamic release of GnRH.[17, 18] It was proposed that sex steroids enhance the activity of endogenous opioids that exert an inhibitory effect on GnRH secretion.[15] The negative effect of opioids on GnRH secretion is clinically explicable because the reduced GnRH secretion associated with hypothalamic amenorrhea may be mediated by an increase in endogenous opioid inhibitory tone.[19]

Estrogen signaling to GnRH neurons appears to be critical for suppressing FSH and LH and for coordinating the preovulatory surge release of LH. The precise roles of estrogen receptors α and β (ERα and ERβ, respectively) within the GnRH neurons or estradiol-sensitive afferent neurons in these negative and positive feedback effects of estrogen are not well understood.[20] Stimulation of FSH release after treatment of premenopausal women with an aromatase inhibitor and in vitro studies suggest that estrogen locally produced by aromatase activity in hypothalamic neurons may regulate gonadotropin secretion.[21,22]

Binding of the peptide kisspeptin to its G protein–coupled receptor KISS1R (previously known as GPR54), which is expressed in GnRH neurons, stimulates GnRH release in the hypothalamus.[23,24] Kisspeptin neurons contact GnRH neurons and act at the cell body and the nerve terminals.[25] Kisspeptin can act directly on GnRH neurons or indirectly through synaptic input from other neurons to inhibit inwardly rectifying potassium channels and activate nonspecific cation channels, producing long-lasting depolarization and an increased action-potential firing rate.[26] Mutations or knockout of KISS1R produces isolated hypogonadotropic hypogonadism in humans and mice, indicating that signaling through this receptor is essential for sexual development and function.[23,24] Moreover, kisspeptin-expressing and KISS1R-expressing neurons may be critical targets for the negative and positive feedback actions of estrogen and progesterone.[27]

Gonadotropin-Releasing Hormone Analogues

The half-life of GnRH is short (2–4 minutes) because it is degraded rapidly by peptidases in the hypothalamus and pituitary gland.[28] These peptidases cleave the bonds between amino acids 5 and 6, 6 and 7, and 9 and 10. Analogues of GnRH with different properties have been synthesized by alteration of amino acids at these positions. Many agonistic and antagonistic GnRH analogues with various biologic effects have been produced.

Peptide Gonadotropin-Releasing Hormone Agonists

Several GnRH agonist peptides are generated by substitution of amino acids at the 6 or 10 position (Fig. 17.5). The increased biologic activity of agonistic peptides has been attributed to their high binding affinity to GnRH receptors and their reduced susceptibility to enzymatic degradation. An amino acid substitution at position 6 gives rise to metabolic stability, whereas replacement of the carboxy-terminal glycinamide residue by an ethylamide group increases strikingly the affinity for the receptors.[28,29]

Peptide GnRH agonists are administered subcutaneously, intranasally, or intramuscularly. An initial agonistic action (i.e., flare effect) is associated with an increase in the circulating levels of LH and FSH. The most prominent agonistic response is observed during the early follicular phase, when the combined effects of GnRH agonist and elevated levels of estradiol create a large reserve pool of gonadotropins.[30]

Administration of a long-acting depot formulation of a GnRH agonist gives rise to an initial flare followed by downregulation of the gonadotropin-gonadal axis within 1 to 3 weeks. The initial downregulation effect is caused by desensitization, whereas the sustained response results from loss of receptors and the uncoupling of the receptor from its effector system.

The US Food and Drug Administration (FDA) approved the use of these agonists for the treatment of GnRH-dependent precocious puberty, endometriosis, and prostate cancer. Another indication is preoperative hematologic improvement of patients with anemia caused by uterine leiomyomas. Off-label indications for GnRH agonists include downregulation of the pituitary during ovulation induction, induction of endometrial atrophy before endometrial ablation surgery, and prevention of menstrual bleeding in patients with coagulation defects. GnRH agonists have also been used to suppress ovarian steroidogenesis in hirsute patients.[31]

The most prominent side effects of long-term use of depot GnRH agonist formulations are caused by estrogen deficiency. Depot GnRH agonists induce a menopause-like state characterized by hot flashes, vaginal dryness, bone resorption, and osteopenia. Osteopenia is reversible in young women if treatment is maintained for no more than 6 months.[32, 33] The risk-benefit ratio must be considered carefully before GnRH agonist treatment is extended for longer periods. Add-back regimens using low-dose estrogens or progestins, or both, administered along with GnRH agonists have provided a means to overcome these side effects and to extend the length of agonist therapy.[34]

Peptide Gonadotropin-Releasing Hormone Antagonists

Inhibition of a premature LH rise by GnRH agonists requires at least 7 days, because it is accompanied by an initial stimulation of GnRH receptors before gonadotroph desensitization is achieved. In contrast, GnRH antagonists compete directly with endogenous GnRH for receptor binding and therefore rapidly inhibit secretion of gonadotropin and steroid hormones (see Fig. 17.5).[35–38] This property conveys a potential advantage over GnRH agonists in the management of ovarian stimulation. However, because of the constant need to block endogenous GnRH, much higher doses of antagonists are required. The GnRH antagonists incorporate a number of amino acid substitutions in the amino-terminal domain (involved in receptor activation) combined with a D-amino acid substitution for Gly6, which enhances the βII-type bend necessary for receptor binding.[3] GnRH antagonists have the advantage of inducing an immediate decrease in circulating gonadotropin levels with rapid reversal.[35–38] GnRH antagonists are alternative drugs to GnRH agonists for the prevention of a natural LH surge during ovulation induction by injectable FSH.[39] Use of GnRH antagonists has become popular in ovulation induction protocols for IVF (see Fig. 17.5).[39]

Nonpeptide Gonadotropin-Releasing Hormone Antagonists

Small molecular compound collections have been screened using mammalian cells that heterologously expressed human GnRH type I receptor. These studies led to the identification of synthetic compounds that bind to the GnRH type I receptor and block signal transduction. Several companies have manufactured orally administered GnRH antagonists intended for various indications, including endometriosis.[1] The FDA approved the oral GnRH antagonist named elagolix to treat endometriosis-associated pelvic pain.[2]

Reproductive Functions of the Anterior Pituitary

Gonadotrophs

Gonadotrophs are specialized cell types of the anterior pituitary that synthesize and secrete LH and FSH. These cells constitute 7% to 15% of the total number of anterior pituitary cells and are detected in this location from early fetal life.[40] Most gonadotrophs are capable of synthesizing both LH and FSH.[40, 41] LH and FSH are each composed of two distinct, noncovalently associated protein α-subunits and β-subunits (see Fig. 17.4). In the gonadotroph, the subunit genes encode the subunit precursors. Gonadotrophs contain cell-surface GnRH type I receptors that mediate the action of GnRH. These receptors belong to the seven-transmembrane domain and G protein–coupled receptor family.

Gonadotropin-Releasing Hormone Receptor

In humans, hypothalamic GnRH regulates gonadotropin secretion through the pituitary GnRH type I receptor by activation of $G_{q/11}$.[42] Although the predominant coupling of the type I GnRH receptor in the gonadotroph is through $G_{q/11}$ stimulation, signal transduction can occur through other G proteins and potentially by G protein–independent means.[3,42] A number of downstream signaling cascades include protein kinase C (PKC)–, Ca^{2+}-, and tyrosine kinase–dependent pathways.[3] In mouse pituitary gonadotrophs, the GnRH receptor activates several mitogen-activated protein (MAP) kinase cascades, including the ERK1/2, the JUN amino-terminal kinase (JNK), the p38 MAP kinase, and the big MAP kinase (BMK1/ERK5).[3] The cross-talk between these pathways remains to be clarified.

Luteinizing Hormone and Follicle-Stimulating Hormone

The α-subunits of human LH, FSH, thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG) have an identical polypeptide structure. In contrast, the β-subunit of each hormone has a unique amino acid sequence and confers the specific activity of the αβ-heterodimer. Each subunit is rich in cysteine and contains multiple disulfide linkages. Each subunit also contains multiple carbohydrate moieties that play important roles in the biologic activity and metabolism of these hormones.

The human common α-subunit gene encodes a precursor polypeptide with a 24–amino acid leader sequence that is cleaved post-translationally to produce the mature 92–amino acid α-subunit. The β-subunits of human FSH, LH, and hCG contain 117, 121, and 145 amino acids, respectively.[43–46] On binding of GnRH to its receptor, the biosynthesis of the gonadotropins proceeds by transcription of the subunit genes, translation of the subunit messenger ribonucleic acids (mRNAs), post-translational modifications of the precursor subunits and subunit folding and combination, mature hormone packaging, and secretion (see Fig. 17.4).

The human LH and hCG β-subunit genes are located on chromosome 19q13.3, which contains a cluster of seven β-subunit–like genes.[44] Five of these sequences are noncoding pseudogenes arranged in groups of tandem and inverted pairs. Only LH and hCG β-subunit genes give rise to two distinct and functional mRNA species. The LH β-subunit mRNA encodes a 145–amino acid precursor protein that is later cleaved to produce a 24–amino acid leader peptide and a 121–amino acid, biologically active, mature peptide. The hCG β-subunit mRNA also encodes a 145–amino acid protein. This protein, however, is not processed post-translationally and functions as the biologically active hCG β-subunit. The amino acid sequences of the human LH and hCG β-subunits are 82% homologous. These two β-subunits confer identical biologic activities when associated with the α-subunit.[44–46]

A single gene encodes the FSH β-subunit.[47] Complementary DNA encoding human FSHβ, LHβ, or hCGβ in combination with the complementary DNA of the α-subunit is expressed in mammalian cells in culture. These cells can synthesize these proteins, modify them after translation, glycosylate and combine the subunits, and secrete them as intact FSH, LH, or hCG.[48] Recombinant gonadotropins are used clinically to stimulate gonadal function.[49]

Regulation of Circulating Levels of Follicle-Stimulating Hormone and Luteinizing Hormone

The molecular mechanisms responsible for formation and combination of the α-subunits and β-subunits of FSH and LH are not completely understood. Production rates of α-subunits and β-subunits are regulated in part by negative feedback by estrogen, which regulates the pulsatile release of GnRH from the hypothalamus.[48,50] The pituitary contains more α-subunit than β-subunit mRNA, and readily detectable levels of free α-subunit are present in serum. The free β-subunit is present at relatively low levels in the pituitary and is rarely found in serum or urine. The specific β-subunit may be the rate-limiting factor in the synthesis of these glycoprotein hormones.

Inhibin, activin, and follistatin were first identified as gonadal hormones that exerted selective effects on FSH secretion.[51] Although the primary source of inhibin remains the ovary, activin and follistatin are produced in extragonadal tissues and can exert effects on FSH through an autocrine-paracrine mechanism. Inhibin-B is secreted by ovarian granulosa cells during the follicular phase (under the control of FSH) and inhibin-A by the corpus luteum in the luteal phase (under the control of LH). Inhibins act synergistically with estradiol to inhibit FSH secretion. Activin can directly stimulate FSH biosynthesis and release from the gonadotroph cells of the pituitary gland.[51] Follistatin can negatively regulate biologic functions of activin via binding and prevent it from interacting with the activin receptor at the cell membrane.[52]

Serum levels of gonadotropins are proportional to their secretion rates and serum half-lives, which are regulated by the number of carbohydrate residues. The sialic acid content of gonadotropic hormones and other glycoproteins has a marked effect on their rate of clearance and influences their apparent molecular size.[53] The higher content of sialic acid in FSH compared with LH is responsible for slower clearance of FSH, which has a half-life of 3 to 4 hours. LH, which has a half-life of 20 minutes, has the most rapid clearance rate. The hCG is highly sialylated and has the longest half-life (24 hours).

Ovary

The ovary is essential for periodic release of oocytes and production of the steroid hormones, estradiol and progesterone. These activities are integrated into the cyclic repetitive process of follicular maturation, ovulation, and formation and regression of the corpus luteum. The ovary fulfills two major objectives: generation of a fertilizable ovum and preparation of the endometrium for implantation through the sequential secretion of estradiol and progesterone.[49] The ovarian follicle comprising the egg and surrounding granulosa and theca cells constitutes the fundamental functional unit of the ovary.

Adult human ovaries are oval bodies with a length of 2 to 5 cm, a width of 1.5 to 3 cm, and a thickness of 0.5 to 1.5 cm. The ovaries lie near the posterior and lateral pelvic wall and are attached to the posterior surface of the broad ligament by the peritoneal fold, called the *mesovarium*.

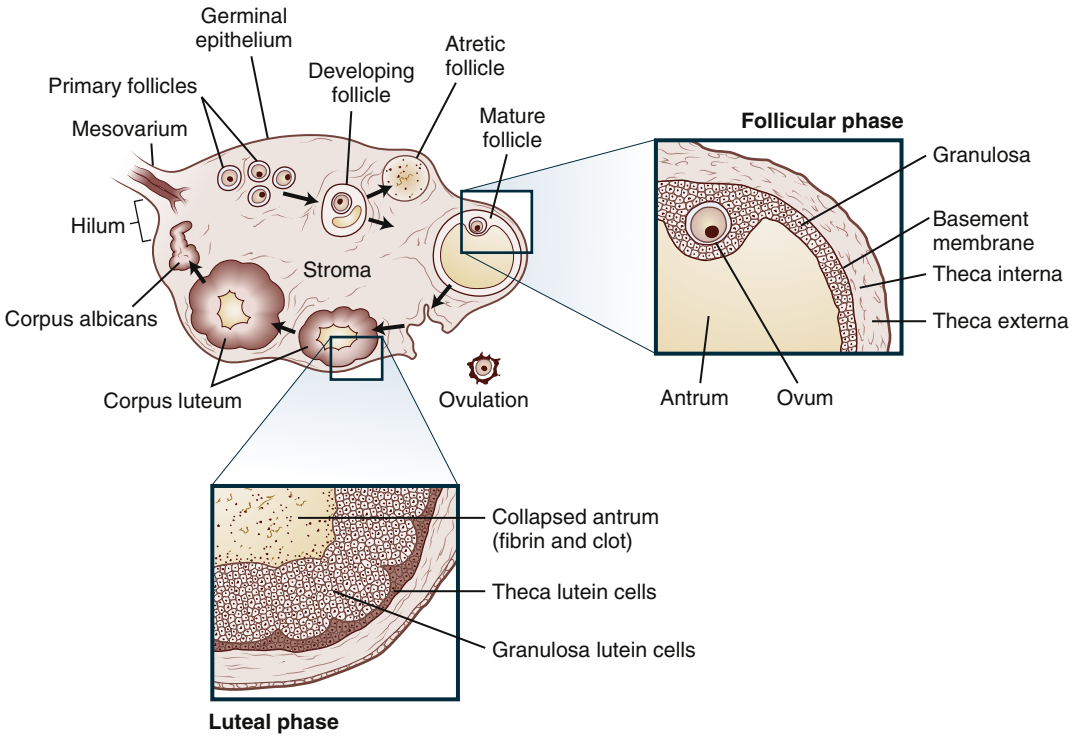
The ovary consists of three structurally distinct regions: an outer cortex containing the surface germinal epithelium and the follicles, a central medulla consisting of stroma, and a hilum around the area of attachment of the ovary to the mesovarium (Fig. 17.6). The hilum is the point of attachment of the ovary to the mesovarium. It contains nerves, blood vessels, and hilus cells, which have the potential to become active in steroidogenesis or to form androgen-secreting tumors. These cells are similar to the testosterone-producing Leydig cells of the testes.

The outermost portion of the cortex, called the *tunica albuginea*, is covered by a single layer of surface cuboidal epithelium called the *germinal epithelium*. The oocytes, enclosed in complexes called *follicles*, are in the inner part of the cortex, embedded in stromal tissue. One dominant follicle is recruited for ovulation during each cycle (see Fig. 17.6). The preovulatory follicle transforms into a corpus luteum after ovulation. In the absence of pregnancy, the corpus luteum regresses to become the corpus albicans (see Fig. 17.6). The stromal tissue is composed of connective tissue and interstitial cells, which are derived from mesenchymal cells and are presumed to have the ability to respond to LH or hCG with the production of androstenedione. The central medullary area of the ovary is derived largely from mesonephric cells.

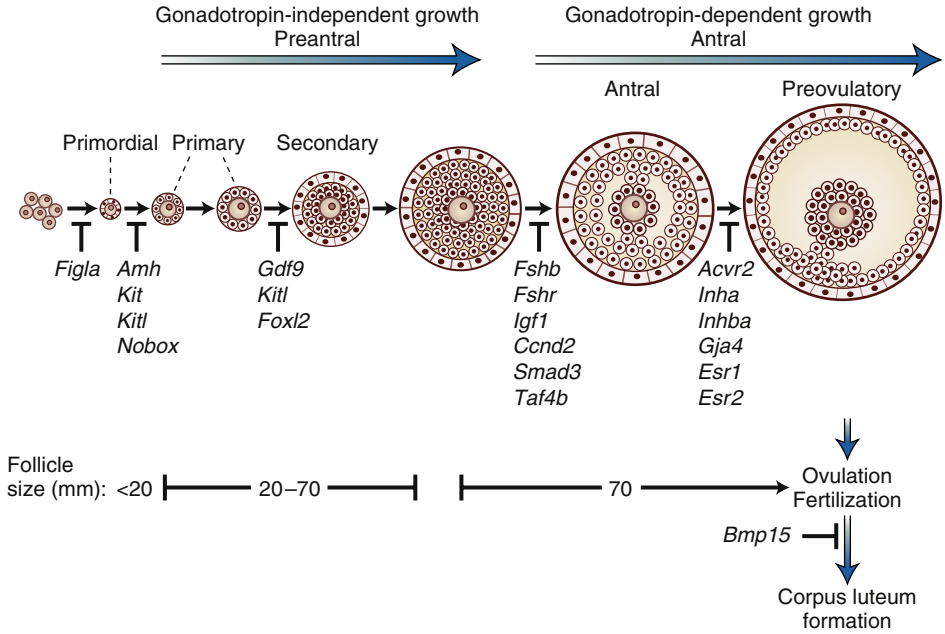
Genetic Determinants of Ovarian Differentiation and Folliculogenesis

Nascent components of the human ovary develop long before a distinct ovary-like organ can be discerned. The female germ cells are formed during embryogenesis when the precursors of primordial germ cells differentiate from somatic lineages of the embryo and take a unique route from the base of the yolk sac along the hindgut to reach the genital ridge. This starts the differentiation of female gonads (ovaries) at the genital ridge. The originally undifferentiated gonad differentiates along a female pathway, and the newly formed oocytes proliferate and subsequently enter meiosis.[49]

Ovarian differentiation and folliculogenesis depend on coordinated expression and interaction of a multitude of genes.[54] Targeted gene disruption or insertion in mice has made it possible to inquire about the function of specific genes in ovarian differentiation and folliculogenesis. Fig. 17.7 summarizes the biologic roles of some of these genes.[54] Genetically altered mice represent a first step in attempts to understand in vivo the various gene interactions that result in a functional ovary. Ovarian pathologic conditions in transgenic mice closely resemble disorders observed in mutant human homologues, as exemplified in cases involving the



• **Fig. 17.6** Functional anatomy and changes in the adult ovary during an ovarian cycle. (From Carr BR, Wilson JD. Disorders of the ovary and female reproductive tract. In: Braunwald E, Isselbacher KJ, Petersdorf RG, et al, eds. *Harrison's Principles of Internal Medicine*. 11th ed. New York, NY: McGraw-Hill; 1987:1818–1837.)



• **Fig. 17.7** Developmental stages at which certain murine genes affect oogenesis. Data from transgenic mice with disruption of various genes have delineated critical roles of several genes during various phases of the follicular development. Preantral follicular growth is thought to be gonadotropin independent, whereas antrum formation and follicular maturation require the action of follicle-stimulating hormone (FSH). *Acvr2,* activin type II receptor; *Amh,* antimüllerian hormone; *Bmp15,* bone morphogenetic protein-15; *Ccnd2,* cyclin d2; *Esr1,* estrogen receptor-α; *Esr2,* estrogen receptor-β; *Figla,* factor in the germline-α; *Foxl2,* forkhead box L2; *Fshb,* FSHβ-subunit; *Fshr,* FSH receptor; *Gdf9,* growth differentiation factor 9; *Gja4,* gap junction protein connexin 37; *Igf1,* insulin-like growth factor 1; *Inha,* inhibin α-subunit; *Inhba,* inhibin subunit-βA; *Kit,* kit receptor; *Kitl,* kit-ligand; *Nobox,* newborn oogenesis homeobox gene; *Smad3,* Sma mothers against decapentaplegic-3; *Taf4b,* TATA-box-binding protein-associated factor-4b. (Modified from Simpson JL, Rajkovic A. Ovarian differentiation and gonadal failure. *Am J Med Genet*. 1999;89:186–2000; Choi Y, Rajkovic A. Genetics of mammalian folliculogenesis. *Cell Mol Life Sci*. 2006;63:579–590.)

FSH β-subunit and FSH receptor. Many mouse models of ovarian pathologic conditions are available.[49,54] They can be divided into mice that have prenatal ovarian insufficiency with disordered gonad formation and diminished number of germ cells or absent germ cells and mice that have postnatal ovarian insufficiency as a result of defects at various stages of folliculogenesis (see Fig. 17.7).[54] These models should lead to the identification of genetic and molecular mechanisms responsible for the development and function of the human ovary.

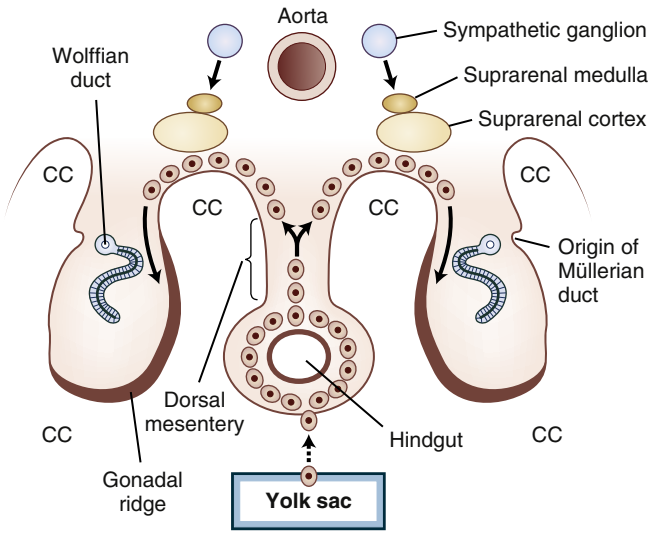
In humans, certain gene defects give rise to specific defects in folliculogenesis. This was demonstrated by the discovery of a heterozygous mutation in the bone morphogenetic protein 15 gene *(BMP15)* that caused ovarian dysgenesis. BMP15 is a growth and differentiation factor that is primarily expressed in the oocyte and stimulates folliculogenesis and granulosa cell growth. In vitro, mutant BMP15 reduced granulosa cell growth and antagonized the stimulatory activity of wild-type protein on granulosa cell proliferation. In vivo, this mutation was associated with familial ovarian dysgenesis, indicating that the action of BMP15 is required for progression of human folliculogenesis.[55] A comprehensive discussion of genes responsible for ovarian development, folliculogenesis, and ovulation is provided in an excellent review article by Edson and colleagues.[49]

Oocytes

The fertilization of an oocyte by a spermatozoon gives rise to a zygote that starts to divide rapidly. An eight-cell embryo is formed usually on the third day after fertilization. Up to that point, all embryonic cells are morphologically identical, truly totipotential, and capable of starting a new individual or any lineage. The formation of a 16-cell morula marks the beginning of the process of differentiation, with cells being allocated to the inside or outside of the embryo. At the next stage, the blastocyst, three lineages are defined: trophectoderm, which is the precursor of the placenta; epiblast, which gives rise to the somatic cells of the embryo; and primitive endoderm, which eventually forms the yolk sac. After the embryo implants, a group of cells within the epiblast form the precursors of the primordial germ cells, the first cells of the future ovary to be defined.[49] The extraembryonic trophectoderm and primitive endoderm, which surround the epiblast cells of the postimplantation egg cylinder, are the sources of signals that instruct this small number of epiblast cells to become primordial germ cells; the rest of the cells commence differentiation into somatic tissues. The first primordial germ cell precursors express a key protein named PRDM1 (PRDI-BF1-RIZ domain containing 1, formerly called BLIMP1); these precursor cells represent the first cells of the mammalian embryo with committed cell fates.[49]

The primordial germ cells first become recognizable as a cluster of cells that stain intensely for alkaline phosphatase activity; these epiblast cells are observed at the base of the yolk sac before formation of the allantois.[49] Studies have confirmed that these cells are the only primordial germ cells because their ablation results in embryos without germ cells, whereas transplantation of these cells leads to their proliferation followed by migration to the genital ridge.[49]

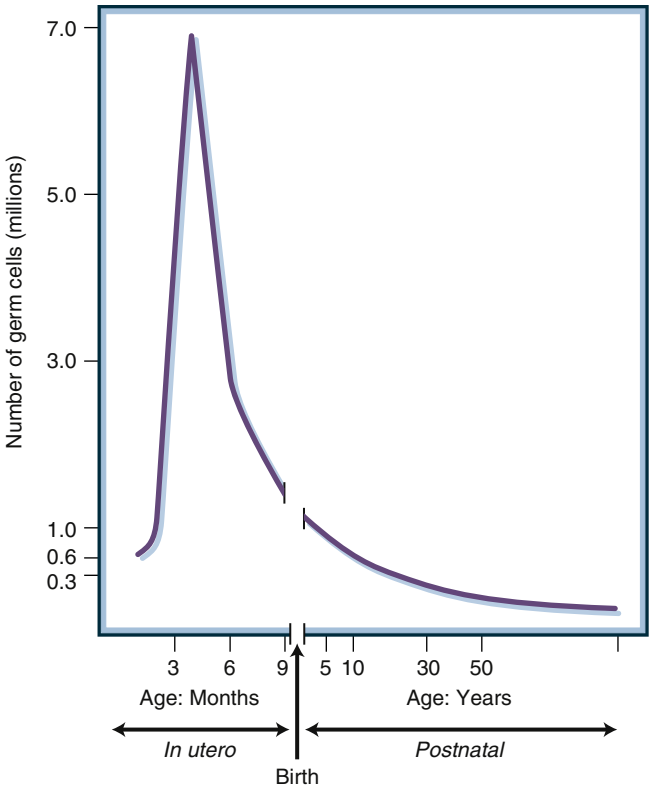
With the use of alkaline phosphatase as a marker, migration of these primordial germ cells from the yolk sac–epiblast junction to the indifferent gonad can be tracked; eventually, the ovary forms and permits the primordial germ cells to differentiate into oocytes. The oocytes enter meiosis and subsequently arrest. Entry into meiosis marks the developmental stage at which any progenitor cells that are capable of differentiating to oocytes disappear. The meiotically arrested oocytes eventually become surrounded by pre-granulosa cells and form individual primordial follicles, the resting pool of oocytes that have the potential to be recruited into the growing follicle pool during the postpubertal stage to be fertilized and to contribute to the next generation. These phenomena have been primarily observed in mice and are thought to be applicable to humans.[49]



• **Fig. 17.8** Transverse section of the caudal region of a 5-week embryo shows the location of gonadal ridges, the primordium of the adrenal glands, and the migration path of primordial germ cells. From the third week on, germ cells of epiblast origin located at the base of the yolk sac cross the dorsal mesentery of the hindgut and migrate to the gonadal ridges. By the end of the fifth week, rapid division of primordial germ cells, gonadal epithelium, and mesenchyme starts the early gonad that differentiates subsequently into the ovary in a 46,XX fetus. *CC,* coelomic cavity. (Modified from Moore K. *The Developing Human.* Philadelphia, PA: WB Saunders; 1983.)

Primordial germ cells of epiblast origin migrate to cross a remarkably long distance from the base of the yolk sac to the genital ridge in the fetus by ameboid movements with the aid of pseudopodia.[56] This long route of migration along the dorsal mesentery of the hindgut is interrupted only by the required lateral crossing of the coelomic angle at the level of the genital ridge (Fig. 17.8). The triggers that initiate primordial germ cell migration and the chemoattractants required for directional movement toward the genital ridge are beginning to be uncovered. A critical trigger may be expression of a key receptor on the primordial germ cell and expression of the secreted chemoattractants from the genital ridge. For example, suppression of transforming growth factor-β (TGFβ) signaling leads to enhanced migration due to reduction in the levels of TGFβ-induced collagen type 1 in the extracellular matrix.[57] An extracellular matrix gradient along the path of migration is important, and if too much matrix is laid down, germ cells show reduced migration. The KIT ligand (KITLG) may function as an effective chemoattractant for the primordial germ cells. The phosphatidylinositol 3-kinase (PI3K)/AKT and SRC kinase pathways are involved downstream of KIT in the primordial germ cell.[58]

Germ cells appear to be unable to persist outside the genital ridge, which may be viewed as the only region competent to sustain gonadal development. By the same token, germ cells play an indispensable role in the induction of gonadal development. No functional gonad can form in the absence of germ cells.



• **Fig. 17.9** Age-dependent changes in germ cell number in the human ovary. The highest number of oocytes is found in the ovaries of a human fetus at midgestation. This number decreases sharply during the third trimester. After birth, the progressive decline in the number of ovarian follicles containing oocytes continues until complete depletion at menopause. (From Baker TG. A quantitative and cytological study of germ cells in the human ovaries. *Proc R Soc Biol Sci*. 1963;158:417–433.)

On arrival at the genital ridge by the fifth week of gestation, the premeiotic germ cells are referred to as *oogonia*.[59] During the subsequent 2 weeks of intrauterine life (weeks 5–7 of gestation, or the *indifferent stage*), the primordial gonadal structure constitutes no more than a bulge on the medial aspect of the urogenital ridge (see Fig. 17.8). This protuberance is created by proliferation of surface (coelomic) germinal epithelium, by growth of the underlying mesenchyme, and by oogonial multiplication. The oogonia total 10,000 by about 6 to 7 weeks of intrauterine life.[59] Because meiosis and oogonial atresia are not occurring, the actual number of germ cells is dictated by mitotic division at this time.[59]

During the indifferent phase, the gonadal cortex and medulla are first delineated. However, short of cytogenetic evidence, the precise sexual identity of the gonadal ridge cannot be ascertained at this point. Nevertheless, the absence of testicular development beyond 7 weeks of gestation is considered presumptive evidence of formation of the ovary. Additional clues to the sexual identity of the gonad can be derived from the detection of oogonial meiosis at about 8 weeks of gestation because no comparable process is observed in the testis until puberty. The sexual identity of the gonadal ridge is histologically clear by 16 weeks of gestation, when the first primordial follicles can be visualized.

By about 8 weeks of intrauterine life, persistent mitosis increases the total number of oogonia to 600,000 (Fig. 17.9). From this point on, the oogonial endowment is subject to three simultaneous processes: mitosis, meiosis, and oogonial atresia. Stated differently, the onset of oogonial meiosis and oogonial atresia is superimposed on oogonial mitosis. As a result of the combined impact of these processes, the number of germ cells peaks at 6 to 7 × 10^6 by 20 weeks of gestation (see Fig. 17.9). At this time, two-thirds of the total germ cells are intrameiotic primary oocytes; the remaining third can still be viewed as oogonial. The midgestational peak and the postpeak decline are accounted for in part by the progressively decreasing rate of oogonial mitosis, a process destined to end entirely by about the seventh month of intrauterine life. Equally relevant is the increasing rate of oogonial atresia, which peaks at 5 months of gestation. During this period, regulation of the ovarian developmental process is complex and probably involves a diverse group of genes (see Fig. 17.7).

From midgestation onward, relentless and irreversible attrition progressively diminishes the germ cell endowment of the gonad.[60] About 50 years later, it is exhausted. For the most part, this is accomplished through follicular atresia rather than oogonial atresia, begins at about month 6 of gestation, and continues throughout life (see Fig. 17.9). In contrast, oogonial atresia is destined to end at 7 months of intrauterine life as follicular atresia sets in. Follicular atresia has a profound effect on germ cell endowment, because only 1 to 2 × 10^6 germ cells are present at birth (see Fig. 17.9).[60] Remarkably, this dramatic depletion of the germ cell mass occurs during a period as short as 20 weeks. No similar rate of depletion occurs earlier or subsequently. Consequently, newborn girls enter life still far from realizing their reproductive potential but having lost as much as 80% of their germ cell endowment. The germ cell mass decreases further to approximately 300,000 by the onset of puberty. Of these follicles, only 400 to 500 (<1% of the total) are recruited for ovulation in the course of a reproductive life span.

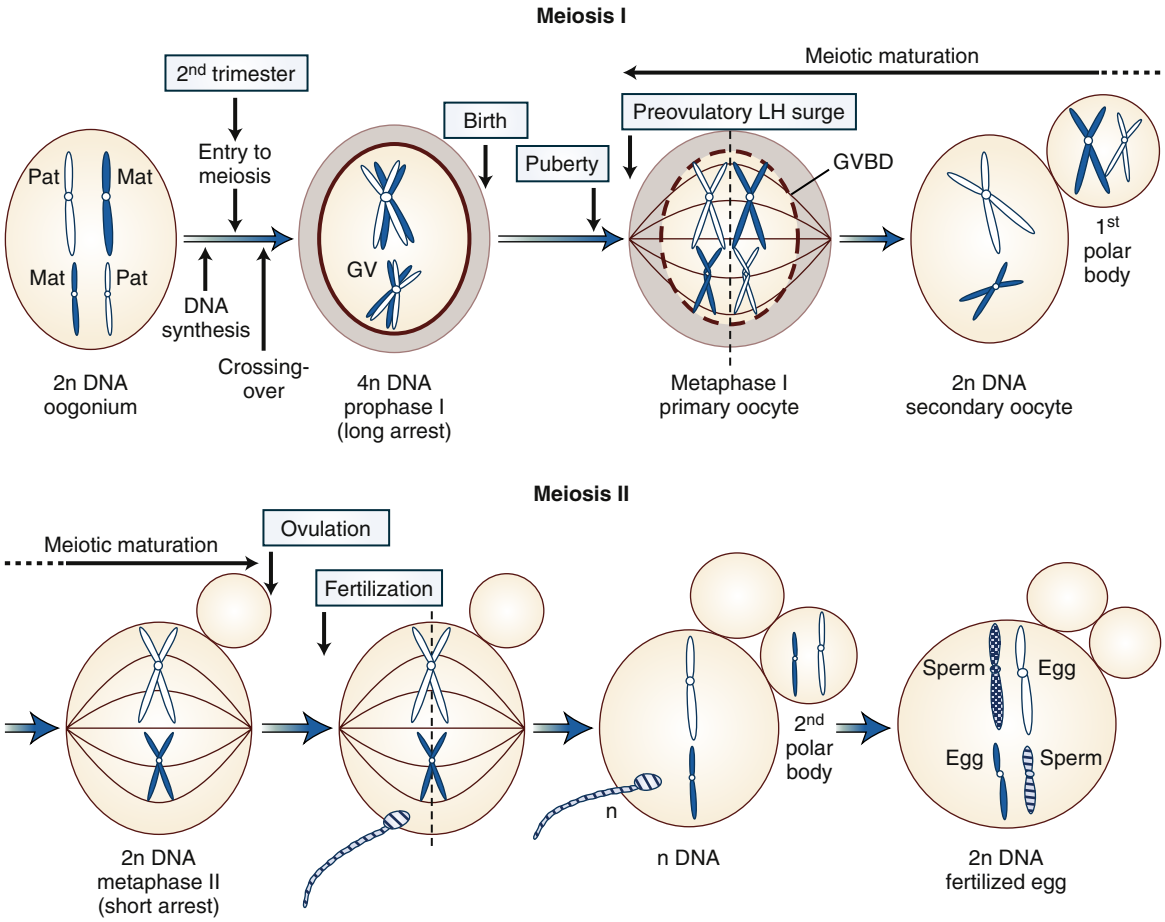
Between weeks 8 and 13 of fetal life, some of the oogonia depart from the mitotic cycle to enter the prophase of the first meiotic division. This change marks the conversion of these cells to primary oocytes well before actual follicle formation. Meiosis (beginning at about 8 weeks of gestation) provides temporary protection from oogonial atresia, allowing the germ cells to invest themselves with granulosa cells and to form primordial follicles. Oogonia that persist beyond the seventh month of gestation and have not entered meiosis are subject to oogonial atresia; consequently, no oogonia are usually present at birth.

Once formed, the primary oocyte persists in prophase of the first meiotic division until the time of ovulation, when meiosis is resumed and the first polar body is formed and extruded (Fig. 17.10). Although the exact cellular mechanisms responsible for this meiotic arrest remain uncertain, it is presumed that a granulosa cell–derived meiosis inhibitor is responsible. This hypothesis is based on the observation that denuded (granulosa-free) oocytes are capable of spontaneously completing meiotic maturation in vitro.

The primary oocyte is converted into a secondary oocyte by completion of the first meiotic metaphase and formation of the first polar body, which occurs before ovulation but after the LH surge. At ovulation, the secondary oocyte and the surrounding granulosa cells (cumulus oophorus) are extruded and enter the fallopian tube. If sperm penetration occurs, the secondary oocyte undergoes a second meiotic division, after which the second polar body is eliminated (see Fig. 17.10).

Granulosa Cell Layer

In the developing ovaries of a human female fetus, oocytes initially exist as germ cell clusters before an ovarian follicle is formed.

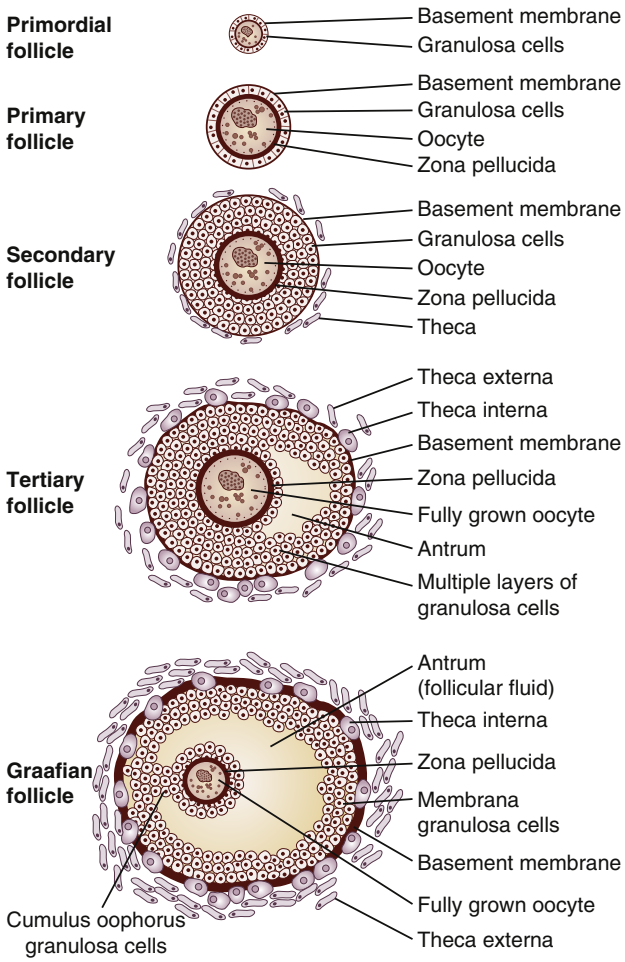


• **Fig. 17.10** Meiotic cell division. During meiosis, the chromosomes that were inherited from the parents of the individual and stored in gonads are processed to prepare their genetic material for transmission to the offspring. Meiosis occurs exclusively in germ cells and serves two critical purposes: generation of germ cells genetically distinct from the somatic cells and generation of a mature egg (or sperm) with a reduction in the number of chromosomes from 46 to 23. Genetic recombination through crossover of genes between homologous chromosomes and random assortment of (grand-) maternal and (grand-) paternal chromosomes into daughter cells during the first meiotic division are responsible for the first function of meiosis, maintenance of genetic diversity. The second function is provided by a reduction in the number of chromosomes so that each daughter cell, or ovum, receives randomly one chromosome from each of the 23 pairs. During fertilization, the fusion of ovum and sperm, each of which contributes 23 chromosomes, produces a genetically novel individual with 46 chromosomes. The chromosome marked as *white* in the oogonium *(upper left corner)* originates from the father of the female fetus, whereas the *blue* chromosome comes from the mother of the fetus. The random exchange of genes (alleles) between homologous chromosomes (crossover) takes place before the meiotic arrest in the prophase I stage before birth. After birth, the oocytes of this child remain in meiotic arrest until puberty. In the developing oocyte in the Graafian follicle, meiosis I is resumed immediately after the preovulatory luteinizing hormone (LH) surge during each ovulatory cycle. Meiotic maturation is defined as the period from the breakdown of the oocyte's nucleus (germinal vesicle [GV]) until the oocyte reaches metaphase II (i.e., transition from oocyte to egg). A second and short meiotic arrest occurs at metaphase II until the oocyte is fertilized by a sperm. *DNA,* deoxyribonucleic acid; *GVBD,* germinal vesicle breakdown; *Mat,* maternal; *n,* amount of DNA material in the haploid number (23) of chromosomes; *Pat,* paternal.

During the second half of in utero life, these germ cell clusters break down, and the surviving oocytes become individually surrounded with squamous pregranulosa cells to give rise to primordial follicles. The transition from primordial to primary follicle is marked histologically by a morphologic change in granulosa cells from squamous to cuboidal. By the secondary stage, there are at least two layers of cuboidal granulosa cells and an additional layer of somatic cells, the theca, which forms outside the basement membrane of the follicle (Fig. 17.11).[49] At puberty, FSH secreted by the pituitary promotes further granulosa cell proliferation and survival.

A basement lamina separates the oocyte and granulosa cells from the surrounding stromal cells.[59] The granulosa cells do not have direct access to the circulation before ovulation (Fig. 17.12).

The avascular nature of the granulosa cell compartment necessitates contact between neighboring cells. The granulosa cells are interconnected by extensive intercellular gap junctions, which result in their coupling to yield an expanded, integrated, and functional syncytium (Fig. 17.13).[61] Gap junctions are composed of proteins called *connexins*. Connexin 37 and other connexins have been demonstrated in gap junctions in follicles. Gap junction



• **Fig. 17.11** Developmental stages of the ovarian follicle. The primordial follicle is composed of a single layer of granulosa cells and a single immature oocyte arrested in the diplotene stage of the first meiotic division. The primordial follicle is separated from the surrounding stroma by a thin basal lamina (i.e., basement membrane). The oocyte and granulosa cells do not have a direct blood supply. The first sign of follicular recruitment is cuboidal differentiation in the spindle-shaped cells inside the basal lamina, which thereafter undergo successive mitotic divisions to form a multilayered granulosa cell zone. The oocyte enlarges and secretes a glycoprotein-containing mucoid substance called the *zona pellucida,* which surrounds the oocyte and separates the granulosa cells from the oocyte. This structure is a primary follicle. The secondary follicle is formed by further proliferation of granulosa cells and by the final phase of oocyte growth, in which the oocyte reaches 120 μm in diameter, coincident with proliferation of layers of cells immediately outside the basal lamina to constitute the theca. The portion of the theca adjacent to the basal lamina is called the *theca interna*. Theca cells that merge with the surrounding stroma are designated the *theca externa*. The secondary follicle acquires an independent blood supply consisting of one or more arterioles that terminate in a capillary bed at the basal lamina. Capillaries do not penetrate the basement membrane, and the granulosa and oocyte remain avascular. The tertiary follicle is characterized by further hypertrophy of the theca and the appearance of a fluid-filled space among the granulosa cells, called the *antrum*. The fluid in the antrum consists of a plasma transudate and secretory products of granulosa cells, some of which (estrogens) are found there in strikingly higher concentrations than in peripheral blood. The follicle rapidly increases in size under the influence of gonadotropins to form the mature or Graafian follicle. In the Graafian follicle, the granulosa and oocyte remain encased by the basal lamina and are devoid of direct vascularization. The antral fluid increases in volume, and the oocyte, surrounded by an accumulation of granulosa cells (i.e., cumulus oophorus), occupies a polar, eccentric position within the follicle. The mature Graafian follicle is then ready to release the ovum by the process of ovulation. (Adapted from Erickson GF, Magoffin DA, Dyer CA. The ovarian androgen producing cells: a review of structure-function relations. *Endocr Rev*. 1985;6:371–379. Copyright © 1985 by The Endocrine Society.)

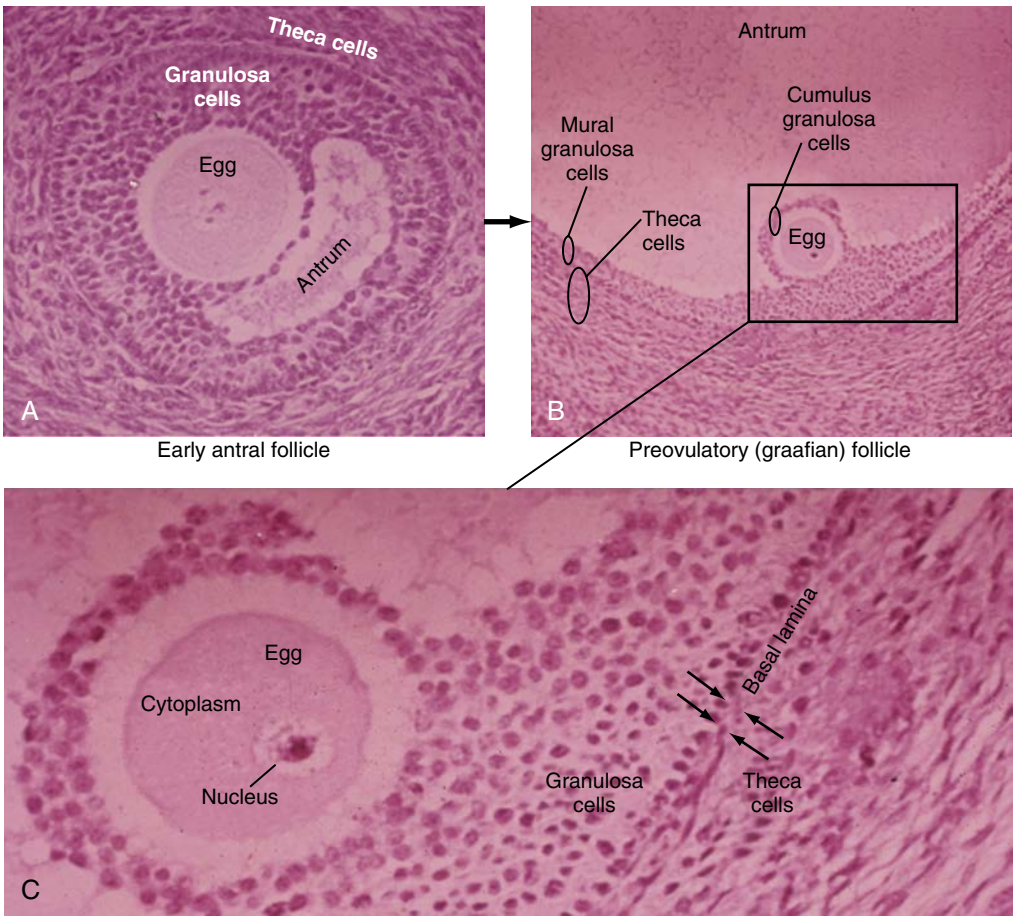
protein connexin 37 (GJA4)–deficient mice lack Graafian follicles, fail to ovulate, and develop inappropriate corpora lutea.[61] These specialized cell junctions may be important in metabolic exchange and in the transport of small molecules between neighboring granulosa cells. Moreover, the granulosa cells extend cytoplasmic processes that penetrate the zona pellucida to form gap junctions with the plasma membrane of the oocyte (see Fig. 17.13). In the GJA4-deficient mice, oocyte development is arrested before meiotic competence.[62] Gap junctions represent a crucial communication system that is needed for the tight control exerted by the cumulus granulosa cells on the resumption of meiosis by the enclosed primary oocyte.

Several gene products regulate the transition from primordial to primary follicle, which is marked by a change in the morphologic appearance of granulosa cells from squamous to cuboidal, followed by an increase in granulosa cell layers in the secondary follicle.[49] These genes are expressed in the oocyte or granulosa cells, emphasizing the active role of the oocyte in granulosa cell differentiation. Newborn oogenesis homeobox (NOBOX), spermatogenesis and oogenesis helix-loop-helix 1 (SOHLH1), and SOHLH2 are critical transcription factors during the transition from primordial to primary follicles.[49] Interactions between KIT ligand expressed in granulosa cells and the KIT tyrosine kinase receptor expressed in oocytes also appear to be critical in early folliculogenesis. The KITLG/KIT pathway induces the PI3K/AKT pathway, leading to phosphorylation and inactivation of forkhead box O3 (FOXO3), an inhibitor of primordial follicle activation.[49] These genetic studies supported a critical role of the PI3K/AKT/FOXO3 pathway in early follicle development and granulosa cell differentiation. Whereas FOXO3 is the key oocyte factor critical for suppressing primordial follicle activation, another forkhead domain transcription factor, forkhead box L2 (FOXL2), is crucial in the transition from squamous to cuboidal granulosa cells.[49]

Antimüllerian hormone (AMH) produced by the granulosa cells of growing follicles appears to inhibit the growth of primordial follicles; in its absence there is a faster depletion of growing follicles, although it is unknown whether this is a direct or indirect effect of AMH.[49] Clinically, serum AMH may be a useful biomarker of ovarian reserve. In humans and mice, serum AMH declines with increasing age. Although it is difficult to establish a direct link between serum AMH and the primordial follicle pool in humans, antral follicle number is positively correlated with AMH levels.[49] More detailed information has been reviewed by Edson and colleagues.[49]

The granulosa cells in the fully developed Graafian follicle shortly before ovulation are stratified in a manner that allows the distinction of a number of populations of cells.[63] Distinct populations of granulosa cells exhibit specific, specialized functions.[63,64] Mural granulosa cells within the outermost layer adjacent to the basement layer contain high levels of gonadotropin hormone receptors and steroidogenic enzymes and account for most of the steroidogenesis in the follicle.[63,64] The *cumulus oophorus* contains the egg and a surrounding mass of granulosa cells that have cell-cell interactions with the egg and seem to have critical roles in oocyte development (Fig. 17.13; see also 17.12).[63,64]

Mural and cumulus granulosa cells also exhibit distinct patterns of gene expression. For example, the tumor suppressor BRCA1 is highly expressed in ovarian granulosa cells of developing follicles.[65] However, in large antral or preovulatory follicles, BRCA1 expression significantly decreases in mural granulosa cells and becomes restricted to cumulus granulosa cells; these cells, unlike the mural ones, do not contain abundant aromatase, so this development gives rise to an intriguing inverse correlation of BRCA1 with aromatase mRNA and protein levels.[65] Stimulation of an FSH-dependent



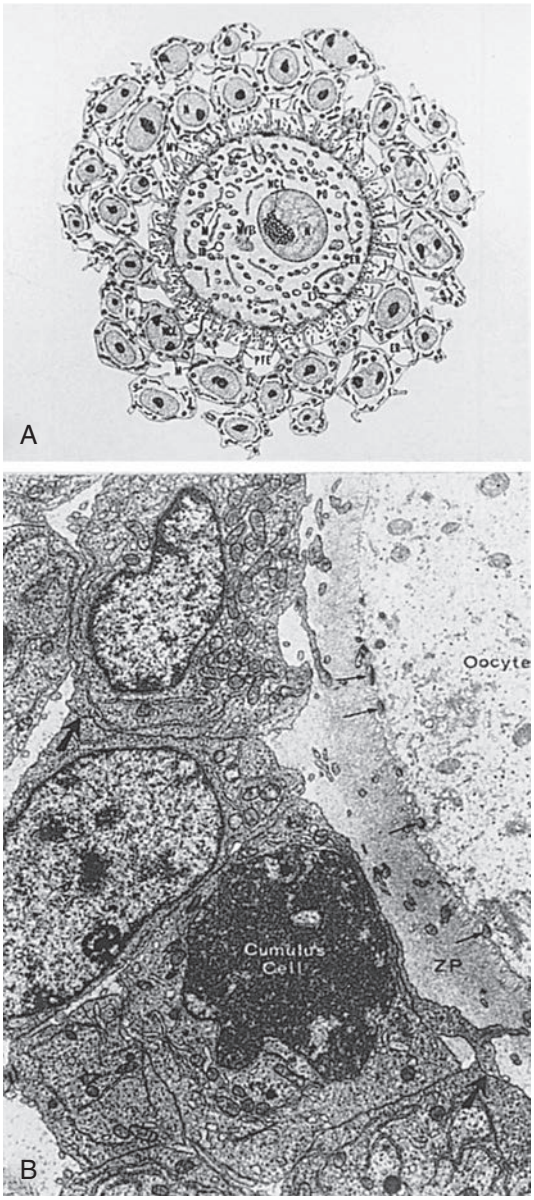
• **Fig. 17.12** Histology of gonadotropin-dependent ovarian follicle development. (A) Development of an antrum marks gonadotropin dependency. Multiple layers of granulosa and theca cells are present. (B) The follicle destined to ovulate distinguishes itself from the rest of the cohort through accumulation of large quantities of antral fluid. The granulosa cells, which accumulate around the oocyte, are called *cumulus granulosa cells* and primarily function to support egg development. The mural granulosa cells in the periphery primarily serve as steroidogenic cells. (C) A membrane called the *basal lamina (arrows),* which has been formed at the primary stage, separates the granulosa cells from the theca component of the follicle.

signaling pathway greatly induces aromatase but suppresses BRCA1 expression in granulosa cells. Moreover, BRCA1 binds to the aromatase promoter and inhibits its activity.[66] Therefore BRCA1 may exert its tumor suppressor activity, in part, by limiting excessive estrogen formation in the ovary. A summary of ovarian follicle development is presented in Fig. 17.11.

Theca Cell Layer

After the follicle achieves two layers of granulosa cells, another morphologically distinct layer of somatic cells, the theca, differentiates from ovarian stroma (see Figs. 17.11, 17.12, and 17.13).[49] The cells making up the theca-interstitial compartment are heterogeneous in nature.[67] Cells of the theca interna layer, which forms just outside the basement membrane surrounding the granulosa cells, show typical steroidogenic features, including mitochondria with tubular cristae, smooth endoplasmic reticulum, and abundant lipid vesicles (see Figs. 17.11 and 17.12). Theca interna cells are responsible for producing the C19 steroids that diffuse into the neighboring granulosa cells and serve as substrates for estrogen production. Theca externa is the outermost layer of the follicle and is composed of fibroblasts, smooth muscle–like cells, and macrophages (see Figs. 17.11 and 17.12). The theca externa is thought to have an important function during ovulation. Cells that contribute to the theca differentiate from mesenchymal precursor cells present in the ovarian stroma, adjacent to developing follicles. Like preantral folliculogenesis, theca formation is gonadotropin independent. Theca precursor cells lack LH receptors, and the theca layer still forms in the ovaries of FSH-deficient mice.[49] After a discernible theca interna layer has developed, theca cell C19 steroid production is regulated primarily by LH.

The differentiated state of theca interna cells is marked by expression of a number of steroidogenic genes, including those that encode the LH receptor *(LHCGR),* steroidogenic acute regulatory protein *(STAR),* side-chain cleavage enzyme *(CYP11A1),* 3β-hydroxysteroid dehydrogenase-$\Delta^{5,4}$ isomerase type 2 *(HSD3B2),* and 17-hydroxylase/17,20-lyase *(CYP17A1)* in the human. Granulosa cells of the developing follicles appear to secrete factors that regulate theca cell differentiation. Candidate factors that may contribute to theca cell differentiation include insulin-like growth factors (IGFs), KITLG, and growth differentiation factor 9 (GDF9). IGF1 induces expression of *Lhcgr*, *Cyp11a1*, and *Hsd3b1* (counterpart of human *HDS3B2*), whereas KITLG stimulates *Star* and *Cyp17a1* expression in rat theca cells.[49] In mice lacking the *Gdf9* gene, a theca layer fails to form in the ovary. Whether *GDF9* regulates theca cell recruitment or differentiation



• **Fig. 17.13** Structural relationship between the cumulus granulosa cell and the oocyte. (A) Microvilli of an oocyte interdigitate with cytoplasmic extensions of granulosa cells, penetrating the zona pellucida. (B) Notice the penetration of the zona pellucida (ZP) by cytoplasmic processes of the granulosa cells. Small gap junctions *(thin arrows)* are observed between processes of the granulosa cell and the oocyte membrane. The *thick arrows* indicate a gap junction between granulosa cells. (From Erickson GF. An analysis of follicle development and ovum maturation. *Semin Reprod Endocrinol*. 1986;4:233, used with permission of Thieme Medical Publishers, New York.)

directly or indirectly through regulation of preantral granulosa cell development is unknown.[49]

Follicles

The follicle represents the key functional unit in the ovary with respect to germ cell development and steroid production. The follicles are embedded in loose connective tissue of the ovarian cortex and can be subdivided into two functional types: nongrowing (primordial) and growing. Between 90% and 95% of follicles are nongrowing throughout reproductive life. Recruitment of a primordial follicle initiates dramatic changes in growth, structure, and function. The growing follicles are divided into four stages: primary, secondary, tertiary, and graafian (see Figs. 17.7 and 17.11). The first three stages of growth can occur in the absence of the pituitary and therefore appear to be controlled by intraovarian mechanisms (see Figs. 17.7 and 17.11). The follicle destined to ovulate is recruited during the first few days of the current cycle.[68]

The early growth of follicles occurs over several menstrual cycles, but the ovulatory follicle is one of a cohort recruited at the time of transition from the previous cycle's luteal phase to the current cycle's follicular phase.[68] The total time to achieve preovulatory status is approximately 85 days.[68] Most of this developmental period is FSH independent. Eventually this cohort of follicles reaches a stage at which, unless recruited by FSH, the next step is atresia. A cohort of follicles measuring 2 to 5 mm in diameter is continuously available for a response to FSH. The late luteal increase in FSH is the critical feature in rescuing this cohort of follicles from atresia; it allows a dominant follicle to emerge and pursue a path to ovulation. The increase in the FSH level must be maintained for a critical period (see Fig. 17.1).[69]

Recruited primordial follicles either develop into dominant, mature Graafian follicles destined to ovulate or degenerate as a result of atresia.[70] The average time for development of a selected follicle to the point of ovulation is 10 to 14 days. If a follicle is not recruited, it goes through a process called *atresia*, during which the oocyte and granulosa cells within the basal lamina die and are replaced by fibrous tissue. There is general agreement that atresia of follicles occurs through apoptosis.[71]

Ovulation

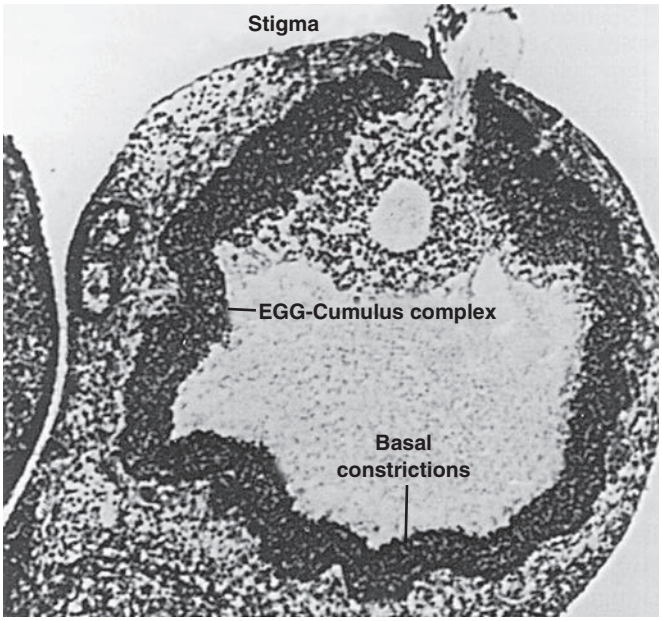
There is a dramatic rise in circulating estradiol level as midcycle approaches. This is followed by a striking LH surge and, to a lesser extent, an FSH surge, which trigger the dominant follicle to ovulate. During each menstrual cycle, usually one follicle ovulates and gives rise to a corpus luteum. In women, LH or its surrogate, hCG, is essential to stimulate rupture of the mature follicle. It has been proposed that increased local prostaglandin biosynthesis in the follicle mediates the ovulatory effect of LH.[72,73]

Ovulation consists of rapid follicular enlargement followed by protrusion of the follicle from the surface of the ovarian cortex. This is followed by the rupture of the follicle and extrusion of an egg-cumulus complex into the peritoneal cavity (Fig. 17.14). Follicular rupture or ovulation occurs predictably 34 to 36 hours after the start of the LH surge. Elevation of a conical *stigma* on the surface of the protruding follicle precedes rupture (see Fig. 17.14). Rupture of this stigma is accompanied by a gentle rather than explosive expulsion of the ovum and antral fluid. A number of transcriptional regulators downstream of the LH receptor are required for ovulation. After the LH surge, progesterone receptor (PR) levels rapidly increase in the mural granulosa cells of the preovulatory follicle.[49] LH-dependent or PR-dependent production of proteases acting locally on protein substrates in the basal lamina may play an important role in stigma formation and follicular rupture.[62,74] In particular, levels of plasminogen activator increase in the follicle before rupture.[75] Plasminogen activator–mediated conversion of plasminogen to plasmin may contribute to the proteolytic digestion of the follicular wall, which is a prerequisite for follicular rupture. Gene knockout studies in mice suggest that other factors important for ovulation or follicular rupture include endothelin 2, peroxisome proliferator-activated

receptor-γ (PPARγ), CCAAT/enhancer-binding protein-β, liver receptor homologue 1 (LRH1), steroidogenic factor 1 (SF1), and nuclear receptor–interacting protein 1.[49]

Corpus Luteum

After ovulation, the dominant follicle reorganizes to become the corpus luteum (Fig. 17.15). After rupture of the follicle, capillaries and fibroblasts from the surrounding stroma proliferate and penetrate the basal lamina (see Fig. 17.11). This rapid vascularization of the corpus luteum may be guided by angiogenic factors, some of which are detected in the follicular fluid.[76] Vascular endothelial growth factor has been isolated from corpora lutea and has been postulated, along with basic fibroblast growth factor, to be a potential angiogenic agent in corpora lutea.[77] Concurrently, the granulosa and theca cells undergo morphologic changes collectively referred to as *luteinization*. The granulosa cells become granulosa-lutein cells (large cells), and the theca cells are transformed into theca-lutein cells (small cells) (see Fig. 17.15).[78] The so-called K cells, scattered throughout the corpus luteum, are believed to be macrophages.

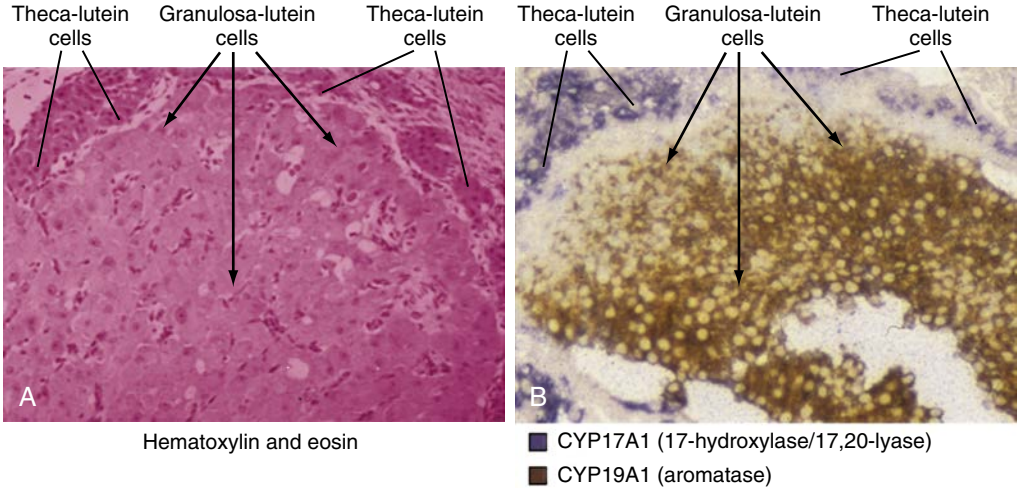


• **Fig. 17.14** Ovulation of the cumulus-oocyte complex through the stigma. (From Erickson GF. An analysis of follicle development and ovum maturation. *Semin Reprod Endocrinol*. 1986;4:233, used with permission of Thieme Medical Publishers, New York.)

The corpus luteum is the major source of sex steroid hormones secreted by the postovulatory ovary. The human corpus luteum secretes as much as 40 mg of progesterone per day during the midluteal phase of the ovarian cycle.[79] In view of the small size of the corpus luteum, it is the most active steroidogenic tissue in humans. An important aspect of corpus luteum formation is the penetration of the follicle basement membrane by blood vessels. This vascularization provides the granulosa-lutein cells with low-density lipoprotein (LDL) cholesterol.[76] LDL cholesterol serves as the primary substrate for corpus luteum progesterone production.

A key regulator of steroidogenesis in the corpus luteum is LH. In humans, the LH receptor is maintained throughout the functional life span of the corpus luteum and is not downregulated during the maternal recognition of pregnancy.[80] The rate-limiting step in LH-mediated progesterone formation in luteinized granulosa cells is the entry of cholesterol into the mitochondria, which is regulated by the steroidogenic acute regulatory (StAR) protein.[81] The availability of LDL cholesterol and the StAR-mediated mitochondrial entry of cholesterol into the mitochondria seem to be the two critical factors that account for the production of large amounts of progesterone in the corpus luteum.

Unless pregnancy occurs, the functional life span of the corpus luteum is normally 14 ± 2 days, after which it spontaneously regresses and is replaced by an avascular scar named the *corpus albicans*. There is little doubt about the central role of LH or hCG in the maintenance of corpus luteum function. Withdrawal of LH support in a variety of experimental circumstances has almost invariably resulted in luteal regression.[82] In pregnancy, however, the LH surrogate, hCG, secreted by the gestational trophoblast, maintains the ability of the corpus luteum to elaborate progesterone; this stimulus helps to maintain the early gestation until the luteoplacental shift.[82] The corpus luteum doubles in size



• **Fig. 17.15** Corpus luteum. (A) Hematoxylin and eosin stain shows the large granulosa-lutein cells occupying the center and smaller theca-lutein cells in the periphery. (B) Immunoreactive aromatase, the product of the *CYP19A1* gene *(brown stain),* is the hallmark of granulosa-lutein cells, whereas immunoreactive 17-hydroxylase/17,20-lyase, the product of the *CYP17A1* gene *(purple stain),* is selectively localized to theca-lutein cells. (Courtesy Dr. Hironobu Sasano, Tohoku University, Sendai, Japan.)

(compared with the pre-pregnancy size) during the first 6 weeks of gestation because of hypertrophy of the luteinized granulosa and theca cells (see Fig. 17.15). This early hypertrophy is followed by regression. The corpus luteum at term is only one-half of its size during the menstrual cycle.

Hormones such as estrogens and prostaglandins have been suggested to be important factors in the promotion of luteal demise.[83] Immune factors may influence luteal life span because corpus luteum regression is associated with a progressive infiltration of lymphocytes and macrophages. In the absence of LH or hCG, apoptosis is a critical endpoint mechanism by which human corpora lutea are deleted.[84]

Ovarian Follicle-Stimulating Hormone and Luteinizing Hormone Receptors

The FSH receptor is expressed exclusively by granulosa cells. The LH or hCG receptor (LHCGR) is expressed primarily by the theca-interstitial cells of all follicles and by granulosa cells of large preovulatory follicles.

Granulosa cells in primary or secondary follicles that are in the early developmental stages before antrum formation primarily bind FSH but not LH. In these preantral follicles, the binding of LH or hCG is confined to theca-interstitial cells.[85] Granulosa cells in more mature tertiary follicles with an antrum appear capable of binding both LH and FSH. The FSH receptors are found in granulosa cells from follicles of all sizes, but LH receptors are found only in granulosa cells of large preovulatory follicles.[86,87] These observations are consistent with the concept that the acquisition of LH receptors on granulosa cells is under the influence of FSH.[88]

The receptors for the glycoprotein hormones have related structures (see Fig. 17.17, later). The receptors belong to the large family of G protein–coupled receptors, whose members all have a transmembrane domain that consists of seven membrane–traversing α-helices connected by three extracellular and three intracellular loops. The glycoprotein hormone receptors form a separate subgroup within this large family by virtue of their large extracellular hormone-binding domain at the amino-terminus. FSH binds to the FSH receptor, and LH and hCG bind to the same LH receptor. The LH and FSH receptor genes are located on chromosome 2 in the p21 region.[47] The carboxy-terminal half of the receptor is encoded by a single last exon and contains the seven transmembrane segments and the G protein–coupling domain. The unusually large extracellular domain of the glycoprotein hormone receptors, on the other hand, is encoded by the first 9 or 10 exons.

Role of Follicle-Stimulating Hormone in Ovarian Function

FSH is the main promoter of follicular maturation. Given that FSH receptors have been exclusively localized to granulosa cells, it is presumed that FSH action in the ovary involves the granulosa cells. The ability of FSH to orchestrate follicular growth and differentiation depends on its ability to exert multiple actions concurrently.

Phenotypes of women with mutations that disrupt the function of the FSH β-subunit gene are in good agreement and demonstrate that FSH is necessary for normal follicular development, ovulation, and fertility. Pubertal development is hampered in the absence of sufficient numbers of later stage follicles with the granulosa cells needed for adequate estrogen production. Treatment of affected patients with exogenous FSH has resulted in follicular maturation, ovulation, and normal pregnancy.[47] The presenting phenotype of FSH β-subunit deficiency is practically identical to that caused by inactivating mutations of the FSH receptor, except that the latter fail to respond to exogenous FSH.[47]

Women with FSH receptor mutations are clinically similar to patients with gonadal dysgenesis; they have absent or poorly developed secondary sexual characteristics and high serum levels of FSH and LH.[47] The notable difference is the presence of ovarian follicles in women with mutated FSH receptors, consistent with the FSH independence of primordial follicle recruitment and early follicular growth and development. Total absence of any follicles, including those in the primordial stage, occurs in women in whom FSH receptor mutations cannot be demonstrated.[47] The ovarian phenotype of FSH receptor deficiency is distinct from the common form of gonadal dysgenesis (Turner syndrome), which is characterized by streak gonads and an absence of growing follicles.[47]

In vivo rodent studies suggest that FSH is capable of increasing the number of its own receptors in the granulosa cell. Whereas estradiol by itself may be without effect on the distribution, number, or affinity of granulosa cell FSH receptors, estrogens synergize with FSH to enhance the overall number of granulosa cell FSH receptors.[89] Changes in the production of estradiol by preantral follicles can increase their response to FSH through regulation of granulosa cell surface FSH receptors. This interaction between FSH and estradiol in follicular development has been well established in rodents. It appears that ERα and ERβ mediate the estrogenic effect on ovarian development and follicular maturation in mice.[90] However, it is not clear whether a similar relationship exists in the human ovary. ERα is not detected in the human ovary in significant quantities. Nevertheless the demonstration of ERβ in the human ovary suggests an interaction between FSH and estrogen in the regulation of normal follicle development and ovulation in women.[91]

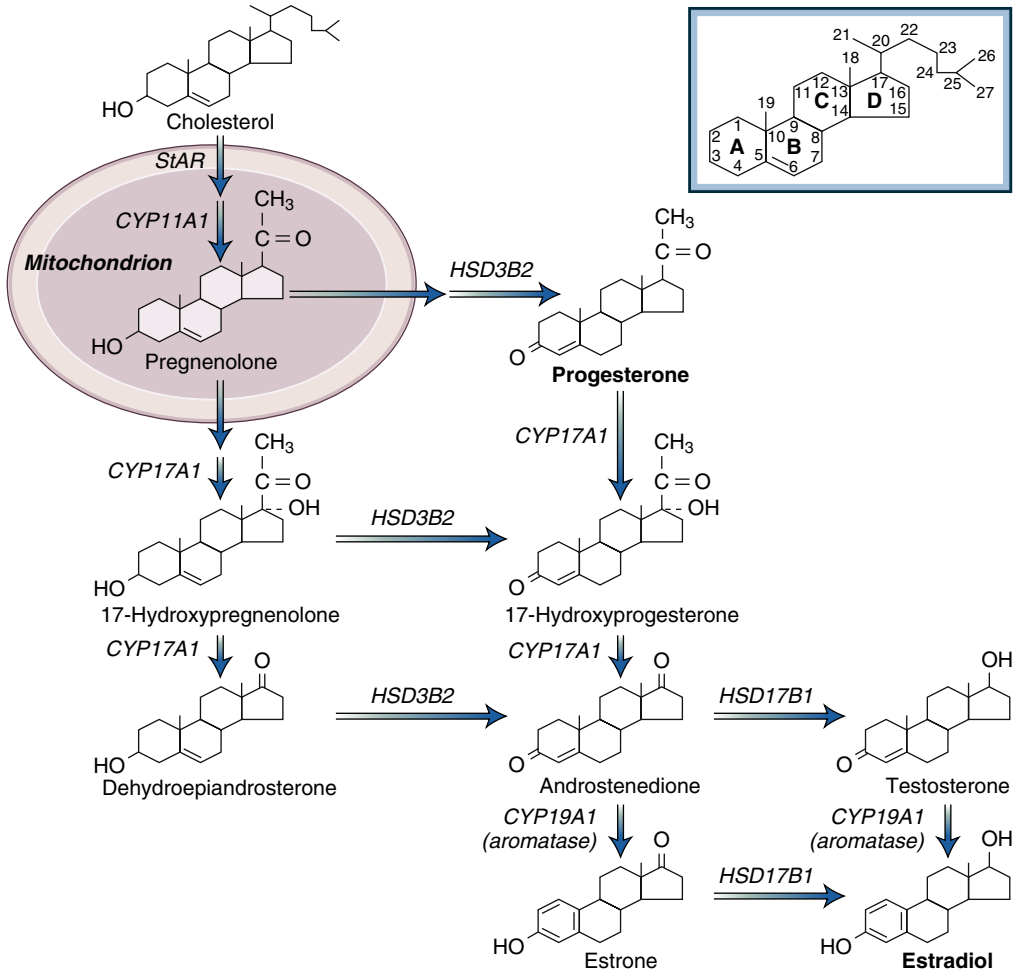
One of the major actions of FSH is induction of granulosa cell aromatase activity.[92] Little or no estrogen can be produced by FSH-unprimed granulosa cells even if they are supplied with aromatizable androgen precursors. Treatment with FSH enhances the aromatization capability of granulosa cells, an effect related to enhancement of the granulosa cell aromatase content.[92]

Treatment with FSH has also been shown to induce LH receptors in granulosa cells. The ability of FSH to induce LH receptors is augmented by the concomitant presence of estrogens.[93] Progestins, androgens, and LH itself may also induce LH receptors. After induction, the granulosa cell LH receptor requires the continued presence of FSH for its maintenance.

Circumstantial evidence, deduced from studies of women with disrupting mutations of the genes that encode FSH and LH receptors and aromatase (CYP19A1), indicates that FSH action, but not estrogen or LH action, is essential for follicular growth in humans.[47,93] Follicular growth and development up to the antral stage were observed in women with deficient LH action or estrogen biosynthesis, although these individuals were anovulatory.[47,93] Women with mutations of the FSH β-subunit or FSH receptor have only primordial follicles in their ovaries.[47] These data indicate that estrogen and LH are not critical for follicular development at least until the tertiary stage (see Figs. 17.11 and 17.12). However, FSH by itself is not sufficient to achieve normal follicular development and ovulation.

Role of Luteinizing Hormone in Ovarian Function

LH is essential for ovulation (follicular rupture) and the sustenance of corpus luteum function; in addition, it plays other important roles in follicular function.[93] First, LH probably plays



• **Fig. 17.16** Steroidogenic pathway in the human ovary. The biologically active steroids progesterone and estradiol are produced primarily in the ovary of a woman of reproductive age. Estradiol production requires the activity of six steroidogenic proteins, including StAR, and six enzymatic steps. 17-Hydroxylase/17,20-lyase, the product of the *CYP17A1* gene, catalyzes two enzymatic reactions. The four rings of the cholesterol molecule and its derivative steroids are identified by the first four letters in the alphabet, and the carbons are numbered in the sequence shown in the *insert*. *CYP17A1,* 17-hydroxylase/17,20-lyase; *CYP19A1,* aromatase; *HSD17B1,* 17β-hydroxysteroid dehydrogenase type 1; *HSD3B2,* 3β-hydroxysteroid dehydrogenase-$\Delta^{5,4}$ isomerase type 2; *StAR,* steroidogenic acute regulatory protein.

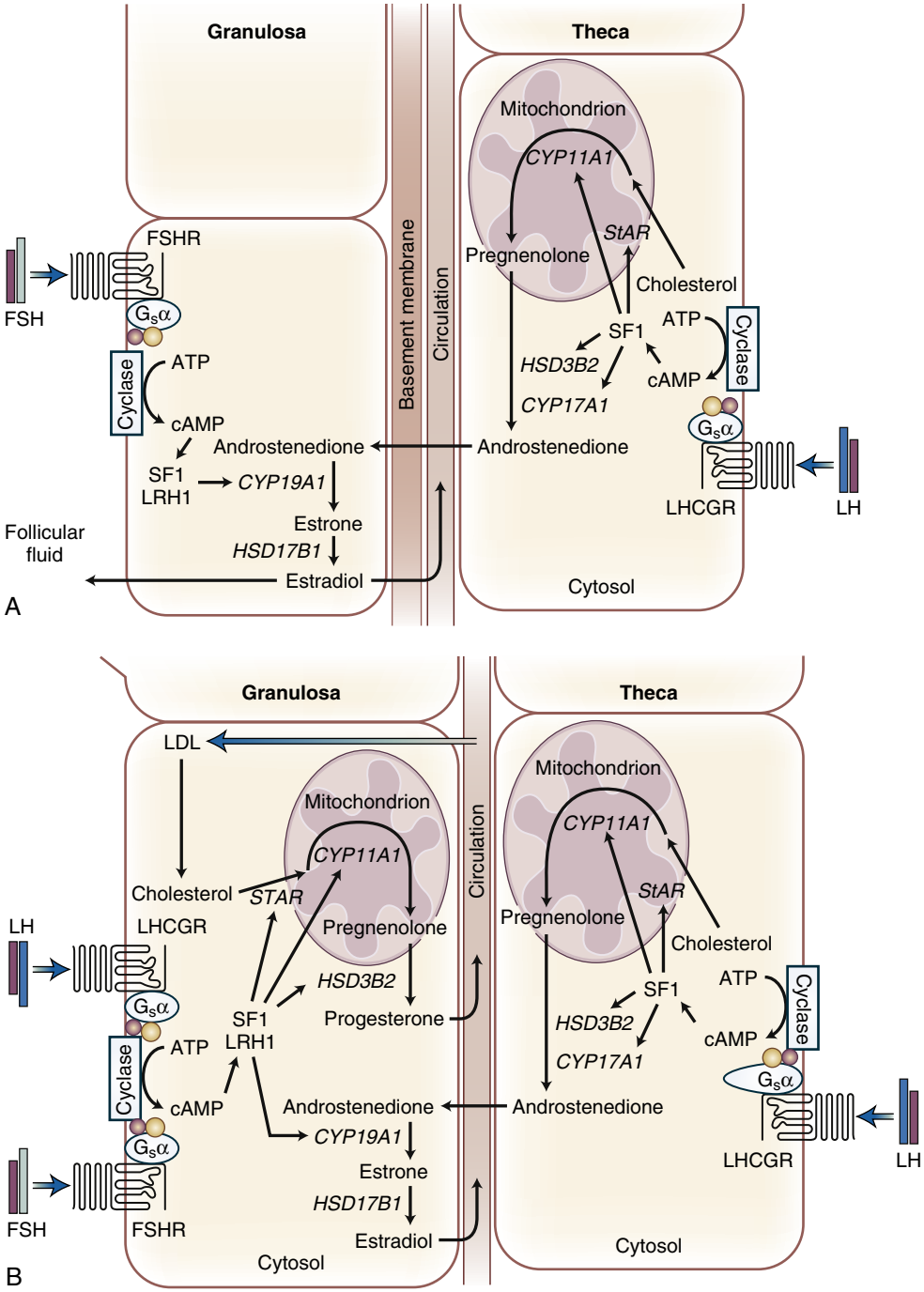
a major role in the promotion of theca-interstitial cell androgen production. Second, LH may well synergize with FSH in the more advanced phases of follicular development. Third, small and sustained increments in the circulating levels of LH are necessary and sufficient to cause small antral follicles to grow and develop to the preovulatory stage.[93]

It is presumed that LH acts on the theca-interstitial cells of small follicles, where it promotes the biosynthesis of C19 steroids.[93] The consequent increase in estrogen production is presumed to contribute to the growth and development of the follicles. Treatment with small doses of LH also results in an increase in LH receptor content and in induction of the key steroidogenic proteins, such as StAR, CYP11A1, HSD3B2, and CYP17A1.[93]

The role of LH action in human ovarian physiology was exemplified by the phenotype of a woman with a disrupting mutation of the LH receptor gene.[47] She presented with amenorrhea, normally developed secondary sexual characteristics, increased circulating FSH and LH levels, and low levels of estradiol and progesterone that were unresponsive to hCG treatment.[47] The ovary contained follicles that developed up to antral stage with a well-developed theca layer but no preovulatory follicles or corpora lutea. These observations collectively support the view that LH is essential for ovulation and sufficient estrogen production, whereas follicular development is initially autonomous but at later stages depends on intact FSH action.[47]

Ovarian Steroidogenesis

The steroid hormone contents of the ovarian vein effluents and peripheral venous blood were compared to distinguish steroids secreted by the ovary from those secreted by the adrenal or produced by peripheral conversion of precursors.[94] These studies revealed that the ovaries secrete pregnenolone, progesterone, 17α-hydroxyprogesterone, dehydroepiandrosterone (DHEA), androstenedione, testosterone, estrone, and estradiol.[95,96] Although such measurements provide insights into the steroidogenic pathways under study, they do not identify the specific ovarian cells involved. Studies using microdissected preovulatory follicles identified estrone and estradiol as the major steroid products (Fig. 17.16). Progesterone and 17α-hydroxyprogesterone proved to be the major products of the corpus luteum (see Fig. 17.16).



• **Fig. 17.17** Two-cell hypothesis for ovarian steroidogenesis. (A) The preovulatory follicle produces estradiol through a paracrine interaction between theca and granulosa cells. In response to stimulation with a gonadotropin, steroidogenic factor 1 (SF1, encoded by NR5A1, a member of the nuclear receptor family) acts as a master switch to initiate transcription of a series of steroidogenic genes in theca cells. In follicular granulosa cells, another nuclear receptor, liver homologue receptor 1 (LRH1, encoded by NR5A2), seems to primarily mediate the downstream effects of follicle-stimulating hormone (FSH) in the rodent ovary. In humans, the roles of SF1 and LRH1 in steroidogenesis in preovulatory granulosa cells are not well understood. Because granulosa cells do not have a direct connection to the circulation, CYP19A1 (aromatase) in granulosa cells depends for substrate on androstenedione that diffuses from theca cells. Two critical steps in estradiol formation are the entry of cholesterol into mitochondria facilitated by steroidogenic acute regulatory protein (StAR) in theca cells and the conversion of androstenedione to estrone catalyzed by CYP19A1 in granulosa cells. (B) In the corpus luteum, granulosa-lutein cells are heavily vascularized, a condition that is critical for entry of abundant quantities of cholesterol into this cell type through primarily low-density lipoprotein (LDL)-cholesterol receptors and for secretion of large amounts of progesterone into the circulation. The entry of cholesterol into mitochondria (mediated by StAR) is probably the most critical steroidogenic step for progesterone formation in granulosa-lutein cells. Androstenedione produced in theca-lutein cells serves as a substrate for estrone, which is further converted to estradiol in granulosa-lutein cells. Human data suggest that LRH1 may mediate at least a portion of gonadotropin-dependent steroidogenesis in the corpus luteum. Gonadotropins, SF1, and possibly LRH1 play key roles for important steroidogenic steps in the ovary. *ATP,* adenosine triphosphate; *cAMP,* cyclic adenosine monophosphate; *FSHR,* FSH receptor; *HSD,* hydroxysteroid dehydrogenase; *LHCGR,* LH receptor.

The general steroidogenic pathway for the production of estrogens and androgens is depicted in Fig. 17.16. The biologically active ovarian steroids are estradiol and progesterone. The major C19 steroid product of the ovary, androstenedione, is not biologically active. However, it acts as a dual precursor and contributes to circulating levels of estrone and testosterone through conversion in extraglandular tissues such as adipose tissue and skin (discussed later).[97] It is likely that estrogenically weak estrone is further converted to the potent estrogen estradiol and that testosterone is converted to the most potent endogenous androgen dihydrotestosterone (DHT) locally in target tissues such as brain, breast, prostate, and genital skin and subsequently exert potent biologic effects. This notion is supported by the presence in many human tissues of multiple proteins with overlapping enzymatic activities that catalyze these conversions (e.g., reductive 17βHSD and 5α-reductase).[98]

The preovulatory follicle secretes estradiol during the first half of the menstrual cycle, and the corpus luteum secretes estradiol and progesterone during the second half of the cycle. The production of these two biologically active steroids is orchestrated in the follicle and corpus luteum in a cell-specific manner that is under the control of LH and FSH.

Steroids formed by the ovary and other steroid-producing organs are derived from cholesterol (see Fig. 17.16). Several sources of cholesterol can provide the ovary with substrate for steroidogenesis, including plasma lipoprotein cholesterol, cholesterol synthesized de novo within the ovary, and cholesterol from intracellular stores of cholesterol esters within lipid droplets. In the human ovary, LDL cholesterol is an important source of cholesterol used for steroidogenesis.[79] LH stimulates the activity of adenylyl cyclase, increasing production of cyclic adenosine monophosphate (cAMP), which serves as a second messenger to increase LDL receptor messenger RNA (mRNA), binding and uptake of LDL cholesterol, and the formation of cholesterol esters.[78,79] LDL-derived cholesterol is particularly essential for normal levels of progesterone production in the granulosa-lutein cells of the corpus luteum.[79]

Steroidogenic Genes and Their Functions in the Ovary

The ovarian granulosa, theca, and corpus luteum cells possess StAR plus five distinct proteins with specific enzyme activities for steroid hormone formation. These steroidogenic enzymes are CYP11A1 (side-chain cleavage of P450), 3βHSD2 (3β-hydroxysteroid dehydrogenase-$\Delta^{5,4}$ isomerase type 2), CYP17A1 (17-hydroxylase/17,20-lyase), CYP19A1 (aromatase), and 17βHSD1 (17β-hydroxysteroid dehydrogenase type 1).[92] These enzymes are responsible for the conversion of cholesterol to the two major biologically active products: estradiol and progesterone.[92,97]

The first and rate-limiting step in the synthesis of all ovarian steroid hormones is the movement of cholesterol into the mitochondrion, which is regulated by a mitochondrial membrane protein encoded by the *STAR* gene (see Fig. 17.16).[99] This movement is followed by conversion of cholesterol to pregnenolone, which is catalyzed by the mitochondrial side-chain cleavage enzyme complex consisting of CYP11A1, adrenodoxin, and flavoprotein. LH induces steroidogenesis by increasing the conversion of cholesterol to pregnenolone in two distinct ways: acute regulation, which occurs over minutes through phosphorylation of preexisting StAR and rapid synthesis of new StAR, and chronic stimulation, which occurs within hours to days through the induction of CYP11A1 expression and consequent increased steroidogenesis (Fig. 17.17). StAR increases the flow of cholesterol to mitochondria, regulating substrate availability to CYP11A1 on the inner mitochondrial membrane.[99] In the absence of StAR, only 14% of the maximal StAR-induced level of steroidogenesis persists.[99]

StAR expression in the preovulatory Graafian follicle is limited primarily to the theca cells (see Fig. 17.17).[100] The most important product of the theca cell during the follicular phase is the estrogen precursor androstenedione, and its production is controlled primarily by StAR. The biologically active steroid product of the ovary during the follicular phase is estradiol, which arises from the granulosa cells located adjacent to theca cells. The rate-limiting step for granulosa cell estradiol production is regulated by the FSH-dependent activity of the aromatase enzyme in a cyclic fashion.[92] During the luteal phase, cells of the corpus luteum, including granulosa-lutein cells, also show intense StAR immunoreactivity with a patchy distribution.[81,100] The delivery of cholesterol to the mitochondrial side-chain cleavage enzyme system in the corpus luteum is the rate-limiting step for progesterone biosynthesis and is regulated by StAR.[81] Thus estradiol production seems to be regulated primarily by StAR and aromatase, whereas progesterone biosynthesis may be primarily under the control of StAR.

Steroidogenesis that depends on LH and FSH in theca and granulosa cells is mediated by common signaling molecules, including cAMP and the specific transcription factors SF1, product of the *NR5A1* gene, and LRH1, product of the *NR5A2* gene, which belong to the nuclear receptor family (see Fig. 17.17).[101,102] SF1 and LRH1 regulate the expression of genes that encode StAR, CYP11A1, 3βHSD2, CYP17A1, and CYP19A1 (see Fig. 17.17). SF1 and possibly LRH1 can be regarded as downstream master switches that orchestrate ovarian steroidogenesis.[102]

All steroid hormones are derived from cholesterol. C27 cholesterol is converted to the carbon steroid hormones 18, 19, and 21 that are secreted by the ovary (see Fig. 17.16).

C21 Steroids

The principal progestogens are C21 steroids and include pregnenolone, progesterone, and 17- hydroxyprogesterone (see Fig. 17.16). Pregnenolone is of primary importance in the ovary because of its key position as a precursor of all steroid hormones.[103] Progesterone, the principal secretory product of the corpus luteum, is responsible for the progestational effects (i.e., cell differentiation and induction of secretory activity in the endometrium of the estrogen-primed uterus).[103] Progesterone is essential for implantation of the fertilized ovum and maintenance of pregnancy. It also induces decidualization of the endometrium, inhibits uterine contractions, increases the viscosity of cervical mucus, promotes lateral (alveolar) development of the breast glands, and increases basal body temperature.[103]

C19 Steroids

The ovary secretes a variety of C19 steroids, including DHEA, androstenedione, and testosterone, all of which primarily serve as distant or immediate precursors for the potent androgen, DHT, or potent estrogen, estradiol (see Fig. 17.16; also see Fig. 17.26, later).[97] C19 steroids are produced by the theca cells and, possibly, to a lesser degree by the ovarian stroma. The major C19 steroid is androstenedione, part of which is secreted directly into plasma, with the remainder converted to estrogen by the granulosa cells.[92] DHEA and androstenedione do not appear to have major androgenic actions. In the ovary and in peripheral tissues, DHEA is converted to androstenedione, which can be converted

to estrone or testosterone.[97] Testosterone is converted locally to DHT at target tissues for full androgenic action or estradiol for estrogenic activity.

C18 Steroids

Estrogen regulates gonadotropin secretion, development of the secondary sexual characteristics of women, uterine growth, thickening of the vaginal mucosa, thinning of the cervical mucus, linear growth of the ductal system of the breast, growth spurt, epiphyseal closure, and bone mineralization.[104] The naturally occurring estrogens are C18 steroids characterized by the presence of an aromatic A ring, a phenolic hydroxyl group at C3, and a hydroxyl group (estradiol) or a ketone group (estrone) at C17.[92,97] Aromatase is the key enzyme for estrogen production in the ovary (see Fig. 17.16).[92] The protein aromatase, encoded by the *CYP19A1* gene, confers the specific activity of the aromatase enzyme complex.

Aromatase mRNA and protein expression and its enzyme activity in the ovarian granulosa cell are regulated primarily by FSH.[92,97] The principal and most potent estrogen secreted by the ovary is estradiol. Although estrone is also secreted by the ovary, another important source of estrone is extraglandular conversion of androstenedione in peripheral tissues.[97] All C18 steroids, including estrone, estradiol, and estriol, are commonly referred to as estrogens. However, estrone is only weakly estrogenic and must be converted to estradiol to show full estrogenic action. At least seven enzymes with overlapping activities are capable of converting estrone to estradiol in the ovary and extraovarian tissues.[105]

Catechol estrogens are formed by hydroxylation of estrogens at the C2 or C4 position. The physiologic role of catechol estrogen is unclear. Estrone sulfate, formed by peripheral conversion of estradiol and estrone, is the most abundant estrogen in blood, but it is not physiologically active. Estrone sulfate is presumed to serve as a reservoir for estrone and eventually estradiol formation in a number of tissues, including those that are targets of estrogen.[97]

Two-Cell Theory for Ovarian Steroidogenesis

The classic two-cell theory is supported by molecular findings. Ovarian steroidogenesis in the preovulatory follicle takes place through LH receptors on theca cells and FSH (possibly also LH) receptors on granulosa cells (see Fig. 17.17). cAMP production and increased SF1 binding to multiple steroidogenic promoters mediate LH action in theca cells.[106] The StAR protein is the primary regulator of production of androstenedione, which subsequently diffuses into granulosa cells to serve as the estrogen precursor.[97] In the preovulatory follicle, cholesterol in theca cells arises from circulating lipoproteins and de novo biosynthesis. FSH is responsible for follicular growth and estrogen formation. FSH induces cAMP formation, activation of protein kinase A and certain MAP kinases, and increased binding activity of LRH1 or SF1 to the *CYP191A1* promoter in preovulatory granulosa cells to form estrone and then estradiol primarily through aromatization of androstenedione (see Fig. 17.17). The relative roles of SF1 and LRH1 in estrogen formation in human ovarian granulosa cells are not well understood.[106]

In the corpus luteum, large stores of cholesterol esters (which provide the yellow color) arise primarily from circulating lipoproteins to support the production of extremely high quantities of progesterone.[107] Other key anatomic events in the formation of the corpus luteum are the disruption of the basement membrane between the granulosa and theca cells and strikingly increased vascularization of granulosa-lutein cells (see Fig. 17.15). Theca-lutein cells possess LH receptors and produce androstenedione. cAMP, SF1, and StAR induced by LH remain as the key regulators for biosynthesis of androstenedione, which serves as the estrogen precursor in neighboring granulosa-lutein cells (see Fig. 17.17).

The granulosa-lutein cell of the corpus luteum is anatomically and functionally different from its counterpart in the preovulatory follicle in several ways. First, these cells are characterized by large and granular cytoplasms, are heavily vascularized, and contain large quantities of cholesterol esters. Second, granulosa-lutein cells contain high levels of LH receptors in addition to FSH receptors. Third, they produce large quantities of progesterone; this function is regulated primarily by LH and StAR. Granulosa-lutein cells also aromatize androstenedione of thecal origin and eventually give rise to estradiol formation through FSH action and CYP19A1. The known mediators of LH and FSH in human granulosa-lutein cells are cAMP and increased LRH1 levels.[106,107] The relative roles of LRH1 and SF1 for progesterone and estradiol production in granulosa-lutein cells are not clear. Specific functions of the two gonadotropins (i.e., differentiation, growth, and progesterone vs estradiol formation) are probably determined by numerous modifying factors (see Fig. 17.17).

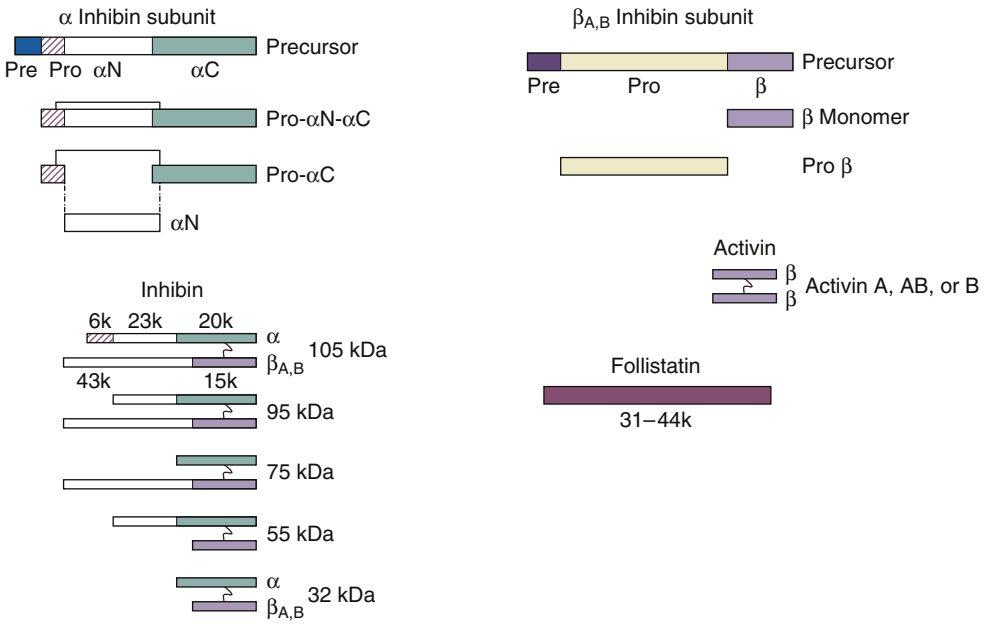
Peptide Hormones Produced by the Ovary

The ovary produces a large number of peptides that can act in an intracrine, autocrine, paracrine, or endocrine fashion.[106,108,109] They include numerous growth factors (e.g., IGFs) and cytokines (e.g., interleukin 1β). IGFs cross-talk with the FSH-dependent signaling cascade to augment the effects of FSH in granulosa cells.[108,109]

Inhibin, activin, and follistatin are produced in ovarian granulosa cells under the control of FSH and LH (Fig. 17.18).[106] Production of inhibin and activin is not limited to the ovary; a number of other tissues, including adrenal, pituitary, and placenta, synthesize these members of the broader TGFβ family of paracrine/endocrine factors. Two isoforms of inhibin have been isolated: inhibin-A and inhibin-B.[106] They contain an identical α-subunit but distinct β-subunits (β_A and β_B), encoded by separate genes. The heterodimers of inhibin, $\alpha\beta_A$ and $\alpha\beta_B$, are called inhibin-A and inhibin-B, respectively (see Fig. 17.18). Although inhibin is produced by a number of tissues in the body, most of it is derived from the gonads. In the ovary, the source of inhibin is granulosa cells. The main role of inhibin is to suppress FSH production in the pituitary.[106]

Although both inhibin isoforms seem to have similar biologic properties, their synthesis is regulated differently during the follicular and luteal phases (see Fig. 17.1A). Under the influence of FSH, inhibin-B is secreted mainly during the early follicular phase, with levels decreasing in midfollicular phase and becoming undetectable after the LH surge.[106] LH-induced inhibin-A levels are low during the first half of the follicular phase but increase gradually during the midfollicular phase and peak during the luteal phase. All three subunits are detected in small antral follicles by immunohistochemistry and in situ hybridization.[106] The α-subunit and β_A-subunit are found in the dominant follicle and in the corpus luteum. All three subunits are expressed in response to gonadotropins or factors that increase intracellular cAMP.[106]

Activin is structurally related to inhibin but exerts opposite actions.[106] Activin contains two subunits that are identical to the β-subunits of inhibin-A and inhibin-B. The three activin isoforms are activin A ($\beta_A\beta_A$), activin B ($\beta_B\beta_B$), and activin AB ($\beta_A\beta_B$). In the pituitary, activin stimulates the release of FSH. In the ovarian follicle, activin enhances FSH action (see Fig. 17.18). As in the case of inhibin, activins are also produced in ovarian granulosa



• **Fig. 17.18** Structures of inhibin subunit precursors and processed forms in serum, the activins and the follistatins. The precise contribution of each molecular-weight form of inhibin to the biologic activity in serum is not known, but it has been established that the 55-kDa and 32-kDa forms are biologically active. *αN,* amino region of alpha-inhibin; *αC,* carboxy region of alpha-inhibin. (Modified from Burger H. Inhibin, activin and neoplasia. In: Yen SC, Jaffe RB, Barbieri RL, eds. *Reproductive Endocrinology*. 4th ed. Philadelphia, PA: Saunders; 1999:669–675.)

cells and pituitary gonadotrophs. Unlike inhibin, locally synthesized activin in the pituitary, rather than the ovarian-derived activin, is responsible for regulating FSH (see Fig. 17.4).[106]

Follistatin is a single-unit peptide that is produced in several human tissues, including the pituitary and ovary (see Fig. 17.18).[106] It binds and neutralizes the biologic functions of activin. It appears that local follistatin levels in tissues modulate the effects of activin. This explains the inhibitory effect of follistatin on pituitary FSH secretion (see Fig. 17.4).[106]

Overview of the Hormonal Changes During the Ovarian Cycle

FSH secretion is suppressed by negative feedback of the ovarian hormones estradiol, inhibin, and progesterone during the early and midluteal phase. The sharp decline of these hormones upon regression of the corpus luteum during the late luteal phase abolishes this negative feedback (see Fig. 17.1A). This permits increased secretion of FSH just before and during menses. This initial increase in FSH is essential for follicle recruitment and growth and steroidogenesis. With continued growth of the follicle, autocrine and paracrine factors produced within the follicle maintain follicular sensitivity to FSH. Continuing and combined action of FSH and activin leads to the appearance of LH receptors on the granulosa cells, a prerequisite for ovulation and luteinization.

Ovulation is triggered by the rapid rise in circulating levels of estradiol. A positive feedback response at the level of the anterior pituitary and possibly at the hypothalamus results in the midcycle surge of LH that is necessary for expulsion of the egg and formation of the corpus luteum (see Fig. 17.1A). A rise in the progesterone level follows ovulation, along with a second rise in the estradiol level, producing the 14-day luteal phase characterized by low FSH and LH levels. Demise of the corpus luteum concomitant with a fall in hormone (progesterone, estradiol, and inhibin-A) levels allows FSH to increase again toward the end of the luteal phase, initiating a new cycle. If pregnancy is established by implantation of a blastocyst, the structural integrity and function (i.e., progesterone and estradiol production) of the corpus luteum are maintained by hCG secreted from the trophoblast. The hCG acts as a surrogate for LH on the corpus luteum.

In addition to FSH and LH, local factors (e.g., activin, inhibin) regulate follicular development and steroidogenesis. In the early follicular phase, activin produced by granulosa cells in immature follicles enhances the action of FSH on aromatase activity and FSH and LH receptor formation while simultaneously suppressing C19 steroid formation in theca cells. In the late follicular phase, increased production of inhibin by the granulosa cells and decreased activin levels promote the synthesis of C19 steroids in the theca layer in response to LH and local growth factors and cytokines; this provides larger amounts of the precursor androstenedione for production of estrone and ultimately of estradiol in the granulosa cells.[110]

LH-mediated androstenedione production in theca cells and FSH-mediated estradiol production in granulosa cells are potentiated by IGFs.[108] The major endogenous IGF produced in the human ovarian follicle is IGF2 (rather than IGF1), which is produced by granulosa and theca cells. The actions of IGF1 and IGF2 are mediated by IGF receptor type I in both cells. IGF receptor type I is structurally similar to the insulin receptor. It appears that gonadotropin-related IGF action in the ovary is regulated primarily by IGF2 and IGF receptor type I.[108,109]

In summary, ovulation is under the control of substances functioning as classic hormones (i.e., FSH, LH, estradiol, and inhibin), which transmit messages between the ovary and the hypothalamic-pituitary axis and of paracrine and autocrine factors such as IGF2, inhibin, and activin, which coordinate sequential activities within the follicle destined to ovulate. The negative feedback relationship between corpus luteum products (i.e., estradiol,

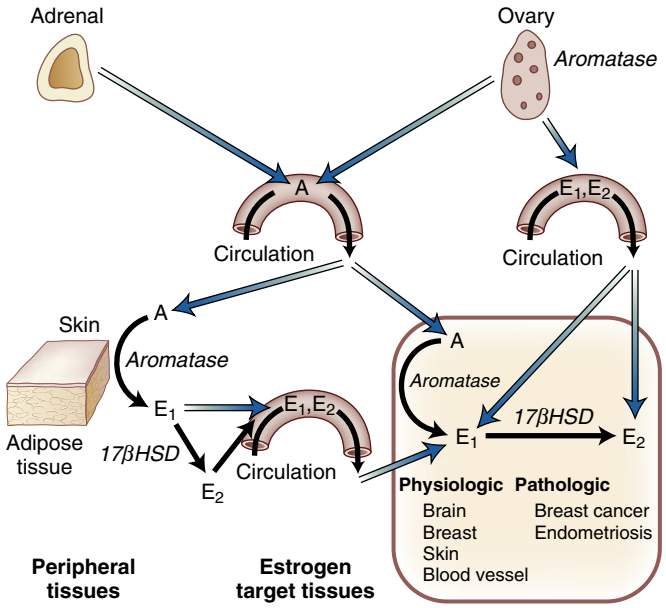
progesterone, and inhibin) and FSH results in the critical initial rise in FSH immediately before and during menses, and the positive feedback relationship between estradiol and LH is responsible for the ovulatory stimulus (see Fig. 17.1). Within the ovary, IGF2, inhibin, and activin modify follicular responses necessary for growth and function. These endocrine, paracrine, and autocrine factors undoubtedly represent only a portion of the complete picture. The causes of anovulation are diverse and may be related to defects in cell surface receptors, intracellular elements of signal transduction, or cell-cell interactions.[111,112]

Extraovarian Steroidogenesis

Estradiol formation takes place in several tissues in the woman of reproductive age, including the ovary, peripheral tissues such as subcutaneous fat and skin, and physiologic and pathologic target sites such as the hypothalamus, breast cancer cells, and the cells of endometriosis (Fig. 17.19).[97] Fat and skin are particularly critical sources of estrogen in anovulatory premenopausal and postmenopausal women. Although only small quantities of estrogen are produced by an individual adipocyte or skin fibroblast in a continuous fashion, these cell types contribute to circulating estradiol levels because of their relative abundance.[97] This effect is more pronounced in obese women because of increased mass of the adipose tissue and skin.[97]

Aromatase (CYP19A1) in adipocytes and skin fibroblasts is responsible for peripheral aromatization of androstenedione that arises from the ovary and the adrenal gland in premenopausal women and primarily from the adrenal in postmenopausal women (see Fig. 17.19).[97] However, the product of this reaction, estrone, is only weakly estrogenic. Estrone is further converted to estrone sulfate, which serves as a reservoir for estrone in blood and other tissues. Estrone (arising from androstenedione and estrone sulfate) is further converted to the biologically active estradiol in target tissues such as the endometrium and breast by a number of enzymatic proteins with overlapping reductive 17βHSD activity (see Fig. 17.19).[97,105] It is likely that local CYP19A1 expression in the hypothalamus is critical for the regulation of gonadotropin secretion.[21,113] Estrogen-dependent pathologic tissues such as those in breast cancer and endometriosis contain extremely high levels of CYP19A1 that enhances tissue growth by increasing local estradiol concentrations (see Fig. 17.19).[97] Circulating androstenedione is the major substrate for aromatase activity in these physiologic and pathologic target tissues.[97]

Significant quantities of circulating androstenedione can also be converted to testosterone in peripheral tissues (discussed later). This is probably accomplished by the presence of multiple 17βHSDs with overlapping reductive activities in peripheral tissues.[105] Androgenic action of testosterone is strikingly amplified by its conversion to DHT in peripheral and target tissues (e.g., skin, prostate). At least two distinct proteins encoded by two separate genes, 5α-reductase type 1 and type 2, catalyze the conversion of testosterone to DHT in liver, prostate, and skin.[98] Local production of DHT in genital skin fibroblasts is critical for normal masculinization of external genitalia of male fetuses in utero.[114] DHT formation in the skin is an important cause of hirsutism.[115]



• **Fig. 17.19** Estrogen biosynthesis in women. The biologically active estrogen, estradiol (E_2), is produced in at least three major sites: (1) by direct secretion from the ovary in reproductive-age women; (2) by conversion of circulating androstenedione (A), originating from the adrenal or ovary or both, to estrone (E_1) in peripheral tissues; and (3) by conversion of A to E_1 in estrogen target tissues. In the latter two instances, estrogenically weak estrone (E_1) is converted to E_2 within the same tissue. The expression of genes that encode the enzymes aromatase and reductive 17β-hydroxysteroid dehydrogenases (17βHSD) is critical for E_2 formation at these sites. Reductive 17βHSD activity in peripheral tissues may be conferred by protein products of several genes with overlapping functions. HSD17B1 is a distinct reductive 17βHSD enzyme that is encoded by a specific gene expressed primarily in the ovary. Aromatase is encoded by a single gene *(CYP19A1)*. E_2 formation by peripheral and local conversion is particularly important in postmenopausal women and in those with estrogen-dependent diseases such as breast cancer, endometriosis, or endometrial cancer.

Endometrium

The endometrium is the mucosal lining of the uterine cavity. The decidua is the highly modified and specialized endometrium of pregnancy. From an evolutionary perspective, the human endometrium is highly developed to accommodate the hemochorioendothelial type of placentation, which requires the presence of spiral arteries (Fig. 17.20). Trophoblasts of the blastocyst invade spiral arteries during implantation and placentation in the establishment of uteroplacental vessels.

Spiral arteries of the human endometrium confer another unique process, *menstruation*. Menstruation is shedding of endometrial tissue with hemorrhage that depends on sex steroid hormone–directed changes in blood flow in the spiral arteries. Spiral arteries are essential for menstruation; only humans and a few other primates that have endometrial spiral arteries experience menstruation. With nonfertile but ovulatory ovarian cycles, menstruation effects desquamation of the endometrium. New endometrial growth and development must be initiated with each ovarian cycle, so endometrial maturation corresponds with the next opportunity for pregnancy. There seems to be a narrow window of endometrial receptivity to blastocyst implantation, comprising the period between days 20 and 24 during a 28-day menstrual cycle.[116]

Functional Anatomy of the Endometrium

The endometrium can be divided morphologically into an upper two-thirds *functionalis* layer and a lower one-third *basalis* layer (see

Fig. 17.20). The purpose of the functionalis layer is to prepare for the implantation of the blastocyst; it is the site of proliferation, secretion, and degeneration. The purpose of the basalis layer is to provide the regenerative endometrium after menstrual loss of the functionalis.[117] Major histologic components of the endometrium include stromal cells, which constitute the skeleton of the tissue; a single layer of epithelial cells, which lines the lumen of the endometrial cavity and invaginations of the stroma; blood vessels; and resident immune cells. The epithelial cells that line the rather deep invaginations of the stroma are also referred to as *glandular cells*. However, these deep crypts represent extensions of the intracavitary lumen and are not true glands. These invaginations lined by epithelial cells extend from the surface of the functionalis layer (i.e., luminal epithelium) deep into the basalis level (i.e., glandular epithelium). After the functionalis layer is shed at the time of menstruation, the tissue stem cells in the basalis layer respond rapidly to estrogen and give rise to a new functionalis layer for the upcoming cycle (Fig. 17.21).[118] In humans and some other primates, the cellular components of the functionalis layer undergo a striking progression during the menstrual cycle, whereas the basalis layer shows only modest alterations.[117,119,120]

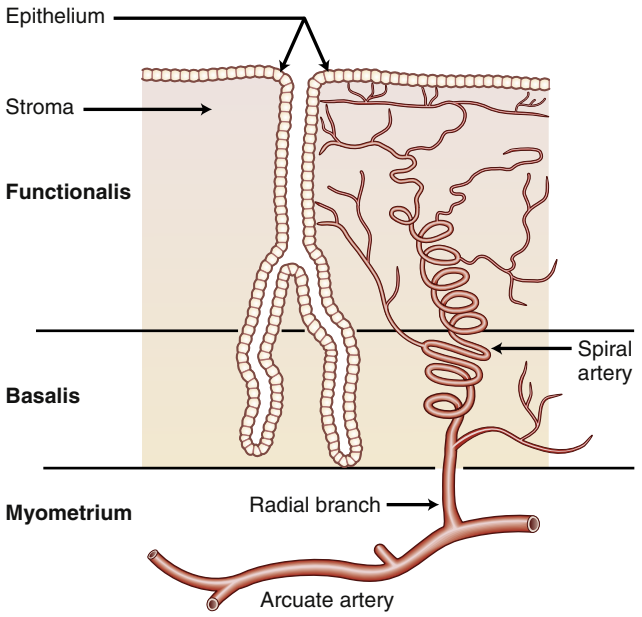
Hormone-Induced Morphologic Changes of the Endometrium

The cyclic changes in endometrial histology are faithfully reproduced during each ovulatory ovarian cycle (see Fig. 17.21). First, during the preovulatory, or follicular, phase of the cycle, estradiol is secreted (principally by a single dominant follicle of one ovary) in increasing quantities until just before ovulation. Second, during the postovulatory, or luteal, phase of the cycle, progesterone is secreted by the corpus luteum in increasing amounts (up to 40–50 mg/day) until the midluteal phase. Third, beginning about 7 to 8 days after ovulation, the rates of progesterone and estradiol secretion by the corpus luteum begin to decline and then diminish progressively before menstruation (see Fig. 17.1).

In response to these cyclic changes in the rates of ovarian sex steroid hormone secretion, there are five main stages of the corresponding endometrial cycle: (1) menstrual-postmenstrual *reepithelialization*; (2) *endometrial proliferation* in response to stimulation by estradiol; (3) abundant *epithelial secretion*, occurring in response to the combined action of estradiol and progesterone; (4) *premenstrual ischemia*, the result of endometrial tissue volume involution, which causes stasis of blood in the spiral arteries; and (5) *menstruation*, which is preceded and accompanied by severe vasoconstriction of the endometrial spiral arteries and collapse and desquamation of all but the deepest layer of the endometrium. In the final analysis, menstruation is the consequence of the withdrawal of factors that maintain endometrial growth and differentiation (see Fig. 17.21).

Commonly, the initiation of menstruation is attributed to progesterone withdrawal. This concept was developed because the administration of estrogen to postmenopausal women and treatment with and then withdrawal of a progestin causes menstruation, even with continued estrogen treatment. Moreover, progesterone facilitates decidualization of the endometrium and the maintenance of pregnancy, whereas progesterone withdrawal favors the initiation of menstruation, lactation, and parturition.

The preovulatory (follicular or proliferative) phase and the postovulatory (luteal or secretory) phase of the ovarian-endometrial cycles are customarily divided into early and late stages (see Fig. 17.21). The normal secretory phase of the menstrual cycle can be subdivided almost daily by histologic criteria, from shortly after ovulation until the onset of menstruation.[121] Some gynecologists use histologic dating of endometrial biopsies obtained during the luteal phase to evaluate ovulation, progesterone production, or the degree of biologic response of the endometrium to progesterone.[121]

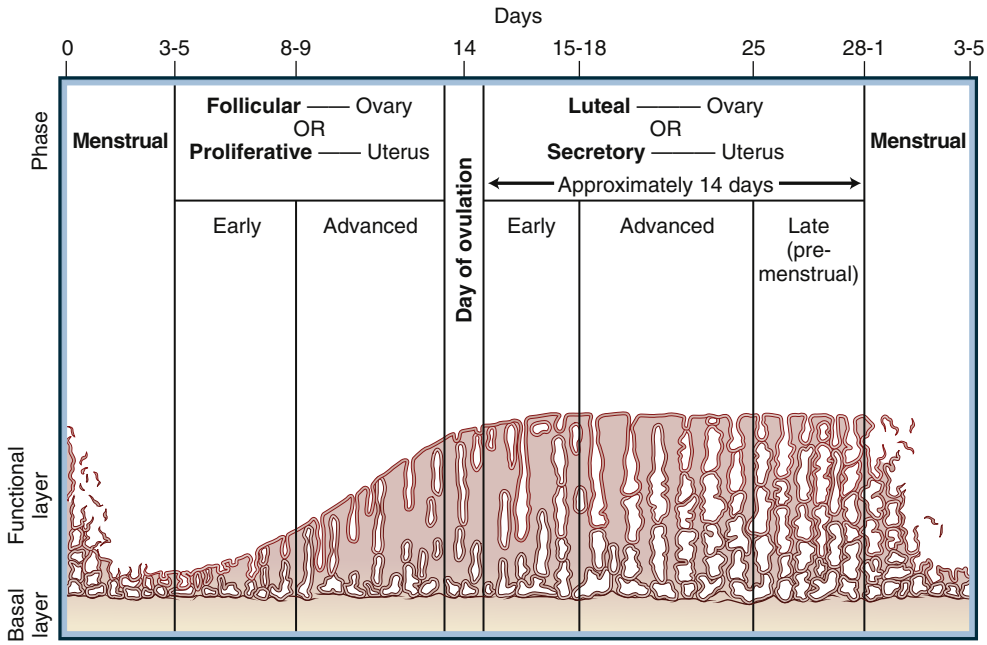


• **Fig. 17.20** Functional anatomy of the endometrium. Endometrium is a multilayered mucosa specialized for implantation and support of pregnancy. A single, continuous layer of epithelial cells lines the surface of the stroma and penetrates the stroma with deep invaginations almost all the way down to the myometrium-endometrium junction. The entire thickness of the endometrium is penetrated by the spiral arteries and their capillaries. Spiral arteries originate from the radial branches of arcuate arteries, which arise from uterine arteries. The superficial layer (functionalis) is shed during menstruation, whereas the permanent bottom layer (basalis) gives rise to the regeneration of endometrium after each menstruation. The striking changes in the spiral arteries (i.e., coiling, stasis, and vasodilatation followed by intense vasoconstriction) are consistently observed before the onset of every menstruation episode. (Courtesy Dr. Kristof Chwalisz, AbbVie, Inc., North Chicago, IL.)

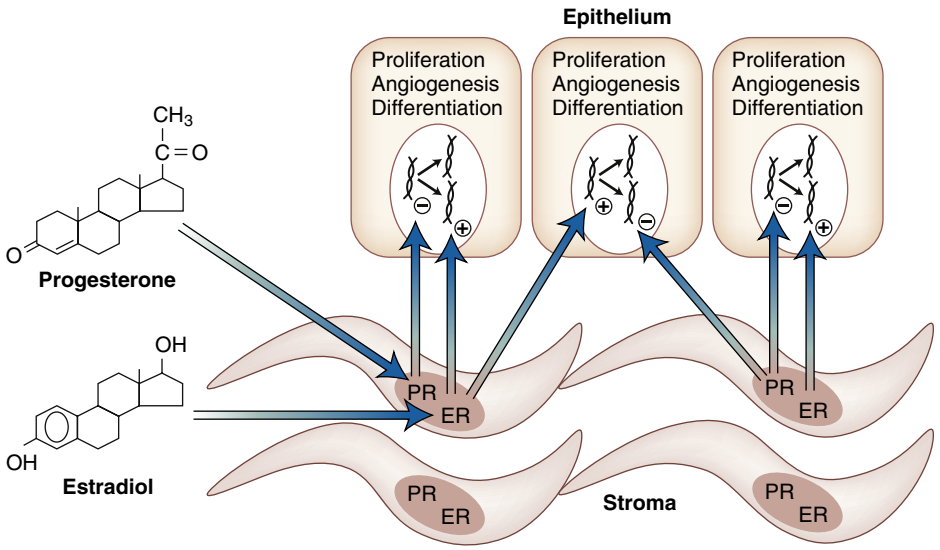
Effects of Ovarian Steroids on Endometrium

The exogenous administration of only estradiol and progesterone is sufficient to prepare the endometrium for implantation in the absence of ovarian function.[122,123] This observation underscores the essential roles of these steroids in uterine physiology. Estradiol or synthetic estrogens such as ethinyl estradiol cause a striking thickening of endometrial tissue. Stromal and epithelial cells of the endometrium proliferate rapidly under the influence of estradiol. Estradiol greatly increases mitotic activity and deoxyribonucleic acid (DNA) synthesis in both cell types (Fig. 17.22). While promoting growth, estradiol also renders endometrial tissue responsive to progesterone by inducing the expression of PRs; progesterone action depends on previous or concurrent estrogen exposure of the endometrium.[124]

In contrast to the proliferative effects of estrogen, progesterone action primarily enables differentiation of the endometrium. Progesterone can inhibit and even reverse the proliferative action of estrogen on epithelial cells in the functionalis layer (see Fig. 17.22). Moreover, progesterone action prepares the endometrium



• **Fig. 17.21** Cyclic changes in thickness and morphologic changes of endometrium and the relationship of these changes to those of the ovarian cycle. (From Cunningham FG, MacDonald PC, Gant NF, et al. The endometrium and decidua: menstruation and pregnancy. In: Cunningham FG, ed. *Williams Obstetrics*. 19th ed. Stamford, CT: Appleton & Lange; 1993:81–109.)



• **Fig. 17.22** Critical epithelial effects of estrogen (i.e., deoxyribonucleic acid [DNA] synthesis, proliferation, and gene expression) are mediated primarily by estrogen receptor-α (ER) in stromal cells in a paracrine manner in the endometrium. This was demonstrated in mice. It was also shown in mice and humans that the antiestrogenic effects of progesterone on epithelial cells (e.g., decreased proliferation, enhanced differentiation) are mediated primarily by progesterone receptors (PRs) in stromal cells.

for implantation of the embryo through differentiation of stromal and epithelial cells. Progesterone induces the production and secretion of a glycogen-rich substance from the epithelial cells. Progesterone also causes an increase in stromal cell cytoplasm, a process called *pseudodecidualization*. The term *decidualization* is reserved for stroma differentiated under the combined influence of progesterone and hCG of placental origin during pregnancy.

Blood vessels that carry estrogen or progesterone come in contact with endometrial stromal cells first. These steroid ligands interact with their nuclear receptors in endometrial stromal cells, which in turn send paracrine signals to neighboring epithelial cells to regulate their functions.[103,124]

Estrogen Action

Estradiol enters endometrial cells from blood by simple diffusion and binds ERs, proteins with a high affinity for estradiol and biologically active synthetic estrogens. Although both ERα and ERβ are present in the endometrium, ERα is the primary mediator of the estrogenic action in that tissue.[125,126] The estradiol-ERα complex is a transcriptional factor that becomes associated

with chromatin.[127] Estradiol-ERα complexes bind thousands of DNA sites across the entire genome and regulate the transcription of hundreds of genes at one time.[127] Most ERα binding sites are outside basal promoters and interact with the transcriptional start sites through DNA looping.[114,127] This interaction brings about ER-specific initiation of gene transcription, which promotes the synthesis of specific mRNAs and, thereafter, specific proteins.[114,127] Estradiol acts in the endometrium and in other estrogen-responsive tissues to promote the responsiveness of that tissue to progesterone via inducing PR expression.[103]

The endometrial epithelial cells are estrogen responsive but probably do not replicate as a result of direct action of estradiol on them. Replication of human endometrial epithelial cells in culture is not increased appreciably, if at all, when estrogen is added to the medium. Estrogen acts on mouse uterine stromal cells to promote the synthesis of growth factors that act on epithelial cells (see Fig. 17.22).[124] These growth factors operate in a paracrine manner to cause increased DNA synthesis and replication in the adjacent epithelial cells.

Progesterone Action

Progesterone enters cells by diffusion and binds to its nuclear receptor (PR). Two PR isoforms, PR-A and PR-B, are present in the human endometrium.[128] Because PR-B but not PR-A levels in the endometrium are tightly regulated during the human menstrual cycle, PR-B is presumed to play a more important biologic role.[128] The cellular content of PRs typically depends on previous estrogen action.

The progesterone-PR complex regulates gene transcription, but the response to progesterone is strikingly different from that evoked by the estradiol-ER complex. Similar to ERα, PR binding sites are also widely distributed across the genome, lie frequently outside basal promoters, and do not involve classically defined progesterone response elements.[129] Interaction of PR binding sites with basal promoters possibly requires DNA looping.[129]

Progesterone acts as an antiestrogen by reducing ERα expression, by decreasing the tissue levels of estradiol through its conversion to estrone via inducing the 17βHSD2 enzyme, and by enhancing estrogen inactivation through sulfation.[130–132] Tissue recombination experiments using uteri of PR knockout and normal mice have demonstrated that many effects of progesterone on epithelial cells are also mediated in a paracrine fashion by PRs in stromal cells but not by those in epithelial cells (see Fig. 17.22).[103,132]

The most striking consequences of progesterone action, stromal predecidualization and epithelial secretion, parallel increased levels of circulating progesterone during the luteal phase. The PR content of human endometrial tissue peaks during the late proliferative phase, just before ovulation, and declines sharply before circulating progesterone levels increase during the luteal phase.[128] This dyssynchrony between endometrial PR expression and circulating progesterone is not well understood. Molecular correlates of progesterone action with respect to differentiation include increased production of lactoferrin and glycodelin in epithelial cells and of prolactin and IGF-binding protein 1 in stromal cells of the endometrium.[103]

The Receptive Phase of the Endometrium for Implantation

Unless the ovum is fertilized within 24 hours after ovulation, it does not survive. Fertilization takes place in the ampullary, the distal one-third of the oviduct. Over the next 2 days, the fertilized ovum remains unattached within the tubal lumen. After this stage, the embryo (which consists of a solid ball of cells called the *morula*) leaves the oviduct and enters the uterine cavity. By this time, endometrial secretions under the influence of luteal progesterone have filled the cavity and bathe the embryo in nutrients. This is the first of many neatly synchronized events that mark the conceptus-endometrial relationship. By 6 days after ovulation, the embryo (now a blastocyst) is ready to implant. It finds an endometrial lining of sufficient depth, vascularity, and nutritional richness to sustain the important events of early placentation that are to follow. Just below the epithelial lining, a rich capillary plexus has been formed and is available for creation of the trophoblast-maternal blood interface. Later, the surrounding superficial portion of the functionalis zone, now occupying more and more of the endometrial cavity, provides a sturdy splint to retain endometrial architecture despite the invasive inroads of the burgeoning trophoblast.

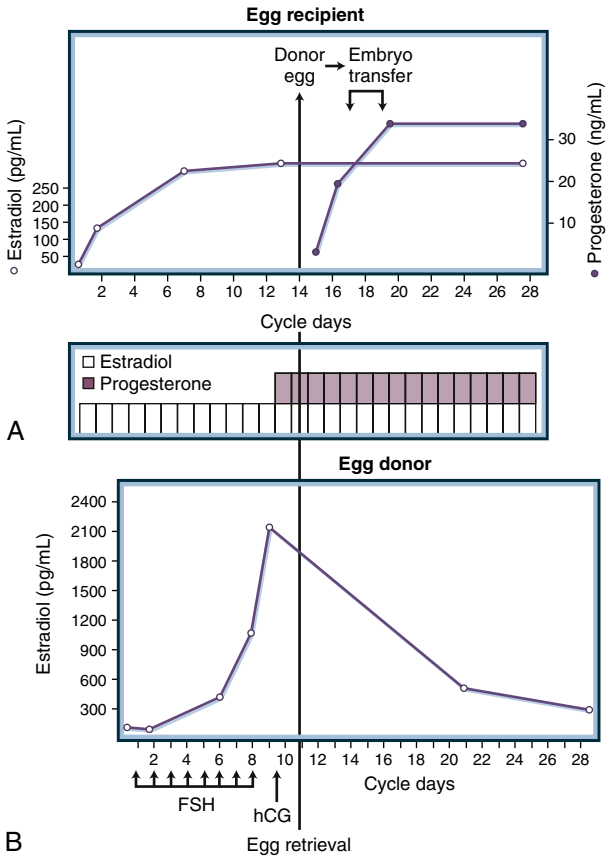
Progesterone is essential for the maintenance of pregnancy. The blastocyst depends on progesterone produced by the corpus luteum at this time. The hCG secreted by the trophoblast acts as a surrogate LH and prevents regression of the corpus luteum that provides a continuous supply of progesterone until the placental tissue starts to produce sufficient quantities of progesterone (6–7 weeks after fertilization).

The receptive phase of the endometrium is the temporal window of endometrial maturation during which the trophectoderm of the blastocyst can attach to the endometrial epithelial cells and proceed to invade the endometrial stroma. The window of uterine receptivity can be inferred from what has been learned from transfer of embryos to uteri of women primed with exogenous estrogen and progesterone preparations (Fig. 17.23). There is a distinct window for embryo transfer leading to implantation, which spans endometrial cycle days 16 to 20. The actual window of implantation follows this window of transfer, because embryos need to develop further, from the four-cell to eight-cell stage to the blastocyst stage, before initiation of attachment and frank invasion can occur. Based on serial measurements of serum hCG as a marker of initial embryonic-maternal interaction, the window of implantation in humans is estimated to be between days 20 and 24 of the cycle.[133] This relatively wide window agrees with the earlier morphologic data.[134]

Control of Endometrial Function With the Use of Exogenous Hormones

The fertility potential of a woman is primarily determined by the biologic quality of her oocytes, reflected in part by a normal chromosomal complement and the capacity of the fertilized ovum to divide at an optimal rate. This biologic quality declines sharply after the age of 35 years. However, the biologic potential of the endometrium for successful implantation remains intact even at advanced ages.[122] Oocyte donation from a fertile woman and fertilization of the donor eggs in vitro with the recipient's male partner's sperm, followed by embryo transfer into the uterine cavity of the recipient woman with nonfunctioning ovaries (e.g., premature ovarian insufficiency [POI]), have been used successfully as a therapeutic strategy to treat infertility (see Fig. 17.23).[122,123] This clinical application has provided unique opportunities to examine the hormonal therapy for endometrial maturation. The success of these procedures (i.e., pregnancy rate) has averaged higher than in conventional IVF.[123]

The follicular phase is mimicked by administration of oral micronized estradiol in daily doses of up to 8 mg for about 10



• **Fig. 17.23** Donation of oocytes by a woman undergoing egg retrieval to a woman with ovarian insufficiency treated with exogenous estrogen and progesterone. The window of implantation in both women is synchronized by different but comparable hormonal treatments. (A) Woman with ovarian insufficiency is initially treated with oral micronized estradiol during days 1 through 14 of the cycle. Exogenous intramuscular progesterone is added to the estradiol treatment on days 15 through 28 and continued if pregnancy is diagnosed. Several donor eggs are fertilized with sperm from the recipient's husband, and one or two embryos are transferred to the uterus on day 16 to 19, depending on the stage of embryo development. These embryos are expected to implant between days 20 and 24. (B) The egg donor is simultaneously treated with human recombinant follicle-stimulating hormone (FSH) with or without menopausal gonadotropin until cycle day 8 to 12, when human chorionic gonadotropin (hCG) is given to induce ovulation, and oocytes are harvested 32 to 36 hours later. One or two fertilized eggs are transferred to the uterus of the recipient. Progesterone supplement to the recipient is started before the embryo transfer. Serum levels of estradiol and progesterone in both women are shown. To convert estradiol values to picomoles per liter, multiply by 3.671. To convert progesterone values to nanomoles per liter, multiply by 3.180.

days. Serum estradiol during the replacement follicular phase reaches sufficiently high levels to stimulate endometrial growth. This is followed by up to 8 mg per day of oral estradiol combined with daily intramuscular (50 mg) or vaginal (200–400 mg) progesterone to promote the secretory transformation. Progesterone supplementation is ordinarily continued until 8 to 10 weeks of gestation.

Mechanism of Menstruation

In the absence of pregnancy, failure of the appearance of hCG despite otherwise appropriate tissue reactions leads to the vasomotor changes associated with estrogen-progesterone withdrawal and menstrual desquamation. A program of endometrial remodeling is initiated; alterations in the extracellular matrix and infiltration of leukocytes lead to hypoxia-reperfusion injury and sloughing of the functionalis, followed by activation of hemostatic and regenerative processes. The main histologic features of the premenstrual phase are degradation of the stromal reticular network, stromal infiltration by polymorphonuclear and mononuclear leukocytes, and secretory exhaustion of the endometrial glands, whose epithelial cells now have basal nuclei. The endometrium shrinks preceding menstruation in part as a result of diminished secretory activity and the catabolism of extracellular matrix.

An ischemic phase caused by vasoconstriction of the arterioles and coiled arteries precedes the onset of menstrual bleeding by 4 to 24 hours.[119] Bleeding occurs after the arterioles and arteries relax, leading to hypoxia-reperfusion injury. The superficial endometrial layers are distended by the formation of hematomas, and fissures develop, leading to the detachment of tissue fragments. Lysis and fragmentation of cells and apoptosis are evident. The menstrual efflux is composed of shed fragments of endometrium mixed with blood and liquefied by the fibrinolytic activity of the cellular debris (see Fig. 17.21). Clots of various sizes may be present if blood flow is excessive. Myometrium contracts to mechanically stop bleeding from the spiral arteries and other endometrial vessels.

Approach to the Woman With Reproductive Dysfunction

Reproductive dysfunction in an adult woman is most often manifested by disruption of cyclic, predictable menses. Efficient diagnosis of the underlying disorder requires a thorough understanding of female reproductive physiology and pathologic conditions and an accurate history and physical examination. Without a critical analysis of clinical findings based on thorough knowledge of normal and abnormal reproductive function, the application of predetermined algorithms of laboratory testing causes unnecessary use of hormone measurements or imaging studies and delays diagnosis.

History

An essential tool for the evaluation of a woman with a reproductive disorder is a carefully recorded history. The history should be obtained from the patient with the aim of assessing the biologic effects of each of the various hormones. Recording the details of pubertal development as a reference for the onset of particular symptoms provides critical clues to the cause of certain reproductive disorders. For example, anovulation manifested by irregular uterine bleeding associated with the polycystic ovary syndrome (PCOS) most often begins during the pubertal years. The early onset of pubic hair and gradually progressing hirsutism at or before the time of puberty suggests nonclassic congenital adrenal hyperplasia or PCOS. In these cases, measurement of serum 17-hydroxyprogesterone may help to differentiate nonclassic congenital adrenal hyperplasia from PCOS. The appearance of hirsutism before puberty or several years after normal pubertal development should alert the clinician to the possibility of ovarian or adrenal neoplasms. Sudden onset of hirsutism at any age or the presence of virilization should prompt the physician to rule out steroid-secreting ovarian or adrenal tumors. Most women with symptomatic endometriosis suffer from severe episodes of painful menses (i.e., dysmenorrhea) that start during pubertal years.

Evaluation of female reproductive function depends on a detailed history of the menses. For example, PCOS is unlikely without a long-standing history of irregular periods since the menarche. A history of a period of cyclic, predictable menses before the onset of menstrual irregularities should draw attention to hypothalamic or other causes of anovulation. The current frequency, regularity, length, and quantity of uterine bleeding should be carefully recorded for several reasons. First, this information reflects tightly regulated interactions of several tissues, including the hypothalamus, pituitary, ovaries, and endometrium. Second, regular, predictable menses imply ovulation. Third, defining the type of menstrual irregularity may help with diagnosis of the underlying cause. For example, prolonged amenorrhea in a thin and estrogen-deficient woman suggests anovulation of hypothalamic origin. Infrequent periods of varying duration and with a varying amount of blood loss in a well-estrogenized, overweight woman suggest a primary ovarian dysfunction such as PCOS. Anovulation in a thin but well-estrogenized woman may also be caused by PCOS. Regular but heavy and prolonged menses with intermittent spotting may result from uterine anatomic disorders such as adenomyosis or leiomyomas. Fourth, neoplastic disorders of the endometrium, including endometrial polyps, hyperplasia, or malignancies, may be manifested by any pattern of irregular bleeding. The combination of vaginal ultrasonography and endometrial biopsy is helpful for the diagnosis of endometrial neoplasia.[135]

Disruption of cyclic and predictable menses is a common and alarming symptom that initially brings the patient to the clinician. After a careful evaluation of the menstrual symptoms, the clinician should identify other obvious symptoms of the endocrine disorder underlying the irregular periods. Pregnancy is the most common cause of amenorrhea (and other menstrual irregularities) in a woman of reproductive age. In a woman presenting with amenorrhea or any other menstrual irregularity, normal pregnancy, ectopic pregnancy, or gestational trophoblastic disease must be excluded at the onset. Careful evaluation of past reproductive history and the patient's sexual activity and contraceptive practices can provide useful indications of the likelihood of pregnancy. The reproductive history may suggest the possibility of Sheehan syndrome of postpartum pituitary necrosis if menses did not resume after a delivery complicated by significant hemorrhage.[136] In such instances, evidence of adrenal and thyroid insufficiency should be sought. A classic symptom of Sheehan syndrome is the absence of postpartum lactation, which is related to prolactin deficiency.

Amenorrhea is traditionally categorized as primary (no history of menstruation) or secondary (cessation of menses after a variable time). The diverse causes of primary amenorrhea are discussed extensively in Chapters 23 and 25. Although the distinction between primary and secondary amenorrhea may be useful for identifying the mechanism of disease and the differential diagnosis, the clinician should be aware that a disorder can initially manifest with either primary or secondary amenorrhea. For example, most women with gonadal dysgenesis have primary amenorrhea, but some patients have residual follicles and ovulate, and in these women with partial gonadal dysgenesis, some menstruation and rare pregnancies may occur before the cessation of ovarian function.[137,138] Patients with PCOS usually have secondary amenorrhea but occasionally have primary amenorrhea. Rarely, pregnancy may manifest as primary amenorrhea.

After pregnancy is ruled out, secondary amenorrhea is most often caused by chronic anovulation, which can be broadly categorized as hypothalamic dysfunction, hyperprolactinemia-associated anovulation, ovarian insufficiency, androgen excess, or chronic illness or primary uterine disease (e.g., intrauterine adhesion formation after a postpartum curettage). Establishing any association of secondary amenorrhea with life events is extremely useful. Strenuous exercise is often associated with amenorrhea. Weight loss often precedes or accompanies secondary amenorrhea and has been suggested as evidence of hypothalamic dysfunction. An unusual dietary history may suggest bulimia or anorexia nervosa. The presence of any signs or symptoms of estrogen deficiency, including painful intercourse, atrophic vagina, emotional lability, and vasomotor instability, suggests anovulation of a central nature with low concentrations of circulating gonadotropins (i.e., hypogonadotropic hypogonadism) or ovarian insufficiency with elevated gonadotropins (i.e., hypergonadotropic hypogonadism).

Galactorrhea in the absence of a recent history of pregnancy suggests a host of diagnostic possibilities and is frequently a manifestation of excessive prolactin secretion, although it may result from increased sensitivity of breast tissue to the hormones necessary for milk production. This history frequently reveals drug ingestion as the cause. Various drugs, including several psychotropic agents, antihypertensive agents, and oral contraceptives, have been implicated. Primary hypothyroidism may be associated with precocious puberty with galactorrhea in the child and with amenorrhea or galactorrhea, or both, in the adult woman. A history of excessive nipple manipulation or chest wall disease should be elicited because it may be the cause of galactorrhea. Prolactinomas, the prolactin-secreting adenomas of the pituitary, are a common cause of galactorrhea related to abnormally high serum levels of prolactin. A history of dilatation and curettage, postpartum endometritis, or disseminated tuberculosis with absent to scant menses suggests the possibility of intrauterine adhesion.[139]

Physical Examination

The quantity and distribution of excessive hair growth should be considered in light of the familial history. Hypertrichosis—excessive growth of hair on the extremities, the head, and the back—must be distinguished from true hirsutism, which is the development of facial hair, chest hair, and a male escutcheon with or without signs of virilization in response to increased production of or sensitivity to biologically active androgens. Some degree of hypertrichosis is not uncommon in women of Mediterranean descent, whereas the occurrence of any facial hirsutism in the relatively hairless Asian woman may require thorough investigation. Hirsutism is best documented and quantified with the help of photographs. Virilization is characterized as deepening of the voice, severe cystic acne, hair loss, increased muscle mass, and clitoromegaly and implies a more severe degree of androgen excess than that found with hirsutism. The syndrome of complete androgen insensitivity is characterized by sparse to absent pubic and axillary hair due to resistance to androgen.

A careful inspection of the breasts is essential for a thorough physical examination. Classification of the stage of breast development according to the method of Marshall and Tanner[140] is a convenient and valuable adjunct. The physician should assess whether the breasts appear to have decreased in size recently (e.g., severe androgen excess), whether the areolae are well formed and pigmented (as they are in pregnancy), and whether a discharge (e.g., galactorrhea) can be expressed.

A woman with PCOS who has never ovulated or taken a progestin-containing medication may have Tanner stage 4 breast development related to adequate estrogen production, whereas the progression to Tanner stage 5 requires exposure to progesterone

through ovulation, ingestion of a progestin (e.g., administration of oral contraceptives), or pregnancy. Chapter 25 discusses Tanner staging of breast development.

The vulva, vagina, and cervix also represent sensitive indicators of gonadal steroid action. Because sensitivity of the genital skin and mucosa to androgen decreases with time from the early stages of fetal development to adulthood, the extent of any virilization can be helpful in suggesting the timing of androgen exposure. The most profound androgenic effects, such as posterior labial fusion with or without formation of a penile urethra, are usually observed in 46,XX fetuses exposed to androgens during the first 8 to 10 weeks of pregnancy. Similar findings have been described in patients with virilizing congenital adrenal hyperplasia, true hermaphroditism, and drug-induced virilization. Postnatal development of significant clitoromegaly in a 46,XX infant requires marked hormonal stimulation and, in the absence of significant exogenous steroids, strongly implicates an androgen-secreting tumor. The size of the glans clitoris can be quantified by determining the clitoral index, which is the product of the sagittal and transverse diameters of the glans. Ninety-five percent of normal women have a clitoral index less than 35 mm^2.

The vagina and uterine cervix are the most sensitive indicators of estrogen action. Under the influence of estrogen, the vaginal mucosa progresses during sexual maturation from a tissue with a shiny, bright red appearance with sparse, thin secretions to a tissue with a dull, gray-pink, rugated surface with copious, thick secretions. Well-estrogenized vaginal mucosa with stretchable cervical mucus (i.e., *spinnbarkeit*) may indicate the proliferative phase of the menstrual cycle in an ovulatory woman or extraovarian estrogen formation in an anovulatory woman with PCOS. The biologic activity of estrogen can also be quantified by vaginal cytologic examination.

To summarize, irregular uterine bleeding is a common symptom that brings the woman with reproductive dysfunction to the physician's office. Various disorders of the hypothalamus, pituitary, ovaries, or uterus or other tissues that affect reproductive function may be responsible for this alarming symptom. After pregnancy is ruled out, a detailed history and physical examination should be carefully recorded. In particular, the physician should pay attention to the salient features in the history and the biologic indicators of hormone action at target tissues during the physical examination. An analysis of these findings most often leads to a tentative diagnosis, which may be confirmed by laboratory testing.

Disorders of the Female Reproductive System

Chronic Anovulation

Chronic anovulation is one of the most common gynecologic problems encountered by the practitioner. Patients may present with secondary amenorrhea, infrequent uterine bleeding (i.e., oligomenorrhea), or irregular episodes of excessive uterine bleeding. Infertility is an obvious consequence of chronic anovulation. Pregnancy, end-organ defects (e.g., intrauterine adhesions, müllerian agenesis), amenorrhea associated with genital ambiguity at birth (e.g., 46,XX, 46,XY, and ovotesticular disorders of sex development), or sexual infantilism due to gonadal dysgenesis should initially be ruled out.

For practical purposes, most of the etiologic factors giving rise to chronic anovulation in a woman of reproductive age fall into five broad categories: hypothalamic anovulation, hyperprolactinemia, androgen excess, POI, and chronic illness (e.g., hepatic or renal insufficiency, acquired immunodeficiency syndrome [AIDS]). Salient features of the history and physical examination help to place a woman with anovulation in one or more of these categories.

One group of anovulatory patients is estrogen deficient. Common disorders in this group include hypothalamic anovulation, galactorrhea-hyperprolactinemia (e.g., hypothyroidism, prolactinoma, nonfunctioning pituitary tumor), and POI in a woman of reproductive age. These patients are usually amenorrheic. All patients present with signs of estrogen deficiency (e.g., vaginal atrophy). Patients with hypothalamic anovulation or galactorrhea-hyperprolactinemia usually do not complain of hot flashes, whereas women with POI present with vasomotor symptoms. One serious consequence of estrogen deficiency is bone loss, giving rise to osteopenia and osteoporosis. If possible, the underlying cause should be corrected. Hormone therapy should be provided if ovulation cannot be restored.

Women with androgen excess constitute the second major group of anovulatory patients. A serious consequence of anovulation in this group is the greater risk for carcinoma of the endometrium because of unopposed action of estrogen formed continuously in extraovarian tissues. The most common disorder of the ovary associated with androgen excess and anovulation is PCOS. Insulin resistance plays a significant role in this condition and, along with hyperandrogenism, increases the risk of developing cardiovascular disease (CVD), diabetes mellitus, or both.[111] The clinician must recognize the long-term impact of PCOS and undertake therapeutic management of these anovulatory patients to avoid unwanted consequences. The clinician should also develop a plan with the patient to address long-term complications of unopposed estrogen formation associated with PCOS (e.g., endometrial neoplasia). Oral contraceptives or periodic progestin supplementation may be provided to prevent endometrial hyperplasia and cancer.

Many mechanisms may be responsible for anovulation in chronic illness. Effective treatment of the primary illness may restore normal menses. Alternatively, anovulatory bleeding may be managed with the use of exogenous hormones in these chronically ill patients (discussed later).

Measurements of FSH and prolactin help to categorize anovulatory patients. An undetectable or low-normal FSH level is consistent with hypothalamic amenorrhea, PCOS, or hyperprolactinemia, whereas high FSH levels suggest ovarian insufficiency. High prolactin levels may indicate a pituitary prolactinoma, pituitary disease, or hypothyroidism. The following sections describe specific disorders that cause chronic anovulation in women of reproductive age.

Hypothalamic Anovulation

Anovulation of hypothalamic origin usually manifests as amenorrhea. The terms *hypothalamic anovulation* and *hypothalamic amenorrhea* are used interchangeably in this chapter. A reduction in GnRH pulse frequency from the characteristic 60 to 120 minutes to intervals longer than 180 minutes leads to lower levels of LH and FSH secretion by the pituitary gland.[13,141] This functional gonadotropin deficiency fails to provide adequate stimulation

to the ovarian follicles, and the normal sequence of follicular growth, maturation, follicular selection, and ovulation becomes attenuated. Downstream ovarian estradiol production remains low, and endometrial growth is reduced or arrested, resulting in a prolonged interval of amenorrhea. The transition from normal menstrual cyclicity to anovulation and amenorrhea can take place gradually and may be characterized by inadequate luteal phases, irregular menstrual bleeding, and amenorrhea. Patients with hypothalamic amenorrhea do not complain of hot flashes, even though circulating levels of estradiol are within the menopausal range. This suggests a significant role for GnRH or gonadotropins in the cause of hot flashes.

Any disorder of the central nervous system that interferes with normal GnRH pulse frequency can cause anovulation. Some of these disorders may be defined genetic or anatomic disorders such as isolated gonadotropin deficiency (with or without anosmia), infection, suprasellar tumors (e.g., pituitary adenomas, craniopharyngioma), and head trauma.[10] These genetic and anatomic disorders affect the function of the hypothalamus, and some of them may be ruled out by the medical history, physical examination, and imaging of the head (Table 17.1).

The most commonly observed form of hypothalamic anovulation is not associated with a demonstrable neuroanatomic finding.[142] This common form is called *functional hypothalamic amenorrhea* because it is presumed to involve aberrant but reversible regulation of otherwise normal neuroendocrine pathways. Other causes of hypothalamic anovulation demonstrable by neuroanatomic or genetic evidence are less common (see Table 17.1).

Functional Hypothalamic Amenorrhea

Anovulation resulting from stress-associated changes is viewed as a functional disorder in which no anatomic or organic abnormalities of the hypothalamic-pituitary-ovarian axis can be identified.[142] This condition typically manifests as amenorrhea of at least a 6-month duration and has also been called *functional hypothalamic amenorrhea*.[142] The overall prevalence of functional hypothalamic amenorrhea among all amenorrhea disorders ranges from 15% to 48%.[143]

Anovulation of hypothalamic origin is characterized by estrogen deficiency and low levels of gonadotropins. No genetic or anatomic disorders are identified in most patients.[142] The concept of functional hypothalamic anovulation (i.e., amenorrhea) was postulated as the failure of the hypothalamic-pituitary pathways to release gonadotropins from the anterior pituitary.[142] Clinical studies have supported this common mechanism associated with an alteration in the pulsatile secretion of GnRH.[14] Diverse etiologic factors such as malnutrition or caloric restriction, depression, psychogenic stress, excessive energy expenditure related to exercise, or combinations of these disorders precede the onset of functional hypothalamic anovulation.[142] Heightened awareness of diet or exercise and unrealistic expectations with respect to body image most likely contributed to the epidemic of this anovulatory disorder.

Diagnosis of Functional Hypothalamic Amenorrhea

Patients most commonly present with secondary amenorrhea characterized by absence of menstrual cycles for longer than 6 months without evidence of an organic disorder. The diagnosis is one of exclusion. There are many neuroanatomic or genetic disorders that can mimic functional hypothalamic anovulation (see Table 17.1), and a careful and complete evaluation is essential to make this diagnosis.

TABLE 17.1 Classification of Anovulation Caused by Disorders of the Hypothalamic-Pituitary Unit

Functional Hypothalamic Anovulation (Amenorrhea)

Stress (psychogenic or physical)
Dieting
Vigorous exercise
Chronic illness (e.g., chronic liver or renal insufficiency, AIDS)

Psychiatric-Medical Emergencies

Anorexia nervosa

Medications

Antipsychotics (e.g., olanzapine, risperidone, amisulpride, clozapine)
Opiates

Hypothyroidism

Anatomically or Genetically Defined Pathologic Conditions of the Hypothalamic-Pituitary Unit

Pituitary tumors
Prolactinoma
Clinically nonfunctioning adenoma
GH-secreting adenoma (acromegaly)
ACTH-secreting adenoma (Cushing disease)
Other pituitary tumors (e.g., metastasis, meningioma)
Pituitary stalk section
Hemorrhagic pituitary destruction, including pituitary apoplexy and Sheehan syndrome
Pituitary aneurysm
Infiltrative disease of the pituitary (e.g., lymphocytic hypophysitis, sarcoidosis, histiocytosis X, tuberculosis)
Empty sella syndrome
Tumors that affect hypothalamic function (e.g., metastasis, craniopharyngioma)
Infiltrative granulomatous disease of the hypothalamus (e.g., sarcoidosis, histiocytosis X, tuberculosis)
Head trauma
Irradiation to the head
CNS infection
Isolated gonadotropin deficiency (including Kallmann syndrome)
Other

ACTH, Adrenocorticotropic hormone; *AIDS,* acquired immunodeficiency syndrome; *CNS,* central nervous system; *GH,* growth hormone.

Women with functional hypothalamic amenorrhea usually present with a history of regular menses for some period after menarche. This period of normal ovulatory function (determined by history) is interrupted by anovulation that usually manifests as secondary amenorrhea. Women with functional hypothalamic anovulation may occasionally present with primary amenorrhea.

Women with functional hypothalamic amenorrhea typically have a normal body weight or are thin. They may be highly driven for success and involved in high-stress occupations. The occupation of the patient (e.g., ballerina, competitive athlete) may be an important clue. A detailed interview may also reveal a variety of emotional crises or stressful events (e.g., divorce, death of a friend) preceding the onset of amenorrhea. During the interview, additional environmental and interpersonal factors may become evident, such as academic pressure, social maladjustment, or psychosexual problems.

When evaluating the patient, the physician should take note of the current diet regimen, the use of any sedatives or hypnotics, and the nature and extent of the patient's exercise habits. Despite

a careful interview, a history of stress, excessive physical exercise, or an eating disorder may not be readily revealed by some women with functional hypothalamic anovulation. These women do not complain of hot flashes, in contrast to women with ovarian insufficiency.

These women usually have normal secondary sexual characteristics. The pelvic examination usually shows a thinning vaginal mucosa accompanied by scant to absent cervical mucus with a normal to small uterus, which are all evidence of estrogen deficiency. Signs of a well-estrogenized vagina and cervix observed during the physical examination make the diagnosis of hypothalamic amenorrhea unlikely. The physician should exclude a possible hyperprolactinemic cause (e.g., prolactinoma, hypothyroidism) and evidence of androgen excess (e.g., PCOS).

Laboratory tests are obtained to exclude other causes of anovulation and secondary amenorrhea. LH and FSH levels are usually lower than the normal values ordinarily found in the early follicular phase. TSH and prolactin levels are obtained to rule out hypothyroidism and hyperprolactinemia. The progestin challenge test (medroxyprogesterone acetate [MPA] at 5 mg/day for 10 days) elicits either a small spotting episode or an absence of withdrawal uterine bleeding in most patients. The administration of combined estrogen (2 mg/day of oral micronized estradiol) with progestin (5 mg/day of MPA for 10 days) will result in endometrial growth followed by vaginal bleeding after one or more cycles of therapy because the uterine compartment remains functionally normal.

These results suggest that there is a scant or absent estrogenic effect on the endometrium, because circulating estradiol levels are typically in the low or early follicular phase range. Measurement of the serum estradiol level is not necessary. Because a suprasellar or large pituitary tumor is in the differential diagnosis, magnetic resonance imaging (MRI) of the head is necessary to rule out these possibilities. Imaging of the head is especially important if amenorrhea develops suddenly or is associated with a neurologic sign, both of which make the presence of a tumor more likely.

Pathophysiology of Functional Hypothalamic Anovulation

A critical defect in hypothalamic amenorrhea is the reduction in GnRH release from the medial basal hypothalamus, which leads to a reduction in GnRH pulse frequency.[14,144] LH pulse frequency is used as a surrogate measure to evaluate GnRH secretion because each GnRH pulse is accompanied by a concomitant LH pulse.[143] A key observation in functional hypothalamic anovulation is the absence of increased gonadotropin secretion despite the lack of inhibitory factors of ovarian origin, such as estradiol and inhibin.

There is considerable variability in the amplitude and frequency of the pulsatile LH secretion in women with functional hypothalamic amenorrhea. When the LH secretory patterns are compared with those of the follicular phase of the menstrual cycle, a characteristic abnormality in LH pulse frequency and amplitude is seen; occasionally, regression to a pronounced variability similar to what is seen in the prepubertal pattern is observed.[14,143,144] In severe cases, the frequency and amplitude of LH pulses are markedly reduced. These LH patterns also suggest that GnRH pulsatile secretion is not altered to the same degree in every patient. During the recovery phase of hypothalamic amenorrhea, reversal to a pattern of LH secretion seen early in puberty often occurs, and it is characterized by a sleep-associated increase in LH amplitude.[143]

The response of the pituitary gland to GnRH with respect to production and release of gonadotropins is not impaired in functional hypothalamic anovulation. Intravenous pulsatile GnRH administration can restore normal levels of LH and FSH.[145]

Norepinephrine, dopamine, and serotonin produced in the brain have been shown to modulate GnRH or LH release in animal studies.[146] Patients receiving medication that alters these neurotransmitters (e.g., sedatives, antidepressants, stimulants, antipsychotics) have presented with abnormalities in their menstrual cycles. These responses to medications provide circumstantial evidence that disruptions of neural pathways can alter GnRH release in humans. It appears that activation of the noradrenergic neurons principally stimulates release of GnRH, whereas dopaminergic and serotoninergic neurons can stimulate or inhibit GnRH-LH secretion.[146]

Another group of substances that have inhibitory influences on GnRH secretion are endogenous opioid peptides.[147,148] Blockade of endogenous opiate receptors by the administration of naloxone, an opiate antagonist, causes an increase in the frequency and amplitude of pulsatile LH release in the majority of women with hypothalamic amenorrhea.[144] Gonadotropin secretion resumes if the activity of the opiate receptor is blocked by long-term naloxone use, and ovulatory function may be regained in some cases.[149] These studies suggest that there is an overall increase in endogenous opiate activity, which can reduce pulsatile GnRH secretion in some cases of functional hypothalamic amenorrhea.

The hypothalamic-pituitary-adrenal axis is dysfunctional in many women with functional hypothalamic amenorrhea, with increased secretion of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol. Activation of the pituitary-adrenocortical system is a common response in patients with chronic stress.[150] In functional hypothalamic amenorrhea, stressors such as exercise or emotional distress can chronically activate the hypothalamic-pituitary-adrenal axis and disrupt reproductive function.

Studies have demonstrated an increase in pulsatile ACTH secretion, increased adrenal sensitivity to ACTH, and increased cortisol secretion with a normal diurnal rhythm.[143] Daytime cortisol levels are markedly elevated, and the pituitary response to CRH is blunted.[151] In an animal model, CRH seems to be an important factor in the inhibition of GnRH pulsatility.[152,153] This inhibitory effect can be prevented by coadministration of a CRH antagonist or reversed by the opiate antagonist naloxone, suggesting that cross-talk occurs between the action of CRH and activation of the opioidergic system. Moreover, ACTH administration blocks the pituitary response to GnRH at the pituitary level.[154,155] In summary, overproduction of CRH and other stress-related hormones in the brain and activation of the pituitary-adrenocortical system by chronic stress seem to play causative roles in the inhibition of gonadotropin secretion in functional hypothalamic anovulation.

The roles of energy balance–regulating peptides such as leptin and ghrelin were investigated in the mechanism of hypothalamic amenorrhea.[143] Leptin is a cytokine produced by the adipocytes and is considered to be an appetite-suppressor peptide. Leptin is secreted in a pulsatile manner with a diurnal rhythm. A decrease in total circulating leptin with loss of the normal diurnal rhythm was reported in women with hypothalamic amenorrhea.[156] This relative hypoleptinemia is a common characteristic of several energy-deficient conditions and is associated with slowing of the LH pulse frequency.[156] Leptin administration to correct the relative leptin deficiency in women with hypothalamic amenorrhea was shown to improve reproductive, thyroid, and growth hormone axes and markers of bone formation, suggesting that

leptin, a peripheral signal reflecting the adequacy of energy stores, is required for normal reproductive and neuroendocrine function.[156] In contrast to leptin, ghrelin is an appetite-inducing peptide secreted from the stomach. During the fasting state, ghrelin serves as the hunger signal from the periphery to the hypothalamic arcuate nucleus, a region that is known to control food intake. Ghrelin levels are reported to be elevated in women with hypothalamic amenorrhea.[157]

Hypothalamic Anovulation and Exercise

Regular vigorous exercise can lead to menstrual disturbances, a delay in menarche, luteal phase dysfunction, and secondary amenorrhea. Thirty percent of adolescent ballet dancers have problems with the progression of puberty. The mean age at menarche is delayed until the age of 15 years. Advancement of pubertal stages seems to coincide with times of prolonged rest or after recovery from an injury.[158–161] The intensity, length, and type of the sport determine the severity of the disease. Activities associated with an increased frequency of reproductive dysfunction are those that favor a lower body weight and include middle-distance and long-distance running, competitive swimming, gymnastics, and ballet dancing.

Competitive athletes show endocrine abnormalities in the central nervous system consistent with those in other forms of functional hypothalamic anovulation. Abnormalities include elevations of central CRH and β-endorphin levels.

The management of exercise-related anovulation depends on the patient's choices and expectations. Side effects such as osteoporosis and delay of puberty must be discussed thoroughly with the patient.[162] Decrease in exercise level and behavioral modification may be sufficient for the return of ovulatory function. Hormone therapy should be provided if sufficient results are not achieved. A low-dose oral contraceptive is a suitable option for women younger than 35 years of age.

Hypothalamic Anovulation Associated With Eating Disorders

Two common eating disorders associated with hypothalamic dysfunction are anorexia nervosa and bulimia. Patients with anorexia nervosa have extreme weight loss (>25% of original body weight) and a distorted body image accompanied by a striking fear of obesity. Bulimia is a related disorder characterized by alternating episodes of binge eating followed by periods of food restriction, self-induced vomiting, or excessive use of laxatives or diuretics. Approximately 90% to 95% of these patients are women. The incidence of classic anorexia nervosa is approximately 1 case per 100,000 people in the general population.[163] Among female high school and college students, bulimia is fairly common. The incidence of anorexia nervosa peaks twice during the teen years, at ages 13 and 17. Bulimia usually begins at a later age, between 17 and 25 years. Anorexia nervosa has an extremely high mortality rate of 9% and is a true medical emergency. Death may result from cardiac arrhythmia, which may be precipitated by diminished heart muscle mass and associated electrolyte abnormalities.[164] These patients are also at increased risk for suicide.[165]

Gonadotropin secretion in anorexic women exhibits a prepubertal pattern that is similar to that observed in other forms of hypothalamic anovulation. Transitional patterns of LH secretion are seen with moderate degrees of weight recovery, and there is a normal or supranormal response to GnRH. Anovulation can persist in up to 50% of anorexic patients, even after normal weight is achieved. Anorexic and bulimic patients exhibit hyperactivation of the hypothalamic-pituitary-adrenal system. Although the diurnal variation is maintained, persistent hypersecretion of cortisol occurs throughout the day.[166] Cushingoid features are not present, in part because of mild hypercortisolemia and a reduction of peripheral glucocorticoid receptors. Levels of CRH and β-endorphin are increased in the central nervous system.[167,168]

In anorexia nervosa, basal metabolism is decreased because peripheral conversion of thyroxine (T_4) to biologically potent triiodothyronine (T_3) is decreased. Instead, T_4 is converted to reverse T_3, an inactive isomer. This alteration is also observed in severely ill patients and during starvation.[169] Anorexics have partial diabetes insipidus and are unable to concentrate urine appropriately because of the impaired secretion of vasopressin.[170]

Anorexia nervosa and bulimia are extremely difficult to treat. The most accepted approaches include individual psychotherapy, group therapy, and behavior modification. Patients with eating disorders should have psychiatric consultation and follow-up. This helps with the diagnosis and treatment. For patients who weigh less than 75% of their ideal body weight, immediate hospitalization and aggressive treatment are recommended. Complications of anorexia nervosa include osteoporosis, estrogen deficiency, and generalized effects of malnutrition.[159] Hormone therapy in the form of a low-dose oral contraceptive should be provided until ovulatory function is achieved.

Treatment and Management of Functional Hypothalamic Anovulation

Treatment of chronic anovulation resulting from central nervous system–hypothalamic disorders should be directed at reversal of the primary cause (e.g., stress management, reduction of exercise, correction of weight loss). The importance of successful treatment of this disease state is underscored because these women are prone to the development of osteoporosis. For a considerable number of patients, spontaneous recovery of menstrual function takes place after a modification of lifestyle, psychologic guidance, or accommodation to environmental stress. The initial treatment should be directed to a change in lifestyle and tailored to the individual patient. For individuals who remain amenorrheic, periodic assessment of reproductive status (every 4–6 months) is prudent.

Modification of the stress response through cognitive-behavioral therapy is a logical approach to lowering the endogenous stress levels in women with hypothalamic amenorrhea. This approach was explored in 16 subjects with hypothalamic amenorrhea randomized to cognitive-behavioral therapy or observation for a 20-week period.[171] The therapy design focused on attitudes and habits concerning eating, exercise, body image, problem-solving skills, and stress reduction. The results were encouraging. Approximately 88% of those who underwent cognitive-behavioral therapy had evidence of ovulation, compared with only 25% of those who were observed.[171] These results suggest that endogenous stress is a major factor in the development and maintenance of hypothalamic amenorrhea and that modification of this stress response can restore normal menses.

If anovulation persists for longer than 6 months or if reversal of the primary cause is not practical (e.g., professional athletes, ballerinas), a major concern is the long-term effect of hypoestrogenism, especially on bone metabolism. In addition to estrogen deficiency, IGF1 deficiency, hypercortisolism, and nutritional factors may contribute to bone loss in this disorder.[172] However, epidemiologic data on the risk of fractures and the benefits of hormone therapy are scant.[162,172] On the basis of studies of reproductive-age women who were ovariectomized or underwent treatment with GnRH agonist for endometriosis, bone density is expected

to decrease significantly, even within the first 6 months of amenorrhea. Because these patients are often reluctant to take medications, serial bone density studies of the lumbar spine and femur may be necessary to convince them of the necessity to begin estrogen replacement therapy. If the patient is not at risk for thromboembolism and does not smoke cigarettes, a low-dose combination oral contraceptive is a reasonable replacement option. Alternatively, a combination of conjugated equine estrogens (0.625 mg) and MPA (2.5 mg) daily may be administered to provide estrogenic support. The progestin (MPA) is added solely to prevent endometrial hyperplasia.

If the patient desires ovulation to achieve pregnancy, the most physiologic approach is ovulation induction with pulsatile GnRH. This is the best physiologic means of induction because the cause of the anovulatory state is decreased endogenous GnRH secretion. Pulsatile intravenous GnRH (5 μg every 90 minutes) was shown to be effective.[173] Monitoring of serum estradiol levels or follicular development can be minimized because the ovarian follicular response and gonadotropin output mimic the natural menstrual cycle. In these patients, continuation of pulsatile GnRH or hCG (1500 units administered intramuscularly every 3 days for a total of four doses) can support the function of the corpus luteum. Intravenous GnRH treatment results in ovulation rates of approximately 90%, pregnancy rates of up to 30%, and hyperstimulation rates of less than 1% per treatment cycle. Because the intravenous GnRH pump is not a practical choice for many women, an alternative strategy is the use of subcutaneous recombinant FSH for the development of one to three follicles and the induction of ovulation with intramuscular hCG followed by luteal support using intramuscular hCG or progesterone in oil.

Chronic Anovulation Associated With Pituitary Disorders

The most common pituitary-related causes of anovulation are associated with hyperprolactinemia caused by prolactinomas or other functional or anatomic disorders of the pituitary. These disorders are frequently associated with dysregulation of gonadotropin secretion. Hyperprolactinemia and other pituitary disorders and their relation to reproduction are discussed in Chapter 9.

Chronic Anovulation Associated With Androgen Excess

The most common ovary-related disorder of chronic anovulation is PCOS. Irregular periods or amenorrhea and androgen excess are the most commonly observed features of PCOS. Other causes of ovary-related anovulation include steroid-secreting ovarian tumors and POI. Androgen excess arising from extraovarian sources (e.g., adrenal disorders) is also associated with anovulation.

Approach to the Patient With Androgen Excess

Two natural androgens are testosterone, which is transported to target tissue by the circulation, and DHT, which is produced primarily by target tissues. Increased levels of these androgens can lead to hirsutism, which is excessive androgenic hair growth, or to virilization, a more severe form of androgen excess. Emerging evidence also suggest that the adrenal steroid 11-hydroxyandrostenedione may be converted to 11-ketotesosterone and 11-keto-DHT in peripheral or target tissues.[174,175] These latter steroids may act as potent androgens.[174,175]

Hirsutism is defined as the presence of terminal (coarse) hair in locations at which hair is not commonly found in women, including facial hair on the cheek, above the upper lip, and on the chin (Fig. 17.24 A and B). The presence of midline chest hair is also significant (see Fig. 17.24C). A male escutcheon, hair on the inner aspects of the thighs, and midline lower back hair entering the intergluteal area are hair growth patterns compatible with androgen excess. A moderate amount of hair on the forearms and lower legs by itself may not be abnormal, although it may be viewed by the patient as undesirable and may be mistaken for hirsutism. Numerous scoring systems are available for quantifying hirsutism. One of the most detailed scales was proposed by Ferriman and Gallwey.[176] A practical and clinically useful means of quantifying hirsutism is recording the hair growth in detail using simple drawings and photographs. In particular, photographs are invaluable for documenting hirsutism accurately.

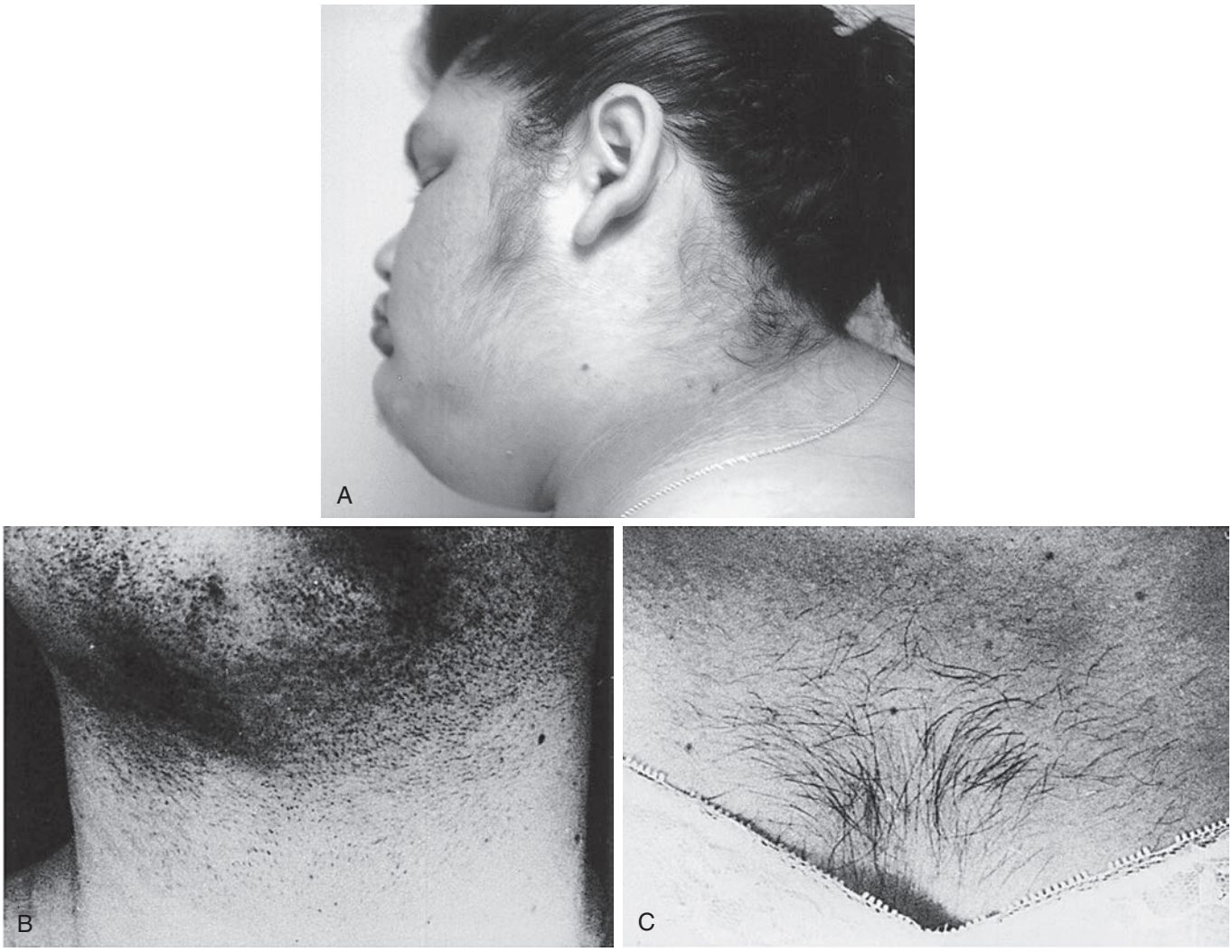
Compared with hirsutism, virilization is a more severe form of androgen excess and implies significantly higher rates of testosterone production. Its manifestations include temporal balding, deepening of voice, decreased breast size, increased muscle mass, loss of female body contours, and clitoral enlargement (Fig. 17.25). Even if testosterone levels are moderately increased (<1.5 ng/mL), temporal balding and clitoromegaly may be observed after a long period (>1 year) in the presence of persistent androgen excess. A marked increase in androgen secretion, such as that which may occur from production by neoplasms, leads to a more full-blown picture of virilization in less than a few months (see Fig. 17.25).

Measurements of an enlarged clitoris may be used for the quantification of virilization. A clitoral length greater than 10 mm is considered abnormal (see Fig. 17.25). Clitoral length is quite variable, however. An increase in clitoral diameter is a much more sensitive indicator of androgen action. Normal values for clitoral diameter are less than 7 mm at the base of the glans (see Fig. 17.25). The most accurate definition of clitoromegaly involves use of the clitoral index (the product of the sagittal and transverse diameters of the glans clitoris). A clitoral index greater than 35 mm^2 is abnormal and correlates statistically with androgen excess.[177]

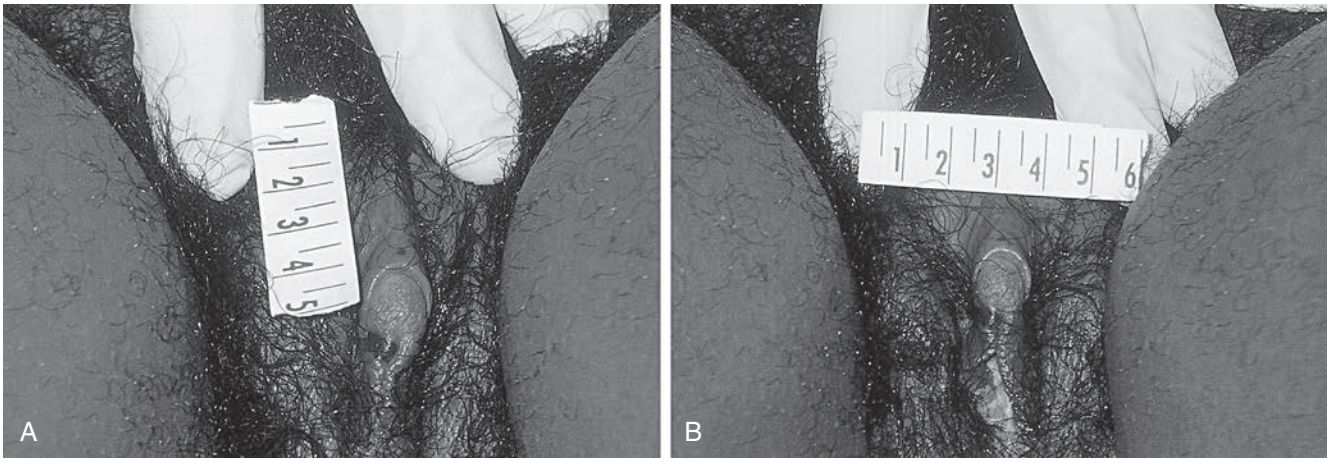
Origins of Androgens

Among the natural C19 steroids, DHT is a biologically potent androgen that is capable of acting through androgen receptors on target cells. Almost all testosterone target tissues contain 5α-reductase activity, which converts testosterone to DHT, or aromatase activity, which produces estradiol in an intracrine fashion. There is no convincing evidence that the other C19 steroids, including androstenedione, DHEA, and dehydroepiandrosterone sulfate (DHEAS), are biologically active.

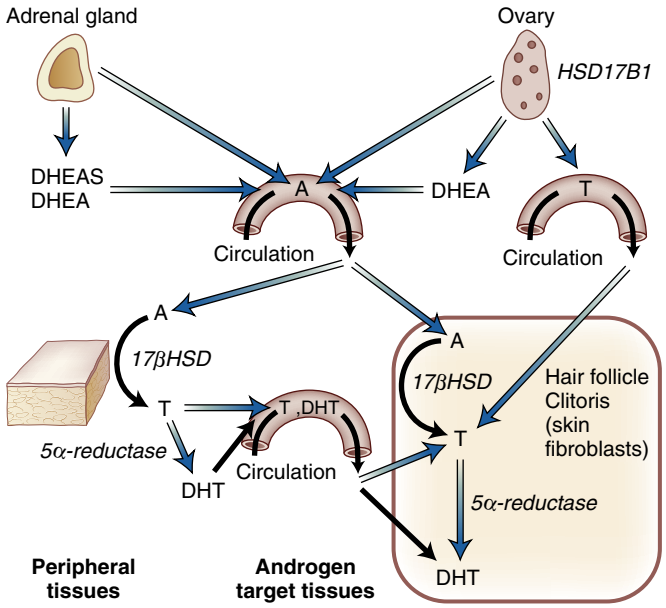
Testosterone in reproductive-age women is produced by two major mechanisms: direct secretion by the ovary, which accounts for roughly one-third of testosterone production, and conversion of the precursor, androstenedione, to testosterone in the peripheral (extragonadal) tissues, which accounts for two-thirds of testosterone production (Fig. 17.26).[178] These peripheral tissues include the skin and adipose tissue. Androstenedione, the direct precursor of testosterone, is produced in the ovary and the adrenal gland. The C19 steroids DHEAS and DHEA of adrenal origin, and DHEA of ovarian origin, indirectly contribute to testosterone formation by first being converted to androstenedione, which is subsequently converted to testosterone (see Fig. 17.26). Androstenedione is the major precursor that is converted directly to testosterone. The conversion fraction of circulating androstenedione to testosterone in extragonadal tissues is about 5% in both men and women.



• **Fig. 17.24** Hirsutism. (A) Mild facial hirsutism. (B) Severe facial hirsutism (chin), which requires regular shaving. (C) Severe hirsutism on chest. (B and C, from Dunaif A, Hoffman AR, Scully RE, et al. The clinical, biochemical and ovarian morphologic features in women with acanthosis nigricans and masculinization. *Obstet Gynecol.* 1985;66:545–552.)



• **Fig. 17.25** Severe clitoromegaly resulting from a testosterone-secreting ovarian tumor. (A) The entire length of the clitoris is approximately 4 cm (normal, <1 cm). (B) The transverse diameter of the clitoris measures 1.5 cm (normal, <0.7 cm).



• **Fig. 17.26** Androgen biosynthesis in women. Depending on the menstrual cycle phase or postmenopausal status, 20% to 30% of testosterone (T) is secreted by the ovary. The rest is accounted for by the conversion of circulating androstenedione (A) to T in various peripheral tissues. Both the adrenal gland and the ovary contribute to circulating A directly or indirectly, depending on the cycle phase or postmenopausal status and chronologic age. T may also be formed locally in androgen target tissues. T is converted to the biologically potent androgen dihydrotestosterone (DHT) within the target tissues and cells. For example, local conversion of T to DHT by 5α-reductase activity, which is conferred by products of at least two genes, in sex skin fibroblasts and hair follicles plays a key role in clitoral enlargement and hirsutism. The enzyme activity of 17βHSD in peripheral tissues may be conferred by protein products of several genes with overlapping functions; HSD17B1, a distinct reductive 17βHSD enzyme, is encoded by a specific gene expressed primarily in the ovary. *DHEAS,* dehydroepiandrosterone sulfate; *17βHSD,* reductive 17β-hydroxysteroid dehydrogenase.

Testosterone is converted to a potent steroid, DHT, to exert full androgenic effects on certain target tissues such as hair follicles and external genitalia.[98,115] This conversion is catalyzed by the enzyme 5α-reductase and takes place in the liver for systemic DHT production and within androgen target cells such as sex skin fibroblasts for an intracrine effect.[98]

The androgenic effects of testosterone in target tissues are determined by the level of local 5α-reductase activity and the androgen receptor content (see Fig. 17.26). Androgen receptors mediate androgenic action in critical target tissues.[115,179] Other local enzymes at target tissues (e.g., aromatase, oxidative 17βHSD) also regulate hormone action by metabolizing testosterone to the androgenically inactive androstenedione or to estradiol, a potent estrogen. There appears to be a balance between potent androgen action when DHT is formed and reduction of androgenicity when inactive C19 steroids or estradiol is formed from testosterone in target tissues and other extragonadal tissues. This may be particularly relevant for androgen-dependent disorders (e.g., hirsutism, virilization) and estrogen-dependent disorders such as malignancies of breast and endometrium (see Figs. 17.19 and 17.26).

Laboratory Evaluation of Androgen Action

Testosterone circulates in three forms: that which is bound to sex hormone–binding globulin (SHBG), the portion not bound to SHBG but loosely associated with albumin, and the fraction not bound by SHBG or albumin (i.e., free or dialyzable testosterone). The blood testosterone that is available to diffuse into target tissues includes the free and albumin-bound fractions and is referred to as bioavailable or non–SHBG-bound testosterone. The remainder is tightly bound to the protein SHBG.

SHBG is one of the primary regulators that determine the amounts of circulating bound and bioavailable testosterone available to act on target tissues. Conditions that decrease SHBG binding (e.g., androgen excess, obesity, acromegaly, hypothyroidism, liver disease) also increase bioavailable testosterone, augmenting the effect of testosterone. SHBG also regulates the circulating amounts of bioavailable estradiol by binding a significant fraction of circulating estradiol. Conditions that decrease SHBG levels give rise to increased bioavailable (non–SHBG-bound) estradiol.

The measurement of non–SHBG-bound (bioavailable) forms of testosterone has been advocated for states of androgen excess to detect more accurately subtle forms of hirsutism. Although the diagnostic yield of this measurement is superior to that of total serum testosterone, the correlation between total and non–SHBG-bound testosterone is excellent, so that bioavailable testosterone can usually be predicted from the total testosterone and SHBG levels.[180] The purpose of measuring serum testosterone is to establish the presence of circulating androgen excess and to detect extremely high values that may originate from an androgen-secreting neoplasm.

The normal serum levels of androgens, especially free testosterone determined by radioimmunoassay (RIA) or other direct methods, vary from laboratory to laboratory. A group of investigators compared serum-free testosterone levels measured by equilibrium dialysis with those measured by direct RIA and with those calculated from the free androgen index (100 × testosterone/SHBG), a simple index that correlates with the free testosterone level.[181] Calculated values for free testosterone using the free androgen index correlated well with those obtained from equilibrium dialysis. In contrast, the direct free testosterone measurements had unacceptably high systematic bias and random variability and did not correlate as well with equilibrium dialysis values. Moreover, the lower limit of detection was higher for the direct RIA than for equilibrium dialysis or calculated free testosterone.[181] The clinician should be aware of the limitations of direct free testosterone measurements performed without rigorous quality control.

Measuring the levels of all C19 steroids is not clinically necessary for most patients presenting with androgen excess. The most useful initial test is a serum total testosterone level. An abnormal level in the presence of hirsutism or virilization may be associated with PCOS, hyperthecosis, nonclassic congenital adrenal hyperplasia, or an androgen-secreting neoplasm. Most androgen-secreting tumors are of ovarian origin. The likelihood of a neoplasm correlates roughly with increasing testosterone levels. The following tests may be added on the basis of the clinical presentation: serum 17-hydroxyprogesterone (i.e., nonclassic congenital adrenal hyperplasia), serum prolactin and TSH (i.e., mild androgen excess associated with hyperprolactinemia), serum FSH and LH (i.e., elevated LH/FSH ratio in PCOS), serum DHEAS (i.e., adrenal tumors), and imaging of ovaries and adrenals (i.e., PCOS and steroid-secreting tumors).

Causes of Androgen Excess

Several disorders give rise to androgen excess. They include unusual causes such as iatrogenic or drug-induced androgen excess, congenital genital ambiguity (e.g., excessive in utero androgen formation

TABLE 17.2 Causes of Androgen Excess in Women of Reproductive Age

Ovarian

Polycystic ovary syndrome (PCOS)
Hyperthecosis (a severe PCOS variant)
Ovarian tumor (e.g., Sertoli-Leydig cell tumor)

Adrenal

Nonclassic congenital adrenal hyperplasia
Cushing syndrome
Glucocorticoid resistance
Adrenal tumor (e.g., adenoma, carcinoma)

Specific Conditions of Pregnancy

Luteoma of pregnancy
Hyperreactio luteinalis
Aromatase deficiency in fetus

Other Causes

Hyperprolactinemia, hypothyroidism
Medications (danazol, testosterone, anabolizing agents)
Idiopathic hirsutism (normal serum testosterone in an ovulatory woman)
Idiopathic hyperandrogenism (patients who do not fall into any of the other categories listed)

in 46,XX disorders of sex development), and conditions unique to pregnancy (e.g., luteoma of pregnancy, hyperreactio luteinalis). These uncommon causes and relatively more prevalent disorders associated with androgen excess are listed in Table 17.2. The term *extraovarian steroid formation* is used synonymously with *extraglandular*, *extragonadal*, or *peripheral steroid formation* in this text.

Overall, the prevalence of androgen-excess disorders was found to be as follows: 72.1% for PCOS (anovulatory patients, 56.6%; mildly affected ovulatory patients, 15.5%), 15.8% for idiopathic hyperandrogenism, 7.6% for idiopathic hirsutism, 4.3% for 21-hydroxylase-deficient nonclassic congenital adrenal hyperplasia, and 0.2% for androgen-secreting tumors.[182]

In most hyperandrogenic disorders, androgen originates from more than one source (see Fig. 17.26). For example, testosterone secretion is somewhat increased from the ovary in PCOS, but the bulk of testosterone comes from extraovarian conversion of significantly elevated circulating androstenedione of ovarian origin to testosterone. Patients with PCOS also show increased adrenal output of DHEAS, which (after peripheral conversion to DHEA that is further converted to androstenedione) contributes indirectly to extraovarian testosterone formation.

If androgen excess is associated with primary amenorrhea, abnormal in utero sexual differentiation should be strongly suspected. These disorders are discussed in Chapter 23. Before embarking on a major workup for hirsutism or virilization, the physician is well advised to rule out exogenous androgen use. It is best to ask the patient to list all prescriptions and over-the-counter medications that she takes on her own, including injections. This is usually more rewarding than asking the patient whether she takes any androgens. Medications that can cause hirsutism or virilization are related to testosterone and include anabolic steroids and similar compounds.

The most common identifiable cause of androgen excess is PCOS, which is discussed elsewhere in this chapter. In this section, we first define some of the other disorders associated with hirsutism or virilization. This is followed by a simplified treatment strategy that may be applied to most hirsute patients within the categories of PCOS, nonclassic congenital adrenal hyperplasia, and idiopathic hirsutism.

Idiopathic Hirsutism

Hirsutism is defined subjectively as the presence in a woman of terminal hair growth in a male-distribution pattern that affects quality of life sufficiently to prompt her to seek medical advice. Hirsutism should be distinguished from hypertrichosis, in which the excessive hair growth is not restricted to androgen-dependent areas and comprises vellus or lanugo-type hair. Hypertricosis is considered to be a phenotype not associated with male pattern hair growth and is unlikely to be modified by the known treatments of hirsutism.

Idiopathic (constitutional) hirsutism is characterized by excessive hair growth in the absence of elevated circulating androgen levels in ovulatory women, and it occurs more frequently in certain ethnic populations, particularly in women of Mediterranean ancestry.[115] It is defined as hirsutism in conjunction with regular menstrual cycles and normal levels of serum testosterone. Idiopathic hirsutism is not associated with any signs of virilization. Its cause is not understood completely. It has been proposed that women with idiopathic hirsutism have significantly increased cutaneous 5α-reductase activity,[183] but this association has not been confirmed. It is also unclear whether a certain 5α-reductase isoenzyme (type 1 or 2) is involved in the development of idiopathic hirsutism.[115]

Idiopathic hirsutism is diagnosed in women who have hirsutism,[115] normal ovulatory function, and normal total or free testosterone levels. Cyclic predictable menses are usually indicative of regular ovulation. If in doubt, ovulatory function may be verified by a luteal phase day 7 progesterone level, which should be at least 5 ng/mL. Luteal phase day 7 corresponds to cycle day 17 for 24-day intervals, cycle day 21 for 28-day intervals, and cycle day 28 for 35-day intervals. The presence of oligo-ovulation or anovulation in hirsute women after exclusion of related disorders (e.g., hypothyroidism, hyperprolactinemia, nonclassic congenital adrenal hyperplasia) is consistent with the diagnosis of PCOS.[115] Thyroid dysfunction or hyperprolactinemia should be excluded by the measurements of TSH and prolactin. The follicular-phase basal 17-hydroxyprogesterone level should be measured to exclude 21-hydroxylase–deficient, nonclassic congenital adrenal hyperplasia. The use of exogenous androgens should also be excluded. In summary, the diagnosis of idiopathic hirsutism is one of exclusion in which ovulatory dysfunction, elevated circulating testosterone levels, and other causes of androgen excess are ruled out.

Androgen-Secreting Tumors of the Ovary and Adrenal

Most androgen-secreting tumors arise from the ovary and secrete large quantities of testosterone or its precursor, androstenedione. These include Sertoli-Leydig cell tumors, hilus cell tumors, lipoid cell tumors, and (infrequently) granulosa-theca tumors. Steroidogenically inert ovarian neoplasms such as epithelial cystadenomas or cystadenocarcinomas may produce factors that stimulate steroidogenesis in adjacent nonneoplastic ovarian stroma and induce production of sufficient amounts of androgen precursors such as androstenedione to give rise to clinically detectable androgen excess. Approximately 5% of androstenedione is converted to testosterone in extraovarian tissues, ultimately producing androgen excess (see Fig. 17.26).

Sertoli-Leydig cell tumors, which account for fewer than 1% of all solid ovarian tumors, tend to occur during the second to fourth

decades of life, whereas hilus cell tumors occur more frequently in postmenopausal women. By the time the signs and symptoms of androgen excess cause the patient to seek medical assistance, Sertoli-Leydig cell tumors are usually so large that they are readily palpable on pelvic examination, whereas hilus cell tumors are still small. In women with either type of tumor, the serum testosterone level is markedly elevated. Granulosa-theca tumors primarily produce estradiol but may occasionally produce testosterone.

Rapidly progressing symptoms of androgen excess suggest the presence of an androgen-producing tumor unless proven otherwise. This rapid progression is typical of both ovarian and adrenal androgen-producing tumors. Progression is usually associated with defeminizing signs, such as loss of female body contour, increased muscle mass, and decreased breast size. As the tumor continues to grow, more and more testosterone is produced, resulting in rapidly worsening hirsutism and progressive virilization. Elevated serum testosterone levels are characteristically associated with ovarian tumors. This change may be mediated by production and secretion of testosterone directly by the tumor or by secretion of large quantities of androstenedione that are converted to testosterone in extragonadal tissues. The testosterone levels produced by certain ovarian tumors (e.g., Sertoli-Leydig cell tumors) may be suppressed by GnRH agonists,[184] so use of a GnRH agonist cannot be relied on to differentiate a neoplasm from another functional state.

In interpreting testosterone levels, the clinician should be familiar with the normal ranges of the clinical laboratory used. A value of three times the upper-normal range (or >2 ng/mL) suggests a neoplasm, particularly if the clinical history supports this diagnosis. Lower serum testosterone levels occasionally may be associated with virilizing ovarian tumors. If an androgen-secreting tumor is suspected, measurement of androstenedione is clinically useful. A severely elevated level of androstenedione is not diagnostic but consistent with an ovarian or adrenal tumor. When an elevated level of testosterone is associated with suggestive clinical history, meticulously performed transvaginal ultrasonography is the most sensitive method to detect an ovarian tumor.

In contrast to testosterone-secreting tumors of the ovary, testosterone-secreting tumors of the adrenal are rare. The cells of some testosterone-producing adrenal tumors may resemble ovarian hilus cells, which are analogous to Leydig cells. These tumor cells produce testosterone and may be stimulated by LH or hCG. In patients with testosterone-producing adrenal adenomas, testosterone secretion usually decreases after LH suppression and increases after hCG stimulation. Testosterone-secreting adrenal carcinomas also have been reported.[185]

Virilizing adrenal tumors commonly secrete large quantities of DHEAS, DHEA, and androstenedione, and testosterone is usually produced by extraovarian conversion of these precursors. Levels of serum DHEAS are highly elevated in most patients with virilizing adrenal tumors.[186] If DHEAS levels exceed 8 μg/mL, adrenal imaging by computed tomography (CT) or MRI should be ordered. Occasionally, such high levels of DHEAS are associated with a functional abnormality such as congenital adrenal hyperplasia caused by an enzymatic defect or an unexplained hyperfunctional adrenal state that is commonly associated with PCOS. These circumstances may explain a negative CT or MRI result, which warrants further investigation.

Levels of a variety of adrenal steroids, including corticosteroids, may be elevated in various combinations in the presence of an adrenal tumor. It is not possible to describe a particular pattern of hormones that defines an adrenal tumor.[186] Very high levels of serum DHEAS (>8 μg/mL) suggest an adrenal tumor. Virilizing ovarian tumors are encountered much more frequently than those of adrenal origin. If the presentation is compatible with an androgen-secreting tumor and the ovaries are normal by transvaginal ultrasonography, the adrenals should be evaluated next by imaging.

Testosterone levels three times the upper-normal range (i.e., >2 ng/mL) and DHEAS levels higher than 8 μg/mL have been used traditionally as guidelines to investigate further whether neoplasms of the ovary or adrenal are the sources of androgen excess. These numbers are provided only as guidelines, not as rules, and there are exceptions. First, because tumors secrete androgens episodically, more than one measurement may be required to detect a significantly elevated level.[187] Second, other precursor steroids are often elevated as well (particularly androstenedione), and their measurement should be considered. Third, some tumors may give rise to milder elevations of DHEAS and testosterone levels. Even mild elevations in a postmenopausal woman are highly suspicious for an androgen-secreting tumor. By the same token, greatly elevated serum testosterone levels may be observed in women with severe ovarian hyperthecosis (a severe variant of PCOS) in the absence of a tumor.

Virilization of recent onset and short duration warrants immediate investigation, even if testosterone and DHEAS levels are mildly elevated. With improvements in imaging techniques—vaginal ultrasonography for the ovary; abdominal ultrasonography, CT, and MRI for the adrenal glands—the diagnosis of even a small ovarian or adrenal tumor may be made. If no neoplasm can be localized, imaging of the ovary or adrenal after intravenous administration of radiolabeled iodomethylnorcholesterol (NP59), which detects active steroid-producing tumors, has proven useful.[188] These diagnostic studies should be pursued aggressively before surgical exploration of a suspected tumor.

The clinician should question whether an ovarian or adrenal tumor detected by imaging is the actual source of androgen excess before resorting to its surgical resection. Occasionally, a hemorrhagic corpus luteum cyst of the ovary may mimic an androgen-secreting tumor, or a woman with androgen excess may have an adrenal incidentaloma, which does not secrete androgen. Intraoperative selective ovarian or adrenal vein catheterization may be considered as a last resort to demonstrate significant steroid gradients before surgical exploration of an adrenal or ovary for a small tumor is undertaken, especially if the clinical picture is not certain.[189]

Nonneoplastic Adrenal Disorders and Androgen Excess

Adrenal disorders such as classic congenital adrenal hyperplasia, Cushing syndrome, and glucocorticoid resistance give rise to androgen excess related to overproduction of testosterone precursors from the adrenal gland. These disorders are discussed elsewhere in this text. In this chapter, we discuss nonclassic congenital adrenal hyperplasia.

The diagnosis and prevalence of nonclassic congenital adrenal hyperplasia continue to be debated, although the disorder clearly exists. Other terms that have been used to describe this syndrome include late-onset, adult-onset, attenuated, incomplete, and cryptic adrenal hyperplasia. This form of adrenal hyperplasia is caused by a partial deficiency in 21-hydroxylase activity. Although deficiencies in 11β-hydroxylase and 3βHSD may result in the disorder, defects in 21-hydroxylase account for more than 90% of cases.[190]

The clinical presentation is almost identical to that of patients with PCOS. The prevalence of this disorder varies according to ethnic background, and the prevalence reported by different

investigators has varied widely. The characteristic presentation consists of anovulatory uterine bleeding and progressive hirsutism of pubertal onset. These individuals are born with normal genitalia, do not exhibit salt wasting, and many have premature pubarche and accelerated linear growth with advanced bone age. Patients of northern European ancestry have a low frequency of this disorder, whereas Ashkenazi Jews, Hispanics, and patients of central European ancestry have a much higher prevalence.[191] The patient with androgen excess from a high-risk ethnic group should be screened. (See Chapter 15 for more complete discussion.)

Screening may first be carried out by obtaining an 8 AM serum level of 17-hydroxyprogesterone in an anovulatory patient on any day. Although most women with nonclassic congenital adrenal hyperplasia are anovulatory, some women with this disorder present with regular periods and hirsutism of pubertal onset or with only unexplained infertility and frequent miscarriage.[190] If nonclassic congenital adrenal hyperplasia is suspected in an ovulatory patient on the basis of clinical presentation, an 8 AM serum level of 17-hydroxyprogesterone should be obtained during the follicular phase, because 17-hydroxyprogesterone levels are higher in the luteal phase in ovulatory women.[190] A level of less than 2 ng/mL effectively rules out this diagnosis.[190]

The diagnosis of nonclassic congenital adrenal hyperplasia can be made if the basal 17-hydroxyprogesterone level is higher than 10 ng/mL. No further testing is required in these cases. Values between 2 and 10 ng/mL are considered increased but not diagnostic of nonclassic congenital adrenal hyperplasia. For example, disease-free women and patients with PCOS may also have basal levels of 17-hydroxyprogesterone in this indeterminate range.[190] In these circumstances, an ACTH stimulation test should be used to distinguish nonclassic congenital adrenal hyperplasia from PCOS.[190] A rise of the 17-hydroxyprogesterone level to at least 10 ng/mL 60 minutes after intravenous injection of ACTH is considered diagnostic of nonclassic adrenal hyperplasia.[192] A higher basal level of 17-hydroxprogesterone within the range of 2 to 10 ng/mL is associated with a higher likelihood of nonclassic congenital adrenal hyperplasia. For example, an 8 AM 17-hydroxyprogesterone level higher than 4 ng/mL had a sensitivity of 90% for the diagnosis of nonclassic congenital adrenal hyperplasia.[190]

In an androgen excess patient from a high-risk ethnic group, a baseline level of 17-hydroxyprogesterone should be measured at 8 AM. A screening baseline level of 17-hydroxyprogesterone should be obtained for patients with premature pubarche, those with androgen excess of early pubertal onset, women with progressive hirsutism or virilization, and patients with strong family histories of severe androgen excess.

Laboratory Testing to Aid the Differential Diagnosis of Androgen Excess

Algorithms exist for the differential diagnosis of anovulation associated with hirsutism or virilization or both. Salient clinical features are of paramount importance to guide laboratory testing. The most important features are the onset and severity of the signs and the rapidity with which they progress. Rapidly progressing severe androgen excess implies an androgen-secreting tumor until proven otherwise. The possibility of a tumor is further underscored in a postmenopausal woman or in a reproductive-age woman with a recent history of cyclic, predictable periods. Ovarian hyperthecosis, a severe variant of PCOS, also gives rise to severe androgen excess that may progress rapidly, especially at the time of expected puberty. Androgen excess emerging at the time of puberty may be indicative of PCOS or nonclassic congenital adrenal hyperplasia.

TABLE 17.3 Laboratory Tests for the Differential Diagnosis of Androgen Excess

Initial Testing
Total testosterone
Prolactin
Thyroid-stimulating hormone
Further Testing Based on Clinical Presentation[a]
17-Hydroxyprogesterone (8 AM)
17-Hydroxyprogesterone 60 min after intravenous ACTH
Cortisol (8 AM) after 1 mg dexamethasone at midnight
DHEAS
Androstenedione
Imaging of ovaries (transvaginal ultrasonography)
Imaging of adrenal glands (abdominal ultrasonography, CT, MRI)
Nuclear imaging after intravenous administration of radiolabeled cholesterol

[a]See text.

ACTH, Adrenocorticotropic hormone; *CT,* computed tomography; *DHEAS,* dehydroepiandrosterone sulfate; *MRI,* magnetic resonance imaging.

The most useful initial test to evaluate androgen excess is the serum level of total testosterone (Table 17.3). Testosterone levels in most normal ovulatory women are lower than 0.6 ng/mL, although the value may vary from laboratory to laboratory. Women with idiopathic hirsutism have cyclic menses and normal testosterone levels. No further testing for androgen excess is required in this group.

If the testosterone level is elevated in an anovulatory woman, serum levels of TSH and prolactin should be obtained to rule out anovulation associated with hyperprolactinemia. Ultrasonography of the ovaries also can help to identify an ovarian tumor or polycystic ovaries. If the ethnic background of the patient (i.e., Ashkenazi Jews, Hispanics, and those of central European ancestry), onset of hirsutism (i.e., puberty), or family history suggests nonclassic congenital adrenal hyperplasia, a baseline serum level of 17-hydroxyprogesterone should be obtained at 8 AM. Rare causes of androgen excess include an adrenal tumor, Cushing syndrome, and glucocorticoid resistance. A serum level of DHEAS and adrenal imaging are required to assess the presence or absence of an adrenal tumor. CT, MRI, or abdominal ultrasonography may be used to assess the adrenals, depending on the expertise of the local radiology laboratory. A screening test for Cushing syndrome and glucocorticoid resistance may be performed to explore rare adrenal causes of androgen excess (see Chapter 15).[193]

Most women with chronic anovulation and mild to moderate hirsutism of pubertal onset fall into the category of PCOS. These women usually have high-normal or mildly elevated testosterone levels and may not have any other laboratory abnormalities. After other diagnoses are ruled out by laboratory testing or on clinical grounds, a diagnosis of PCOS can be made.

Treatment of Hirsutism

Therapy for androgen excess should be directed toward its specific cause and suppression of abnormal androgen secretion. Neoplasms warrant surgical intervention and are not discussed in greater detail here. Suppression with a GnRH analogue may be tried initially for ovarian hyperthecosis. However, bilateral oophorectomy may become necessary to control androgen excess arising from hyperthecosis (see later discussion). Patients with adrenal disease are treated specifically. For Cushing syndrome, treatment correlates with the

source of hypercortisolism. When treating androgen excess associated with nonclassic congenital adrenal hyperplasia, an antiandrogen (e.g., spironolactone) in combination with an oral contraceptive is used. Although a glucocorticoid may be considered, particularly if fertility is desired, the doses of glucocorticoids needed to suppress the adrenal can cause symptoms and signs of glucocorticoid excess during long-term treatment. Thus a combination oral contraceptive plus spironolactone is favored to treat androgen excess if the patient responds to this treatment with decreased hirsutism. Several classes of medications are reviewed in detail later in the chapter for the treatment of androgen excess and hirsutism.

Oral Contraceptives

Oral contraceptives reduce circulating testosterone and androgen precursors by suppression of LH and stimulation of SHBG levels, thereby reducing hirsutism in hyperandrogenic patients.[115] Oral contraceptives decrease circulating androgen in patients with PCOS and synergize with the effects of antiandrogens. Oral contraceptives may further improve the results of antiandrogen therapy in patients with idiopathic hirsutism or nonclassic congenital adrenal hyperplasia. It is advisable to use an oral contraceptive containing 30 or 35 μg of ethinyl estradiol to achieve effective suppression of LH.[115] A meta-analysis showed that treatment with oral contraceptives for 6 months reduced Ferriman-Gallwey scores of hirsutism by an average of 27%.[194]

Spironolactone

The most commonly used androgen blocker for the treatment of hirsutism in the United States is spironolactone, an aldosterone antagonist structurally related to progestins. Spironolactone is effective for abnormal hair growth associated with PCOS, nonclassic congenital adrenal hyperplasia, or idiopathic hirsutism. Treatment with spironolactone for 6 months reduces Ferriman-Gallwey scores of hirsutism by an average of 38.4%.

Because spironolactone acts through mechanisms different from those of oral contraceptives, overall effectiveness is improved by combining these two medications in patients with hirsutism, including those with PCOS, idiopathic hirsutism, or nonclassic congenital adrenal hyperplasia. Apart from inhibiting steroidogenesis and acting as an androgen antagonist, spironolactone has a significant effect in inhibiting 5α-reductase activity.[115,195] Basic experimental and several clinical studies have confirmed the efficacy of spironolactone for hyperandrogenism and suggest that the principal effect is related to its ability to block peripheral androgen production and action.[115]

Doses of spironolactone used in clinical studies have varied from 50 to 400 mg daily. Although doses of 100 mg/day are usually effective for the treatment of hirsutism, higher doses (200–300 mg/day) may be preferable in extremely hirsute or markedly obese women.[115,195] The initial recommended dosage is 100 mg/day, which is gradually increased by increments of 25 mg/day every 3 months up to 200 mg/day on the basis of the response. This approach may be helpful to minimize side effects such as gastritis, dry skin, and anovulation. In patients with normal renal function, hyperkalemia is almost never seen. Hypotension is rare except in older women. Monitoring for electrolytes and blood pressure is imperative within the first 2 weeks at each dose level. Adjustments in dose should be made only after 3 to 6 months, as with other antiandrogens, to account for the slow changes in the hair cycle.

Patients usually notice an initial transient diuretic effect. Some women with normal cycles complain of menstrual irregularity with spironolactone; this is remedied by a downward dose adjustment or the addition of an oral contraceptive. The mechanism for abnormal bleeding is unclear. In women with oligomenorrhea, such as those with PCOS, resumption of normal menses may occur. This change may be caused in part by an alteration in levels of circulating androgens; LH levels have only occasionally been reported to decrease.[196] Another important consideration is the potential in utero feminizing effect of this antiandrogen on the genitalia of a 46,XY fetus. Effective contraception should always be provided in women taking spironolactone.

Cyproterone Acetate

Cyproterone acetate is a 17-hydroxyprogesterone acetate derivative with strong progestagenic properties. Cyproterone acetate acts as an antiandrogen by competing with DHT and testosterone for binding to the androgen receptor. There is also some evidence that cyproterone acetate and ethinyl estradiol in combination can inhibit 5α-reductase activity in skin.[197] Cyproterone acetate is not available in the United States but has been used in other countries. The drug is usually administered daily in doses of 50 to 100 mg on days 5 through 15 of the treatment cycle. Because of its slow metabolism, it is administered early in the treatment cycle; when ethinyl estradiol is added, it is usually administered in 50-μg doses on days 5 through 26. This regimen is needed for menstrual control and is usually referred to as the *reverse sequential regimen*. Cyproterone acetate in doses of 50 to 100 mg/day, combined with ethinyl estradiol at 30 to 35 μg/day, is as effective as the combination of spironolactone (100 mg/day) and an oral contraceptive in the treatment of hirsutism.[115] In smaller doses (2 mg), cyproterone acetate has been administered as an oral contraceptive in daily combination with 50 or 35 μg of ethinyl estradiol. This regimen is primarily suited for individuals with a milder form of hyperandrogenism.[115]

Finasteride

Finasteride inhibits 5α-reductase activity and has been used primarily for the treatment of prostatic hyperplasia. It can also be used in the treatment of hirsutism.[198,199] At a dose of 5 mg/day, a significant improvement of hirsutism is observed after 6 months of therapy, without significant side effects. In hirsute women, the decline in circulating DHT levels is small and cannot be used to monitor therapy. Although this treatment regimen increases testosterone levels, SHBG levels remain unaffected.[198] A meta-analysis showed that finasteride treatment for 6 months reduced Ferriman-Gallwey scores of hirsutism by an average of 20.3%.[194]

Finasteride primarily inhibits 5α-reductase type 2. Because hirsutism results from the combined effects of type 1 and type 2, this agent is only partially effective. Although prolonged experience with finasteride is lacking, one of the potential advantages of this agent is its benign side effect profile. One study showed efficacy with 1 year of hirsutism treatment.[200] It was also reported that finasteride is less effective than spironolactone with respect to the reduction of hirsutism in women.[115] Nevertheless, finasteride at a dose of 5 mg/day for prolonged periods represents a useful option because of its benign side effect profile and good tolerance by patients. Like spironolactone, finasteride may cause congenital genital ambiguity in a 46,XY fetus, and effective contraception should be provided during its use.

Flutamide

Flutamide is a potent antiandrogen used in the treatment of prostate cancer. It has been shown to be effective in the treatment of

hirsutism.[201,202] The mean Ferriman-Gallwey score is reduced by 41.3%.[194] Nevertheless, occasional severe hepatotoxicity makes this drug unsuitable for the indication of hirsutism.[203]

Metformin and Thiazolidinediones

Because PCOS is often associated with insulin resistance, drugs that mitigate insulin resistance have been used in this disorder.[110] Metformin (1500–2700 mg/day) for 6 months significantly reduces hirsutism by 19.1% as assessed by the Ferriman-Gallwey scoring system.[194] In obese adolescent women with PCOS, metformin in combination with lifestyle modification (i.e., diet with a 500 kcal/day deficit and exercise 30 minutes/day) and oral contraceptives reduced the total testosterone level and waist circumference.[204] The thiazolidinediones (4 mg/day of rosiglitazone or 30 mg/day of pioglitazone) also reduced Ferriman-Gallwey scores significantly.[194] These studies suggested that insulin-sensitizing agents may be used in the treatment of hirsutism of PCOS, especially for women who do not wish to use other oral agents.

Lifestyle Modification

In obese adolescent women with PCOS, lifestyle modification (i.e., diet with a 500 kcal/day deficit and exercise 30 minutes/day) alone resulted in a 59% reduction in the testosterone/SHBG ratio, with a 122% increase in SHBG.[204] A moderate diet and exercise program should be recommended as part of hirsutism management, particularly for obese women.

A Comprehensive Treatment Strategy for Hirsutism

The medications described in the previous paragraphs may be effective when administered as individual treatments. Patients with the most common form of hirsutism (i.e., PCOS, nonclassic congenital adrenal hyperplasia, or idiopathic hirsutism) are often initially treated with a combination of two agents, one that suppresses the ovary (e.g., an oral contraceptive) and another that suppresses the extraovarian (peripheral) action of androgens (e.g., spironolactone). An oral contraceptive containing 30 to 35 μg of ethinyl estradiol combined with spironolactone (100 mg/day) is the initial treatment of choice. Even in women with idiopathic hirsutism, the addition of an oral contraceptive to the antiandrogen spironolactone can improve efficacy and prevent abnormal bleeding. For women with only minor complaints of hirsutism, the use of an oral contraceptive alone may be an appropriate first approach. Moderate lifestyle modification (i.e., diet with a 500-kcal/day deficit and 30 minutes/day of exercise) should be a part of hirsutism management in obese patients.

Because the growth phase of body hair lasts 3 to 6 months, a response should not be expected before 6 months after onset of treatment. Objective means should be used to assess changes in hair growth. Scoring systems and evaluation of anagen hair shafts are difficult; taking photographs is the simplest and most objective tool. Patients are often unaware that change is taking place unless there is some objective measurement. Pictures of the face and selected midline body areas before and during therapy are especially useful for the encouragement of the patient and compliance with the treatment.

Suppression of androgen production and action inhibits only new hair growth. Existing coarse hair should be removed mechanically. Plucking, waxing, and shaving are ineffective for hair removal and cause irritation, folliculitis, and ingrown hairs. Electrolysis and laser epilation are more effective and preferred methods.[115]

Most patients with PCOS, nonclassic congenital adrenal hyperplasia, or idiopathic hirsutism respond to this strategy within 1 year. Patients should be encouraged to continue treatment for at least 2 years. Then, depending on the wishes and clinical responses of patients, therapy can be stopped and the patient reevaluated. Many patients require continuous treatment for suppression of hirsutism. Patients with clitoromegaly may be referred to a urologist for clitoral reduction surgery after the source of virilization has been effectively eliminated.

Polycystic Ovary Syndrome

PCOS is the most common form of chronic anovulation associated with androgen excess; it occurs in perhaps 5% to 10% of reproductive-age women.[205] The diagnosis of PCOS is made by excluding other hyperandrogenic disorders (e.g., nonclassic congenital adrenal hyperplasia, androgen-secreting tumors, hyperprolactinemia) in women with chronic anovulation and androgen excess.[205]

During the reproductive years, PCOS is associated with important reproductive morbidity, including infertility, irregular uterine bleeding, and increased pregnancy loss. The endometrium of the patient with PCOS must be evaluated by biopsy because long-term unopposed estrogen stimulation leaves these patients at increased risk for endometrial cancer. PCOS is also associated with increased metabolic and cardiovascular risk factors.[206] These risks are linked to insulin resistance and are compounded by the common occurrence of obesity, although insulin resistance also occurs in nonobese women with PCOS.[111]

PCOS is considered to be a heterogeneous disorder with multifactorial causes. PCOS risk is significantly increased with a positive family history of chronic anovulation and androgen excess, and this complex disorder may be inherited in a polygenic fashion.[207,208]

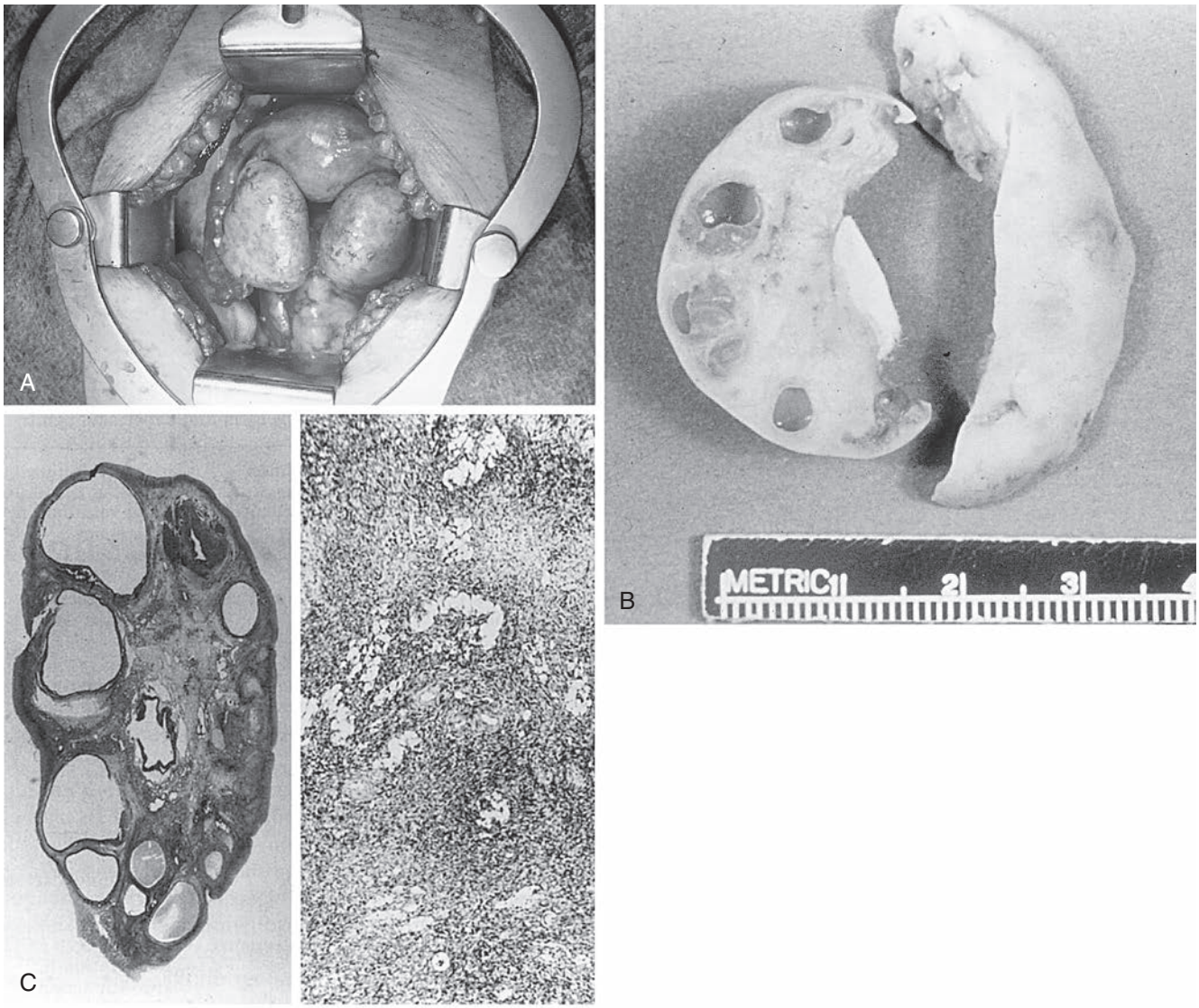
Historical Perspective

In their pioneering studies, Stein and Leventhal described an association between the presence of bilateral polycystic ovaries and signs of amenorrhea, oligomenorrhea, hirsutism, and obesity (Fig. 17.27).[209] At the time, these signs were strictly adhered to in the diagnosis of what was then known as *Stein-Leventhal syndrome*. These investigators also reported the results of bilateral wedge resection of the ovaries, in which at least half of each ovary was removed as a therapy for PCOS; most of their patients resumed menses and achieved pregnancy after ovarian wedge resection. The exact mechanism responsible for the therapeutic effect of removal or destruction of part of the ovarian tissue is still not well understood.

On the basis of Stein and Leventhal's original work, a primary ovarian defect was inferred. Subsequent clinical, morphologic, hormonal, and metabolic studies uncovered multiple underlying pathologies, and the term *polycystic ovary syndrome* was introduced to reflect the heterogeneity of this disorder. One of the most significant discoveries regarding the pathophysiology of PCOS was the demonstration of a unique form of insulin resistance and associated hyperinsulinemia.[111,209]

Diagnosis of Polycystic Ovary Syndrome and Laboratory Testing

One of the most prominent features of PCOS is the history of ovulatory dysfunction (i.e., amenorrhea, oligomenorrhea, or other forms of irregular uterine bleeding) of pubertal onset. A clear history of cyclic predictable menses of menarchal onset makes the diagnosis of PCOS unlikely. Acquired insulin resistance associated

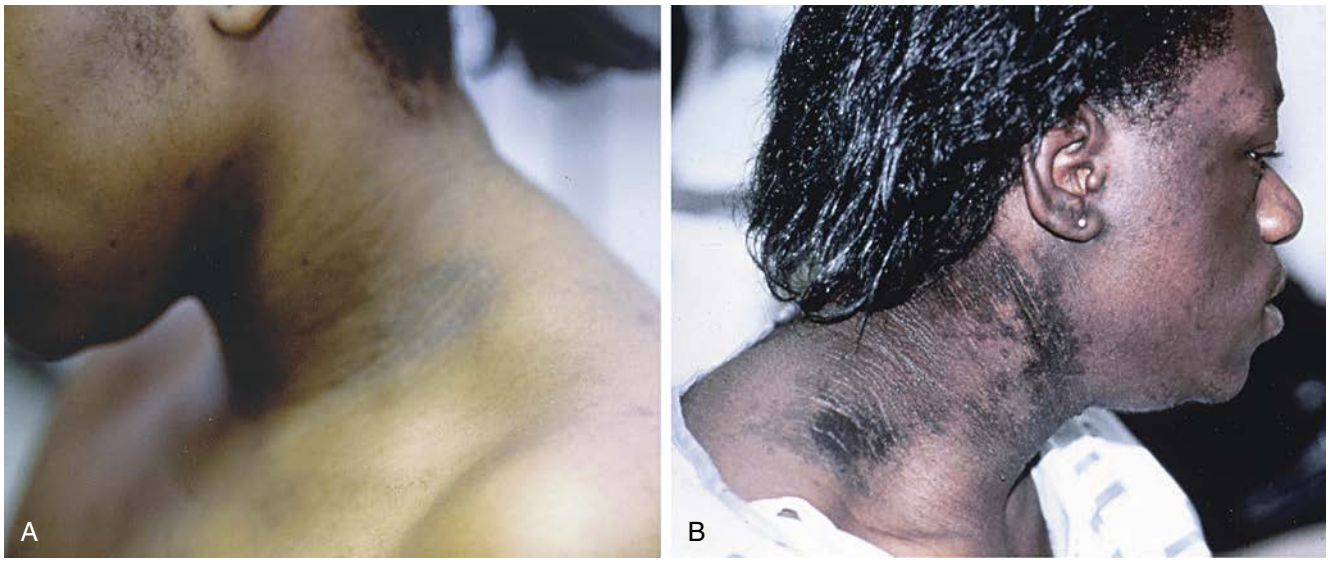


• **Fig. 17.27** Polycystic ovaries. (A) Operative findings of classic enlarged polycystic ovaries. The uterus is located adjacent to the two enlarged ovaries. (B) Sectioned polycystic ovary with numerous follicles. (C) Histologic section of a polycystic ovary with multiple subcapsular follicular cysts and stromal hypertrophy at low power *(left)*. At higher power (×100), islands of luteinized theca cells are visible in the stroma *(right)*. This morphologic change is called *stromal hyperthecosis*, and it appears to correlate directly with circulating insulin levels. (C, from Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev.* 1997;18:774–800. Copyright © 1997 by The Endocrine Society.)

with significant weight gain or an unknown cause may induce the clinical picture of PCOS in a woman with a history of previously normal ovulatory function. Hirsutism may develop prepubertally or during adolescence, or it may be absent until the third decade of life. Seborrhea, acne, and alopecia are other common clinical signs of androgen excess. In extreme cases of ovarian hyperthecosis (a severe variant of PCOS), clitoromegaly may be observed. Nonetheless, a history of rapid progression of androgenic symptoms and virilization is unusual. Some women may never have signs of androgen excess because of hereditary differences in target tissue sensitivity to androgens.[115] Infertility related to the anovulation may be the only presenting symptom.

During the physical examination, it is essential to search for and document signs of androgen excess (hirsutism, virilization, or both), insulin resistance (acanthosis nigricans) (Fig. 17.28), and the presence of unopposed estrogen action (well-rugated vagina and stretchable, clear cervical mucus) to support the diagnosis of PCOS. None of these signs is specific for PCOS, and each may be associated with any of the conditions listed in the differential diagnosis of PCOS (Table 17.4).

PCOS was previously defined according to the proceedings of an expert conference sponsored by the National Institutes of Health (NIH) in 1990, which described the disorder as including hyperandrogenism or hyperandrogenemia (or both), oligo-ovulation, and exclusion of known disorders of androgen excess and anovulation (Table 17.5).[210,211] Another expert conference held in Rotterdam in 2003 defined PCOS, after the exclusion of related disorders, by the presence of two of the following three features: oligo-ovulation or anovulation, clinical or biochemical signs of hyperandrogenism (or both), and polycystic ovaries (Fig. 17.29).[212] In essence,



• **Fig. 17.28** Acanthosis nigricans. (A) Moderate acanthosis nigricans (i.e., darkening and thickening of skin) at the lateral lower fold of the neck. Notice facial hirsutism (sideburns) in the same patient. (B) Severe acanthosis nigricans in another patient with severe insulin resistance. (B, courtesy Dr. R. Ann Word, UT Southwestern Medical Center, Dallas, TX.)

TABLE 17.4 Differential Diagnosis of Polycystic Ovary Syndrome

Idiopathic hirsutism
Hyperprolactinemia, hypothyroidism
Nonclassic congenital adrenal hyperplasia
Ovarian tumors
Adrenal tumors
Cushing syndrome
Glucocorticoid resistance
Other rare causes of androgen excess

TABLE 17.5 Criteria for the Definition of Polycystic Ovary Syndrome (PCOS)

NIH Statement (1990)[211]

To include all of the following:
1. Hyperandrogenism and/or hyperandrogenemia
2. Oligo-ovulation
3. Exclusion of related disorders[a]

ESHRE/ASRM Statement (Rotterdam, 2003)[212]

To include two of the following, in addition to exclusion of related disorders[a]:
1. Oligo-ovulation or anovulation (e.g., amenorrhea, irregular uterine bleeding)
2. Clinical and/or biochemical signs of hyperandrogenism (e.g., hirsutism, elevated serum total or free testosterone)
3. Polycystic ovaries (by ultrasonography)

AES Suggested Criteria for the Diagnosis of PCOS (2006)[213]

To include all of the following:
1. Hyperandrogenism: hirsutism and/or hyperandrogenemia
2. Ovarian dysfunction: oligo-anovulation and/or polycystic ovaries
3. Exclusion of other androgen excess or related disorders[a]

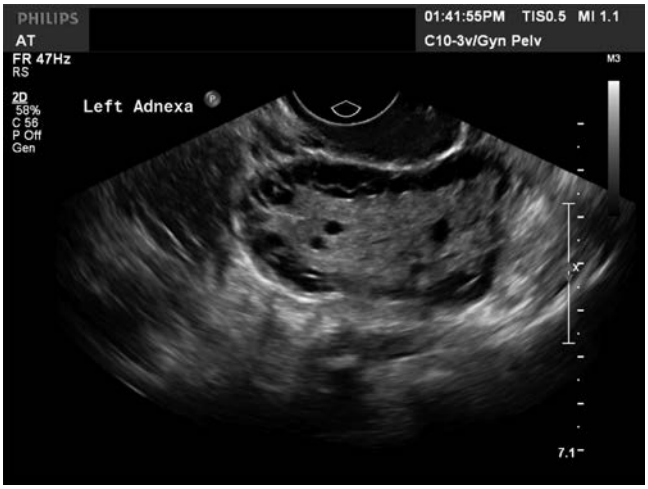
[a]Including but not limited to 21-hydroxylase-deficient nonclassic adrenal hyperplasia, thyroid dysfunction, hyperprolactinemia, neoplastic androgen secretion, drug-induced androgen excess, the syndromes of severe insulin resistance, Cushing syndrome, and glucocorticoid resistance. Superscript numbers in table indicate references at the end of the chapter.

AES, Androgen Excess Society; *ASRM,* American Society for Reproductive Medicine; *ESHRE,* European Society for Human Reproduction and Embryology; *NIH,* National Institutes of Health.

the Rotterdam 2003 criteria expanded the NIH 1990 definition by creating two new phenotypes: ovulatory women with polycystic ovaries plus hyperandrogenism and oligo-anovulatory women with polycystic ovaries but without hyperandrogenism. The clinical usefulness of including these new groups with respect to increased risk of infertility, insulin resistance, and long-term metabolic complications is not clear at this time.[213] More recently, the Androgen Excess Society published a broad consensus statement that included discussions of the merits and disadvantages of the NIH and Rotterdam criteria and suggested a practical definition that integrates both sets of diagnostic criteria (see Table 17.5).[212]

The exclusion of hyperprolactinemia, hypothyroidism, nonclassic congenital adrenal hyperplasia, and tumors requires a careful history, physical examination, and laboratory testing, as detailed previously (see Table 17.4). Cushing syndrome and glucocorticoid resistance may give rise to androgen excess and anovulation after a period of normal ovulatory function in teens. An 8 AM cortisol level after dexamethasone (1 mg) administration at midnight is a useful screening test for both conditions. Cushing syndrome may be recognized by its typical signs, whereas 8 AM and 4 PM cortisol levels are essential to confirm the diagnosis of glucocorticoid resistance.[193] Glucocorticoid resistance is characterized by preserved diurnal rhythm despite significantly elevated cortisol, ACTH, and adrenal C19 steroid levels and absence of cushingoid symptoms and signs.[193]

Elevated total testosterone is the most direct evidence for androgen excess. Various levels of testosterone are found in women with PCOS. Rarely, serum testosterone levels higher than 2 ng/mL may be encountered in association with the most severe form of PCOS, ovarian hyperthecosis. Overall, it is much more common to observe high-normal levels or borderline elevations of testosterone in women with PCOS.



• **Fig. 17.29** Transvaginal ultrasound image of a polycystic ovary. Notice the multiple, midsized follicles in the periphery and the increased solid area in the middle.

Prolactin and TSH concentrations should be measured routinely to rule out mild androgen excess and anovulation that may be associated with hyperprolactinemia. If basal LH levels are used as a marker for PCOS, a significant number of patients will slip through the cracks because they do not manifest elevated LH levels or increased LH/FSH ratios. The NIH-sponsored consensus conference on diagnostic criteria for PCOS in 1990 recommended that LH and the LH/FSH ratio are not required for the diagnosis of PCOS.[211,214] The heterogeneity of LH values in PCOS may be caused by the pulsatile nature of LH secretion and negative effects of obesity on LH levels. An elevated LH/FSH ratio supports the diagnosis of PCOS and may be useful in differentiating mild cases of nonobese PCOS without prominent androgen excess from hypothalamic anovulation. However, failure to exhibit an elevated LH level is of no diagnostic value. By definition, nonclassic congenital adrenal hyperplasia does not manifest as congenital virilization of external genitalia. Hyperandrogenic symptoms most commonly appear peripubertally or postpubertally. The clinical evaluation and laboratory-based diagnosis of nonclassic congenital adrenal hyperplasia were discussed earlier. Chapter 15 describes the ACTH stimulation test. A screening test for Cushing syndrome or glucocorticoid resistance should be performed as clinically indicated (see Chapter 15).

Serum DHEAS levels may be increased (up to 8 μg/mL) in about 50% of anovulatory women with PCOS. DHEAS originates almost exclusively from the adrenal gland.[215] The cause of adrenal hyperactivity in PCOS is unknown. Obtaining a DHEAS level routinely in a patient with PCOS is not recommended because it does not change the diagnosis or management. If an adrenal tumor is suspected, a DHEAS level should be obtained. DHEAS levels higher than 8 μg/mL may be associated with steroidogenically active adrenal tumors, and imaging is then indicated.

The Rotterdam 2003 criteria include the use of ultrasound as a diagnostic tool. The use of ultrasonography in the diagnosis of PCOS must be tempered by an awareness of the broad spectrum of women with ultrasonographic findings characteristic of polycystic ovaries. The typical polycystic-appearing ovary may emerge in a nonspecific fashion when a state of anovulation of any cause persists for any length of time (see Fig. 17.29).[216] Thus the polycystic-appearing ovary is the result of a functional derangement but not a specific central or local defect.

Biochemical evidence of insulin resistance or glucose intolerance is not necessary for the diagnosis of PCOS. Nonetheless, glucose intolerance should be investigated. Plasma glucose levels should be measured after a 75-g glucose load as a screen for glucose intolerance.

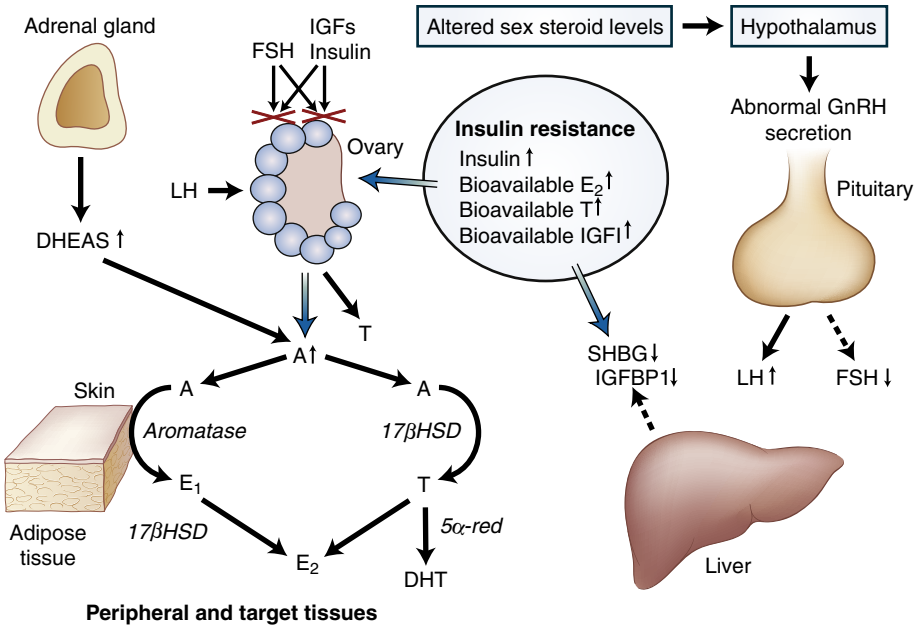
Women with PCOS commonly present with irregular uterine bleeding in the form of infrequent periods (i.e., oligomenorrhea) or amenorrhea. It is not necessary to document anovulation by ultrasonography, progesterone levels, or otherwise, especially if menstrual cycles are irregular with periods of amenorrhea. To confirm the diagnosis of chronic anovulation and unopposed estrogen exposure, most clinicians perform a progestin challenge test after a negative urine pregnancy test. Because endometrium is exposed to estradiol chronically in PCOS, these women respond to a challenge with a progestin (e.g., 5 mg/day of MPA given orally for 10 days) by uterine bleeding within a few days after the last pill of progestin. Reasons for lack of uterine bleeding after a progestin challenge include pregnancy, insufficient prior estrogen exposure of the endometrium, or an anatomic defect. If uterine bleeding does not follow progestin challenge, pregnancy should be ruled out again, along with other causes of chronic anovulation, as described in this chapter. An anatomic defect such as intrauterine adhesions may be ruled out with a hysterosalpingogram or hysteroscopy.

During the initial workup, an endometrial biopsy specimen should be obtained with the use of a plastic minisuction cannula (e.g., Pipelle) in the physician's office. If chronic anovulation persists, endometrial biopsies should be repeated periodically. Pregnancy should be ruled out by a urine or serum pregnancy test before each biopsy. Response to oral contraceptives or periodic progestin treatment with predictable withdrawal bleeding episodes is reassuring, and patients with predictable bleeding patterns do not need endometrial sampling during these treatments. In untreated patients, the risk of endometrial hyperplasia and malignancy is significantly increased even in young women with PCOS because of unopposed estrogen exposure.

Gonadotropin Production in Polycystic Ovary Syndrome

Women with PCOS have higher mean concentrations of LH but low or low-normal levels of FSH compared with levels found in normal women in the early follicular phase.[217] The elevated LH levels in PCOS are presumed to be primarily caused by accelerated GnRH-LH pulsatile activity (Fig. 17.30).[218–220] Central opioid tone appears to be suppressed because the pattern of LH secretion does not change in response to naloxone.[221] The enhanced pulsatile secretion of GnRH has been attributed to a reduction in hypothalamic opioid inhibition caused by the chronic absence of progesterone.[189] An increase in amplitude and frequency of LH secretion also correlates with steady-state levels of circulating estrogen.

In obese women with PCOS, LH levels may not be increased. The increase in LH pulse frequency is characteristic of the anovulatory state regardless of body fat content.[222] LH pulse amplitude, however, is comparatively normal in overweight women with PCOS, whereas it is increased in nonobese women with PCOS.[223] The overall LH reduction in obese women with PCOS may result from factors other than changes in LH pulse amplitude.[224] A low



• **Fig. 17.30** Pathologic mechanisms in polycystic ovary syndrome (PCOS). A deficient in vivo response of the ovarian follicle to physiologic quantities of follicle-stimulating hormone (FSH), possibly because of an impaired interaction between signaling pathways associated with FSH and insulin-like growth factors (IGFs) or insulin, may be an important defect responsible for anovulation in PCOS. Insulin resistance associated with increased circulating and tissue levels of insulin and bioavailable estradiol (E_2), testosterone (T), and IGF1 gives rise to abnormal hormone production in a number of tissues. Oversecretion of luteinizing hormone (LH) and decreased output of FSH by the pituitary, decreased production of sex hormone–binding globulin (SHBG) and IGF-binding protein 1 (IGFBP1) in the liver, increased adrenal secretion of dehydroepiandrosterone sulfate (DHEAS), and increased ovarian secretion of androstenedione (A) all contribute to the feed-forward cycle that maintains anovulation and androgen excess in PCOS. Excessive amounts of E_2 and T arise primarily from the conversion of A in peripheral and target tissues. T is converted to the potent steroids estradiol or DHT (dihydrotestosterone). Reductive 17β-hydroxysteroid dehydrogenase (17βHSD) enzyme activity may be conferred by protein products of several genes with overlapping functions; 5α-reductase (5α-red) is encoded by at least two genes, and aromatase is encoded by a single gene. *GnRH,* gonadotropin-releasing hormone.

LH value does not rule out the diagnosis of PCOS, whereas a high LH-FSH ratio supports this diagnosis in an anovulatory woman.

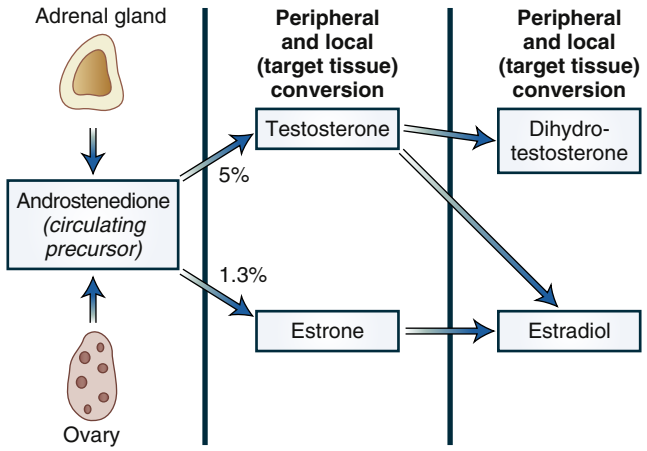
Insulin has been implicated as a potential regulator of LH secretion in PCOS. Insulin enhances the transcription of the LHβ gene *(LHB)*.[225,226] This laboratory observation was supported by an in vivo human study showing that insulin infusion suppresses pituitary response to GnRH in normal women and in women with PCOS.[227] These studies support the concept that insulin resistance or hyperinsulinemia may be responsible for abnormal gonadotropin release (see Fig. 17.30).

Steroid Production in Polycystic Ovary Syndrome

Ovulatory cycles are characterized by cyclic fluctuating hormone levels that regulate ovulation and menses (see Fig. 17.1A). Anovulation in women with PCOS is associated with steady-state levels of gonadotropins and ovarian steroids. In patients with persistent anovulation, the average daily production of estrogen and androgens is increased and depends on LH stimulation (see Fig. 17.30).[228] This is reflected in higher circulating levels of testosterone, androstenedione, DHEA, DHEAS, 17-hydroxyprogesterone, and estrone.[229] Testosterone, androstenedione, and DHEA are secreted directly by the ovary, whereas DHEAS, which is elevated in about 50% of anovulatory women with PCOS, is almost exclusively an adrenal contribution.[215] Circulating levels of androstenedione, secreted by polycystic ovaries, are particularly high.

Local conversion of steroid precursors to estradiol is an important physiologic process for certain estrogen target tissues, such as normal breast and genital skin, and can also promote the growth of pathologic estrogen-dependent tissues, such as endometrial or breast cancer (Fig. 17.31).[97,105,229–231] Androstenedione of ovarian origin is the most strikingly elevated steroid in PCOS. Androstenedione is not biologically active but serves as a dual precursor for androgen (i.e., testosterone that is further converted to the biologically far stronger androgen DHT) and estrogen (i.e., estrone that is further converted to biologically active estradiol in target tissues) (see Fig. 17.31). Increased androstenedione of ovarian origin in women with PCOS gives rise to circulating levels of estradiol higher than those measured during the first few days of an ovulatory cycle.

Estradiol is an extremely potent steroid. Biologically effective circulating levels of estradiol are measured in units of picograms per milliliter (pg/mL) or picomoles per liter (pmol/L); biologically effective levels of testosterone are measured in units of nanograms per milliliter (ng/mL) or nanomoles per liter (nmol/L) and circulate at 10 to 100 times the physiologic levels of estradiol. Even small rates of conversion of androstenedione to estrone may have a significant biologic impact, whereas markedly elevated production of androstenedione is required to produce significant amounts of testosterone and manifestations of androgen excess (see Fig. 17.31). Because elevated production of androstenedione does occur in PCOS,



• **Fig. 17.31** Extraovarian conversion of androstenedione to androgen and estrogen. Androstenedione of adrenal or ovarian origin, or both, acts as a dual precursor for androgen and estrogen. Approximately 5% of circulating androstenedione is converted to circulating testosterone, and approximately 1.3% of circulating androstenedione is converted to circulating estrone in peripheral tissues. Testosterone and estrone are further converted to biologically potent steroids, dihydrotestosterone and estradiol, in peripheral and target tissues. Biologically active amounts of estradiol in serum are measured in picograms or picomoles per milliliter (pg/mL or pmol/L), whereas biologically active levels of testosterone in serum are measured in nanograms or nanomoles per milliliter (ng/mL or nmol/L). The 1.3% conversion of normal quantities of androstenedione to estrone may have a critical biologic impact in settings such as postmenopausal endometrial or breast cancer. Significant androgen excess is observed in conditions with abnormally increased androstenedione formation (e.g., polycystic ovary syndrome).

extraovarian production of testosterone is biologically significant in this disease. In postmenopausal women, who have much lower levels of androstenedione, extraovarian production of testosterone is less important. Relatively small quantities of estrone (and estradiol) produced primarily by peripheral aromatization of androstenedione have a biologic impact in men and postmenopausal women.

Production of Sex Hormone–Binding Globulin in Polycystic Ovary Syndrome

SHBG binds testosterone and estradiol and decreases their biologic activities. In PCOS, the net production of androgen and estrogen is increased. Amplified estrogenic and androgenic effects in PCOS also are caused by a decreased SHBG concentration, giving rise to increased free or biologically active circulating levels of estradiol and testosterone (see Fig. 17.30). The levels of SHBG are controlled by a balance of hormonal influences on its synthesis in the liver. Testosterone and insulin inhibit, and estrogen and thyroid hormone stimulate, SHBG formation.[232] In anovulatory women with PCOS, circulating levels of SHBG are reduced by approximately 50%; this effect may be a hepatic response to increased circulating levels of testosterone and insulin (see Fig. 17.30).[232]

Testosterone decreases serum SHBG levels, giving rise to a vicious feedback circle favoring low SHBG and high bioavailable testosterone levels (see Fig. 17.30). Insulin directly decreases serum SHBG concentrations in women with PCOS independent of any action of sex steroids.[232] Insulin increases free testosterone levels in PCOS by two mechanisms: increasing ovarian secretion of testosterone precursors (e.g., androstenedione) and suppressing SHBG.[232]

Follicular Fate in Polycystic Ovary Syndrome

Under the influence of relatively low but constant levels of FSH, follicular growth is continuously stimulated, although not to the point of full maturation and ovulation.[233] Although full growth potential is not realized, the follicular life span may be extended by several months in the form of multiple follicular cysts. Most of these follicles in polycystic ovaries are 2 to 10 mm in diameter, and some can be as large as 20 mm. Hyperplastic theca cells, often luteinized in response to the high LH levels, surround these follicles (see Fig. 17.27). The accumulation of follicles arrested at various stages of development allows increased and relatively constant production of steroids in response to steady-state levels of gonadotropins.

These follicles are subject to atresia and are replaced by new follicles of similar limited growth potential. Steady-state turnover of stromal cells contributes to the stromal compartment of the ovary, and it is sustained by tissue derived from follicular atresia. A degenerating granulosa compartment, leaving the theca cells to contribute to the stromal compartment of the ovary, accompanies atresia (see Fig. 17.27). This functioning stromal tissue secretes significant amounts of androstenedione under the influence of increased LH. Androstenedione, by the mechanisms discussed previously, leads to increases in free testosterone and free estradiol levels and decreases in SHBG concentrations (see Fig. 17.30). From the point of view of steroidogenesis and steroid action, PCOS is the result of a complex vicious circle that includes a number of positive and negative feedback mechanisms.

Fig. 17.30 summarizes the postulated mechanisms underlying PCOS. Because FSH, insulin, and IGF pathways can synergize, it was postulated that this synergy does not occur in the presence of insulin resistance and might lead to relative resistance of the ovarian follicle to FSH. However, in vitro studies provide mixed results with respect to this hypothesis. For example, cultured granulosa cells obtained from the small follicles of polycystic ovaries produce negligible amounts of estradiol but show a dramatic increase in estrogen production when FSH or IGF1 is added to the culture medium. When FSH and IGF1 were added together in vitro, they synergized to increase estrogen biosynthesis in granulosa cells from polycystic ovaries.[234]

Induction of ovulation in PCOS is achieved by increasing FSH levels that are hypothesized to overcome this postulated in vivo block to FSH at the granulosa cell level. Two popular treatments, oral clomiphene citrate and injectable recombinant FSH, can provide increased levels of endogenous or exogenous FSH that may lead to ovulation at various doses. Some PCOS patients require large doses of clomiphene citrate or FSH to achieve ovulation. Paradoxically, the polycystic ovary may overreact to pharmacologic levels of FSH by recruiting a large number of developing follicles at once; this occasionally gives rise to the ovarian hyperstimulation syndrome (OHSS) (discussed later).[235] The therapeutic window between ovarian nonresponsiveness and hyperreactivity is usually narrow. There are significant gaps of knowledge that do not permit reconciliation of clinical postulates derived from in vitro or in vivo studies of PCOS.

Ovarian Hyperthecosis

Ovarian hyperthecosis is a severe variant of PCOS. The term refers to significantly increased stromal tissue with luteinized theca-like cells scattered throughout large sheets of fibroblast-like cells. The clinical and histologic findings and pathophysiology represent an exaggerated version of PCOS.[236] This diagnosis can be made on clinical grounds; an ovarian biopsy is not necessary except to rule out an ovarian tumor.

Increased androgen production leads to the clinical picture of more intense androgenization. The higher testosterone levels may also lower LH levels by blocking estrogen action at the hypothalamic-pituitary level.[224] The severity of hyperthecosis correlates with the degree of insulin resistance.[224] Because insulin and IGF1 stimulate proliferation of thecal interstitial cells, hyperinsulinemia may be an important pathophysiologic factor in the cause of hyperthecosis.

It is not uncommon to encounter markedly high levels of testosterone, even above 2 ng/mL, in patients with ovarian hyperthecosis. Virilization is common. These patients do not usually ovulate in response to clomiphene or recombinant FSH. It is usually difficult to suppress testosterone production, even using a GnRH analogue. Bilateral oophorectomy should be a last resort, but it may be necessary to control testosterone production in some of these patients.

Genetics of Polycystic Ovary Syndrome

The strong trend of PCOS to aggregate in families suggests an underlying genetic basis.[237,238] Some key clinical features of PCOS are genetically transmitted. In particular, there is familial aggregation of hyperandrogenemia (with or without oligomenorrhea) in PCOS kindreds, suggesting that it is a genetic trait.[208] Another study showed that hyperinsulinism may be a familial characteristic in daughters of women with PCOS.[239]

The PCOS phenotype is a consequence of genes and environment. For example, obesity associated with unhealthy lifestyle choices aggravates the PCOS phenotype in genetically susceptible women. The variable PCOS phenotypes add further challenge to genetic studies of PCOS. Several genomic loci have been proposed to account for the PCOS phenotype. These include *CYP11A1*, the insulin gene, and the follistatin gene; however, no convincing evidence regarding any of these loci has been published.[240] Although independent studies identified a dinucleotide repeat marker near the insulin receptor gene that maps to chromosome 19p13.2, the particular PCOS gene in this locus has not been isolated.[241] In the Han Chinese population, genome-wide PCOS association signals showed evidence of enrichment for candidate genes related to insulin signaling *(INSR)*, gonadotropin receptors *(FSHR, LHCGR),* and type 2 diabetes *(HMGA2, THADA, DENND1A)*.[242]

Insulin Resistance and Polycystic Ovary Syndrome

Insulin resistance is a major factor in the pathogenesis of non–insulin-dependent diabetes mellitus (NIDDM). The term *insulin resistance* can be defined as impaired whole-body insulin-mediated glucose disposal, as determined with the use of techniques such as the hyperinsulinemic glucose clamp technique.[111] Insulin resistance is defined clinically as the inability of a known quantity of exogenous or endogenous insulin to increase glucose uptake and use in an affected individual as much as it does in a normal person. Insulin resistance is frequently observed in lean and obese women with PCOS. More severe degrees of insulin resistance or impaired glucose tolerance are more common in obese women with PCOS.[111]

Androgen excess and insulin resistance are often associated with acanthosis nigricans. Acanthosis nigricans is a gray-brown, velvety discoloration and increased thickness of the skin, usually seen at the neck, groin, and axillae and under the breasts; it is a marker for insulin resistance (see Fig. 17.28). Hyperkeratosis and papillomatosis are the histologic characteristics of acanthosis nigricans. Acanthosis nigricans in hyperandrogenic women depends on the presence and severity of hyperinsulinemia and insulin resistance.[243] The mechanism responsible for the development of acanthosis nigricans is uncertain. This abnormal growth response of the skin may be mediated through receptors for various growth factors, including those for insulin and IGF1. Acanthosis nigricans can also be observed in the absence of insulin resistance or androgen excess.

Insulin resistance is characterized by an impaired glucose response to a specific amount of insulin. In many of these patients, normal glucose levels are maintained at the expense of increased circulating insulin to overcome the underlying defect. More severe forms of insulin resistance in PCOS range from impaired glucose tolerance to frank NIDDM. Resistance to insulin-stimulated glucose uptake is a relatively common phenomenon in the general population and is sometimes referred to as *syndrome X* or *metabolic syndrome*. The fundamental abnormality leading to the manifestations that make up the metabolic syndrome is resistance to insulin-mediated glucose uptake in muscle and increased lipolysis, which produces elevated levels of circulating free fatty acids.[244] These individuals also have dyslipidemia, hypertension, and increased risk of developing CVD. Not surprisingly, the incidences of dyslipidemia and cardiovascular risk are increased significantly in women with PCOS.[245,246] The incidence of hypertension increases significantly after menopause in women with a history of PCOS.[111] There is a significant clinical and pathologic overlap between the metabolic syndrome and PCOS.[247]

The clinical presentation of patients with insulin resistance depends on the ability of the pancreas to compensate for the target tissue resistance to insulin. During the first stages of this condition, compensation is effective, and the only metabolic abnormality is hyperinsulinemia. In many patients, the beta cells of the pancreas eventually fail to meet the challenge, and declining insulin levels lead to impaired glucose tolerance and eventually to frank diabetes mellitus. Beta-cell dysfunction is demonstrable in women with PCOS before the onset of glucose intolerance.[248]

Studies of well-characterized causes of hyperinsulinemia and androgen excess have illuminated various mechanisms of insulin resistance. Factors such as a decrease in insulin binding related to autoantibodies to insulin receptors, postreceptor defects, and a decrease in insulin receptor sites in target tissues are all involved in insulin resistance.[249] These rare syndromes are found in an extremely small portion of women with anovulation, androgen excess, and insulin resistance, leaving most PCOS patients without any demonstrable abnormalities in the number or quality of receptors or in antibody formation. The exact nature of insulin resistance in most women with PCOS is not well understood.

To understand the molecular defect underlying insulin resistance in PCOS, Dunaif and coworkers studied the differences between skin fibroblasts from women with and without PCOS with respect to insulin-dependent signal transduction.[111] The fibroblasts of women with PCOS showed no change in insulin binding or receptor affinity, but a postreceptor defect was observed in one-half of the women with PCOS.[111] This defect is characterized by increased basal insulin receptor serine phosphorylation and decreased insulin-dependent tyrosine phosphorylation of the insulin receptor.[111] At about the same time, Miller and coworkers evaluated whether post-translational modification of the product of the *CYP17A1* gene alters the ratio of its hydroxylase to lyase activity; they found that serine phosphorylation of CYP17A1 dramatically increases the enzyme's 17,20-lyase but not its 17α-hydroxylase activity. These observations led the investigators to hypothesize that a dominantly inherited aberrant kinase

activity phosphorylates serine residues at the insulin receptor-β and CYP17A1 product, leading to insulin resistance and increased androgen production, respectively. The cause of this abnormal phosphorylation pattern and consequences for insulin action and androgen production are important topics for further study.[250]

Within the context of a unified hypothesis, insulin resistance seems to be a critical defect that explains most of the endocrine abnormalities observed in PCOS (see Fig. 17.30). Insulin resistance is associated with abnormal responses of the ovarian follicle to FSH, which lead to anovulation and androgen secretion. This results in noncyclic formation of estrogen from androgens in peripheral tissues. Estradiol together with elevated androgen and insulin levels gives rise to abnormal gonadotropin secretion. This creates an anovulatory state favoring continuous excess of LH, steroid precursors, androgen, and estrogen (see Fig. 17.30).

Role of Obesity in Insulin Resistance and Anovulation

Increased waist-to-hip ratio compounded by significantly increased body mass index is called *android obesity* because this type of adipose tissue distribution is observed more commonly in men. Overweight women with anovulatory androgen excess commonly have this particular body fat distribution.[251] Android obesity is the result of fat deposition in the abdominal wall and in visceral mesenteric locations. This fat is more sensitive to catecholamines, less sensitive to insulin, and more active metabolically. Android obesity is associated with insulin resistance, glucose intolerance, diabetes mellitus, and an increase in androgen production rate and results in decreased levels of SHBG and increased levels of free testosterone and estradiol.[251] Android obesity is associated significantly with cardiovascular risk factors, including hypertension and dyslipidemia, and it has been tied to a notable increase in the risk of poor-prognosis breast cancer.[252,253] However, no direct association has been reported between PCOS and breast cancer risk.[254]

Although the combination of insulin resistance and androgen excess is often observed in obese women overall, women with android-type obesity appear to be at a significantly higher risk for insulin resistance and androgen excess. However, insulin resistance and androgen excess are not confined to obese anovulatory women but also occur in nonobese anovulatory women.[222] Although obesity by itself causes insulin resistance, the combination of insulin resistance and androgen excess is a specific feature of PCOS. Not surprisingly, the combination of obesity and PCOS is associated with more severe degrees of insulin resistance than those found in nonobese women with PCOS.[222,255] Android-type obesity, in contrast to general obesity, is a more specific risk factor for PCOS.

Laboratory Evaluation of Metabolic Syndrome in PCOS

In everyday clinical practice, the criteria for diagnosing insulin resistance in an individual patient have not been standardized and present extremely complex issues. One-fourth of the normal population has fasting and glucose-stimulated insulin levels that overlap with those of insulin-resistant individuals because of the great variability of insulin sensitivity in normal subjects.[111] Clinically available measures of insulin action, such as fasting or glucose-stimulated insulin levels, do not correlate well with more detailed measurements of insulin sensitivity in research settings.

In view of these constraints, it is reasonable to consider all women with PCOS at risk for insulin resistance and the associated abnormalities of the metabolic syndrome—dyslipidemia, hypertension, and CVD.[247] A lipid profile should be obtained in all cases of PCOS. Especially for obese women with PCOS, fasting glucose levels and glucose levels 2 hours after a 75-g glucose load should be obtained as a screen for glucose intolerance. The clinician should encourage the patient to take every possible measure (e.g., weight reduction, exercise) to reduce insulin resistance.

Use of Antidiabetic Drugs to Treat Anovulation and Androgen Excess

A logical approach to the management of PCOS includes using medications that improve insulin sensitivity in target tissues, achieving reductions in insulin secretion, and stabilizing glucose tolerance. Antidiabetic medications in the classes of biguanides (metformin) and thiazolidinediones (pioglitazone and rosiglitazone) have been used to reduce insulin resistance. Although metformin appears to influence ovarian steroidogenesis directly, this effect does not appear to be primarily responsible for the attenuation of ovarian androgen production in women with PCOS. Rather, metformin inhibits the output of hepatic glucose, necessitating a lower insulin concentration and thereby probably reducing androgen production by theca cells.[256]

Metformin at a dose of 500 mg three times daily reduced hyperinsulinemia, basal and stimulated levels of LH, and free testosterone concentrations in overweight women with PCOS.[257,258] Some anovulatory women ovulated and achieved pregnancy; however, clomiphene is superior to metformin in achieving live births in infertile women with PCOS.[259–261] Among published studies of metformin use in women with PCOS, subject characteristics and control measures for effects of weight change, dose of metformin, and outcome vary widely. A meta-analysis of 13 studies in which metformin was administered to 543 participants reported that patients taking metformin had an odds ratio for ovulation of 3.88 (95% confidence interval [CI], 2.25–6.69) compared with placebo and an odds ratio for ovulation of 4.41 (95% CI, 2.37–8.22) for metformin plus clomiphene compared with clomiphene alone.[262] Although the addition of metformin to clomiphene seems to increase the ovulation rate, it does not result in a higher rate of live births.[260] Metformin also improved fasting insulin levels, blood pressure, and levels of LDL cholesterol. These effects were judged to be independent of any changes in weight that were associated with metformin treatment, but controversy persists about whether the beneficial effects of metformin are entirely independent of the weight loss that is typically seen early in the course of therapy.[261,262]

The thiazolidinediones are pharmacologic ligands for the nuclear receptor PPARγ. They improve the action of insulin in the liver, skeletal muscle, and adipose tissue and have only a modest effect on hepatic glucose output. As with metformin, the thiazolidinediones are reported to affect ovarian steroid synthesis directly, although most evidence indicates that the reduction in insulin levels is responsible for decreased concentrations of circulating androgen.[256]

Women with PCOS who took troglitazone had consistent improvements in insulin resistance, pancreatic beta-cell function, hyperandrogenemia, and glucose tolerance.[263,264] In a double-blind, randomized, placebo-controlled study, ovulation was significantly greater for women with PCOS who received troglitazone than for those who received placebo; free testosterone levels decreased, and levels of SHBG increased in a dose-dependent fashion.[265] Although troglitazone is no longer available because of its hepatotoxicity, subsequent studies using rosiglitazone and pioglitazone had similar results.[266–268] Because of concern about the use of thiazolidinediones in pregnancy and recent evidence

linking these drugs to serious side effects such as heart failure and stroke, they have been less readily adopted for routine treatment of PCOS. The success of the strategy of reversing insulin resistance as a way to correct the critical abnormalities in PCOS argues for this defect as central to the pathogenesis of the disorder.

Management of Long-Term Deleterious Effects of Polycystic Ovary Syndrome

The long-term consequences of PCOS include irregular uterine bleeding, anovulatory infertility, androgen excess, chronically elevated levels of free estrogen associated with an increased risk of endometrial cancer, and insulin resistance associated with an increased risk of CVD and diabetes mellitus. Treatment must aid in achieving a healthy lifestyle and normal body weight, protect the endometrium from the effects of unopposed estrogen, and reduce testosterone levels.

Any woman with PCOS should be counseled to maintain a healthy lifestyle. In obese PCOS women, permanent lifestyle modification should be emphasized as the primary preventive measure to minimize short-term and long-term deleterious effects. Simple measures such as decreasing daily food intake by 500 kcal and introducing any type of moderate exercise for 30 minutes daily for 6 months can decrease hyperandrogenemia and diastolic blood pressure.[204] Because insulin resistance contributes to the abnormal lipid profile and increased cardiovascular risk in women with PCOS, weight loss is a high priority for patients who are overweight.[269] Insulin resistance and androgen excess can be reduced with a weight reduction of at least 5%.[270,271] Significant weight loss has also resulted in ovulation and pregnancy in a number of patients with PCOS.[272] Nutritional counseling and an emphasis on lifestyle changes are essential components of the long-term management of PCOS.

If the patient does not wish to become pregnant, medical therapy is directed toward the interruption of the effect of unopposed estrogen on the endometrium. Nonfluctuating levels of unopposed estradiol in the absence of progesterone cause irregular uterine bleeding, amenorrhea, and infertility and increase the risk of endometrial cancer. Anovulatory women with PCOS may have endometrial cancer even in their early 20s.[273] Endometrial biopsy should be performed periodically in untreated women with PCOS regardless of age. Pregnancy should be ruled out before each endometrial biopsy. The uterine bleeding pattern should not influence the decision to perform an endometrial biopsy. The presence of amenorrhea does not rule out endometrial hyperplasia. The critical factor that determines the risk of endometrial neoplasia is the duration of anovulation and exposure to unopposed estradiol. Long-term treatment with a progestin or oral contraceptive significantly decreases the risk of endometrial cancer.

One of the simplest and most effective ways to administer a progestin in the long term is to use an oral contraceptive. Oral contraceptives provide two additional benefits: reduction of androgen excess and contraception. Oral contraceptive pills reduce circulating androgen levels through suppression of circulating LH and stimulation of SHBG levels, and they have been shown to reduce hirsutism.[152] Oral contraceptive treatment for anovulation and hyperinsulinemia in women with androgen excess does not increase cardiovascular risk.[274]

For the patient who does not complain of hirsutism but is anovulatory and has irregular bleeding, treatment with progestin alone may be attempted as an alternative to oral contraceptive use. Progestin therapy is directed toward interruption of the chronic exposure of endometrium to unopposed effects of estrogen. MPA may be administered intermittently (e.g., 5 mg daily for the first 10 days of every other month) to ensure withdrawal bleeding and prevent endometrial hyperplasia. This treatment does not decrease androgen excess, nor does it provide contraception. Because an oral contraceptive with an ethinyl estradiol content of 30 μg or less can suppress androgen excess of ovarian origin, provide contraception, and protect the endometrium and does not increase insulin resistance, a low-dose oral contraceptive is the treatment of choice for nonsmokers with PCOS. An oral contraceptive together with the antiandrogen spironolactone (100 mg/day) is the recommended starting treatment for a hirsute woman with PCOS. The dose of spironolactone can be increased in increments to suppress hair growth, as described earlier.

Treatment with an oral contraceptive, with or without spironolactone, may not be effective in androgen suppression in severe cases of PCOS. In patients resistant to oral contraceptives, suppression of the ovary using a depot GnRH agonist may be required. Spironolactone does not affect insulin sensitivity in anovulatory women and can be used safely without causing adverse effects on carbohydrate or lipid metabolism.[275]

The clinician must counsel women with PCOS regarding their increased risk of future diabetes mellitus. The age at onset of NIDDM is significantly earlier in these women than in the general population.[206] Women with PCOS are more likely to experience gestational diabetes.[276] Long-term follow-up studies have shown a significantly increased risk for frank diabetes mellitus in anovulatory patients with PCOS.[111] It is therefore important to monitor glucose tolerance with periodic measurements of glucose levels after fasting and after a 75-g glucose load. The place of metformin in the long-term treatment of PCOS remains to be determined.[261,277]

The physician should alert the patient with PCOS that up to one-half of first-degree relatives and sisters may be affected by PCOS or at least by androgen excess in the presence of regular menses.[208] These individuals may be at higher than average risk for CVD and may benefit from preventive measures that reduce this risk.

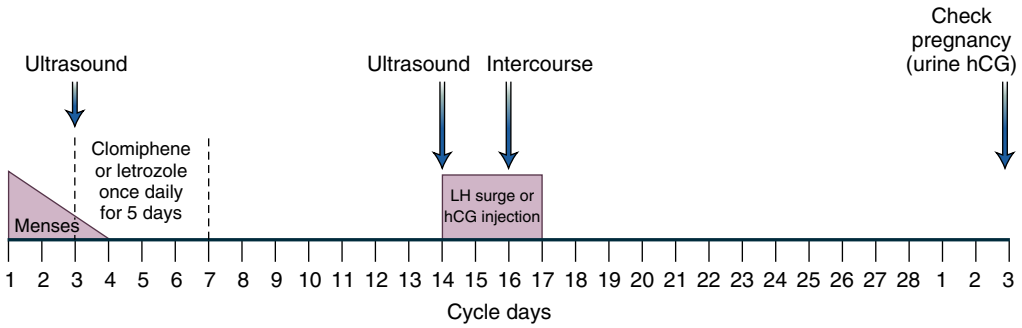
Ovulation Induction in Polycystic Ovary Syndrome

When pregnancy is achieved, patients with PCOS appear to have an increased risk of spontaneous miscarriage.[278] This increased risk may be related to elevated levels of LH that may produce an adverse environment for the oocyte and the endometrium. LH levels should be suppressed with oral contraceptives before ovulation is induced. Suppression can be achieved in most patients with PCOS by the use of an oral contraceptive for 4 to 6 weeks before ovulation induction with clomiphene citrate, an aromatase inhibitor, or recombinant FSH.

To induce ovulation in women with PCOS, FSH levels may be increased by oral administration of an antiestrogen (clomiphene citrate) or an aromatase inhibitor (letrozole, anastrozole) or by subcutaneous injection of recombinant FSH. Presumably, pharmacologic levels of FSH overcome the ovarian defect that is responsible for anovulation in PCOS.

Clomiphene Citrate

Clomiphene citrate is a nonsteroidal, ovulation-inducing ER ligand with mixed agonistic-antagonistic properties.[279] Acting as an antiestrogen, clomiphene citrate is thought to displace endogenous estrogen from hypothalamic ERs, thereby removing the negative feedback effect exerted by endogenous estrogens. The resultant change in pulsatile GnRH release is thought to normalize the release



• **Fig. 17.32** Monitoring of clomiphene citrate or letrozole-initiated ovulation. On cycle day 2 or 3, a baseline ultrasound examination is performed to rule out any large ovarian follicular cyst. Oral administration of clomiphene citrate or letrozole is started on day 3 of the cycle and continued for 5 days. Ultrasonography is performed on cycle day 13 or 14 to ensure follicular development. Upon observing at least one mature follicle by ultrasound, ovulation may be triggered by a human chorionic gonadotropin (hCG) injection followed by intercourse in 24 to 34 hours. Alternatively, the patient may be encouraged to have intercourse every other day during the 12-day period after the last clomiphene citrate or letrozole dose. Urinary hCG levels are checked to determine whether pregnancy has occurred. *LH,* luteinizing hormone.

of pituitary FSH and LH, which is followed by follicular recruitment and selection, assertion of dominance, and ovulation.[279]

Clomiphene citrate treatment can be started at any time to induce ovulation in an amenorrheic and anovulatory patient provided that a pregnancy test is performed beforehand (Fig. 17.32). Alternatively, uterine bleeding may be induced after a 10-day treatment with a combination oral contraceptive or MPA (5 mg/day). On cycle day 2 or 3 (day 1 is the first day of uterine bleeding), a baseline ultrasound study is performed to rule out any ovarian follicular cyst of more than 25-mm average diameter. If one or more large cysts are seen, ovulation induction should be delayed until after gonadotropin suppression by continuous oral contraception treatment for 4 to 6 weeks to eliminate these cysts or decrease their size. Clomiphene citrate is started at 50 mg/day orally on day 3 of the cycle and continued for 5 days. Ultrasonography is performed on cycle day 13 or 14 to ensure follicular development (i.e., at least one new follicle measuring at least 16 mm in diameter). The patient should be encouraged to have intercourse every other day during the 10-day period following the last clomiphene citrate dose. Alternatively, measurement of urinary LH to detect an LH surge can be used to time intercourse. Intercourse is recommended on the day of a positive urinary LH peak and on the next day. If an hCG injection is used to induce ovulation, intercourse is recommended within 24 to 34 hours after the injection.

If follicular development does not occur after the first course of therapy with clomiphene citrate at 50 mg/day, a second course of 100 mg daily for 5 days may be started. Lack of response at doses of 150 to 200 mg daily for 5 days should be an indication for a change of treatment. Most patients destined to conceive do so with the starting dose of clomiphene citrate (50 mg/day for 5 days). Most clomiphene citrate–initiated conceptions occur within the first 6 ovulatory cycles.[279] The incidence rate for multiple gestation in clomiphene citrate–induced pregnancies is 6% (4% for twins and 2% for triplets).[260] Failure to achieve pregnancy after three clomiphene cycles despite sonographic evidence of follicular development should prompt the clinician to perform a comprehensive infertility workup, including a semen analysis and evaluation of the uterine cavity and tubal patency.

Aromatase Inhibitors

Via decreasing aromatization of the estrogen precursor steroids in the brain and other body sites, aromatase inhibitors reduce hypothalamic-pituitary estrogen feedback, and this leads to increased GnRH secretion, concomitant elevations in LH and FSH, and increased ovarian follicular development in premenopausal women.[104,280] The gonadotropin-stimulating aromatase inhibitors letrozole and anastrozole have been used off-label in the treatment of patients with ovulatory dysfunction, such as PCOS, and for increasing the number of ovarian follicles recruited for ovulation in women who are already ovulatory.[281] Oral administration of letrozole (2.5 or 5 mg/day) or anastrozole (1 mg/day) on days 3 to 7 after uterine bleeding is effective for ovulation induction in women with anovulatory infertility (see Fig. 17.32).[281,282] In a randomized study, as compared with clomiphene (50 mg/day), letrozole (2.5 mg/day) was associated with higher live-birth and ovulation rates among infertile women with PCOS.[282] A retrospective study did not show any difference in the overall rates of major and minor congenital malformations among newborns from mothers who conceived after letrozole or clomiphene treatment.[283]

Metformin

Head-to-head randomized trials showed that clomiphene alone is clearly superior to metformin only with respect to achieving ovulation and live births in women with PCOS.[260] However, the ovulatory response to clomiphene was increased in obese women with PCOS by decreasing insulin secretion with the addition of metformin.[277] Another randomized study showed that the higher rate of ovulation in the users of clomiphene plus metformin seemed to be offset by a higher rate of pregnancy losses, producing similar live-birth rates in clomiphene-only and clomiphene plus metformin groups.[260] The benefit of the addition of metformin to clomiphene in obese clomiphene-nonresponders with PCOS needs to be assessed further.

Low-Dose Gonadotropin Therapy

For women who do not ovulate in response to clomiphene citrate or letrozole, FSH injections are started on day 3 of spontaneous or progestin-induced uterine bleeding. Recombinant FSH is administered subcutaneously starting with a daily dose of 75 IU for up to 10 days, if necessary, and using small incremental dose increases (12.5–37.5 IU) from then on at intervals of 3 to 7 days until serum estradiol concentrations begin to increase. The dose is then maintained until follicular rupture, which is induced by

subcutaneous injection of recombinant hCG (250 μg). Follicular growth is monitored by transvaginal ultrasonography and blood estradiol levels, which serve as biochemical markers for the granulosa cell mass in the growing follicle.[235] This regimen induces development of a single follicle in most cycles and has succeeded in reducing the rate of multiple pregnancies to as low as 6% in some series.[235] The low-dose regimen also practically eliminated the complication of severe OHSS.[235] Conception rates are comparable to those achieved with conventional therapy. The miscarriage rate remains somewhat higher than that after spontaneous conceptions (20–25%).

Conventional-dose gonadotropin therapy (starting FSH dose, 150 IU daily) should not be used as first-line treatment in patients with PCOS because it causes an alarming number of multiple pregnancies (14–50% of treatment cycles) and a significantly increased risk of OHSS (1.3–9.4% of treatment cycles).[284] OHSS is a more common complication of conventional-dose compared with low-dose gonadotropin treatment. Milder forms are relatively common and are characterized by weight gain, abdominal discomfort, and enlarged ovaries. Home bed rest and increased oral intake of fluids are sufficient to manage this form. Severe OHSS occurs in 0.1% to 0.2% of stimulation attempts and is accompanied by severe ascites, pleural effusion, electrolyte imbalance, and hypovolemia with oliguria. The most dreaded complication is deep venous thrombosis and embolism. Its cause is poorly understood. Large numbers of follicles, peak estradiol levels greater than 2000 pg/mL, and pregnancy are associated with a higher likelihood of OHSS. Prevention includes withholding of the hCG injection and intrauterine insemination. Treatment of severe OHSS includes hospitalization, maintenance of fluid and electrolyte balance, prophylaxis for thromboembolism by heparin, and drainage of severe ascites or pleural effusions. Frequently, supportive measures are sufficient to manage this self-limited condition.

Premature Ovarian Insufficiency

On average, menopause occurs at the age of 50 years, with 1% of women continuing to menstruate beyond the age of 60 years and another 1% whose menopause occurs before age 40 years. Premature menopause or ovarian insufficiency has been arbitrarily defined as the cessation of menses before 40 years of age.[285]

POI, which is defined as early depletion of ovarian follicles (before the age of 40 years), is a state of hypergonadotropic hypogonadism. These patients go through a normal puberty and a variable period of cyclic menses followed by oligomenorrhea or amenorrhea accompanied by hot flashes and urogenital atrophy. POI should always be included in the differential diagnosis of chronic anovulation. History and physical examination may reveal menstrual irregularity or secondary amenorrhea accompanied by symptoms and signs of estrogen deficiency, such as hot flashes and urogenital atrophy.[286]

The cause or genetic basis of POI is not well understood. Two genetic syndromes associated with POI are gonadal dysgenesis with primarily mosaic X-chromosome defects and *FMR1* gene premutation, a variant of fragile X syndrome.[287] Syndromes resulting from single-gene mutations (e.g., blepharophimosis-ptosis-epicanthus inversus syndrome [*FOXL2* mutation], galactosemia [*GALT* mutation]) may be associated with POI.[287] However, the cause of POI remains unknown in most cases.

The underlying ovarian defect may manifest at various ages, depending on the number of functional follicles left in the ovaries. If loss of follicles occurs rapidly before puberty, primary amenorrhea and lack of secondary sexual development ensue. The degree to which the adult phenotype develops and the age at which secondary amenorrhea actually occurs depend on whether follicle loss took place during or after puberty. In cases of primary amenorrhea associated with sexual infantilism, the ovarian remnants exist as streaks, and transvaginal ultrasonography usually cannot detect any ovaries. Many gene defects (e.g., *FSHR*, *CYP17A1*, *CYP19A1*) involve ovarian failure at the expected time of puberty, and the phenotypes manifest with primary amenorrhea and lack of secondary sexual development (see Chapter 25).[287]

POI can also result from an autoimmune process and may be associated with an autoimmune polyendocrine syndrome.[288] Other causes of premature insufficiency can be related to the sudden destruction of follicles by chemotherapy, irradiation, or infections such as mumps oophoritis. The effect of irradiation depends on the patient's age and the x-ray dose.[289] Steroid levels begin to fall and gonadotropins rise within 2 weeks after irradiation of the ovaries. Younger women exposed to radiation are less likely to have permanent ovarian insufficiency because of the higher number of oocytes present at earlier ages. When the radiation field excludes the pelvis or the ovaries are transposed out of the pelvis by laparoscopic surgery before irradiation, the risk for POI is minimized.[290] Most chemotherapeutic agents used for eradication of malignancies are toxic to the ovaries and cause ovarian insufficiency.[291] Resumption of menses and pregnancy have been reported after radiotherapy or chemotherapy,[292] but POI may occur years after these therapies.[272]

Diagnosis and Management of Premature Ovarian Insufficiency

POI should be suspected in a woman younger than 40 years of age who presents with amenorrhea, oligomenorrhea, or another form of menstrual irregularity accompanied by hot flashes. Menopausal serum FSH levels (40 IU/L) on at least two occasions are sufficient for the diagnosis of POI.

Case reports of pregnancies in affected women occurring during hormone replacement therapy have been published.[293,294] In particular, young women with POI may experience intermittent periods of ovarian function, with antral follicles present at ovarian ultrasonography and ovulation described in cases that followed up with regular endocrine assessments. A randomized trial of hormone therapy in this setting showed that folliculogenesis occurred often but was less frequently followed by ovulation and even less frequently by pregnancy (up to 14%); conventional-dose estrogen therapy did not improve the rate of folliculogenesis, ovulation, or pregnancy.[286] Later pilot studies or case reports suggested that lowering FSH levels to less than 15 IU/L by use of high-dose estrogen or a GnRH antagonist in young women with POI may trigger ovulation or permit ovulation induction and pregnancy in a small number of patients.[295,296]

The clinician should inform patients diagnosed with POI that there is a small but significant likelihood of spontaneous pregnancy or pregnancy after ovulation induction. Women who desire pregnancy are still best served by assisted reproductive technology using donor oocytes, because the probability of pregnancy with an autologous egg is low. Use of donor oocytes followed by IVF with the partner's sperm and intrauterine embryo transfer after synchronization of the recipient patient's endometrium with the donor's cycle using exogenous estrogen and progesterone is offered to the patient who wishes to carry a pregnancy in her uterus (see Fig. 17.23). This approach offers an excellent chance of achieving live birth (>50% per donor oocyte IVF cycle).

Patients with POI have an increased incidence of an abnormal complement of chromosomes.[297] The risk of having an abnormal karyotype increases with decreasing age at onset of the POI. A chromosomal analysis is recommended for the POI patients younger than 30 years of age because of the increased risk of a gonadal tumor associated with the presence of a Y chromosome.[298] It is extremely rare to encounter a gonadal tumor in patients with POI after the age of 30 years.[299]

Mosaicism that includes a Y chromosome has been associated with a high incidence of gonadal tumors.[298] These malignant tumors arise from germ cells and include gonadoblastomas, dysgerminomas, yolk sac tumors, and choriocarcinoma. Secondary virilization in patients with karyotypic abnormalities and POI is associated with a significantly increased risk of a gonadal tumor. The precise risk of a tumor in various subsets of these patients is not well known because a significant number of women carrying a Y chromosome do not have symptoms of virilization. The frequency of Y chromosomal material determined by polymerase chain reaction is high in those with Turner syndrome (12.2%), but the occurrence of a gonadal tumor among these Y-positive patients is about 7% to 10%.[300]

Fragile X–associated disorders are caused by a CGG trinucleotide repeat expansion in the promoter region of the *FMR1* gene. Expansion of the CGG trinucleotide repeats to more than 200 copies induces methylation of the *FMR1* gene, with an outcome of transcriptional silencing.[301] This so-called full mutation was linked to mental retardation or autism. Individuals who carried the premutation (defined as >55 but <200 CGG repeats) have increased *FMR1* mRNA levels with decreased levels of fragile X mental retardation protein (FMRP). Convincing evidence relates the *FMR1* premutation to altered ovarian function and loss of fertility.[302] The natural history of the altered ovarian function in women who carry the *FMR1* premutation is still not well understood. Women with POI are at increased risk for an *FMR1* premutation and should be informed of the availability of fragile X testing.

POI usually occurs as an isolated autoimmune disorder. Rarely, it may be associated with hypothyroidism, diabetes mellitus, hypoadrenalism, hypoparathyroidism, or systemic lupus erythematosus (SLE).[303] It can be part of an autoimmune polyendocrine syndrome.[288] Thyroid insufficiency, adrenal insufficiency, and diabetes mellitus are the endocrine disorders most frequently associated with POI.[304] Periodic endocrine testing for glucose intolerance, adrenal or parathyroid function, and autoimmune disease (e.g., SLE) should be considered based on the clinical presentation (Table 17.6). Because hypothyroidism is more commonly found than other endocrine disorders associated with POI, this author prefers to check a TSH level during the initial evaluation (see Table 17.6).

Treatment of POI should be directed toward its specific cause. In most cases, however, it is not possible to identify a specific cause if there are no karyotypic anomalies. Besides infertility, long-term ovarian steroid deficiency has far-reaching health implications. Early menopause has been associated with increased cardiovascular mortality and stroke, bone fracture, and colorectal cancer risks.[287] Despite reduced risks for development of breast cancer, overall quality of life and life expectancy decline with early menopause.[287] Hormone therapy, using combined estrogen and progestin or a low-dose oral contraceptive, is the cornerstone of the management for these women. The added value of androgen replacement remains uncertain.[287]

TABLE 17.6 Laboratory Evaluation of Premature Ovarian Insufficiency

Follicle-stimulating hormone (to establish the diagnosis of premature ovarian insufficiency)
Karyotype (<30 yr of age or sexual infantilism)
Testing for *FMR1* gene premutation carrier state
Thyroid-stimulating hormone (hypothyroidism)

FMR1, Fragile X mental retardation 1.

Diagnosis and Management of Anovulatory Uterine Bleeding

Acyclic production of estrogen during anovulatory cycles gives rise to irregular shedding of the endometrium. These bleeding manifestations of anovulatory cycles in the absence of uterine pathology or systemic illness are commonly referred to as *dysfunctional uterine bleeding*. Anovulatory uterine bleeding, which is the most common cause of chronic menstrual irregularities, is a diagnosis of exclusion. Pregnancy, uterine leiomyomas, endometrial polyps, and adenomyosis should be ruled out as anatomic causes of irregular or excessive uterine bleeding. Malignancies of the vagina, cervix, endometrium, myometrium, fallopian tubes, and ovaries or coagulation abnormalities should also be ruled out before a diagnosis of anovulatory uterine bleeding is made.

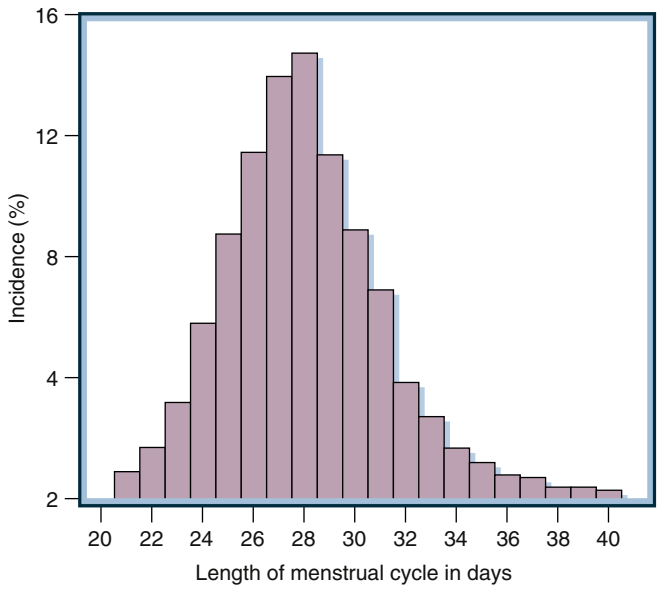
Anovulatory uterine bleeding can be managed without surgical intervention by either restoring ovulation or mimicking the ovulatory hormonal profile by providing exogenous steroids. The rationale for use of exogenous steroids is based on the knowledge of predictable responses of the endometrium to estrogen and progesterone. Physiologic responses of the endometrium to natural ovarian steroids have been uncovered by observation of the gross and microscopic changes occurring in the endometrium during thousands of normal ovulatory cycles in humans and other primates.[119,120,305] The pharmacologic application of exogenous estrogens and progestins in women with anovulatory bleeding aims to correct the production of local tissue factors that mediate physiologic steroid action and reverse the excessive and prolonged flow typical of anovulatory cycles.

Clinical management of irregular uterine bleeding with exogenous hormones is a time-honored method, and it has diagnostic value. Failure to control vaginal bleeding with hormonal therapy, despite appropriate application and use, makes the diagnosis of anovulatory uterine bleeding considerably less likely. In such cases, attention is directed to an anatomic pathologic entity within the reproductive axis as the cause of abnormal bleeding.

Heavy but regular menstrual bleeding (i.e., hypermenorrhea) can be encountered in ovulatory women. It may have an anatomic cause, such as a leiomyoma impinging on the endometrial cavity or the diffuse and pathologic presence of benign endometrial glands in the myometrium (i.e., adenomyosis). In the absence of a specific pathologic cause, it is presumed that hypermenorrhea reflects subtle disturbances in the endometrial tissue mechanism. In essentially all cases, evaluation and treatment are identical to the approach detailed in this section.

Characteristics of Normal Menses

Normal menstruation takes place about 14 days after each ovulation episode as a consequence of postovulatory estrogen-progesterone



• **Fig. 17.33** Variation of the duration of the menstrual cycle in women with regular cycles. (From Cunningham FG, MacDonald PC, Gant NF, et al. The endometrium and decidua: menstruation and pregnancy. In: Cunningham FG, ed. *Williams Obstetrics*. 19th ed. Stamford, CT: Appleton & Lange; 1993:81–109.)

withdrawal. The quantity and duration of bleeding are quite reproducible. This predictability leads many women to expect a certain characteristic flow pattern. Any slight deviations, such as plus or minus 1 day in duration or minor deviation from expected tampon use, are causes for major concern in the patient. Most women of reproductive age can predict the timing of their flows so accurately that even minor variability may require reassurance by the clinician. Although variability of menstrual cycles is a common feature during the teenage years and perimenopausal transition, the characteristics of menstrual bleeding do not undergo appreciable change between ages 20 and 40.[306]

For ovulatory women, the changes in the length of the menstrual cycle over the period of reproductive age are predictable. Between menarche and age 20, the cycle length for most ovulatory women is relatively longer. Between 20 and 40 years, there is increased regularity as cycles shorten. In the 40s, cycles begin to lengthen again. The highest incidence of anovulatory cycles occurs before age 20 and after age 40.[307] In these age groups, the average length of a cycle is between 25 and 28 days. Among ovulatory women, the frequency of a cycle of less than 21 days or more than 35 days is rare (<2%).[308] Overall, most women have cycles that last 24 to 35 days (Fig. 17.33).[306] Between ages 40 and 50, menstrual cycle length increases and anovulation becomes more prevalent.[309]

The average postovulatory bleeding lasts from 4 to 6 days. The normal volume of menstrual blood loss is 30 mL. More than 80 mL is considered abnormal. Most of the blood loss occurs during the first 3 days of a period, so excessive flow may exist without prolongation of flow.[310,311]

During an ovulatory cycle, the duration from ovulation to menses is relatively constant and averages 14 days (see Fig. 17.1A). Greater variability in the length of the proliferative phase, however, produces a distribution in the duration of the menstrual cycle. Menstrual bleeding more often than every 24 days or less often than every 35 days requires evaluation.[306,309] Flow that lasts 7 or more days also requires evaluation. A flow that totals more than 80 mL/month usually leads to anemia and should be treated.[312] In clinical practice, however, it is difficult to quantify menstrual flow because evaluation and treatment are based solely on the patient's perceptions regarding the duration, amount, and timing of her menstrual bleeding. Despite this difficulty in quantifying menstrual blood loss, the clinician should evaluate the cause of excessive uterine bleeding. Anemia should be ruled out by a complete blood cell count.[313] A low hemoglobin value accompanied by microcytic and hypochromic red blood cells suggests excessive blood loss during menses. These patients should be provided with iron supplementation. The likely presence of coagulation defects, uterine leiomyomas, or adenomyosis underlying prolonged menses should be evaluated in anemic patients through a meticulous history and physical examination followed by relevant laboratory tests.

Terminology Describing Abnormal Uterine Bleeding

Oligomenorrhea is defined as intervals between episodes of uterine bleeding longer than 35 days, and the term *polymenorrhea* is used to describe intervals shorter than 24 days. *Hypermenorrhea* refers to regular intervals (24–35 days) but excessive flow or duration of bleeding, or both. An alternative term for hypermenorrhea is *menorrhagia*. *Hypomenorrhea* refers to diminution of the flow or shortening of the duration of regular menses, or both.

Uterine Bleeding in Response to Steroid Hormones

Estrogen Withdrawal Bleeding

Uterine bleeding follows acute cessation of estrogen support to the endometrium. This type of uterine bleeding can occur after bilateral oophorectomy, irradiation of mature follicles, or administration of estrogen to a woman who previously underwent removal of both of her ovaries, followed by discontinuation of therapy. Similarly, the bleeding that occurs after bilateral removal of ovaries can be delayed by concomitant estrogen therapy. Flow occurs on discontinuation of exogenous estrogen. Estrogen withdrawal by itself (in the absence of progesterone) almost invariably causes uterine bleeding.

Estrogen Breakthrough Bleeding

Chronic exposure to various quantities of estrogen stimulates the growth of endometrium continuously in the absence of progesterone, as in the case of excessive extragonadal estrogen production in PCOS. After a certain point, the amount of estrogen produced in extraovarian tissue remains insufficient to maintain structural support for the endometrium. This gives rise to unpredictable episodes of shedding of the surface endometrium. Relatively low doses of estrogen yield intermittent spotting that may be prolonged, but the quantity is light. High levels of estrogen and sustained availability lead to prolonged periods of amenorrhea followed by acute, often profuse episodes of bleeding with excessive loss of blood.

Progesterone Withdrawal Bleeding

Typical progesterone withdrawal bleeding occurs after ovulation in the absence of pregnancy. Removal of the corpus luteum also leads to endometrial desquamation. Pharmacologically, a similar event can be achieved by administration and then discontinuation of progesterone or a synthetic progestin. Progesterone withdrawal

bleeding occurs only if the endometrium is initially primed by endogenous or exogenous estrogen. If estrogen therapy is continued as progesterone is withdrawn, the progesterone withdrawal bleeding still occurs. Only when estrogen levels are increased markedly is progesterone withdrawal bleeding delayed.[314] Progesterone withdrawal bleeding is quite predictable in the presence of previous or concomitant estrogen exposure.

Progestin Breakthrough Bleeding

The pharmacologic phenomenon of breakthrough bleeding occurs in the setting of an unfavorably high ratio of progestin to estrogen. In the absence of sufficient estrogen, continuous progestin therapy leads to intermittent bleeding of variable duration, similar to the low-dose estrogen breakthrough bleeding described previously. This type of bleeding is associated with combination oral contraceptives that contain low-dose estrogen and the long-acting, progestin-only contraceptive methods such as Norplant and Depo-Provera.[315] Progestin breakthrough bleeding is highly unpredictable and is characterized by extensive variability among women.

Causes of Irregular Uterine Bleeding

The most common cause of disruption of a normal menstrual pattern is pregnancy or a complication of pregnancy. Pregnancy and pregnancy-related problems such as ectopic pregnancy or spontaneous miscarriage are extremely common causes of abnormal uterine bleeding (Table 17.7). Pregnancy should be ruled out by a urine test in any woman of reproductive age who presents with irregular bleeding (Table 17.8).

Anovulatory uterine bleeding is a diagnosis of exclusion for several reasons. Vulvar, vaginal, or uterine malignancies or an estrogen-secreting or androgen-secreting ovarian tumor may cause abnormal uterine bleeding (see Table 17.7). Anovulatory uterine bleeding arising from responses of the endometrium to inappropriate production of ovarian steroids has also been called *dysfunctional uterine bleeding* because treatments that restore ovulatory function potentially reverse the irregular bleeding pattern. Common examples of anovulatory bleeding include those associated with exercise-related anovulation, hyperprolactinemia, hypothyroidism, or PCOS.[316] In these cases, restoration of ovulatory menses by correction of the underlying disorder or use of exogenous hormones can achieve predictable uterine bleeding.

Another common cause of irregular uterine bleeding is observed in oral contraceptive users in the form of progestin breakthrough bleeding. Progestin breakthrough bleeding during postmenopausal hormone therapy is also common. Patients may be unknowingly using other hormonal medications with an impact on the endometrium. For example, the use of ginseng, an herbal root, has been associated with estrogenic activity and abnormal bleeding.[317] Although uterine bleeding is a common benign side effect of various long-term hormone treatments, the clinician should always be convinced first that no other pathologic condition is present. Anatomically demonstrable pathologic disorders of the menstrual outflow tract include endometrial hyperplasia and cancer, endometrial polyps, uterine leiomyomas, adenomyosis, and endometritis. Irregular uterine bleeding may be associated with chronic illness, such as renal insufficiency, liver insufficiency, or AIDS. Careful examination may discover genital injury or a foreign object (see Table 17.7).

At puberty, the most common cause of irregular uterine bleeding is anovulation. Approximately 20% of adolescents with excessive

TABLE 17.7 Causes of Irregular Uterine Bleeding

Complications of Pregnancy
- Threatened miscarriage
- Incomplete miscarriage
- Ectopic pregnancy

Anovulation
- Physiologic
  - Uncomplicated pregnancy (amenorrhea)
  - Pubertal (postmenarchal) anovulation
  - Anovulation immediately before menopause
- Medications (e.g., oral contraceptives, GnRH agonists, danazol)
- Hypothalamic (frequently presents as amenorrhea)
  - Functional (e.g., diet, exercise, stress)
  - Anatomic (e.g., tumor, granulomatous disease, infection)
- Hyperprolactinemia, other pituitary disorders
  - Prolactinoma
  - Other pituitary tumors, granulomatous disease
  - Hypothyroidism
  - Medications
  - Other
- Androgen excess
  - PCOS, hyperthecosis
  - Ovarian tumor (e.g., Sertoli-Leydig cell tumor)
  - Nonclassic congenital adrenal hyperplasia
  - Cushing syndrome
  - Glucocorticoid resistance
  - Adrenal tumor (e.g., adenoma, carcinoma)
  - Medications (e.g., testosterone, danazol)
  - Other
- Premature ovarian insufficiency (frequently presents as amenorrhea)
- Chronic illness
  - Liver insufficiency
  - Renal insufficiency
  - AIDS
- Other

Anatomic Defects Affecting the Uterus
- Uterine leiomyomas
- Endometrial polyps
- Adenomyosis (usually manifests as hypermenorrhea)
- Intrauterine adhesions (usually manifests as amenorrhea)
- Endometritis
- Endometrial hyperplasia, cancer
- Chronic estrogen exposure (e.g., PCOS, medication, liver insufficiency)
- Estrogen-secreting ovarian tumor (e.g., granulosa cell tumor)
- Advanced cervical cancer
- Other

Coagulation Defects (Usually Manifest as Hypermenorrhea)
- Von Willebrand disease
- Factor XI deficiency
- Other

Extrauterine Genital Bleeding (May Mimic Uterine Bleeding)
- Vaginitis
- Genital trauma
- Foreign body
- Vaginal neoplasia
- Vulvar neoplasia
- Other

AIDS, Acquired immunodeficiency syndrome; *GnRH*, gonadotropin-releasing hormone; *PCOS*, polycystic ovary syndrome.

TABLE 17.8 Diagnostic Tests to Evaluate Irregular Uterine Bleeding

Commonly Used Tests

Urine hCG test
Serum hCG level (incomplete miscarriage, ectopic pregnancy)
Transvaginal pelvic ultrasonography (intrauterine or ectopic pregnancy, uterine leiomyoma, endometrial polyp or neoplasia, ovarian tumor)
Serum FSH, LH (anovulation; ovarian insufficiency)
Serum prolactin, TSH (anovulation; hyperprolactinemia)
Complete blood count, PT, PTT (evaluation for anemia, coagulation defect)
Liver and renal functions, HIV (anovulation; chronic disease)
Endometrial biopsy (endometrial disease; polyp, neoplasia, endometritis)

Less Commonly Used Tests

Evaluation for PCOS, ovarian or adrenal tumor, nonclassic congenital adrenal hyperplasia, Cushing syndrome, and glucocorticoid resistance (androgen excess)
Head CT or MRI scan (hypothalamic anovulation, hyperprolactinemia)
Pelvic MRI scan (adenomyosis, uterine leiomyoma)
Hysterosonography with intrauterine saline instillation (endometrial polyp, uterine leiomyoma)
Hysteroscopy (endometrial polyp, uterine leiomyoma)
Dilatation and curettage (endometrial disease not diagnosed by ultrasonography or biopsy)

CT, Computed tomography; *FSH,* follicle-stimulating hormone; *hCG,* human chorionic gonadotropin; *HIV,* human immunodeficiency virus; *LH,* luteinizing hormone; *MRI,* magnetic resonance imaging; *PCOS,* polycystic ovary syndrome; *PT,* prothrombin time; *PTT,* partial thromboplastin time; *TSH,* thyroid-stimulating hormone.

irregular uterine bleeding have a coagulation defect.[318,319] Among all women of reproductive age with hypermenorrhea, the prevalence of a coagulation disorder is 17%; von Willebrand disease is the most common defect, and factor XI deficiency is the second most common diagnosis. Bleeding because of a coagulation defect usually consists of a heavy flow with regular, cyclic menses (i.e., hypermenorrhea), and the same pattern can be seen in patients being treated with anticoagulants.[320] Bleeding disorders are usually associated with hypermenorrhea since menarche and a history of bleeding with surgery or trauma. Hypermenorrhea may be the only sign of an inherited bleeding disorder.[321]

Early pregnancy or its complications should always be ruled out first by a sensitive urine hCG measurement in any reproductive-age woman presenting with irregular bleeding. Other tests should be ordered on the basis of the initial clinical evaluation, including tests to evaluate anovulatory disorders of various causes (see Table 17.8). In patients with a history of prolonged heavy menses (i.e., hypermenorrhea) of pubertal origin, coagulation studies (e.g., prothrombin time, partial thromboplastin time, bleeding time) and a complete blood cell count should be obtained.

Pelvic ultrasonography through a vaginal probe is an extremely useful test for the evaluation of normal or abnormal pregnancy, uterine leiomyomas, endometrial neoplasia, and ovarian tumors (see Table 17.8). Other imaging studies may be used judiciously to rule out pathologic involvement of the hypothalamus, pituitary, and adrenal (discussed earlier). Pelvic MRI or ultrasound is used to assess adenomyosis, a uterine disorder characterized by the abnormal presence of diffuse endometrial tissue in the myometrial layer. Advanced adenomyosis is associated with diffuse enlargement of the uterus, hypermenorrhea, and anemia.

Endometrial histologic evaluation should be determined by an endometrial biopsy performed in the physician's office in patients at risk for endometrial hyperplasia or cancer (e.g., PCOS, liver insufficiency, obesity, diabetes mellitus, hormone therapy). A benign endometrial polyp or a uterine leiomyoma protruding into the uterine cavity can be diagnosed by hysterosonography using intrauterine saline installation or by hysteroscopy. Hysterosonography and hysteroscopy are not appropriate tests to evaluate endometrial hyperplasia or cancer because these procedures may cause dissemination of malignant cells. If malignancy is suspected, it should be ruled out by an office endometrial biopsy (see Table 17.8). Occasionally an office endometrial biopsy cannot be performed or is not diagnostic of endometrial neoplasia. In these rare instances, endometrial curettage under anesthesia is performed for a reliable tissue diagnosis.

A careful history and physical examination may eliminate the need for most of these diagnostic tests. Before ordering a certain diagnostic study, it is useful to consider whether a particular test result would alter the ultimate clinical management.

Management of Anovulatory Uterine Bleeding

If ovulatory function can be restored, anovulatory bleeding usually gives way to predictable cyclic periods. Because restoration of ovulatory function may not be possible or practical in many of these women, exogenous estrogen and progestin are administered for several purposes. The indications for hormonal treatment of uterine bleeding include the need to stop acute uterine bleeding, to maintain predictable bleeding episodes, or to prevent endometrial hyperplasia.

Anovulatory uterine bleeding is a diagnosis of exclusion. Various anatomically demonstrable pathologic involvements of the genital tract (see Table 17.7) should be ruled out before administration of estrogen, progestin, or GnRH analogues.

Oral Contraceptives

Use of combination oral contraceptives in an acute or chronic fashion is the most common treatment for irregular uterine bleeding. The estrogen component of the combination pill stabilizes the endometrial tissue and stops shedding within hours; it decreases ovarian secretion of sex steroids by suppression of gonadotropins within several days. The progestin component of the pill directly affects endometrial tissue to decrease shedding over days and potentiates ovarian suppression induced by estrogen. The progestin (in the presence of estrogen) induces differentiation of the endometrial tissue into a stable form called *pseudodecidua*. Typically, a monophasic oral contraceptive preparation that contains 30 or 35 μg of ethinyl estradiol is preferred. Triphasic oral contraceptives and those with less than 30 μg of ethinyl estradiol are not suitable for the treatment of excessive anovulatory uterine bleeding. A combination oral contraceptive in high doses (two or three pills per day) can be used for short intervals (i.e., weeks) to treat an acute episode of excessive uterine bleeding. The usual dose of one pill per day may be administered for years to manage chronic anovulatory bleeding associated with PCOS or hyperprolactinemia.

Oral Contraceptives and Acute Excessive Uterine Bleeding Associated With Anemia

Unopposed estrogen exposure in women with anovulatory uterine bleeding is commonly associated with chronic endometrial buildup and heavy bleeding episodes. Therapy is administered as

one combination oral contraceptive pill twice daily for 1 week. In obese women, the oral contraceptive may be given three times daily. This therapy is maintained despite cessation of flow within 2 days. If flow does not abate, other diagnostic possibilities (e.g., previously missed diagnosis of polyps, incomplete abortion, or neoplasia) should be reevaluated. In case of anovulatory bleeding, the flow does diminish rapidly within 2 days after the beginning of high-dose oral contraceptive treatment (i.e., one pill two or three times daily). Specific causes of anovulation and possible coagulation disorders should be evaluated during the next few days. The physician should also consider whether blood replacement or initiation of iron therapy is necessary. The patient should also be warned of possible nausea that may be caused by high-dose oral contraceptive treatment.

At the end of a week of high-dose oral contraceptive treatment, the pill is stopped temporarily. A heavy flow usually starts within a few days. On the third day of this withdrawal bleeding, a regular dose of combination oral contraceptive medication (one pill/day) is started. This is repeated for several 3-week treatments interrupted by 1-week withdrawal intervals. A decrease in volume with each successive cycle is expected. Oral contraceptives reduce menstrual flow by more than one-half in most women.[322]

Because oral contraceptive use does not treat the underlying cause of anovulation but provides symptomatic relief by directly affecting the endometrium, its cessation results in the return of erratic uterine bleeding. Regardless of the requirement for contraception, use of oral contraceptives represents the best choice for hormonal management of heavy anovulatory bleeding and should be offered as long-term management.

Oral Contraceptives and Chronic Irregular Uterine Bleeding

PCOS is a common form of anovulation associated with chronic steady-state levels of unopposed estrogen that may give rise to endometrial hyperplasia and cancer (discussed earlier). Hypothalamic anovulation and hyperprolactinemia are associated with low estrogen levels that are insufficient to prevent bone loss. A combination oral contraceptive is a suitable long-term treatment for both forms of chronic anovulation.

Before the administration of an oral contraceptive, pregnancy should be ruled out. One pill per day is ordinarily administered for 3-week periods interrupted by 1-week hormone-free intervals. Withdrawal bleeding is expected during the hormone-free interval. The progestin component serves to prevent endometrial hyperplasia associated with steady-state unopposed estrogen exposure in PCOS. In cases of anovulation associated with hypoestrogenism (e.g., hypothalamic anovulation, hyperprolactinemia), the estrogen component of the pill provides sufficient replacement to prevent bone loss. The risk of thromboembolism, stroke, or myocardial infarction associated with long-term administration is extremely low in current nonsmokers and in the absence of a history of thromboembolism. Provided that the oral contraceptive controls the abnormal uterine bleeding effectively, a chronically anovulatory woman can continue this regimen until menopause.

Synthetic Progestins

Synthetic progestins enhance endometrial differentiation and antagonize the proliferative effects of estrogen on the endometrium (see Fig. 17.22).[130,323] The effects of progestins or natural progesterone include limitation of estrogen-induced endometrial growth and prevention of endometrial hyperplasia. The absence of naturally synthesized progesterone in anovulatory states is the rationale for administering a progestin.

The most common indication for long-term cyclic progestin administration is to prevent endometrial malignancy in a patient with PCOS and unopposed chronic estrogen exposure of the endometrium. A combination oral contraceptive is the treatment of choice in these cases. If the patient cannot use an oral contraceptive for some reason (e.g., history of thromboembolism), a progestin may be administered in a cyclic fashion to prevent endometrial hyperplasia. Before the administration of a progestin (or oral contraceptive), pregnancy should be ruled out. In the treatment of oligomenorrhea associated with PCOS, orderly, limited withdrawal bleeding can be accomplished by administration of a progestin such as MPA (5 mg/day) for at least 10 days every 2 months. Alternatively, norethindrone acetate at 5 mg/day or megestrol acetate at 20 mg/day may be administered for 10 days every 2 months. Absence of withdrawal bleeding requires further workup.

In the treatment of excessive uterine bleeding (i.e., hypermenorrhea or polymenorrhea), these progestins at higher daily doses (20 mg/day of MPA, 10 mg/day of norethindrone acetate, or 40 mg/day of megestrol acetate) are prescribed for 2 weeks to induce predecidual stromal changes in the endometrium. A heavy progestin withdrawal flow usually follows within 3 days after the last dose is administered. Thereafter, repeated progestin treatment (5 mg/day of MPA, 5 mg/day of norethindrone acetate, or 20 mg/day of megestrol acetate) is offered cyclically for at least the first 10 days of every other month to ensure therapeutic effect. Failure of progestin to correct irregular bleeding requires diagnostic reevaluation such as endometrial biopsy. Predictable withdrawal bleeding within several days after each cycle of progestin administration suggests the absence of endometrial malignancy.

High-Dose Estrogen for Acute Excessive Uterine Bleeding

An oral contraceptive given two or three times daily is the treatment of choice to stop heavy anovulatory bleeding. A high-dose oral contraceptive regimen should be offered to women with heavy uterine bleeding with or without asymptomatic anemia after anatomically demonstrable pathology of the genital tract has been ruled out (see Table 17.7). A patient with acute and severe anovulatory bleeding accompanied by symptomatic anemia represents a medical emergency. These patients should be hospitalized immediately and offered a blood transfusion. After genital tract disease has been ruled out by history, physical examination, and pelvic ultrasonography, intravenously administered high-dose estrogen is the treatment of choice to stop life-threatening bleeding. A well-established regimen is to administer 25 mg of conjugated estrogen intravenously every 4 hours until bleeding markedly slows down or for at least 24 hours.[324] Estrogen most likely acts on the capillaries to induce clotting.[325] Before intravenous estrogen treatment is discontinued, an oral contraceptive pill is started three times daily. Oral contraceptive treatment is then continued as described previously.

Because high-dose estrogen is a risk factor for thromboembolism, taking two or three oral contraceptive pills per day for a week or large doses of intravenous conjugated equine estrogens for 24 hours should be regarded as presenting a significant risk. However, no data are available to evaluate any risk associated with this type of acute use of hormonal therapy for such short intervals. The physician and patient should make a decision regarding high-dose hormone therapy after considering its risks and benefits. Alternative treatment options may be offered to patients with significant risk factors. Exposure to high doses of estrogen should be avoided in women with a past episode or a strong family history of

idiopathic venous thromboembolism. High-dose hormone treatment should also be avoided in women with severe chronic illness such as liver insufficiency or renal insufficiency. One alternative for these patients is dilatation and curettage, followed by treatment with an oral contraceptive (one pill per day) until the uterine bleeding is under control.

Gonadotropin-Releasing Hormone Analogues for Excessive Anovulatory Uterine Bleeding

A GnRH analogue may be given to women with excessive anovulatory bleeding or hypermenorrhea related to severe chronic illness such as liver insufficiency or coagulation disorders. Monthly depot injections of GnRH agonists are not effective for acute excessive uterine bleeding and may increase uterine bleeding for the first 2 weeks. GnRH antagonists downregulate FSH and LH without a delay and achieve amenorrhea more rapidly. The GnRH agonist leuprolide acetate depot (3.75 mg/month intramuscularly) may be administered for 6 months or longer to control uterine bleeding due to chronic illness. GnRH antagonists can probably be used to halt acute or chronic anovulatory bleeding; however, insufficient published data are available to provide dose recommendations. Long-term side effects of GnRH analogues, including osteoporosis, make this an undesirable choice for long-term therapy. If long-term treatment with GnRH analogues is chosen, norethindrone acetate (2.5 mg daily) should be added back. This add-back regimen is usually sufficient to prevent osteoporosis and does not ordinarily worsen the uterine bleeding.

Hormone-Dependent Benign Gynecologic Disorders

Endometriosis

Endometriosis is defined as the presence of endometrium-like tissue in ectopic sites outside the uterine cavity, primarily on pelvic peritoneum and ovaries, and it is associated with chronic pelvic pain, pain during intercourse, and infertility.[326] This estrogen-dependent inflammatory disease affects 5% to 10% of US women of reproductive age.[326] This classic presentation may represent a common phenotype resulting from diverse anatomic or biochemical aberrations of uterine function. As cellular and molecular mechanisms in endometriosis are uncovered, this condition is coming to be viewed as a systemic and chronic complex disease, much like diabetes mellitus or asthma.[327] Endometriosis may be inherited in a polygenic manner, because its incidence is increased by up to sevenfold in relatives of women with endometriosis.[328]

Pathology

There are three clinically distinct forms of endometriosis: endometriotic implants on the surface of pelvic peritoneum and ovaries (i.e., peritoneal endometriosis); ovarian cysts lined by endometrioid mucosa (i.e., endometriomas); and a complex, solid mass composed of endometriotic tissue blended with adipose and fibromuscular tissue and residing between rectum and vagina (i.e., rectovaginal nodule). These three types of lesions may be variant phenotypes of the same pathologic process, or they may be caused by different mechanisms.[329,330] Their common histologic characteristic is the presence of endometrial stromal or epithelial cells, along with chronic bleeding and inflammatory changes. These lesions may occur singly or in combination and are associated with significantly increased risk of infertility and chronic pelvic pain.[329,330] The inflammatory process in endometriosis may stimulate nerve endings in the pelvis to cause pain, impair the function of the uterine tubes, decrease receptivity of the endometrium, and negatively affect development of the oocyte and embryo. Endometriosis may also cause infertility by physically blocking the tubes. An ovarian endometrioma may decrease the quality of the eggs or become sufficiently large to interfere with the ovulation process.

Clinical evidence points to a deleterious effect of uninterrupted ovulatory cycles on the development and persistence of endometriosis.[327] First, symptoms of endometriosis usually appear after menarche and vanish after menopause. Occasionally, a rectovaginal nodule remains symptomatic in a postmenopausal woman, suggesting that its persistence is independent of ovarian estrogen. Second, multiparity is associated with a decreased risk of endometriosis. Third, disruption of ovulation by GnRH analogues, oral contraceptives, or progestins reduces pelvic disease and associated pain. In line with these observations, basic and clinical research findings indicate major roles of the ovarian steroids estrogen and progesterone in the pathologic development of endometriosis. In humans and primate models, estrogen stimulates the growth of endometriotic tissue, whereas aromatase inhibitors that block estrogen formation and antiprogestins are therapeutic.[327] Levels of nuclear receptors for estrogen and progesterone in endometriotic tissue are strikingly different from those in normal endometrium.[327] Fourth, biologically significant quantities of progesterone and estrogen are produced locally by an abnormally active steroidogenic cascade that includes aromatase.[327]

Mechanism of Disease

A number of hypotheses have been proposed regarding the histologic origin of endometriosis. Sampson suggested that fragments of menstrual endometrium pass retrograde through the tubes and then implant and persist on peritoneal surfaces.[331] This mechanism has been demonstrated in primate models and observed naturally in human disease, and it is supported by the observation that spontaneous endometriosis occurs exclusively in species that menstruate. Alternatively, the coelomic-metaplasia hypothesis describes the genesis of endometriotic lesions within the peritoneal cavity by differentiation of mesothelial cells into endometrium-like tissue. A third hypothesis argues that menstrual tissue from the endometrial cavity reaches other body sites through veins or lymphatic vessels.[327] Finally, it has been proposed that circulating blood cells originating from bone marrow differentiate into endometriotic tissue at various body sites.[332] Sampson's implantation hypothesis offers a plausible mechanism for most endometriotic lesions but does not explain why only some women develop endometriosis. Although most women of reproductive age have reflux menstruation into the peritoneal cavity, endometriosis is encountered in only 5% to 10% of this population.

Two possible mechanisms may explain the successful implantation of refluxed endometrium on the peritoneal surface or in a hemorrhagic corpus luteum cyst of the ovary. First, the eutopic endometrium of women with endometriosis exhibits multiple subtle but significant molecular abnormalities, including activation of oncogenic pathways or biosynthetic cascades favoring increased production of estrogen, cytokines, prostaglandins, and metalloproteinases.[327] When this biologically distinct tissue attaches to mesothelial cells, the magnitude of these abnormalities is amplified dramatically to enhance implant survival.[327] A second mechanism suggests that a defective immune system fails to clear implants off the peritoneal surface.[327] It is possible that both mechanisms may contribute to the same phenotype.

Clear molecular distinctions, such as overproduction of estrogen, prostaglandins, and cytokines, are observed between endometriotic tissue and endometrium (Fig. 17.34).[327] Subtler forms of these abnormalities are also observed in endometrium from a patient with endometriosis compared with endometrium from a disease-free woman. Inflammation is a hallmark of endometriotic tissue that overproduces prostaglandins, metalloproteinases, cytokines, and chemokines.[327] Increased levels of acute inflammatory cytokines such as interleukin 1β (IL1β), IL6, and tumor necrosis factor (TNF) likely enhance adhesion of shed endometrial tissue fragments on peritoneal surfaces, and proteolytic membrane metalloproteinases may further promote their implantation.[327] Monocyte chemoattractant protein 1, IL8, and RANTES (i.e., *r*egulated on *a*ctivation, *n*ormal *T* cell *e*xpressed and *s*ecreted) attract the granulocytes, natural killer cells, and macrophages typically observed in endometriosis.[333] Autoregulatory positive feedback loops ensure further accumulation of these immune cells, cytokines, and chemokines in established lesions.

Basic biologic functions such as inflammation, immune response, angiogenesis, and apoptosis are altered in favor of survival and replenishment of endometriotic tissue.[327] These functions depend in part on estrogen or progesterone action. Excessive formation of estrogen and prostaglandin and development of progesterone resistance have emerged as clinically useful concepts, because targeting of aromatase in the estrogen biosynthetic pathway, cyclooxygenase 2 (COX2) in the prostaglandin pathway, or the PR significantly reduces laparoscopically visible endometriosis and pelvic pain (see Fig. 17.34).[327] These three critical mechanisms have been linked by specific epigenetic (hypomethylation) defects that cause overexpression of the nuclear receptors SF1 and ERβ.[327] The genome-wide unique epigenetic fingerprint in endometriosis suggests DNA methylation is an integral component of the disease and identifies a novel role for the GATA family as key regulators of uterine physiology; aberrant DNA methylation in endometriotic cells correlates with a shift in GATA isoform expression that facilitates progesterone resistance and disease progression.[334]

Diagnosis

A history of severely painful menses during the teenage years, which eventually progressed into chronic pelvic pain experienced both during and between periods, is suggestive of endometriosis. Reliable diagnosis of peritoneal endometriosis can be made only by direct visualization of these lesions by laparoscopy or laparotomy. Ovarian endometriotic cysts filled with a thick, bloody fluid (i.e., endometriomas) can be diagnosed accurately by vaginal ultrasonography.

Treatment

Treatment of infertility caused by endometriosis consists of surgical removal with or without assisted reproductive technology, whereas pain is usually treated with a combination of medical suppression of ovulation and surgery. Peritoneal implants are resected or vaporized by electric current or laser. Ovarian endometriomas and rectovaginal endometriotic nodules may be effectively removed only by full dissection. Epidemiologic and laboratory data suggest a link between ovarian endometriosis and distinct types of ovarian cancers.[327]

Although current hormonal therapy for infertility associated with endometriosis is not of proven value, it is somewhat successful for pelvic pain associated with endometriosis. Various agents used are comparable in terms of efficacy. Most of the current medical treatments were designed to suppress ovulation (e.g., GnRH agonists and antagonists, oral contraceptives, danazol, progestins). A possible alternative mechanism of action of the androgenic steroid danazol or a progestin is a direct growth-suppressive effect on endometriotic tissue.

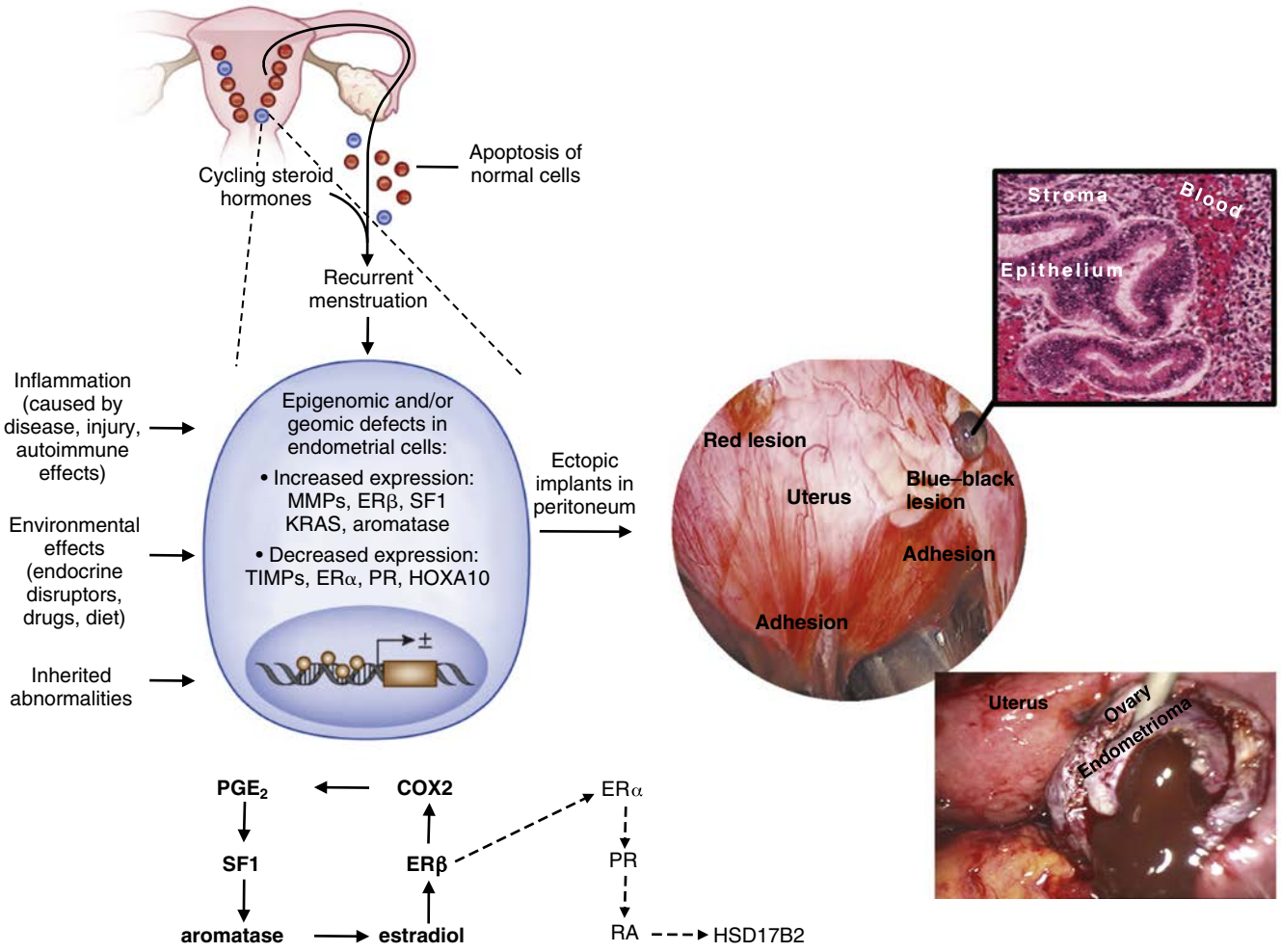
Many patients and physicians do not favor danazol because of its anabolic and androgenic side effects of weight gain and muscle cramps and occasional irreversible virilization (e.g., clitoromegaly, voice changes).[335] Up to 50% of patients with endometriosis fail to complete 6 months of treatment with danazol.[336] The rest of the hormonal agents—oral contraceptives, progestins, and GnRH agonists—show comparable efficacy for control of endometriosis-associated pain.[337–339] A 6-month course using any one of these agents results in a significant reduction of pain in more than 50% of patients.[337–339] Induction of pain relief with a continuously administered oral contraceptive or progestin takes longer than with a GnRH agonist. Recently, an oral GnRH antagonist, elagolix, has been introduced to treat endometriosis-associated pain.[2] Both higher and lower doses of elagolix decreased dysmenorrhea and nonmenstrual pelvic pain and were approved in 2018 for 6-month and 24-month periods, respectively, in women with endometriosis-associated pain. Greater duration is not recommended due to a dose-dependent decrease in bone mineral density. Both doses of elagolix were also associated with other hypoestrogenic adverse effects.[2]

There is a high incidence of recurrence or persistence of the disease and pain after all of these medical therapies.[340] Thus successful medical management of pain requires long-term ovarian suppression for years. Because it is practical to maintain these patients on a combination oral contraceptive for years with a relatively benign side effect profile, birth control pills as a class has been the most suitable long-term management option.[34]

We are still far from the cure of endometriosis, and current treatments are not satisfactory for effective control of pain. The radical treatment is removal of both ovaries, and even this was not found to be effective in a number of cases of postmenopausal endometriosis.[341] New alternative strategies are needed to offer women with endometriosis a reasonable chance to live without suffering from chronic pelvic pain for decades.

There are two important caveats about ovulation suppression–based treatments. First, large quantities of estrogen can be produced locally within the endometriotic cells. This represents an intracrine mechanism of estrogen action, in contrast to ovarian secretion, which is an endocrine means of supplying this steroid to target tissues (see Fig. 17.19).[97,338] Second, estradiol produced in peripheral tissue sites (e.g., adipose tissue, skin fibroblasts) may give rise to pathologically significant circulating levels of estradiol in a subset of women.[97] GnRH agonists do not inhibit peripheral estrogen formation or local estrogen production within the estrogen-responsive lesion. Moreover, endometriosis is resistant to selective effects of progesterone and progestins.[327]

Aromatase inhibitors and selective progesterone response modulators are candidate therapeutic agents for endometriosis refractory to existing treatment options. Aromatase expression and local estrogen biosynthesis in endometriotic implants prompted pilot studies to target aromatase in endometriosis using its third-generation inhibitors. Among these inhibitors, anastrozole and letrozole were used successfully to treat endometriosis in postmenopausal and premenopausal women.[336,342–345] An aromatase inhibitor is the medical treatment of choice for persistent postmenopausal endometriosis. Use of aromatase inhibitors in premenopausal women with endometriosis requires concomitant



• **Fig. 17.34** Molecular mechanisms in endometriosis. Endometriosis is defined as the presence of endometrium-like tissue on the pelvic peritoneum (red and blue-black lesions) or in the ovary (blood-filled cyst, i.e., endometrioma). These lesions are thought to originate from abnormal endometrial tissue stem cells *(blue),* which have migrated retrograde during menstruation. Normal endometrial cells *(red)* without such survival capabilities are thought to go through apoptosis in the peritoneal or ovarian environments. It is possible that women with endometriosis have higher numbers of the abnormal cells in their eutopic endometrial tissues. Thus recurrent menstruation seems to be a significant risk factor for developing endometriosis. These abnormal cells *(blue)* contain genome-wide epigenetic abnormalities, such as DNA methylation, that affect the expression of a wide variety of genes. These epigenetic abnormalities may be inherited or caused by environmental influences such as inflammation and endocrine disruptors. Two nuclear receptors, steroidogenic factor 1 (SF1) and estrogen receptor-β (ERβ), play significant roles in the pathology of endometriosis. In normal endometrial stromal cells, cytosine-phosphate-guanine islands located at the SF1 and ERβ promoters are robustly methylated and silenced. A lack of promoter methylation is associated with promoter activation and the presence of extraordinarily large quantities of these nuclear receptors in endometriotic stromal cells. Prostaglandin E_2 (PGE_2) induces multiple steroidogenic genes, including aromatase, and formation of estradiol from cholesterol. SF1 mediates this steroidogenic action of PGE_2. ERβ suppresses ERα and progesterone receptors (PR). This results in defective retinoic acid *(RA)* production and action, leading to 17β-hydroxysteroid dehydrogenase 2 (HSD17B2) deficiency and failure to metabolize estradiol. ERβ also induces cyclooxygenase 2 (COX2) and formation of PGE_2. Estradiol and PGE_2 are produced in large quantities and enhance cell survival and inflammation in endometriotic tissue. *HOXA10,* homeobox A10; *KRAS,* V-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue; *MMPs,* matrix metalloproteinases; *TIMPs,* tissue inhibitors of metalloproteinases.

ovarian suppression by the addition of a GnRH analogue, progestin, or combination oral contraceptive.

For the medical management of pain in premenopausal women with endometriosis, this author favors the following simple algorithm. Unless contraindicated, the continuous use of a combination oral contraceptive is the initial treatment of choice. The patient is reassured that the majority of women will have minimal or no breakthrough bleeding after 6 months of continuous oral contraceptive treatment. If pain relief is adequate, the patient can remain on this regimen for years. If adequate pain relief is not achieved after 6 months of use, a daily oral aromatase inhibitor (anastrozole 1 mg/day or letrozole 2.5 mg/day) is added to the

continuous oral contraceptive regimen. This combination may be maintained for at least 1 year. If pain relief is still not satisfactory, conservative laparoscopic surgery is considered.

Uterine Leiomyomas

Uterine fibroids (leiomyomas) represent the most common tumor in women.[346] These lesions disrupt the functions of the uterus and cause excessive uterine bleeding, anemia, defective implantation of an embryo, recurrent pregnancy loss, preterm labor, obstruction of labor, pelvic discomfort, and urinary incontinence and may mimic or mask malignant tumors. By the time they reach 50 years of age, nearly 70% of white women and more than 80% of black women will have had at least one fibroid; severe symptoms develop in 15% to 30% of these women. Uterine fibroids in black women are significantly larger at diagnosis than those in white women, are diagnosed at an earlier age, and are characterized by more severe symptoms and a longer period of sustained growth. Approximately 200,000 hysterectomies, 30,000 myomectomies, and thousands of selective uterine-artery embolizations and high-intensity focused ultrasound procedures are performed annually in the United States to remove or destroy uterine fibroids.[346]

Each fibroid seems to originate from the transformation and monoclonal expansion of a single somatic stem cell of the myometrium under the influence of ovarian hormones. Human uterine fibroid tissue contains fewer stem cells than normal myometrium. However, stem cells derived from fibroid tissue—not the myometrium—carry *MED12* mutations, which suggests that at least one genetic hit initially transforms a myometrial stem cell, which subsequently interacts with the surrounding myometrial tissue to give rise to a fibroid tumor.[346] Each fibroid tumor seems to contain a unique driver mutation. *MED12* mutations are found in approximately 70% of the fibroids.[346] Somatic mutations that affect the *HMGA2* gene represent the second most common genetic disruption in a fibroid tumor.[346]

Estrogen stimulates the growth of uterine fibroids through its receptor ERα. The primary roles of estrogen and ERα in fibroid growth are permissive in that they enable tissue to respond to progesterone by inducing the expression of PR, which is essential and sufficient for tumor growth, as indicated by the stimulation of cell proliferation, the accumulation of extracellular matrix, and cellular hypertrophy.[346] Since the stem cell population expresses much lower levels of PR than the population of mature cells but serves as the key source of tissue growth, a paracrine signal originating from PR-rich differentiated cells may mediate the proliferative effects of progesterone on fibroid stem cells.[346]

Diagnosis can be made by abdominal or transvaginal ultrasonography. Transvaginal ultrasonography is a sensitive method for determining the size, number, and location of uterine leiomyomas.

The therapeutic choices depend on the goals of therapy, with hysterectomy most often used for definitive treatment and myomectomy used when preservation of childbearing capability is desired. Intracavitary and submucous leiomyomas can be removed by hysteroscopic resection. Laparoscopic myomectomy is technically possible but involves an increased risk of uterine rupture during pregnancy. The overall recurrence rate after myomectomy varies widely, from 10% to 50%. Other FDA-approved treatment options include selective uterine artery embolization and extracorporeal ablation of uterine fibroids with MRI-guided, high-intensity, focused ultrasound.[347]

Although GnRH agonist–induced hypogonadism can reduce the overall volume of the uterus containing leiomyomas and tumor vascularity, the severe side effects and prompt recurrences make GnRH agonists useful only for short-term goals such as reducing anemia related to uterine bleeding or decreasing tumor vascularity before hysteroscopic resection. Trials have consistently demonstrated that treatment with an antiprogestin such as mifepristone or ulipristal acetate reduces fibroid size.[346] This observation underscores the role of progesterone in the cause of uterine fibroids and opens a new area of therapeutic investigation.[346]

Management of Menopause

Consequences of Menopause

Perimenopause Stage

Menopause is the cessation of ovulatory menses as a result of the irreversible loss of a number of ovarian functions, including ovulation and estrogen production. Perimenopause is a critical period of life during which striking hormonal, somatic, and psychologic alterations occur in the transition to menopause. Perimenopause encompasses the change from ovulatory cycles to cessation of menses and is marked by irregularity of menstrual bleeding.

The most sensitive clinical indication of perimenopause is the progressively increasing occurrence of menstrual irregularities. The menstrual cycle for most ovulatory women lasts 24 to 35 days, and approximately 20% of all reproductive-age women experience irregular cycles.[306] When women are in their 40s, anovulation becomes more prevalent and the menstrual cycle length increases, beginning several years before menopause.[309] The median age at the onset of perimenopause is 47.5 years.[348] Regardless of the age at onset, menopause (i.e., cessation of menses) is consistently preceded by a period of prolonged cycle intervals.[349] Elevated circulating levels of FSH mark this menstrual cycle change before menopause and are accompanied by decreased inhibin levels, normal levels of LH, and slightly elevated levels of estradiol.[350] These changes in serum hormone levels reflect a decreasing ovarian follicular reserve and can be detected most reliably on day 2 or 3 of the menstrual cycle.

Serum estradiol levels do not begin to decline until less than a year before menopause. The average circulating estradiol levels in perimenopausal women are estimated to be somewhat higher than those in younger women because of an increased follicular response to elevated FSH levels.[351] The decline in inhibin production by the follicle, which allows a rise in FSH levels, in the later reproductive years reflects diminishing follicular reserve and competence. Ovarian follicular output of inhibin begins to decrease after 30 years of age, and this decline becomes much more pronounced after age 40. These hormonal changes parallel a sharp decline in fecundity, which starts at age 35.

Perimenopause is a transitional period during which postmenopausal levels of FSH can be observed despite continued menses; LH levels remain in the normal range. Pregnancy is still possible in the perimenopausal woman, because occasional ovulation and functional corpus luteum formation can occur. Until complete cessation of menses is observed or FSH levels higher than 40 IU/L are measured on two separate occasions, some form of contraception should be recommended to prevent unwanted pregnancy.

Perimenopause represents an optimal period in which to evaluate the general health of the mature woman and introduce measures to prepare her for the striking physiologic changes that come with menopause. The patient and her clinician should attempt to achieve several important aims during perimenopause. The long-term goal is to maintain an optimal quality of physical and social

life. Another immediate objective is the detection of any major chronic disorders that occur with aging. The benefits and risks of hormone therapy should be discussed thoroughly at this time.

Menopause Features

The median age at menopause is approximately 51 years.[352] The age at menopause is probably determined in part by genetic factors, because mothers and daughters tend to experience menopause at about the same age.[353–355] Environmental factors may modify the age at menopause. For example, current smoking is associated with an earlier menopause, whereas alcohol consumption delays menopause.[352] Oral contraceptive use does not affect the age at which menopause begins.

The symptoms frequently seen and related to decreased estrogen production in menopause include irregular frequency of menses followed by amenorrhea, vasomotor instability manifested as hot flashes and sweats, urogenital atrophy giving rise to pain during intercourse and a variety of urinary symptoms, and consequences of osteoporosis and CVD. The combination and extent of these symptoms vary widely for each patient. Some patients experience multiple severe symptoms that may be disabling, whereas others have no symptoms or only mild discomfort associated with perimenopause.

Biosynthesis of Estrogen and Other Steroids in the Postmenopausal Woman

No follicular units can be detected histologically in the ovaries after menopause. In reproductive-age women, the granulosa cell of the ovulatory follicle is the major source of inhibin and estradiol. In the absence of these factors that inhibit gonadotropin secretion, FSH and LH levels increase sharply after menopause. These levels peak a few years after menopause and decrease gradually and slightly thereafter.[356] The postmenopausal serum level of either gonadotropin may be more than 100 IU/L. FSH levels are usually higher than LH levels because LH is cleared from the blood much more quickly and possibly because the low levels of inhibin in menopause selectively lead to increased FSH secretion. Nevertheless, increased LH is a major factor that maintains significant quantities of androstenedione and testosterone secretion from the ovary, although the total production rates of both steroids decline after menopause.

The primary steroid products of the postmenopausal ovary are androstenedione and testosterone.[97] The average premenopausal rate of production of androstenedione of 3 mg/day is decreased by one-half to approximately 1.5 mg/day.[97] The decrease primarily results from a substantial reduction in the ovarian contribution to the circulating androstenedione pool. Adrenal secretion accounts for most of the androstenedione production in the postmenopausal woman, with a smaller amount secreted from the ovary.[97] DHEA and DHEAS originate almost exclusively from the adrenal gland and decline steadily with advancing age independent of menopause. The serum levels of DHEA and DHEAS after menopause are about one-fourth of those in young adult women.[357]

Testosterone production is decreased by approximately one-third after menopause.[97] Total testosterone production can be approximated by the sum of ovarian secretion and peripheral formation from androstenedione (see Fig. 17.26). In the premenopausal woman, significant amounts of testosterone are produced by conversion of androstenedione in extraovarian tissues. Because ovarian androstenedione secretion is substantially decreased after menopause, the decrease in postmenopausal testosterone production is accounted for in large measure by a decrease in the relative contribution of extraovarian sources.[97] With the disappearance of follicles and decreased estrogen, the elevated gonadotropins drive the remaining stromal tissue in the ovary to maintain testosterone secretion at levels observed during the premenopausal years. The contribution of the postmenopausal ovary to the total testosterone production is increased in the presence of seemingly unaltered ovarian secretion.

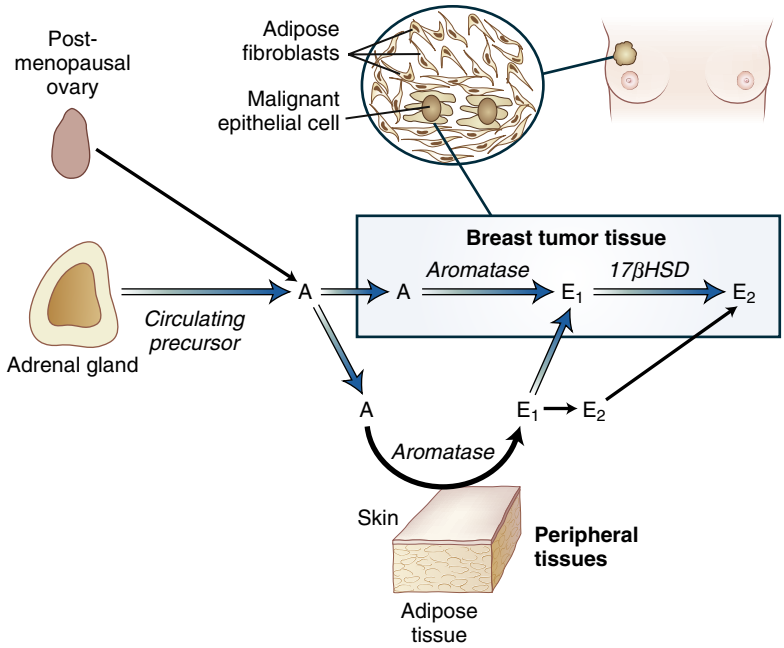
The most dramatic endocrine alteration of perimenopause involves the decline in the circulating level and production rate of estradiol. The average menopausal level of circulating estradiol is less than 20 pg/mL. The estradiol and estrone levels in postmenopausal women are usually slightly less than those in adult men. Circulating estradiol in postmenopausal women (and men) is derived from the peripheral conversion of androstenedione to estrone, which is converted peripherally to estradiol (see Fig. 17.19).[97] The mean circulating level of estrone in postmenopausal women (37 pg/mL) is higher than that of estradiol. The average postmenopausal production rate of estrone is approximately 42 μg every 24 hours. After menopause, almost all estrone and estradiol is derived from the peripheral aromatization of androstenedione. There is a dramatic change in the androgen-to-estrogen ratio because of the sharp decrease in estradiol level and the only slightly reduced testosterone. The frequent onset of mild hirsutism after menopause reflects this striking shift in the hormone ratio. During the postmenopausal years, DHEAS and DHEA levels continue to decline steadily with advancing age, whereas serum androstenedione, testosterone, estrone, and estradiol levels do not change significantly.[356]

The aromatization of androstenedione to estrone in extraovarian tissues correlates positively with weight and advancing age (see Figs. 17.19 and 17.31).[97] Body weight correlates positively with the circulating levels of estrone and estradiol. Because aromatase enzyme activity is present in significant quantities in adipose tissue, increased aromatization of androstenedione in overweight individuals may reflect the increased bulk of tissue containing the enzyme.[97] There is a twofold to fourfold increase in the specific activity of aromatase per cell with advancing age.[97] An increased overall number of adipose fibroblasts with aromatase activity and a decrease in the levels of SHBG increase the free estradiol level and contribute to the increased risk of endometrial cancer in obese women.[97]

In postmenopausal women, estrogen produced from androstenedione peripherally in fat and skin and locally in breast cancer tissue promotes the growth of this malignancy (Fig. 17.35).[97] The clinical relevance of extraovarian estrogen formation is exemplified by the successful use of aromatase inhibitors as the current endocrine treatment for postmenopausal breast cancer.[97]

Postmenopausal Uterine Bleeding

Perimenopausal or postmenopausal bleeding can be caused by hormone administration or excessive extraovarian estrogen formation. Irregular uterine bleeding is commonly observed during the perimenopausal transition as anovulatory cycles alternate with ovulatory cycles. Uterine bleeding after menopause is less common if the patient is not receiving hormone therapy. Obese women are more likely to experience postmenopausal bleeding because of increased peripheral aromatization of adrenal androstenedione. Patients receiving a continuous combination regimen of hormone therapy may experience unpredictable uterine bleeding. The major objective in these circumstances is to rule out endometrial malignancy. This can be best achieved by tissue diagnosis through an office endometrial biopsy using a plastic cannula.



• **Fig. 17.35** Tissue sources of estrogen in postmenopausal breast cancer. The important pathologic roles of extraovarian (peripheral) and local estrogen biosynthesis are shown for an estrogen-dependent disease in postmenopausal women. The estrogen precursor androstenedione (A) originates primarily from the adrenal gland in the postmenopausal woman. Aromatase expression and enzyme activity in extraovarian tissues such as fat increase with advancing age. The aromatase activity in skin and subcutaneous adipose fibroblasts gives rise to formation of systemically available estrone (E_1) and, to a smaller extent, estradiol (E_2). The conversion of circulating A to E_1 in undifferentiated breast adipose fibroblasts compacted around malignant epithelial cells and subsequent conversion of E_1 to E_2 in malignant epithelial cells provide high tissue concentrations of E_2 for tumor growth. The clinical relevance of these findings is exemplified by the successful use of aromatase inhibitors to treat breast cancer in postmenopausal women. *17βHSD,* reductive-type 17β-hydroxysteroid dehydrogenase.

Transvaginal ultrasonographic measurement of endometrial thickness may be used in postmenopausal women to avoid unnecessary biopsies.[135] A biopsy is required if an endometrial thickness of 5 mm or greater is observed.

Unpredictable irregular uterine bleeding is observed in approximately 20% of postmenopausal women receiving a long-term (>1 year) continuous estrogen-progestin combination. Before using ultrasonography and endometrial biopsy to explore the cause of bleeding that is assumed to arise from the intrauterine cavity, the clinician should rule out diseases of the vulva, vagina, and cervix.[135] Careful inspection of these organs along with a normal cervical Papanicolaou (Pap) smear within the past year is sufficient to rule out the vulva, vagina, and cervix as potential sources of bleeding. The causes of postmenopausal uterine bleeding are benign most of the time. Endometrial malignancy is encountered in patients with bleeding in only about 1% to 2% of postmenopausal endometrial biopsies.[358] Approximately three-fourths of endometrial biopsies from postmenopausal women reveal no pathologic change or an atrophic endometrium. Other histologic findings include hyperplasia (15%) and endometrial polyps (3%). Persistent unexplained uterine bleeding requires repeated evaluation, biopsy, hysteroscopy, or dilatation and curettage.

Hot Flashes

The most frequent and striking symptom during perimenopause is the hot flash. It typically occurs during the transition from perimenopause to postmenopause. The hot flash is also a major symptom of postmenopause, and it can occur up to 5 years after menopause.[359] More than four-fifths of postmenopausal women experience hot flashes within 3 months after the cessation of ovarian function, whether natural or surgical in origin. Of these women, more than three-fourths have hot flashes for more than 1 year, and approximately one-half have them for up to 5 years.[359] Hot flashes lessen in frequency and intensity with advancing age, unlike other sequelae of menopause, which progress with time.

A hot flash is a subjective sensation of intense warmth of the upper body, which typically lasts for approximately 4 minutes but may range in duration from 30 seconds to 5 minutes. It can follow a prodrome of palpitations or headache and is frequently accompanied by weakness, faintness, or vertigo. The episode usually ends in profuse sweating and a cold sensation. Hot flashes may occur rarely or recur every few minutes. At night, hot flashes are more frequent and can be severe enough to awaken a woman from sleep. They are also more intense during times of stress. In a cool environment, hot flashes are fewer, less intense, and shorter in duration than in a warm environment.[360]

The hot flash results from a sudden reduction of estrogen levels rather than from hypoestrogenism itself. Regardless of the cause of menopause—natural, surgical, or estrogen withdrawal caused by a GnRH agonist—hot flashes are associated with an acute and significant drop in estrogen level. The consistent association between the onset of flashes and acute estrogen withdrawal is supported by the effectiveness of estrogen therapy and the absence of flashes in prolonged hypoestrogenic states, such as gonadal dysgenesis or hypothalamic amenorrhea. Hypogonadal women experience

hot flashes only after estrogen is administered and withdrawn.[361] Higher body mass index, and body fat in particular, is associated with greater vasomotor symptom reporting, primarily hot flashes.[362]

Other conditions cause symptoms similar to the menopausal hot flash. Sudden episodes of sweating and/or flushing may be caused by catecholamine-secreting or histamine-secreting tumors (e.g., pheochromocytoma, carcinoid), hyperthyroidism, or chronic infection (e.g., tuberculosis). The hot flash may also be psychosomatic in origin. In these circumstances, the clinician should obtain a serum FSH level to confirm perimenopause or menopause before initiating hormone therapy.

Urogenital Atrophy

The urogenital sinus gives rise to development of the lower vagina, vulva, and urethra during embryonic development, and these tissues are estrogen responsive. The decrease in estrogen at menopause causes the vaginal walls to become pale because of diminished vascularity and to thin down to only three or four squamous epithelial cell layers. Loss of this protective mechanism leaves the thin, friable tissue vulnerable to infection and ulceration. The vagina also loses its rugae and becomes shorter and inelastic. Postmenopausal women may complain of symptoms caused by vaginal dryness, such as pain during intercourse, vaginal discharge, burning, itching, or bleeding. Genitourinary atrophy leads to a variety of symptoms that affect the ease and quality of living. Intravaginal estrogen treatment can effectively alleviate these vaginal symptoms in the postmenopausal patient.[363]

Postmenopausal Osteoporosis

Osteoporosis is a disorder characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk. The World Health Organization criteria for defining and diagnosing osteoporosis in postmenopausal women is the finding of a T-score of –2.5 or lower at the lumbar spine, femur neck, or total hip by bone mineral density (BMD) testing.[364] Osteoporosis is a public health concern that is associated with more than 2 million fractures per year in the United States.[364] The most frequent sites of fracture are the vertebral bodies, distal radius, and femoral neck. Most osteoporotic patients are postmenopausal women.

Osteoporosis in postmenopausal women is a function of advancing age and estrogen deficiency. Seventy-five percent or more of the bone loss in women during the first 15 years after menopause is attributed to estrogen deficiency rather than to aging.[365,366] For the first 20 years after the cessation of ovarian estrogen secretion, postmenopausal osteoporosis accounts for a 50% reduction in trabecular bone and 30% loss of cortical bone.[365,366] Vertebral bone is especially vulnerable because the trabecular portion of the vertebral bodies is metabolically very active and decreases dramatically in amount in response to estrogen deficiency. Vertebral bone mass is already significantly decreased in perimenopausal and early postmenopausal women who have rising FSH and decreasing estrogen levels, whereas bone loss from the radius is not detected until at least 1 year after menopause.[366]

The risk of fracture depends on two factors: the peak bone mass achieved at maturity (at approximately age 30) and the subsequent rate of bone loss. An accelerated rate of bone loss after menopause strongly predicts an increased risk of fracture. The unfavorable effects of low premenopausal bone mass and accelerated loss of bone after menopause are additive, and these individuals are at the highest risk for fracture. An increased rate of average bone loss during menopause is an indicator of lower endogenous estrogen levels, possibly because postmenopausal bone loss is considerably slower in women with increased adipose tissue mass and consequent increased peripheral estrogen formation.[230]

Numerous studies showed that hormone therapy started at perimenopause prevents postmenopausal bone loss.[367] Hormone therapy started at any age in a postmenopausal woman prevents additional bone loss. The Women's Health Initiative (WHI) trial results revealed the most conclusive evidence for a decreased number of vertebral and hip fractures in the group of postmenopausal women receiving estrogen plus progestin (E+P) or estrogen (E) only.[368] The risk reductions were attenuated in both trials after the intervention was stopped; however, a significant hip fracture benefit persisted over 13 years for women assigned to conjugated equine estrogens (CEEs) plus MPA.[368]

Postmenopausal Hormone Therapy

A key and complex decision facing postmenopausal women is whether to use menopausal hormone therapy (HT). HT is the most effective treatment for vasomotor symptoms and may also improve vaginal dryness, sleep, and quality of life.[369] Postmenopausal women who have undergone a hysterectomy ordinarily receive HT with estrogen only (HT-E). A progestin is added to estrogen (HT-EP) in the postmenopausal woman with a uterus to prevent endometrial hyperplasia and cancer. Although either form of HT was originally prescribed primarily to treat vasomotor symptoms, starting from the 1950s, HT-E and later HT-EP had been increasingly viewed as a way to forestall many chronic diseases of aging, including CVD, cognitive impairment, and osteoporotic fractures. Approximately 40% of postmenopausal women in the United States were using HT shortly before the publication of the initial findings from the WHI.[368,370] A number of observational studies had suggested benefits of HT for CVD, including coronary heart disease (CHD) and all-cause mortality, and an overall favorable benefit-risk profile.[371] There had been, however, no large-scale randomized prevention trials to address the balance of risks and benefits of HT. In these observational studies, the apparent benefits of HT may result in part from differences between women who choose to take postmenopausal HT and women who do not; HT users tend to be healthier and have better access to medical care.[372]

Until the late 1990s, the most common practice was to treat all women disturbed by the symptoms of hormone deprivation (e.g., hot flashes) with HT and to use long-term hormonal prophylaxis against osteoporosis. The assumption that HT was cardioprotective played an important role in encouraging postmenopausal women to stay on this regimen indefinitely.[373] This trend changed dramatically in the early 2000s, after publication of the principal results of two large randomized trials, the Heart and Estrogen/Progestin Replacement Study (HERS) in 1998 and the WHI trial in 2002.[374–376] The WHI results constituted the direct cause of discontinuation of HT in approximately 30% of postmenopausal women.[376] Debate continues regarding the applicability of the WHI results to all or various subsets of postmenopausal women.[373] Although the use of HT has dramatically decreased, the WHI trials raised a number of important issues that require further studies to address.[373,377]

The WHI investigators sought to determine the benefits and risks of HT taken for chronic disease prevention in predominantly

healthy postmenopausal women aged 50 to 79 years at enrollment.[368,370] The WHI trials elected to include the most popular HT formulations in the United States at that time, which were CEEs (0.625 mg/day) plus MPA (2.5 mg/day) and CEEs (0.625 mg/day) alone.[368,370] Starting from 2002, a large number of original and review articles came from the WHI trials. A comprehensive integrated overview of findings from the two WHI HT trials with extended postintervention follow-up for a median of 13 years was published.[368] Here the WHI investigators reported the key outcomes and stratified results by age and by time since menopause onset.[368] A discussion of the WHI and other large trials and how they changed HT practices is provided next.

The Long-Term Benefits and Side Effects of Hormone Therapy

Unless there are contraindications, HT should be offered to every menopausal woman suffering from vasomotor symptoms, vulvovaginal atrophy, or dyspareunia. For these indications, HT may be maintained for up to 5 years based on the preference of the woman appropriately informed of the risks and benefits.

Menopause is also associated with increased risks for chronic disorders, including heart disease, osteoporosis, cognitive impairment, or certain malignancies. Previously, hormone therapy was used for the primary prevention of these chronic conditions, but results from the WHI and other randomized clinical trials provided a clearer picture regarding the benefits and risks of HT and have provided insights to improve decision making in HT practices.[368,370,373–376] A number of clinical characteristics are useful in identifying postmenopausal women for whom benefits of HT are likely to outweigh the risks.[368] Age and time since menopause were considered as predictors of risks of HT.[368] The role of age was particularly prominent in the HT-E (estrogen only, for women without uteri) trial.[368] Younger women (50–59 years) taking HT-E had more favorable results for all-cause mortality, myocardial infarction, and the global index but not for stroke and venous thrombosis.[368] The influence of age was less clear for HT-EP (women with intact uteri, taking estrogen/progestin), owing to increased risks of breast cancer, stroke, and venous thrombosis in all age groups. Overall, risks of adverse events were lower in younger than in older women among both HT-E and HT-EP users.[368]

A recent benefit versus harm analysis of 18 recent trials, including the two WHI trials (HT-EP and HT-E), is summarized in Table 17.9, showing intervention-phase findings such as absolute risks per 10,000 women per year, rate differences, and relative risks for a wide range of chronic disease outcomes.[378] HT-E users compared with placebo had significantly lower risks, per 10,000 person-years, for diabetes (–19 cases [95% CI, –34 to –3]) and fractures (–53 cases [95% CI, –69 to –39]). On the other hand, increased risks for HT-E users (per 10,000 person-years) included gallbladder disease (30 more cases [95% CI, 16–48]), stroke (11 more cases [95% CI, 2–23]), venous thromboembolism (11 more cases [95% CI, 3–22]), and urinary incontinence (1261 more cases [95% CI, 880–1689]) (see Table 17.9).[378]

HT-EP users compared with placebo had lower risks, per 10,000 person-years, for colorectal cancer (–6 cases [95% CI, –9 to –1]), diabetes (–14 cases [95% CI, –24 to –3), and fractures (–44 cases [95% CI, –71 to –13).[378] Increased risks for HT-EP users, per 10,000 person-years, included invasive breast cancer (9 more cases [95% CI, 1–19]), probable dementia (22 more cases [95% CI, 4–53]), gallbladder disease (21 more cases [95% CI, 10–34]), stroke (9 more cases [95% CI, 2–19]), urinary incontinence (876 more cases [95% CI, 606–1168]), and venous thromboembolism (21 more cases [95% CI, 12–33]).[378] The authors of this recent meta-analysis and review, including 18 trials (including the WHI trials), stated "the available evidence regarding benefits and harms of early initiation of hormone therapy is inconclusive."[378]

An analysis of WHI results indicated that neither HT modality altered the mortality rate.[368,370] Interestingly, breast cancer findings were divergent between the two trials, and for both cancer and CVD outcomes, results tended to be more adverse for HT-EP than for HT-E.[368,370] There was a clear and sizable benefit for reducing vasomotor symptoms; the results for other quality of life measures, however, varied widely.[368,370]

The age of the woman and time since menopause seemed to be the key modifiers of most of the WHI results.[368,370] In the HT-E trial, younger women 50 to 59 years of age had more favorable results for all-cause mortality, myocardial infarction, colorectal cancer, and the global index.[368] Age, however, did not affect increased risks of stroke, venous thrombosis, gallbladder disease, or urinary incontinence associated with both HT regimens. Among HT-EP users, breast cancer was an additional adverse effect. Although risk of myocardial infarction varied by time since menopause, the overall risks of chronic disease events outweighed benefits across all age groups for HT-EP.[368] In general, variations in hazard ratios by age were more apparent among the HT-E users than in the HT-EP users.[368,370] However, risks of unfavorable outcomes were much lower in younger women than in older women in both HT-E and HT-EP trials. Absolute risks measured by the global index per 10,000 women per year on HT-EP varied from 12 excess cases for ages 50 to 59 years to 38 excess cases for ages 70 to 79 years and, for HT-E, from 19 fewer cases for ages 50 to 59 years to 51 excess cases for the age group 70 to 79 years.[370] Overall, findings from both WHI trials suggest that HT has a harmful effect on CHD risk in older women and those at higher baseline risk of CVD, whereas the results in younger and low-risk women tend to be neutral for HT-EP and in a favorable direction for HT-E.[370] HT use had a negative impact on cognitive function among women older than 65 years, whereas HT effect was neutral for women younger than 55 years of age.[370,379]

Risks and Contraindications of Hormone Therapy

Coronary Heart Disease

Data suggest that initiation of HT many years after menopause is associated with excess CHD risk, whereas HT given for a limited period soon after menopause is not. HT-EP or HT-E does not prevent CHD as was previously proposed. To the contrary, there may be a small but significant increase in the rate of CHD among HT-EP users; these women with preexisting CHD and healthy women are at risk.[368,380] HT-E, on the other hand, does not increase the risk for CHD in healthy women.[368]

Stroke

Another outcome that was consistent across the two WHI trials and HERS is the increased risk of stroke among women assigned to HT-E or HT-EP. Increased stroke risk may be attributable to the estrogen component of the hormone regimen because its increase is statistically significant among both HT-E and HT-EP users.[368,370,380]

Pulmonary Embolism

A pattern of increased pulmonary embolism was observed in all randomized studies, although the risk was attenuated and was not statistically significant in the WHI HT-E trial.[368,370,380]

TABLE 17.9 Absolute Risk Reductions or Increases for Women Treated With Estrogen Alone and With Estrogen Plus Progestin

ESTROGEN ALONE				
Outcome	**No. of Trials**	**Strength of Evidence**	**RR (95% of CI)**	**Absolute Risk Difference, Events per 10,000 Women (95% CI)**
Breast cancer (invasive)	1	Moderate	0.79 (0.61–1.01)	-52 (-97–3)
Colorectal cancer	1	Low	1.15 (0.81–1.63)	16 (-20–73)
Lung cancer	1	Low	1.04 (0.73–1.48)	5 (-30–54)
Coronary heart disease	3	High	0.95 (0.79–1.14)	-20 (-82–55)
Dementia (probable)	1	Low	1.49 (0.84–2.66)	63 (-21–213)
Diabetes	1	Moderate	0.87 (0.77–0.98)	-137 (-242–-21)
Fractures (osteoporotic)	1	High	0.73 (0.65–0.80)	-382 (-495–-283)
Gallbladder disease	1	Moderate	1.63 (1.33–2.00)	213 (111–338)
Stroke	1	Moderate	1.33 (1.06–1.67)	79 (14–160)
Urinary incontinence	1	Moderate	1.53 (1.37–1.71)	1261 (880–1689)
Venous thromboembolism	1	Moderate	1.43 (1.11–1.85)	78 (20–153)
All-cause mortality	3	High	1.01 (0.88–1.17)	6 (-74–96)
ESTROGEN + PROGESTIN				
Outcome	**No. of Trials**	**Strength of Evidence**	**RR (95% of CI)**	**Absolute Risk Difference, Events per 10,000 Women (95% CI)**
Breast cancer (invasive)	1	High	1.27 (1.0–1.56)	52 (6–107)
Cervical cancer	1	Low	1.52 (0.50–4.66)	3 (-3–23)
Colorectal cancer	1	Moderate	0.64 (0.44–0.91)	-33 (-52–-8)
Endometrial cancer	1	Low	0.86 (0.51–1.44)	-5 (-18–6)
Lung cancer	1	Moderate	1.06 (0.77–1.46)	5 (-20–40)
Ovarian cancer	1	Low	1.43 (0.76–2.69)	9 (-5–33)
Coronary heart disease	3	High	1.23 (1.00–1.52)	41 (7–93)
Dementia (probable)	1	Moderate	1.97 (1.16–3.33)	88 (15–213)
Diabetes	1	Moderate	0.84 (0.72–0.97)	-77 (-135–-15)
Fractures (osteoporotic)	5	High	0.80 (0.68–0.94)	-221 (-353–-66)
Gallbladder disease	1	Moderate	1.59 (1.29–1.97)	116 (57–190)
Stroke	1	High	1.39 (1.09–1.77)	53 (12–104)
Urinary incontinence	1	Moderate	1.39 (1.27–1.52)	876 (606–1168)
Venous thromboembolism	1	Moderate	1.95 (1.54–2.47)	120 (68–185)
All-cause mortality	3	Moderate	1.01 (0.88–1.17)	4 (-48–68)

Modified from Gartlehner G, Patel SV, Feltner C, et al. Hormone therapy for the primary prevention of chronic conditions in postmenopausal women: evidence report and systematic review for the us preventive services task force. *JAMA*. 2017;318(22):2234–2249. © 2017 American Medical Association.

Breast Cancer

Findings of the WHI HT-E trial were markedly different from those of the HERS and WHI HT-EP trials with respect to breast cancer risk.[370] The WHI HT-E trial indicated a trend, albeit statistically not significant, toward a lower breast cancer risk during the intervention phase. This protective effect was statistically significant during cumulative (intervention plus postintervention) follow-up (HR 0.79, CI 0.65–0.97).[368] This result was in contrast to findings of an observational literature that mostly reports a moderate increase in risk with estrogen-alone preparations.[381]

However, after control for prior use of postmenopausal HT and additional control for time from menopause to first use of postmenopausal HT, the hazard ratios agreed closely between the observational and randomized trial data.[381] Nonetheless, the higher risk for breast cancer observed in the HT-EP trials probably represents a harmful additional effect of MPA, the progestin used in these studies.[368] The increased breast cancer risk was statistically significant in the WHI HT-EP trial, which demonstrated an attributable risk of 9 cases per 10,000 person-years during the 5.6-year intervention phase.[368] This risk persisted and remained significant during the postintervention phase, giving rise to an overall risk of 9 cases per 10,000 person-years during the 13 years of cumulative follow-up.[368] This result was matched by a trend of the same magnitude in the HERS study and was supported by evidence from large observational studies suggesting that the addition of MPA or another progestin to estrogen may significantly increase the risk for breast cancer (see Table 17.9).[382]

Dementia

In postmenopausal women 65 years of age or older, HT-EP significantly increased risk and resulted in an additional 23 cases of probable dementia per 10,000 women per year.[383] Alzheimer disease was the most common classification of dementia. A similar trend was observed for HT-E but did not reach statistical significance.[383] When the data were pooled, HT significantly increased probable dementia risk.[383]

Hypertriglyceridemia

Worsening of hypertriglyceridemia is a rare side effect of HT, particularly in patients with severe familial hypertriglyceridemia. An oral estrogen regimen can hasten development of severe hypertriglyceridemia in women with previously elevated triglyceride levels, which can precipitate pancreatitis.[384] Estrogen replacement is relatively contraindicated in women with substantially increased triglyceride levels.

Gallbladder Disease

Both WHI trials (HT-E and HT-EP) showed substantially greater risk for any gallbladder disease or surgery with estrogen.[385] Both trials indicated a higher risk for cholecystitis and for cholelithiasis. Women on either HT regimen were more likely to undergo cholecystectomy.[368,370] These data suggest an increase in risk of biliary tract disease among postmenopausal women using estrogen therapy. The morbidity and cost associated with these outcomes may need to be considered in decisions regarding the use of estrogen therapy. Preexisting gallbladder disease is a relative contraindication for estrogen replacement.

Urinary Incontinence

Both HT-E and HT-EP users are at strikingly higher risk for urinary incontinence.[378] The type of incontinence or the underlying mechanisms for this side effect are not well understood. Thus, until these issues are resolved, urinary incontinence should be considered at least a relative contraindication.

Indications for Hormone Therapy

Hot Flashes

HT-E or HT-EP reliably treats hot flashes in most women.[386] Currently, hot flashes constitute the most common indication for a short course of HT (<5 years).

Fractures

HT-EP or HT-E significantly decreased the incidence of hip, vertebral, and other osteoporotic fractures.[368] In this case, the results of observational studies of estrogen and fracture risk and of trials using a surrogate endpoint (i.e., BMD) agree with the results of clinical trials of fracture prevention.[378]

Diabetes

Diabetes was significantly less common with both HT-E and HT-EP users.[368,370,378] Thus diabetes prevention should be considered in menopausal women starting either form of HT.[378]

Post-WHI Recommendations for Hormone Therapy

A number of publications with flow charts are available to clinicians for decision making about HT use.[370] Here, a number of useful principles are offered to guide the clinician and the patient for an optimal plan that suits her short-term and long-term needs and expectations. Decision making for women interested in HT involves balancing the potential benefits of HT against the potential risks.[370,387] HT is extremely effective for treatment of hot flashes, and for this indication women with intact uteri should receive HT-EP, whereas women without uteri should be prescribed HT-E because the only known benefit of adding a progestin is to prevent endometrial cancer. Overall, HT-E seems to be associated with less risk than HT-EP.[386] Nonetheless, HT-E also has adverse effects, and it is prudent to keep the dose low and the duration of treatment short for all HT regimens.[386]

Many clinicians and epidemiologists agree that short-term estrogen therapy using the lowest effective estrogen dose is a reasonable option for recently menopausal women who have moderate to severe symptoms and no previous history or elevated risk of CHD, stroke, breast cancer, or venous thromboembolism.[370,387] HT usually lasts for 2 to 3 years but rarely more than 5 years, because menopausal symptoms diminish after several years, whereas the risk of breast cancer increases with longer duration of HT.[386]

A small group of women may need long-term therapy for severe, persistent symptoms after stopping HT. These few women may be encouraged to first try nonhormonal options such as selective serotonin reuptake inhibitors (SSRIs); estrogen treatment should be resumed only if these alternatives are not helpful.[369,386] For isolated symptoms of genitourinary atrophy, low-dose vaginal estrogen with relatively lesser systemic absorption or endometrial effects is highly beneficial.[387]

In the absence of evidence for an overall net benefit of postmenopausal treatment with HT-E and with the evidence that HT-EP is harmful, neither therapy should be used for preventing CHD or improving mental function.[378]

Target Groups for Hormone Therapy

In women with gonadal dysgenesis and surgical menopause, the duration of estrogen deprivation is prolonged. Estrogen replacement is recommended for these patients for reduction of hot flashes and for long-term prophylaxis against osteoporosis and target organ atrophy. A low-dose contraceptive may be offered to nonsmoking women until the age of 45 years. After that point,

doses of estrogen equivalent to 0.625 mg of conjugated equine estrogens may be more appropriate because of a sharp age-related increase in risk for thromboembolic events. The physician should recommend a continuous or cyclical estrogen-progestin combination to those women with a uterus and an estrogen-only regimen to women without a uterus.

During perimenopause, hot flashes can be suppressed with an estrogen-progestin combination. Because bone loss related to estrogen deprivation also begins during this period, a benefit for women who take HT to prevent hot flashes is that bone loss will not start for the few years of therapy.[388] In perimenopausal women, unexplained uterine bleeding should be evaluated with an endometrial biopsy before the start of HT.

Estrogen Preparations and Beneficial Dose of Estrogen

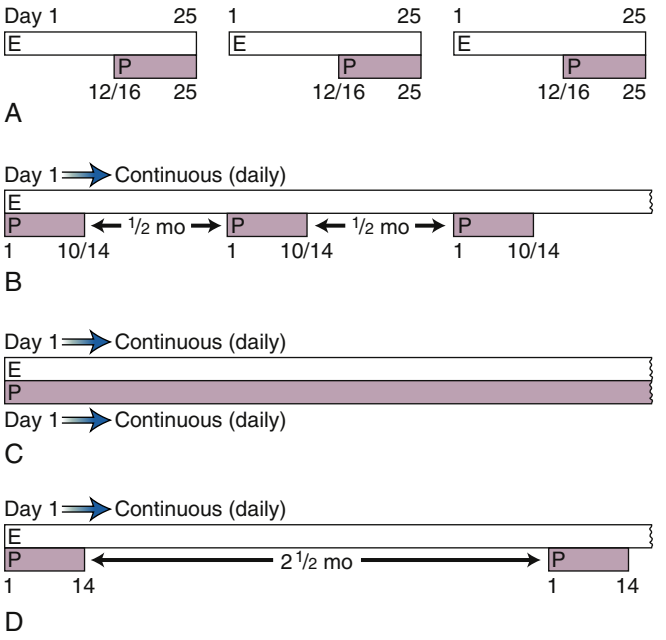
Oral Estrogens: Combined Conjugated Equine Estrogens

The amount of estrogen that provides effective treatment for hot flashes varies. For this purpose, it is reasonable to start with CEEs at 0.3 mg/day (or micronized estradiol at 0.5 mg/day) and gradually increase this dose to CEEs at 0.625 mg/day (equivalent to micronized estradiol at 1 mg/day) and 1.25 mg/day (equivalent to micronized estradiol at 2 mg/day). If hot flashes in a postmenopausal woman are not alleviated by 1.25 mg/day of CEEs or an equivalent transdermal estradiol dose, it is unlikely that higher doses will be effective. In this case, alternative diagnoses (e.g., tuberculosis, depression, thyroid disease) should be ruled out.

Low-dose estrogen (0.3 mg/day of CEEs or 0.5 mg/day of micronized estradiol) maintains blood estradiol levels between 17 and 32 pg/mL (average, 22 pg/mL) and may be sufficient for preserving bone density and for alleviating menopausal symptoms in early postmenopausal women.[389] Osteoporosis and fracture risk should be assessed via periodic dual-energy x-ray absorptiometry (DXA) scans, especially because low-dose estrogen preparations may not prevent bone loss. The effect of estrogen on arterial thrombosis is probably dose related.[389] When choosing a dose for HT, it is imperative to achieve and maintain the lowest beneficial levels of circulating estradiol and to avoid higher levels to minimize the risk of thrombosis.

The addition of a progestin, either cyclically or continuously, to concomitant estrogen replacement reduces the risk of estrogen-induced endometrial hyperplasia or carcinoma but poses additional problems, which include regular withdrawal bleeding in up to 90% of women treated with cyclic therapy and irregular spotting in 20% of women treated with continuous estrogen plus progestin.[390] Progestins appear to reduce the beneficial effects of estrogen on high-density lipoprotein (HDL) and LDL cholesterol and to increase the risks of pulmonary embolism, CHD, and breast cancer.[368,370,380]

A time-honored sequential regimen involves oral administration of 0.625 mg of CEEs or the equivalent dose of a variety of available products on days 1 through 25 of each month (Fig. 17.36). A daily dose of 5 mg of MPA is added on days 12 through 25 or on days 16 through 25. Withdrawal bleeding is expected on or after day 26 of each month. Another common cyclic regimen involves continuous oral administration of 0.625 mg of CEEs or the equivalent daily dose (see Fig. 17.36). A daily dose of 5 mg of MPA is added for the first 10 to 14 days of every month. One-year randomized trial data indicate that the 5-mg dose protects the endometrium as well as the 10-mg dose does.[391] Progestin



• **Fig. 17.36** Regimens of hormone therapy. Estrogen (E) is replaced in a postmenopausal woman to prevent osteoporosis, urogenital atrophy, and hot flashes. In the postmenopausal woman with a uterus, a progestin (P) should be added to estrogen to prevent endometrial hyperplasia and cancer. E and P can be administered in several ways. (A, B) Postmenopausal women receiving hormone therapy have predictable withdrawal bleeding episodes after each P course. (C) E and P are administered together. After a year of continuous combination therapy, the rate of unpredictable breakthrough spotting is 20%. (D) This relatively new regimen was introduced to minimize the harmful effects of a progestin. Its long-term safety for endometrial hyperplasia or cancer risk is unknown. Predictable bleeding after each P course every 3 months is presumed to be reassuring.

withdrawal bleeding occurs in 90% of women with a sequential or cyclic regimen.[392,393] These regimens can also cause adverse symptoms related to the relatively high daily doses of progestin, including breast tenderness, bloating, fluid retention, and depression. The lowest possible dose of a progestin is recommended.

The continuous combined method of treatment has the potential benefit of reduced bleeding and amenorrhea, but it is occasionally complicated by breakthrough bleeding (see Fig. 17.36).[392,393] In this regimen, a combination of 0.625 mg of CEEs and 2.5 mg of MPA is given orally every day. The continuous combination regimen is simple and convenient, and it is associated with an incidence of amenorrhea in 80% of patients after at least 6 months of use. The other 20% of patients continue to experience some degree of unpredictable spotting. Overall compliance is much better in users of the continuous combination regimen. Moreover, the lower daily dose of MPA is associated with a lower incidence of breast tenderness with this regimen. Other estrogen-progestin combinations are also available for similar continuous use.

Cyclic progestin has also been used at less frequent intervals, such as every 3 to 6 months. When added to standard-dosage estrogen, 10 mg of MPA every 3 months for 14 days produced a 1.5% rate of hyperplasia (a rate low enough to be interpreted as endometrial protection), and long-term use of MPA at 6-month intervals was associated with a low rate of endometrial cancer.[394,395] However, clinicians have not determined the optimal progestin dosage and schedule to use with low-dosage estrogen. Low-dosage estrogen use can reasonably be assumed to require less progestin for protection of the endometrium.

Most postmenopausal women can switch their HT regimen from standard-dosage HT to low-dosage estrogen combined with a 15-day MPA treatment at 3-month intervals or start HT at this low dose.[389] Although its long-term safety has not been proven with respect to endometrial hyperplasia, the following regimen appears to be a reasonable compromise for treating hot flashes and preventing osteoporosis while minimizing the harmful effects of progestins and high-dose estrogen[389]: 0.3 mg/day of CEEs or 0.025 mg of transdermal estradiol is administered continuously. Every 3 months, a 14-day course of 5 mg of MPA is added (see Fig. 17.36D). Endometrial biopsy is not required in the presence of withdrawal bleeding after each periodic progestin intake and in the absence of irregular bleeding. This regimen may be continued for up to 5 years. After discontinuation of HT, the postmenopausal woman can be switched to a bisphosphonate or a selective estrogen receptor modulator (SERM) for bone protection if needed.

Transdermal Estrogen

Transdermal estrogen preparations appear to be as effective as oral estrogens for treating hot flashes and maintaining BMD, but they have different metabolic profiles. Oral estrogens seem to have favorable effects on lipoprotein profiles. However, oral estrogens are associated with several disadvantages, including unfavorable changes in serum levels of triglycerides, C-reactive protein, fibrinogen, factor VII, and plasminogen activator inhibitor type 1.[387] A meta-analysis of clinical trials suggested a higher risk of venous thromboembolic events among oral HT users compared with transdermal estrogen users.[387] Clinical trial data that address the effect of transdermal estrogen on CHD and stroke risk are limited.[387]

A daily dose of 0.05 mg of transdermal estradiol is equivalent to 0.625 mg of oral CEEs or 1 mg of oral micronized estradiol. A lower-dose transdermal preparation includes 0.025 mg of estradiol (equivalent to 0.3 mg of oral CEEs). An ultralow-dose transdermal estradiol preparation (0.014 mg) is also available. In women with symptoms not responding to smaller doses, high-dose transdermal estradiol at 0.1 mg/day (equivalent to CEEs at 1.25 mg/day) may be used. For the average menopausal woman with hot flashes, it is reasonable to start with a daily dose of 0.025 mg, which should be accompanied by a progestin in a woman with a uterus.

Vaginal Estrogen

Vaginal estrogen formulations are the first choice for the initial management of menopause-related vaginal atrophy symptoms. Low-dose vaginal tablets, rings, and creams administered via the vagina are equally effective when used for relief of vulvovaginal symptoms. A usual starting dose is 0.625 mg CEEs (in 1 g cream) applied vaginally daily for 1 week; the following maintenance dose is twice weekly. A 12-week study designed to elucidate the lowest effective dose of estradiol cream for relieving vaginal symptoms reported that 100% of women responded to the lowest dose tested (10 μg daily). Circulating estradiol levels remained in the postmenopausal range (3–10 pg/mL, 13.6–36.7 pmol/L) using a highly sensitive assay, and improvements in vaginal cytologic features and decreases in vaginal pH were significant. No endometrial hyperplasia was noted during the course of the study.[396]

Management of Breakthrough Bleeding During Postmenopausal Hormone Therapy

Approximately 90% of women receiving estrogen plus cyclic administration of a progestin have monthly progestin withdrawal bleeding in a predictable fashion. Continuous combined estrogen-progestin therapy causes breakthrough bleeding in approximately 40% of women during the first 6 months, with the remaining 60% being amenorrheic. The pattern of vaginal bleeding in women taking the continuous combined regimen is unpredictable and causes anxiety in most patients, but the incidence of breakthrough bleeding decreases to 20% after 1 year of treatment.[392,393,397] Nevertheless, breakthrough bleeding remains the most important reason for discontinuance of this therapy. A significant number of patients find it unacceptable and prefer to switch to a cyclic progestin regimen or to discontinue HT altogether. There is no effective pharmacologic method to manage the breakthrough bleeding associated with continuous combined estrogen-progestin regimens. The physician can only reassure the patient that the bleeding is likely to subside within 1 year from the start of HT. If breakthrough bleeding continues beyond 1 year, the regimen may be changed to daily estrogen plus cyclic progestin.

HT can be started in the amenorrheic postmenopausal patient at any time. Perimenopausal women with oligomenorrhea, hot flashes, or other associated symptoms can also be treated with HT. In the oligomenorrheic patient, an HT regimen may be initiated on day 3 of one of the infrequent menses. If the candidate for HT does not have irregular uterine bleeding, it is not essential to perform an endometrial biopsy routinely before beginning treatment. Studies indicate that asymptomatic postmenopausal women rarely have endometrial abnormalities.[341,397,398] Pretreatment biopsies using a thin plastic biopsy cannula in the office may be limited to patients who are at higher risk for endometrial hyperplasia (e.g., unpredictable uterine bleeding, history of PCOS or chronic anovulation, obesity, liver disease, diabetes mellitus).

Prescribing a combined estrogen-progestin regimen does not preclude the development of endometrial cancer.[399] Therefore it is necessary to rule out endometrial malignancy in women receiving HT who are experiencing irregular uterine bleeding. The important task is to differentiate breakthrough bleeding from bleeding induced by hyperplasia or cancer. Because breakthrough bleeding is extremely common, many biopsies must be performed to detect a rare case of endometrial abnormality during HT. To decrease the number of endometrial biopsies, a screening method using transvaginal ultrasonography has been introduced.[135] The thickness of the postmenopausal endometrium as measured by transvaginal ultrasonography in postmenopausal women correlates with the presence or absence of pathologic changes.[135] Patients receiving a cyclic or daily combination HT regimen who have an endometrial thickness of less than 5 mm can be managed conservatively.[400–402] An endometrial thickness equal to or greater than 5 mm requires biopsy. Based on this algorithm, it is estimated that 50% to 75% of bleeding patients receiving HT and evaluated by ultrasonography require biopsy.[135]

Management of Menopausal Symptoms in Breast Cancer Survivors

Vasomotor symptoms constitute a major problem for survivors of breast cancer. Approximately 65% of women become symptomatic with hot flashes (mostly severe) after treatment for breast cancer.[403] Hot flashes are encountered more frequently among tamoxifen users and women treated with chemotherapy. Up to 90% of premenopausal women who receive chemotherapy and tamoxifen have vasomotor symptoms.[403]

Breast cancer survivors often seek relief from hot flashes.[404] HT is typically withheld from women with breast cancer because of concerns that estrogen may stimulate recurrence. One randomized

study showed that after extended follow-up, there was a statistically significant increased risk of a new breast cancer event in survivors who took HT.[405] In this randomized, non–placebo-controlled study, 442 women assigned to receive HT-EP/HT-E or best symptomatic management without hormones were followed for a median of 4 years. Thirty-nine of the 221 women in the HT arm and 17 of the 221 women in the control arm had a new breast cancer diagnosed (hazard ratio, 2.4; 95% CI, 1.3–4.2). Cumulative breast cancer incidences at 5 years were 22.2% in the HT arm and 8% in the control arm. No difference in mortality rate from breast cancer was found.[405]

Because of higher breast cancer recurrence associated with HT, many breast cancer survivors have sought nonhormonal alternatives, including other pharmaceutic agents, herbal or dietary remedies, and mind-body or behavioral therapies.[403] Mind-body or behavioral treatments for hot flashes are particularly attractive to survivors of breast cancer because they do not have the side effects caused by pharmaceutic agents, but it is not yet known whether they are effective.[386,403,404]

Several nonhormonal pharmaceutic agents have been used off-label for women who cannot take or elect not to take HT. Among the non-HT treatments, SSRIs, serotonin-norepinephrine reuptake inhibitors (SNRIs), clonidine, and gabapentin were found to be more effective than placebo.[387] Although all of these nonsteroidal medications could reduce the number of hot flashes per day, their beneficial effects were far less than what has been observed with estrogen, and these drugs are all associated with significant side effects that can limit their use in some women. Extracts of black cohosh or red clover were found to be ineffective, and the results for soy isoflavone extracts were mixed.[387] The beneficial doses for hot flashes include paroxetine (an SSRI) at 10 to 20 mg/day, paroxetine controlled release at 12.5 to 25 mg per day, venlafaxine (an SNRI) at 75 mg/day, desvenlafaxine (a SNRI) at 100 mg/day, and gabapentin at 900 mg/day.[386] Gabapentin should be started with a smaller dose given at bedtime, and the dose should be gradually increased. Paroxetine reduces the metabolism of tamoxifen to its most active metabolite, endoxifen, and should be avoided in women with breast cancer who are receiving tamoxifen.[387]

For long-term prophylaxis against osteoporosis in breast cancer survivors, tamoxifen, raloxifene, or a bisphosphonate are viable options. However, tamoxifen and raloxifene intensify hot flashes.[404]

Selective Estrogen Receptor Modulators and Bisphosphonates for Osteoporosis Prevention

Postmenopausal women who are at risk for osteoporosis should be screened at least once for osteoporosis by DXA scan. Based on the initial results and other risk factors, DXA should be repeated periodically, preferably annually or every other year, to monitor the effectiveness of osteoporosis treatment and prevention.[406]

SERMs are compounds that act like estrogen in some target tissues but antagonize estrogenic effects in others (see Chapter 29).[407] One of the first SERMs was tamoxifen, for which estrogen-like agonist activity on bone was observed to occur simultaneously with estrogen antagonist activity on the breast.[407] Tamoxifen had been approved initially for treatment and prevention of breast cancer. An unwanted effect of tamoxifen is its estrogen-like action on the endometrium. Second-generation compounds have since been developed, most notably raloxifene, which has estrogen-like actions on bone, lipids, and the coagulation system; estrogen antagonist effects on the breast; and no detectable action in the endometrium.[408] In 2007 the FDA approved raloxifene for reducing the risk of invasive breast cancer in postmenopausal women with osteoporosis and in postmenopausal women who are at high risk for invasive breast cancer. Raloxifene is most often used to prevent and treat osteoporosis in postmenopausal women.[409]

In placebo-controlled trials, raloxifene reduced vertebral fractures, whereas tamoxifen reduced nonvertebral fractures.[408] Tamoxifen and raloxifene had similar effects on fractures at multiple sites in a randomized, head-to-head trial.[408] Neither drug has been shown to prevent hip fractures, however. Tamoxifen or raloxifene reduced risk for invasive breast cancer compared with placebo by approximately 7 to 10 cases per 1000 women per year.[408] Tamoxifen and raloxifene reduced ER-positive breast cancer but not ER-negative breast cancer, noninvasive breast cancer, or mortality rate.[408] Tamoxifen and raloxifene increase thromboembolic events by 4 to 7 cases per 1000 women per year; raloxifene causes fewer events than tamoxifen. Tamoxifen increases risk for endometrial cancer compared with placebo by 4 cases per 1000 women per year and causes cataracts.[408] Raloxifene use is not associated with endometrial cancer or cataract risk.[408] The most common side effects for tamoxifen are hot flashes and other vasomotor symptoms and vaginal discharge, itching, or dryness. For raloxifene, vasomotor symptoms and leg cramps are most common. In a head-to-head trial, raloxifene users reported more musculoskeletal problems, dyspareunia, and weight gain, whereas tamoxifen users had more gynecologic problems, vasomotor symptoms, and bladder control symptoms.[408] Tamoxifen and raloxifene may be used to reduce the incidence of vertebral fractures and invasive breast cancer in postmenopausal women. The major drawbacks include hot flashes and increased thromboembolic events for both drugs and endometrial cancer for tamoxifen.

SERMs are effective for managing certain aspects of estrogen deficiency in postmenopausal women, but recent data suggest that pairing a SERM with estrogens may provide a more optimal therapeutic profile for women with a uterus. The drug combination bazedoxifene/CEE is a medication approved by the FDA in 2013 for the treatment of menopause symptoms and the prevention of postmenopausal osteoporosis but not for the treatment of osteoporosis.[409] It is a fixed-dose combination drug containing the SERM bazedoxifene and CEE.[410] This new agent demonstrated efficacy in postmenopausal women with a uterus, while allowing these women to avoid progestins and their possible adverse effects.[410]

The major treatment goal in postmenopausal osteoporosis is to prevent fractures by maintaining or increasing BMD and reducing excessive bone turnover.[411] Bisphosphonates suppress resorption by inhibiting the attachment of osteoclasts to bone matrix and enhancing programmed cell death in osteoclasts. They have increased BMD and reduced the risk for osteoporotic fractures in numerous clinical trials.[411] The FDA has approved a number of bisphosphonates for treatment. Oral alendronate and oral risedronate were approved in 1995 and 2000, respectively. In 2003, oral ibandronate was approved, followed by intravenous ibandronate in 2006. Intravenous zoledronic acid was approved in 2007. A 5-mg dose of zoledronic acid is infused over 15 minutes once each year.[409] Alendronate is given as a once-weekly 35-mg or 70-mg tablet or a once-daily 5-mg or 10-mg tablet; risedronate is given as a once-daily 5-mg tablet, a once-weekly 35-mg tablet, or a once-monthly 150-mg tablet; ibandronate is given as a once-monthly 150-mg tablet or as 3 mg intravenously every 3 months.[412]

Compared with placebo control subjects, all approved bisphosphonates reduce the relative risk of new vertebral fractures by on

average 50% in women with postmenopausal osteoporosis.[412,413] Alendronate, risedronate, and zoledronic acid reduce the relative risk of new nonvertebral and hip fractures.[411] Clinical trial extensions of up to 10 years with alendronate and 7 years with risedronate have shown that efficacy is maintained during long-term treatment.[411] Moreover, discontinuation of long-term (≥5 years) alendronate therapy results in minimal bone loss over the ensuing 3 to 5 years.[414] As in the case of SERMs, definitive data are lacking on optimal doses, duration, timing, long-term effects, and effects in nonwhite women. Once-yearly zoledronic acid infusions appear to be an attractive choice because of better compliance and lack of esophagitis, a side effect associated with oral bisphosphonates.[404]

Osteonecrosis of the jaw is a rare but serious side effect of bisphosphonate therapies. Bisphosphonate users who plan to undergo tooth extraction should discuss this side effect with their dental surgeons, because tooth extraction may predispose them to osteonecrosis of the jaw.[415] In premenopausal patients with estrogen-responsive early breast cancer, the addition of zoledronic acid to adjuvant endocrine therapy reduced disease recurrence in all body sites and improved disease-free survival.[416] Further studies are required to explore the potential of bisphosphonates in preventing breast cancer in postmenopausal women with no previous history of breast cancer.

A number of case reports and a recent retrospective study suggested that the risk of esophageal cancer increased with oral bisphosphonate use over about a 5-year period. In Western countries, the incidence of esophageal cancer in patients over the age of 60 is estimated to increase from 1 to 2 per 1000 population with 5 years' use of an oral bisphosphonate.[417,418] Another retrospective study, however, did not find such a link.[419] Until more accurate data are available, health care providers should avoid prescribing oral bisphosphonates to patients with Barrett esophagus, a known risk factor for esophageal cancer.[417]

Tibolone for Osteoporosis Prevention

Tibolone, a synthetic steroid with estrogenic, androgenic, and progestagenic properties, is approved in many countries for the treatment of menopausal symptoms and prevention of osteoporosis. Tibolone preserves BMD, reduces hot flashes, and may increase libido and vaginal lubrication in postmenopausal women.[420] A randomized study showed that tibolone reduced the risks of fracture and breast cancer and possibly colon cancer but increased the risk of stroke in older women with osteoporosis.[420] Tibolone should not be used in breast cancer survivors, as it increases breast cancer recurrence.[404,421] Tibolone is not available in the United States.

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The complete list of references is available online at ExpertConsult.com.

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18

Hormonal Contraception

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CHAPTER OUTLINE

KEY POINTS

- Reducing health care system barriers to contraceptive initiation and continuation is important to reduce unintended pregnancy rates. Such barriers include requiring unnecessary health screenings, waiting until menstruation to begin methods, inappropriate contraindications, and failure to provide adequate refills for prescription-based methods. The US Selected Practice Recommendations for Contraceptive Use from the Centers for Disease Control and Prevention offers evidence-based guidelines to assist providers in caring for women who need contraception.
- Long-acting reversible contraceptive methods, which include the copper intrauterine device (IUD), the levonorgestrel IUD, and the etonogestrel subdermal implant, provide superior contraceptive effectiveness, equivalent to sterilization, and higher continuation and satisfaction rates compared with shorter-acting methods. These methods can be offered to all women, including adolescents seeking contraception.
- Emergency contraception should ideally be available to women at risk for unplanned pregnancy. The copper IUD is the most effective emergency contraceptive, followed by oral ulipristal acetate and oral levonorgestrel.
- Besides offering highly effective contraception, the higher-dose levonorgestrel IUD represents a first-line treatment for heavy menstrual bleeding and an effective alternative to endometrial ablation and hysterectomy.
- Pregnancy in women with underlying medical conditions is associated with higher risks of maternal and perinatal morbidity and mortality; therefore, achieving effective preconception and family planning care is particularly important in this setting. Health care providers should consult the US Medical Eligibility Criteria for Contraceptive Use from the Centers for Disease Control and Prevention when caring for these women.

Prevention of unplanned pregnancy continues to challenge women, couples, and clinicians in developed and developing countries. For all couples, access to contraception is critical to be able to plan the timing and spacing of their childbearing for social, economic, and health reasons.[1] Most women in the United States and other developed countries desire to have two children.[2] Therefore, on average, sexually active women devote more than three decades of their lives to avoiding unintended pregnancy. However, nearly half (45%) of all pregnancies in the United States are unintended.[3] Women who use contraception consistently and correctly account for only 5% of unintended pregnancies, but women who use contraception inconsistently or incorrectly account for 41%, and women who do not use any method of contraception account for 54% of unintended pregnancies. Pregnancies that are unintended or too closely spaced can lead to adverse maternal and child health outcomes.[4] In developing countries, childbirth and unsafe abortion are important causes of maternal death and morbidity, and the unintended pregnancy rate is estimated at 65 per 1000 women.[5]

Contraceptive advice and provision should start before the commencement of sexual activity and continue through the reproductive years. Although the incidence is declining, the United States still has one of the highest teenage pregnancy rates in the developed world.[6] Teenage parenting can have adverse consequences for the adolescent, as well as the adolescent's children and family.[7] Programs to prevent unplanned pregnancy by promoting abstinence have largely been ineffective. Nonhormonal contraceptives, such as male condoms, may offer benefits in terms of protection against sexually transmitted infection (STI) but are highly user dependent and do not reliably prevent unplanned pregnancy in many populations. Female hormonal contraception, along with the copper intrauterine device (IUD), represents the most effective and acceptable reversible contraceptive options; male hormonal contraception has not achieved this goal. The ideal contraceptive would be safe, highly effective, discreet, inexpensive, long acting, easily reversible, and under the woman's control. It would not need to be activated at or around the time of intercourse. The

ideal contraceptive would also protect against STIs. Because an ideal contraceptive does not exist, the challenge for clinicians is to tailor available methods to the medical, personal, and social needs of the woman and her partner and changing approaches when necessary as these needs evolve throughout her reproductive life span. The clinician must also learn to recognize and address barriers to safe and effective implementation of the selected methods. Hormonal contraceptives are most effectively used by women who are well informed about the advantages and common side effects of the method, and who have actively participated in selecting the method. Guidance on medical eligibility for contraceptives can be found in two companion documents from the Centers for Disease Control and Prevention (CDC), entitled "U.S. Selected Practice Recommendations for Contraceptive Use, 2016"[8] and "U.S. Medical Eligibility Criteria for Contraceptive Use, 2016."[9]

In addition to enhancing quality of life by allowing couples to choose whether and when they wish to bear children, effective contraception lowers health care costs.[10] Male and female sterilization and long-acting reversible methods (e.g., IUDs, subdermal implants) constitute the most cost-effective contraceptive options, followed by other hormonal methods (e.g., oral contraceptives). Depot medroxyprogesterone acetate (DMPA) injection is more cost effective than oral contraception. Barrier and behavioral methods (i.e., male condom and withdrawal, respectively) are the least cost effective compared with other contraceptive options. Nevertheless, when compared with no method, they still prevent a large number of unintended pregnancies, leading to important cost savings.

Choosing a Contraceptive Method

Given the number of contraceptive options available to women, it is important providers concentrate their efforts on helping women choose the best contraceptive method for them and focus on counseling that helps improve continuation. The best birth control method is one that provides the safest and most effective contraceptive for a woman and is the method she chooses to use and has access to. This approach not only places a strong value on medical considerations but also includes consideration of a woman's preferences, values, and level of prevention desired, as well as the affordability of the method given her access to health insurance or ability to pay. When helping women make sound contraceptive decisions, clinicians should consider the patient's age, lifestyle, and other relevant circumstances, including the recognition that contraceptive needs are likely to change during different phases of reproductive life, and that the risks and benefits may alter according to age and background health factors. For those considering pregnancy in the future, contraceptive reversibility and time to return of fertility should be discussed. Affordability must be considered, because it may affect continuation rates and therefore affect efficacy.

Long-acting reversible contraceptives (i.e., IUDs and the implant) offer women the advantages of high contraceptive efficacy and high rates of continuation. When discussing contraception, clinicians should present all suitable options to their patients but outline the options in terms of tiers of effectiveness (Fig. 18.1). Tier 1 includes long-acting reversible contraception and sterilization. Tier 2 includes combined methods (pill, patch, and ring), DMPA, and the progestin-only pill (POP). Tier 3 includes barrier methods, and tier 4 includes withdrawal and use of spermicides.

As with all medications, contraceptives have potential side effects. Candid discussion of these effects and other areas of anticipatory guidance may increase acceptability. The clinician should offer information about adverse events that are individualized and provided in the context of how they compare with the effects of an unplanned pregnancy. Information about contraceptive failure and access to emergency contraception should be given. For those at risk for STIs, clinicians should encourage consistent condom use and minimizing the number of partners, regardless of contraceptive choice. No method of contraception is perfect. Each woman must consider the advantages and disadvantages of each method in making her decision. The most effective method is likely to be the one that she can use successfully.

Combined Estrogen and Progestin Contraceptives

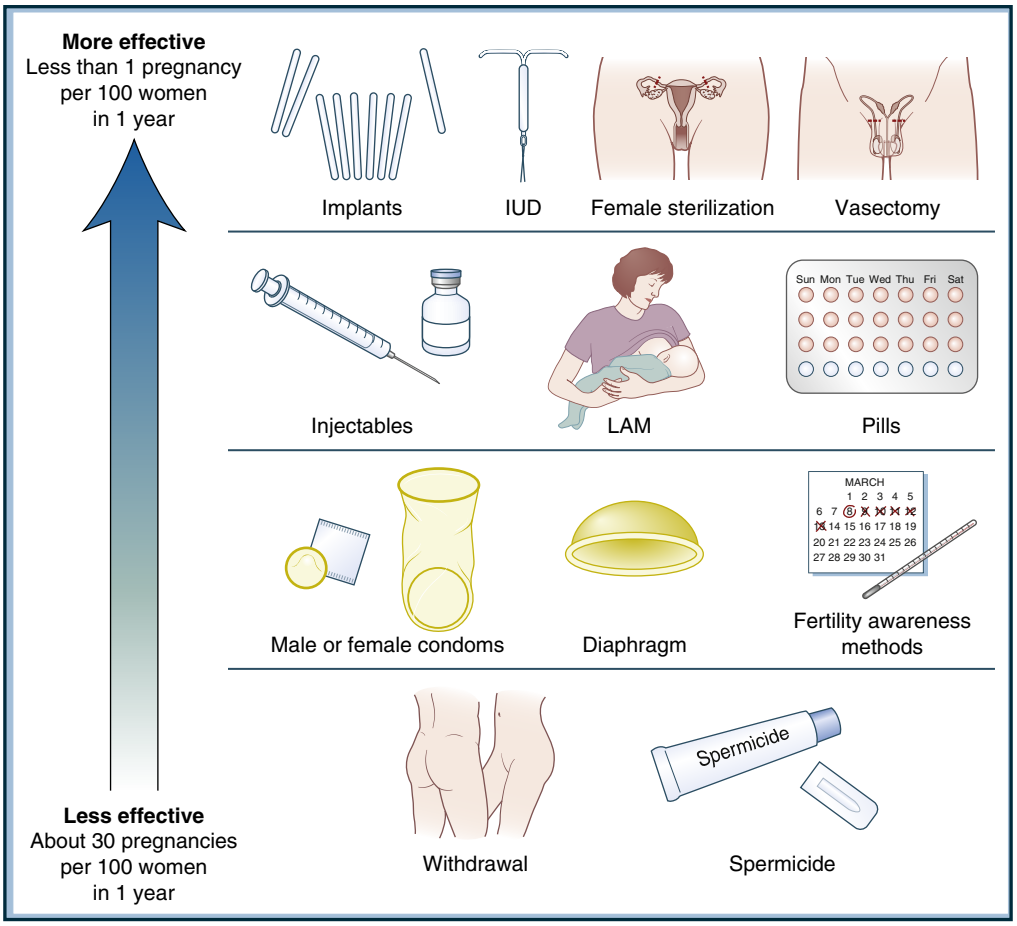
Methods combining estrogen and progestin offer the advantage of relatively regular bleeding patterns and high efficacy if used consistently. These combined methods are available in oral, transdermal, and transvaginal preparations providing increased flexibility in choice of delivery system.

The Combined Oral Contraceptive Pill

Combined oral contraceptives (COCs) offer safe, reversible, and convenient birth control that is highly effective for those who take pills correctly and consistently. In many settings, oral contraception provides important noncontraceptive benefits (mentioned later) that should be discussed during counseling.[11] By individualizing counseling and follow-up strategies based on relevant behavioral and medical considerations, clinicians can help women maximize efficacy of COCs. COCs have been available for more than 50 years and have gradually been refined to improve safety, efficacy, and acceptability. COCs are used by almost 10 million women in the United States, making them the most widely used hormonal contraceptive method.[12] Currently used methods contain estrogen, usually as ethinyl estradiol, at doses of 10 to 35 μg/day and progestins in different forms and doses. This section describes COCs available in the United States, focusing on education, counseling, and management measures to maximize contraceptive efficacy.

Composition and Formulations

Over time, the dose of estrogen and progestin in COCs has gradually decreased, and the types of progestins have changed. Currently, the highest-dose formulations marketed in the United States contain 50 μg of estrogen, but the great majority of COCs prescribed contain 35 μg or less. Most modern COCs formulated with 35 μg of estrogen or less use ethinyl estradiol, which is a potent synthetic estrogen with long half-life and slow metabolism and similar metabolic effects (e.g., liver protein production) regardless of the route of administration (oral, transdermal, or vaginal ring).[13] Estradiol valerate has been marketed as a component of one newer COC, Natazia (Bayer Healthcare Pharmaceuticals, Wayne, NJ). Estradiol valerate is a synthetic hormone extensively metabolized to estradiol and valeric acid before reaching the systemic circulation. A dose of estradiol valerate (2 mg daily) has biologic effects on the uterus, ovary, and hypothalamic-pituitary-ovarian axis similar to those of a 20-μg dose of ethinyl estradiol.[14] A COC formulated with micronized estradiol (1.5 mg) and the progestin nomegestrol acetate has been approved for use in some European countries but is not currently marketed in the United States.[15] Older COC preparations marketed in the United States contain one of five progestins: norethindrone, norethindrone acetate, ethynodiol diacetate, norgestrel, or levonorgestrel. Newer formulations contain more potent progestins: norgestimate, desogestrel, drospirenone, and dienogest.



• **Fig. 18.1** Comparing effectiveness of family planning methods. *IUD,* intrauterine device; *LAM,* lactational amenorrhea method. (Redrawn from World Health Organization Department of Reproductive Health and Research (WHO/RHR) and Johns Hopkins Bloomberg School of Public Health/Center for Communication Programs (CCP), Knowledge for Health Project. Family Planning: A Global Handbook for Providers (2011 update). Baltimore and Geneva: CCP and WHO; 2011.)

Many COC formulations are marketed in the United States (Table 18.1). These products do not appear to be different in terms of safety or efficacy if used correctly.[16] COCs were originally formulated to mimic the normal menstrual cycle by providing 21 days of combined estrogen and progestin, followed by a 7-day hormone-free interval (HFI) when withdrawal bleeding occurred. Although there is no evidence that an HFI provides any health benefits, most modern COCs continue to follow this approach and contain active pills for 21 to 24 days in each 28-day pack; in most formulations, pills containing no active medication are taken during the HFI.[17] Less pituitary-ovarian activity during the HFI is noted in the 24/4 regimen compared with the 21/7 regimen.[18,19] COC formulations with a shorter HFI allow less opportunity for follicle development and possible ovulation; accordingly, such formulations may be more effective.[20] Some women may ovulate if the HFI is extended beyond 7 days.[20,21]

The HFI can be modified (shortened to 4 days) or eliminated to reduce hormone-withdrawal symptoms including pelvic pain, breast tenderness, and mood symptoms, and to improve treatment of problems such as dysmenorrhea, pelvic pain, and anemia. This approach may enhance convenience for some women.[22] A recent meta-analysis showed lower rates of menstrual-related symptoms, including headache, bloating, and pelvic pain with extended-dosing regimens.[17] Extended oral contraceptive formulations are available as packs of 84 pills, followed by 7 days of inert tablets or tablets containing ethinyl estradiol 10 μg in place of the conventional HFI. These formulations offer women only four scheduled withdrawal bleeds per year. In addition, a continuous COC with no HFI is available.

COC regimens also differ with respect to the dose of sex steroid and whether these remains constant throughout the hormonally active pills. Monophasic preparations have a constant dose of estrogen and progestin in each of the 21 or 24 active hormone tablets in each cycle pack. Phasic preparations alter the dose of the progestin and, in some formulations, the estrogen component within the active tablets. There is no evidence phasic preparations are superior to monophasic formulations in terms of efficacy or bleeding patterns.[23-25]

Mechanism of Action, Efficacy, Administration, and Effect on Pregnancy

Estrogen and progestin components of COCs contribute to inhibition of ovulation through suppression of the hypothalamic-pituitary-gonadal axis. Suppression of pituitary release of follicle-stimulating hormone and luteinizing hormone (LH) discourages folliculogenesis and prevents ovulation. Additionally, the exogenous progestin increases cervical mucous viscosity, causes endometrial atrophy, and impairs function of the fallopian tube cilia to prevent fertilization.[16]

TABLE 18.1 Oral Contraceptive Formulations Available in the United States

Description	Name	Estrogen	Progestin	Progestin Dose (mg)
EE, 50 μg monophasic	Ovcon 50	EE	Norethindrone	1
	Ogestrel 0.5/50	EE	Norgestrel	0.5
	Zovia 1/50	EE	Ethynodiol diacetate	1
	Norinyl 1/50[a]	Mestranol	Norethindrone	1
EE, 35 μg monophasic	Femcon Fe chewable	EE	Norethindrone	0.4
	Modicon[a]	EE	Norethindrone	0.5
	Brevicon[a]	EE	Norethindrone	0.5
	Ovcon 35[a]	EE	Norethindrone	0.4
	Ortho-Cyclen[a]	EE	Norgestimate	0.25
	Zovia 1/35[a]	EE	Ethynodiol diacetate	1
	Ortho-Novum 1/35a[a]	EE	Norethindrone	1
	Norinyl 1 + 35[a]	EE	Norethindrone	1
EE, 35 μg biphasic	Ortho-Novum 10/11[a]	EE	Norethindrone	0.5/1
EE, 35 μg triphasic	Ortho-Novum 7/7/7[a]*	EE	Norethindrone	0.5/0.75/1
	Ortho Tri-Cyclen[a,b]	EE	Norgestimate	0.18/0.215/0.25
	Tri-Norinyl[a]	EE	Norethindrone	0.5/1.0/0.5
	Estrostep[a,b]	EE (20/30/35)	Norethindrone acetate	1
EE, 30 μg monophasic	Loestrin 1.5/30[a]	EE	Norethindrone acetate	1.5
	Ortho-Cept[a]	EE	Desogestrel	0.15
	Desogen[a]	EE	Desogestrel	0.15
	Lo-Ovral[a]	EE	Norgestrel	0.3
	Nordette[a]	EE	Levonorgestrel	0.15
	Levora[a]	EE	Levonorgestrel	0.15
	Yasmin[a]	EE	Drosperinone	3.0
EE, 30 μg triphasic	Triphasil[a]	EE (30/40/30)	Levonorgestrel	0.05/0.075/0.125
	Trivora[a]	EE (30/40/30)	Levonorgestrel	0.05/0.075/0.125
EE, 30 μg extended cycle (84 estrogen/progestin tablets)	Seasonale[a]	EE	Levonorgestrel	0.15
EE, 30 μg extended cycle (84 estrogen/progestin tablets, 7 tablets 10 μg EE)	Seasonique[a]	EE	Levonorgestrel	0.15
EE, 30 μg extended cycle triphasic (84 estrogen/progestin tablets, 7 tablets 10 μg EE)	Quartette	EE (20/25/30)	Levonorgestrel	0.15
EE, 25 μg monophasic (24/4)	Generess Fe chewable	EE	Norethindrone	0.8
EE, 25 μg triphasic	Cyclessa[a]	EE	Desogestrel	0.10/0.125/0.15
	Ortho Tri-Cyclen Lo[a]	EE	Norgestimate	0.18/0.215/0.25
EE, 20 μg monophasic	Loestrin 1/20[a]	EE	Norethindrone acetate	1
	Lutera[a]	EE	Levonorgestrel	0.1
EE, 20 μg biphasic	Mircette[a]	EE (20/10)	Desogestrel	0.15
EE, 20 μg 24/4 (24 estrogen/progestin tablets)	Yaz[a,b,c]	EE	Drospirenone	3.0
	Minastrin 24 Fe chewable	EE	Norethindrone acetate	1
EE, 20 μg extended cycle (84 estrogen/progestin tablets, 7 tablets 10 μg EE)	LoSeasonique[a]	EE	Levonorgestrel	0.1
EE, 20 μg extended cycle (all estrogen/progestin tablets)	Lybrel[a]	EE	Levonorgestrel	0.09
EE, 10 μg 24/4 (24 estrogen/progestin tablets, 2 tablets 10 μg EE)	Lo Loestrin Fe[a]	EE	Norethindrone	1
Estradiol valerate, quadriphasic	Natazia[d]	EV (mg) (3/2/2/1)	Dienogest	2/3
Progestin only	Micronor[a]	N/A	Norethindrone	0.35

[a]Generic versions available.

[b]Indicated for the treatment of acne in women desiring to use oral contraception.

[c]Indicated for the treatment of premenstrual dysphoric disorder in women desiring to use oral contraception.

[d]Indicated for the treatment of heavy menstrual bleeding in women desiring to use oral contraception.

EE, Ethinyl estradiol; *EV*, estradiol valerate.

Daily use is critical to contraceptive efficacy of COC, so failure rates are largely attributable to poor adherence but may also be influenced by age and frequency of sexual intercourse. The Pearl index is a method of measuring contraceptive effectiveness in clinical trials, with a lower Pearl index representing a lower chance of unintended pregnancy. Failure rates range from less than 1 per 100 woman-years (Pearl index) with excellent adherence to more than 15 pregnancies per 100 woman-years with low adherence. Typical first-year combination oral contraception failure rates are estimated at 7 per 100 women.[26] There does not appear to be any significant difference in contraceptive efficacy of COCs formulated with different progestins.[27]

Traditionally, COCs have been started on the first day of menses, but the pills can be safely started at any time if pregnancy has been excluded (the quick-start method).[8] It is unclear whether this approach reduces unplanned pregnancies occurring while women are waiting until menses to start the pills.[28] If the COCs are inadvertently taken during pregnancy, they do not appear to increase the rate of miscarriage or adversely affect the developing fetus.[29] Of course, use of COCs may delay the diagnosis of an early pregnancy.

The importance of daily administration for contraceptive efficacy must be communicated to patients. Some women link pill taking with a daily ritual (e.g., tooth brushing) or use daily reminders through email or text messaging. Clear instructions for managing missed pills are an essential component of COC counseling. If a woman misses one tablet, she should take the missed pill as soon as possible even if it means taking two pills on the same day. She should then continue to take one tablet daily; no additional contraceptive protection is needed.[8] If she has missed two or more consecutive tablets, she should take the most recent pill and continue taking the remaining pills at the usual time, even if it means taking two pills on the same day. In this instance, she should also use an additional form of contraception (e.g., condoms) for 7 days. If two or more pills are missed in the third week of the 28-day pack, she can omit the HFI in the current pack and start a new pack. Emergency contraception can also be considered in these cases. These instructions also apply to women with vomiting and severe diarrhea while taking COCs. COCs are not suitable for women who consistently miss pills, because this undermines contraceptive efficacy. In these cases, a method that does not require daily adherence (e.g., contraceptive rings; patches; an injectable, intrauterine, or implantable method) should be considered.

After discontinuation of COCs, most women rapidly resume ovulation. In some women, ovulation may be temporarily delayed for several months after discontinuing oral contraception; however, 12-month conception rates are no different in former pill users compared with women who discontinue other contraceptive methods.[30]

Noncontraceptive Health Benefits

Educating women regarding potential noncontraceptive benefits of COC can assist patients in making a prudent contraceptive choice and can increase oral contraception adherence and continuation. For many users, COCs offer substantial noncontraceptive health benefits, including enhanced menstrual regularity; improved pelvic pain; reduced premenstrual symptoms; reduction in benign breast diseases, including fibroadenoma and fibrocystic changes; management of acne; and preservation of bone mineral density (BMD).[11,31] The benefits to bone health are an important consideration in perimenopause for women who have been hypoestrogenic and those with hypothalamic amenorrhea, where accelerated bone loss occurs in addition to the need for contraception.[32] As with most contraceptives, COC use reduces the incidence of ectopic pregnancy, which is a common and potentially life-threatening condition.[33]

Reducing the risk of epithelial ovarian and endometrial cancer is an important noncontraceptive benefit of COC use. Although the incidence is low, ovarian cancer is the most common cause of death from gynecologic malignancy and the fifth most common cause of overall cancer death in women in the United States.[34] Meta-analysis data of cohort and case-control studies demonstrated a reduction in ovarian cancer risk in women who have ever used COC, although 185 women needed to use COC to prevent one ovarian cancer. COC use of more than 10 years appeared to reduce the incidence of ovarian cancer by more than 50%. These results do not appear to be dependent on the dose of sex steroids administered.[35] COCs appear to reduce risk of ovarian cancer in women at increased inherited risk of ovarian cancer through *BRCA1* and *BRCA2* genes.[36-40] However, this benefit must be carefully weighed against the possible increase in breast cancer risk associated with COC use in *BRCA1* and *BRCA2* carriers, which is discussed further in the following.

Endometrial adenocarcinoma is the most common gynecologic cancer in US women, and rising obesity rates put more women at risk. The risk of endometrial adenocarcinoma is reduced with COC use, with several meta-analyses demonstrating reduced risk of more than 50%.[41-43] This protective effect appears to be time dependent, with risk of endometrial cancer reduced by almost 70% with 8 years of COC use and with protection persisting for at least 20 years after oral contraception discontinuation.[43] It is unclear whether the level of protection varies among distinct COC formulations.

Around 6% of reproductive-age women meet diagnostic criteria for polycystic ovary syndrome (PCOS), and prevalence is likely to be higher in women considering COC use due to clinical features such as irregular menstruation and hyperandrogenism.[44] Use of COC leads to more regular vaginal bleeding patterns, prevents endometrial hyperplasia, and may reduce clinical and biochemical hyperandrogenism in PCOS women.[45] The impact of COC on carbohydrate metabolism and cardiovascular risk in this population is unclear.

Side Effects

Contrary to popular perception, randomized, placebo-controlled trials have not shown COCs to cause weight gain, nausea, breast tenderness, or mood changes.[46,47] Consequently, COCs are well tolerated by most users. Nevertheless, some individuals report side effects they attribute to COCs that may affect quality of life, contraceptive continuation, and patient satisfaction. Therefore, counseling about side effects or lack thereof is an important aspect of contraceptive care and may improve patient tolerance and adherence to COCs when they occur.[48]

Unscheduled vaginal bleeding is a common side effect attributable to COC. Unscheduled bleeding affects 30% to 50% of COC users during the initial 3 months of use, but the incidence declines with ongoing use. Unscheduled bleeding is more common with lower-dose (20 μg) than standard-dose (30 or 35 μg) ethinyl estradiol preparations, which increases discontinuation rates.[49] The influence of progestin type is unclear. In addition, unscheduled bleeding is more common in women using extended COC formulations in early cycles, but this bleeding will lessen over time. If unscheduled bleeding during extended COC use is bothersome, women may opt to stop active tablets for 3 days, thereby inducing

withdrawal bleeding, and then continue with active pills. This strategy has been shown to reduce subsequent unscheduled bleeding in this setting.[50]

Irregular bleeding may be a manifestation of pregnancy or disease such as cervical or endometrial infection, polyps, or neoplasia. Persistent or new-onset bleeding should therefore be investigated. Amenorrhea (absence of withdrawal bleeding) may occur with long-term COC use, and it may be more acceptable with counseling that provides appropriate reassurance.[48] Women who experience absence of withdrawal bleeding or who for other reasons suspect that they could be pregnant should use a urine pregnancy test.

Headaches are common, but there is a lack of high-quality evidence that COC use contributes to headaches.[51] Some users may experience headaches in early cycles, which tend to improve with ongoing use. Furthermore, estrogen withdrawal headaches that occur during the HFI may respond to a shorter duration HFI of 3 days.[52,53] Any new-onset or worsening headache with COC use must be evaluated. A history of migraine with aura is a contraindication for use of COC because of increased risk of stroke (described in the following).[54] COC use in women with a history of migraine without aura should be avoided in women with other risk factors for stroke.[52] Similarly, any COC users who experience increased frequency or intensity of any type of migraine headache should discontinue estrogen-containing contraceptives.

Health Risks

Extensive studies in large populations have established the risks and benefits of COCs for most women.[55] For the majority of women, use of COCs represents a safe contraceptive choice. However, clinicians should be aware of circumstances in which COC may pose health risks. The US Medical Eligibility Criteria for Contraceptive Use (USMEC), most recently updated in 2016,[9] were adapted from the World Health Organization (WHO) document *Medical Eligibility Criteria for Contraceptive Use*, 5th edition (Tables 18.2 and 18.3).[55a] The guidelines provide evidence-based advice on eligibility for use of hormonal contraceptives. Although the WHO document covered many conditions, such as postpartum status, breastfeeding, smoking, obesity, cardiovascular disease, diabetes, and cancer, the USMEC added new categories: endometrial hyperplasia, rheumatoid arthritis, solid organ transplantation, inflammatory bowel disease, bariatric surgery, and peripartum cardiomyopathy. The USMEC assists providers in deciding what contraceptive methods are appropriate for their patients to improve access to contraception, especially among women with medical problems for which providers may have been hesitant to prescribe contraception in the past.[9]

The safety of contraception in the USMEC is considered in four categories:

1. Conditions with no restriction on the use of the contraceptive method.
2. Conditions in which the advantages of the method usually outweigh the theoretical or proven risks. The method can generally be used, but careful follow-up may be required.
3. Conditions in which the theoretical or proven risks usually outweigh the advantages; examples of estrogen-containing methods include current gallbladder disease, diabetes with end-organ damage, controlled hypertension, and taking medications that may interfere with COC efficacy. The method is generally not recommended unless other more appropriate methods are not available or acceptable.
4. Conditions that present an unacceptable health risk if the contraceptive method is used; examples of estrogen-containing methods include delivery during the past 21 days, a personal history of deep venous thrombosis or pulmonary embolism, ischemic heart disease, stroke, known thrombogenic mutations, and migraine headaches with aura or other neurologic signs.

TABLE 18.2 US Medical Eligibility Criteria (USMEC) for Contraceptive Use Categories for the Use of Estrogen-Containing Contraception According to Medical Condition

Condition	USMEC
Smoking age ≥35 years	
<15 cigarettes/day	Risks outweigh benefits
≥15 cigarettes/day	Unacceptable risk
Obesity (body mass index ≥30)	Benefits outweigh risks
Hypertension	
Controlled hypertension	Risks outweigh benefits
Elevated blood pressure	
Systolic 140–159 mm Hg or diastolic 90–99 mm Hg	Risks outweigh benefits
Systolic ≥160 mm Hg or diastolic ≥100 mm Hg	Unacceptable risk
Vascular disease	Unacceptable risk
Diabetes	
No vascular disease	Benefits outweigh risks
Vascular disease or >20 years' duration	Either risks outweigh benefits or unacceptable risk (based on severity of condition)
Stroke	Unacceptable risk
Current or history of ischemic heart disease	Unacceptable risk
Multiple risk factors for cardiovascular disease (older age, smoking, obesity, diabetes, hypertension)	Either risks outweigh benefits or unacceptable risk (based on severity of condition)
Breast cancer	
Current	Unacceptable risk
Past and no evidence of disease for 5 years	Risks outweigh benefits
Migraines	
Without aura	Benefits outweigh risks
With aura	Unacceptable risk

Thromboembolic Disease

The established increased risk of venous thromboembolism (VTE) is related to the estrogenic component, and although modern low-dose preparations (≤35 μg) carry a lower risk than the original oral contraceptives, the incidence of VTE is still increased.[56] COC use increases risk of VTE by more than twofold, with an incidence of approximately 9 to 10 per 10,000 women per treatment year[57]

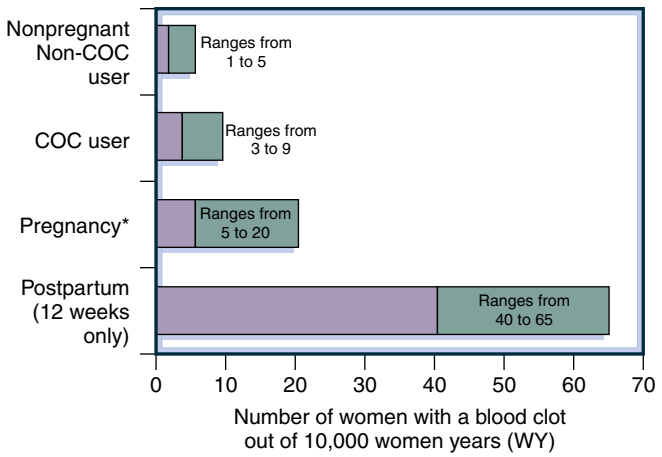
TABLE 18.3 US Medical Eligibility Criteria for Contraceptive Use Categories for the Use of Progestin-Only Methods of Contraception According to Medical Condition

Condition	POP	DMPA	Implant	Levonorgestrel IUD
Smoking age ≥35 years	No restriction	No restriction	No restriction	No restriction
Obesity ≥30 BMI	No restriction	No restriction	No restriction	No restriction
Menarche to age 18 years and ≥30 BMI	No restriction	Benefits outweigh risks	No restriction	No restriction
Hypertension				
Controlled hypertension	No restriction	Benefits outweigh risks	No restriction	No restriction
Elevated BP				
Systolic 140–159 mm Hg or diastolic 90–99 mm Hg	No restriction	Benefits outweigh risks	No restriction	No restriction
Systolic ≥160 mm Hg or diastolic ≥100 mm Hg	Benefits outweigh risks	Risks outweigh benefits	Benefits outweigh risks	Benefits outweigh risks
Vascular disease	Benefits outweigh risks	Risks outweigh benefits	Benefits outweigh risks	Benefits outweigh risks
Diabetes				
No vascular disease	Benefits outweigh risks	Benefits outweigh risks	Benefits outweigh risks	Benefits outweigh risks
Vascular disease or >20 years' duration	Benefits outweigh risks	Risks outweigh benefits	Benefits outweigh risks	Benefits outweigh risks
Stroke	I: Benefits outweigh risks C: Risks outweigh benefits	Risks outweigh benefits	I: Benefits outweigh risks C: Risks outweigh benefits	Benefits outweigh risks
Current or history of ischemic heart disease	I: Benefits outweigh risks C: Risks outweigh benefits	Risks outweigh benefits	I: Benefits outweigh risks C: Risks outweigh benefits	I: Benefits outweigh risks C: Risks outweigh benefits
Multiple risk factors for cardiovascular disease (older age, smoking, obesity, diabetes, hypertension)	Benefits outweigh risks	Risks outweigh benefits	Benefits outweigh risks	Benefits outweigh risks
Breast cancer				
Current	Contraindicated	Contraindicated	Contraindicated	Contraindicated
Past and no evidence of disease for 5 years	Risks outweigh benefits	Risks outweigh benefits	Risks outweigh benefits	Risks outweigh benefits
Migraines				
Without aura	No restrictions	No restrictions	No restrictions	No restrictions
With aura	No restrictions	No restrictions	No restrictions	No restrictions

BMI, Body mass index; *BP*, blood pressure; *C*, continuation; *DMPA*, depot medroxyprogesterone acetate; *I*, initiation; *IUD*, intrauterine device; *POP*, progestin-only pill.

(Fig. 18.2). Comparatively, in otherwise healthy reproductive-age women, the risk of VTE ranges from 1 to 5 per 10,000 women per year, whereas VTE risk in pregnancy is almost 29 per 10,000 women per year and even higher in the postpartum period.[58] Other risk factors for VTE include, but are not limited to, age, obesity, smoking, and thrombogenic mutations.[59-61] The best-quality prospective epidemiologic studies have not shown any increased risk of VTE with newer progestins (desogestrel and drospirenone) and the vaginal ring (etonogestrel) compared with older progestins (levonorgestrel and norethindrone).[62-65] These prospective cohort studies addressed important baseline confounders, including age, family history, and body mass index (BMI); categorized contraceptives users by duration of use; maintained regular contact with users; and individually validated each diagnosis of VTE.[66-68] Because COCs containing estradiol valerate are relatively new, there is limited data on their effects on risk of VTE.

National guidelines contraindicate COC use in those at increased risk for VTE, including those with a personal history of VTE, women immediately after delivery, women undergoing surgery with prolonged immobilization, and those with known inherited thrombophilic conditions.[9] In the context of inherited thrombophilias, clinicians need to consider VTE risk based on the type of thrombophilia present and its association with other risk factors, such as coexistence of multiple thrombophilias, obesity, age, and any VTE events during prior periods of hormonal exposure, such as pregnancy and estrogen-containing contraceptives.[69] Routine screening of the general population for familial thrombophilic markers is not recommended before initiating COC use.[9]



• **Fig. 18.2** Venous thromboembolism risk among women of reproductive age by various conditions. Pregnancy data based on actual duration of pregnancy in the reference studies. Based on a model assumption that pregnancy duration is 9 months, the rate is 7 to 27 per 10,000 women-years. *COC,* combined oral contraceptives. (From U.S. Food and Drug Administration. FDA Drug Safety Communication: Updated information about the risk of blood clots in women taking birth control pills containing drospirenone. Available at https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-updated-information-about-risk-blood-clots-women-taking-birth-control.)

Myocardial Infarction and Thrombotic Stroke

Although arterial events are substantially less common than VTE in reproductive-age women, the sequelae of strokes and myocardial infarctions are more devastating than those of VTE. The risk of arterial thrombosis including myocardial infarction and stroke is increased with COC use. The risk is estrogen dose dependent, with a 1.6-fold increase in thromboses with estrogen doses of 20 μg and up to a 2.4-fold increase in risk with estrogen doses greater than 50 μg; risk appears to be unrelated to progestin type.[70,71] This effect is estimated to account for 19 thrombotic strokes and seven myocardial infarctions per 100,000 women per treatment year.[71] Risk of arterial thrombosis does not appear to be increased with past COC use.[72]

The absolute risk of myocardial infarction and stroke increases with age. Therefore, COC should be avoided in women older than 35 years with risk factors for myocardial infarction or stroke, including smoking, hypertension, long-standing diabetes, and migraine headache with aura.[9]

Breast Cancer

Findings that exogenous estrogen-progestin combination hormone therapy increases breast cancer risk in postmenopausal women raises concerns that COCs may increase breast cancer risk in premenopausal women. Recent findings from a large national registry in Denmark including approximately 1.8 million women 15 to 49 years of age over a period of almost 11 years reported a modest increased relative risk of invasive breast cancer in current hormonal contraception users (most of which were COC users) of 1.20 (95% confidence interval [CI], 1.14–1.26) compared with never users.[73] The absolute increased risk of breast cancer was small, translating into approximately one extra breast cancer for every 7690 women using hormonal contraception for 1 year. The study adjusted for duration of hormonal contraceptive use, age, calendar year, education level, parity, PCOS, endometriosis, and family history of breast or ovarian cancer. However, methodologic limitations should be considered when interpreting these findings. The Danish researchers utilized a database that did not include information addressing several potential confounders, including age at menarche, history of breastfeeding, and alcohol consumption. In addition, investigators did not address possible differences between users and nonusers in breast cancer surveillance, such as screening mammography. Although the database had information on women 15 to 79 years of age, the analysis was limited to women younger than 50 years. This is unfortunate given that more than three quarters of invasive breast cancers are diagnosed in women age 50 years and older; not including this population eliminated the opportunity to assess the potential association of hormonal contraceptive use and risk of breast cancer during women's postmenopausal years.[74] A large prospective cohort study in the United Kingdom including more than 46,000 women followed for more than 40 years also reported an increased risk of breast cancer but also a reduced overall cancer risk (lower rates of colorectal, endometrial, ovarian, hematopoietic cancers) in ever users of COC compared with nonusers.[75] The authors concluded that most women who choose to use oral contraceptives do not expose themselves to long-term cancer harms; instead, with some cancers, many women benefit from important reductions of risk that persist for many years after stopping. These findings are consistent with a systematic review of oral contraceptive use and cancer risk that reported a reduction in cancer risk.[41]

In women with a family history of breast cancer, a systematic review of retrospective cohort studies and case-control studies show that COC use does not confer an increased risk of breast cancer.[76] However, recent data suggest that COC use may increase risk of early-onset breast cancer in women carrying *BRCA* gene mutations,[77] although previous reports have not found this association.[37,78] According to the current USMEC guideline, there are no restrictions on use of hormonal contraception in women with a family history of breast cancer or women with breast cancer susceptibility genes (e.g., *BRCA1* and *BRCA2*).[9]

Cervical Cancer

For current COC users, the risk of invasive cervical cancer increases with increasing duration of use (relative risk for ≥5 years' use vs. never use is 1.90; 95% CI, 1.69–2.13), as demonstrated in a meta-analysis of pooled data from 24 studies including more than 16,000 women with cervical cancer and 35,000 without cervical cancer. The risk declined after use ceased; however, after 10 or more years, it returned to the risk level of never users. A similar pattern of risk was seen for invasive and in situ cancer, as well as for women who tested positive for high-risk human papillomavirus strains.[79]

A more recent meta-analysis of case-control and cohort studies demonstrated an increased risk of cervical cancer beyond 5 years of treatment, but the effect was not statistically significant and the evidence was of insufficient strength.[41] Regular cervical cancer screening according to national protocols is advised for all sexually active women, regardless of contraceptive use. A history of cervical intraepithelial neoplasia or genital human papillomavirus infection is not a contraindication for COC use.[9]

Use of Concomitant Medications With Combined Oral Contraceptives Pills

Certain anticonvulsants (phenytoin, carbamazepine, barbiturates, primidone, topiramate, and oxcarbazepine) that induce hepatic

enzymes may reduce contraceptive efficacy of COCs.[9] Rifamycin antibiotics, including rifampicin and rifabutin, can also interact with contraceptive hormones and reduce the efficacy of the contraceptive during and up to 4 weeks after use.[80] With the exception of those in the rifamycin class, concomitant use of antibiotics has not been found to reduce efficacy of any hormonal contraceptives.[81] In addition, antiretrovirals may reduce efficacy of hormonal contraception, including some non-nucleoside reverse transcriptase inhibitors (efavirenz) and some ritonavir-boosted (/r) protease inhibitors (darunavir/r, fosamprenavir/r, lopinavir/r, saquinavir/r, and tipranavir/r).[9] If oral contraceptives are used with these medications, a formulation containing at least 30 μg of ethinyl estradiol and backup contraception in the form of condoms should be used.[9] Alternatively, high-dose progestin-only methods such as DMPA or intrauterine contraceptives may be preferable because their efficacy is not reduced by medications that induce liver enzymes.

Contraceptive Vaginal Ring and Transdermal Patch

The transdermal and transvaginal routes are safe and acceptable alternatives to COCs to deliver combined estrogen-progestin contraception. The contraceptive patch (Xulane, Mylan Pharmaceuticals, Morgantown, WV) and the contraceptive vaginal ring (NuvaRing, Merck & Co. Inc., Rockville, MD) offer women combination estrogen-progestin contraception without the need to take pills daily. In consistent users, contraceptive failure rates with the patch and ring are similar to those for oral contraceptives.[82] There is currently insufficient evidence to determine how body weight affects the contraceptive efficacy of the vaginal ring and contraceptive patch.[83]

It is important to note that all ethinyl estradiol contraceptives elevate VTE risk, regardless of whether the route of administration is oral, vaginal, or transdermal.[84] The contraceptive patch generates higher circulating levels of ethinyl estradiol than either COC or the vaginal ring.[85] It is uncertain whether use of the patch is associated with a higher risk of VTE than use of COC.[67,86] Contraindications to the patch and the vaginal ring are the same as those for COCs. In the absence of adequate evidence to determine how risks and noncontraceptive benefits of the patch and the ring compare with those of COC, they are usually considered to be equivalent.

As with COC pills, the contraceptive patch and vaginal ring act primarily by suppression of ovulation. The patch and ring are immediately effective if commenced within the first 5 days of the onset of menstruation, but because therapeutic steroid levels are achieved over the course of several days, starting at any other time requires 7 days of backup contraception, such as condoms or abstinence.[8]

Transdermal Contraceptive Patch

Xulane, the only contraceptive patch available in the United States, is a 4.5-cm tan square that releases 20 μg of ethinyl estradiol daily along with norelgestromin, the biologically active metabolite of the progestin norgestimate[87] (Fig. 18.3). A new patch is applied each week on the same day for 3 weeks, followed by a patch-free week, during which withdrawal bleeding is anticipated. Sweating associated with vigorous exercise, swimming, and use of a hot tub or sauna should not result in patch detachment. Unscheduled bleeding in patch users is similar to that seen with COCs.[82] Although patch users are more compliant than pill users, they are more likely to report breast discomfort, dysmenorrhea, nausea, and vomiting.[82,88] Mild local skin reactions are common. An investigational contraceptive patch is being developed that releases less ethinyl estradiol than the Xulane transdermal contraceptive.[89]

• **Fig. 18.3** The contraceptive patch.

• **Fig. 18.4** The contraceptive vaginal ring.

Contraceptive Vaginal Ring

The vaginal mucosa offers excellent absorption of sex steroids. The only contraceptive vaginal ring available in the United States is the NuvaRing, a flexible plastic ring that is 4 mm thick and has an outside diameter of 54 mm.[90] The ring releases 15 μg of ethinyl estradiol daily along with etonogestrel, the biologically active metabolite of the progestin desogestrel (Fig. 18.4). The contraceptive vaginal ring is inserted in the vagina for 3 weeks and then withdrawn for 1 week, during which withdrawal bleeding is anticipated. A new ring is required every 4 weeks. The ring does not require individual fitting; as long as it remains in the vagina, appropriate absorption of steroids occurs. The ring is well tolerated, with increased physiologic vaginal discharge being the main bothersome side effect.[88] Rates of unscheduled bleeding and spotting are lower with the ring than with oral COC.[82] Spontaneous expulsion is uncommon. The ring can also be used in extended-cycle regimens.[91]

Women's interest in using a contraceptive vaginal ring varies; some women are highly motivated and comfortable with this method. Some users keep the ring in place during sexual relations; in this setting, male discomfort is uncommon. Other users prefer to remove the ring before intercourse, and removal for less than 48 hours does not appear to impair efficacy.[8] However, frequent removal for long periods is not recommended, and the label states a maximum of 3 hours is allowed.[92] Backup contraception is required for 7 days if the ring is removed for 48 hours or longer.

Progestin-Only Contraceptive Methods

Progestin-only contraceptives offer many advantages over estrogen-containing methods. There are fewer contraindications to progestin-only contraception (see Table 18.3). Progestin-only contraceptives may be appropriate for many women with contraindications to contraceptive doses of estrogen, including those at elevated baseline risk for VTE, women 35 years or older who smoke, who have hypertension, or who have long-standing diabetes. In addition, they can be used immediately after delivery, whether or not the patient is breastfeeding.

Progestin-only methods include the following:

- the POP (minipills),
- DMPA injections (DMPA-IM [Depo-Provera, Pfizer, New York, NY], DMPA-SC [Depo-subQ Provera]),
- the etonogestrel implant (Nexplanon [Merck & Co., Whitehouse Station, NJ]), and
- levonorgestrel IUDs (LNG IUDs; the larger-frame devices Mirena [Bayer Healthcare Pharmaceuticals] and Liletta [Medicines360, San Francisco, CA] and the smaller-frame devices Kyleena and Skyla [Bayer Healthcare Pharmaceuticals]).

These methods comprise various types of progestins, doses, and routes of administration. The dose of progestin in the "minipill" available in the United States (0.35-mg norethindrone) is one third that typically found in combined hormonal contraceptives formulated with this progestin. A stable serum concentration of 150 to 200 pg/mL of levonorgestrel occurs after insertion of the 52-mg LNG IUD,[93] a concentration substantially lower than that seen in women using a 150-µg levonorgestrel-containing oral contraceptive pill (6.2 ng/mL).[94]

Although POPs, implants, and the LNG IUD do not appear to increase risk of VTE, data for DMPA are less clear.[95-99] A twofold increased risk of VTE with progestin-only injectables was found in meta-analysis[96] based on two studies; however, residual confounding weakens the result.[100-102] If DMPA does increase VTE risk, the magnitude of this increase is likely less than that seen with COCs. The USMEC allows the use of DMPA in women with VTE and those at elevated risk for thromboembolism.

Progestin-Only Oral Contraceptive Pill

Only one POP formulation is marketed in the United States: norethindrone in 0.35-mg tablets (e.g., Micronor, Nor-QD, and generics). The progestin dose is lower than the dose in any combination oral contraceptive. Due to the short half-life of POPs, serum steroid levels decline to near baseline as quickly as 24 hours after administration, and a dose is considered missed if it has been more than 3 hours beyond an expected dose.[8] Norethindrone POPs are dispensed in packs of 28 active pills, which are taken continuously (i.e., no HFI) (Table 18.4). Contraindications to POPs are few (see Table 18.3).

TABLE 18.4 Summary and Recommendations for Progestin-Only Oral Contraceptive Use

1. Progestin-only contraception is an option for women in whom an estrogen-containing contraceptive is either contraindicated or causes additional health concerns.
2. Ovulation is not consistently suppressed; the main contraceptive actions of progestin-only oral contraception are effects on cervical mucus and the endometrium.
3. The typical user failure rate with progestin-only oral contraception is estimated to be more than 7%. Women choosing progestin-only oral contraception are often subfertile as a result of breastfeeding or older reproductive age, so the failure rate in these populations may be lower than in more fertile populations.
4. It is essential that the pill be taken at the same time each day to maximize contraceptive efficacy.
5. Menstrual irregularities are common in users of progestin-only oral contraception and represent the most frequent cause for contraceptive discontinuation.

Mechanism of Action

The mechanism of action of progestin-only oral contraceptives may include suppression of ovulation, thickening of cervical mucus, and induction of endometrial atrophy. Unlike with COCs, ovulation is not consistently suppressed with the 0.35-mg norethindrone POP, and the progestin effects on cervical mucus and the endometrium are critical factors in preventing conception.[103] Within hours of administration, progestin-only oral contraceptives thicken cervical mucus, which acts as a barrier to stop sperm at the cervical canal; 48 hours of norethindrone POP use is necessary to achieve full contraceptive effects on cervical mucus.[8] In European countries, a POP containing 75 µg of desogestrel is available. In contrast with the norethindrone POP available in the United States, the desogestrel POP inhibits ovulation, and contraceptive efficacy is as high as with COCs.[104]

Efficacy

The efficacy of progestin-only oral contraceptives is not well established.[105] National survey data used to estimate contraceptive failure rates with typical use have failed to distinguish between COCs (the larger group) and POPs. A potential confounding factor is that women using progestin-only oral contraceptives may have reduced fertility as a result of breastfeeding or older reproductive age. It is possible that the typical first-year failure rates (7%) with progestin-only oral contraceptives are higher than those seen with COCs.[26] In general, norethindrone 0.35-mg POPs should not be considered a first-line contraceptive except in women who should not or do not wish to use estrogen. Given concerns regarding the efficacy of norethindrone 0.35-mg pills in women with normal fertility, some experts recommend that women with normal fertility who choose to use POPs (e.g., a 35-year-old hypertensive woman) take *two* daily tablets (two tablets as a single dose, off-label). A tablet combining 1 mg of norethindrone acetate and 5 µg of ethinyl estradiol is marketed for treatment of menopausal symptoms.[106] This dose of norethindrone acetate appears adequate to consistently suppress ovulation; in addition, the dose of estrogen is small. Accordingly, some clinicians prescribe this combination medication off-label to women requiring contraception who medically are not candidates for conventional combination estrogen-progestin contraceptives. Such patients should be advised that the 1-mg norethindrone acetate/5-µg ethinyl estradiol combination is not approved for contraceptive use, and condom backup is

recommended. Of note is that the progestational impact of 1-mg norethindrone acetate is similar to that of 1-mg norethindrone.

Starting the Progestin-Only Pill

Progestin-only oral contraceptives can be initiated within the first 5 days of menses, without need for backup contraception. Some clinicians initiate progestin-only oral contraceptives at any time in the cycle, as long as the clinician is reasonably certain that the patient is not pregnant. With this strategy, backup contraception should be used for the first 2 days of use.[8] Because of the short duration of action and the short half-life of POPs, the pill must be taken at the same time each day to maximize contraceptive efficacy. A backup contraceptive (e.g., condoms) should be used for at least 2 days if the progestin-only oral contraceptive is taken more than 3 hours late or forgotten on any day. The patient should also resume taking daily progestin-only oral contraceptives as soon as possible. Progestin-only oral contraceptives may be initiated immediately or within 7 days after induced or spontaneous abortion. If not administered immediately after abortion, the woman must use alternative contraception or abstinence for 2 days. After delivery, POPs can be started at any time, including immediately postpartum in breastfeeding and nonbreastfeeding women.[107] If the woman is more than 21 days postpartum and is not using POPs to augment lactational amenorrhea, then backup contraception should be used for 2 days.[8]

Side Effects of Progestin-Only Oral Contraceptives

Sonographic studies have shown that prominent ovarian follicular activity is more common among progestin-only oral contraceptive users than among other women, but this may wax and wane over time.[108] Transvaginal ultrasound follow-up of women during a 3-year clinical trial of the small-frame LNG IUD (Skyla) found that ovarian cysts (defined as >3 cm) were present in 1.6% of participants at screening and 1.1% to 2.4% of participants at each subsequent visit. Eighty-eight percent of these cysts were 5 cm or less; none were greater than 8 cm. No ovarian cysts persisted beyond 9 months, which is consistent with a functional etiology.[109] Other than reassurance and sonographic follow-up, no intervention is required in asymptomatic women found to have asymptomatic incidental ovarian cysts during use of progestin-only contraceptives. If a follow-up sonogram in 6 to 8 weeks demonstrates resolution or decrease in size of ovarian cysts, no further evaluation is required.

Side effects with POPs other than changes in bleeding patterns are relatively uncommon.[103] Weight gain has not been objectively documented, and headaches are uncommon. Unscheduled bleeding, spotting, and amenorrhea are common menstrual patterns during progestin-only oral contraceptive use, and users should be counseled accordingly. Interpreting signs and symptoms of pregnancy, whether intrauterine or extrauterine, can be challenging. Pregnancy testing is appropriate for POP users experiencing nausea, breast tenderness, a change in menstrual pattern, or lower abdominal pain (see Table 18.2).

Other Effects

Most studies have reported that progestin-only oral contraceptives have little impact on carbohydrate metabolism.[110] However, one study conducted in Latina women observed that lactating women with a history of gestational diabetes who used progestin-only oral contraceptives after delivery had an almost tripled risk of diabetes compared with those who used low-dose COCs.[111] All women with gestational diabetes, regardless of contraceptive used, should be screened for diabetes postpartum with a 75-g, 2-hour oral glucose tolerance test.[112]

As with all contraceptives, POPs lower overall risk for ectopic pregnancy. A history of ectopic pregnancy does not contraindicate progestin-only oral contraceptive use. However, if pregnancy occurs, the likelihood that the pregnancy is ectopic is higher in POP users than in nonusers of contraceptives.[103] Bone density does not appear to be adversely affected by the POP. The only study assessing skeletal health in progestin-only oral contraceptive users was conducted in breastfeeding women; progestin-only oral contraceptives, in fact, protected against small, reversible losses in BMD occurring during lactation.[113] Finally, progestins usually suppress endometrial growth.[108] However, few epidemiologic data address the effect of progestin-only oral contraceptives on endometrial cancer risk or on any cancer risk.

Progestin-Only Oral Contraceptives During Lactation

Progestin-only oral contraceptives do not interfere with the quality or quantity of breast milk.[107] Very little progestin is passed from nursing mothers into their breast milk, and no adverse impact on infant growth has been observed. Package labeling advises delaying administration in lactating women until 6 weeks after delivery. A trial conducted in the United States randomized breastfeeding women to initiate progestin-only compared with combination estrogen-progestin oral contraceptives 2 weeks postpartum. At 8 weeks postpartum, approximately two-thirds of women continued to breastfeed in both groups.[114] Based on the absence of data suggesting harm to the infant or mother and the contraceptive benefits of early initiation, some experts recommend initiation of POPs before hospital discharge and no later than the third postpartum week, regardless of lactation status.

Depot Medroxyprogesterone Acetate for Contraception

DMPA is an injectable, progestin-only contraceptive that provides effective, private, and reversible contraception. It is discrete, avoids the need for user action daily or near the time of sexual intercourse, and avoids the need for partner cooperation (Table 18.5).

Formulations and Pharmacology

DMPA is available in two formulations: 150 mg/1 mL for intramuscular injection and 104 mg/0.65 mL for subcutaneous injection. The injections can be given every 3 months because low solubility of the microcrystals at the injection site allows pharmacologically active drug levels to persist for several months. DMPA primarily acts by inhibiting follicular maturation and ovulation through inhibition of gonadotropin secretion. Unlike other progestin-only contraceptives, mean estradiol levels may be lower than normal for cycling premenopausal women.[115]

DMPA is an effective contraceptive. Following a 150-mg intramuscular injection, failure rates in clinical trials have ranged from 0.0 to 0.7 per 100 woman-years. In clinical practice, typical-user failure rates are 4 failures per 100 woman-years, reflecting the fact that some users do not return for their injections as scheduled.[26] Because progestin levels are high, efficacy is not reduced by obesity or use of concurrent medications, such as anticonvulsants. For the 104-mg subcutaneous injection, no contraceptive failures were reported in phase III clinical trials.[116] This formulation is relatively new, so typical-user failure rates are not available, but they are expected to be similar to those for the intramuscular preparation.

TABLE 18.5 Summary and Recommendations for DMPA Use

1. DMPA is an excellent method of contraception for women who desire a long-term, reversible contraceptive method.
2. DMPA primarily acts by inhibiting follicular maturation and ovulation through inhibition of gonadotropin secretion; it also affects cervical mucus.
3. DMPA is available in two formulations: 150 mg/1 mL for IM injection and 104 mg/0.65 mL for SC injection.
4. DMPA can be administered as long as the provider is reasonably certain the patient is not pregnant. The dose is repeated every 3 months (13 weeks), with a 2-week grace period.
5. Although DMPA does not permanently affect endocrine function, return of fertility may be delayed.
6. Thorough, candid counseling about side effects is important. Women who are well informed when they choose this method of contraception are much more likely to become highly satisfied users with high continuation rates.
7. Menstrual changes occur in all women using DMPA and are the most frequent cause for discontinuation.
8. Because DMPA induces amenorrhea, it can be used for managing a variety of gynecologic and nongynecologic disorders, such as heavy menstrual bleeding, dysmenorrhea, and iron deficiency anemia.
9. There is no high-quality evidence that use of DMPA increases the risk of developing cancer, cardiovascular disease, or sexually transmitted infection. DMPA use significantly reduces the risk of developing endometrial cancer.
10. There is an association between current DMPA use and decreased bone mineral density; losses in bone mineral density are temporary, reverse after discontinuation of DMPA, and have not been linked to postmenopausal osteoporosis or fractures.

DMPA, Depot medroxyprogesterone acetate; *IM*, intramuscular; *SC*, subcutaneous.

Although users may be hypoestrogenic, vasomotor symptoms and vaginal atrophy are uncommon. Because of its progestin effect, DMPA also causes changes in cervical mucus that are hostile to sperm migration and endometrial atrophy. The newer subcutaneous injection is less painful and is available in a prefilled syringe, offering the potential for self-administration.[117] Intramuscular DMPA is available as a generic formulation that is less costly than subcutaneous DMPA. Otherwise, benefits and risks are similar for intramuscular and subcutaneous administration.[118]

Administration of DMPA

Starting Injections

The ideal time to initiate DMPA is within 7 days of the onset of menses.[8] This approach ensures that the patient is not pregnant at the time of injection and prevents ovulation during the first month of use, so backup contraception is unnecessary. Most women have pharmacologically active drug levels and a poor cervical mucus score within 24 hours after injection.[119] The "same-day," "quick-start," or "Depo-now" approach when a pregnancy test result is negative facilitates DMPA initiation for many users, and it may prevent some pregnancies.[120] However, there is a small possibility of undiagnosed pregnancy despite a negative pregnancy test result. When the quick-start approach is used for initiating DMPA, backup contraception or abstinence should be used for 7 days and a repeat pregnancy test should be performed in 2 to 4 weeks. Nevertheless, DMPA given inadvertently during pregnancy does not appear to be teratogenic.[121] DMPA may be initiated immediately after spontaneous or induced abortion or within the first 7 days.[8] If given in the first 7 days after the event, then backup contraception should be used. After delivery, DMPA can be started at any time, including immediately postpartum in breastfeeding and nonbreastfeeding women.[107,122] If the woman is more than 21 days postpartum and not breastfeeding exclusively, backup contraception should be used for 7 days after the first DMPA injection.[8]

Repeat Injections

Repeat injections of DMPA should be scheduled every 3 months (13 weeks). After a 150-mg injection, ovulation does not occur for at least 14 weeks. A 2-week grace period (repeat injection without pregnancy testing, up to 15 weeks following the prior injection) is appropriate for women receiving injections every 3 months.[8] In women more than 2 weeks late for an injection, a urine pregnancy test should be performed before administering further DMPA, and backup contraception for 7 days is advised.

Side Effects of DMPA

Candid counseling regarding the side effects of DMPA and the need for timed injections must be provided. Women who are well informed when they choose this method of contraception are more likely to become satisfied users with high continuation rates.[123] Menstrual changes occur in almost all women using DMPA and are the most frequent cause for discontinuation of injectable and all other progestin-only contraceptives.[124] Proactive patient education before the initiation of DMPA and supportive follow-up can improve tolerance of menstrual changes. During the first months of use, episodes of unpredictable bleeding and spotting lasting 7 days or longer are common. Bleeding decreases with longer duration use, and at 1 year, 50% of women experience amenorrhea; this rate increases to 75% with long-term use.[115] Similar bleeding patterns are reported with the subcutaneous preparation.[125] Many women view amenorrhea (along with a reduction or elimination of menstrual cramps) as one of the advantages of using this method.

There are no established methods for predicting, preventing, or treating unscheduled bleeding in DMPA users. Small studies have shown that estrogen supplements (e.g., 1.25 mg of conjugated estrogen orally, 1–2 mg of micronized estradiol orally, or 0.1-mg estradiol patches for 10–20 days) may terminate a bleeding episode.[126] Another treatment option endorsed by the US Selected Practice Recommendations for Contraceptive Use is nonsteroidal anti-inflammatory drugs for 5 to 7 days. However, a systematic review concluded that there is a lack of high-quality data to support routine clinical use of any interventions to treat persistent unscheduled bleeding with progestin-only contraceptives.[125]

Observational studies have not reported any consistent effects of DMPA on mood.[127] Progestins may cause or exacerbate depressive symptoms in certain subpopulations of women, including those with a history of premenstrual syndrome or mood disorders. However, depression is not a contraindication to DMPA use.[128] The impact of DMPA on weight has been controversial.[129] Randomized controlled trials, although limited, fail to show that DMPA causes weight gain.[115,130] Observational studies are difficult to interpret given that individuals tend to gain weight over time irrespective of contraceptive use. On average, the weight gain with DMPA use is small (2 kg); however, there is marked individual variation. Weight gain with the use of DMPA may be associated with user subgroups at particular risk for obesity, including adolescents and ethnic minorities.[131] In the US CHOICE study, women who chose DMPA were more likely to be African-American

than those who chose other contraceptives. Weight changes varied among women choosing different progestin-only methods; however, being of the black race predicted weight gain regardless of the contraceptive chosen.[132,133] It is reasonable to monitor weight over time in women using DMPA.[8]

Risks and Benefits of DMPA

DMPA has been used to manage a variety of gynecologic and nongynecologic disorders. The tendency of DMPA to cause amenorrhea makes it a particularly appropriate contraceptive choice for women with heavy menstrual bleeding, dysmenorrhea, or irondeficiency anemia. DMPA is a useful means of suppressing menstrual bleeding and managing menstrual hygiene in individuals with special needs (e.g., cognitive impairment, military personnel, wheelchair-bound individuals).[115,134]

Progestins inhibit endometriotic tissue growth by directly causing initial decidualization and eventual atrophy, and by inhibiting pituitary gonadotropin secretion and ovarian estrogen production. Randomized trials show that DMPA is more effective than oral contraceptives and danazol and as effective as leuprolide injections for treatment of pain associated with endometriosis.[130] In the United States, subcutaneous DMPA is approved for the treatment of pain associated with endometriosis.

Effect on Cancer Risk

Large case-control studies conducted by the WHO have shown that use of DMPA is associated with an 80% reduced risk of endometrial cancer and does not affect the incidence of cervical cancer.[135-137] A more recent Thai case-control study found that use of DMPA is associated with protection against epithelial ovarian cancer, similar to that seen with use of COCs.[138] Multicountry data from the WHO and data from the United States, South Africa, and New Zealand provide reassurance that use of DMPA is not associated with an increased risk of breast cancer.[139]

Effect on Cardiovascular Risk

DMPA has an adverse effect on circulating lipids but does not increase production of coagulation factors and has no adverse effect on blood pressure. No adverse clinical effects on cardiovascular disease have been observed.[102] Based on these findings, the USMEC allows DMPA and other progestin-only contraceptives for use in women with a history of VTE and those in whom use of combination estrogen-progestin contraceptive is contraindicated.[9] This recommendation is different from package labeling for DMPA (written in the 1960s), which indicates that a prior history of VTE is a contraindication to DMPA use. In women with multiple risk factors for cardiovascular disease (e.g., smoking, older age, hypertension, diabetes), the USMEC classifies DMPA as category 3 (see Table 18.3), indicating that the risks of use may exceed the benefits. The basis for this caution, which the authors do not support, seems to be the hypoestrogenic effects of DMPA and reduced high-density lipoprotein (HDL) levels. In addition, the effects of DMPA might persist for some time after discontinuation, so it would not be immediately reversible if there were an adverse event.

Effect on Skeletal Health

DMPA's impact on BMD has generated much controversy. *Bone mineral density* is defined as the amount of mineral matter per volume of bone and directly correlates with bone strength.[140] DMPA injections suppress the secretion of gonadotropins, decreasing ovarian production of estrogen, which results in a decline in BMD. In 2004, the US Food and Drug Administration (FDA) added a "black box" warning to DMPA labeling about declines in BMD, which might discourage health care providers from initiating DMPA or limit long-term use.[141] Pregnancy, breastfeeding, menopause, and use of hormonal contraceptives can all impact BMD by affecting sex hormones.

Both cross-sectional and longitudinal studies using dual-energy x-ray absorptiometry technology to evaluate current users of DMPA have observed lower BMD in current DMPA users compared with nonusers.[142-149] Longitudinal studies report BMD losses of –3.1% to –5.8% and –4.1% to –5.7% at the hip and spine, respectively, after 2 years of use, and losses of –4.5% to –7.7% and –4.9% and –6.6% after 4 years of use.[147,148] Most of the decline in BMD is observed within the first 2 years of use, suggesting that longer use may not progressively increase risk of osteoporosis.[147] Decline in BMD appeared to be substantially or completely reversible, as seen in studies including both adults and adolescents, with a duration of DMPA use of 2 to 5 years and follow-up to 5 years after discontinuation.[145,147,150-152]

DMPA use and breastfeeding are both accompanied by hypoestrogenemia. The reversible declines in BMD associated with DMPA use, followed by recovery when the DMPA is discontinued, parallels the BMD trends seen with breastfeeding.[153,154] Cross-sectional studies demonstrate that BMD in adult women who previously used DMPA is similar to that of women who never used injectable contraception, an observation that provides further reassurance that loss of BMD associated with DMPA use is reversible.[155-157]

Changes in BMD resulting from use of DMPA are clinically important if they increase risk for fracture. No published data address whether premenopausal DMPA impacts subsequent fracture risk in postmenopausal women. Although the association between decline in BMD and risk of fracture is well established in postmenopausal women,[158] the association is less robust in healthy premenopausal women.[134] Observational studies have mixed findings on the association of DMPA use and fracture risk in reproductive-age women. Three observational studies used large national databases to examine the association between DMPA or LNG IUD use and fracture.[159-161] Two of these were based on the same large United Kingdom database.[159,160] Using case-control methods, ever users of DMPA had a higher risk of fracture compared with never users (odds ratio [OR], 1.44).[159] Using the same British database but a retrospective cohort analysis, a second group also observed that DMPA users had an increased risk for fracture (OR, 1.41). However, this second report noted that the elevated risk was present at baseline, *prior* to DMPA use; accordingly, the elevated fracture risk could not have been caused by DMPA.[160] A Danish case-control study also noted that ever use of DMPA was associated with increased risk for fracture (OR, 1.44). However, the authors of this Danish report suggested that the subgroup of women choosing DMPA, 0.1% of the study sample, were not representative of the larger Danish population, limiting attribution of their findings.[161] These British and Danish studies raise the issue that women who choose DMPA are different from women who choose other methods of contraception (as noted also in the US CHOICE study[132]) and hypothesize that fracture risk associated with DMPA exposure may be due to unmeasured confounders in women who choose injectable contraception, including behavioral differences. For example, authors of the Danish study point out the prevalence of alcoholism (a condition known to be associated with fractures from motor vehicle and other accidents) in women using DMPA was 14%, sevenfold higher than in women

not using DMPA; furthermore, the Danish investigators note that cases with fractures were some threefold more likely to be classified as alcoholics as control women.

When counseling women considering initiation or continuation of injectable contraception, clinicians should discuss the benefits and the risks of DMPA, including the FDA black box warning, and use clinical judgment and shared decision making to assess appropriateness of use. The effect of DMPA on BMD on skeletal health should not prevent clinicians from recommending DMPA initiation or continuing use beyond 2 years (USMEC category 2 for adolescents <18 years of age and women >45 years of age; category 1 for women age 18–45 years).[9] Routine assessment of BMD is not recommended in adolescents and young women using DMPA. Although low-dose estrogen supplementation has been observed to limit BMD loss in adolescent DMPA users,[162,163] estrogen supplementation during DMPA use is not recommended due to potential adverse effects and a paucity of data from clinical trials assessing skeletal health outcomes.

For patients with comorbidities and conditions that may impact skeletal health, including an elevated risk of falls, wheelchair use, chronic corticosteroid use, renal disease, or malabsorption, individualized counseling and clinical management along with shared decision making is appropriate. Age-appropriate calcium and vitamin D dietary intake, regular weight-bearing exercise, and smoking cessation should be encouraged for all women. Although these recommendations may benefit the health of patients in general, studies demonstrating that these measures will improve skeletal health outcomes in women using injectable contraception are lacking.

Effect on Sexually Transmitted Infections

The association between use of DMPA and risk of STIs is uncertain. Some studies show an increased risk of STI in DMPA users compared with other contraceptives, but it is unclear whether this reflects differences in sexual practices, such as lower condom use, in DMPA users.[164,165] The relationship between DMPA use and human immunodeficiency virus (HIV) acquisition and transmission is also unclear, but several clinical trials show an increased risk.[166] There are several plausible biologic mechanisms through which DMPA may increase HIV transmission risk.[167,168] DMPA is widely used in the areas of high HIV prevalence where alternative contraceptive options are limited. A systematic review of progestin injectables found a possible increased risk for HIV acquisition (adjusted hazard ratio, 1.4; 95% CI 1.2–1.6) among women at increased risk.[166] It is unknown whether this association is due to a real biologic effect, because there were methodologic issues causing the evidence to be inconclusive. The current USMEC recommendation states that the advantages of DMPA continue to outweigh the theoretical or proven risks (USMEC category 2) among women at high risk for HIV infection but that women should be counseled regarding these issues.[165] The USMEC emphasizes that women should not be denied DMPA because of these concerns, as there is a very real risk that unintended pregnancies and maternal morbidity and mortality would result. Women with HIV or those at risk for HIV should be counseled to always use condoms to prevent HIV and other STI transmission.[165]

Effect on Return of Fertility

Although DMPA does not permanently impact endocrine function, return of fertility may be delayed after stopping DMPA use. Within 10 months of the last injection, 50% of women who discontinue DMPA to become pregnant will conceive. However, in some women, fertility is not reestablished until up to 18 months after the last injection.[115] The persistence of ovulation suppression after DMPA discontinuation is not related to the duration of use, but it is related to weight, as clearance is slower in heavier women.[169] Before initiating DMPA contraception, clinicians should counsel candidates about the possible prolonged duration of action. Women who may want to become pregnant within the next year should choose an alternative contraceptive.

Progestin-Releasing Intrauterine Devices

LNG IUDs provide highly effective, safe, convenient, and reversible contraception.[170] In the 1980s, intrauterine contraception use in the United States fell dramatically after earlier flawed studies reported an association between intrauterine contraception use and later tubal infertility. It is now acknowledged that modern intrauterine contraception not only is highly effective but also is safe for most women to use.[171] Use of IUDs has increased in the United States, with the prevalence of use rising from 2% to more than 10% from 2002 to 2012 among women using contraception. Most IUDs used by US women are progestin-releasing devices.[172] The addition of progestin to the IUD increases contraceptive efficacy, and the 52-mg LNG IUD is approved in the United States not only for contraception but also for treatment of heavy menstrual bleeding. The LNG IUD provides other off-label therapeutic benefits, including treatment of pain associated with endometriosis, treatment of symptoms associated with uterine adenomyosis, treatment of endometrial hyperplasia or carcinoma, and protection of the endometrium in women during use of menopausal estrogen therapy.

Contraceptive Uses

Mirena, Liletta, Kyleena, and Skyla are the four LNG IUDs marketed in the United States. Mirena and Liletta are larger higher-dose T-shaped devices with a reservoir containing 52 mg of levonorgestrel. These devices deliver 20 μg of levonorgestrel per day. Although Mirena is approved for up to 5 years of use, it provides contraceptive efficacy for a longer duration. In a US study of 496 women who had used the LNG IUD for 5 years, overall, two pregnancies occurred in the LNG IUD group during the sixth and seventh years of use. The failure rate in the sixth year of use was 0.25 per 100 women-years (95% CI, 0.04–1.42); in the seventh year of use, the failure rate was 0.43 per 100 women-years (95% CI, 0.08–2.39).[173] Accordingly, women, particularly women older than 35 years, can be offered the opportunity to retain their 52-mg LNG IUDs for up to 6 or 7 years of use, recognizing that such use is off-label.[174] Although Liletta is approved for up to 4 years of use (as of early 2018) but given that it has the same quantity of levonorgestrel as Mirena, it is expected to eventually be approved for 5 or more years of use.

Kyleena and Skyla are smaller and lower-dose LNG IUDs, containing 19.5 mg and 13.5 mg and releasing 17.5 μg/day and 14 μg/day of levonorgestrel, respectively. Kyleena and Skyla are approved for up to 5 and 3 years of use, respectively. These small-frame devices are distinguished from Mirena and Liletta by their smaller size (30 mm × 28 mm compared with 32 mm × 32 mm) and a silver ring at the top of the vertical stem (visible with ultrasound). The inserter diameter is also narrower than Mirena (3.8 mm vs. 4.5 mm). The smaller dimensions of the Kyleena and

Skyla devices make them particularly suitable for women with smaller uterine cavities or tight cervices.[175] Data from large international studies confirm extremely low pregnancy rates with the various LNG IUD devices, ranging between 0.1 and 0.3 per 100 woman-years.[26,176,177]

Despite endometrial suppression, fertility returns rapidly after contraceptive removal.[178] The high contraceptive efficacy of LNG IUDs reflects the thickening cervical mucus and profound endometrial suppression caused by locally high concentrations of progestin. Most LNG IUD users continue to ovulate, even when amenorrhea is present.[176] There are few contraindications to IUDs, and most women are appropriate candidates—including adolescents and nulliparous women.[170] Contraindications to IUD use are pregnancy, active cervicitis or uterine infection, malignancy in the uterus or cervix, a distorted uterine cavity, unexplained abnormal bleeding, and adverse reaction to product ingredients.[179] Although systemic concentrations of levonorgestrel are very low in LNG IUD users, use of hormonal contraceptives is generally avoided after breast cancer.

Expanding the Use of IUDs

There is growing consensus that women in the United States will benefit from broader use of IUDs, including the LNG IUD.[171] The Contraceptive CHOICE Project in St. Louis, Missouri, provided women (n = 5000) who did not desire pregnancy for at least 1 year with free reversible contraceptives for up to 3 years. This project has shown that with standardized counseling and no financial barriers to contraceptive acquisition, the majority of women (68%) will choose long-acting reversible contraceptive methods (45% LNG IUD, 10% copper IUD, and 13% etonogestrel implant).[180] Continuation rates at 12 and 24 months were 88% and 79% for the LNG IUD, 84% and 77% for the copper IUD, and 83% and 69% for the etonogestrel implant, respectively.[181] Satisfaction rates were also higher for long-acting reversible methods compared with other methods of contraception, such as oral contraceptives and the injectable DMPA. In addition, among women participating in the Contraceptive CHOICE Project, rates of unintended pregnancy, birth, and abortion were substantially lower than for other women in St. Louis and compared with women in the United States overall.[182] Adolescents in this study also frequently chose either IUDs or implants.[183] With appropriate counseling and management regarding STI prevention and anticipated side effects, it is now accepted that IUDs can be used safely and effectively in adolescents.[184,185] Adolescents and young women have higher continuation rates and lower repeat unintended pregnancy rates when using a method that does not require ongoing adherence.[132] Additionally, the use of long-acting reversible contraception immediately after abortion has been shown to decrease repeat abortion rates.[186,187]

Abnormal Bleeding, Expulsion, and Uterine Perforation

As with DMPA and other progestin-only contraceptives, unpredictable uterine bleeding is the most common reason for discontinuation of LNG IUDs.[188] Unscheduled bleeding is most common during the early months of LNG IUD use and tends to resolve with time. By 12 months, up to 50% of women have amenorrhea or infrequent bleeding. With the Kyleena and Skyla LNG IUD, by 12 months, approximately 38% and 26% of women have amenorrhea or infrequent bleeding, respectively.[189,190] Addressing patient preferences and assessing acceptance of menstrual disturbances are integral to efforts aimed at reducing early discontinuation rates. Adequate and specific counseling about likely changes in bleeding patterns before placement is essential for increasing patient acceptability.

Expulsion is the most common cause of IUD failure. The cumulative expulsion rate was 10.2 per 100 IUD users over 36 months and did not vary among LNG and copper IUDs in a US study.[191] Increased risk for expulsion occurs in nulliparous women, women with severe dysmenorrhea or uterine adenomyosis, those with uterine cavity abnormalities, and women with insertions immediately after delivery. The authors' anecdotal impression is that appropriate high fundal IUD insertion minimizes the likelihood of expulsion.

The USMEC allows immediate insertion after first-trimester (category 1) and second-trimester (category 2) abortions.[9] Postpartum insertion is recommended after a 4-week interval (category 1). Immediate postpartum insertion (following vaginal or cesarean birth) is also an option (category 2) within 10 minutes of placental delivery as long as there is no evidence of chorioamnionitis or puerperal sepsis.[192] Expulsion rates with immediate postpartum insertion range from 10% to 20%, with lower rates seen after cesarean delivery. Because expulsion usually occurs within the first few months, women are encouraged to follow up with their care provider within 12 weeks of insertion.

Uterine perforation is an uncommon but potentially serious complication of LNG IUD placement. A large European prospective cohort study found a perforation incidence of 0.6 for nonlactating women and 4.5 for lactating women per 1000 insertions.[193] No perforations caused injury of intra-abdominal or pelvic structures or serious morbidity.[194]

Upper Genital Tract Infection and Infertility

The use of intrauterine contraception does not increase the risk of pelvic infection.[195] Similarly, there is no evidence intrauterine contraception increases the risk of subsequent infertility.[196] Intrauterine methods are suitable for nulliparous and adolescent women.[184] Prophylactic antibiotics are not recommended for IUD insertion. A clinical history (including sexual history) should be taken as part of the routine assessment for intrauterine contraception to identify women at high risk for STIs; testing before insertion should be performed selectively, not routinely. Even among high-risk women, testing on the same day of insertion was found to be as safe as testing prior to insertion in terms of subsequent pelvic inflammatory disease (PID) risk.[197] An IUD should not be inserted in women with current PID, purulent cervicitis, or current chlamydial or gonorrheal infection.[9] However, cervical or vaginal infection is not an indication to remove an IUD.[8] If a woman contracts gonorrhea, chlamydia, or PID with an LNG IUD in place, treatment follows the CDC Sexually Transmitted Diseases Treatment guidelines, and if the patient responds to therapy, the IUD can remain in place.[198]

Metabolic and Systemic Effects

Low but detectable circulating levels of progestin in LNG IUD users have raised concerns that glucose control, the lipid profile, and blood pressure may be negatively affected. However, these concerns have not been substantiated in high-quality clinical trials.[176] The data support that the LNG IUD is safe to use in patients with diabetes, hyperlipidemia, or hypertension. Progestins are not thought to increase the risk of thromboembolic disease, and the LNG IUD has not been associated with venous or arterial events.[102]

Noncontraceptive Uses of the Levonorgestrel-Releasing Intrauterine System

Heavy Menstrual Bleeding

The high-dose LNG IUD is a well-established and highly effective treatment intervention for heavy menstrual bleeding. In comparative studies, it offers efficacy equal to or greater than other surgical uterine-conserving treatments (e.g., global endometrial ablation, transcervical endometrial resection), is an acceptable alternative to hysterectomy for many women, and offers comparable improvement in health-related quality of life for menstrual disorders.[199-202] Although up to 43% of women using the LNG IUD to treat abnormal uterine bleeding will eventually undergo hysterectomy, the system still offers lower direct and indirect health care costs at 5 years compared with immediate hysterectomy.[201,203]

Symptomatic Fibroids and Uterine Adenomyosis

Data support that the LNG IUD is also an effective treatment for heavy menstrual bleeding associated with uterine fibroids.[204,205] However, submucous fibroids may be more likely to cause heavy bleeding and are more likely to lead to LNG IUD expulsion because they distort the uterine cavity.[206] Therefore, effectiveness of the LNG IUD likely depends on fibroid number, size, and location in the uterus.

Uterine adenomyosis also is a common, benign condition that may be associated with heavy menstrual bleeding and pelvic pain. Limited data suggest that the LNG IUD reduces bleeding and pain in women with this condition.[207-209] As with uterine fibroids, expulsion rates appear elevated when IUDs are placed in women with uterine adenomyosis.[200]

Endometriosis

A Cochrane review concluded that the 52-mg LNG IUD reduces pelvic pain and dysmenorrhea associated with endometriosis.[210] Advantages of the LNG IUD in this setting include the absence of hypoestrogenic effects associated with the use of gonadotropin-releasing hormone agonists, as well as lower medication costs. Disadvantages of the LNG IUD include irregular bleeding associated with this approach.

Endometrial Protection With Estrogen Replacement Therapy

Good-quality evidence supports long-term use of the LNG IUD for endometrial suppression during estrogen replacement therapy, and it is approved for this indication in other countries.[211,212] However, the 52-mg LNG IUD is relatively large and may be more difficult to insert into a postmenopausal woman's uterus. The two smaller LNG IUDs may be better choices in menopausal women. Acceptability studies are needed, and more studies are necessary to determine the lowest effective dose of levonorgestrel required to achieve effective endometrial suppression.[213]

Endometrial Protection With Tamoxifen Use

Tamoxifen is commonly used as adjuvant endocrine therapy in the treatment of estrogen receptor–positive breast cancer in premenopausal women and in selected postmenopausal women. In postmenopausal women, tamoxifen increases the risk of endometrial polyps, hyperplasia, and cancer. A systematic review of the 52-mg LNG IUD for prevention of endometrial disease in tamoxifen users concluded that the device reduced the risk of endometrial polyps, but it is not known whether LNG IUD reduces the risk of endometrial hyperplasia or cancer.[214] LNG IUD users had a higher incidence of unscheduled bleeding, which may increase the need for diagnostic intervention in this high-risk group. The FDA lists a personal history of breast cancer as a contraindication to use of all progestational medications, including the LNG IUD. Reflecting the uncertain safety of LNG IUD use in women with breast cancer, guidance released in 2014 from the American College of Obstetricians and Gynecologists does not recommend that this intrauterine system be used in this patient population.[215]

Treatment for Endometrial Hyperplasia or Carcinoma

Although hysterectomy represents the best treatment for early endometrial cancer, progestins are sometimes used to treat endometrial hyperplasia with atypia or early endometrial cancer in women who wish to preserve future fertility or who represent poor surgical candidates due to comorbidities. The 52-mg LNG IUD reverses endometrial neoplastic changes in most women with complex atypical hyperplasia and early-grade endometrial cancer.[216]

Contraceptive Implants

Contraceptive implants provide long-acting, highly effective, convenient, and reversible contraception. All subdermal contraceptive implants for clinical use in humans employ progestins. These methods offer an excellent contraceptive option for women who have contraindications to combined hormonal methods and an option for any woman who desires long-term protection against pregnancy that is rapidly reversible. The only subdermal implant currently available to women in the United States is Nexplanon (Fig. 18.5), released in 2006 as Implanon. In 2011, Nexplanon (a radiopaque implant) replaced Implanon.

Nexplanon contains 68 mg of etonogestrel as a single-rod subdermal implant.[217] The device is approved for 3 years' duration, provides excellent efficacy throughout its use, and is easy to insert and remove by trained practitioners. Nexplanon can be used during lactation; may improve dysmenorrhea; does not significantly affect weight, acne, lipids, or liver enzymes; and has only modest effects on BMD.[218] Like other progestin-only contraceptives, the etonogestrel implant commonly causes irregular vaginal bleeding.

• **Fig. 18.5** The subdermal etonogestrel implant.

Description and Pharmacology

The Nexplanon rod releases the gonane progestin etonogestrel, formerly known as 3-ketodesogestrel, the biologically active metabolite of desogestrel.[218] Desogestrel is used in some oral contraceptives, and etonogestrel is the same progestin used in the contraceptive vaginal ring. The implant is 4 cm long and 2 mm in diameter, is radiopaque, and has a core made from a nonbiodegradable solid composed of ethylene vinyl acetate impregnated with 68 mg of etonogestrel (see Fig. 18.5). The ethylene vinyl acetate copolymer of Nexplanon allows controlled hormone release over at least 3 years of use. Each implant is provided in a disposable sterile inserter for subdermal application. Maximum serum concentrations of etonogestrel are usually seen by day 4 after implant insertion. Etonogestrel levels decrease slightly by 1 year and further by 3 years but remain above the threshold needed to suppress ovulation.[217] After removal, serum levels are undetectable by 1 week in most users, who resume ovulation within 6 weeks of implant removal. Despite effective suppression of ovulation, estradiol levels only fall into the early follicular level range, and the implant does not cause hypoestrogenism. Consistent with that observation, based on limited data from clinical trials, there does not appear to be a clinically significant adverse effect of the etonogestrel implant on BMD.[218]

Mechanism of Action and Efficacy

Nexplanon prevents fertilization primarily by inhibiting ovulation, although some thickening of cervical mucus may also occur.[219] The etonogestrel implant provides highly effective contraception. In an integrated analysis of 11 international clinical trials that included more than 900 healthy women between 18 and 40 years old, no pregnancies were reported while the etonogestrel implants were in place. Six pregnancies occurred during the first 14 days after etonogestrel implant removal. Including these six pregnancies, the cumulative Pearl index (number of pregnancies per 100 woman-years) was 0.38 (Pearl indices were 0.27 and 0.30 at 1 and 2 years, respectively).[219] After the implant has been removed, normal ovulation and fertility rapidly return. Reported pregnancies among implant users have primarily been due to unrecognized pregnancies at the time of insertion and failure to insert the device. Postmarketing data regarding the etonogestrel implant from Australia found a real-world failure rate of 1.07 per 1000 insertions.[220] Of the 218 pregnancies identified in the Australian study, data were insufficient to assess the reason for contraceptive failure in 45 women, and 46 women were determined to have been already pregnant prior to implant insertion. Of the remaining 127 cases, failure to insert the implant resulted in pregnancies in 84 women. Other reasons for contraceptive failure included incorrect timing of insertion (19 cases), expulsion of the implant out of the insertion site (3 cases), and interaction with hepatic enzyme–inducing medications (8 cases). The remaining 13 cases were classified as product failures. However, even when these method failures are accounted for, Nexplanon continues to have one of the highest efficacies of any available reversible or permanent contraceptive.

There are reports of implant failure in women using anticonvulsants, particularly carbamazepine. Accordingly, contraceptive implants are not recommended in women taking anticonvulsants, certain antiretrovirals, or other medications that induce hepatic enzymes.[220-222] Overweight and obese women were not included in the trials for approval of the etonogestrel implant. However, a prospective cohort study that examined 1168 women (28% overweight and 35% obese) found that failure rates were not impacted by BMI.[223]

Package labeling indicates that Nexplanon can be used for up to 3 years. However, the implant appears to retain its efficacy for up to 5 years.[224] In a US study among 291 women who had used the implant for 3 years, no pregnancies occurred during a fourth or fifth year of use.[173] Accordingly, and recognizing that such use is off-label, individuals can be offered the opportunity to extend use to as long as 5 years.

Safety and Side Effect Profile

Nexplanon users commonly experience irregular and unpredictable bleeding patterns, similar to the experience of users of other continuous progestin-only contraceptives. Combined data from 11 clinical trials show that the most common bleeding patterns with the etonogestrel implant are amenorrhea (22%), infrequent bleeding (34%), frequent bleeding (7%), and frequent or prolonged bleeding or both (18%).[225] The number of bleeding days are usually not increased, but the pattern is unpredictable. Of clinical relevance is that bleeding patterns experienced during the initial 3 months predict future patterns for most women. Women with favorable bleeding patterns during the first 3 months tend to continue this pattern throughout the first 2 years of use. Women with unfavorable initial patterns have at least a 50% chance that the pattern will improve. Only 11.3% discontinue use because of bleeding irregularities, mainly because of prolonged flow and frequent irregular bleeding.[225] Most women (77%) with baseline dysmenorrhea experience complete resolution of symptoms due to ovulation suppression. Effective preinsertion counseling on the possible changes in bleeding patterns may improve continuation rates.[126,226] A small, short-term clinical trial found that adding COCs reduces bothersome bleeding in implant users.[227] If a patient using a contraceptive implant desires treatment to reduce bothersome irregular bleeding, the US Selected Practice Recommendations for Contraceptive Use present two options: (1) nonsteroidal anti-inflammatory drugs for 5 to 7 days, or (2) low-dose COCs or estrogen for 10 to 20 days.[8] These treatments may shorten bleeding episodes and offer temporary relief but do not change long-term bleeding patterns. In selected patients experiencing bothersome bleeding due to the contraceptive implant and without contraindications to COC use, the authors recommend long-term use of COCs to reduce such bleeding if the patient desires this therapy.

Several studies observe a small (<1 kg) weight increase in etonogestrel implant users.[219] However, only 3% to 7% of women choose to remove the implant because of weight changes. Similar to the effect seen in women using POPs, ovarian cysts occur in up to 15% of users. Most cysts regress spontaneously and do not need additional treatment.[228]

Nexplanon appears safe for use during lactation.[108] A 2015 Cochrane review did not identify concerns regarding milk volume or composition of infant growth in implant users.[229] Likewise, a Brazilian randomized trial of breastfeeding women found that infant growth was similar with early (prior to hospital discharge) implant placement compared with delayed placement, and that Nexplanon does not appear to change the content of breast milk and does not influence infant growth up to 3 years.[230]

Lipid measurements demonstrate an overall decrease in serum total cholesterol, HDL cholesterol, and low-density lipoprotein cholesterol. Some studies also find a decrease in triglyceride levels. Minor reductions in the HDL/low-density lipoprotein ratio have been observed, although not into ranges thought to be clinically significant. Etonogestrel implant use does not significantly increase the risk of cardiovascular disease.[231]

Migration of contraceptive implants to the systemic vasculature, particularly the pulmonary artery, represents a rare complication of implant placement. Proper superficial placement of implants represents the key to avoiding this serious complication.[232,233]

Patient Selection

Before recommending the contraceptive implant, providers should review the indications and contraindications for its use. Contraindications to etonogestrel implant use are few and include breast cancer, use of hepatic enzyme–inducing drugs, unexplained and unevaluated abnormal vaginal bleeding, severe cirrhosis, systemic lupus erythematosus (SLE) with positive or unknown antiphospholipid antibodies, and liver tumors.[9] There are also reports describing etonogestrel implant failure in a woman taking the antiretroviral drug efavirenz.[234] When counseling women regarding the etonogestrel implant, the clinician should address concerns and fears a woman may have about this method of contraception. Women may have particular concerns about implant removal, although removal problems with single-rod devices such as Nexplanon are uncommon. Side effects (particularly irregular bleeding) should be discussed, as an unexpected side effect may cause women to request early removal of the implant. The implant does not provide protection against STIs. Accordingly, and as is appropriate for all sexually active women, implant users should be reminded about safe sexual practices. Appropriate candidates for implantable contraception are women who desire long-term reversible birth control, have no contraindications to etonogestrel implant use, accept implant insertion and removal, and are ready to accept a change in menstrual bleeding patterns.

Insertion and Removal

Proper insertion and removal techniques are essential for clinical efficacy and for prevention of complications. Timing of insertion depends on the patient's prior use of contraception and the clinician's evaluation of the appropriateness for the individual.[8] In the United States, Nexplanon has been made available only to clinicians who have completed insertion and removal training provided by the manufacturer. Proper training of clinicians reduces the incidence of complications at insertion and removal. Complications of Nexplanon insertion are rare (<2%) but may include local pain, infection, and bleeding. Migration of the implant into the systemic vasculature is described earlier.[232,233] A follow-up visit following implant placement is not routinely indicated.

Before removal, the clinician needs to palpate the implant. Under sterile conditions and after injection of a local anesthetic, a 2- to 3-mm incision is made vertically over the implant. The rod is then removed using the pop-out technique described by Pymar and associates[235] for Norplant System removal. If inserted correctly, removal is usually simple and should take less than 5 minutes. The most common reason for difficulty in removal is placement of the implant too deeply. If the clinician cannot palpate the implant, imaging techniques may be necessary before proceeding.

Emergency Contraception

Emergency contraception (EC) is defined as a drug or device used to prevent pregnancy after unprotected sexual intercourse (including sexual assault) or after a recognized contraceptive failure.[236] Although EC is sometimes called the morning-after pill, this label is confusing because it implies that EC can be taken only the morning after unprotected sexual intercourse. EC, or postcoital contraception, can actually reduce the risk of unintended pregnancy up to 5 days after unprotected sex.

Oral EC is intended as a backup method for occasional use rather than a regular method of contraception. Discussions with patients regarding EC offer an opportunity to discuss longer-term contraception, sexual health and safety, and other preventative women's health measures such as Papanicolaou smears. EC should be readily available to all women at risk for unplanned pregnancy, including adolescents and victims of sexual assault.

Research shows that increased access to EC improves use, but it is not yet clear whether this access translates into a reduction in unintended pregnancy or abortion.[237] Although access is an important factor, how women use EC may be a stronger determinant of its ultimate effect. Even when women have EC at home, they often fail to use it after unprotected sexual intercourse. The most common reason for this is a lack of recognition of the risk of pregnancy or neglect of the perceived risk.[238] Although increasing knowledge of and access to EC is a public health priority, use of regular, ongoing contraception is a more effective way to reduce unplanned pregnancy and abortion.

Emergency Contraception Regimens

The EC currently available to women in the United States includes 1.5-mg levonorgestrel (Plan B One-Step [Duramed Pharmaceuticals, Pomona, NY], Next Choice [Watson Laboratories, Inc., Corona, CA]), 30-mg ulipristal acetate (UPA, Ella, Afaxys Pharma, LLC., Charleston, SC), and the copper T 380A IUD (Paragard [CooperSurgical, Trumbull, CT]).

In 2009, a one-dose 1.5-mg levonorgestrel tablet was introduced (preferred over the previous 0.75-mg two-dose regimen), which should be taken as soon as possible after unprotected intercourse and is labeled for use up to 72 hours following intercourse.[236] The medication is available without a prescription. A prescription was previously required for those younger than 17 years, but there are now no age restrictions for access to levonorgestrel EC without a prescription following FDA approval in 2014. The progestin-only oral EC regimen is better tolerated than the older Yuzpe method of EC that used high doses of COCs.

UPA was approved in 2010 for EC in the United States and is marketed as Ella.[239] UPA is a progesterone receptor modulator in the same class as mifepristone and is more effective than levonorgestrel EC.[236] UPA requires a prescription, and although it should be taken as soon as possible after unprotected intercourse, it has been shown to be effective for up to 5 days.[240]

The copper T 380A IUD may be inserted for EC up to 5 days after unprotected intercourse, although some evidence suggests that it may still be effective when inserted up to 10 days post intercourse.[241-243] Although the copper T is a highly effective form of EC, with the advantage of providing ongoing longer-term contraception for women,[244] the requirement for specialist insertion and the cost of the device may act as a barrier to access.

Mechanism of Action

The mode of action of hormonal EC is multifactorial and not completely understood. Because sperm are viable in the female reproductive tract for up to 5 days but eggs can be fertilized only within 1 day of ovulation, the mechanism of action depends on when the oral EC regimen is given in relation to the time of intercourse and time of ovulation.

Levonorgestrel EC prevents or delays ovulation if administered before the commencement of the LH surge and is ineffective if taken afterward.[236,245] UPA is a selective progesterone receptor modulator with different proposed mechanisms when taken at different times of the menstrual cycle. UPA administered before the commencement of the LH surge inhibits follicular development, and when taken during the LH increase but prior to the peak, it delays follicular rupture.[246] Neither oral agent has been shown to interfere with implantation directly. Therefore, they are not considered abortifacients or teratogenic to a developing pregnancy.[247,248]

The mechanism of action of the copper IUD is thought to be the release of copper ions toxic to sperm function while associated inflammation potentially impairs transport of ovum or implantation of the fertilized egg.[249]

Efficacy

The effectiveness of EC depends on the mechanism of action and when the EC regimen is given in relation to the time of unprotected intercourse. The probability of pregnancy after a single act of unprotected intercourse varies (3–8%) according to the day of the menstrual cycle and the couple's fertility status.[250] Calculating the efficacy of EC is complex because it is impossible to know how many pregnancies would otherwise have occurred. Therefore, an estimation of efficacy is the failure rate of the contraceptive method—that is, the rate of pregnancy following administration.

The most effective EC is the copper IUD, with a failure rate of less than 0.1%.[241] With regard to oral EC, UPA has greater efficacy over levonorgestrel because it more effectively delays follicular rupture and has a longer action well into the LH surge.[251] A recent systematic review directly comparing UPA and levonorgestrel demonstrated that UPA almost halved the risk of pregnancy compared with levonorgestrel when given within 120 hours of unprotected sexual intercourse; the failure rate of UPA when administered within 120 hours is 1.3%.[252] Moreover, when given within 24 hours of intercourse, UPA reduces the risk of pregnancy by almost two thirds compared with levonorgestrel, with a failure rate of 0.9%.[253]

After a single episode of unprotected intercourse, the use of levonorgestrel-only EC is more effective than no treatment at all.[254] Taking a total of 1.5 mg of levonorgestrel in a single dose is as effective as the 0.75-mg split dose, and single dosing is more user-friendly.[252] The timing of levonorgestrel EC influences its effectiveness. Waiting 12 hours to initiate treatment after unprotected intercourse increases the odds of pregnancy by almost 50%, and its efficacy decreases linearly with time.[255]

The efficacy of oral EC may be reduced with increased BMI. One study used a subanalysis to demonstrate the effect of BMI on contraceptive efficacy; levonorgestrel was shown to be ineffective in overweight women (with a BMI >26 kg/m^2) compared with UPA, which was ineffective in obese women (with a BMI >35 kg/m^2).[256] Women should not be denied access to EC based on BMI, but the potential impact of weight on EC efficacy should be discussed and considered in agent selection. Some European authorities have recommended increasing the dose of levonorgestrel EC to 3 mg in the setting of obesity and also when a woman is taking concomitant liver enzyme–inducing drugs.[257] The efficacy of the copper IUD is not affected by BMI.

Indications

EC is indicated for any woman at risk for unintended pregnancy from an episode of contraceptive failure or unprotected intercourse. Experts recommend the advance provision of EC to women.[258] However, surveys have found that the level of knowledge and use of EC in the United States by women and their health care providers is low.[259]

No clinical examination or pregnancy testing is required before using oral hormonal EC.[9] Given the infrequent use and short duration of action of EC, the medical contraindications to regular oral contraceptives are not applicable. USMEC recommendations state that women with previous ectopic pregnancy, cardiovascular disease, migraines, or liver disease can use EC.[9] Current guidelines advise that breastfeeding should be avoided for 1 week after using UPA; breastfeeding women should continue to express and discard milk during this time.[260]

The only contraindication to the oral EC regimens is allergy to the active substance. Pregnancy is a relative contraindication because EC is ineffective if a pregnancy is already established; as discussed earlier, there is no evidence demonstrating teratogenicity to a developing pregnancy. There may be reduced efficacy of oral EC in women with severe malabsorption syndromes and those taking hepatic enzyme–inducing drugs and certain antiretroviral drugs. Contraindications for the copper IUD for EC are the same as those for regular contraceptive use.[9]

Side Effects

The FDA has determined that levonorgestrel EC is safe enough to be available without a prescription in states with direct pharmacy access. UPA has an excellent safety profile and is available over the counter throughout Europe but requires a prescription in the United States. The side effects of the copper IUD for EC are the same as those for its use for long-term contraception.

No deaths or serious complications have been directly linked to EC. The most common short-term side effects of oral EC include nausea, vomiting, and irregular bleeding, which affect up to 20% of users. Irregular bleeding caused by hormonal EC typically resolves by the next menstrual cycle. Other minor adverse effects reported by women in clinical trials include dizziness, fatigue, breast tenderness, headache, and abdominal pain.[252] If vomiting occurs within 3 hours of taking oral EC, most experts recommend that the dose be repeated.[236]

After hormonal EC is taken, menses usually occurs within 1 week before or after the expected time. Women using levonorgestrel are more likely to experience early menstruation, whereas women using UPA are more likely to experience delayed menstruation.[252] If the delay in the onset of menses is greater than 1 week or if the expected menses is lighter than usual, a pregnancy test should be performed. A woman should also be advised to seek medical attention for continued irregular bleeding or abdominal pain, as these symptoms may be a sign of a spontaneous abortion or ectopic pregnancy.

Ongoing Contraception

As outlined previously, hormonal EC can postpone ovulation, making a woman vulnerable to pregnancy later in the cycle. Women should therefore be counseled to begin a regular method of contraception after using EC. However, there is preliminary data demonstrating that hormonal contraception, particularly oral progestins, could reduce the efficacy of UPA.[261] Further research is needed into the mechanism and risk profiles of various therapies. It has been suggested that commencement of regular hormonal contraception should be delayed for 5 days after the administration of UPA, and that women should either abstain from sexual intercourse or use barrier protection for that duration.[8,261,262]

Clinical Challenges in Contraceptive Care

Hormonal Contraception for Adolescents

Although on the decline, rates of teenage pregnancy are higher in the United States than in other Western industrialized countries.[6,263] Approximately 75% of these pregnancies are unplanned, of which about 38% end in induced abortion.[3] Teenage pregnancy and parenting are risk factors for adverse medical, educational, and psychosocial outcomes for mother and child.[264] Children of teenage mothers are at greater risk for preterm birth, low birth weight, neonatal death, and later behavioral problems and poor academic performance.[265] Teenage parenting contributes to the intergenerational transmission of poverty.[266] Sexually active adolescents are more motivated to use contraception if they are academically successful, believe that pregnancy would be an impediment to their goals, and are involved in a stable relationship with a sexual partner.[267]

Considerations for clinicians advising adolescents about contraception should include the potentially high fertility rates in these young women, their high rates of unprotected intercourse, elevated risk of sexual assault, and increased risk of STIs.[268] Consistent and correct use of contraceptive methods can be challenging for adolescents, and long-acting methods increase efficacy.[132] These methods, including the DMPA injection, contraceptive implants, and IUDs, should be made available to teens.[184,269] With proper patient selection and counseling, IUDs and implants can be successful contraceptive options for adolescents.[184,270] Clinicians should also be aware of the legal conditions surrounding prescribing contraception to minors in the state in which they are practicing.

Sexually active adolescents benefit from health guidance annually regarding responsible health behaviors, including abstinence, latex condoms to prevent STIs, and appropriate methods of birth control, along with instructions on ways to use them effectively.[268] Adolescents should also be counseled in a nonjudgmental manner about the need to use condoms for all types of intercourse, including anal and oral intercourse. Barriers to effective contraception use by adolescents include lack of forward planning, nonconsensual intercourse, lack of confidential care, fear of disapproval by parents and doctors, absence of adolescent-friendly services, language and cultural barriers, fear of pelvic examination, and cost.[271] Misconceptions about contraception, including effects on weight gain, future fertility, acne, and risk of cancer, may also prevent adolescents from using effective contraception and should be addressed in counseling. The National Campaign to Prevent Teen and Unplanned Pregnancy, now called *Power to Decide*, operates a website that provides excellent information, decision tools, and reminders specifically designed for young women at https://www.bedsider.org.

Combined Hormonal Contraceptives in Adolescents

Although COCs, the patch, and the ring represent safe and effective methods for adolescents, many young women are not effectively educated about correct use and anticipated side effects. They may use these short-acting hormonal contraceptives inconsistently, which will lead to very high pregnancy rates.[271] During counseling, it is important to provide clear oral and written instructions about initiating these methods, ways to ensure correct use, and what to do if one or more pills are missed or if the patch or ring is not used correctly. By the same token, POPs are not favored for adolescents because they require stricter adherence for efficacy.

Initiating or continuing hormonal contraception does not require pelvic examination, cervical cancer screening, or STI screening in adolescents or older reproductive-age women.[8] A history excluding contraindications, blood pressure measurement, and being reasonably sure the patient is not pregnant is sufficient before initiating hormonal contraception.[272] Age-appropriate screening for STIs or cervical cancer should be considered regardless of contraceptive use. Screening for chlamydial infection and gonorrhea, as recommended by the CDC for women under the age of 26 years, can be accomplished through urine tests or a vaginal swab without a speculum.[198] The use of condoms should be encouraged in conjunction with the use of hormonal contraceptives to prevent STIs.

Initial follow-up should be 8 to 12 weeks after commencing the COCs to monitor correct use and adverse events and should be done thereafter at 6- to 12-month intervals. Education and ongoing counseling are essential to ensure correct usage. Many adolescents are concerned that the COCs will cause weight gain or acne, and these issues should be directly addressed.[271] Chewable pills are available and may be more acceptable to young women who find it difficult to swallow pills. Unscheduled bleeding may be less acceptable to adolescents than adult women. Clinicians must consider the possibility that unscheduled bleeding represents cervicovaginal infection in this high-risk population and investigate and treat it accordingly.

Injectable Contraceptives in Adolescents

Discontinuation rates for DMPA in adolescents are high, with half of adolescents discontinuing the method by 12 months.[273] However, because DMPA suppresses ovulation for an extended period of time, prior use of DMPA protects many adolescents from unintended pregnancy despite inconsistent use. Weight gain is the most commonly cited reason for adolescents to discontinue DMPA, and it may be more common in obese adolescents.[115] Although loss of bone density has been a concern in adolescents using DMPA, the position statement of the Society for Adolescent Medicine is that DMPA represents an extremely effective contraceptive, and that clinical concerns about loss of BMD must be placed within the context of likely bone recovery on discontinuation, low risk of fractures, and benefits of preventing unintended pregnancy among adolescents.[274]

Hormonal Contraception in Postpartum and Lactating Women

The postpartum period is a critical time period for initiating contraception to help women achieve optimal interpregnancy intervals.[275] The immediate postpartum period offers an ideal time for women to initiate contraception because of patient access and convenience. Delaying initiation of contraception until the standard 6-week postpartum visit places many women at risk for unintended pregnancy. By 6 weeks postpartum, up to 40% of women will have had unprotected intercourse, and nearly 50% will have ovulated if they are not breastfeeding.[276,277] Furthermore, not all women seek postpartum medical care, resulting in a large proportion of women with unmet contraceptive needs.[278] Return of fertility is, of course, postponed in breastfeeding women who meet criteria for the lactational amenorrhea method of contraception.

Postpartum women remain in a hypercoagulable state for weeks after childbirth.[279,280] This elevated risk does not resolve until 12 weeks postpartum, although after 6 weeks the risk is low (22 cases per 100,000 deliveries within 6 weeks and falling to three cases

per 100,000 deliveries in weeks 7–12).[281] Risk factors for VTE in the postpartum period include age 35 years or older, previous VTE, thrombophilia, immobility, transfusion at delivery, peripartum cardiomyopathy, BMI 30 kg/m^2 or higher, postpartum hemorrhage, cesarean delivery, preeclampsia, and smoking.[282] The USMEC recommends deferring use of estrogen-containing methods (category 4) until 3 weeks after delivery in all women and until 4 weeks in breastfeeding women.[9] The guidelines further state that the risks of using estrogen-containing methods outweigh the benefits in those with risk factors for VTE starting 6 weeks postpartum.

Traditionally, combined hormonal contraception has not been recommended as the first choice for lactating women because of concerns that the estrogenic component can reduce the volume of milk production and the caloric and mineral content of breast milk.[229] However, use of COCs by well-nourished breastfeeding women does not appear to result in problems with infant development. Data regarding this concern is limited and inconsistent, with two recent systematic reviews unable to draw firm conclusions regarding the effect of COCs on breastfeeding duration and infant outcomes.[229,283] On an individual level, it is important to note that some women might be at risk for breastfeeding difficulties, and if full breastfeeding is desired, counseling regarding these concerns is warranted. USMEC guidelines allow use of estrogen-containing methods after 30 days postpartum in breastfeeding women without risk factors for VTE.[9]

Evidence from limited studies suggests that progestin-only contraception does not interfere with lactation or infant development and does not increase risk of thromboembolic disease.[102,107] It appears reasonable to initiate progestin-only contraception, including DMPA, POPs, and implants, immediately after delivery, regardless of whether mothers are nursing their infants. There is a theoretical concern, nevertheless, that initiation of progestin methods immediately postpartum could preempt lactogenesis, given that progesterone withdrawal after delivery of the placenta is thought to trigger prolactin secretion.[229] Although long-term data are limited, observational studies of progestin-only contraceptives suggest that they have no effect on successful initiation and continuation of breastfeeding or on infant growth and development even when started immediately postpartum.[107] IUDs are also an option either immediately after delivery of the placenta or as an interval insertion (4–6 weeks postpartum) provided that there is no evidence of chorioamnionitis or postpartum endometritis or sepsis. Higher risk of expulsion with postplacental IUD insertion compared with interval insertion should be balanced against the benefit of immediate access to a highly effective contraceptive method, especially in women at risk for not returning for postpartum follow-up.[284]

Hormonal Contraception in Women Older Than 35 Years

There are no contraindications to use of hormonal contraceptives on the basis of age alone.[9] However, risks associated with the use of some methods may increase with age and additional comorbid conditions. For example, although the incidence of VTE, myocardial infarction, and stroke is low, these risks increase with age, obesity, smoking, diabetes, migraine headaches with aura, and hypertension.[72,285] However, because lean, healthy, nonsmoking women are at low risk for these rare events, they can use any method, including combined (estrogen-progestin pills, patch, and vaginal ring) methods, until menopause.[286] Large US population-based case-control studies have found no increased risk of myocardial infarction or stroke among healthy, nonsmoking women older than 35 years who use COCs formulated with less than 50 μg of estrogen.[287,288] Perimenopausal women may benefit from a positive effect on BMD,[289] abnormal uterine bleeding,[290] and a reduction in vasomotor symptoms[291] offered by COCs. In addition, COC use is associated with a reduced risk of endometrial cancer and ovarian cancer, which is of particular importance to older reproductive-age women. Progestin-only hormonal contraceptives and IUDs are preferred for older women at elevated risk for VTE, myocardial infarction, and stroke.[292-294] It is unknown if long-term older DMPA users can regain BMD levels to baseline before entering menopause. For this reason, DMPA in women older than 45 years is USMEC category 2, and the other progestin-only methods are category 1. The LNG IUD is particularly effective for perimenopausal bleeding.[295,296]

Discontinuation of Hormonal Contraception at Menopause

When deciding to stop use of a contraceptive method, the benefits and risks of the method must be evaluated in the context of the diminishing risk of pregnancy as the woman ages. The median age of menopause in North America is 51 years, and about 90% of women will have reached menopause by age 55.[297] Although pregnancy is uncommon after age 44, spontaneous conception can occur. Assessment of follicle-stimulating hormone levels to determine when hormonal contraceptive users have become menopausal and thus no longer need contraception may be misleading and should not be routinely performed.[8] Until a well-validated tool to confirm menopause is available, it is appropriate for healthy, nonsmoking women using a hormonal contraceptive to continue until age 50 to 55 years, after weighing the risks and benefits.[9] Thus, most women will be able to use contraception safely until they are assured of menopause. At the time they discontinue hormonal contraception, some midlife women will choose to initiate menopausal hormonal therapy.

Contraception in Women With Underlying Medical Conditions

Because pregnancy in women with underlying medical conditions is associated with higher risks of maternal and perinatal morbidity and mortality, achieving effective contraception is particularly important in this setting[298] (Table 18.6). Providers should remember that the risk of using a contraceptive must be balanced with the risk of pregnancy. Although numerous studies have addressed safety and effectiveness of hormonal contraceptive use in healthy women, data unfortunately are far less complete for women with underlying medical problems or other special circumstances. The US Medical Eligibility Criteria for Contraceptive Use from the CDC provides an important evidence-based resource for clinicians.[9]

Decisions regarding contraception for women with coexisting medical problems may be complicated. In some cases, medications taken for certain chronic conditions may alter the effectiveness of hormonal contraception, and pregnancy in these cases may pose substantial risks to the mother and her fetus. Differences in content and delivery methods of hormonal contraceptives may affect patients with certain conditions differently. Because transdermal and vaginal ring contraception are relatively new, less data address their use in women with medical concerns. In the absence

TABLE 18.6 Conditions Associated With Increased Risk for Adverse Health Events as a Result of Unintended Pregnancy

- Breast cancer
- Complicated valvular heart disease
- Diabetes: insulin dependent; with nephropathy/retinopathy/neuropathy or other vascular disease; or of >20 years' duration
- Endometrial or ovarian cancer
- Epilepsy
- Hypertension (systolic >160 mm Hg or diastolic >100 mm Hg)
- History of bariatric surgery in the past 2 years
- HIV/AIDS
- Ischemic heart disease
- Malignant gestational trophoblastic disease
- Malignant liver tumors (hepatoma) and hepatocellular carcinoma of the liver
- Peripartum cardiomyopathy
- Schistosomiasis with fibrosis of the liver
- Severe (decompensated) cirrhosis
- Sickle cell disease
- Solid organ transplantation within the past 2 years
- Stroke
- Systemic lupus erythematosus
- Thrombogenic mutations
- Tuberculosis

AIDS, Acquired immunodeficiency syndrome; *HIV*, human immunodeficiency virus.

From Curtis KM, Tepper NK, Jatlaoui TC et al. US medical eligibility criteria for contraceptive use, 2016. *MMWR Recomm Rep*. 2016;65:1–104.

of more extensive data, contraindications to the use of estrogen-progestin COCs should also be considered as contraindications to the use of transdermal and vaginal ring contraception. Practitioners should recognize that use of nonhormonal forms of contraception, such as the copper IUD, remain a safe, effective choice for many women with medical conditions.

Hormonal Contraception in Obese Women

The proportion of American adult women who are obese (BMI of 30 kg/m^2 or higher) has increased to 38%.[299] Studies show that obese women have similar levels of sexual activity and unintended pregnancy compared with normal weight women.[300] No methods of contraception are contraindicated in obese women.[9,301] As noted earlier, oral EC may be less effective in obese women. Although the efficacy of oral contraceptives and the transdermal patch in obese women has been questioned, a 2016 Cochrane review found that there was generally no association between BMI and the effectiveness of hormonal contraceptives.[302] Similarly, the overall risk for unintended pregnancy in women using the combined hormonal pill, patch, or ring was not significantly different across BMI categories, with an overall range of 8.4% to 11.0% at 3 years of use in a prospective cohort study of 1500 women.[303] This study was not able to evaluate efficacy separately among pill, patch, and ring users.[304] A recent large, prospective cohort study of more than 52,000 women reported a slight increase in failure rates of COCs as BMI increased (hazard ratio of 1.5 for BMI higher than 35; 95% CI, 1.3–1.8), adjusting for age, parity, and education.[20] However, this difference disappeared when evaluating pills dosed in a 24/4 fashion compared with 21/7 regimens. The ability to detect small differences in contraceptive efficacy between normal weight and obese women may be obscured by adherence issues with short-term methods.[305]

Efficacy of COCs, contraceptive rings, and transdermal patches primarily reflects suppression of ovulation resulting from the dose of progestin. Pharmacokinetic studies show that, compared with normal weight women, it takes twice as long for obese women to reach steady-state therapeutic levels of contraceptive steroids when starting the pill or after the HFI due to changes in clearance.[306,307] Therefore, these episodes might represent a vulnerable time period for this population. It is possible that continuous oral contraceptive use or oral contraceptives with a shorter HFI may be more effective in obese women. Evidence evaluating the effectiveness of the patch and vaginal ring among obese women is limited, but both provide more effective contraception than barrier methods alone.[89,301,308,309] Among overweight and obese women, higher pregnancy rates have not been observed with use of the 150-mg intramuscular DMPA or 104-mg subcutaneous formulations[116,310] or the etonogestrel implant.[311] Considering that IUDs work locally on the uterus and do not rely on systemic drug levels, their efficacy is not affected by BMI.[305]

Combined hormonal contraceptives are rated USMEC category 2 for obese women due to theoretical concerns about arterial and VTE. Although obesity and use of combined hormonal contraceptives represent independent risk factors for VTE,[285,312,313] the absolute risk of VTE with combined contraceptives in obese women is still less than the risk of VTE during pregnancy and the puerperium in obese women.[314,315] The evidence of an increased risk of acute myocardial infarction or stroke in obese users of COC is conflicting,[316] but the overall absolute risk of these events in reproductive-age women is low.[72] That being said, obese women are more likely to have other comorbidities, such as hypertension, hypercholesterolemia, and diabetes. No published studies address the safety of combined hormonal contraceptives in obese women with these comorbidities. Accordingly, consideration should be given to progestin-only and intrauterine methods when counseling obese individuals regarding contraceptive choices, particularly when such patients are older than 35 years. Because obese women experience an elevated risk for abnormal uterine bleeding and endometrial neoplasia, use of the LNG IUD represents a particularly sound choice in this patient population.[317,318]

Individuals who undergo bariatric surgery that may compromise the absorption of oral medications (Roux-en-Y gastric bypass or biliopancreatic diversion) should use oral contraceptives with caution (USMEC category 3).[319] There are no similar concerns for women who have had restrictive types of bariatric surgery (vertical banded gastroplasty, laparoscopic adjustable gastric band, or laparoscopic sleeve gastrectomy).

Hormonal Contraception in Women Taking Antiepileptic Drugs

Effective contraception is a critical component in management of the female patient with epilepsy because of increased risk of seizures in pregnancy, teratogenic effects of some antiepileptic drugs (AEDs), and the multitude of interactions between AEDs and hormonal contraception.[320] AEDs that induce hepatic enzymes can decrease serum concentrations of one or both of the estrogen or progestin component of oral contraceptives.[321] Risk of COC failure may be increased in the presence of CYP3A4 enzyme inducing, such as carbamazepine, felbamate, lamotrigine, phenobarbital, phenytoin, oxcarbazepine, primidone, topiramate, and rufinamide. Concomitant use of AEDs that induce hepatic enzymes with combined hormonal contraceptives is rated USMEC category 3, given that the increased risk of pregnancy

generally outweighs the benefits. Lamotrigine is a special case in that estrogen-containing contraceptives increase metabolism of lamotrigine, reducing drug levels.[322,323] Therefore, dose adjustments of lamotrigine will be needed if the two medications are used together. In addition, during the HFI, lamotrigine levels may increase.

As a high-dose contraceptive, injectable contraception (DMPA) appears effective with concomitant AED use, and IUDs are highly effective in women taking anticonvulsants. The USMEC recommends that when women taking liver enzyme–inducing medications use COC, a formulation containing a minimum of 30 μg of ethinyl estradiol should be used. In addition, expert opinion recommends selecting an oral contraceptive with a progestin that has a longer half-life (drospirenone, desogestrel, levonorgestrel) or using formulations with HFIs shorter than 7 days, or active pills in a continuous fashion, to potentially improve efficacy.[20,324] Given that the norethindrone POP available in the United States is dosed too low to consistently suppress ovulation, this contraception does not represent a sound choice for women taking enzyme-inducing AEDs. Data are limited for the contraceptive patch and vaginal ring. Contraceptive failures have also been reported in women taking enzyme-inducing AEDs and using the etonogestrel implant system (Nexplanon).[220-222]

Hormonal Contraception in Women Taking Antibiotics

Although there have been many retrospective case series and anecdotal reports of COC failure in women taking concomitant antibiotics, pharmacokinetic evidence of lower serum steroid levels exists only for rifampin.[80,81] Women taking rifampin should not rely on oral, transdermal, vaginal ring, or implantable contraception alone for protection. In contrast to rifampin, use of ampicillin, doxycycline, fluconazole, metronidazole, miconazole, quinolones, and tetracycline have not lowered steroid levels in women using COCs. The use of combined hormonal methods is not restricted among women taking broad-spectrum antibiotics, antifungals, or antiparasitics.[9]

Hormonal Contraception in HIV-Positive Women

More than 17 million women, many of reproductive age, are infected with HIV.[325] HIV transmission increasingly is linked to heterosexual intercourse. The role of hormonal contraception in HIV-positive women has been controversial. There may be an increased risk for acquiring or transmitting HIV in DMPA users, but DMPA is a needed contraceptive in areas of high HIV prevalence (sub-Saharan Africa) where there are limited alternative contraceptive options.[166] The USMEC recently revised its recommendation for the use of DMPA among women at increased risk for HIV acquisition based on a systematic review of progestin injectables that found a possible increased risk for HIV acquisition (adjusted hazard ratio 1.4; 95% CI, 1.2–1.6) in this population.[166] The current recommendation is that the advantages of DMPA still outweigh the theoretical or proven risks (category 2) among women at high risk for HIV infection, but women should be counseled regarding these issues.[165] POPs, combined hormonal methods, and implants are safe (category 1) for women at high risk for HIV infection or infected with HIV. Hormonal contraceptives, including DMPA, do not appear to have an impact on HIV disease progression.[326] For women on antiretroviral therapy, it is important to note there are several drug-drug interactions with hormonal contraceptives (particularly some non-nucleoside reverse transcriptase inhibitors and ritonavir-boosted protease inhibitors). These interactions might alter the safety and efficacy of both the hormonal contraceptive and the antiretroviral drug. The website https://www.hiv-druginteractions.org is kept up to date as new evidence emerges and new drugs are released. Case reports suggest that the antiretroviral drug efavirenz reduces contraceptive implant protection; IUDs and DMPA represent preferable contraceptive choices for women taking this medication or others that induce hepatic enzymes.[234]

IUDs are allowed by the USMEC for women with HIV (category 1), as there is no evidence that they are at higher risk for infectious complications than HIV-uninfected women.[166] The IUD has not been shown to affect disease progression or increase risk of transmission to sex partners. For women with acquired immunodeficiency syndrome, IUDs can be used if they are clinically well on antiretroviral therapy (category 1) and also considered if they are not (category 2). All IUD users with acquired immunodeficiency syndrome should be closely monitored for pelvic infection.

Hormonal Contraception and Chronic Hypertension

Blood pressure should be measured prior to initiating combined hormonal contraceptives.[8,327] Although few women develop overt hypertension after starting combined hormonal contraceptives, blood pressure should be checked at follow-up visits.[328] Modern low-dose COCs may increase systolic and diastolic blood pressure, on average, by 8 and 6 mm Hg, respectively.[329] This increase is thought to be secondary to increased activation of the renin-angiotensin system.[330] Nevertheless, a systematic review of the literature found that only a small percentage of women developed incident hypertension in up to 2 years of follow-up after starting a COC.[331]

For women with preexisting hypertension, the concern with combined hormonal methods is the increased risk of arterial thrombosis leading rarely to myocardial infarction and stroke. In women with stage 1 hypertension (systolic 140–159 mm Hg or diastolic 90–99 mm Hg), combined hormonal contraceptives should not be used unless no other method is appropriate or acceptable to the patient (USMEC category 3).[9] Women with stage 2 hypertension (systolic ≥160 mm Hg or diastolic ≥100 mg Hg) or vascular disease should not use combined hormonal contraceptives (USMEC category 4). Women on antihypertensive medication represent a separate category (see Table 18.2). Although the risk of cardiovascular disease in women with hypertension adequately controlled with medications should be reduced, there are no data on the use of combined hormonal contraceptives in this population. Therefore, use of combined hormonal contraceptives in these women requires clinical judgment (USMEC category 3). Women with well-controlled and monitored hypertension who are age 35 years or younger may be appropriate candidates for a trial of combined contraceptives, provided that they are otherwise healthy, show no evidence of end-organ vascular disease, and do not smoke cigarettes. If blood pressure remains well controlled with careful monitoring several months after contraceptive initiation, use can be continued.

Progestin-only contraceptives, such as DMPA, POPs, the etonogestrel implant, or the LNG IUD, are appropriate options in women with hypertension. Unlike other progestins, the use of DMPA in women with stage 2 hypertension (systolic ≥160 mm Hg or diastolic ≥100 mm Hg) is generally not

advised because of the theoretical risk of unfavorable lipoprotein changes (USMEC category 3).[9] However, risks and benefits of the method need to be weighed against risks of adverse pregnancy outcomes in women with hypertension. Blood pressure measurement prior to or during the use of progestin-only methods is not necessary.[8]

Hormonal Contraception in Women With Diabetes

A systematic review concluded that hormonal contraceptives have limited effect on carbohydrate metabolism in women without diabetes.[332] Likewise, COCs do not appear to impair carbohydrate metabolism or affect vascular disease in diabetic women. A history of gestational diabetes is not a contraindication to hormonal contraceptives. However, studies are limited and do not inform the management of diabetic women who are overweight.[333] Additionally, clinicians should consider the presence of other cardiovascular risk factors in a diabetic patient, such as obesity, smoking, hypertension, and age, when evaluating for hormonal contraception.

For women with diabetes of more than 20 years' duration or evidence of microvascular disease (retinopathy, nephropathy, or neuropathy), combined hormonal contraceptives are generally contraindicated (USMEC category 3 or 4 depending on the severity of the condition).[9] Due to the concern about possible hypoestrogenism during use of injectable contraception and effect on HDL levels,[334] DMPA is given a category 3 rating for women with diabetes of more than 20 years or evidence of microvascular disease, indicating that the risks usually outweigh the benefits.

The POP, IUDs, and implant are suitable for women with diabetes. A clinical trial found that metabolic control was similar in women with uncomplicated diabetes randomized to a copper IUD or the LNG IUD.[335]

Hormonal Contraception in Women Awaiting Surgery

VTE with pulmonary embolism remains a major cause of fatalities associated with surgical (including gynecologic) procedures.[336] There is concern that combined hormonal contraception use around the time of surgery may increase this risk. The procoagulant changes of COCs take 6 weeks or longer to resolve after discontinuation.[337] The benefits associated with stopping combined hormonal contraceptives 1 month or more before major surgery should be balanced against the risks of an unintended pregnancy. The USMEC recommends that COCs be discontinued before major surgery with an anticipated prolonged immobilization postoperatively (category 4).[9] In addition, patients with risk factors for VTE, such as prior VTE or undergoing high-risk procedures (e.g., major abdominal-pelvic surgeries, major orthopedic surgeries, or cancer surgery), may consider discontinuing COCs. Otherwise, for any major surgery for which the patient is expected to be ambulatory immediately after surgery, it is not necessary to discontinue combined hormonal contraceptives. Because of the low perioperative risk of VTE, it is not considered necessary to discontinue combined hormonal contraceptives before laparoscopic tubal sterilization or other minor surgical procedures not known to be associated with an elevated VTE risk. Progestin-only and intrauterine contraceptives are not expected to be associated with an increased risk of perioperative VTE.

Hormonal Contraception in Women With a History of Thromboembolism

Women with an acute deep vein thrombosis or pulmonary embolism, documented history of unexplained VTE, recurrent VTE or VTE associated with pregnancy, exogenous estrogen use, known thrombophilia or antiphospholipid antibody syndrome, or active cancer should not use combined hormonal contraceptives (USMEC category 4).[9,338] With respect to women not using anticoagulants, the risk of recurrent VTE depends on whether the initial deep vein thrombosis was associated with a permanent (e.g., factor V Leiden) or reversible (e.g., surgery) risk factor.[339] Therefore, a COC candidate who had experienced a single episode of VTE years earlier associated with a nonrecurring risk factor (e.g., VTE occurring after immobilization following a motor vehicle accident) may not currently be at increased risk for VTE. Accordingly, the decision to initiate estrogen-containing contraceptives in such patients can therefore be individualized. Most studies have not found an increased VTE risk with use of progestin-only contraception; therefore, these methods and IUDs are not contraindicated in women at increased risk of thromboembolism.[102]

Hormonal Contraception in Women Taking Anticoagulation Therapy

Long-term risks of warfarin for reproductive-age women include heavy or prolonged menstrual bleeding and rarely include hemoperitoneum after rupture of ovarian cysts. Warfarin is also a teratogen. Because the risk of recurrent thrombosis is reduced in women on therapeutic anticoagulation, some authorities recommend that use of estrogen-containing contraceptives can be considered for anticoagulated women with a prior history of venous thrombosis on a case-by-case basis, especially if alternative methods are not acceptable to the woman or contraindicated and they have a low risk of recurrence (USMEC category 3).[340] Among women with a history of thromboembolic disease participating in clinical trials of anticoagulants, use of hormonal contraceptives, including combination methods, does not elevate the risk of recurrent thrombosis among women using anticoagulants.[340] Accordingly, among chronically anticoagulated women, use of combination hormonal contraceptives may be appropriate. Because of its high contraceptive efficacy and ability to suppress uterine bleeding, use of LNG IUDs also represents a useful option for some anticoagulated women and is preferred over the copper IUD in this setting.[341-345] Intramuscular injection of DMPA consistently suppresses ovulation, and limited evidence has not revealed injection site problems, such as hematoma, in anticoagulated women.[346] Similar injections (e.g., influenza vaccine) in anticoagulated women have not shown risk of hematoma.[347] Accordingly, DMPA represents a useful contraceptive for anticoagulated women. There are no data on the risk of hematoma formation at the insertion site of contraceptive implants; however, it is unlikely to be a greater risk than that associated with intramuscular DMPA injections.

Hormonal Contraception for Women With Migraine Headaches

Headaches are common in women of reproductive age. Most of these headaches are tension headaches, not migraines.[348] Some women with migraines experience improvement in their symptoms with use of hormonal contraceptives, and some women's symptoms worsen. Because the presence of true migraine

headaches with aura affects the decision to use estrogen-containing contraception, careful consideration of the diagnosis is important. Most migraines occur without aura. Nausea, vomiting, photophobia, phonophobia, or visual blurring occurring before or during a migraine headache do not constitute aura. According to the International Headache Society, an aura is the complex of neurologic symptoms that occurs usually before the headache, but it may begin after the headache has begun.[349] A typical aura is reversible, lasts less than 60 minutes, and can consist of visual symptoms such as a zigzag figure (fortification spectrum, so named due to its resemblance to the walls of a medieval fortress) spreading across the visual field, sensory symptoms such as pins and needles, speech disturbances, or motor weakness.

Although the absolute risk of ischemic stroke is low in women of reproductive age, migraine with aura is an independent risk factor for stroke. Most studies note a higher risk of stroke in women who have migraine with aura than in those who have migraine without aura.[350,351] The assumption is that aura is associated with ischemic changes.[352] Use of combined hormonal contraception by itself also increases the risk of stroke in women, although the absolute risk is still low.[353] The elevated risk of ischemic stroke is likely due to the hypercoagulable state caused by estrogen. The risk of stroke among women using combined hormonal contraception rises with increasing age from 3.4 per 100,000 women-years in adolescents to 64.4 events per 100,000 women-years among women age 45 to 49 years.[72] In a recent case control study, investigators found combined hormonal contraceptive use by women with migraines with aura synergistically increased the risk of stroke.[54] In contrast, use of combined hormonal contraception by women with migraines without aura did not increase the risk over baseline. Although cerebrovascular events occur rarely among women with migraine with aura who use COCs, the impact of a stroke can be so devastating that clinicians should consider the use of progestin-only or intrauterine contraceptives in this setting. According to the USMEC, estrogen-containing contraceptives are contraindicated (category 4) in women migraine sufferers with aura and should be discontinued in patients suffering from migraine without aura if aura symptoms appear. Of note, some headache specialists are suggesting that today's ultralow-dose COC formulations (e.g., the COC formulated with ethinyl estradiol dosed at 10 μg) may be safe and also useful in preventing menstrual-related migraines in migraineurs who are otherwise healthy, normotensive nonsmokers.[354] There are no contraindications to the use of any progestin-only method or IUD in women with migraines with or without aura (USMEC category 1).[9]

Hormonal Contraception in Women With Systemic Lupus Erythematosus

Although effective contraception is important for women with lupus, concerns about increasing disease activity and thrombosis have resulted in clinicians rarely prescribing combination estrogen-progestin oral contraceptives to women with this disease. Indeed, women with SLE are at higher risk for ischemic heart disease, stroke, and VTE, especially in the presence of antiphospholipid antibodies.[355-357] Prior to initiating contraceptives in women with SLE, the level of disease activity and presence of antiphospholipid antibodies and thrombocytopenia should be established. Combined hormonal contraception is contraindicated in women with SLE and positive antiphospholipid antibodies due to the increased risk of venous and arterial thromboembolism (USMEC category 4). In other SLE patients, decisions regarding use of combined hormonal contraceptives should consider the presence of other cardiovascular disease risk factors (e.g., older age, smoking, hypertension, diabetes, and hypercholesterolemia) and is rated USMEC category 2. Use of COC does not appear to worsen disease activity in women with inactive or stable active disease.[358] Although progestin-only methods are not considered to increase the risk of VTE, they are all rated category 3 (risks outweigh benefits) for SLE patients with antiphospholipid antibodies. This is because the propensity for VTE is quite high in these patients, and one randomized controlled trial reported the development of thrombosis in two such women taking POPs.[359,360] Although the category 3 classification also applies to the LNG IUD, systemic drug levels with this device would be minimal. In other SLE patients without antiphospholipid antibodies, progestin-only methods are rated USMEC category 2. There are no restrictions on the use of the copper IUD in women with SLE and positive antiphospholipid antibodies. For women with thrombocytopenia, the LNG IUD is preferred over the copper IUD. Many women with SLE take immunosuppressant medications, and, in the past, the use of IUDs among immunocompromised women has been controversial. However, immunosuppression is no longer considered a contraindication to IUD use.[361,362]

Hormonal Contraception in Women With Sickle Cell Disease

Similar to pregnancy in SLE patients, pregnancy in women with sickle cell disease increases maternal and fetal morbidity and mortality rates. The safety of hormonal contraceptives in women with homozygous (SS) sickle cell disease has been controversial.[363] Only one small randomized controlled trial has addressed this issue. Twenty-five patients were given DMPA or intramuscular saline placebo injections once every 3 months in a crossover design. DMPA users were less likely to experience painful sickle episodes (OR, 0.23; 95% CI, 0.05–1.02).[364] No randomized studies have addressed estrogen-containing products.[365] These limited data suggest that DMPA and other progestin-only methods are a safe contraceptive option for women with sickle cell disease.[363]

No well-controlled study has assessed whether VTE risk in oral contraception users with sickle cell disease is higher than in other combination oral contraception users. However, a small case-control study conducted in the United States found that COC use was associated with a nonsignificantly elevated risk of VTE.[366] Cross-sectional studies of women with sickle cell disease have observed no differences in markers for platelet activation, thrombin generation, fibrinolysis, or red blood cell deformability between users of combination oral contraception methods, users of progestin-only methods, and nonusers of hormonal contraception.[367] On the basis of these observations, studies of pregnant women with sickle cell disease, and small observational studies of women with sickle cell disease who use COCs, and on theoretical considerations, the USMEC concludes that pregnancy carries a greater risk than estrogen-containing contraceptive use.[9] This recommendation would change, however, if the woman had concomitant cardiovascular risk factors or pulmonary hypertension—a complication of sickle cell disease.

Although the lack of evidence on IUD use among women with sickle cell disease represents a major gap in the literature, theoretical concerns about IUD use in this population are few. There is no current evidence to support limiting IUD use among women with sickle cell disease.[363] The USMEC rates the LNG IUD category 1, indicating that there are no restrictions for this method in women with sickle cell disease. The copper IUD is considered category 2,

in which benefits outweigh risks, because of the theoretical concern about increased blood loss with menstruation.

Hormonal Contraception in Depression

Depression and mood disorders are extremely common in women of reproductive age.[128] The issues to consider are the impact of hormonal contraceptives on mood and the potential impact of treatments for depression on contraceptive efficacy. The use of any method of contraception is acceptable for women who suffer from depression, and there are no contraindications (USMEC category 1).[9] There is no evidence that hormonal contraception including DMPA worsens a woman's mood, regardless of whether or not she has baseline depression.[127,368] Selective serotonin reuptake inhibitors and serotonin norepinephrine reuptake inhibitors, the most commonly used antidepressants, do not appear to interact with hormonal contraceptives.[369,370] In contrast, a clinical trial observed that use of the herbal remedy St. John's wort, a hepatic enzyme inducer, increased progestin and estrogen metabolism, as well as breakthrough bleeding and the likelihood of ovulation, in women using COCs.[371,372] For this reason, concomitant use of St. John's wort is rated USMEC category 2 for combined hormonal contraception, POPs, and the etonogestrel implant.

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The complete list of references is available online at ExpertConsult.com.

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19

Testicular Disorders

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CHAPTER OUTLINE

KEY POINTS

- The testes are critical for normal development of internal and external genitalia in the fetus; for secondary sexual characteristics, sexual function, and initiation of spermatogenesis during puberty; and for development and maintenance of adult male phenotype, sexual function, and fertility.
- Knowledge of the anatomy, physiology, and regulation of the hypothalamic-pituitary-testicular axis forms the basis for understanding the clinical manifestations, diagnosis, and management of the main disorders of testis function, androgen deficiency and infertility.
- Male hypogonadism is a clinical syndrome that results from the failure of the testes to produce adequate amounts of testosterone (androgen deficiency) and sperm, or from an isolated impairment of spermatogenesis.
- The consequences of androgen deficiency vary depending on the stage of sexual development. In the fetus, androgen deficiency causes ambiguous genital development; in the child, it causes delayed puberty or eunuchoidism; and in adults, it causes sexual dysfunction, gynecomastia, infertility, and changes in body composition, muscle mass and strength, and bone density and strength that adversely affect health.
- Male hypogonadism is common, but the diagnosis should only be made in men with symptoms and signs of androgen deficiency and consistently low serum testosterone concentrations, in the absence of conditions that transiently suppress testosterone.
- In men with low serum total testosterone concentrations and conditions that alter sex hormone–binding globulin, an accurate assessment of serum free testosterone is useful.
- In men with hypogonadism, gonadotropin concentrations should be measured to distinguish between primary and secondary hypogonadism.
- Primary hypogonadism is associated with infertility that cannot be treated with medical therapy.
- Men with secondary hypogonadism should be evaluated for causes of hypothalamic or pituitary dysfunction, such as hyperprolactinemia, Cushing syndrome, sella mass, and iron overload syndromes.
- Gonadotropin replacement therapy generally improves spermatogenesis and fertility in men with secondary hypogonadism.
- Prior to initiating testosterone replacement in men with obesity or metabolic syndrome, low serum testosterone concentrations, and symptoms of possible androgen deficiency, it is important to initiate lifestyle changes for weight loss. Drugs that might suppress the hypothalamic-pituitary-testicular axis should be stopped, if possible.
- Efficacy and safety monitoring should be performed during testosterone treatment.

The testes have critical physiologic roles during various stages of development. During early fetal life, production of testosterone and antimüllerian hormone (AMH) by the fetal testes is required for the differentiation and development of normal male internal and external genitalia. During puberty, activation of the hypothalamic-pituitary-testicular axis and testosterone production by the testes are necessary for the induction of secondary (adult) male sexual characteristics, stimulation of sexual function, and initiation of sperm production. In adults, testis production of testosterone is required for the maintenance of adult male characteristics (virilization), sexual function, spermatogenesis, and fertility potential. Therefore, disorders of the testis may result in abnormalities in sexual development and function, body habitus and function, and fertility that have profound effects on health and well-being.

Disorders of the testis are common. Klinefelter syndrome is the most common human sex chromosome abnormality and the most common cause of primary hypogonadism, occurring in 1 in 500 to 600 male births.[1,2] Isolated disorders of sperm production are the main causes of male infertility, affecting approximately 5% to

6% of otherwise healthy men in the reproductive age group.[3] Testicular disorders resulting in testosterone deficiency may contribute to complaints of reduced libido (sexual interest and desire), erectile dysfunction, gynecomastia (benign breast enlargement), and reduced bone mass (osteoporosis), all of which are common in men, particularly as they age. Finally, disordered hypothalamic-pituitary-testicular function is commonly associated with chronic systemic illnesses, wasting syndromes, morbid obesity, chronic use of certain medications (e.g., opioids and glucocorticoids), and aging. These conditions often result in testosterone deficiency that, if severe and prolonged, may contribute to clinical manifestations and morbidity.[4,5]

The treatment of testicular disorders usually results in significant clinical improvements in function and quality of life. In prepubertal boys and adults with severe testosterone deficiency, testosterone therapy results in dramatic transformations in body composition and function.[6] In men with low serum testosterone, infertility, and impaired spermatogenesis due to gonadotropin deficiency, gonadotropin replacement therapy generally increases sperm and testosterone production and restores fertility. Finally, advances in assisted reproductive technology (ART) have permitted previously infertile men with testicular disorders to have children. For example, although men with Klinefelter syndrome and azoospermia (no spermatozoa in their ejaculate) were once thought to have untreatable infertility, testicular sperm extraction (TESE) using microsurgical techniques combined with intracytoplasmic sperm injection (ICSI) may permit some of these men to father children.[7]

Functional Anatomy and Histology

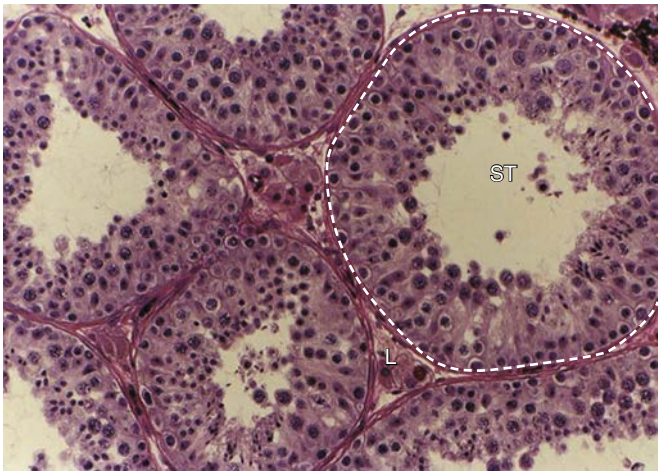
The Testis

Adult testes are paired, ovoid organs that hang from the inguinal canal by the *spermatic cord* (which is composed of a neurovascular pedicle, vas deferens, and cremasteric muscle); they are located outside the abdominal cavity within the scrotum. The left testis hangs lower in the scrotum than the right in about 60% of men, and the right testis hangs lower in approximately 30% of men. Each testis has a volume of 15 to 30 mL and measures 3.5 to 5.5 cm in length by 2.0 to 3.0 cm in width.[8,9]

The testis comprises two structurally and functionally distinct compartments: the *seminiferous tubule compartment*, which is composed of Sertoli cells and developing germ cells at various stages of spermatogenesis and accounts for 80% to 90% of the volume of the testis, and the *interstitial compartment*, which is composed of Leydig cells that secrete testosterone, the main male sex steroid hormone, as well as peritubular myoid cells, fibroblasts, neurovascular cells, and macrophages[10] (Fig. 19.1). Because germ cells constitute most of the testis volume, small testes are usually an indication of significantly impaired spermatogenesis.

The testis is surrounded by a fibrous capsule, the *tunica albuginea.* Fibrous septa that emanate from the tunica albuginea separate the parenchyma of the testis into lobules. The arterial blood supply of the testes is derived primarily from the testicular (internal spermatic) arteries that arise from the abdominal aorta and descend through the inguinal canal in the spermatic cord. Collateral blood supply is provided by the cremasteric and deferential arteries. This collateral supply permits survival of the testis after a testicular artery ligation associated with surgical fixation of a high undescended testis into the scrotum (orchiopexy). However, twisting of the spermatic cord, known as *testicular torsion*, results in strangulation of the blood supply to the testis and causes testicular necrosis and infarction after 6 to 8 hours, making this condition a surgical emergency.[11] A testis that has a *bell-clapper deformity* (i.e., is not attached to the scrotal wall) is more susceptible to testicular torsion. Lymphatic drainage from the testes follows the testicular arteries to periaortic lymph nodes; this is a common route for metastasis of testicular cancer.

A network of veins that compose the pampiniform plexus provides venous drainage from the testes. The pampiniform plexus coalesces into the testicular (internal spermatic) vein. The right testicular vein drains into the inferior vena cava, and the left testicular vein empties at a right angle into the left renal vein. One-way valves in testicular veins prevent backflow of blood into the scrotum. Abnormal enlargement of the venous plexus draining a testicle, known as a *varicocele*, occurs if valves are defective or absent or if there is extrinsic venous compression impeding normal venous drainage.[12] Increased pressures associated with the backflow of blood and altered temperature regulation may contribute to testicular dysfunction associated with a varicocele. Ninety-eight percent of varicoceles occur in the left scrotum, possibly because of absent or defective valves in the left testicular vein. The presence of a prominent unilateral right-sided varicocele or new-onset varicocele on either side should prompt evaluation for venous obstruction by an abdominal or pelvic malignancy (e.g., renal cell carcinoma) or lymphadenopathy; a chronic right-sided varicocele may also indicate *situs inversus*. Rarely, an anatomic anomaly of the superior mesenteric artery that compresses the left renal vein causes a left-sided varicocele; this is known as the *nutcracker syndrome*.



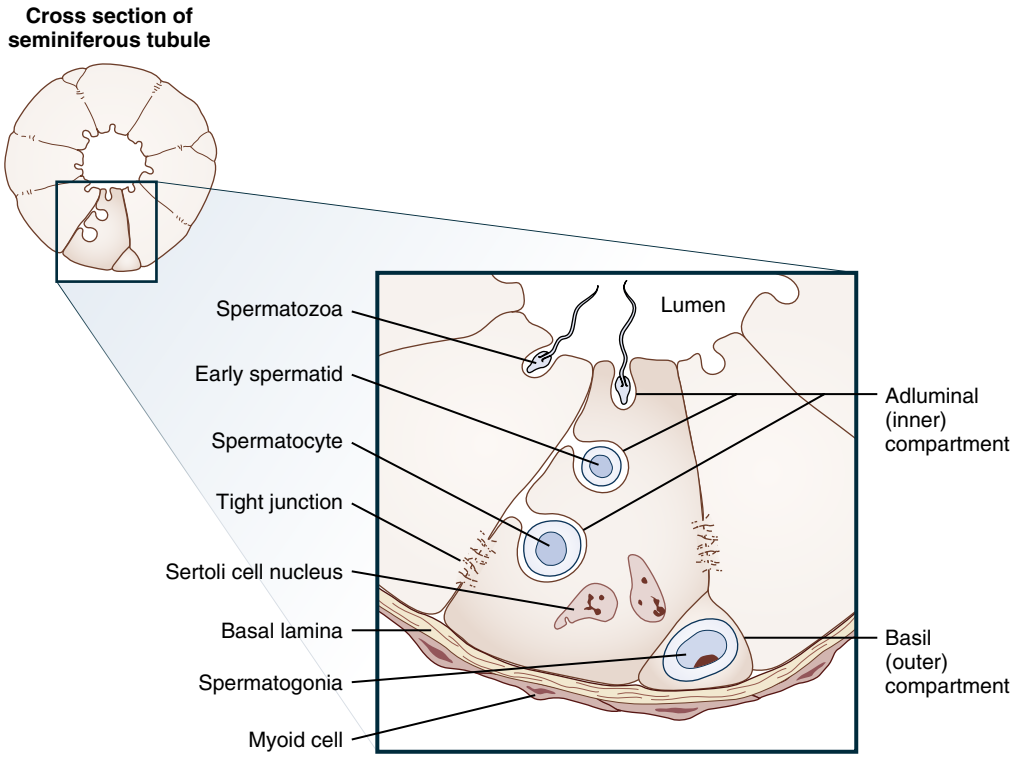
• **Fig. 19.1** Light photomicrograph of the seminiferous tubule and interstitial compartments of the human testes. The seminiferous tubule (ST) compartment makes up the majority of the testis and is composed of developing germ cells enveloped by Sertoli cells. Spermatogonia line the basal lamina of the seminiferous tubules, spermatocytes at various stages of development are present in the middle layers of the tubules, and spermatids at various steps of maturation are present in the luminal aspect of the seminiferous tubules. Within each tubule, there are germ cells at different stages of spermatogenesis. In the interstitial compartment, there are prominent clusters of Leydig cells (L) nestled between seminiferous tubules, peritubular myoid cells within the basal lamina of the tubules, and scattered blood vessels and macrophages. (From Matsumoto AM. Spermatogenesis. In: Adashi EY, Rock JA, Rosenwaks Z, eds. *Reproductive Endocrinology, Surgery, and Technology.* Philadelphia, PA: Lippincott-Raven; 1996:359–384.)

Because the testes are located outside the abdominal cavity, they are exposed to temperatures approximately 2°C lower than core body temperature. The position of the testes within the scrotum and the testicular temperature are regulated by the cremasteric muscle. The cremasteric muscle contracts when warming is needed, resulting in shortening of the spermatic cord and drawing of the testis toward the abdomen; when cooling is needed, the muscle relaxes, resulting in lowering of the testis into the scrotum. In addition, the pampiniform venous plexus provides a countercurrent heat exchange mechanism to cool the testis by surrounding the testicular artery with cooler venous blood. A testis temperature slightly lower than core body temperature is important for normal spermatogenesis. Exposure of the testes to higher temperatures, such as with failure of the testes to descend normally into the scrotum (*cryptorchidism*) or excessive external heat exposure due to frequent, prolonged hot tub use may impair spermatogenesis.

Seminiferous Tubule

Seminiferous tubules contain epithelium consisting of Sertoli cells that envelop and support germ cells undergoing progressive differentiation and development into mature spermatozoa. Once released into the lumen, mature spermatozoa are transported within seminiferous tubules, which measure up to 70 cm in length and are tightly coiled within lobules of the testis, to the rete testis, the efferent ducts, the epididymis, and, finally, the vas deferens for ejaculation. The seminiferous tubules are surrounded by a basal lamina composed of an extracellular matrix that serves to separate them from the interstitial compartment, provides structural integrity to the tubules, and regulates the function of cells in contact with it. Histologic examination of a testis biopsy specimen in cross section reveals many different seminiferous tubules surrounded by basal lamina and clusters of Leydig cells in the interstitial compartment between each tubule[10] (see Fig. 19.1).

Sertoli cells extend from the basal lamina to the lumen of tubules, and adjacent Sertoli cells envelop and provide a structural scaffold for germ cells as they differentiate within the tubule[9] (Fig. 19.2). Undifferentiated spermatogenic stem cells, called *spermatogonia*, lie along the basal lamina at the periphery of tubules, interspersed between Sertoli cells. Adjacent Sertoli cells surround spermatogonia and form specialized junctional complexes or tight junctions that divide the seminiferous tubule into the *basal compartment*, in which spermatogonia reside, and the *adluminal compartment*, which is occupied by differentiating germ cells. Sertoli cell tight junctions impede the passage of large molecules, steroids, and ions into the seminiferous tubule and constitute the cytologic basis of the *blood-testis barrier*, analogous to the blood-brain barrier. In the adluminal compartment, *spermatocytes* derived from spermatogonia in the basal compartment undergo meiosis to form *spermatids* that progressively mature (*spermiogenesis*), with



• **Fig. 19.2** Schematic diagram of the cells in the seminiferous tubule (*top*). The seminiferous tubule consists of Sertoli cells that surround developing germ cells (*middle*). Sertoli cells extend from the basal lamina to the lumen. Tight junctions between adjoining Sertoli cells separate the seminiferous tubule into basal and adluminal compartments and are the anatomic basis for the blood-testis barrier (*bottom*). The basal compartment, which contains spermatogonia lining the basal lamina and peritubular myoid cells, is exposed to the interstitial compartment, which contains Leydig cells and blood vessels that deliver endocrine regulators of testis function (e.g., gonadotropins). The adluminal compartment contains developing spermatocytes, spermatids, and mature spermatozoa that are released into the lumen of the seminiferous tubule. (From Matsumoto AM. The testis. In: Felig P, Frohman LA, eds. *Endocrinology and Metabolism.* 4th ed. New York, NY: McGraw-Hill; 2001:635–705.)

the more mature germ cells occupying positions closer to the lumen, until mature spermatozoa are released into the lumen of the tubule (*spermiation*).

Because of the blood-testis barrier, only Sertoli cells and spermatogonia are directly accessible to endocrine and paracrine regulation from the circulation and cells of the interstitial compartment, respectively. Sertoli cells need to synthesize and secrete many products, some of which are present in the circulation but not accessible to developing germ cells in the adluminal compartment, to nurture and regulate spermatogenesis. Sertoli cells contain receptors for follicle-stimulating hormone (FSH) and androgens, and they mediate the regulation of spermatogenesis by circulating FSH and testosterone produced locally by Leydig cells in response to stimulation from circulating luteinizing hormone (LH). Sertoli cells produce extracellular matrix components and AMH that causes regression of müllerian ducts and prevents the development of female accessory sex organs in the male fetus and inhibin B, which is the most important testicular contributor to negative feedback suppression of FSH.

Spermatogenesis

In male humans, the process of spermatogenesis supports a production rate of approximately 120 million mature spermatozoa per day by the testis (approximately 1000 per heartbeat!).[13] Spermatogenesis, the process by which stem cells (spermatogonia) differentiate into mature spermatozoa, proceeds in three functionally distinct phases: (1) the *mitotic* or *proliferative phase*, during which the majority of spermatogonia undergo mitosis to renew the stem cell pool and a minority become committed to further differentiation to produce spermatocytes; (2) the *meiotic phase*, during which spermatocytes undergo successive meiotic divisions to produce haploid germ cells (spermatids); and (3) *spermiogenesis*, during which immature, round spermatids differentiate into mature spermatozoa[10,14] (Fig. 19.3).

Proliferative Phase

Based on chromatin staining and pattern, spermatogonia may be classified as A dark (A_d), A pale (A_p), or B spermatogonia. Because of their relatively low mitotic rate, A_d spermatogonia are thought to be the spermatogonial stem cells. A_d spermatogonia are relatively resistant to external insults (e.g., ionizing radiation), and in response to such insults, they undergo mitotic proliferation. However, severe or complete depletion of A_d spermatogonia, such as that which occurs with high-dose x-irradiation or vascular compromise, results in irreversible impairment or loss of sperm production.

A small number of A_d spermatogonia undergo mitotic divisions to form A_p and then B spermatogonia. In humans, the rate of formation of B spermatogonia is low, so only a small number of B spermatogonia are available to enter meiosis and undergo further differentiation.[15] This rate limits the efficiency of spermatogenesis in humans. B spermatogonia are the most sensitive germ cells to the effects of ionizing radiation, and their numbers are reduced after irradiation of the testes.[16]

B spermatogonia that become committed to further differentiation undergo mitotic division to form *preleptotene* or *resting* spermatocytes, which enter a prolonged meiotic phase of 24 days. Spermatogonia do not completely separate after mitosis (*incomplete cytokinesis*). Groups of spermatogonia remain connected via cytoplasmic bridges, forming a syncytium, and undergo meiosis and spermiogenesis in synchrony.

Meiotic Phase

Preleptotene primary spermatocytes contain a diploid complement of chromosomes (46 chromosomes or 2N, where N is the number of haploid chromosomes), and they are the last germ cells to undergo DNA synthesis. Preleptotene spermatocytes undergo an initial round of meiotic division (*meiosis I*), lasting longer than 2 weeks, to form secondary spermatocytes that contain a haploid complement of chromosomes (1N). Secondary spermatocytes, which are present for only about 8 hours, undergo a second meiotic division (*meiosis II*) to form haploid spermatids.

Improper segregation of chromosomes (*meiotic nondisjunction*) resulting in an abnormal number of chromosomes (*aneuploidy*) occurs in 0.7% of live births and 50% of first-trimester abortuses.[17,18] Klinefelter syndrome is classically associated with a 47,XXY karyotype caused by paternal meiotic nondisjunction in 50% of cases.[19,20]

Spermiogenesis

The final phase of spermatogenesis is the maturation of spermatids from round to elongated spermatids and then to mature spermatozoa; this process, known as spermiogenesis, is followed by release of spermatozoa into the lumen of seminiferous tubules (spermiation). The major changes that occur during spermiogenesis include formation of the sperm head with condensation of chromosomes (DNA and nucleoproteins) and formation of the *acrosomal cap*, which contains proteolytic enzymes needed for sperm penetration of the ovum; formation of the sperm tail or *flagellum* (pointing into the lumen) that permits motility; phagocytic removal of excess spermatid cytoplasm (known as the *residual body*) by Sertoli cells; and release of mature spermatozoa into the lumen. Progressive maturation of spermatids is accompanied by progressive movement of more mature spermatids toward the lumen of the seminiferous tubule. Under the regulation of FSH and intratesticular testosterone, Sertoli cells nurture spermiogenesis by supporting spermatid maturation.

Germ Cell Loss

Compared with most other species, the efficiency of spermatogenesis in humans is relatively poor, and the germ cell degeneration and loss that occur predominantly during mitosis and meiosis are major contributors to the low efficiency of sperm production.[21] Significant degrees of germ cell degeneration occurring during meiosis results in a loss of about 60% during formation of spermatids from preleptotene spermatocytes. As men age, increased germ cell degeneration may result in lower daily sperm production. It is hypothesized that germ cell degeneration prevents abnormal germ cells from further development, thereby serving an important quality control function.

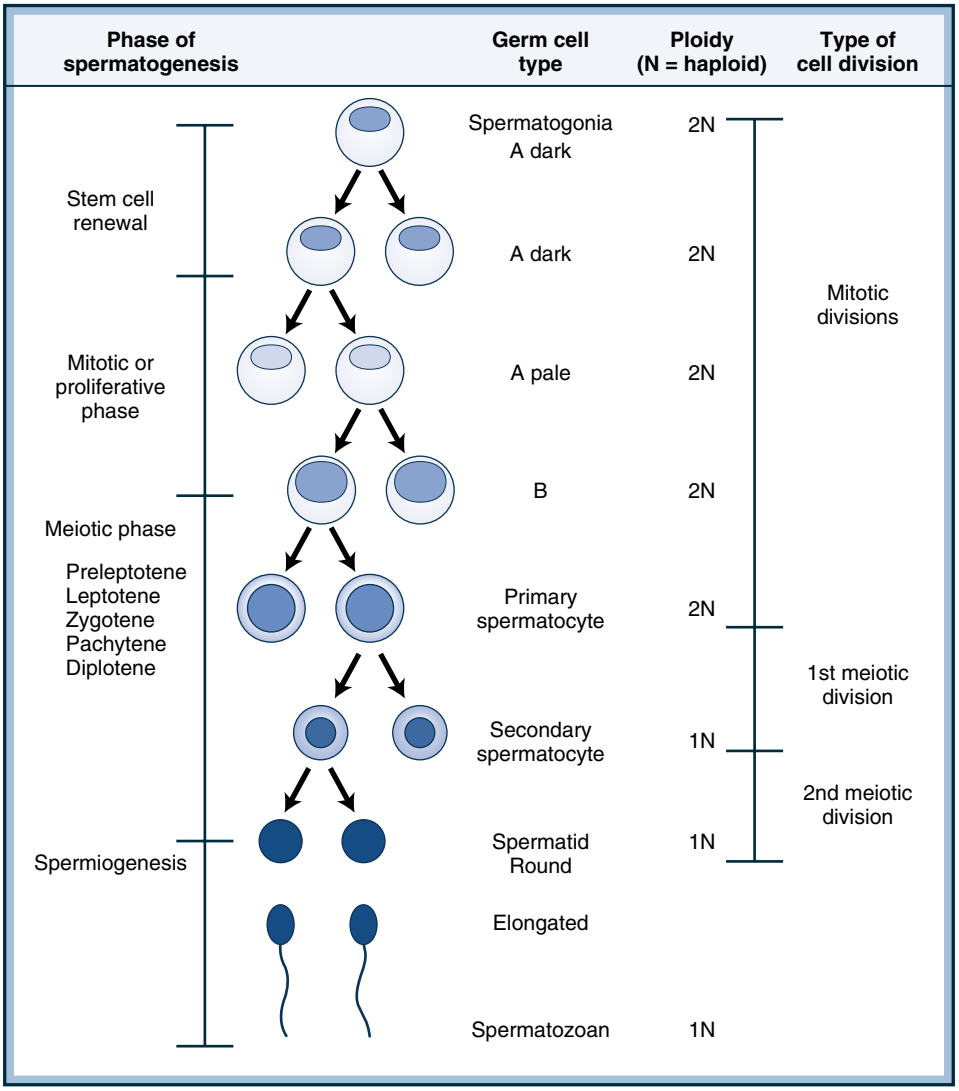
Organization of Spermatogenesis

Histologic examination of a human testis in cross section reveals that germ cells at particular phases of development cluster in six cellular associations, referred to as *stages*, which together constitute a complete *cycle* of spermatogenesis. In most mammals, stages are organized sequentially along the longitudinal axis of the tubules so that all of the germ cells present in a cross section of a tubule are in the same stage of spermatogenesis.[22,23] In contrast, three or more different stages of a cycle may variably be present in a cross section of human testis. Although some have proposed a helical pattern of stages along the tubule to explain this seemingly chaotic arrangement, this has not been confirmed by others.

In humans, the time for completion of spermatogenesis (from A_p spermatogonia to release of mature spermatozoa) is 74 ± 4 days.[24] The epididymal transit time of spermatozoa is approximately 12 to 21 days.[25] Therefore, external insults to the testis (e.g., ionizing radiation) or induction of gonadotropin deficiency (e.g., by male hormonal contraceptive regimens) that inhibits early germ cell development and reduces spermatogenesis are not reflected in reduced sperm counts in the ejaculate for 3 months or more.

Sperm Transport and Fertilization

Mature spermatozoa released into the lumen of the seminiferous tubule are transported to the rete testis, to the efferent ducts of the testis, and then to the caput epididymis primarily by peristaltic contractions and intratubular fluid flow. In the epididymis, sperm undergo biochemical and functional modifications that result in the capacity for sustained forward motility. After ejaculation from the vas deferens and penis into the female reproductive tract, human sperm interact with uterine secretory products and undergo *capacitation* in the uterus; the resulting biochemical alterations in the acrosomal cap increase the sperm membrane fluidity, calcium influx, and sperm motility ("hyperactivation") so that the spermatozoa acquire the capacity to fertilize an ovum.[26,27] After capacitation, as the spermatozoon meets an ovum in the ampulla of the fallopian tube, the sperm binds to the egg and releases hyaluronidase to penetrate the zona pellucida that surrounds the ovum, a process known as the *acrosome reaction*. Fertilization then occurs as the plasma membranes of sperm and ovum fuse.

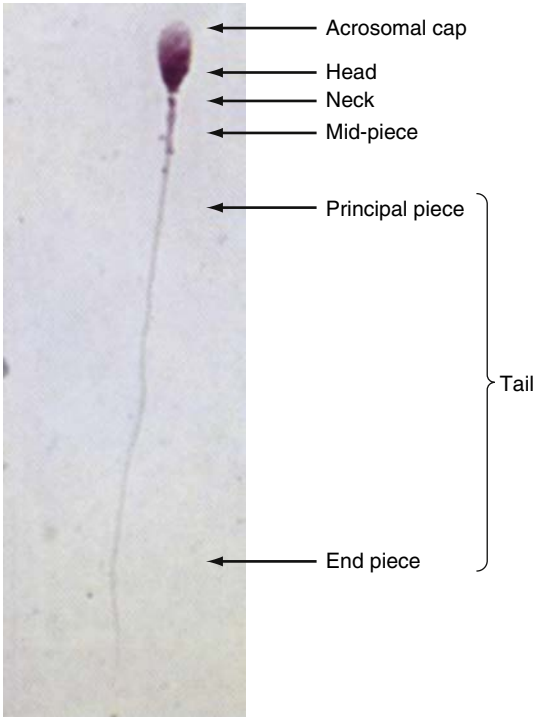


• **Fig. 19.3** Schematic diagram of human spermatogenesis. Spermatogonial stem cells undergo self-renewal by mitotic division. At the initiation of spermatogenesis, some spermatogonia undergo differentiation into primary spermatocytes, which contain a diploid number of chromosomes (2N = 46 chromosomes). The primary spermatocytes then undergo two successive meiotic divisions to form spermatids, which contain a haploid number of chromosomes (1N = 23 chromosomes). Spermatids undergo spermiogenesis from round to elongated spermatids to form mature spermatozoa, which also contain a haploid number of chromosomes. (Modified from Matsumoto AM. Spermatogenesis. In: Adashi EY, Rock JA, Rosenwaks Z, eds. *Reproductive Endocrinology, Surgery, and Technology.* Philadelphia, PA: Lippincott-Raven; 1996:359–384.)

Spermatozoa

Morphologically, most ejaculated human spermatozoa are composed of an oval-shaped head that contains condensed chromatin and nucleoproteins, an acrosomal cap that covers approximately the anterior two thirds of the head, a short neck that contains centrioles important for attachment of the tail and cleavage of the zygote after fertilization, a middle piece that consists of axial filaments surrounded by a spiral of mitochondria containing oxidative enzymes that provide energy for motility, and a long tail or flagellum that permits normal progressive forward motility of the spermatozoa[10] (Fig. 19.4). The flagellum consists of a microtubule-based cytoskeleton, the *axoneme*, which has a characteristic structure composed of two central microtubules surrounded by nine microtubule doublets (9 × 2 + 2 pattern) that serves as a scaffold for motor protein complexes (i.e., *dynein arms*).[28]

In humans, the normal sperm concentration in the ejaculate is greater than 15 million/mL, with 4% or more having normal morphologic appearance by strict criteria (≥30% by previous criteria) and 40% or more having total motility, according to the most recent World Health Organization (WHO) criteria.[29,30] Men with infertility may exhibit morphologic abnormalities of the sperm head (tapered, amorphous, or double-headed forms) or of the tail (coiled forms) and reduced or absent motility. Alterations in the structural or functional components of the axoneme (e.g., absence of dynein arms) result in altered motility, and deficiency of dynein adenosine triphosphatase results in primary ciliary dyskinesia (also known as immotile cilia syndrome), a syndrome associated with lung disease, recurrent sinusitis, and otitis media, as well as male infertility.[31]



• **Fig. 19.4** Light photomicrograph of an ejaculated mature human spermatozoon, composed of a head, neck, mid-piece, and tail (principal piece and end piece). (From Matsumoto AM. Spermatogenesis. In: Adashi EY, Rock JA, Rosenwaks Z, eds. *Reproductive Endocrinology, Surgery, and Technology.* Philadelphia, PA: Lippincott-Raven; 1996:359–384.)

Interstitium

The interstitial compartment of the testis contains clusters of Leydig cells, the primary sex steroid–producing cells of the testis, which make up only about 5% of testis volume[23,32] (see Fig. 19.1). Leydig cells produce testosterone, which acts as a paracrine regulator within the seminiferous tubules of the testis on Sertoli cells in close proximity to stimulate spermatogenesis. Testosterone is secreted into adjacent testicular capillaries and then into the general circulation to act as an endocrine signal on androgen target organs throughout the body. Leydig cells also produce *insulin-like factor 3* (INSL3), a peptide hormone in the relaxin-insulin family, which plays an important role in the first phase of testicular descent from the abdomen into the scrotum during development.[33] INSL3 also may be an important autocrine regulator of Leydig cells and a paracrine regulator of germ cells directly.[34]

Peritubular myoid cells that surround the seminiferous tubules are contractile, smooth muscle-like cells that serve to facilitate forward transport of spermatozoa and testicular fluid within the tubular lumen, provide structural integrity to the tubule, secrete extracellular matrix components and putative regulatory factors such as growth factors, and are involved in retinol metabolism.[23,35] These cells contain androgen receptors (ARs) and are thought to mediate some of the paracrine effects of testosterone on Sertoli cells within the seminiferous tubules, but their precise role in human testicular physiology remains unclear.

The interstitial compartment also contains macrophages that may regulate Leydig cell steroidogenesis by secretion of cytokines and may play a role in phagocytosis of degenerating cells and necrotic debris. The interstitium contains arterioles and a rich network of capillaries that permit secretion of testosterone and other products into the circulation and delivery of circulating gonadotropins (e.g., LH and FSH).

Testis Development

Fetal Development

During embryogenesis, the Y chromosome directs the development of the testis from an undifferentiated anlage that has the potential to develop into either a testis or an ovary.[36] The *SRY (sex-determining region of the Y chromosome) gene*, located on the pseudoautosomal region of the Y chromosome, encodes a transcription factor that increases the expression of SRY-box 9 (*SOX9*), which in turn drives the formation of Sertoli cells and testis differentiation. *SRY* gene expression is activated by several factors, including steroidogenic factor 1 and the DNA-binding protein GATA4.[37] *SRY*-independent *SOX9* expression may also be driven by steroidogenic factor 1. *SOX9* directs the expression of other genes that are essential in testis differentiation, such as fibroblast growth factor 9 (*FGF9*) and *AMH*, and in repression of ovarian differentiation, such as *WNT4* and *NR0B1* (formerly known as *DAX1*). In the absence of *SRY* or *SRY* action, *SOX9* is repressed by many factors, including *β-catenin*, and development of the follicular cells and ovaries follows.

Primordial germ cells originate in the yolk sac and migrate to the genital ridges. Together with coelomic epithelial and mesenchymal cells that eventually differentiate, respectively, into Sertoli cells and interstitial cells (Leydig and peritubular myoid cells), they form the *genital blastema* by 6 weeks of gestation. Primordial germ cells that fail to migrate normally explain the location of extragonadal germ cell cancers in men. Under the influence

of gene products activated by *SRY*, primordial germ cells become surrounded by primitive Sertoli cells to form seminiferous or sex cords that eventually develop into seminiferous tubules.

Under the influence of maternal human chorionic gonadotropin (hCG) initially and later LH and FSH from the fetal pituitary gland, immature Leydig cells, Sertoli cells, and germ cells undergo differentiation, proliferation, and organization. Testosterone production from fetal Leydig cells increases progressively and induces development of the epididymis, vas deferens, and seminal vesicles from wolffian or mesonephric ducts. Conversion of testosterone to 5α-dihydrotestosterone (DHT) in the urogenital tract leads to the formation of the prostate from the urogenital sinus, the penis from the genital tubercles and folds, and the scrotum from the urogenital swelling.[38] In the absence of testosterone production or action, female internal and external genitalia develop. AMH secretion from the fetal Sertoli cells causes regression of the müllerian or paramesonephric ducts and prevents the formation of a uterus and fallopian tubes.

Male phenotypic development is complete by about 15 weeks of gestation, after which the proliferation of Sertoli and germ cells arrests. The Leydig cells produce testosterone in the third trimester when fetal gonadotropin secretion rises, and this phase of androgen action is required for normal penis size at birth.

Testis Descent

The developing testis is attached to the diaphragm by the craniosuspensory ligament and anchored to the inguinal region by a caudal ligament known as the *gubernaculum*. Descent of the testis occurs in two phases.[33] During the initial *transabdominal phase*, the testis descends within the abdomen to the inguinal region; this occurs between 10 and 23 weeks of gestation. Studies in animals suggest that testis descent during this phase depends on two processes: (1) regression of the craniosuspensory ligament, induced by testosterone, which frees the testes to descend, and (2) thickening of the gubernaculum, which is controlled by INSL3 and its cognate receptor, relaxin-family peptide receptor 2 (RXFP2, also known as leucine-rich repeat–containing G protein–coupled receptor 8 or G protein–coupled receptor affecting testis descent). During the *inguinoscrotal phase*, which begins at 26 to 28 weeks of gestation, the testis descends into the scrotum; this process is largely controlled by the effects of testosterone on gubernacular shortening and contractions. The effects of testosterone may be mediated in part by the neurotransmitter, calcitonin gene–related peptide, which is released by the genitofemoral nerve. The importance of testosterone, gonadotropins, and INSL3 in testis descent in humans is suggested by the occurrence of undescended testes (cryptorchidism) associated with fetal androgen deficiency or resistance, gonadotropin deficiency, and *INSL3* or *RXFP2* mutations.[34]

Testis descent is usually complete, with the testes entirely within the scrotum, by 7 months of gestation to birth. During testis descent, a herniation of the abdominal cavity, the *processus vaginalis*, develops along the course of the gubernaculum, forming the inguinal ring and canal and descending with the testis into the scrotum. As the abdominal wall and muscles develop, the inguinal rings close, and the processus vaginalis obliterates to form the *tunica vaginalis*, which covers the anterior and lateral portion of the testes. Incomplete closure of the inguinal ring predisposes an individual to *inguinal hernia*, and incomplete obliteration of the processus vaginalis with accumulation of serous fluid results in a *hydrocele*; either of these conditions can manifest as a scrotal mass.

Postnatal Development

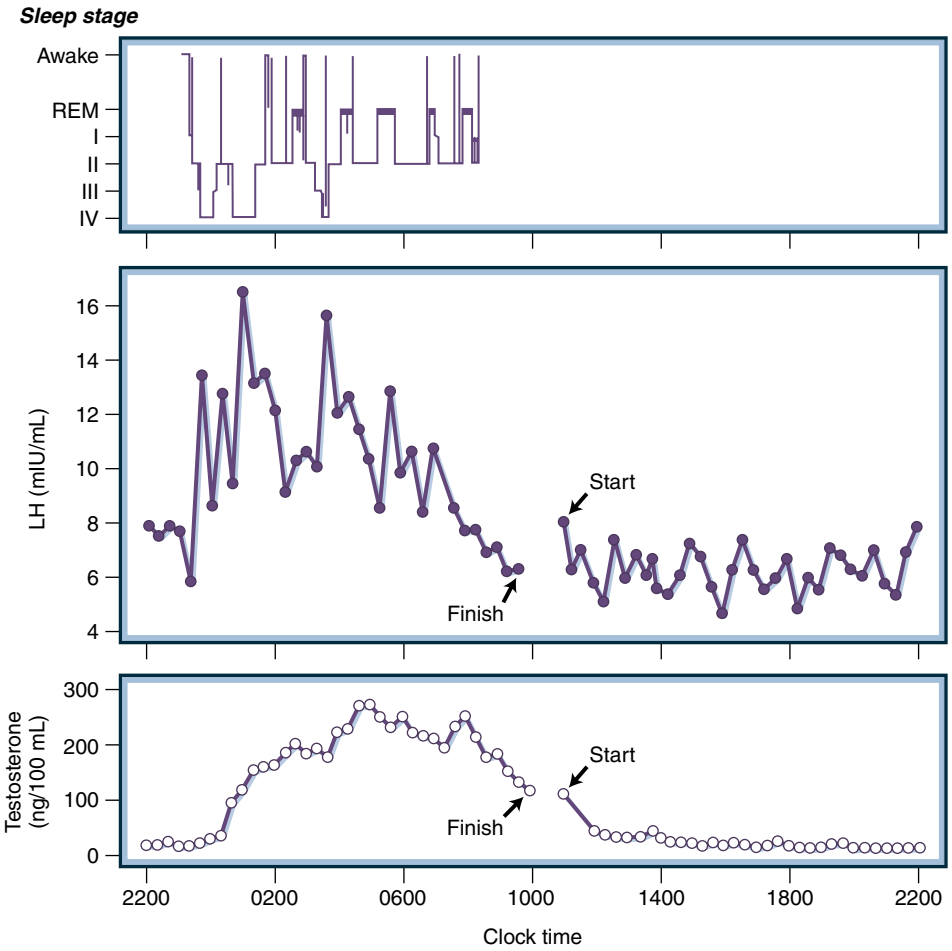
During late gestational life, the male fetus is exposed to high concentrations of maternal estrogens from the placenta. With the decline in estrogen concentrations after birth, the hypothalamic-pituitary-testicular axis is released from negative feedback suppression, resulting in a postnatal surge of gonadotropin that stimulates the testes to produce testosterone and inhibin B.

LH and testosterone concentrations begin to increase at about 1 week of life and peak 1 to 2 months later, reaching the equivalent of adolescent concentrations in association with an increase in the number of Leydig cells; LH and testosterone then decline to prepubertal concentrations by about 6 months of age. Infants with complete androgen insensitivity who lack androgen action due to an AR mutation (see later discussion) do not demonstrate a postnatal gonadotropin surge and have low or undetectable LH and testosterone concentrations postnatally, suggesting that AR expression is required for the surge.[39] In humans, there is no evidence that the postnatal surge of LH and testosterone has an effect on adult Leydig cell function. However, in infants with hypogonadotropic hypogonadism, the lack of third trimester fetal gonadotropins and the neonatal surge in testosterone play an important role in the development of micropenis and cryptorchidism.[40,41] In infants with micropenis, postnatal hormone testing may permit early identification and possible treatment of isolated hypogonadotropic hypogonadism or hypopituitarism.

FSH and inhibin B concentrations also begin to rise at 1 week of life, in association with an increase in proliferation of Sertoli cells, and peak at 3 months. FSH declines to prepubertal concentrations by 9 months of age. Because Sertoli cells continue to proliferate for some time after FSH declines, the inhibin B concentration declines more slowly and plateaus at approximately 15 months of age. Sertoli cell number determines spermatogenic potential, and the postnatal gonadotropin surge is likely important for quantitatively normal sperm production in adults. The postnatal testosterone surge also increases the formation of A_d spermatogonia (spermatogonial stem cells) from gonocytes during the first 3 months and increases testis size and seminiferous tubule length during the first year of life, providing further evidence for the importance of the gonadotropin surge on normal spermatogenesis and fertility as an adult. In male infants with congenital gonadotropin deficiency, the lack of a postnatal surge in serum gonadotropin concentrations results in lower numbers of Sertoli cells and spermatogonia. The lack of a postnatal gonadal surge explains why gonadotropin replacement therapy fails to produce normal sperm counts in the majority of men with congenital forms of hypogonadotropic hypogonadism who are treated as adults.

Pubertal Development

At the onset of puberty, reactivation of hypothalamic GnRH secretion stimulates pituitary LH and FSH, initially only during nocturnal sleep (Fig. 19.5) and subsequently throughout the day.[42,43] Increased circulating LH increases Leydig cell secretion of testosterone that induces male secondary sexual characteristics. Increased serum LH and intratesticular testosterone concentrations together with increased FSH concentrations stimulate Sertoli cells to initiate spermatogenesis. With increasing germ cell numbers and expansion of seminiferous tubules, testis size increases progressively. Increase in testis size is the first clinical sign of puberty. In addition, with the release of mature spermatozoa into the lumen of seminiferous tubules and transport of sperm to



• **Fig. 19.5** Sleep-associated secretion of luteinizing hormone (LH) (*middle*) and testosterone (*bottom*) related to sleep stage (*top*) in a boy entering puberty. *REM,* rapid eye movement sleep. (From Boyar RM, Rosenfeld RS, Kapen S, et al. Human puberty: simultaneous augmented secretion of luteinizing hormone and testosterone during sleep. *J Clin Invest.* 1974;54:609–618.)

the genitourinary tract, sperm begin to appear in the urine (*spermarche*) during early puberty (usually at 12–15 years of age).[44]

Adult Physiology

Hypothalamic-Pituitary-Testicular Axis

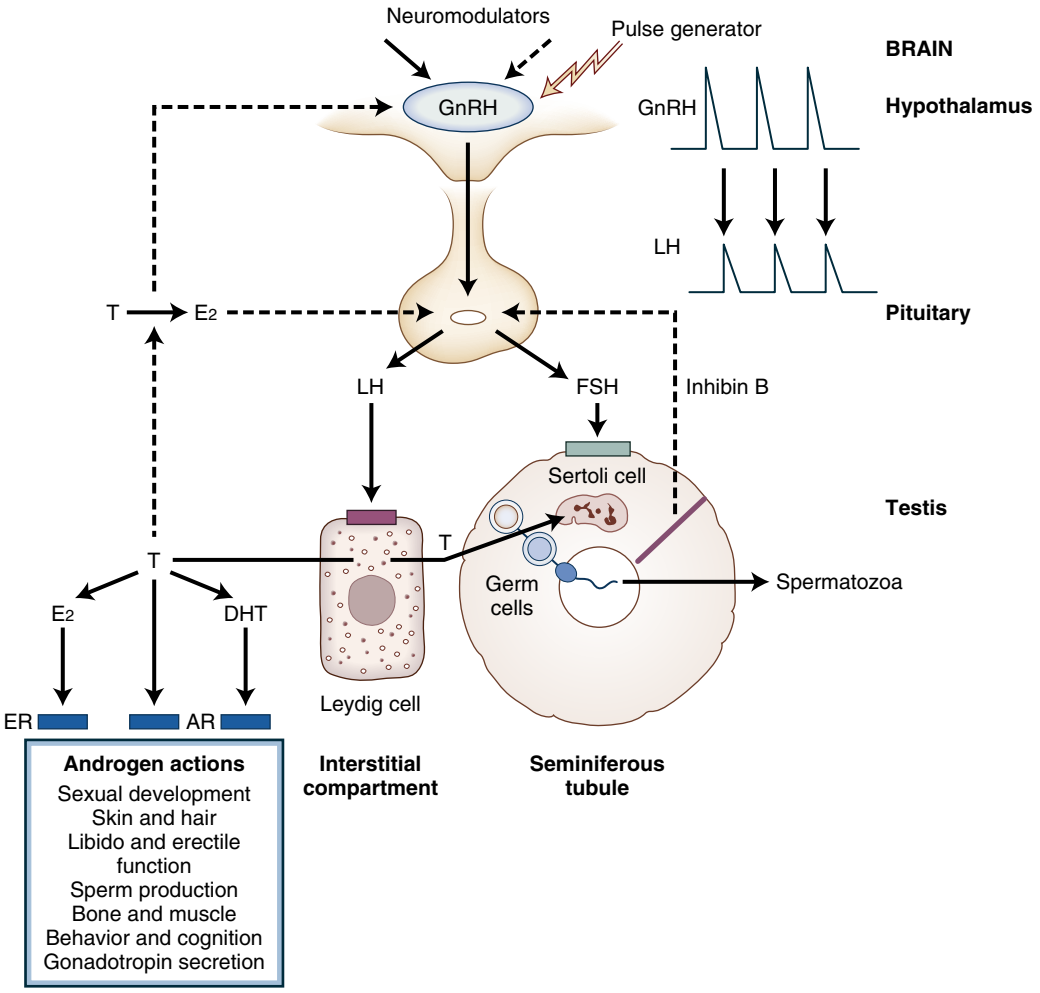
The testis is controlled by classic positive feed-forward and negative feedback mechanisms (Fig. 19.6). The major positive regulators of testis function are LH and FSH, which are synthesized and secreted from the anterior pituitary gland. Secretion of LH and, to a lesser extent, FSH, is pulsatile and driven primarily by episodic release of GnRH from neurons in the hypothalamus.[45] GnRH stimulates gonadotropin-producing cells of the anterior pituitary (gonadotrophs) to secrete LH and FSH every 90 to 120 minutes in men.

LH acts on Leydig cells of the testes to stimulate production of testosterone, the main sex steroid hormone in males. In concert with FSH, testosterone acts locally on Sertoli cells within the seminiferous tubules of the testes to initiate and maintain spermatogenesis. Testosterone secreted into the circulation acts to mediate and promote androgen action on almost every tissue in the body, including negative feedback inhibition of pituitary LH and FSH secretion (primarily via conversion to estradiol) and suppression of GnRH production by the hypothalamus. FSH stimulates Sertoli cells to produce inhibin B, a peptide hormone that causes negative feedback inhibition of FSH secretion by the anterior pituitary.

Knowledge of the hypothalamic-pituitary-testicular axis is essential in understanding the causes, classification, differential diagnosis, clinical consequences, and treatment of testicular disorders.

Central Nervous System Regulation of Gonadotropin-Releasing Hormone Secretion

The brain plays a vital role in regulation of the testis and of reproductive function through production of the decapeptide, gonadotropin-releasing hormone (GnRH), by a relatively small number of neurons located primarily in the arcuate nucleus of the medial basal hypothalamus. GnRH is released episodically from axon terminals in the median eminence into capillaries of the hypothalamic hypophyseal portal system, through which it is carried to the anterior pituitary to stimulate synthesis and the release of LH and FSH. Like all hypothalamic trophic hormones, the concentration of GnRH in portal blood is low. As a result, GnRH concentration in peripheral blood is very low and cannot be measured for clinical purposes (e.g., to diagnose GnRH deficiency).

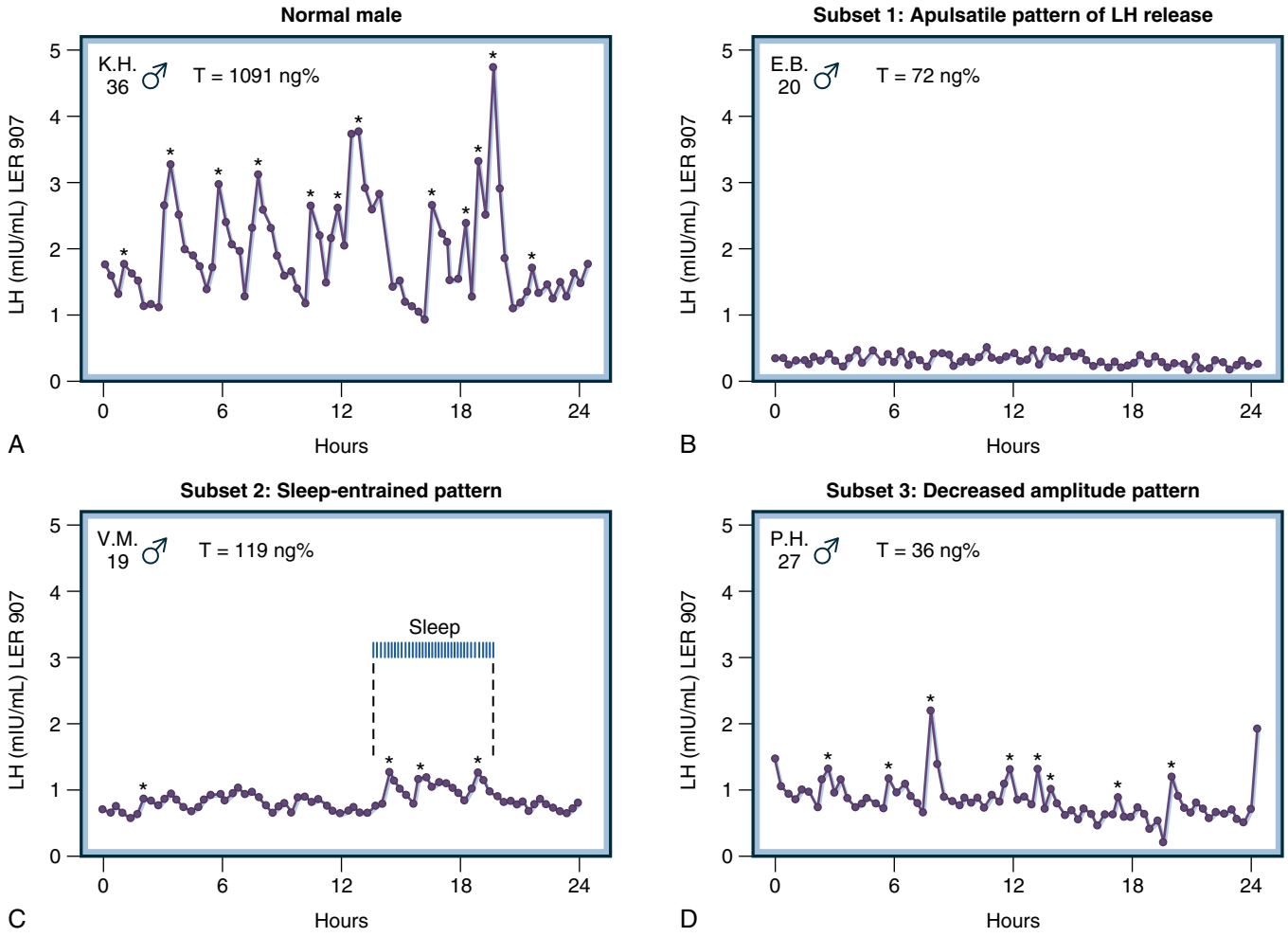


• **Fig. 19.6** Schematic diagram of the hypothalamic-pituitary-testicular axis. Hypothalamic gonadotropin-releasing hormone (GnRH) stimulates the pituitary to produce luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH stimulates *(solid lines)* Leydig cells of the testes to produce testosterone (T), which is actively metabolized to estradiol (E_2) and dihydrotestosterone (DHT), which interact with androgen receptor (AR) and estrogen receptor (ER), respectively, resulting in several direct and indirect androgen actions. FSH stimulates Sertoli cells of the testes, which, together with LH-stimulated testosterone, increases spermatogenesis. LH-stimulated testosterone and E_2 exert negative feedback suppression *(dashed lines)* of GnRH production at the hypothalamus and LH and FSH production at the pituitary, and FSH-stimulated inhibin B exerts negative feedback suppression on FSH secretion by the pituitary. (Modified from Matsumoto AM. The testis. In: Felig P, Frohman LA, eds. *Endocrinology and Metabolism.* 4th ed. New York, NY: McGraw-Hill; 2001:635–705.)

The precise mechanism of synchronous episodic release of GnRH from many separate GnRH neurons into the hypophyseal portal system to provide pulsatile stimulation of pituitary gonadotrophs is not clear. There is evidence that pulse generation is a direct consequence of intrinsic periodicity of GnRH or other neurons that synapse on GnRH neurons within the medial basal hypothalamus, such as neurons containing kisspeptin or excitatory or inhibitory neurotransmitters.

The frequency of pulsatile GnRH secretion is temporally coupled with the episodic release of LH, free α-subunit (co-secreted with intact gonadotropins), and FSH.[46] Because the half-lives of LH and free α-subunit in the circulation are shorter than that of FSH, concentrations of LH and α-subunit demonstrate discrete pulses, which are evident with frequent blood sampling (e.g., every 10 minutes for 12–24 hours), whereas FSH pulses are not as apparent. The frequency of LH or free α-subunit pulses reflects GnRH pulse frequency and serves as an indicator of synchronous GnRH neuronal activity (pulse generation) in the brain. The amplitude of LH or free α-subunit pulses reflects both the amplitude of GnRH pulses and the responsiveness of the gonadotrophs to GnRH stimulation. Normal men usually demonstrate 12 to 16 LH pulses of varying amplitude over 24 hours (Fig. 19.7). In men with GnRH deficiency (congenital hypogonadotropic hypogonadism [CHH], also referred to as idiopathic hypogonadotropic hypogonadism [IHH]), there is an absence of LH pulses (most commonly) or abnormalities in LH pulsatility.[47]

Treatment of men who have GnRH deficiency with low-dose pulsatile GnRH normalizes LH and FSH secretion and testicular function. In contrast, continuous low-dose GnRH administration does not stimulate normal gonadotropin secretion in these men.[48] Administration of potent GnRH receptor agonists providing continuous, high-dose GnRH stimulation of the pituitary initially



• **Fig. 19.7** Endogenous pulsatile secretion of luteinizing hormone (LH) in a normal man (A) and in men with idiopathic hypogonadotropic hypogonadism (B–D), assessed by blood sampling every 20 minutes for 24 hours. In normal men (A), discrete pulses (*) of LH occur approximately every 2 hours, reflecting pulsatile release of gonadotropin-releasing hormone from the hypothalamus, and stimulate normal adult concentrations of testosterone (T). Most men with idiopathic hypogonadotropic hypogonadism (B) demonstrate no detectable LH pulses and have prepubertal testosterone concentrations. Others exhibit primarily sleep-entrained LH pulses of reduced amplitude without significant LH pulsatility during the waking hours (C) or LH pulses of reduced amplitude throughout sleep and waking hours (D) with pubertal or prepubertal testosterone concentrations, respectively. (From Santoro N, Filicori M, Crowley WF Jr. Hypogonadotropic disorders in men and women: diagnosis and therapy with pulsatile gonadotropin-releasing hormone. *Endocr Rev.* 1986;7:11–23.)

stimulates but then downregulates and profoundly suppresses gonadotropin secretion and testosterone production. This effect has been the basis for the use of potent GnRH agonists to produce medical castration (androgen deprivation therapy) in men with advanced prostate cancer. These findings underscore the critical importance of pulsatile GnRH control of reproductive function in men.

GnRH neurons receive many excitatory and inhibitory inputs from other brain regions (e.g., from kisspeptin neurons), as well as feedback signals from the testes and other circulating endocrine signals. Therefore, the GnRH neuronal system serves an important integrative role in the regulation of reproductive and testis function. A large and complex ensemble of neuroregulators mediates GnRH secretion, acting directly on GnRH neurons themselves or indirectly on other neurons that in turn regulate GnRH neurons to stimulate or inhibit GnRH secretion. These central nervous system (CNS) neuromodulatory systems, together with peripheral endocrine regulators, provide the means by which GnRH secretion and testicular function may be altered by environmental factors such as stress (e.g., via corticotropin-releasing hormone, glucocorticoids), nutritional compromise (e.g., via leptin), and medications (e.g., opioid drugs).

During embryogenesis, GnRH and olfactory neurons originate outside the CNS in the olfactory placode and migrate together along olfactory axons through the cribriform plate of the ethmoid bone to the olfactory bulb, where GnRH neurons diverge and continue to migrate to the medial basal hypothalamus.[49] Abnormalities in the development of the olfactory bulbs and migration of these neurons explain the association between CHH due to GnRH deficiency and the loss or impairment of the sense of smell (anosmia or hyposmia, respectively) that occurs in patients with Kallmann syndrome. Loss-of-function mutations occur in

genes that play important roles in the migration and embryologic development of GnRH neurons, such as the genes for Kallmann syndrome 1 (*ANOS1*), *KAL2* (now called *fibroblast growth factor receptor 1* [*FGFR1*]), and prokineticin receptor 2 (*PROKR2*) and its ligand, prokineticin 2 (*PROK2*). Similarly, genes that are important in the regulation of GnRH neurons may sustain loss-of-function mutations; examples include the genes for kisspeptin 1 receptor (*KISS1R* [formerly *GPR54*]) and its ligand, kisspeptin 1 (*KISS1*), also called *metastin*; the neurokinin B (tachykinin 3) receptor (*TACR3*) and its ligand (*TAC3*); and the GnRH receptor (*GNRHR*) and its ligand (*GNRH*). All of these defects result in isolated GnRH deficiency associated with impaired pubertal development, often in combination with anosmia or hyposmia or other morphologic defects.[50]

GnRH Regulation of Gonadotropin Secretion

GnRH released from the hypothalamus into the hypophyseal portal system binds to G protein–coupled GnRH receptors on anterior pituitary gonadotrophs.[51] In humans, GnRH receptors are coupled primarily to $G_{q/11}$ proteins, which activate phospholipase C-β to produce 1,2-diacylglycerol and inositol 1,4,5-trisphosphate (IP3). Diacylglycerol activates protein kinase C (PKC), and IP3 mobilizes intracellular calcium, which binds to the calcium-binding protein, calmodulin. Both PKC and calmodulin-dependent kinases phosphorylate and activate several transcription factors, resulting in increased synthesis of the gonadotropin subunits LHβ, FSHβ, and the common α-subunit and release of intact LH and FSH and free α-subunits into the circulation. The GnRH receptor may also be coupled to G_s protein, which activates protein kinase A (PKA), resulting in the synthesis and release of gonadotrophs.

LH and FSH, together with another anterior pituitary hormone, thyroid-stimulating hormone (TSH), and a placental hormone, hCG, are members of the glycoprotein hormone family. Glycoprotein hormones are heterodimers in which a common α-subunit is noncovalently linked to a unique β-subunit; this structure confers their ability to bind to their cognate receptors and their biologic specificity. In the pituitary gonadotroph, the common α-subunit and the LHβ- and FSHβ-subunits are products of different genes that are synthesized and regulated differentially.[52] After the subunits are synthesized, an α-subunit combines noncovalently with either an LHβ- or FSHβ-subunit. After translation, the heterodimer undergoes variable glycosylation wherein oligosaccharide chains (glycans) are attached covalently to specific amino acids, resulting in LH and FSH molecules with a high degree of microheterogeneity (i.e., many LH and FSH isoforms characterized by different glycosylation patterns). The gonadotropin α-subunit is produced in excess relative to the LHβ- and FSHβ-subunits; it too is glycosylated, and free α-subunit is cosecreted into the circulation with LH and FSH. Many nonfunctional and gonadotropin-secreting pituitary adenomas secrete excessive amounts of free α-subunit into the circulation.[53]

The degree of glycosylation of gonadotropins and other glycoprotein hormones alters their clearance rate from the circulation and their signal transduction after receptor binding, thereby affecting their biologic activity in vivo. The half-life in the circulation of gonadotropins increases with greater degrees of glycosylation: hCG > FSH > LH > free α-subunit. In humans, the initial half-life of disappearance for LH is about 40 minutes, and the secondary half-life of disappearance is about 120 minutes; for FSH, these periods are approximately 4 and 70 hours, respectively.[54,55]

Variations in glycosylation of LH and FSH result in substantial microheterogeneity among circulating gonadotropin isoforms, which vary in half-life and biologic activity and may be altered by particular physiologic conditions, such as during puberty, with aging, and with androgen deprivation.

Clinically, serum LH and FSH concentrations are measured with rapid, nonradioactive, highly sensitive immunoassays that use monoclonal antibodies recognizing two separate epitopes on the gonadotropin molecule. Gonadotropin measurements are essential in the evaluation of men with hypogonadism to distinguish those who have a primary testicular disorder (*primary hypogonadism*, in which gonadotropins are high) from those who have a secondary hypothalamic or a pituitary disorder (*secondary hypogonadism*, in which gonadotropins are low or normal). Specific immunoassays for free α-subunit are used to diagnose and monitor patients with nonfunctional and gonadotropin-secreting pituitary adenomas.

Gonadotropin Control of Testicular Function

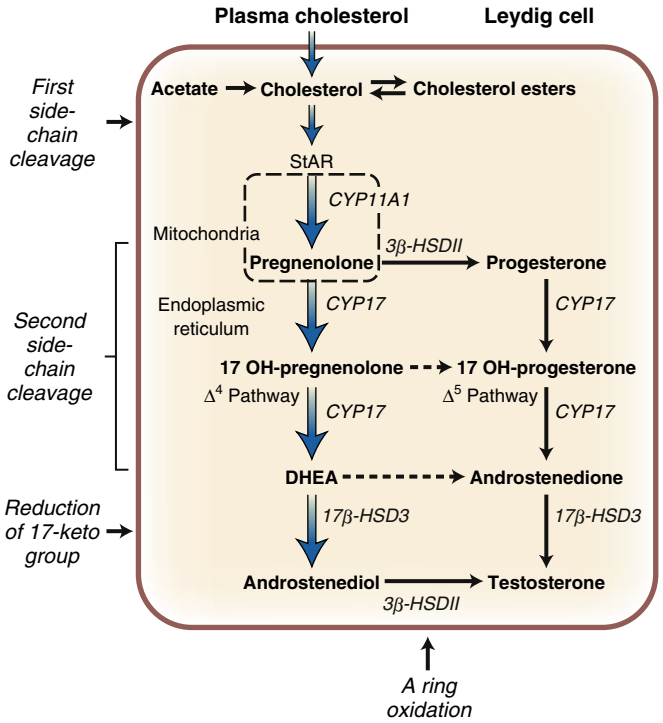
LH Regulation of Leydig Cells

Circulating LH binds to the G protein–coupled receptor for LH and hCG (termed *LHCGR*) on the surface of Leydig cells, resulting in receptor aggregation and a conformational change that activates G_s protein. G_s protein, in turn, results primarily in cyclic adenosine monophosphate (cAMP)-dependent activation of PKA.[56] Activated PKA increases the production of proteins that regulate steroidogenesis and testosterone biosynthesis (Fig. 19.8). The main proteins regulated by LH-stimulated PKA are as follows:

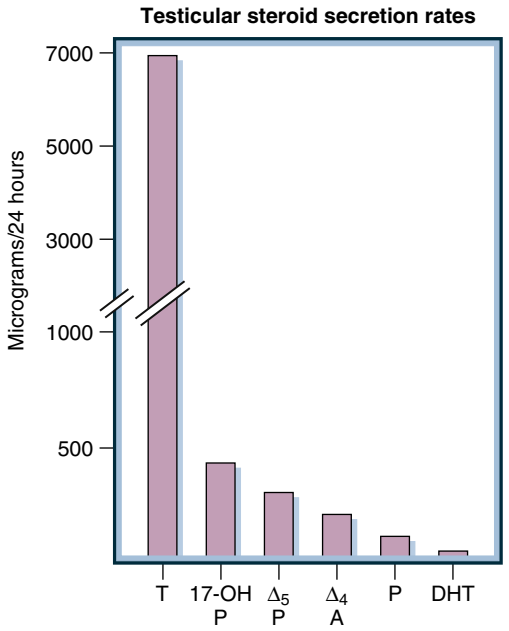
1. *Steroidogenic acute regulatory protein* (StAR), a transport protein that regulates transfer of cholesterol from the outer to the inner mitochondrial membrane—the rate-limiting step for steroid production
2. *Cytochrome P450 isoenzyme 11A1 (P450 11A1)*, also called *cholesterol side-chain cleavage enzyme*, within the inner mitochondrial membrane, which catalyzes the conversion of cholesterol delivered by StAR protein to pregnenolone—the first and rate-limiting enzymatic step in steroidogenesis
3. *P450 17A1*, also called *17α-hydroxylase/17,20-lyase* in the endoplasmic reticulum, which catalyzes the conversion of pregnenolone to 17α-hydroxypregnenolone—the second enzymatic step in testosterone biosynthesis.[57]

In humans, cholesterol is synthesized within Leydig cells from acetate by *3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase*, or it is derived from circulating low-density lipoprotein (LDL) cholesterol.

Clinically, rare inactivating mutations of the LHCGR cause Leydig cell hypoplasia, resulting in impaired male genital development and *46,XY disorder of sex development* (46,XY DSD, previously known as male pseudohermaphroditism; see Chapter 23) resulting from insufficient testosterone production during fetal development.[58] Rare LHβ mutations cause failure of normal male pubertal development with micropenis but otherwise normal genital development at birth, evidence that normal endogenous LH secretion is not required for male sexual differentiation during fetal development and that hCG stimulation of testosterone production by fetal Leydig cells is the main driver.[59] Activating mutations of the LHCGR have been found in boys with familial precocious puberty (*testotoxicosis*).[60] Inhibitors of HMG-CoA reductase (statins) used to treat hypercholesterolemia do not significantly affect serum testosterone concentrations.



• **Fig. 19.8** Testosterone biosynthetic pathways in the Leydig cell of the human testis. Cholesterol may be synthesized de novo from acetate within the Leydig cell or derived from hydrolysis of cholesterol esters or circulating cholesterol. Cholesterol is transported into the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR); there it is converted to pregnenolone by the cholesterol side-chain cleavage enzyme P450 11A1 (CYP11A1). Biosynthesis of testosterone in the human testis proceeds predominantly through the Δ^5 pathway in the endoplasmic reticulum, where pregnenolone is converted to 17-hydroxypregnenolone and then to dehydroepiandrosterone (DHEA) by 17α-hydroxylase/17,20 lyase P450 17A1 (CYP17), which is converted to androstenediol by 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3 or HSD17B3) and then to testosterone by 3β-hydroxysteroid dehydrogenase type II (3β-HSDII or HSD3B2). In the Δ^4 pathway, pregnenolone is converted successively to 17-hydroxyprogesterone, androstenedione, and testosterone. (Modified from Bhasin S. Testicular disorders. In: Kronenberg HM, Melmed S, Polonsky KS, et al, eds. *Williams Textbook of Endocrinology,* 11th ed. Philadelphia, PA: Elsevier; 2008:645–698.)



• **Fig. 19.9** Relative steroid hormone secretion rates from the human testis. Secretion rates were calculated from arteriovenous (AV) differences across the testis of testosterone and other steroids, assuming a testosterone secretion rate of 7000 μg per 24 hours and using the following formula: Secretion rate = Assumed testosterone secretion rate/Testosterone AV difference × AV difference of steroid in question. Testosterone (T) is the main steroid secreted by the testis; much lower amounts of 17-hydroxyprogesterone (17-OHP), pregnenolone (Δ^5P), androstenedione (Δ^4A), progesterone (P), and dihydrotestosterone (DHT) are also secreted. (From Hammond GL, Ruokonen A, Kontturi M, et al. The simultaneous radioimmunoassay of seven steroids in human spermatic and peripheral venous blood. *J Clin Endocrinol Metab.* 1977;45:16–24.)

Leydig Cell Production of Testosterone and INSL3

In the human testis, LH-stimulated transport of cholesterol into the inner mitochondrial membrane is followed by conversion of cholesterol to pregnenolone by CYP11A1 and conversion of pregnenolone to 17α-hydroxypregnenolone by CYP17A1. Testosterone biosynthesis then proceeds via a series of further enzymatic steps in the endoplasmic reticulum, initially through the Δ^5 steroid biosynthesis pathway[57,61] (see Fig. 19.8).

The *17,20-lyase (desmolase)* activity of P450 17A1 catalyzes the further conversion of 17α-hydroxypregnenolone to DHEA. DHEA then is converted to Δ^5-androstanediol by *17β-hydroxysteroid dehydrogenase 3* (*17β-HSD3* or *HSD17B3*). DHEA and Δ^5-androstanediol are converted to the Δ^4 steroids, Δ^4-androstenedione and testosterone, respectively, by the enzyme *3β-hydroxysteroid dehydrogenase/Δ^{5-4} isomerase type 2* (*3β-HSD2* or *HSD3B2*).

The early steroid precursors, pregnenolone and 17α-hydroxypregnenolone, may also be converted to progesterone and 17α-progesterone, respectively, by HSD3B2 and then proceed down the Δ^4 pathway to testosterone synthesis. However, in the human testis, the Δ^5 pathway is the predominant steroid biosynthetic pathway for testosterone production. Testosterone may be converted in the testes to the active metabolites, estradiol and DHT, by the enzymes *aromatase* (*P450 19A1*) and *steroid 5α-reductase type 1* (*SRD5A1*, the predominant isoform found in the testes), respectively.

Mutations in testosterone biosynthetic enzymes result in abnormalities of sexual differentiation and varying degrees of 46,XY DSD, depending on the severity of androgen deficiency.[62]

Testosterone is the major androgen produced by the testis. In humans, the average secretion rate of testosterone is approximately 7000 μg/day. The testes also secrete significant but quantitatively smaller amounts of 17α-hydroxyprogesterone, pregnenolone, Δ^4-androstenedione, and progesterone. Very little estradiol (about 10 μg/day) or DHT (about 69 μg/day) is secreted by the testes[9,63] (Fig. 19.9).

In response to pulsatile LH stimulation, testosterone is secreted episodically into the spermatic vein and then into the general circulation. However, testosterone pulses are less discrete, relatively low in amplitude, and concordant with LH pulses only after a lag time of 80 to 120 minutes, suggesting a relatively sluggish response of Leydig cells to LH stimulation.[45] In addition to this ultradian variation, testosterone concentrations in young men exhibit a circadian variation characterized by a maximum excursion of 140 ng/dL, with peak testosterone concentrations occurring at approximately 8 AM and nadir concentrations at 8 PM.[64] The circadian variation in testosterone concentrations is blunted

but still present in older men, with a maximum excursion of 60 ng/dL. The testosterone response to hCG (LH-like) stimulation is greater in the morning than in the evening, suggesting that diurnal variation in Leydig cell responsiveness may contribute to the circadian variation in testosterone concentrations.[65] Both the ultradian and the circadian variation in testosterone concentrations contribute to the variability in testosterone measurements within an individual; together with assay variability, this underscores the importance of repeating testosterone measurements during the clinical evaluation of patients with clinical manifestations of male hypogonadism.

INSL3 is a peptide hormone in the relaxin-insulin family that is produced by Leydig cells and secreted into the circulation.[66] Serum concentrations of INSL3 reflect the number and differentiation status of Leydig cells. During puberty, LH induces proliferation and differentiation of Leydig cells and production of INSL3. Serum INSL3 concentrations increase progressively during puberty, reaching adult concentrations at about 18 years of age and remaining stable until 35 to 40 years of age, after which they decline steadily with age.

Men with anorchia and bilateral orchidectomy, in whom Leydig cells are absent, and men with chronic gonadotropin suppression induced by GnRH analogues or androgens have undetectable or very low concentrations of INSL3. In men with hypogonadotropic hypogonadism, INSL3 concentrations are undetectable; in these patients, hCG (LH-like) stimulation increases serum testosterone concentrations within 72 to 96 hours but has no stimulatory effect on INSL3 concentrations. However, chronic hCG treatment, presumably because of induction of Leydig cell differentiation by longer-term LH-like stimulation, increases both testosterone and INSL3 concentrations in these men.[67] In men with unilateral orchidectomy, INSL3 concentrations are intermediate between those in men with bilateral orchidectomy and those in normal men, but testosterone concentrations are normal, supporting the importance of Leydig cell number for circulating INSL3 concentrations.

FSH and Testosterone Regulation of Sertoli Cells

Circulating FSH binds to G protein–coupled FSH receptors on the surface of Sertoli cells, activating G_s protein that increases cAMP production.[68] cAMP then activates PKA and other signal-transduction proteins (e.g., phosphatidylinositol 3-kinase, phospholipase A_2, calcium channel proteins, mitogen-activated protein kinase [MAPK]). Activated PKA activates many proteins, including the transcription factor, *cAMP response element–binding protein*; these proteins, in turn, regulate gene expression and production of Sertoli cell proteins that play important roles in supporting and regulating spermatogenesis within the seminiferous tubules. In rodents, the expression of FSH receptors in Sertoli cells varies cyclically with the stage of spermatogenesis, being highest in stages XIII through I and lowest in stages VII and VIII.[69] The precise roles of Sertoli cell products in human spermatogenesis are poorly understood and derive from studies using Sertoli cells obtained primarily from immature animals, mostly rodents.

Testosterone produced locally by Leydig cells binds to intracellular ARs in the cytoplasm of Sertoli cells; ligand-bound AR translocates to the nucleus, where it binds to androgen response elements and interacts with coregulator proteins to regulate gene expression and production of Sertoli cell proteins that play vital roles in supporting and regulating spermatogenesis. The expression of the AR also varies cyclically with the stage of spermatogenesis, being highest in stage VII, when FSH receptor expression is the lowest.[70]

The major functions of Sertoli cells[71,72] are (1) to maintain seminiferous tubule structure and compartmentalization; (2) to provide nutrients and growth factors to developing germ cells and spermatozoa; (3) to translocate, sculpt, and release developing germ cells; (4) to secrete seminiferous tubule fluid; and (5) to produce reproductive hormones.

Maintenance of Seminiferous Tubule Structure and Compartmentalization

The blood-testis barrier is formed by basal tight junctions between adjacent Sertoli cells; these serve to compartmentalize the seminiferous tubule into basal and adluminal compartments. Compartmentalization provides an environment in which developing germ cells are protected from external insults and the immune system.

Sertoli cells produce many junctional complex, structural, and extracellular matrix proteins, such as cell adhesion molecules (e.g., claudin 3, which is particularly important for the integrity of Sertoli cell tight junctions), cadherins, laminins, type I and type IV collagen, and proteoglycans including chondroitin and heparin. These proteins are important in maintaining the structural integrity and support for developing germ cells, forming the blood-testis barrier, mediating cell-to-cell interactions, and maintaining polarized secretion of products by Sertoli cells.

Provision of Nutrients and Growth Factors to Developing Germ Cells and Spermatozoa

Although it is protective, the blood-testis barrier also isolates developing germ cells from nutrients, hormones, and growth factors that are present in the systemic circulation. The Sertoli cell has an essential role in producing vital nutrients, cofactors, and proteins that are needed for the normal progression of spermatogenesis and support of spermatozoa being transported within the seminiferous tubule lumen. Sertoli cells produce pyruvate and contain lactate dehydrogenase, which catalyzes the conversion of pyruvate to lactate, the preferred energy substrate of germ cells.

Most of the proteins produced by Sertoli cells are binding or transport proteins for substances (e.g., metals, vitamins, sphingolipids, androgens, hormones, growth factors) that serve as cofactors and regulators of germ cell development within the seminiferous tubule. Binding proteins produced by Sertoli cells include transferrin, an iron-binding protein; ceruloplasmin, a copper-binding protein; glycosphingolipid-binding protein; sulfated glycoprotein 2, also called *clusterin*, a lipid-binding protein with other biologic activities; androgen-binding protein (ABP); α_2-macroglobulin, which serves as a binding protein for inhibin and activin; follistatin, a potent binding protein for activin; and insulin-like growth factor–binding proteins (IGFBPs), which bind insulin-like growth factor type 1 (IGF1).

ABP is a testicular homologue of *sex hormone–binding globulin* (SHBG), the major circulating ABP, which is synthesized by the liver and encoded by the same gene.[73] Based on studies in rodents, ABP is thought to play a role in regulating local testosterone concentrations in the seminiferous tubule and epididymis. However, one study reported that human SHBG is expressed in germ cells but not in Sertoli cells and that a smaller isoform of SHBG is located between the outer acrosomal membrane and the sperm plasma membrane and is released during capacitation.[74] These findings suggest a potentially different role of SHBG/ABP in humans and rodents and underscore the hazard of extrapolating results from animal studies.

The Sertoli cell also produces several growth factors, such as IGF1, basic fibroblast growth factor, activin A, transforming

growth factor-α (TGFα) and TGFβ, interleukin 1α (IL1α) and IL6, stem cell factor (the c-KIT ligand), glial cell–derived neurotrophic factor, and polyamines (putrescine, spermine, and spermidine), which act both as paracrine regulators of stem cell renewal, germ cell development, and Leydig cell and peritubular myoid cell function and as autocrine regulators.

Translocation, Sculpting, and Release of Developing Germ Cells

Sertoli cells actively move developing germ cells from the basal compartment through the adluminal compartment and release spermatozoa from the seminiferous epithelium into the lumen (spermiation). During translocation, Sertoli cells remove degenerating germ cells, residual cytoplasm from late elongated spermatids (residual body), and seminiferous tubule fluid and contents by phagocytosis and pinocytosis. The Sertoli cell produces proteases and protease inhibitors (e.g., testibumin or sulfated glycoprotein 1, tissue plasminogen activators, type IV collagenase, cystatin, α_2-macroglobulin) that are involved in germ cell translocation, removal of degenerating germ cells, and spermiation.

Secretion of Seminiferous Tubule Fluid

Seminiferous tubule fluid serves important roles in the delivery of nutrients to developing germ cells within the seminiferous epithelium, transportation of regulatory factors and nutrients within the seminiferous tubule lumen, and transportation of spermatozoa released into the seminiferous tubule lumen to the rete testis, efferent ducts, and epididymis.

Production of Reproductive Hormones

Sertoli cells produce hormones that are important in male reproductive differentiation and function. These include AMH, which causes regression of the müllerian ducts, preventing uterine and fallopian tube formation during embryogenesis; inhibin B and activin A, peptide hormones that, respectively, participate in negative feedback regulation of FSH secretion and potentially act as a paracrine regulator of spermatogenesis; and estradiol, a potent estrogen that is produced via aromatization of testosterone in immature Sertoli cells.

FSH acts directly and testosterone (secreted by Leydig cells in response to LH) acts directly and also indirectly (e.g., by stimulating peritubular myoid cells) to control Sertoli cell function and regulate spermatogenesis. Gene expression profiling studies using microarray analysis in rat Sertoli cells treated with FSH, in Sertoli cell–specific AR mutant mice, and in GnRH-mutant mice treated with testosterone have provided insight into specific Sertoli cell genes that are regulated directly by FSH and testosterone.[75] However, similar studies in humans investigating the regulation of Sertoli cell gene expression, specifically by FSH and testosterone, have yet to be performed.

Paracrine and Autocrine Regulation of Testis Function

As described earlier, with regard to testicular function, gonadotropins, LH, and FSH secreted by the pituitary are the major endocrine regulators, and testosterone produced by Leydig cells in response to LH stimulation is the main paracrine regulator. However, there is evidence, mostly from studies in experimental animals and in vitro studies using isolated testis cell types from animals, that Leydig, Sertoli, and peritubular myoid cells and macrophages in the testis secrete other paracrine and autocrine factors that may be important modulators of testosterone and sperm production.

One of the most important examples of paracrine regulation within the testis is the effect of testosterone, produced locally by Leydig cells, on Sertoli cell function and spermatogenesis. There is evidence that testosterone has both a direct effect on Sertoli cell function and spermatogenesis and an indirect effect on peritubular myoid cell ARs. In GnRH- and gonadotropin-deficient *hpg* mice in which the AR has been knocked out specifically in Sertoli cells, DHT treatment is not able to stimulate spermatogenesis, suggesting that direct androgen action on Sertoli cells is needed to stimulate spermatogenesis.[76] Animals with a peritubular myoid cell–specific AR knockout demonstrate impaired Sertoli function (i.e., reduced seminiferous tubule fluid and Sertoli cell androgen-dependent gene expression), azoospermia, and infertility not explained by alterations in testosterone, LH, and FSH concentrations.[77] These findings suggest that the paracrine effect of Leydig cell–produced testosterone on Sertoli cell function and regulation of spermatogenesis is mediated in part by androgen-driven interactions between peritubular myoid cells and Sertoli cells. Whether these stromal-epithelial cell interactions occur in humans is not known. At present, the roles of paracrine and autocrine factors other than testosterone in the regulation of human testis function are not clear.

Hormonal Control of Spermatogenesis

LH and FSH are the main hormonal regulators of spermatogenesis in humans. The effect of FSH on spermatogenesis is mediated by a direct endocrine action on Sertoli cells, whereas that of LH is mediated by an action on Leydig cells to produce testosterone, which in turn acts locally within the testes in a paracrine manner, with direct effects on Sertoli cells and possibly indirect effects through peritubular myoid cell regulation of Sertoli cells. The gonadotropin requirements necessary for the initiation of spermatogenesis at the time of puberty differ from those needed for the maintenance of sperm production, once initiated, in adults.[10,78]

Initiation of Spermatogenesis

Usually, both LH and FSH are required to initiate spermatogenesis at the time of puberty. In men with prepubertal gonadotropin deficiency (e.g., CHH), treatment with LH (hCG) is needed to stimulate sufficient intratesticular testosterone production to support spermatogenesis and seminal fluid production by accessory sex glands (seminal vesicles and prostate gland). However, most such patients also require FSH treatment to initiate and complete the first wave of spermatogenesis and to produce sperm in the ejaculate.[79] In some men with incomplete gonadotropin deficiency (usually with evidence of endogenous FSH secretion, e.g., larger testis volume), LH treatment alone is sufficient to initiate and complete spermatogenesis. FSH treatment without LH (hCG) does not stimulate sperm production in men with prepubertal complete gonadotropin deficiency—but might be sufficient in men with partial gonadotropin deficiency.

Natural inactivating mutations of gonadotropin β-subunits and receptors provide some insight into the roles of LH and FSH in initiating spermatogenesis. Men with inactivating mutations of LHβ usually have a lack of pubertal development and arrested spermatogenesis or azoospermia and infertility.[59] Recently, however, a man with an LHβ mutation resulting in a partially active LH molecule (as evidenced by expression of steroidogenic enzymes in a few mature Leydig cells and low intratesticular testosterone concentrations) was reported to have complete and quantitatively normal spermatogenesis.[80] This finding suggests that complete spermatogenesis may be initiated by low concentrations of LH

and intratesticular testosterone in the presence of high serum FSH concentrations, as were present in this case.

Men with inactivating LH receptor mutations present with varying degrees of impaired sexual differentiation or 46,XY DSD, ranging from ambiguous genitalia to perineoscrotal hypospadias, and azoospermia, although defects in sperm production are confounded by the presence of cryptorchidism in many of these men.[58] Recently, a man with a partially inactivating mutation of the LH receptor was reported to have micropenis, delayed puberty, low serum testosterone concentrations, and normal FSH concentrations but bilaterally normal-size, descended testes and normal sperm production, albeit with low sperm concentration (oligozoospermia).[81] This finding suggests that spermatogenesis may be initiated qualitatively by very low LH activity and intratesticular testosterone in the presence of normal FSH concentrations.

Men with inactivating mutations of FSHβ have been found generally to have azoospermia with low or low-normal testosterone and high LH concentrations.[82-85] In contrast, men with inactivating FSH receptor mutations have been reported to have moderate to severely reduced sperm counts (but not azoospermia) with normal testosterone and normal to high LH concentrations.[58,86] The reason for the apparent discrepancy in degree of spermatogenic impairment exhibited by men with FSHβ versus FSH receptor mutations is not clear. It is possible that residual receptor function in men with FSH receptor mutations results in persistent small amounts of FSH signaling or that men with FSHβ mutations have greater Leydig cell dysfunction, as evidenced by lower serum concentrations of LH and intratesticular testosterone that result in greater impairment of spermatogenesis.

In summary, findings from the small number of reports of men with inactivating mutations of gonadotropin β-subunits and receptors suggest that initiation of the first wave of spermatogenesis may require only very low concentrations or activity of either LH (intratesticular testosterone) or FSH in the presence of adequate amounts of the other gonadotropin. Clinically, however, most men with prepubertal gonadotropin deficiency require treatment with both LH and FSH to initiate spermatogenesis during puberty. Because FSH stimulates Sertoli cell proliferation and number during testis development, it plays a vital role in determining the capacity for quantitatively normal spermatogenesis.

Maintenance of Spermatogenesis

In men with prepubertal gonadotropin deficiency (e.g., CHH), once spermatogenesis has been initiated with LH (hCG) and FSH treatment, sperm production may be maintained with LH treatment alone without continued FSH administration.[79] However, spermatogenesis is not stimulated by administration of FSH in combination with testosterone (that maintains normal concentrations of serum testosterone but with continued low LH and intratesticular testosterone concentrations) in men with CHH. Spermatogenesis may be reinitiated with LH (hCG) alone in previously gonadotropin-treated men with CHH after a period of gonadotropin deficiency associated with exogenous testosterone replacement therapy. Furthermore, in men with gonadotropin deficiency and azoospermia acquired as an adult (e.g., secondary to a pituitary adenoma), spermatogenesis may be reinitiated and maintained with LH (hCG) treatment alone.[79]

In normal men with experimental gonadotropin deficiency induced by high-dose testosterone administration, spermatogenesis may be reinitiated and maintained by either LH or hCG alone, despite markedly suppressed FSH concentrations, or by FSH alone, despite severely suppressed LH (and presumably low intratesticular testosterone) concentrations. However, sperm production was not stimulated by either LH or FSH alone to the baseline concentrations that existed before experimental gonadotropin suppression.[87] In this model of gonadotropin deficiency, treatment with both LH (hCG) and FSH restored sperm counts fully to baseline values. Finally, in support of the ability of FSH alone to stimulate sperm production, spermatogenesis was maintained despite undetectable serum gonadotropin concentrations in a hypophysectomized man who had an activating FSH receptor mutation.[88]

Together, these findings suggest that a normal concentration of either FSH or LH is sufficient for maintenance of qualitatively normal sperm production, but both gonadotropins are necessary for quantitatively normal spermatogenesis in male humans.

The effect of gonadotropins on specific stages of spermatogenesis has been studied in normal men with experimental gonadotropin suppression induced by the administration of high-dose progestin and testosterone. In these gonadotropin-deficient men, selective replacement of either FSH or LH (increasing intratesticular testosterone) supported all stages of spermatogenesis, including spermatogonial maturation, meiosis, spermiogenesis, and spermiation, but each agent had predominant actions on specific stages.[89] FSH exerted a relatively greater effect on maturation of spermatogonia (conversion of spermatogonia A_p to spermatogonia B), early meiosis, and maintenance of pachytene spermatocytes (conversion of spermatogonia to pachytene spermatocytes). LH (stimulating intratesticular testosterone) had predominant effects on the completion of meiosis (conversion of pachytene spermatocytes to round spermatids) and on spermiation (release of mature spermatozoa). LH and FSH (intratesticular testosterone) exert similar effects on spermiogenesis (conversion of round to elongated spermatids).

In normal men, LH stimulates intratesticular testosterone concentrations that are approximately 100- to 200-fold higher than serum testosterone concentrations and correlate with circulating LH concentrations. Administration of various combinations of exogenous testosterone, progestin, and GnRH antagonist to induce gonadotropin deficiency in male contraception trials suppressed intratesticular testosterone by 98%, to concentrations comparable to those in serum, and reduced sperm production, producing severe oligozoospermia or azoospermia.[90] Short-term administration of hCG (LH-like activity) in normal men with experimental gonadotropin deficiency resulted in a dose-dependent increase in intratesticular testosterone.[91]

Testosterone replacement therapy in gonadotropin-deficient men does not increase intratesticular testosterone sufficiently to support spermatogenesis. In fact, testosterone treatment suppresses endogenous gonadotropin concentrations and may suppress sperm production. However, this cannot be assumed to occur in all testosterone-treated gonadotropin-deficient men, especially if testosterone replacement is not adequate. In one study involving a small number of men with acquired hypogonadotropic hypogonadism due to hypothalamic-pituitary disease, half had detectable sperm counts ranging from very low (1 million/mL) to normal (120 million/mL) on testosterone replacement therapy, mostly because of incomplete gonadotropin suppression associated with an subtherapeutic testosterone regimen (200–250 mg given by intramuscular [IM] injection every 3–4 weeks).[92]

Spermatogenesis is maintained with intratesticular testosterone concentrations that are 10% of normal, but the minimum concentration needed to support sperm production is not known. Intratesticular testosterone is converted within the testes to its

active metabolites, estradiol and DHT, by P450 19A1 (aromatase) and 5α-reductase (SRD5A1), respectively. As with testosterone, intratesticular concentrations of estradiol are about 100-fold higher than serum estradiol concentrations; however, intratesticular concentrations of DHT are only approximately 15-fold higher than concentrations in the circulation.[93] The roles of these relatively high concentrations of estradiol and DHT within the testes in the maintenance of spermatogenesis are not clear.

Negative Feedback Regulation of Gonadotropin Secretion

As described earlier, the feed-forward regulation of testicular function involves hypothalamic GnRH stimulation of pituitary gonadotropin secretion, which in turn stimulates the testes to secrete testosterone and increase sperm production (see Fig. 19.6). An important aspect of hypothalamic-pituitary-testicular axis regulation is the negative feedback suppression of hypothalamic GnRH and pituitary gonadotropin secretion by steroid and peptide hormones produced by the testes. Testosterone, produced by Leydig cells of the testis, and estradiol, its active metabolite, act at both the hypothalamus and the pituitary gland to inhibit GnRH and gonadotropin secretion. Inhibin B, produced by Sertoli cells within seminiferous tubules of the testis, acts primarily on the pituitary to suppress FSH secretion.

In a series of elegant prospective studies, normal men and GnRH-deficient men with CHH treated with physiologic doses of pulsatile GnRH (i.e., a GnRH clamp) underwent medical castration induced by high-dose ketoconazole and treatment with physiologic doses of testosterone or estradiol. The effects of these interventions on production of LH and FSH were measured. These studies have helped to define the relative roles of testosterone and estradiol in regulating gonadotropin secretion and the sites of negative feedback by these steroids.[94,95] It appears that both testosterone and estradiol (derived from aromatization of testosterone) exert negative feedback effects at the hypothalamus to suppress pulsatile GnRH secretion. These studies also demonstrated that negative feedback inhibition of pituitary LH and FSH secretion by testosterone requires aromatization of testosterone to estradiol. The suppression of FSH by estradiol is modest when inhibin B concentrations are normal and testes are normal, suggesting that inhibin B is the main physiologic negative feedback regulator of FSH secretion.[96] When inhibin B concentrations are low, as in men with seminiferous tubule failure or anorchia, the negative feedback effect of estradiol derived from testosterone assumes a greater role in suppressing FSH.

Although active metabolism of testosterone to estradiol is important in the negative feedback actions of testosterone, conversion of testosterone to DHT by 5α-reductase types 1 and 2 does not play a major role in steroid feedback. Men with mutations in *SRD5A2* exhibit only modest elevations in gonadotropins and increases in LH pulse amplitude but not frequency.[97] Men with benign prostatic hyperplasia (BPH) and normal men treated with finasteride (an SRD5A2 inhibitor) or dutasteride (an SRD5A1 and SRD5A2 inhibitor) do not demonstrate increases in serum LH and FSH concentrations.[98] These findings suggest a relatively minor role for DHT in physiologic negative feedback regulation of gonadotropins. However, administration of supraphysiologic amounts of DHT does suppress concentrations of LH (by 30–60%) and FSH (by 15–30%).[99]

The pituitary gonadotrophs contain estrogen receptor α (ERα), but GnRH neurons appear to lack both ERα and AR. The negative feedback actions of testosterone and estradiol are thought to be mediated indirectly by other neuronal systems that relay steroid feedback signals to GnRH neurons. Studies in animals suggest that neurons that produce *kisspeptin*, a 54–amino acid peptide product of the *KISS1* gene, may be candidate mediators of steroid negative feedback.[100,101] These neurons interact directly with GnRH neurons in the medial basal hypothalamus, the majority of which contains the kisspeptin receptor, *KISS1R*, and the release of kisspeptin thereby stimulates GnRH secretion. Kisspeptin neurons may also interact with other neurons (e.g., γ-aminobutyric acid neurons) to indirectly regulate GnRH secretion. Kisspeptin neurons contain both AR and ERα. In experimental animals, castration increases kisspeptin expression, coinciding with an increase in GnRH and gonadotropin secretion; sex steroid treatment with testosterone, estradiol, or DHT reverses these changes, and kisspeptin antagonists block the postcastration increase in LH secretion. In humans, mutations in *KISS1* or *KISS1R* cause hypogonadotropic hypogonadism and impaired pubertal development,[102] and there is accumulating evidence in animals that kisspeptin may have a key role in the initiation of puberty.

Inhibins are heterodimeric glycoproteins belonging to the TGFβ superfamily of proteins, which includes activins, inhibins, TGFβ, bone morphogenetic proteins, and growth and differentiation factors such as AMH and myostatin.[103] Inhibins are composed of an α-subunit connected by a disulfide bridge to either a β_A or a β_B subunit to form inhibin A or inhibin B, respectively. *Inhibin B* (α-β_B heterodimer) is the physiologically relevant inhibin species in humans. Unlike most proteins in the TGFβ family, which act as local paracrine or autocrine regulators of diverse cellular functions, inhibin B acts as a circulating hormone. Inhibin B is produced by the Sertoli cell in response to FSH stimulation. It binds to a coreceptor composed of the type III TGFβ receptor (TGFBR3 or betaglycan) and the type IIB activin receptor (ACVR2B) and is thought to be the main endocrine negative feedback suppressor of FSH secretion by pituitary gonadotrophs.

In humans, inhibin B concentrations rise progressively at the time of puberty, correlating with FSH concentrations and FSH-stimulated Sertoli cell proliferation.[103] Adult concentrations are reached by midpuberty. At that time, Sertoli cell function becomes intimately linked to the onset of spermatogenesis, and inhibin B concentrations assume an inverse relationship with FSH concentrations as the inhibin B–mediated negative feedback regulation becomes activated. For example, in boys with Sertoli cell–only syndrome, inhibin B concentrations are normal before puberty as a function of Sertoli cell proliferation but become undetectable at the time of puberty, reflecting the absence of germ cells and Sertoli cell dysfunction. In adults, inhibin B concentrations are inversely related to the degree of germ cell damage or loss and Sertoli cell dysfunction. This relationship suggests that germ cells regulate Sertoli cell function, although the precise cellular and molecular mechanisms underlying this regulation are not clear. Inhibin B concentrations have been used as biomarkers of spermatogenesis and Sertoli cell function in research and by some practitioners, but they have not yet been used in routine clinical practice.

Activins include homodimers consisting of two β_A subunits (activin A) or two β_B subunits (activin B) and a heterodimer of one β_A and one β_B subunit (activin AB).[103,104] Activins are produced by gonadotrophs and bind to ACVR2B receptors. They act primarily as autocrine regulators to stimulate FSHβ synthesis and sensitize gonadotrophs to GnRH stimulation, resulting in increased FSH secretion. Inhibin B acts as a selective antagonist of activins in gonadotrophs by binding to ACVR2B receptors.

Follistatins are glycoproteins produced by gonadotrophs and by folliculostellate cells of the pituitary gland that bind and antagonize the actions of activin. They act as autocrine and paracrine regulators of FSH secretion. Activins and follistatins are also produced in Sertoli cells and germ cells and may act as autocrine and paracrine regulators of testis function.

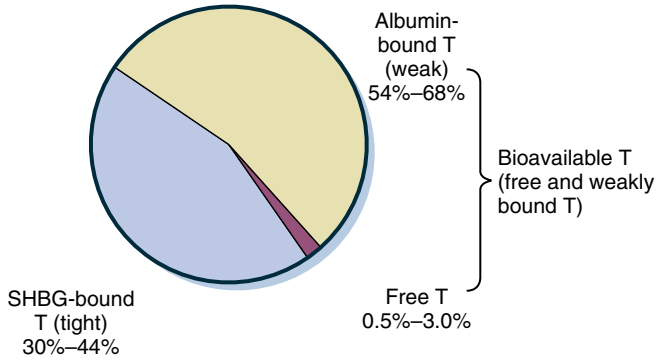
Negative feedback suppression of gonadotropin production (by pharmacologic doses of androgens or combinations of androgens and progestins or by GnRH antagonists) that is sufficient to result in suppression of sperm production has been the basis for male hormonal contraceptive development strategies.[105]

Testosterone Transport, Metabolism, and Actions

Circulating Testosterone

Like other steroid and thyroid hormones, testosterone secreted into the circulation by Leydig cells is mostly bound to plasma proteins, primarily to SHBG and albumin. In the circulation, *total testosterone* is composed of 0.5% to 3.0% *free testosterone* unbound to plasma proteins, 30% to 44% SHBG-bound testosterone, and 54% to 68% albumin-bound testosterone[106,107] (Fig. 19.10).

Clinically, biologic actions of testosterone, like those of other steroid hormones, are thought to conform to the free hormone hypothesis—that is, the biologic activity of testosterone is mediated only by its free (unbound) concentration or the concentration that is easily dissociable from plasma proteins in the circulation.[107,108] Testosterone is tightly bound to SHBG with such high affinity (1.6×10^{-9} mol/L) that it is not easily dissociable and available to target tissues for biologic action. In contrast, testosterone is loosely bound to albumin, with a binding affinity (1.0×10^{-4} mol/L) that is several orders of magnitude less than that of SHBG binding. Therefore, albumin-bound testosterone is dissociable and available to target tissues for action. Together, free testosterone and albumin-bound testosterone are referred to as *bioavailable testosterone*, because these fractions are available to diffuse into target tissues, bind AR, and affect gene transcription, resulting in androgen action in those tissues. One case was reported of a man who had undetectable SHBG and very low total testosterone concentrations due to a missense mutation of the *SHBG* gene that blocked its secretion.[109] His free testosterone and gonadotropin concentrations and semen analysis were normal, and he had normal objective evidence of sexual development. Although he did have subtle symptoms of male hypogonadism (sexual dysfunction, weakness, and fatigue), the overall findings in this man provide support for the free hormone hypothesis. In addition, data from the European Male Aging Study, a longitudinal cohort study, indicate that low serum free testosterone concentrations are associated with symptoms and objective findings of male hypogonadism in men with normal total testosterone concentrations.[110] In addition, incident symptoms of hypogonadism occur in men who have low serum free testosterone and total testosterone concentrations but not in obese men with normal serum free testosterone and low serum total testosterone concentrations.[111] Although the free testosterone hypothesis remains controversial, the limited available data generally support it.[112]



• **Fig. 19.10** Fractions of circulating testosterone in blood. The majority of circulating testosterone (T) is bound to serum proteins: approximately 54% is weakly bound to albumin, and 44% is tightly bound to sex hormone–binding protein (SHBG). Only about 2% of circulating T is free of protein binding. The combination of free and weakly bound (albumin-bound) T is referred to as bioavailable testosterone.

SHBG, which is synthesized by hepatocytes of the liver, is a homodimeric β-globulin protein composed of a heavy and a light subunit that are identical in peptide sequence and encoded by a single gene but differ in their degree of glycosylation.[73] The *SHBG* gene is also expressed in the testes and in most mammals, Sertoli cells produce a homologue of SHBG, known as ABP, under the control of FSH. In the human testis, however, *SHBG* appears to be expressed in germ cells rather than Sertoli cells and to produce a truncated form of SHBG (not ABP) that localizes to the acrosome of spermatozoa.[74] SHBG has two binding sites per monomer; these sites bind DHT and testosterone with high affinity and estradiol less avidly. Previous studies have assumed identical binding affinities of SHBG monomers for testosterone and other ligands. Recent studies suggest that binding of testosterone to one monomer of the SHBG dimer affects the binding affinity of testosterone to the second monomer and that binding can only be characterized by a complex dynamic, multistage, allosteric model.[113] Glycosylation of SHBG is not involved in steroid binding but may prolong the plasma half-life of the protein in the circulation. SHBG production by the liver is increased under the influence of estrogens and thyroxine and decreased by androgens and insulin.

Counter to the free hormone hypothesis, there is evidence that testosterone bound to SHBG may affect androgen action in some target tissues. In some tissues, such as the human prostate, SHBG may bind to a cell surface receptor; testosterone then binds to the SHBG-receptor complex, activating cAMP and affecting target organ function.[114] Glycosylation of SHBG may be important for the interaction of steroid-SHBG complexes with plasma membranes. *Megalin* is a member of the LDL receptor superfamily of proteins that serve as endocytic proteins facilitating the entry of steroids into cells (most notably 25-hydroxyvitamin D into proximal tubule cells of the kidney). Megalin is found in the kidney and also in the epididymides, prostate, ovaries, and uterus. In vitro studies in cells that expressed megalin but no other endocytic receptors found that testosterone, DHT, and estradiol bound to SHBG were endocytosed into cells and activated AR-mediated transcription; these effects were blocked by a megalin antagonist.[115] Furthermore, megalin-knockout mice demonstrated impaired testicular descent, an androgen-mediated process. These findings suggest that megalin may be important in mediating cellular uptake of androgens into some tissues. However, the importance of testosterone bound to SHBG or endocytic proteins such as megalin in human physiology remains to be determined.

In normal men with an intact hypothalamic-pituitary-testicular axis, alterations in SHBG concentrations and testosterone bound to SHBG do not influence the physiology and action of androgens at steady state. Any acute effects on free testosterone

concentration caused by changes in SHBG would alter the negative feedback regulation of gonadotropins, resulting in normalization of free testosterone concentrations. For example, an acute increase in SHBG concentrations may transiently decrease free testosterone concentrations, but the consequent decrease in testosterone negative feedback increases pituitary LH secretion, which increases testosterone production by the testes to restore normal free testosterone concentrations. In contrast, alterations in SHBG concentrations may alter free testosterone concentrations in men with reproductive disorders who have impaired negative feedback regulation or are receiving testosterone replacement therapy.

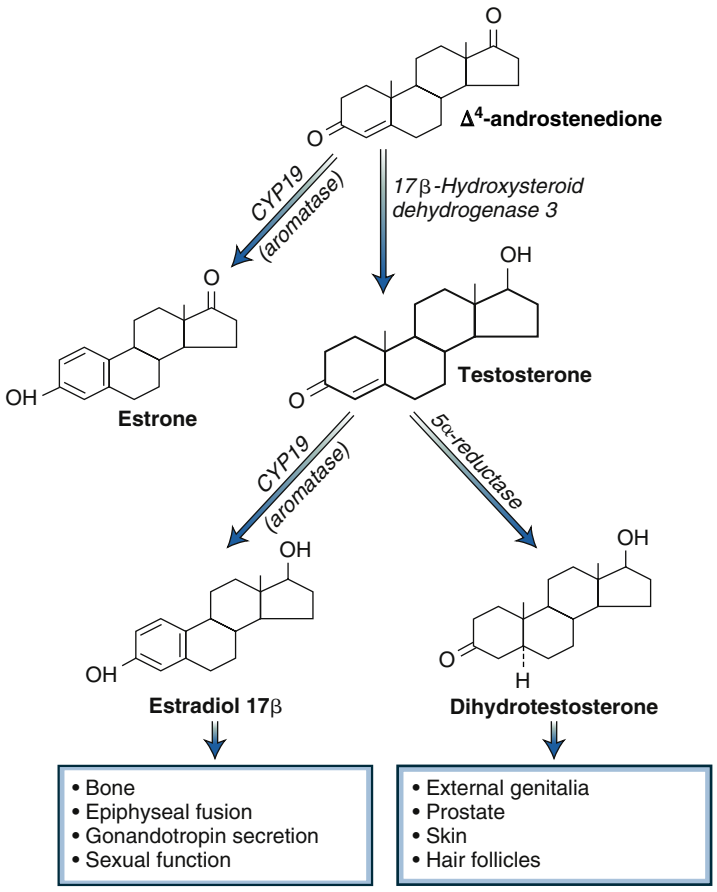
SHBG concentrations may be decreased or increased in a variety of commonly encountered clinical conditions.[116] Clinically, alterations in SBHG are extremely important to consider in the diagnosis of male hypogonadism (see later discussion). Because serum total testosterone measurements are affected by changes in SHBG concentrations, accurate measurements of free or bioavailable testosterone are needed to assess the adequacy of Leydig cell function, to determine whether a patient is hypogonadal, and to monitor testosterone replacement in patients with alterations in circulating SHBG concentrations.

Active Metabolism and Catabolism of Testosterone

An important aspect of the effects of testosterone action on target tissues is its active metabolism to *17β-estradiol* (estradiol) by P450 19A1 and to *DHT* by SRD5A1 and SRD5A2; these are the most potent of the endogenous estrogens and androgens[9] (Fig. 19.11). Many of the biologic actions of testosterone are mediated by these active metabolites, acting through mechanisms that are dependent on ERα and ERβ (estradiol) or on AR (DHT). These active metabolites are formed and act locally as paracrine or autocrine regulators, and they also are secreted and act as endocrine regulators of target tissue function.

Aromatization of Testosterone to Estradiol

Aromatase (P450 19A1) catalyzes the conversion of testosterone to estradiol, as well as the conversion of the weaker androgen, Δ^4-androstenedione, to the weaker estrogen, estrone. In men,



• **Fig. 19.11** Active metabolism of testosterone. Testosterone may be converted to the potent estrogen 17β-estradiol by the enzyme aromatase or P450 19A1 (CYP19) or to the more potent androgen dihydrotestosterone (DHT) by the enzyme 5α-reductase. The effects of testosterone on external genital differentiation, prostatic growth, skin, and hair follicles in androgen-sensitive areas require 5α-reduction of testosterone to DHT. The effects of testosterone that require aromatization to estradiol are prevention of bone resorption, increase in bone mineral density, epiphyseal fusion, and gonadotropin secretion. Testosterone and DHT, but not estradiol, increase and maintain muscle mass and strength; testosterone, DHT, and estradiol play a role in normal male sexual function; and testosterone and DHT have opposing effects to estradiol on high-density lipoprotein cholesterol concentrations. (Modified from Bhasin S. Testicular disorders. In: Kronenberg HM, Melmed S, Polonsky KS, et al, eds. *Williams Textbook of Endocrinology,* 11th ed. Philadelphia, PA: Elsevier; 2008:645–698.)

these conversions occur predominantly in adipose tissue but also in other tissues, including brain, bone, breast, liver, blood vessels, and testes (Sertoli cells and Leydig cells). Approximately 40 to 50 μg of estradiol is produced daily, primarily by extratesticular aromatization of testosterone to estradiol and of Δ^4-androstenedione to estrone (which is then converted to estradiol by various isoforms of the 17β-HSD enzymes). Approximately 15% to 25% of circulating estradiol is produced by the testes, primarily by Leydig cells.[117]

Aromatization of testosterone to estradiol mediates the effects of testosterone on epiphyseal closure at the time of puberty, inhibition of bone resorption and maintenance of bone mineral density (BMD), regulation of fat mass accumulation, negative feedback suppression of hypothalamic GnRH secretion and pituitary gonadotropin secretion, sexual function, and possibly regulation of high-density lipoprotein (HDL) cholesterol concentrations and some aspects of cognitive function and mood.[118,119] Healthy young to middle-age men with experimental hypogonadism induced by a GnRH agonist were treated with placebo or various dosages of testosterone with or without coadministration of an aromatase inhibitor to investigate the relative effects of experimental androgen and estrogen deficiency, respectively.[120,121] Decreases in lean body mass, muscle size, and strength were induced by androgen deficiency; fat mass accumulation and bone loss was induced by estrogen deficiency; and decline in sexual function was induced by both androgen and estrogen deficiency. In addition, estradiol deficiency appears to be the primary cause of vasomotor symptoms (hot flashes) in men with acute severe hypogonadism.[122]

In men, circulating estradiol is regulated primarily by the amount of androgen substrates, testosterone and Δ^4-androstenedione, and by aromatase activity in adipose tissue and other peripheral tissues. Aromatase activity in Leydig cells is controlled primarily by LH. Stimulation of aromatase activity in gonadotropin-deficient men treated with hCG may result in relatively higher concentrations of serum estradiol compared with testosterone and may contribute to benign breast tenderness and enlargement (gynecomastia) during treatment. As in women, men with ER-positive breast cancer may be treated with aromatase inhibitors to reduce estrogen synthesis.

The few men reported with inactivating mutations of the aromatase gene (*CYP19A1*) have demonstrated tall stature, persistent linear growth after puberty, eunuchoidal body proportion, delayed bone age, osteopenia or osteoporosis and progressive *genu valgum*, and variable impairments in glucose and lipid metabolism, including insulin resistance, elevated triglyceride and low HDL cholesterol concentrations, abnormal liver enzymes and fatty liver, variably low sperm counts and infertility, and undetectable estradiol concentrations with normal to elevated serum testosterone and gonadotropin concentrations.[123] Furthermore, estradiol treatment results in closure of the epiphyses, increased BMD, and increased bone age. Men with a rare inactivating mutation of ERα had a similar phenotype, but in contrast to men with aromatase deficiency, they had high serum estradiol, testosterone, and gonadotropin concentrations, consistent with estrogen resistance.[118] These findings support potentially important roles of estradiol in bone, glucose, and lipid metabolism; liver function; and pituitary and testis function.

5α-Reduction of Testosterone to DHT

Testosterone is converted to DHT, an androgen that is 2.5 to 3.0 times more potent than testosterone, by SRD5A1 and SRD5A2. These two isoenzymes of 5α-reductase differ in the optimal pH for their activity and in their expression patterns.[124] SRD5A2 is expressed most highly in prostate, epididymis, seminal vesicles, genital skin, and liver and at lower concentrations in other tissues, such as certain brain regions, nongenital skin, testis, and kidney. SRD5A1 is expressed most highly in nongenital skin (hair follicles), liver, and certain brain regions and at lower concentrations in prostate, epididymis, seminal vesicles, genital skin, testis, adrenal, and kidney. Approximately 200 to 300 μg of DHT is produced daily, mostly from 5α-reduction of testosterone in peripheral tissues (predominantly skin and liver). The prostate and testis contribute relatively little to concentrations of DHT in blood.

Men with inactivating mutations of *SRD5A2* are born with severe 46,XY DSD with ambiguous genitalia (clitoris-like phallus, bifid scrotum, pseudovaginal hypospadias, and rudimentary prostate gland) but normal wolffian duct differentiation (normal seminal vesicles, epididymides, and vas deferens) and no müllerian duct structures, supporting the vital role of DHT in external genital differentiation and prostate development.[125] Individuals with SRD5A2 deficiency are usually raised as girls. With the onset of puberty and increase in testosterone to adult male concentrations, the phallus grows, the scrotum develops, libido and erections are stimulated, and the gender role may change from female to male. Cryptorchidism is common but not invariable and is associated with oligozoospermia or azoospermia. Testes may descend at the time of puberty. Normal sperm counts may occur in individuals with descended testes, and fertility has been reported in men with SRD5A2 deficiency. However, in adults, the prostate remains underdeveloped and is not palpable, facial and body hair are diminished, sebum is not produced, and male-pattern baldness does not occur, supporting the importance of normal SRD5A2 activity and DHT for hair growth, sebaceous function, and prostate development. Serum DHT concentrations are low, testosterone concentrations are normal to slightly elevated, and gonadotropin concentrations are normal to modestly elevated.

Within the prostate gland, conversion of testosterone to DHT produces concentrations of DHT that are approximately 10-fold higher than those in serum, serving to amplify androgen activity in the prostate. Intraprostatic androgen concentrations may contribute to prostate disease, such as BPH or prostate cancer.[126]

Inhibitors of SRD5A2 (finasteride) or of both SRD5A1 and SRD5A2 (dutasteride) are used to treat lower urinary tract symptoms (LUTS), improve urinary flow, and prevent complications related to BPH, as well as to treat male-pattern baldness and androgenic alopecia.[127] Treatment with finasteride or dutasteride reduces, respectively, the prevalence or the incidence of prostate cancer found on biopsy but is possibly associated with a greater number of cancers with high Gleason grade.[128,129] An important aspect of the effect of androgens on the prostate is that intraprostatic androgen concentrations are not reflected in serum concentrations, underscoring the importance of local paracrine and autocrine actions of androgens in the physiology and pathology of the prostate and probably other androgen target tissues.

Catabolism of Testosterone

The primary site of catabolism of circulating testosterone and 5α-DHT is the liver.[130] Testosterone and 5α-DHT are taken up in the liver, and testosterone is converted to an inactive metabolite, 5β-DHT, by the enzyme 5β-reductase. Both 5α- and 5β-DHT then undergo 3α-reduction by the 3α-HSD enzymes to form 3α,5α-androstanediol (also called *3α-diol*) and 3α,5β-androstanediol, respectively; this is followed by 17β-oxidation by the enzyme 17β-HSD type 2 to form androsterone and etiocholanolone as

catabolic products. In peripheral tissues such as skin, 5α-DHT may also be converted to 3α-diol, which is further metabolized in the liver.

In the liver, testosterone, DHT, androsterone, etiocholanolone, and the 3α-androstanediols undergo glucuronidation and, to a lesser degree, sulfation, to form more hydrophilic conjugates that are released into the circulation and excreted in urine and bile. Metabolic inactivation of testosterone primarily involves its conversion to metabolites such as androstenedione (about 50%), androsterone (20%), and etiocholanolone (20%) glucuronides (as well as sulfates) and lesser conversion to 3α-diol glucuronides (3α-diol Gs). Because 3α-diol comes mostly from skin, blood and urine measurements of 3α-diol G have been used as a marker of peripheral androgen action.[130] In men with 5α-reductase deficiency, 3α-diol G concentrations are reduced. The amount of body hair and acne correlates with 3α-diol G concentrations.

Epitestosterone (17α-hydroxy-4-androsten-3-one) is a biologically inactive 17α-hydroxy epimer of testosterone (17β-hydroxy-4-androsten-3-one) that is produced by the testes in response to LH.[131] The production rate of epitestosterone is about 3% that of testosterone, but its clearance rate is 33% that of testosterone, and there is no interconversion of epitestosterone and testosterone. Like testosterone, epitestosterone is conjugated in the liver, primarily to glucuronides and sulfates, and excreted in the urine. Because epitestosterone conjugates are rapidly cleared in the urine, excretion rates of testosterone and epitestosterone are similar, and the ratio of urinary testosterone to epitestosterone (T/E ratio) is approximately 1:1.

Measurements of the T/E ratio and other metabolites in urine by sensitive gas chromatography/mass spectrometry methods are used to detect androgenic anabolic steroid doping, particularly testosterone, by competitive athletes.[131] Administration of exogenous testosterone suppresses LH and the production and clearance of epitestosterone relative to the administered testosterone, resulting in an elevated T/E ratio in urine. The World and United States Anti-Doping Agencies have set a threshold T/E ratio of greater than 4:1 as suspicious for testosterone doping.

Testosterone is glucuronidated primarily by the enzyme uridine diphosphate glucuronyl transferase 2B17 (UGT2B17), whereas epitestosterone is glucuronidated mostly by another UGT isoform, UGT2B7. Although testosterone may be glucuronidated by other UGT isoforms (e.g., UGT2B15), individuals with deletion of UGT2B17 (common in Asian populations) have reduced testosterone glucuronidation and clearance, resulting in lower T/E ratios with testosterone administration.[130-133] Realization that there were populations with naturally low T/E led to a reduction in the T/E ratio cutoff from the previous threshold for suspicion of doping of (>6:1) to the present one (>4:1). In addition, there are individuals with naturally high T/E ratios, perhaps because of other genetic polymorphisms or environmental factors, such as excessive alcohol consumption, that may increase T/E ratio transiently, particularly in women.[134] In the absence of environmental perturbations, the T/E ratio in a single individual is remarkably stable over time, and longitudinal measurements of urinary T/E ratio are used to detect illicit androgen use (known as the athlete biologic passport). Coadministration of epitestosterone with testosterone (to maintain a normal T/E ratio) has been used by athletes to avoid detection.

If a urinary T/E ratio is suspicious for doping, exogenous androgen use is confirmed by gas chromatography combustion isotope ratio mass spectrometry, which can detect small differences in the ratio of carbon-13 to carbon-12 ($^{13}C/^{12}C$) isotopes of testosterone or its metabolites.[131] Because synthetic androgens are synthesized from plant sources (yams or soy), their $^{13}C/^{12}C$ ratio is lower than that of endogenously produced testosterone and other steroids that reflect an animal source or dietary ingestion of both animal and plant products. However, $^{13}C/^{12}C$ isotope ratio mass spectrometry will not detect doping by administration of hCG or LH-like activity to stimulate endogenous testosterone production or administration of androgens derived from animal sources that have a $^{13}C/^{12}C$ ratio like that of endogenous testosterone.

Mechanisms of Androgen Action

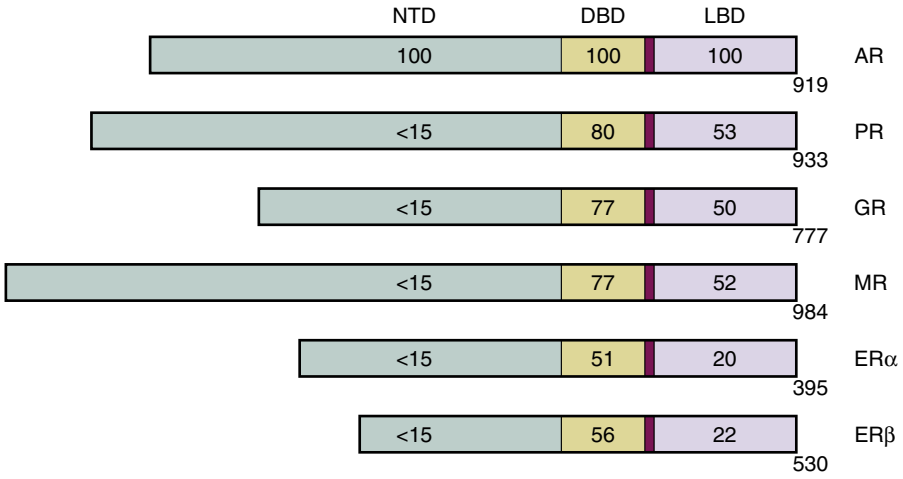
In androgen target tissues, testosterone and DHT in the circulation diffuse through the plasma membrane and bind to intracellular ARs.[135] Binding of androgen to the AR induces a conformational change in the AR that causes dissociation of heat shock proteins bound to AR, permitting translocation into the nucleus, induction of phosphorylation and homodimerization, and interaction with DNA, specifically on androgen response elements located in regulatory sites of target genes. The AR dimer actively recruits tissue-specific coregulators (coactivators and corepressors) to form the transcriptional apparatus necessary to control androgen-regulated gene transcription and subsequent androgen-regulated protein synthesis.

The *AR* gene is located on the long arm of the X chromosome (Xq11-12). *AR* is organized into functional domains[135] (Fig. 19.12): (1) an *N-terminal domain* comprising two transactivation domains (AF1 and AF5) that mediate the majority of *AR* transcriptional activity and coregulator interaction and two trinucleotide repeat segments (CAG and GGN, encoding polyglutamine and polyglycine tracts, respectively) of varying number that modify *AR* transactivation; (2) a *DNA-binding domain* comprising two zinc finger motifs, the first mediating DNA recognition and binding and the second stabilizing DNA interaction and mediating dimerization of *AR*; (3) a small *hinge region* (H); and (4) a *ligand-binding domain* that mediates high-affinity binding of androgen to the AR and also contains another transactivation domain (AF2).

The AR is a member of the nuclear receptor superfamily that includes other steroid hormone receptors. It shares approximately 80% sequence homology in the DNA-binding domain and 50% in the ligand-binding domain, with its most closely related steroid receptors being the progesterone receptor, the glucocorticoid receptor, and the mineralocorticoid receptor.[135] This similarity may explain why, for example, some progestins (e.g., medroxyprogesterone acetate) have AR agonist activity, others (e.g., cyproterone acetate) have AR antagonist activity, and a mineralocorticoid antagonist, spironolactone, has AR antagonist activity. Compared with the binding of testosterone, DHT binds to AR with higher affinity, greater stability, and a slower rate of dissociation, conferring greater androgen activity to DHT, the most potent endogenous androgen in humans.

Inactivating mutations of the *AR* may cause qualitative or quantitative abnormalities of receptor function, resulting in variably impaired androgen action.[136] *AR* mutations manifest phenotypic variability, ranging from that of a 46,XY phenotypic female with normal female external genitalia and breast development (*complete androgen insensitivity*, formerly *testicular feminization*), which occurs in individuals with complete androgen insensitivity or resistance, to that of an otherwise normal male with incomplete hypospadias, mild undervirilization, or infertility.

In a normal population, the number of trinucleotide CAG repeats in the first exon of the *AR* gene varies from 11 to 35. In



• **Fig. 19.12** Schematic diagram of the structure of the human androgen receptor (AR) gene and homology to other steroid hormone receptors: progesterone receptor (PR), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), estrogen receptor α (ERα), and estrogen receptor β (ERβ). The AR is a 919–amino acid protein that is composed of three functional domains: a ligand-binding domain (LBD), a DNA-binding domain (DBD), and an N-terminal transactivation domain (NTD). The DBD shares the greatest degree of homology (>51% vs. AR) and the NTD the least degree of homology (<15% vs. AR) among steroid hormone receptors. (From Li J, Al-Azzawi F. Mechanism of androgen receptor action. *Maturitas.* 2009;63:142–148.)

general, the number of CAG repeats appears to correlate inversely with AR function and action, both in vitro and in vivo, in transgenic mice and humans. *Kennedy disease*, or *X-linked spinal and bulbar muscular atrophy*, is a rare adult-onset neurodegenerative disease of motor neurons that results in progressive muscle weakness; it is associated with a markedly expanded number of CAG repeats, varying from 40 to 62, which does not overlap with the normal population.[137] Neurodegeneration in this disorder is thought to be caused by toxicity from intracellular aggregation of the AR and associated cofactors that is worsened by androgen binding to the mutant AR and translocation into the nucleus.

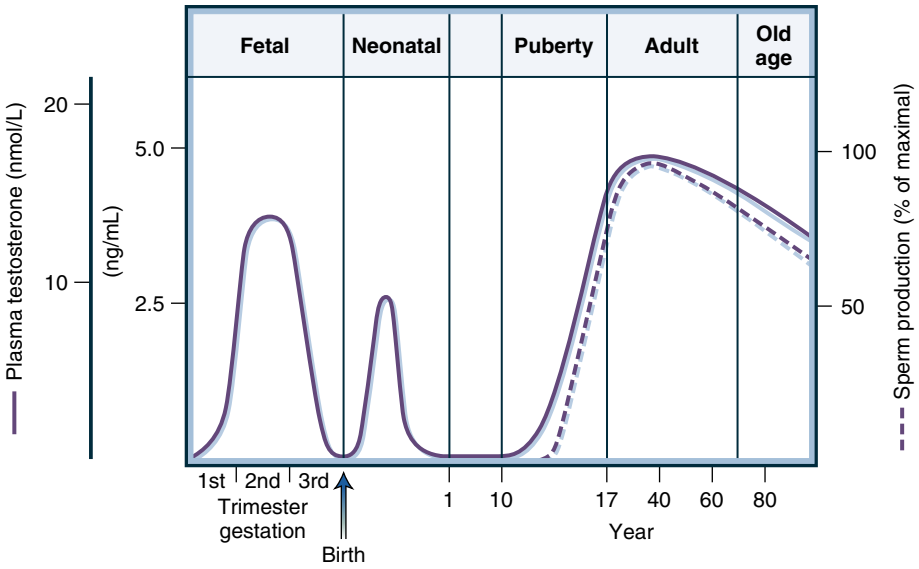
Most men with Kennedy disease also manifest clinical findings of partial androgen resistance, including gynecomastia, reduced libido, erectile dysfunction, decreased facial hair, testicular atrophy, and oligozoospermia or azoospermia in association with high testosterone and high or normal gonadotropin concentrations.[138] The severity of the latter biochemical indices of androgen insensitivity is directly related to CAG repeat length. Although it is not found consistently, some studies have reported an association between CAG repeat number and manifestations of androgen action in normal men.[139] In these studies, a low number of CAG repeats within the normal range was associated with higher androgenicity (e.g., earlier onset of prostate cancer, male-pattern baldness, lower HDL cholesterol), and a high number of repeats within the normal range was associated with lower androgenicity (e.g., gynecomastia, impaired spermatogenesis, lower bone density, depressive symptoms). CAG repeat number also seems to be associated with the clinical manifestations of men with androgen deficiency due to Klinefelter syndrome and their response to testosterone treatment; men with Klinefelter syndrome with higher CAG repeat numbers require relatively high doses of testosterone for full androgen effects.

Studies in vitro and in experimental animals suggest that some actions of androgens may occur within seconds to minutes, too rapidly to be caused by classic genomic effects of androgens acting through the AR on gene transcription and subsequent protein synthesis, which usually take hours to produce effects.[140] Rapid, nongenomic (also called *nonclassical*) effects of androgens may be mediated by cell surface interactions and receptors and by the activation of conventional signal transduction mechanisms, including activation of PKA and PKC, which increase intracellular calcium and MAPK pathways. Binding of androgen to intracellular AR may also activate coregulators that do not require gene transcription to signal, such as the tyrosine kinases (e.g., MAPK, ERK, and Akt) that are AR coactivators. Nongenomic actions of androgens have been described in testis (Sertoli cells), brain, muscle, cardiovascular tissue, prostate, and immune cells. In humans, the rapid vasodilatory effect of testosterone on myocardial ischemia in patients with coronary artery disease is attributed to a direct nongenomic effect of androgens (or estrogenic metabolites) on vascular cells.

Androgen Effects at Various Stages of Sexual Development

Concentrations of testosterone and its actions differ at various stages of sexual development[141] (Fig. 19.13). During *fetal life*, testosterone is secreted by the fetal testis beginning at 7 weeks of gestation.[36] Testosterone secretion is primarily under the control of maternal hCG, initially, and then LH secreted by the fetal pituitary. During this time, testosterone concentrations increase to almost adult male concentrations, and testosterone and its conversion to DHT are critical for normal male internal and external genital differentiation (e.g., development of primary sexual characteristics). Testosterone concentrations remain elevated through most of the second trimester and decline in the third trimester.

Shortly after birth, during *neonatal life*, LH secretion increases and stimulates a second rise in testosterone concentrations, almost to adolescent concentrations, between 3 and 6 months of age; this is followed by a fall to low prepubertal concentrations.[142] The neonatal surge in testosterone concentrations may have a role in the development of normal phallus size and completion of testicular descent. The neonatal increase in testosterone and FSH concentrations at this time also stimulates Sertoli cell proliferation and



• **Fig. 19.13** Schematic diagram of changes in serum testosterone concentration and sperm production during different phases of life. During fetal life, testosterone concentrations increase almost to adult male concentrations, peaking during the first trimester and remaining elevated throughout the second trimester, after which they decline. During neonatal life, testosterone increases almost to adolescent concentrations at 3 to 6 months of age, then declines to prepubertal concentrations. During puberty, testosterone concentrations and sperm production increase to adult male concentrations over several years. With aging, there is a variable, gradual, and progressive decline in serum testosterone concentrations and sperm production, beginning at age 40 years. (From Griffin JE, Wilson JD. The testis. In: Bondy PK, Rosenberg LE, eds. *Metabolic Control and Disease,* 8th ed. Philadelphia, PA: WB Saunders; 1980:1535–1578.)

spermatogonial development, which may play a role in determining spermatogenic capacity.

During *puberty*, testosterone concentrations increase to adult male concentrations (or higher) in response to activation of hypothalamic GnRH secretion and its stimulation of pituitary gonadotropin secretion.[43,143] The progressive increase in testosterone and its active metabolites, estradiol and DHT, is responsible for the development of secondary sexual characteristics (*virilization* or *masculinization*) and other changes. Pubertal changes induced by testosterone may be categorized as those related to body, brain, and sexual function.

Body function changes mediated by testosterone and its active metabolites include the following:

- Growth and development of the penis and scrotum and the appearance of rugal folds and pigmentation in scrotal skin
- Enlargement of the prostate and seminal vesicles and production of accessory sexual gland secretion and seminal fluid
- Androgen-dependent hair growth and development of a male hair distribution—facial (moustache and beard), external auditory canal, chest, axillary, pubic and lower abdomen (male escutcheon), perianal, inner thigh, and leg and arm hair growth and frontal scalp hair recession
- Increase in sebum production
- Stimulation of IGF1 production together with that of growth hormone (GH)
- Long bone growth and eventually closure of long bone epiphyses resulting in cessation of growth
- Increase in BMD and accretion of peak bone mass
- Increase in skeletal muscle mass and strength, especially in shoulder and pectoral muscles
- Decrease and redistribution of body fat
- Enlargement of the larynx and thickening of vocal cords, resulting in a lower-pitched voice
- Stimulation of erythropoiesis resulting in an increase in hematocrit, primarily by direct bone marrow induction of erythroid differentiation and stimulation of erythropoietin secretion
- Reduction of HDL cholesterol.

Brain function changes mediated by testosterone and its active metabolites include stimulation of libido (sexual interest, desire, and motivation); increase in motivation, initiative, and social aggressiveness; and increase in aspects of cognitive function (e.g., visuospatial abilities). *Sexual function changes* mediated by testosterone and its active metabolites include initiation of spermatogenesis and acquisition of fertility potential and increase in spontaneous erections.

In *adult life*, normal adult male concentrations of testosterone serve to maintain many of the changes induced during puberty. This includes maintenance of body function changes such as normal amounts of androgen-dependent hair and male hair distribution, sebum production, BMD, muscle mass and strength, hematocrit in the male range (higher than the female range), and HDL cholesterol in the male range (lower than the female range); brain function changes such as libido, motivation, initiative, social aggressiveness, energy and vitality, mood, and possibly some aspects of cognitive function (e.g., visuospatial ability); and sexual function changes such as sperm production and fertility potential and spontaneous erections. Some of the masculinizing changes induced by testosterone during puberty are permanent. Once some body changes are developed, testosterone is not necessary for maintenance of penis size, scrotal development, linear growth, laryngeal size, vocal cord thickness, or voice pitch.[116]

With *aging*, there is a gradual and progressive decline in serum testosterone concentrations associated with reductions in muscle mass and strength, BMD, libido, energy and vitality, mood,

aspects of cognitive function, sperm production and fertility, and erections.[144] However, the contribution of the age-related decline in testosterone concentrations to these age-associated changes in function is not clear.

Male Hypogonadism

The two major functions of the testis are to produce sufficient amounts of testosterone and of sperm to support the development and maintenance of male sexual function, body function, and fertility. *Male hypogonadism* is a clinical syndrome that results from a failure of the testes to produce adequate amounts of testosterone; this syndrome is almost always associated with impaired sperm production (*androgen deficiency and impairment of sperm production*), or an *isolated impairment of sperm production or function* with normal testosterone production. Hypogonadism is the most common disorder of testis function encountered in clinical practice.

Because testis function is controlled by the hypothalamus and the pituitary, male hypogonadism may be caused by a primary disorder of the testis (*primary hypogonadism*); it may be secondary to a disorder of the pituitary or hypothalamus (*secondary hypogonadism*); or in some instances, there may be defects at both levels (*combined primary and secondary hypogonadism*).

Identifying men with secondary hypogonadism has important clinical implications that may affect management.[116] For example, secondary hypogonadism can be caused by a pituitary adenoma that may be associated with clinical manifestations related to tumor mass (e.g., headaches, visual field defects), to deficiency or excessive secretion of other anterior pituitary hormones, or to diabetes insipidus (polyuria) resulting from hypothalamic antidiuretic hormone deficiency. Such patients require management of the underlying hypothalamic or pituitary disorder in addition to testosterone replacement therapy. Secondary hypogonadism may be reversible with treatment of the underlying condition (e.g., nutritional deficiency) or discontinuation of an offending medication (e.g., glucocorticoids, opioids), or it may be associated with a chronic systemic illness that is not curable, such as chronic kidney disease (CKD). Impaired spermatogenesis and infertility caused by gonadotropin deficiency in men with secondary hypogonadism may be treated with gonadotropin or GnRH therapy, and sperm production and fertility may be restored. In contrast, infertility caused by primary testicular disease is usually not treatable with hormone therapy and requires other fertility options, such as the use of donor sperm, ART (e.g., ICSI), or adoption.

Clinical Manifestations

Because testosterone has different roles during fetal, prepubertal, and adult life, the manifestations of androgen deficiency differ depending on the stage of sexual development.[9,116]

Fetal Androgen Deficiency

During fetal development, testosterone and its conversion to DHT have vital roles in directing male internal and external genital differentiation and development. Fetal androgen deficiency (e.g., from congenital defects in testosterone biosynthesis enzymes) or androgen resistance/insensitivity (e.g., from AR mutations or 5α-reductase deficiency) manifests at birth with varying degrees of ambiguous genitalia and 46,XY DSD[32,62,145] (Table 19.1). Depending on the severity of androgen deficiency or resistance/insensitivity, the phenotype of individuals with these disorders may range from that of a normal female to that of an otherwise normal male with microphallus, pseudovaginal perineoscrotal hypospadias, bifid scrotum, and/or cryptorchidism of varying severity. These disorders are described in greater detail in Chapter 23.

Prepubertal Onset of Androgen Deficiency

The increase in testosterone concentrations that occurs at the time of puberty is responsible for development of secondary sexual characteristics; an increase in muscle mass and reduction and redistribution of body fat; long bone growth and eventually closure of epiphyses resulting in cessation of growth; stimulation of sexual interest (libido), spontaneous erections, and sexual activity; and initiation of spermatogenesis and seminal fluid production.[146,147] Prepubertal onset of androgen deficiency causes *eunuchoidism*[146,147] (Fig. 19.14; see Table 19.1), which is characterized most notably by infantile genitalia with a small penis and a poorly developed scrotum that lacks rugal folds and pigmentation. The testes are small, usually less than 2 cm in length and from 2 mL to less than 4 mL in volume. Hair is thin and fine, and there is a lack of androgen-dependent hair growth (i.e., absence of a male hair pattern in all body areas) and no temporal hair recession. The pubic hair pattern is more typical of females, with the shape of an inverted triangle in the pubic area (female escutcheon) rather in a diamond shape with hair extending from the pubic area to the umbilicus (male escutcheon), and there is little hair extending to the thighs. Acne does not develop because sebum production is not stimulated due to androgen deficiency.

Eunuchoidism is typified by a distinctive body habitus, characterized by poor muscle mass development (especially in the shoulders and chest), prepubertal fat distribution (predominantly in the face, chest, and hips), and excessively long arms and legs relative to height. Arm span exceeds height by greater than 5 cm, and the distance from the symphysis pubis to the floor exceeds the distance from the crown of the head to the symphysis pubis by greater than 5 cm. The voice is high pitched in the absence of androgen-dependent laryngeal enlargement and vocal cord thickening. Relatively long arms and legs result from a failure of long bone epiphyses to close; epiphyseal closure is mediated normally by increased estradiol derived from aromatization of the increased testosterone produced at the time of puberty. In preliminary studies, aromatase inhibitors that inhibit the conversion of testosterone to estradiol and delay epiphyseal closure have been used in combination with GH treatment in boys with idiopathic short stature; however, the long-term risks and benefits of this approach are not known.[148]

Androgen deficiency of prepubertal onset may not be recognized or diagnosed until adulthood. Compromise in peak bone mass accrual due to androgen deficiency may manifest as low BMD for age, and prolonged severe androgen deficiency increases the risk of osteoporosis and fractures as these men become older. Despite the absence of pubertal development, these individuals may develop gynecomastia (benign breast enlargement) that is caused by androgen deficiency rather than by the relatively high production of estradiol concentrations associated with pubertal gynecomastia. (Both prepubertal and pubertal gynecomastia result from relatively high ratios of serum estradiol to testosterone concentrations but by different mechanisms.) Psychological motivation and initiative may be reduced and, together with poor muscle mass and strength, may contribute to poor physical performance (e.g., in athletics or the military). These men have reduced sexual interest or desire (libido) and lack spontaneous erections at night or on awakening in the morning. Hematocrit

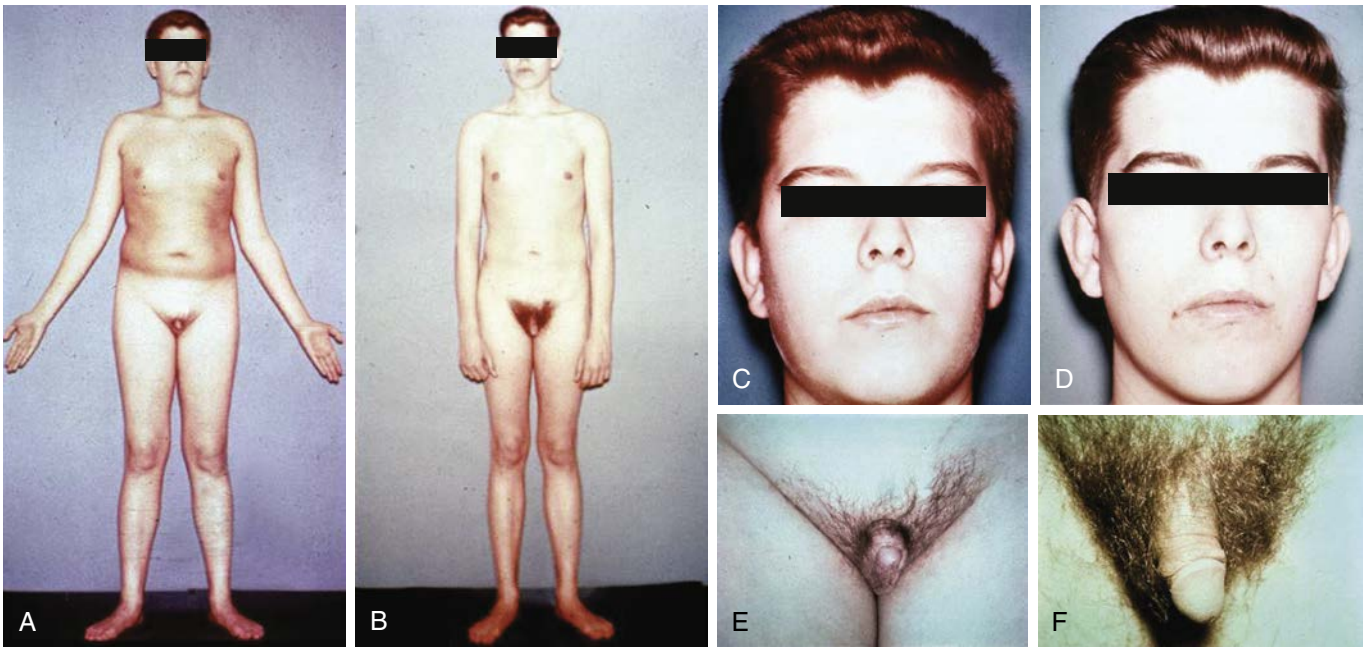
TABLE 19.1 Clinical Manifestations of Androgen Deficiency

FETAL ANDROGEN DEFICIENCY	
Symptoms	**Signs**
Ambiguous genitalia	Ambiguous genitalia (46,XY DSD) Normal female genitalia Microphallus (resembling clitoromegaly) Pseudovaginal perineoscrotal hypospadias Bifid scrotum Cryptorchidism

ANDROGEN DEFICIENCY OF PREPUBERTAL ONSET	
Symptoms	**Signs**
Delayed puberty	Eunuchoidism
Lack of sexual interest or desire (libido)	Infantile genitalia
Reduced nighttime or morning spontaneous erections	Small testes
Breast enlargement and tenderness	Lack of male hair pattern growth, no acne
Reduced motivation and initiative	Disproportionately long arms and legs relative to height
Diminished strength and physical performance	Prepubertal fat distribution
No ejaculate or ejaculation (spermarche)	Poorly developed muscle mass
Inability to father children (infertility)	High-pitched voice Reduced peak bone mass, osteopenia or osteoporosis Gynecomastia Small prostate gland Aspermia, severe oligozoospermia or azoospermia

ADULT ANDROGEN DEFICIENCY	
Symptoms	**Signs**
Incomplete sexual development	Eunuchoidism
Lack of sexual interest or desire (libido)	Small or shrinking testes
Reduced nighttime or morning spontaneous erections	Loss of male hair (axillary and pubic hair)
Breast enlargement and tenderness	Gynecomastia
Inability to father children (infertility)	Aspermia or azoospermia or severe oligozoospermia
Height loss, history of minimal-trauma fracture	Low bone mineral density (osteopenia or osteoporosis)
Hot flushes, sweats	Height loss, minimal-trauma or vertebral compression fracture
Reduced shaving frequency	Unexplained reduction in prostate size or PSA
Less Specific Symptoms	**Less Specific Signs**
Decreased energy, vitality	Mild normocytic, normochromic anemia (normal female range)
Decreased motivation, self-confidence	Depressed mood, mild depression or dysthymia
Feeling sad or blue, irritability	Reduced muscle bulk and strength
Weakness, decreased physical or work performance	Increased body fat or body mass index
Poor concentration and memory	Fine facial skin wrinkling (lateral to orbits and mouth)

DSD, Disorder of sex development; *PSA,* prostate-specific antigen.



• **Fig. 19.14** A 19-year-old patient with androgen deficiency of prepubertal onset caused by congenital anorchia before (A, C, and E) and after (B, D, and F) 5 years of testosterone treatment. Before testosterone treatment, the patient had features of eunuchoidism, characterized by infantile genitalia (small penis and poorly developed scrotum); lack of chest, pubic, and facial hair; long arms and legs relative to height; and poorly developed muscle mass in the upper body with accumulation of fat in the face, chest, and hips. After testosterone treatment, there was an increase in penis size; an increase in chest, pubic, and facial hair with scalp recession and development of acne; an increase in muscle mass, particularly in the upper body; and loss of fat in the face, chest, and hips. (From Matsumoto AM. The testis. In: Felig P, Frohman LA, eds. *Endocrinology and Metabolism.* 4th ed. New York, NY: McGraw-Hill; 2001:635–705.)

remains in the female range due to inadequate androgen stimulation of erythropoiesis. The prostate and seminal vesicles remain small without androgen stimulation, and seminal fluid production is absent, resulting in aspermia (lack of ejaculate) and failure to undergo spermarche (first ejaculation). Seminal fluid may be present in men with mild or partial androgen deficiency of prepubertal onset or in those treated with androgens. However, these men usually have severe oligozoospermia or azoospermia, and most are infertile.

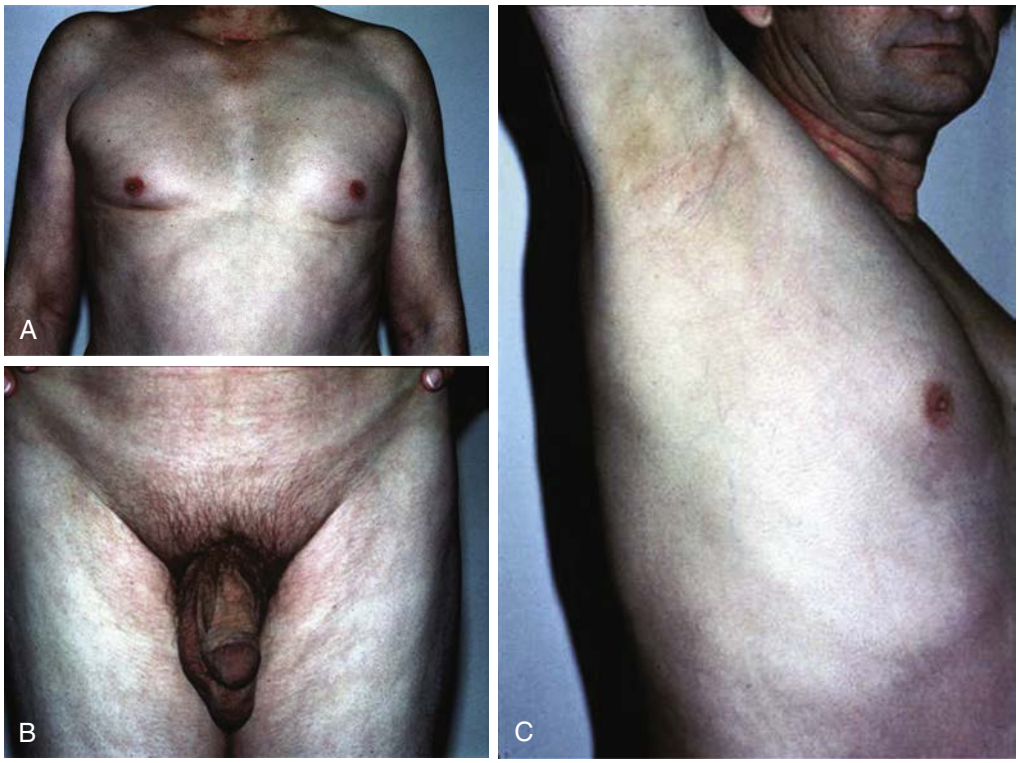
Adult Androgen Deficiency

Some individuals with androgen deficiency of prepubertal onset who are not diagnosed or are inadequately treated as boys present as adults with features of eunuchoidism and other manifestations of androgen deficiency of prepubertal onset (see Table 19.1). Their condition is usually clinically obvious because of inadequate sexual development for their chronologic age.

In adults, testosterone is needed to maintain sexual function, some secondary sexual characteristics, muscle and bone mass, and sperm production. Clinical manifestations of androgen deficiency are nonspecific and may be modified by the severity and duration of androgen deficiency, the presence of comorbid illnesses, previous testosterone treatment, or variations in target-organ sensitivity to androgens. Therefore, the clinical diagnosis of androgen deficiency acquired as an adult can be challenging, particularly in older men.[116]

Some clinical symptoms and signs are suggestive of androgen deficiency. Adults most commonly present with *sexual dysfunction* (diminished libido as manifested by reduced sexual interest or desire, reduced spontaneous and sexually evoked erections, and erectile dysfunction), *gynecomastia* (benign breast enlargement that may be accompanied by tenderness), and *infertility* (inability to father children despite unprotected intercourse) associated with oligozoospermia or azoospermia and small or shrinking testes with severe impairment in spermatogenesis. Secondary sexual characteristics do not regress to a prepubertal state; however, with long-standing, severe androgen deficiency, there may be loss of androgen-dependent hair, such as reduced facial hair associated with reduced shaving frequency and loss of axillary and pubic hair (Fig. 19.15). Men with rapid and profound decreases in testosterone concentrations (e.g., from GnRH agonist treatment of prostate cancer) may have hot flushes and sweats due to vasomotor instability (like those experienced by menopausal women) due mostly to low estradiol production related to extremely low testosterone concentrations. Because testosterone and its active metabolite, estradiol, have an important role in the maintenance of bone mass, men with chronic androgen deficiency may present with osteopenia or osteoporosis on BMD measurement (e.g., by dual-energy x-ray absorptiometry scanning) or with a minimal-trauma bone or vertebral compression fracture that may be associated with height loss. An unexplained reduction in prostate size or in the level of prostate-specific antigen (PSA) is uncommon but may occur as a result of long-term, severe acquired androgen deficiency.

Other symptoms and signs are much less specific for androgen deficiency but may occur, commonly in conjunction with clinical manifestations described previously that are more suggestive of androgen deficiency. Men with low testosterone



• **Fig. 19.15** A 54-year-old man with adult androgen deficiency caused by hypopituitarism who presented with sexual dysfunction (reduced libido and erectile dysfunction); loss of chest, axillary, and pubic hair (A, B, and C); and gynecomastia (A). His penis and testes were normal in size (B). He had normal facial hair (C), but his shaving frequency was less.

concentrations often complain of diminished energy and vitality, poor motivation and social aggressiveness, depressed mood and irritability, or poor concentration and memory. Men with severe androgen deficiency may have a mild hypoproliferative normocytic, normochromic anemia within the female range in the absence of androgen stimulation of erythropoiesis. With long-standing deficiency, reduced muscle bulk and strength associated with weakness and reduced physical and work performance may occur. The latter symptoms may occur in conjunction with an increase in body fat, but androgen deficiency is not a cause of clinically obvious obesity per se. Skin changes and reduced sebum production with severe, long-standing androgen deficiency may be associated with fine facial wrinkling that is particularly noticeable on the lateral corners of the orbits (lateral canthus) and mouth. Testis size may be small, especially with severe impairment of spermatogenesis, but in most men with acquired adult androgen deficiency, testis size is normal to slightly reduced.

Because clinical manifestations are nonspecific, older men may have several medical or comorbid conditions and medications that contribute to symptoms and signs that are consistent with androgen deficiency, presenting a particular diagnostic challenge (Fig. 19.16). Symptoms and signs of comorbid illnesses may mask, mimic, or contribute to clinical manifestations of androgen deficiency in older men. Elderly men may present with muscle loss and mobility impairment, fragility fracture or osteoporosis, and reduced vitality and depressed mood. On close examination, however, older men with severe, long-standing androgen deficiency usually manifest objective evidence of androgen deficiency.

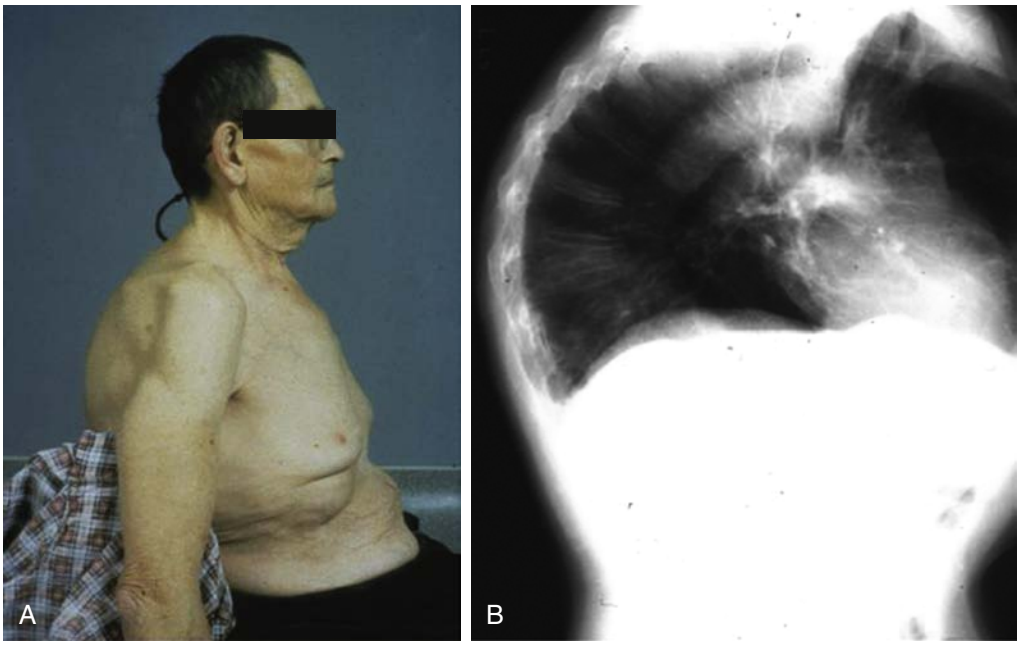
Isolated Impairment of Sperm Production or Function

Most men with male infertility have hypogonadism manifested by an isolated impairment of sperm production with normal androgen production. These men present as adults with infertility and demonstrate oligozoospermia or azoospermia, sperm with abnormal morphologic appearance (*teratospermia*) or reduced or absent motility (*asthenospermia*), or a combination of abnormalities on seminal fluid analysis. They do not have manifestations of androgen deficiency, and serum testosterone concentrations are normal. Testes may be small (if spermatogenesis is severely impaired) or normal size. Testes may not be palpable if cryptorchidism or anorchia is present.

History and Physical Examination

Clinical evaluation of male hypogonadism involves a careful history and physical examination directed at determining whether there are symptoms and signs of androgen deficiency or isolated impairment of sperm production and at identifying potential common causes of hypogonadism.[9] Because adults with androgen deficiency commonly present with sexual dysfunction, gynecomastia, and infertility, the differential diagnosis of these conditions and causes other than hypogonadism of these presenting complaints should be considered. Laboratory evaluation of serum testosterone, gonadotropins, and seminal fluid (in men who are concerned with infertility) are performed to confirm the diagnosis of hypogonadism and to determine whether there is predominantly primary or secondary hypogonadism.

The *history* should include inquiry regarding symptoms of androgen deficiency. Questions about the onset of these symptoms may be useful in determining whether the cause of androgen



• **Fig. 19.16** A 70-year-old man with severe androgen deficiency caused by Kallmann syndrome (hypogonadotropic hypogonadism and anosmia) who presented to a geriatric evaluation and management unit with functional and mobility disability caused by upper (A) and lower extremity muscle wasting and severe back pain from multiple vertebral compression fractures (B) due to osteoporosis. He was noted to have gynecomastia and absence of chest, axillary, and pubic hair.

deficiency occurred before birth, between birth and puberty, or after puberty. Note that symptoms or signs that suggest reduced androgen effect compared with baseline generally indicate postpubertal-onset of hypogonadism. These questions may be grouped in the following areas:

1. *Development:* Developmental markers of congenital androgen deficiency include a history of genital abnormalities (hypospadias, microphallus, cryptorchidism); developmental markers of congenital or androgen deficiency of prepubertal onset include a history of delayed sexual development or growth and testosterone therapy to induce puberty; family history of delayed puberty or reproductive disorders; psychological impact of delayed puberty or growth; difficulty in school or learning disability (often associated with Klinefelter syndrome); inability or reduced ability to smell (suggesting CHH due to Kallmann syndrome)
2. *Sexual function:* Poor erections; reduced spontaneous, nighttime, or morning erections; inability to perform sexually; decreased sexual activity; inability to father children despite unprotected sexual intercourse (>1 year); small (suggesting congenital or prepubertal onset of hypogonadism) or shrinking testes
3. *Brain function:* Poor general well-being; reduced sexual desire, interest, and motivation (libido); poor energy and vitality and excessive fatigue; poor motivation and initiative, passivity, low self-confidence, and low self-esteem; depressed mood and irritability; difficulty sleeping; hot flashes and sweats; poor concentration and memory
4. *Body function:* Decreased muscle bulk and strength; reduced physical activity or performance; breast enlargement or tenderness, especially if recent in onset; height loss, history of low-trauma or vertebral compression fractures, osteopenia, or osteoporosis; body hair loss (chest, axillary, or pubic); reduced beard growth and shaving frequency.

The initial history may also include inquiry concerning the potential cause of hypogonadism. With *primary hypogonadism*, there may be a history of mumps involving the testes; testicular trauma, irradiation, or surgery; complications of pelvic or scrotal surgery; medication use (spironolactone, ketoconazole, cytotoxic agents); or chronic liver or kidney failure. With *secondary hypogonadism*, headaches, visual complaints or reduced peripheral vision, history of pituitary disease, chronic lung disease or congestive heart failure (CHF), wasting conditions (e.g., acquired immunodeficiency syndrome [AIDS], cancer), nutritional deficiencies, recent acute illness, morbid obesity, or use of certain medications (e.g., opioids, glucocorticoids, anabolic steroids, megestrol acetate, medroxyprogesterone acetate, nutritional supplements) may be noted.

The patient should be questioned regarding conditions that are relative or absolute contraindications to testosterone treatment, including a history of severe BPH and LUTS as assessed by the American Urological Association (AUA) symptom score or International Prostate Symptom Score (IPSS), history of prostate or breast cancer, history or symptoms of untreated obstructive sleep apnea syndrome (daytime sleepiness, snoring with sleep disruption, witnessed apnea episodes), history of CHF, and polycythemia.

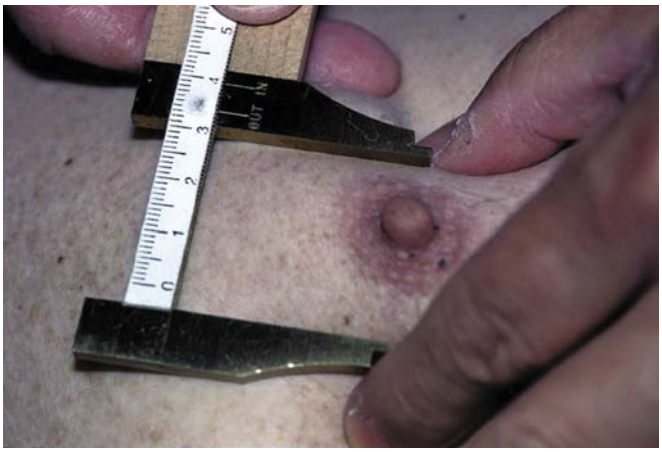
In patients with suspected androgen deficiency of prepubertal onset, *physical examination* should include measurements of total arm span, height, and the distances from the crown of the head to the symphysis pubis and from the symphysis pubis to the floor to determine whether the patient has excessively long arms and legs (see Fig. 19.14). Eunuchoidal body proportions are characterized by an arm span that is at least 5 cm greater than height and a symphysis-to-floor distance that is at least 5 cm greater than crown-to-symphysis distance; such proportions are indicative of androgen deficiency of prepubertal onset. Men with Klinefelter syndrome may have disproportionately long legs relative to arms

and a greater ratio of lower- to upper body segment measurements but a relatively normal ratio of arm span to height. Eunuchoidism is also characterized by infantile genitalia (micropenis or small penis, unrugated and nonpigmented scrotum); small testes or, rarely, absence of the testes; cryptorchidism; sparse or absent facial, axillary, chest, extremity, and pubic hair; poorly developed upper body musculature; fat predominance in the face, chest, and hips; and gynecomastia. Patients with Kallmann syndrome may have anosmia or hyposmia that may be tested with an odor identification and threshold test using readily identifiable, common household odorants (e.g., peppermint, cinnamon, cocoa, coffee, cigarette, orange, soap), or more formally, such as with the University of Pennsylvania scratch and sniff test.

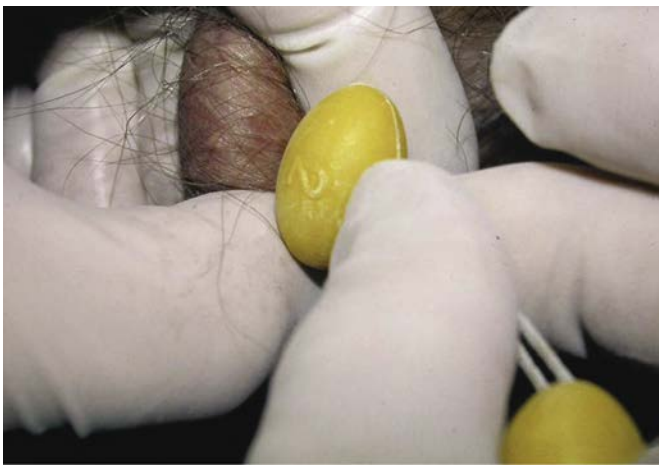
The physical findings of androgen deficiency acquired in adulthood are usually subtler than those of androgen deficiency of prepubertal onset (see Fig. 19.15). In patients with severe, long-standing adult androgen deficiency, there may be loss of androgen-dependent facial, axillary, chest, extremity, and pubic hair; however, there are ethnic variations in body hair in androgen-dependent areas (e.g., less in Asians and Hispanics). The skin may be dry, and there may be fine wrinkling lateral to orbits or the mouth in patients with severe, long-standing androgen deficiency. Patients should be carefully examined for the presence of palpable breast tissue or gynecomastia; presence, size, and consistency of the testes; and palpable abnormalities in the scrotum, such as varicocele, epididymal enlargement, or tenderness or absence of the vas deferens. In older men, particularly those at high risk for prostate cancer (e.g., African-American ethnicity, family history of prostate cancer), a digital rectal examination (DRE) to screen for prostate cancer should be offered. If performed, the focus of the examination is to detect palpable abnormalities, such as a prostate nodule or induration and to assess the size of the prostate. Careful examination for kyphosis and measurement of height are useful for detecting significant height loss (>5 cm) associated with osteoporotic vertebral compression fractures that may be asymptomatic.

Proper technique is needed to examine the male breast. The thumb and index finger (or the fingers of both hands) are used to grasp and gently grasp the periareolar area of the breast and to palpate glandular breast tissue, which is rubbery in consistency and firmer than the surrounding adipose tissue (Fig. 19.17). Breast tissue is generally discoid and has a firm edge that can be palpated and "flipped up" to distinguish it from adipose tissue. With this technique, gynecomastia can usually be distinguished from excessive breast adipose tissue, called *pseudogynecomastia*, which is often associated with generalized obesity. Gynecomastia is usually bilateral and relatively symmetric, but occasionally it is asymmetric and more prominent on one side. If present, asymmetric gynecomastia may suggest breast carcinoma, which is usually rock hard and irregular and may be associated with skin dimpling (*peau d'orange*), nipple retraction or discharge, and axillary lymphadenopathy. The diameter of palpable breast tissue is used as an objective measure of gynecomastia. Gynecomastia of recent onset is usually tender on palpation, and men usually complain of nipple irritation associated with rubbing against clothing.

Examination of the testes and scrotum may be performed with the patient either lying on his back or standing, but the latter position is preferred because it relaxes the scrotum, making some abnormalities (e.g., varicocele) easier to detect. In patients with retractile testes positioned high in the scrotum, it may be possible to palpate the testes only after placing the scrotum in warm water, after a warm bath, or by having the patient assume a squatting position. The testes may be very difficult to examine and palpate in morbidly obese men who have excessive folds of fat overlying the scrotum, in the presence of a large hydrocele, if the testis is tender (e.g., with epididymo-orchitis or testicular torsion), or occasionally in some men who are sensitive to palpation for unclear reasons. In these instances, testicular ultrasound may be required to confirm the presence of the testis, estimate its size, and detect abnormalities.



• **Fig. 19.17** The proper method of examining the male breast is to use the thumb and index finger to grasp the periareolar area of the breast and to gently pinch the thumb and index finger together on either side of the breast toward the nipple. Glandular breast tissue feels like a rubbery disc of tissue that extends concentrically from under the nipple and subareolar area and is firmer than the surrounding adipose tissue. The size of gynecomastia is estimated by measurement of the diameter of palpable breast tissue. (From Matsumoto AM. The testis. In: Felig P, Frohman LA, eds. *Endocrinology and Metabolism.* 4th ed. New York, NY: McGraw-Hill; 2001:635–705.)



• **Fig. 19.18** The assessment of testis size using a Prader orchidometer in a man with classic 47,XXY Klinefelter syndrome is characteristically very small (e.g., 2 mL). Care must be taken not to include the head of the epididymis when estimating testis size.

Although ultrasonographic size estimates are more accurate, testis size can be estimated by measuring the length and width with a ruler or calipers or by comparing testis volume with that of ellipsoid models of known volume (Prader orchidometer) (Fig. 19.18). Normal testis size varies with age and ethnicity. Normal prepubertal testis size is approximately 1.6 to 2.9 cm in length and 1.0 to 1.8 cm in width, or 1 to 4 mL in volume. Testis size greater

than 3 mL (by orchidometer) suggests the onset of puberty.[149] In adults, normal testes usually measure 3.5 to 5.5 cm in length and 2.0 to 3.0 cm in width or 15 to 30 mL in volume.[8,9] In addition to size, testes should be palpated for consistency or firmness and for presence of a mass representing a benign or malignant testicular tumor. The testicular examination in men with classical Klinefelter syndrome is notable for very small (usually <3 mL), firm testes.

Differential Diagnosis

Because sexual dysfunction, gynecomastia, and infertility are often presenting complaints in adults with androgen deficiency, it is important to consider the differential diagnosis of these conditions and to be familiar with other common causes of these manifestations when evaluating men who present with these complaints.

Sexual Dysfunction

Normal sexual function requires successive, coordinated physiologic events—libido, erection, ejaculation, orgasm, and detumescence—that occur in a defined sequence and require normal psychological, CNS, peripheral nerve, vascular, and genital function.[150]

Sexual dysfunction may involve specific disorders of libido or sexual desire, erectile dysfunction, ejaculatory disorders, orgasmic dysfunction, or failure of detumescence. These components may occur in isolation, but specific disorders of sexual function commonly occur together because these processes are interrelated and because a specific cause (e.g., testosterone and/or estrogen deficiency due to hypogonadism) can affect more than one of the physiologic processes that mediate normal sexual function. Male sexual dysfunction is detailed in Chapter 20. Men with androgen deficiency often present with sexual dysfunction, and it is important to consider the differential diagnosis of this complaint in the evaluation.

Androgen deficiency often results in reduced libido or sexual desire (hypoactive sexual desire disorder); loss or reduction of spontaneous evening and morning or sexually stimulated erections (erectile dysfunction); and, if severe, reduced or absent ejaculation. In many men with androgen deficiency, erectile response to intense erotic stimuli (and, occasionally, spontaneous erections) may be preserved, suggesting that the androgen requirement for sexual function is variable.[151] However, persistent erectile dysfunction may cause performance anxiety, and, together with hypoactive sexual desire and depressed mood associated with androgen deficiency, this may contribute to the eventual loss of erotically stimulated erections and, secondarily, to orgasmic dysfunction. Androgen deficiency may also affect nitric oxide (NO) production and maximal smooth muscle relaxation and vasodilatation within the penis, reducing the ability to produce an erection that is sufficient to satisfactorily complete sexual intercourse and further contributing to the severity of erectile dysfunction.[152,153]

Clinically, men with androgen deficiency most commonly present with hypoactive sexual desire disorder and erectile dysfunction. Men with severe androgen deficiency may present with reduced ejaculation, but these individuals usually also complain of hypoactive sexual desire disorder and erectile dysfunction.

Hypoactive Sexual Desire Disorder and Erectile Dysfunction

Libido, the desire or drive for sexual activity, is generated by external visual, auditory, and tactile stimuli, as well as internal psychic stimuli acting on cortical and subcortical brain regions such as the limbic system (amygdala, hippocampus, anterior thalamic nuclei, and prefrontal cortex) and the temporal lobe. Stimuli from these areas are relayed to the medial preoptic area, which serves to integrate central inputs and sends impulses to the paraventricular nuclei; these, in turn, send projections to the thoracolumbar and sacral spinal cord centers that regulate penile erection. This neural pathway explains why brain disorders that cause hypoactive sexual desire disorder are usually accompanied by varying degrees of erectile dysfunction (see later discussion).[150] In particular, there is a loss of the spontaneous evening and morning erections that are associated with brain activation of sexual neural pathways during rapid eye movement sleep and dreaming. Clinically, libido may be influenced by previous or recent sexual activity and by experiences, psychosocial background, overall state of general health, androgen sufficiency, and brain function.

The neurotransmitter systems that regulate the physiology of normal libido are not precisely known. However, there is evidence that central dopamine neurotransmission may be important in mediating CNS regulation of sexual desire and erections. In humans, treatment with dopamine receptor agonists (e.g., bromocriptine, pergolide) may stimulate spontaneous erections (and even hypersexual behavior), and in 20% to 30% of men with Parkinson disease, levodopa therapy is associated with stimulation of libido and spontaneous erections. The use of pharmacologic agents with dopamine receptor antagonist activity is frequently associated with reduced libido and erectile dysfunction. However, these agents also affect many other neurotransmitter systems. Dopamine antagonism (e.g., by neuroleptic or antipsychotic agents) results in elevated prolactin concentrations that suppress endogenous gonadotropin and testosterone secretion and may contribute to reduced libido and erectile dysfunction.

Hypoactive sexual desire disorder is defined as persistent or recurrent deficiency or absence of desire for sexual activity resulting in marked personal distress, interpersonal difficulty, or both[150,154,155] and is estimated to affect more than 15% of men. The causes of hypoactive sexual desire disorder are primarily disorders that affect normal brain function and are usually associated with erectile dysfunction, particularly with the loss of spontaneous evening or morning erections (Table 19.2). *Erectile dysfunction* is defined as the inability to achieve or maintain penile erection that is adequate for completion of satisfactory sexual intercourse or activity.[156] Erectile dysfunction is a common condition that increases with aging. It is estimated to affect fewer than 10% of men younger than 40 years of age but approximately 50% of men between 40 and 70 years of age, with 35% of men in the latter age group having moderate or complete erectile dysfunction.

Hypoactive Sexual Desire Disorder and Erectile Dysfunction Due to Brain Disorders. *Psychogenic disorders* commonly cause hypoactive sexual desire and erectile dysfunction. These disorders include stress or preoccupation associated with life circumstances or situations, illness, marital discord, or underlying maternal transference or gender identity issues; performance anxiety associated with fear of failure or preoccupation with the adequacy of erections during sexual intercourse; major depression or dysthymia (moderate or complete erectile dysfunction occurs in 60% to 90% of men with moderate to severe depression); and major psychiatric illness such as psychotic or personality disorders.[150,154,155]

Chronic systemic illness (chronic heart disease, respiratory illness, kidney or liver failure, or cancer) and poor general health are usually associated with reduced libido and spontaneous erections.[150,154,155] Several *CNS-active medications* may cause hypoactive sexual desire disorder and erectile dysfunction, including alcohol, centrally acting antihypertensive medications, narcotics,

TABLE 19.2 Causes of Hypoactive Sexual Desire Disorder and Erectile Dysfunction

Cause	Examples
Brain Disorders	
Psychogenic disorders	Stress or preoccupation, performance anxiety, depression, major psychiatric illness
Chronic systemic illness	Heart, respiratory, kidney, or liver failure; cancer
CNS-active drugs	Alcohol; antihypertensive, narcotic, sedative-hypnotic, anticonvulsant, antidepressant, antipsychotic medications
Structural brain disease	Temporal lobe or limbic system disorders, Parkinson or other neurodegenerative brain disease, vascular brain disorders
Androgen deficiency	Primary and secondary hypogonadism
Other endocrine disorders	Hyperprolactinemia, Cushing syndrome, hyperthyroidism, hypothyroidism
Spinal Cord and Peripheral Disorders	
Spinal cord disorders	Trauma, vascular compromise, spinal stenosis, epidural abscess, tumor, transverse myelitis, multiple sclerosis, other spinal cord lesions
Peripheral nerve disorders	Diabetes mellitus; pelvic, prostate, or retroperitoneal surgery or damage; other causes of peripheral neuropathy
PNS-active drugs	Anticholinergic, antihistamine, antidepressant, sympathomimetic, α-adrenergic agonist, β-adrenergic antagonist medications
Peripheral vascular disease	Aortoiliac atherosclerosis, diabetes mellitus, trauma, surgery, vasculitis, venous incompetence (venous leakage), smoking
Antihypertensive drugs	Diuretics, β-adrenergic antagonists, calcium channel antagonists
Penile abnormalities	Peyronie disease, chordee, trauma, priapism, phimosis

CNS, Central nervous system; *PNS*, peripheral nervous system.

sedative-hypnotic drugs, anticonvulsants, antidepressants, and antipsychotic medications. In addition to their direct effects on brain neurotransmitter function, both chronic illness and CNS-active medications may also be associated with androgen deficiency. *Structural brain disease*, such as infiltrative or destructive lesions of the temporal lobe or limbic system, Parkinson or other neurodegenerative brain disease, or vascular brain disorders such as stroke or vasculitis, may reduce libido and spontaneous erections.

Androgen deficiency is commonly associated with reduction or loss of libido and spontaneous erections.[157,158] Sexual dysfunction is usually a prominent presenting complaint in young men who are severely androgen deficient and in older men who are treated with medical therapies (e.g., GnRH agonist treatment) or surgical castration for advanced prostate cancer. In contrast, older men with less severe androgen deficiency may have sexual dysfunction that is also related to underlying depression, chronic systemic illness, or use of certain medications.[159] Comorbid conditions contribute to the nonspecificity of presenting complaints of androgen deficiency (e.g., sexual dysfunction) as men age. Testosterone treatment of severe androgen deficiency in young men usually improves sexual desire, interest, and thoughts; attentiveness to erotic stimuli; and the frequency, duration, and rigidity of spontaneous evening and morning erections.[158,160,161] In older hypogonadal men with reduced libido, 1 year of a physiologic dose of transdermal testosterone improved sexual activity and desire compared with placebo.[162]

Other endocrine disorders can cause hypoactive sexual desire disorder and erectile dysfunction; examples include hyperprolactinemia, Cushing syndrome (glucocorticoid excess), hyperthyroidism, and hypothyroidism. In addition to their direct effects on brain function, hyperprolactinemia and glucocorticoid excess also suppress GnRH and gonadotropin secretion and induce androgen deficiency that contributes to sexual dysfunction. Anecdotally, some men with androgen deficiency due to severe hyperprolactinemia who are treated with testosterone alone do not fully recover sexual function and may require additional therapy with dopamine receptor agonists, but this has not been demonstrated conclusively. Dopamine receptor agonists lower elevated prolactin concentrations and may also have direct affects in the brain to activate neuronal systems involved in stimulating libido and erections.

Erectile Dysfunction Due to Spinal Cord or Peripheral Disorders. External and internal erotic stimuli from the brain are relayed via descending neural pathways in the lateral spinal columns to stimulate the parasympathetic sacral (S2-S4) spinal erection center, resulting in *psychogenic erections*. Efferent parasympathetic nervous system stimulation from the sacral center travels via the nervi erigentes (pelvic splanchnic nerve) and the pelvic plexus and enters the penis via the cavernosal nerve. This stimulation causes relaxation of the smooth muscles that form sponge-like interconnected trabecular spaces within the corpora cavernosa of the penis and vasodilation of the cavernosal arterioles and vascular sinusoids. As a result, blood flow and pressure into the trabecular spaces within the corpora increase several fold and cause engorgement of the penis (tumescence). Expansion of the trabecular spaces against the thick fibrous sheath (tunica albuginea) surrounding the corpora compresses subtunical venules and impedes venous outflow, resulting in sustained penile tumescence (i.e., an erection).[150,156]

Afferent somatic (via the pudendal nerve) and parasympathetic impulses in response to sensory stimulation of the penis with sexual intercourse or masturbation also act to stimulate erections via a reflex arc through the sacral spinal erection center, resulting in *reflexogenic erections*. Pudendal nerve stimulation also triggers the reflex contraction of the ischiocavernosus and bulbocavernosus muscles, resulting in vascular compression at the base of the penis, further increasing cavernosal blood pressure and maximal penile rigidity, leading to the plateau phase of erection.

The primary neurotransmitter that mediates penile smooth muscle relaxation and erection is NO. In response to parasympathetic cholinergic (acetylcholine-mediated) stimulation, NO is synthesized from its precursor, L-arginine, by the enzyme nitric oxide synthase and is released by corporal sinusoidal endothelial cells and postganglionic noncholinergic, nonadrenergic nerve terminals. NO then enters adjacent smooth muscle cells, where it activates guanylyl cyclase and increases intracellular cyclic guanosine monophosphate (cGMP). cGMP activates cGMP-dependent protein kinase, which phosphorylates many proteins, including myosin light chains and ion channels that ultimately decrease intracellular calcium concentrations, causing smooth

muscle relaxation, increase in penile blood flow, and erection. cGMP is primarily hydrolyzed and inactivated by the enzyme phosphodiesterase type 5 (PDE5). In addition to cGMP, other neurotransmitters induce cavernosal smooth muscle relaxation, including prostaglandin E_1 (PGE_1) that activates adenylyl cyclase and increases cAMP and cAMP-dependent protein kinase.

Knowledge of the neurotransmitter systems that control erections has been used to design pharmacologic treatments for erectile dysfunction[156,163,164] (detailed in Chapter 20). The most commonly used treatments are oral PDE5 inhibitors, such as sildenafil, vardenafil, and tadalafil, which act to inhibit the breakdown of cGMP, resulting in more sustained smooth muscle relaxation and improved penile erection after erotic stimulation. Injection of intracavernosal alprostadil (PGE_1) or insertion of intraurethral alprostadil pellets acts to increase cavernosal cAMP concentrations and induce smooth muscle relaxation and penile erection even in the absence of sexual stimulation. Intracavernosal injections of papaverine, a nonspecific PDE inhibitor (which inhibits the breakdown of both cGMP and cAMP), combined with phentolamine, an α_1- and α_2-adrenergic receptor antagonist vasodilator (bi-mix), or the two combined together with alprostadil (tri-mix), are also used to induce smooth muscle relaxation and erection.

Nonpharmacologic therapies include vacuum pumps that draw blood into the penis and induce tumescence. Vacuum pumps are often used with veno-occlusive elastic rings to maintain the erection. Some men are best treated with surgically implanted penile prostheses.

Studies in experimental animals and in vitro have found that androgen deficiency impairs penile nerve, trabecular smooth muscle, vascular endothelial, and tunica albuginea structure and function; reduces both endothelial and neuronal NO synthase synthesis and activity; and causes accumulation of adipocytes in the subtunical region of the corpora cavernosa.[153] These changes are reversed with androgen administration, suggesting a direct penile effect of androgens in addition to their central role in maintaining penile erections. In humans, ARs are expressed in the corpora cavernosa tissue. However, there is no conclusive evidence to support the notion that androgen treatment has a direct effect in the penis to enhance the response to PDE5 inhibitor therapy in androgen-deficient men with erectile dysfunction. In practice, symptomatic men with androgen deficiency and sexual dysfunction are usually treated with testosterone replacement, which can variably improve erectile dysfunction, particularly in younger hypogonadal men with severe androgen deficiency and no comorbid illness. In the placebo-controlled Testosterone Trials, 1 year of physiologic transdermal testosterone therapy improved erectile function in older men with hypogonadism, although the improvement was less than that observed with PDE5 inhibitors.[162] If erectile dysfunction does not improve with testosterone treatment alone, patients are given additional therapy for erectile dysfunction (e.g., addition of a PDE5 inhibitor). In some hypogonadal men, PDE5 inhibitor treatment alone may be sufficient to improve erectile dysfunction but is not adequate to treat reduced libido or other symptoms of androgen deficiency.[161]

In addition to the brain disorders that cause hypoactive sexual desire disorder and erectile dysfunction, spinal cord and peripheral disorders (e.g., peripheral nervous system disorders, peripheral vascular disease, medications that affect peripheral nerve and vascular function, penile abnormalities) may also cause erectile dysfunction that is usually not associated with hypoactive sexual desire (see Table 19.2). However, long-standing, severe erectile dysfunction may cause performance anxiety or depression, which may secondarily reduce libido. Furthermore, peripheral disorders that cause erectile dysfunction may also affect brain function and alter sexual interest and drive, contributing to erectile dysfunction. Tricyclic antidepressants may affect both peripheral nervous system and CNS function.

Spinal cord disorders, such as spinal cord injury due to trauma, vascular compromise, spinal stenosis, epidural abscess, tumor, transverse myelitis, multiple sclerosis, or other spinal cord lesions, usually cause erectile dysfunction. In general, the severity of erectile dysfunction associated with spinal cord injury and the response to treatment vary with the cord level involved, the severity of the lesion (i.e., complete vs. incomplete), and the time since the injury. *Peripheral nerve disorders*, particularly those that affect the autonomic nervous system, may disrupt the normal regulation of penile erectile tissue and cause erectile dysfunction. For example, erectile dysfunction may be caused by diabetes mellitus or other diseases that cause peripheral neuropathy (e.g., amyloidosis, vasculitis, heavy metal toxicity, renal failure, multiple system atrophy, acute intermittent porphyria) or by pelvic, prostate, or retroperitoneal surgery or damage (e.g., abdominoperineal resection of the rectum, pelvic lymph node dissection, prostatectomy, aortoiliac bypass, lumbar sympathectomy). Peripheral nervous system medications, including anticholinergic agents, antihistamines, antidepressants, sympathomimetic medications, α-adrenergic agonists, and β-adrenergic antagonists, often impair erectile function by affecting peripheral nervous system regulation of erectile tissue of the penis, and many also cause erectile dysfunction by altering neurotransmitter function in the nervous system and penis.

The blood supply of the penis is derived from the internal iliac (hypogastric) artery, a branch of the common iliac artery that bifurcates from the aorta.[150] The internal iliac artery gives rise to the internal pudendal artery, which branches into the dorsal penile, bulbourethral, and cavernosal arteries. The cavernosal arteries run through the middle of the corpora cavernosa and give off corkscrew-shape branches, the helicine arteries, that open directly into the lacunar spaces. Smooth muscle relaxation of lacunar spaces increases blood flow into the corpora cavernosa, resulting in penile tumescence. Blood from the lacunar spaces or cavernosal sinusoids collects in the subtunical plexus and is delivered via emissary veins to the deep dorsal vein, which ultimately drains into the internal and common internal iliac veins and then into the inferior vena cava. With filling of the lunar spaces of the corpora cavernosa and penile tumescence against the fibrous tunica albuginea, venous outflow from the subtunical venous plexus is impeded, and sustained tumescence or erection ensues. Disorders of arterial inflow or venous output may cause erectile dysfunction.

Peripheral vascular disease due to aortoiliac atherosclerosis is probably the most common cause of erectile dysfunction in aging men.[150,156] These men usually have absent or severely diminished femoral artery pulses, and some present clinically with Leriche syndrome (absent femoral pulses, buttock or leg claudication, and erectile dysfunction). Other men with iliac atherosclerosis may be able to achieve an erection, but with penetration and use of the hip muscles for thrusting during sexual intercourse, blood is diverted from the penis to the hips, resulting in premature detumescence and loss of erection; this is known as the *pelvic steal syndrome*. Atherosclerotic large- and small-vessel disease may contribute to erectile dysfunction in men with diabetes mellitus, hypertension, CKD, smoking, and other atherosclerotic risk factors. Erectile dysfunction occurs in about 50% of men with diabetes mellitus. Smoking, specifically nicotine, also causes direct vasoconstriction of the corpora cavernosum and erectile dysfunction. Other

conditions that compromise aortoiliac circulation, such as pelvic trauma, irradiation, and vasculitis, are less common causes of erectile dysfunction. Chronic pressure on the pudendal artery from bicycle riding, especially with some bicycle seats, may cause penile ischemia and erectile dysfunction; in addition, pressure on the pudendal nerve may cause penile numbness and contribute to sexual dysfunction. *Penile venous incompetence* (venous leakage) may cause premature loss of erections and inability to maintain erections sufficient to complete intercourse.

Many *antihypertensive medications*, including diuretics, α- and β-adrenergic antagonists, angiotensin-converting enzyme inhibitors, and calcium channel antagonists, have been implicated as causes of erectile dysfunction. *Penile abnormalities*, such as Peyronie disease or chordee (fibrosis or scarring of the tunica albuginea resulting in bending of the penis), micropenis or microphallus, penile trauma, phimosis (inability to retract the foreskin over the penis), and priapism (painful extended erections) may also cause erectile dysfunction.

Evaluation of Erectile Dysfunction. The cause of erectile dysfunction is usually strongly suspected based on a careful medical, psychiatric, and medication history and physical examination.[150,156] Erectile dysfunction of psychogenic origin usually occurs abruptly, is transient, is intermittent or associated with a stressful situation, occurs with only some partners but not with others, or does not occur with masturbation. Spontaneous evening and morning erections are usually maintained in psychogenic erectile dysfunction but lost with organic causes. Spontaneous erections may be detected by formal measurements of nocturnal penile tumescence in a sleep laboratory or by breakage of wires of different tensile strength in a snap gauge (RigiScan), but these assessments are not routinely performed in practice and usually are not necessary.

Patients with nonpsychogenic brain disorders, spinal cord or peripheral nervous system disorders, vascular disorders, or penile abnormalities that cause organic erectile dysfunction usually exhibit clinical manifestations of the underlying disorder, and offending drugs that impair erectile function are revealed with a careful review of medications. Androgen deficiency is a cause of reduced libido and erectile dysfunction and occurs in 15% to 20% of men who complain of sexual dysfunction in a general medical clinic.[165] Therefore, evaluation of men who present with sexual dysfunction should include inquiry regarding other symptoms of androgen deficiency, examination for signs such as small testis size and gynecomastia, and confirmation of androgen deficiency by measurement of serum testosterone concentrations (see later discussion).

Peripheral pulses, particularly the presence of femoral pulses, should be tested to assess for peripheral vascular disease. Diagnosis of penile vascular insufficiency may be suspected by Doppler ultrasound measurement of the ratio of penile to brachial systolic blood pressure (penile/brachial index). A penile/brachial index greater than 0.75 is normal, whereas an index of less than 0.60 is suggestive of vascular erectile dysfunction. If there is a clinical suspicion of spinal cord disease, perineal and penile sensation should be assessed. A cremasteric reflex (stroking of the inner thigh associated with contraction of the ipsilateral cremasteric muscle and pulling up of the scrotum and testis) and a bulbocavernosus reflex (squeezing of the glans penis associated with contraction of the anal sphincter) should be elicited to assess spinal cord levels L1-L2 and S2-S4, respectively. Finally, the penis should be examined for abnormalities, such as penile plaques, angulation, or tight and unretractable foreskin.

Ejaculatory Disorders and Orgasmic Dysfunction

After the plateau phase of erection is achieved, sympathetic nervous system stimulation from the thoracolumbar (T10-L2) spinal erection center travels via the hypogastric nerve and pelvic plexus, enters the penis via the cavernosal nerve, and causes α-adrenergic receptor–mediated contraction of the cauda epididymis, vas deferens, accessory sex glands (the bulbourethral or Cowper glands and the urethral glands or glands of Littre), prostate, seminal vesicles, and ejaculatory ducts that moves sperm and semen into the posterior urethra (*emission*). It also stimulates closure of the internal urethral sphincter to prevent retrograde ejaculation of sperm into the bladder.[150] After emission, continued sensory stimulation of the penis with sexual intercourse or masturbation stimulates reflex rhythmic contractions of the ischiocavernosus and bulbocavernosus muscles, resulting in expulsion of semen from the urethra (*ejaculation*).[150]

Like erection, ejaculation is under considerable control by higher brain centers, with both voluntary and involuntary regulation.[150] *Premature ejaculation* is ejaculation that occurs before or shortly after vaginal penetration during sexual intercourse and is followed by a rapid loss of erection.[166] The cause of premature ejaculation is usually a psychological disturbance such as performance anxiety; it is rarely the result of an organic cause. There is evidence that serotoninergic neurotransmission inhibits sexual function and ejaculation. Selective serotonin reuptake inhibitors retard ejaculation, an effect that is used therapeutically to treat premature ejaculation.[166,167] Other men with psychological disorders such as excessive anxiety may have *retarded ejaculation* (inability to ejaculate), either in isolation or in combination with impaired libido and erections. The ejaculate is composed of spermatozoa (10%) and seminal fluid (90%), the latter derived mostly from the seminal vesicles (65%) and the prostate gland (30%). Because secretions from these accessory sex glands are androgen dependent, severe androgen deficiency may result in *absent* or *reduced ejaculation*. Absent or reduced ejaculation may also be caused by urethral abnormalities. Autonomic neuropathy, such as that caused by diabetes mellitus, sympatholytic drugs, thoracolumbar sympathectomy, extensive retroperitoneal or pelvic surgery, or bladder neck surgery, may be associated with absent or reduced ejaculation by causing *retrograde ejaculation* into the bladder.

Orgasm, the pleasurable sensation associated with ejaculation, usually occurs simultaneously with ejaculation and is mediated by CNS activation via ascending pathways from the spinal cord erection centers to regions of the temporal lobe and limbic system.[150] Because of impaired libido and erectile dysfunction, men with androgen deficiency may also fail to achieve an orgasm. Isolated absence of orgasm in the presence of normal libido, erections, and ejaculation is relatively rare and is almost always caused by a psychological disorder.

Disorders of Detumescence. After ejaculation, the thoracolumbar sympathetic outflow acts via α-adrenergic receptor stimulation to cause contraction of trabecular smooth muscle, which results in collapse of lacunar spaces, vasoconstriction of arterioles of the corpora cavernosa (reducing blood flow into the penis), and decompression of subtunical venules, leading to an increase in venous outflow and a flaccid penis (*detumescence*).[150] Premature detumescence may contribute to erectile dysfunction, such as that caused by penile venous incompetence. Intracorporal injection of an α-adrenergic receptor antagonist, phentolamine, together with papaverine and PGE_1, causes sustained lacunar smooth muscle relaxation, arteriole vasodilatation, and penile tumescence and is used to treat erectile dysfunction caused by premature detumescence.

Priapism is failure of detumescence with persistence of erection lasting for longer than 4 hours that is unrelated to sexual stimulation and is usually painful.[150,168] An erection that persists for more than 4 hours is an emergency and may be complicated by ischemia, thrombosis, and vascular damage that contribute further to erectile dysfunction; if ischemia is severe, it can cause gangrene and eventual loss of the penis. Priapism may be idiopathic, or it may be caused by medications (e.g., intracavernosal injection therapy for erectile dysfunction, phenothiazines, trazodone, cocaine), by hematologic disorders such as sickle cell disease or chronic myelogenous leukemia, by neurologic disorders such as spinal cord injury, or by infiltrative diseases such as amyloidosis. The initial treatment is administration of the α-adrenergic receptor agonist pseudoephedrine; if this is unsuccessful, aspiration of blood from the corpora cavernosa is performed with local anesthesia.

Gynecomastia

Gynecomastia is benign enlargement of the male breast caused by proliferation of glandular breast tissue.[169-171] On inspection, it is difficult to distinguish gynecomastia from increased adipose tissue deposition within the breast in the absence of glandular proliferation (pseudogynecomastia), which is commonly present in obese men and boys. Detection of glandular breast tissue requires a careful and properly performed physical examination (see earlier discussion), feeling for a firm, rubbery, finely lobular, freely mobile disc of tissue that extends concentrically from under the nipple and areola. Initially, gynecomastia of relatively recent and rapid onset may be painful and associated with tenderness. With time, glandular tissue is replaced by fibrous tissue and tenderness resolves, although palpable tissue remains. In contrast, pseudogynecomastia is soft, nondiscrete, and irregularly lobular, similar to subcutaneous (SC) fat in the abdomen.

Gynecomastia is usually present bilaterally but may be asymmetric in size and variably symptomatic. If palpable breast tissue is present unilaterally, the major concern is male breast cancer. Breast cancer is usually rock hard and indurated, eccentrically located from the nipple and areola, and fixed to underlying tissue; it may be associated with skin dimpling with retraction of hair follicles (*peau d'orange*), nipple retraction, nipple bleeding or discharge, or axillary lymphadenopathy.[172] Other chest wall tumors may cause unilateral breast enlargement, including lipomas, sebaceous or dermoid cysts, hematomas, fat necrosis, lymphangiomas, neurofibromas, and lymphomas.

The primary hormones that regulate breast tissue development are estrogens, which stimulate the growth and differentiation of breast epithelium to form ducts (ductal hyperplasia), and progesterone, which controls acinar development and the formation of glandular buds (glandular formation).[169,171] GH, IGF1, insulin, thyroid hormone, and cortisol play permissive roles in breast development. Androgens inhibit the growth and differentiation of breast tissue. Prolactin stimulates differentiated breast acinar cells to produce milk, but high progesterone (and to a lesser extent high estradiol) concentrations inhibit lactogenesis. Therefore, milk production requires a reduction in high progesterone (and to a less extent reduction in high estradiol) concentrations in the presence of high prolactin concentrations, as occurs in the first few days after parturition. Milk production (galactorrhea) is rarely seen in men with hyperprolactinemia and gynecomastia, because progesterone concentrations are not usually high enough for breast acinar development to occur, and progesterone and estradiol concentrations do not decline from baseline in men in the presence of high prolactin concentrations to stimulate lactogenesis.

Gynecomastia develops in clinical situations in which the concentrations or activity of estrogens is relatively high in comparison with androgens (i.e., high estrogen to androgen ratio). This hormonal milieu may result from high estrogen or low androgen concentrations or activity. Androgen deficiency, because it decreases the inhibitory influence of androgens on breast development, is a major cause of gynecomastia. However, the differential diagnosis of other causes of gynecomastia should be considered in patients who present with breast enlargement with or without tenderness.

Causes of Gynecomastia

Physiologic gynecomastia occurs normally in neonatal and pubertal boys. Transient gynecomastia (neonatal gynecomastia) occurs in 60% to 90% of neonatal boys as a result of exposure in utero to high concentrations of maternal estrogens; it resolves within several weeks after delivery[169-171] (Table 19.3). At the time of puberty, breast enlargement greater than 0.5 cm in diameter, which is often tender, initially occurs in 60% to 70% of boys by 14 years of age and then regresses within 1 to 2 years. This pubertal gynecomastia is thought to be caused by a transient rise in serum concentrations of estrogen relative to testosterone during puberty.

Pathologic gynecomastia may result from excessive estrogen concentrations or action or from androgen deficiency or androgen resistance/insensitivity in isolation. In some conditions, both estrogen excess and androgen deficiency contribute to proliferation of glandular breast tissue.[169-171] For example, in most conditions that cause gynecomastia as a result of excessive estrogen exposure, high circulating estrogen concentrations inhibit endogenous gonadotropin and testosterone secretion and cause secondary hypogonadism, which also contributes to the growth of breast tissue. In addition, some disorders of the testes that cause androgen deficiency (i.e., primary hypogonadism), such as Klinefelter syndrome, result in high circulating LH concentrations that stimulate aromatase activity in Leydig cells, leading to higher concentrations of estradiol relative to testosterone and contributing to the pathogenesis of gynecomastia.

Estrogen excess disorders that cause gynecomastia include exposure to exogenous estrogens (e.g., diethylstilbestrol treatment of prostate cancer, contact with an estrogen-containing cream or cosmetic, accidental occupational exposure to estrogens, ingestion of estrogen-containing nutritional supplements or excessive amounts of phytoestrogens) and exposure to ER agonists such as marijuana smoke (unidentified phenolic components but not active cannabinoids[173]) or digitoxin. Ingestion of normal dietary amounts of phytoestrogens (e.g., soybean isoflavones) does not usually cause gynecomastia.[174] Uncommonly, administration of testosterone or other aromatizable androgens, usually to prepubertal boys or men with long-standing, severe androgen deficiency, induces or worsens gynecomastia by initially causing relatively higher estradiol than testosterone concentrations.

Increased peripheral aromatase activity with increased conversion of androgens to estrogens in excessive amounts of adipose tissue is thought to cause mild to moderate gynecomastia in men with obesity.[169-171] In addition, increased aromatization of androgens to estrogens with increasing amounts of adipose tissue (including that within the breast) probably contributes substantially to the increased prevalence of gynecomastia with aging, which occurs in up to 65% of men from 50 to 80 years of age.[169-171] Familial gynecomastia, an autosomal dominant or X-linked genetic disorder caused by constitutive activation of the *CYP19A1* (aromatase) gene that results in increased peripheral conversion of androgen to estrogen, is a very rare cause of gynecomastia that manifests as prepubertal gynecomastia persisting into adulthood.

TABLE 19.3 Causes of Gynecomastia

Cause	Examples
Physiologic Causes	
Maternal estrogen exposure	Neonatal gynecomastia
Transient increase in estrogen to androgen concentrations	Pubertal gynecomastia
Estrogen Excess	
Estrogens or estrogen receptor agonists	Estrogens, digitoxin, testosterone or other aromatizable androgens
Increased peripheral aromatase activity	Obesity, aging, familial
Estrogen-secreting tumors	Adrenal carcinoma, Leydig or Sertoli cell tumor
hCG-secreting tumors	Germ cell, lung, hepatic carcinoma
hCG treatment	
Androgen Deficiency or Resistance	
Androgen Deficiency	Primary or secondary hypogonadism
Hyperprolactinemia causing androgen deficiency	
Androgen Resistance Disorders	Congenital and acquired androgen resistance
Drugs that interfere with androgen action	Spironolactone, androgen receptor antagonists, 5α-reductase inhibitors, histamine 2 receptor antagonists
Systemic Disorders	
Organ failure	Hepatic cirrhosis, chronic kidney disease
Endocrine disorders	Untreated hyperthyroidism, acromegaly, growth hormone treatment, Cushing syndrome
Nutritional disorders	Refeeding, recovery from chronic illness (hemodialysis, insulin, isoniazid, antituberculous medications, efavirenz)
Idiopathic Causes	
Drugs	HAART, calcium channel antagonists, amiodarone, antidepressants (SSRIs, tricyclic antidepressants), alcohol, amphetamines, penicillamine, sulindac, phenytoin, omeprazole, theophylline
Adult-onset idiopathic gynecomastia	
Persistent prepubertal macromastia	

HAART, Highly active antiretroviral therapy; *hCG,* human chorionic gonadotropin; *SSRIs,* selective serotonin reuptake inhibitors.

Estrogen-secreting tumors of the adrenal gland or testis are uncommon causes of gynecomastia. Feminizing adrenal tumors are usually malignant and large, manifesting with a palpable abdominal mass. In contrast, estrogen-secreting Leydig or Sertoli tumors are usually small and benign. Feminizing Sertoli tumors (particularly the large cell calcifying variety) may occur in isolation or in association with autosomal dominant disorders such as Peutz-Jeghers syndrome (multiple intestinal polyps and mucocutaneous pigmented macules) or the Carney complex (cardiac or cutaneous myxomas, pigmented skin lesions, and endocrinopathy, including functioning endocrine tumors of the adrenal and testis). *hCG-secreting tumors* (e.g., germ cell, lung, gastric, renal cell, or hepatic carcinomas in adults; hepatoblastomas in boys) or *hCG treatment* of gonadotropin deficiency increases aromatase activity in Leydig cells and stimulates excessive secretion of estradiol relative to testosterone, causing relative rapid onset of symptomatic gynecomastia.

Disorders and drugs that cause *androgen deficiency*, such as conditions that cause either primary or secondary hypogonadism (including medications such as cytotoxic agents) or androgen resistance, are major causes of gynecomastia.[169-171] Although prolactin acts on the breast to facilitate milk production in developed glandular tissue, the major mechanism by which hyperprolactinemia causes gynecomastia is inhibition of endogenous gonadotropin and testosterone production (inducing androgen deficiency), which acts indirectly to stimulate breast development by reducing the inhibitory influence of androgens on the breast. Hyperprolactinemia is the main reason that many CNS-active medications, such as antipsychotics, antidepressants, and sedatives, are associated with gynecomastia. Drugs that interfere with androgen action, such as spironolactone (in contrast to eplerenone, a selective aldosterone receptor antagonist that does not cause gynecomastia), AR antagonists (e.g., flutamide, bicalutamide, nilutamide and enzalutamide), marijuana, and histamine 2 (H_2) receptor antagonists, may cause gynecomastia.

Androgen deficiency contributes to the pathogenesis of gynecomastia in *systemic disorders* such as major organ failure—and particularly in hepatic cirrhosis and CKD, which are commonly associated with combined primary and secondary hypogonadism—and in *endocrine disorders* such as acromegaly and Cushing syndrome, which may be associated with secondary hypogonadism.[169-171] In hepatic cirrhosis, there is reduced catabolism of Δ^4-androstenedione, resulting in increased peripheral conversion of Δ^4-androstenedione to estrone and increased circulating estrogen concentrations. In addition, in both hepatic cirrhosis and hyperthyroidism, increased serum concentrations of SHBG, which binds testosterone with greater affinity than estradiol, result in relatively higher free estradiol compared with free testosterone concentrations and thereby contribute to stimulation of breast tissue and development of gynecomastia. LH concentrations are often elevated in men with hyperthyroidism, which stimulates relatively more estradiol than testosterone secretion by Leydig cells of the testes. Excessive GH with acromegaly or GH treatment and excessive cortisol with Cushing syndrome directly stimulate breast tissue growth in addition to causing secondary hypogonadism, both of which contribute to the pathogenesis of gynecomastia.

Gynecomastia often accompanies *nutritional disorders*, particularly during nutritional repletion after a period of starvation and weight loss (refeeding gynecomastia) and, analogously, during recovery from chronic illness.[169-171] In both starvation and severe chronic illness that is commonly associated with anorexia and weight loss, central GnRH production and concomitant

gonadotropin and testosterone secretion are markedly suppressed. With refeeding or restitution of appetite and weight gain, there is activation of the hypothalamic-pituitary-testicular axis and restoration of gonadal function, similar to what occurs during puberty but occurring more rapidly (a "second puberty"), resulting in transiently higher concentrations of estrogen relative to androgen concentrations and inducing gynecomastia. Refeeding gynecomastia was described initially in World War II prisoners who developed painful gynecomastia after liberation and nutritional repletion. Analogously, refeeding-like gynecomastia may occur in stage 5 CKD with the initiation of hemodialysis, in type 1 diabetes mellitus (T1DM) with insulin therapy, in tuberculosis with antituberculosis medications, and in human immunodeficiency virus (HIV) infection or acquired immunodeficiency syndrome with highly active antiretroviral treatment (HAART). As mentioned, these chronic systemic disorders also cause androgen deficiency that may contribute to the pathogenesis of gynecomastia. HAART also may cause lipohypertrophy and fat accumulation in the breast (pseudogynecomastia); efavirenz, a component of some HAART regimens, has estrogenic activity that might cause true gynecomastia.

The mechanisms of gynecomastia's causal relationship with many *drugs* are not entirely clear, and these cases are usually classified as idiopathic. Such drugs include calcium channel blockers (e.g., nifedipine, verapamil), amiodarone, antidepressants (selective serotonin reuptake inhibitors, tricyclic antidepressants), alcohol, amphetamines, penicillamine, sulindac, phenytoin, omeprazole (much less commonly than H_2-receptor antagonists), and theophylline.[169-171,175,176]

In many cases of adult-onset gynecomastia, the cause remains *idiopathic*. Most of these cases are probably caused by increased aromatization of androgens to estrogens associated with increased peripheral adiposity, enhanced breast production of estrogens, enhanced sensitivity to estrogens, or some combination of these factors. Rarely, boys may develop severe pubertal gynecomastia (female size breast development, Tanner stages III through V) that persists to adulthood (*persistent pubertal macromastia*). This disorder is not associated with specific hormonal or receptor abnormalities.

Evaluation

Most gynecomastia is asymptomatic and of mild degree but can be appreciated on a properly performed, careful physical examination (as described earlier). Asymptomatic gynecomastia (<5 cm) found incidentally on examination does not warrant evaluation. However, breast enlargement that is recent and rapid in onset, large (≥5 cm), symptomatic (i.e., associated with breast pain, tenderness, or galactorrhea), asymmetric, or suspicious for malignancy (eccentrically located, rock hard, fixed to overlying or underlying tissues, or associated with bloody nipple discharge or lymphadenopathy) should evaluated.

A careful history, including medication history, and physical examination usually identify potential predisposing conditions or medications causing gynecomastia that in older men may be mulifactorial.[169-171] Clinical evaluation should focus on evidence of androgen deficiency; assessment of prescription and over-the-counter medications, substance abuse, herbal or nutritional supplement intake, cosmetic use, and usual dietary intake; symptoms and signs of systemic illness (e.g., hepatic or renal disease), malignancy, or endocrine disorders (e.g., thyroid, GH, cortisol excess); and history of recent recovery from malnutrition, severe weight loss, or chronic illness. At a minimum, the initial laboratory evaluation comprises serum testosterone (calculated free testosterone if SHBG perturbation is suspected), LH, FSH, TSH, and renal and liver function tests. Evaluation also usually includes measurements of estradiol, prolactin, and βhCG, although clinically important elevations of these hormones suppress serum gonadotropin concentrations. Breast enlargement suspicious for malignancy should be evaluated by mammography and biopsy.

Treatment

Pubertal gynecomastia usually regresses spontaneously without treatment in 1 to 2 years and by 18 years of age in about 90% of cases. In adults, spontaneous regression of symptoms (breast pain and tenderness, nipple sensitivity) associated with inflammatory glandular proliferation usually occurs within 6 months, after which progressive stromal fibrosis causes permanent palpable breast tissue and only partial regression of gynecomastia by 1 year.

Initial treatment of gynecomastia is directed at correction of the underlying cause of breast enlargement or discontinuation or replacement of a potentially offending medication.[177] No treatment is effective for gynecomastia of 6 months or more; absence of breast tenderness generally indicates the chronic fibrotic stage of gynecomastia that will not remit. Prophylactic low-dose breast irradiation (10–15 Gy over 1–3 days) may be used before androgen deprivation therapy in men with prostate cancer to prevent the development of gynecomastia, which is more common in surgical orchidectomy and in AR antagonist monotherapy than in combined therapy with a GnRH agonist or antagonist. Testosterone replacement therapy in androgen-deficient men may result in partial regression of gynecomastia if breast enlargement is of recent onset. For gynecomastia of recent onset (<6 months), selective ER antagonists (tamoxifen, 10–20 mg daily, or raloxifene, 60 mg daily) are effective in treating pubertal and adult gynecomastia and preventing gynecomastia induced by androgen deprivation therapy. For unclear reasons, aromatase inhibitors (e.g., anastrozole) generally are not effective. Although tamoxifen is not approved for treatment of gynecomastia, it has been shown to be effective in the treatment of idiopathic gynecomastia, resulting in partial regression in approximately 80% and complete regression in about 60% of cases. A gel formulation of DHT, a nonaromatizable androgen, is used to treat gynecomastia in some countries outside the United States.

Gynecomastia of recent onset, during the initial phase of ductal proliferation, periductal inflammation and edema, and subareolar fat accumulation, is usually responsive to medical therapy (e.g., androgen replacement in hypogonadal men, ER antagonist therapy). With long-standing gynecomastia (>6 months), there is progressive stromal fibrosis of the breast that is not responsive to medical treatment. In these cases, surgical reduction mammoplasty (i.e., removal of breast tissue [SC mastectomy] with or without periareolar adipose tissue [liposuction]) is necessary, especially if breast enlargement is severe, painful, socially embarrassing, or disfiguring.

Infertility

Infertility is defined as the inability of a sexually active couple to achieve conception despite 1 year of unprotected intercourse. The probability of conception in a sexually active young couple (both younger than 35 years) is approximately 85% by 1 year. Approximately 15% of couples in the reproductive age group are infertile, and a male factor contributes to the cause (either in isolation or in combination with a female factor) in about half of the cases. Therefore, male infertility is a common condition, affecting approximately 7% of men.[178]

Causes of Male Infertility

In about 80% to 90% of infertile men, infertility is caused by primary or secondary hypogonadism, manifested most commonly by an isolated impairment of sperm production or function; much less commonly by androgen deficiency and impaired spermatogenesis; and rarely by androgen resistance[179,180] (Table 19.4). The evaluation and specific causes of hypogonadism are discussed in detail in subsequent sections. Most men with isolated impairment in sperm production have a primary disorder of the testes that is idiopathic in 60% to 70% of cases (if one includes idiopathic oligozoospermia or azoospermia and varicocele, given that relationship of varicocele to the pathogenesis of infertility is unclear). If isolated impairment of spermatogenesis is severe in men with primary hypogonadism, serum FSH concentrations may be selectively elevated (FSH >8 IU/L) as a result of reduced negative feedback by inhibin B from Sertoli cells of the testis.

Disorders of spermatogenesis caused by primary hypogonadism may be associated with chromosomal or genetic disorders. There is an 8- to 10-fold increase in the prevalence of chromosomal abnormalities among infertile men with impaired spermatogenesis—specifically, sex chromosomal aneuploidy (e.g., Klinefelter syndrome) or Robertsonian translocations of two nonhomologous chromosomes, most commonly involving chromosomes 13 and 14 or chromosomes 14 and 21.[181] The long arm of the Y chromosome (Yq), specifically the azoospermia factor (*AZF*) region (Yq11), contains many genes that encode for proteins that have important roles in spermatogenesis. This region contains highly homologous palindromic DNA repeat sequences that are susceptible to rearrangement and deletions. Small deletions in the *AZF* region (*Y chromosome microdeletions*) are the most common genetic cause of impaired sperm production and male infertility; they are found in 5% to 10% of men with azoospermia or severe oligozoospermia (sperm concentration <5 million/mL).[181] Y chromosome microdeletions have been identified in three regions: in the *AZFa* region, microdeletions are uncommon but are usually associated with azoospermia and Sertoli cell–only histology; in the *AZFb* region, they are usually associated with severe oligozoospermia and germ cell arrest at the pachytene primary spermatocyte stage; and in the *AZFc* region, where the majority of Y chromosome microdeletions reside, they are usually associated with germ cell arrest at the spermatid stage or hypospermatogenesis with some mature spermatids present. Occasionally, microdeletions in the *AZFb* and *AZFc* regions are associated with azoospermia and Sertoli cell–only histology. Genes encoding a number of candidate proteins for male infertility include *DDX3Y* (DEAD box Y, an ATP-dependent RNA helicase), *RBMY* (RNA-binding motif Y-linked, an RNA-binding protein), and *DAZ* (deleted in azoospermia, another RNA-binding protein) in the *AZFa, AZFb,* and *AZFc* regions, respectively.[181,182]

Approximately 15% to 20% of male infertility is caused by *disorders of sperm transport* from the testes to the urethra, most commonly by genital tract obstruction. *Congenital bilateral absence of the vas deferens* (CBAVD) is present in 1% to 2% of men with infertility.[181,183,184] Seventy-five percent of men with CBAVD are heterozygous for the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) that encodes for an epithelial chloride channel. They do not have obvious clinical manifestations of cystic fibrosis, although some manifest abnormalities on sweat chloride testing and sinopulmonary infections. Conversely, almost all men with clinical cystic fibrosis have CBAVD. CBAVD is also commonly associated with absence of the seminal vesicles, ejaculatory ducts, and epididymides due to fetal atrophy of these wolffian duct derivatives; in 10% of cases, there is also renal agenesis or hypoplasia.

Other causes of genital tract obstruction include other congenital defects of the epididymides and vas deferens (e.g., epididymal cysts associated with prenatal diethylstilbestrol exposure); vasectomy (surgical ligation of the vas deferens); postinfectious fibrosis (e.g., associated with gonorrhea, *Chlamydia* infection, other sexually transmitted diseases; tuberculosis; leprosy); and Young syndrome, a rare, congenital primary ciliary dyskinesia syndrome characterized by bronchiectasis, recurrent sinopulmonary infections, and obstructive azoospermia caused by thickened, inspissated mucous secretions obstructing the epididymides.

Although a causal link to infertility has not been clearly established, other genital tract abnormalities may contribute to impaired sperm transport and the pathogenesis of infertility in some men. *Accessory gland dysfunction*, such as reduced seminal vesicle and prostate secretions associated with disorders that cause severe androgen deficiency or resistance, may contribute to reduced fertility, although the main effects of these disorders are

TABLE 19.4 Causes of Male Infertility

Cause	Examples
Hypogonadism	
Isolated impairment of sperm production or function	
Androgen deficiency and impaired sperm production	
Androgen resistance	
Disorders of Sperm Transport	
Genital tract obstruction	Congenital bilateral absence of the vas deferens, cystic fibrosis, other congenital defects, vasectomy, postinfectious fibrosis, Young syndrome
Accessory gland dysfunction	Androgen deficiency or resistance, infection or inflammation, antisperm antibodies (immunologic)
SNS dysfunction	Autonomic neuropathy, sympatholytic drugs, sympathectomy, retroperitoneal or abdominopelvic surgery, spinal cord injury or disease, vasovasostomy
Ejaculatory Dysfunction	
Premature or retarded ejaculation	
Retrograde ejaculation	Prostatectomy, bladder neck surgery, autonomic neuropathy, SNS dysfunction
Reduced ejaculation	Androgen deficiency or resistance, SNS dysfunction, ureteral abnormalities
Coital Disorders	
Erectile dysfunction	
Defects in coital technique	Infrequent intercourse (< once weekly), poor timing in relation to ovulation, premature withdrawal of penis

SNS, Sympathetic nervous system.

to impair spermatogenesis and cause sexual dysfunction. *Infection or inflammation* of the epididymides, seminal vesicles, or prostate gland may affect fertility directly by impairing sperm maturation or function or secondarily by causing scarring of the genital tract or induction of *antisperm antibodies* in semen (resulting in sperm agglutination and impaired sperm function).[185,186] *Sympathetic nervous system dysfunction* (e.g., associated with autonomic neuropathy, sympatholytic drugs, sympathectomy, retroperitoneal or abdominopelvic surgery, spinal cord injury or disease, vasovasostomy) may contribute to impaired sperm transport and male infertility.

Ejaculatory dysfunction may cause or contribute to male infertility by preventing normal or efficient deposition of sperm into the vagina and female genital tract. Premature or delayed ejaculation may contribute to infertility if ejaculation occurs during arousal or foreplay before vaginal penetration or after withdrawal from the vagina. Retrograde ejaculation of semen into the bladder, rather than the urethra, occurs with neuromuscular failure of normal bladder sphincter contraction during ejaculation. Retrograde ejaculation may be associated with prostatectomy or bladder neck surgery (e.g., transurethral resection of the prostate), autonomic neuropathy (e.g., complicating diabetes mellitus), or sympathetic nervous system dysfunction, and particularly with sympatholytic drugs (e.g., α-adrenergic receptor antagonists like prazosin or terazosin), retroperitoneal or abdominopelvic surgery (e.g., retroperitoneal lymph node dissection), and spinal cord injury or disease. Reduced ejaculation caused sympathetic nervous system dysfunction or urethral abnormalities may contribute to reduced sperm delivery to the female genital tract.

Erectile dysfunction may contribute to male infertility by causing unsuccessful vaginal intercourse. Coital disorders are uncommon causes of male infertility, but they are potentially correctable with proper education. Infrequent vaginal intercourse (less than weekly), intercourse during menses rather than just before or around the time of ovulation, and premature withdrawal of the penis during intercourse may contribute to reduced fertility.

Evaluation

Because a coexisting female factor contributes to infertility in 30% of cases, it is important for the female partner to undergo evaluation for ovulation (menstrual periods, androgenization) and for cervical disorders (postcoital testing) and uterine and tubal disorders (hysterosalpingogram, pelvic ultrasound). In men, the history and physical examination are usually able to identify the potential cause of male infertility.[179-181]

In addition to an assessment of general health and medical comorbid conditions, the initial clinical evaluation should focus on the following:

- Symptoms and signs of androgen deficiency or resistance (as detailed elsewhere in this chapter)
- Scrotal examination for presence of a large varicocele, presence and size of the testes, and presence or absence of firm, fibrous cords of the vas deferens
- Family history or evidence of cystic fibrosis
- Previous vasectomy or vasovasostomy
- History or manifestations of genitourinary infections
- Medications, particularly ones that cause androgen deficiency or resistance and sympatholytic agents
- Ejaculatory problems, particularly absent or reduced ejaculate
- Autonomic neuropathy (e.g., complicating diabetes mellitus)
- Retroperitoneal or abdominopelvic surgery
- Spinal cord injury or disease
- Erectile dysfunction
- Coital practices and techniques.

The initial laboratory evaluation of male infertility should begin with at least two or three seminal fluid analyses performed over a period of a few months (see later discussion) to assess semen volume, sperm count and concentration, and sperm motility and morphologic appearance, with the aim of identifying men who have impaired sperm production or function—the major cause of male infertility. The presence of leukocytes in semen ($>10^6$/mL, termed *leukospermia* or *pyospermia*) may suggest genitourinary inflammation or infection, but routine cultures are not helpful in guiding treatment. Agglutination of spermatozoa in semen (i.e., sticking of motile sperm to each other) suggests the presence of *antisperm antibodies* that can be measured in semen and might indicate an immunologic cause of male infertility, but it is unclear whether antisperm antibodies affect fertility.[187]

Seminal fluid fructose is derived mostly from the seminal vesicles (60%) and to a lesser extent from the prostate gland (30%). Absent or low seminal fluid fructose and low semen volume suggest either congenital absence of the vas deferens and seminal vesicles or obstruction of the ejaculatory ducts. Transrectal or scrotal ultrasonography to detect dilated seminal vesicles should be done to confirm ejaculatory duct obstruction. In men who have little or no ejaculate, a postejaculatory urine specimen should be collected and examined for the presence of sperm, indicating retrograde ejaculation.

Serum testosterone concentrations should be measured on at least two occasions to confirm androgen deficiency, and measurements of LH and FSH should be performed to determine whether the patient has primary or secondary hypogonadism (see later discussion). Men with primary hypogonadism have low testosterone with elevated serum LH and FSH concentrations (FSH higher than LH concentrations). Identification of infertile men with secondary hypogonadism (low testosterone with low or inappropriately normal LH and FSH concentrations) potentially has important therapeutic implications. In men with impaired sperm production due to gonadotropin deficiency, spermatogenesis may be stimulated and fertility restored with the use of gonadotropin or GnRH therapy. Secondary hypogonadism is one of few treatable causes of male infertility. Elevated concentrations of testosterone, LH, and FSH suggest androgen resistance: LH is usually higher than FSH.

Measurements of FSH concentrations specifically as a marker of Sertoli cell and seminiferous tubule function are useful in identifying men with severe defects in spermatogenesis and impairment of seminiferous tubule and Sertoli cell function; such patients often demonstrate selective elevation in serum FSH concentrations with normal or high-normal LH concentrations due to loss of negative feedback inhibition of pituitary FSH secretion by inhibin B.[188] In young men with proven fertility, the upper limit of normal for serum FSH is 8 IU/L.[189] Many commercial laboratories have higher upper limits of normal because they have included men without proven fertility. However, men with less severe seminiferous tubule dysfunction and impairment of spermatogenesis and those with azoospermia due to genital tract obstruction (obstructive azoospermia) have normal serum FSH concentrations.

Genetic disorders make up a small but significant proportion of the causes of male infertility. Because ART, and specifically ICSI, which involves direct injection of spermatozoa into the cytoplasm of an ovum (discussed later), is commonly used to treat male

infertility, the potential exists for transmission of genetic defects to offspring. Therefore, genetic testing and counseling should be offered to men who are considering ICSI, particularly for those with severe oligozoospermia or azoospermia.[179-181]

Men in whom bilateral congenital absence of the vas or genital tract obstruction is confirmed by transrectal or scrotal ultrasound and those who have unexplained obstructive azoospermia should undergo genetic testing for *CFTR* mutations and genetic counseling before ICSI. In men with severe oligozoospermia (sperm concentration <5 million/mL) or azoospermia, testing for Y chromosome microdeletions in the *AZF* region should be performed routinely. There is a high prevalence of sex chromosome and autosomal chromosome defects, often in the absence of other phenotypic abnormalities, in men with moderately impaired spermatogenesis and infertility. Therefore, karyotyping is recommended before ICSI for infertile men with impaired sperm production, particularly for those with a sperm concentration of less than 10 million/mL.

In azoospermic men with normal semen volume, a normal fructose level, and a normal FSH level in whom it is unclear whether azoospermia is caused by germ cell failure, genital tract obstruction, or both, surgical exploration of the scrotum and testis biopsy are needed. Microsurgical biopsy is also used to harvest sperm for ICSI, even in men with known severe impairment in spermatogenesis, such as those with Klinefelter syndrome.[179,180,190,191]

Specialized tests of in vitro sperm function, such as detailed examination of sperm motility using computer-assisted semen analysis, cervical mucus penetration tests, acrosome reaction, and human zona pellucida binding tests, may be useful in some men who are considering intrauterine insemination (IUI) or in vitro fertilization (IVF). However, these tests should be performed only in highly specialized laboratories that have demonstrated excellent quality control. Even in such laboratories, there is a high rate of clinical false-positive and false-negative results, limiting the clinical utility of these tests.

Treatment

In men with infertility caused by primary hypogonadism (whether due to androgen deficiency and impairment of sperm production or to isolated impairment of sperm production or function), defects in sperm production are not effectively treated with drugs, although spontaneous recovery of spermatogenesis may occur at variable times after discontinuation of cytotoxic drugs or ionizing radiation. Because intratesticular testosterone concentrations are normally approximately 100-fold higher than serum concentrations, exogenous testosterone treatment of men with androgen deficiency cannot deliver sufficient amounts of testosterone to support sperm production in the testis.

In men with secondary hypogonadism, however, sperm production can be stimulated with gonadotropin or GnRH treatment, or spermatogenesis may recover sufficiently to restore fertility after discontinuation of drugs that suppress gonadotropins, such as androgenic anabolic steroids, progestins, glucocorticoids, opioids, and drugs causing hyperprolactinemia.

Most men with a varicocele and infertility have abnormal seminal fluid. However, varicocele repair has not been demonstrated to be effective in restoring fertility to these men. Therefore, unless a varicocele is very large or symptomatic, surgical repair is not recommended.[12,192] Although clinical trials have failed to demonstrate effectiveness,[193] infertile men with leukospermia or sperm agglutination are sometimes treated empirically with a 14-day course of antibiotics, such as doxycycline, trimethoprim-sulfamethoxazole, or a fluoroquinolone. Although high-dose prednisone (40–60 mg for several months) has been recommended to treat infertility in men with sperm in the ejaculate and serum antisperm antibodies, a recent systematic review concluded that antisperm antibodies do not cause male infertility. It is likely that the "response" to corticosteroid therapy is due to the fact that some of these men eventually conceive without any therapy.[187] Empiric high-dose corticosteroid therapy is not justified because of the high risk of toxicity.

Although ICSI is costly, it is used increasingly to treat male infertility, and it dramatically improves the prognosis for men with impaired sperm production regardless of the cause.[190,191] Spermatozoa that are ejaculated or obtained by testicular biopsy (TESE) or from the epididymis (*microsurgical epididymal sperm aspiration* [MESA]) are used for ICSI and other ARTs. With ICSI, fertilization rates of about 60% and pregnancy rates of approximately 20% are achieved, irrespective of the cause of male infertility or source of spermatozoa. ICSI after TESE using microsurgical testis biopsy or fine-needle aspiration to retrieve sperm has been successful in restoring fertility to men with primary hypogonadism who had severe impairments in spermatogenesis and azoospermia that were previously thought to be untreatable (e.g., Klinefelter syndrome, prolonged azoospermia after chemotherapy).[194] Because of the potential for chromosomal abnormalities and transmission of Y chromosome microdeletions and *CFTR* mutations that cause infertility in male offspring, genetic testing and counseling should be conducted if ICSI is being considered (see earlier discussion).[181]

Obstruction of the epididymides or the ejaculatory ducts can be corrected surgically, such as with end-to-end anastomosis of the epididymides or transurethral resection of the ejaculatory ducts. More commonly, MESA is used to obtain spermatozoa that can be incubated with ova in vitro (IVF) or directly injected into an ovum (ICSI), and this method is more successful in restoring fertility than surgical options. In contrast, microsurgical reanastomosis of the vas (vasovasostomy) to reverse vasectomy is less costly and more successful in restoring fertility than MESA followed by IVF or ICSI. Return of sperm in the ejaculate occurs in approximately 90% of men who undergo vasectomy reversal, but restoration of fertility occurs in only about 50%, probably because of stenosis or blockage of the previous vasovasostomy, epididymal blockage, or the development of antisperm antibodies in response to the vasectomy.[195]

In men with retrograde ejaculation, collection of spermatozoa in alkalinized postejaculation urine, followed by extensive washing and IUI or ICSI, has been used successfully to treat infertility. With proper education, coital disorders that contribute to infertility may be corrected. In addition, the timing of intercourse may be optimized to occur a few days before and after ovulation (the period of highest probability for conception) based on basal body temperature measurements or, more accurately, on commercially available rapid urinary LH kits to estimate the timing of ovulation in the female partner.

If the treatment options described previously are not available or affordable to infertile couples who desire children, artificial insemination with donor sperm or adoption may be considered.

Diagnosis of Male Hypogonadism

Clinical Manifestations of Androgen Deficiency

The diagnosis of male hypogonadism requires clinical manifestations consistent with androgen deficiency and unequivocally low serum testosterone concentrations. In community-dwelling middle-age and older men, the crude prevalence of symptomatic

androgen deficiency is 2% to 9%, depending on the constellation of symptoms and signs and the biochemical definition of androgen deficiency used (i.e., the threshold used for a single low testosterone concentration).[196-198] This prevalence increases with age and is much higher in a primary care setting.[199] In community populations, the prevalence of low testosterone concentrations alone, without consideration of symptoms and signs of androgen deficiency, is much higher than that of clinical androgen deficiency. This underscores the importance of making a diagnosis of hypogonadism only in men who have clinical manifestations and consistently low testosterone concentrations. Both the clinical and the biochemical diagnosis of androgen deficiency can be challenging, especially in older adults.

Although the manifestations of fetal androgen deficiency or resistance (ambiguous genitalia and 46,XY DSD) and those of androgen deficiency of prepubertal onset (eunuchoidism) are usually clinically obvious, the clinical diagnosis of androgen deficiency in adults is more difficult. As described previously, the symptoms and signs of androgen deficiency are nonspecific and have a broad differential diagnosis. Moreover, clinical manifestations may be modified by several factors, such as the severity and duration of androgen deficiency, age, comorbid illnesses, medications, previous testosterone treatment, and individual variations in androgen sensitivity, all of which contribute to variability in clinical presentation that may confound the diagnosis.[116] Because the manifestations of androgen deficiency in adults are nonspecific, other potential causes (e.g., depression, comorbid illness, or medications) should be considered in the differential diagnosis to explain clinical features in any individual patient.[200]

The degree and duration of androgen deficiency have impressive effects on clinical manifestations. The severe and relatively rapid suppression of testosterone concentrations in men with prostate cancer treated with a GnRH agonist or orchidectomy results in prominent clinical manifestations with notable reductions in erectile function, libido, energy, and mood; hot flushes and sleep disturbances; infertility; decreases in muscle mass and strength, BMD (associated with an increase in fracture risk), and body hair; gynecomastia; increases in body fat; anemia; and possibly increases in the risks for diabetes mellitus and cardiovascular events.[201,202] In contrast, men with mild androgen deficiency may have few or no referable manifestations; such patients have "subclinical" androgen deficiency that may or may not be associated with clinically significant outcomes. The latter situation is analogous to that observed in other endocrine disorders such as subclinical hypothyroidism or asymptomatic primary hyperparathyroidism.

Aging is associated with alterations in body functions, such as declines in sexual function, muscle mass and strength, and BMD, that result in clinical manifestations similar to those of androgen deficiency.[144] These alterations associated with aging may also be caused in part by age-related androgen deficiency. To add to the clinical complexity in older men, age-associated comorbid illnesses and medications used to treat these illnesses may modify the symptoms and signs of androgen deficiency, and in many instances, they may also contribute to the cause of androgen deficiency. Therefore, it is understandable why the diagnosis of clinical androgen deficiency is challenging in older men, particularly in frail elderly men who have multiple comorbid illnesses and are taking numerous medications.

Previous testosterone treatment that has been discontinued may affect the clinical manifestations of androgen deficiency, depending on the duration of therapy and the time since discontinuation. It is also likely that clinical manifestations of androgen deficiency are affected by individual variations in androgen action on specific target organs. Alterations in androgen sensitivity may result from individual or tissue-specific differences in the activity of the AR or the ER and associated coregulators, or from differences in active metabolism of testosterone to estradiol or DHT or inactivation of testosterone.

In men with clinical manifestations suggestive of androgen deficiency, the diagnosis of hypogonadism is confirmed biochemically by measurement of consistently low serum testosterone concentrations[116] (Fig. 19.19).

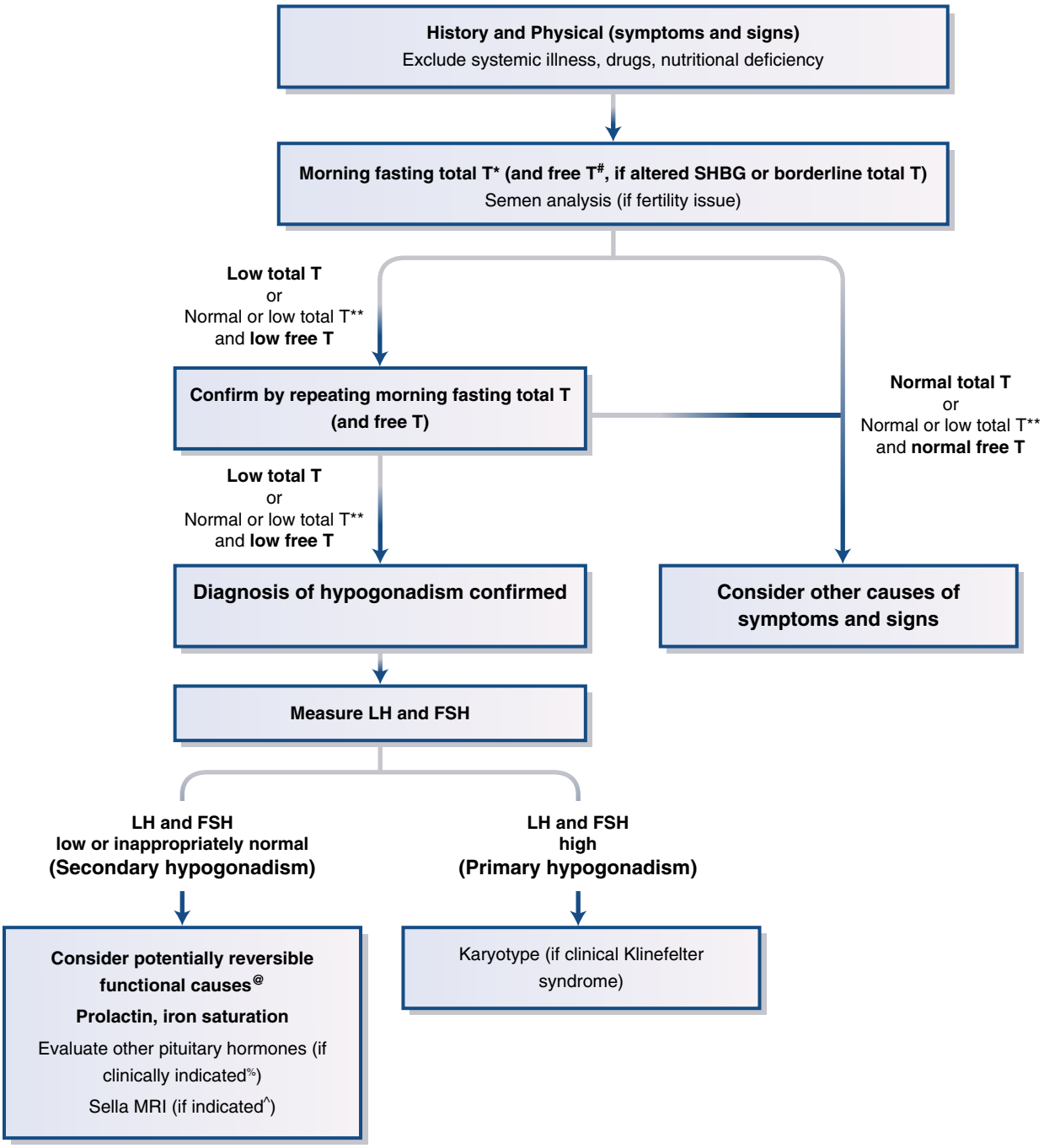
Testosterone Measurements

As with the clinical manifestations, the biochemical confirmation of androgen deficiency presents its own difficulties. Testosterone concentrations exhibit both biologic and assay variability. Total testosterone concentrations are affected by alterations in SHBG, and testosterone concentrations may be suppressed transiently with illness, certain medications, and some nutritional deficiencies.[116] Therefore, the biochemical diagnosis of androgen deficiency requires demonstration of consistently and unequivocally low serum testosterone concentrations in at least two blood samples obtained between 7 AM and 10 AM, preferably fasting. In men who have conditions that alter SHBG, accurate and reliable free or bioavailable testosterone measurements are needed to confirm the diagnosis of hypogonadism. Finally, the diagnosis of hypogonadism should not be made during acute or subacute illness.

The threshold level of circulating total or free testosterone below which symptoms and signs of androgen deficiency occur and for which testosterone treatment will improve clinical manifestations is not known. However, the concept of a single threshold testosterone level is probably not valid, nor is it clinically useful, because thresholds vary with the specific symptom and the androgen target organ or tissue. In general, symptoms and signs of androgen deficiency are more likely to occur with a total testosterone level that is below the lower limit of the normal range for young, nonobese, healthy men (approximately 265–280 ng/dL or 2.65–2.8 ng/mL [9.2–9.7 nmol/L], when using an accurate and reliable assay).[203] The likelihood and severity of clinical manifestations of androgen deficiency increase with a greater decline in testosterone level below normal.

Variability in Testosterone Concentrations

Because serum testosterone concentrations exhibit both biologic and assay variability, a single measurement is not a reliable indicator of an individual's average concentration. Serum testosterone concentrations exhibit both ultradian and circadian variation, providing physiologic sources of biologic variability. Ultradian fluctuations in serum testosterone concentrations, characterized by peaks of incremental amplitude that average approximately 240 ng/dL (40% fractional amplitude) with a 95-minute duration,[45] have been reported in a small number of young men; more chaotic peaks with lower amplitude have been reported in older men.[204] As described previously, the circadian variation in serum testosterone peaks at about 8 AM and has a maximum excursion averaging 140 ng/dL.[64] The circadian variation in testosterone is blunted but still present in older men, with a maximum excursion averaging 60 ng/dL. In young men, serum testosterone concentrations are 20% to 25% lower at 4 PM than at 8 AM (i.e., over the course of usual clinic hours).[205] This difference decreases with age: in 70-year-old men, testosterone concentrations are 10% lower at 4 PM than at 8 AM. Most importantly, many young and old men who have testosterone concentrations that are below normal



• **Fig. 19.19** Algorithm for the diagnosis and evaluation of suspected androgen deficiency. In men with clinical manifestations (symptoms and signs) consistent with androgen deficiency, a morning fasting total testosterone (T), and if a condition is present that causes an alteration in the level of sex hormone–binding globulin (SHBG; see Table 19.5) or if total T is borderline (e.g., between 200 and 400 ng/dL), an accurate measurement of free T level should be obtained initially. Acute systemic illnesses, drugs, or nutritional deficiency that could lower T concentrations should be excluded. If the initial total and/or free T level is low, they should be repeated to confirm a diagnosis of male hypogonadism. *The lower limit of the normal range for total T harmonized to Centers for Disease Control and Prevention (CDC) standards in healthy nonobese men age 19 to 39 years is 264 ng/dL (9.2 nmol/L); this limit could be used for total T assays that are CDC certified. Depending on the assay and reference population used, the lower limit of the normal range established in non–CDC-certified laboratories may not accurately identify men with hypogonadism. #Free T should be measured by an accurate method, such as the equilibrium dialysis method or estimated from total T, SHBG with or without albumin concentrations, using a formula that accurately reflects free T by equilibrium dialysis. The normal range for free T in healthy young men is usually between 5 and 6 ng/dL (0.17–0.31 nmol/L) in most laboratories. **Total T may also be high in some conditions in which SHBG concentrations are very high, such as human immunodeficiency virus disease or with use of some anticonvulsants. Concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) should be obtained (in same sample as T measurements) to determine whether T deficiency is caused by secondary hypogonadism (low T with low or normal LH and FSH) or primary hypogonadism (low T with high LH and FSH). @In men with secondary hypogonadism, potentially reversible or treatable functional causes of primary and secondary hypogonadism that might be managed without T treatment should be considered (denoted in Tables 19.7 and 19.8); serum prolactin and iron saturation should be assessed; and %if there is clinical indication of hypopituitarism or sella abnormality on imaging, evaluation of other pituitary hormones (e.g., free T4 and TSH) should be performed. ^Pituitary imaging (sella magnetic resonance imaging [MRI]) to exclude pituitary and/or hypothalamic tumor or infiltrative disease is indicated if there is severe secondary hypogonadism (e.g., serum total T < 150 ng/dL [5.2 nmol/L]), panhypopituitarism, persistent hyperprolactinemia, or symptoms or signs of tumor mass effect (e.g., new-onset headache, visual impairment, visual field defect, or cerebrospinal fluid rhinorrhea) are present. A sella computed tomography scan may be sufficient if macroadenoma is suspected or to assess parasellar bone involvement. (Redrawn from Bhasin S, Ponce JP, Cunningham GC, et al. Testosterone therapy in men with hypogonadism: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2018;103:1715–1744.)

in the afternoon have consistently normal concentrations in the morning. Testosterone concentrations are also lowered by glucose infusion or food intake,[206,207] and thus measurements should preferably be done in the fasting state. Given these findings and the fact that normal ranges of testosterone concentration are usually based on morning blood samples, testosterone measurements to confirm the diagnosis of hypogonadism should preferably be performed in the morning in the fasting state.

There is also substantial day-to-day variation in serum testosterone concentrations, underscoring the need to repeat the measurement to confirm low concentrations, particularly if the first result was only moderately below normal. Among men with serum testosterone concentrations of less than 300 ng/dL on an initial test, 30% to 35% were found to have a normal level on repeat testing.[208] Among community-dwelling middle-age and older men who had an initial serum testosterone concentration of less than 250 ng/dL, 20% were found to have an average testosterone level higher than 300 ng/dL (i.e., within the normal range) when six samples were drawn over the subsequent 6 months.[209] However, none of the subjects who had an initial average serum testosterone concentration of less than 250 ng/dL on two separate samples obtained 1 to 3 days apart had an average level higher than 300 ng/dL in six samples drawn over the next 6 months. These findings support the need to measure testosterone on at least two occasions to confirm the diagnosis of androgen deficiency.

Total Testosterone Assays

Total testosterone assays are performed in most local laboratories and are readily available to clinicians. Therefore, total testosterone is recommended as the initial measurement for the assessment of androgen deficiency. In local clinical laboratories, total testosterone is usually measured by automated platform-based direct immunoassays on unextracted serum or plasma. However, there is substantial variability in results from different assays, mostly because the accreditation of laboratories has been based on the reproducibility of results in comparison to other laboratories using the same method or kit, rather than on the accuracy of results. For example, when identical quality control samples were assayed by different methods or kits, the reported measured values ranged from 160 to 508 ng/dL. Moreover, the lower limit of the normal range in some assays was as low as 132 to 210 ng/dL (clearly in the hypogonadal range for most conventional assays).[210] In contrast, the lower limit of the normal range based on conventional radioimmunoassays after extraction is approximately 280 to 300 ng/dL. Most commercial reference laboratories now measure testosterone by liquid chromatography tandem mass spectrometry methods after solid phase extraction that are more accurate and precise than immunoassays.

To address the problems in the accuracy, quality control, and lack of standardization of total testosterone assays, the US Centers for Disease Control and Prevention (CDC) has developed a program to standardize and harmonize testosterone assays using accuracy-based quality control standards to which most commercial reference laboratories participate.[203,211] Recently, the College of American Pathologists also instituted an accuracy-based quality control program, but, unfortunately, neither of these programs is mandatory for certification. Recently, a harmonized reference range was established for total testosterone, cross-calibrated to the CDC reference method and standards, in more than 9000 community-dwelling men from four large cohorts in the United States and Europe.[203] The harmonized reference range in healthy, nonobese men 19.39 years of age was 264 to 916 ng/mL. This range could be used for laboratories that perform a CDC-certified total testosterone assay but not necessarily for total testosterone assays that are not CDC certified for accuracy.

The prevalence of low testosterone concentrations is high in several clinical conditions, namely in the presence of a pituitary or sellar mass; irradiation; disease; use of certain medications, such as opiates or high-dose glucocorticoids; HIV disease with weight loss; late-stage CKD; withdrawal from long-term androgenic anabolic steroid use; infertility; BMD concentrations that reveal osteoporosis or low-trauma fracture; and low libido or erectile dysfunction. If clinical manifestations consistent with androgen deficiency are present in men with these conditions, testosterone measurements should be performed.[116]

Total Testosterone Affected by Alterations in SHBG

Because a substantial proportion (30–40%) of circulating testosterone is bound tightly to SHBG, alterations in SHBG concentration may affect total testosterone concentrations without altering free or bioavailable testosterone. Conditions that suppress SHBG concentrations (even within the broad normal range) lower total testosterone (sometimes to below the normal range) without affecting circulating free or bioavailable testosterone concentrations[116] (Table 19.5). Common conditions that lower SHBG concentrations include moderate obesity, often associated with type 2 diabetes mellitus (T2DM); protein-losing states, such as nephrotic syndrome; administration of glucocorticoids, progestins, or androgens; untreated hypothyroidism; acromegaly; familial SHBG deficiency; and SHBG gene polymorphisms. SHBG concentrations are increased with increasing age, hepatic cirrhosis and inflammation (hepatitis of any cause), estrogens, hyperthyroidism, anticonvulsants, HIV disease, and SHBG gene polymorphisms.

If conditions that affect SHBG concentration are present in a patient or if total testosterone concentrations are close to the lower limit of the normal range (e.g., total testosterone concentration 200–400 ng/dL), serum free or bioavailable testosterone measurements should be obtained to confirm androgen deficiency. Unfortunately, accurate and reliable assays for free or bioavailable

TABLE 19.5 Conditions Associated With Alterations in SHBG Concentrations

Decreased SHBG Concentrations	Increased SHBG Concentrations
Moderate obesity, type 2 diabetes mellitus	Aging
Nephrotic syndrome	Hepatic cirrhosis and hepatitis
Glucocorticoids, progestins, and androgens	Estrogens
Hypothyroidism, untreated	Hyperthyroidism, untreated
Acromegaly	Anticonvulsants
Familial SHBG deficiency	HIV disease
Polymorphisms in SHBG gene	Polymorphisms in SHBG gene

HIV, Human immunodeficiency virus; *SHBG*, sex hormone–binding globulin.

From Bhasin S, Brito JP, Cunningham GR, et al. Testosterone therapy in men with hypogonadism; an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2018;103:1715-1744.

testosterone are not performed routinely in most local clinical laboratories. Although direct free testosterone assays using analogue immunoassay methods (that may be automated) are widely available and inexpensive, these assays are inaccurate and are affected by alterations in SHBG, so they are not recommended.[212-214]

The gold standard method for measurement of free testosterone concentrations is equilibrium dialysis or centrifugal ultrafiltration. Free testosterone concentrations may be calculated accurately from measurements of total testosterone, SHBG, and albumin using affinity constants for binding of testosterone to its binding proteins and published formulas. Calculated free testosterone values are comparable to those measured by equilibrium dialysis.[213] However, calculated values depend on the specific testosterone and SHBG assays employed and the formula used to estimate free testosterone.[215] There are several formulas used for calculation of free testosterone that all demonstrate some bias relative to the equilibrium dialysis method, largely as a result of assuming a single or two identical, noninteracting binding sites on SHBG.[113] However, they all provide reasonable approximations of free testosterone in the normal to low range of values that are less prone to misinterpretation and overdiagnosis of hypogonadism than total testosterone measurements when alterations in SHBG concentrations are present.

Bioavailable testosterone is measured by ammonium sulfate precipitation of SHBG-bound testosterone and measurement of free and albumin-bound testosterone in the supernatant. Bioavailable testosterone concentrations may also be calculated from measurements of total testosterone, SHBG, and albumin. In general, fewer studies used bioavailable versus free testosterone measurements, so many clinicians use free rather than bioavailable testosterone measurements to diagnose hypogonadism. These accurate and reliable measurements of free and bioavailable testosterone are available in commercial laboratories and should be used to confirm androgen deficiency in men who have conditions that affect SHBG and in those with total testosterone concentrations near the lower limit of the normal range. In a recent report, 60% of more than 3600 men in a single health care system who had low total testosterone concentrations were found to have normal calculated free testosterone concentrations using the laboratory reference range that was utilized by practitioners to make clinical decisions. These findings highlight the potential clinical importance of using free testosterone to confirm a biochemical diagnosis of hypogonadism.[216]

Transient Suppression of Testosterone

In evaluating men for a diagnosis of male hypogonadism, it is important to recognize that serum testosterone concentrations may be suppressed transiently during acute (particularly critical) and subacute illness and recovery; with the short-term use of certain medications, such as opioids, high-dose glucocorticoids, and CNS-active medications or recreational drugs that suppress gonadotropin and testosterone production; and during transient malnutrition, such as that associated with illness, eating disorders, or excessive or prolonged strenuous exercise (resulting in low energy intake relative to energy expenditure). In such situations, measurement of serum testosterone should be delayed until the patient is completely recovered from the illness, the offending drugs are discontinued, the malnutrition is corrected, or the excessive exercise is stopped.[116]

Screening and Case Finding for Androgen Deficiency

In the absence of evidence for long-term clinically meaningful health benefits greater than risks for testosterone treatment of androgen deficiency, screening for androgen deficiency in the general population or in all elderly men is not indicated. In addition, existing screening instruments lack sufficient specificity and sensitivity to be clinically useful.[116] In certain clinical conditions, case finding is warranted because hypogonadism is common. These conditions include hypothalamic-pituitary disease, mass, surgery, or radiation therapy; medications that suppress testosterone production (e.g., opioids, glucocorticoids); withdrawal from long-term androgenic anabolic steroid use; HIV-associated weight loss and other wasting syndromes; infertility; osteoporosis or minimal-trauma fragility fracture, especially in young men; and low libido or erectile dysfunction.[116]

Seminal Fluid Analysis

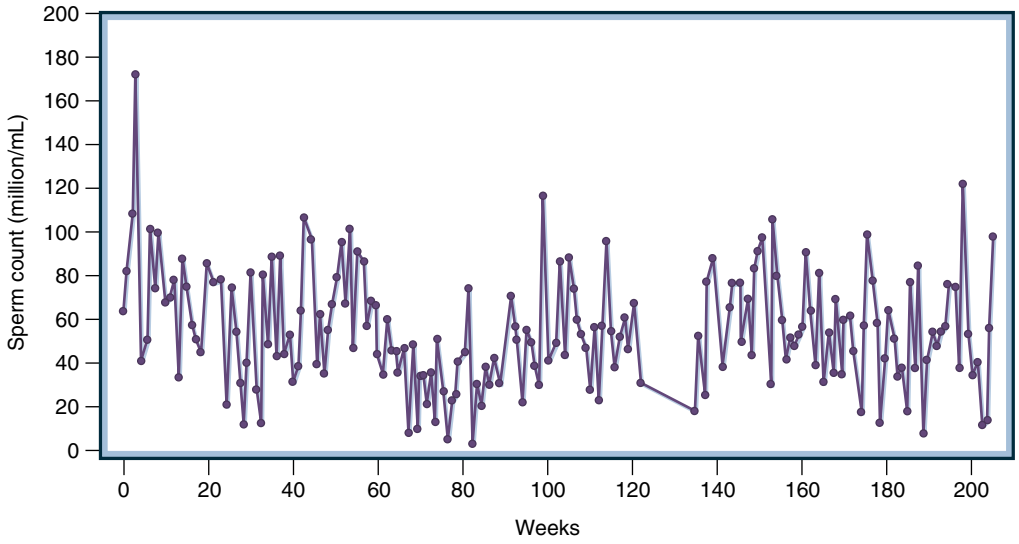
If infertility is a main complaint, whether or not androgen deficiency is also present, seminal fluid analysis should be performed to determine the presence and degree of impairment of sperm production. Seminal fluid analyses are performed on ejaculated semen samples obtained by masturbation after a standardized period (of at least 48 hours and not more than 7 days) of abstinence from ejaculation. Semen collection after withdrawal of the penis from the partner just before ejaculation during sexual intercourse (coitus interruptus) is usually incomplete and is not recommended, but it may be an option if masturbation is not possible or is not permitted for personal or religious reasons. Seminal fluid analyses should be performed in a specialized laboratory that employs standardized procedures, such as those outlined by the WHO, and is certified and qualified to carry out these procedures.[29]

According to recently revised WHO criteria based on approximately 1800 to 1900 men from 14 countries whose partners became pregnant in 12 months or less (Table 19.6), the lower limit of normal sperm concentration is 15 million/mL; semen volume is 1.5 mL; total sperm count is 39 million per ejaculate; total sperm motility (both progressive and nonprogressive) is 40% and progressive sperm motility is 32%; and the percentage of sperm with normal morphologic forms, using strict criteria to eliminate spermatozoa with even mild abnormalities, is at least 4%.[29] Values below these lower limits may be classified as falling into the subfertile range, and such values are found in independent (unscreened) populations. In another study, the subfertile ranges were defined as follows: sperm concentration, less than 13.5 million/mL; motility, less than 32%; and strict morphologic structure, less than 9%. The respective fertile ranges were greater than 48 million/mL for sperm concentration, 63% for motility, and 12% for strict morphologic structure. Values between these ranges indicated indeterminate fertility.[217]

TABLE 19.6 Normal Seminal Fluid Analysis

Parameter	Normal Value
Sperm concentration	≥15 million/mL
Semen volume	≥1.5 mL
Sperm count	≥39 million per ejaculate
Sperm motility	≥40% (progressive sperm motility >32%)
Sperm morphology	≥4% normal forms (by strict criteria excluding sperm with mild abnormalities)

From *WHO Laboratory Manual for the Examination and Processing of Human Semen*. 5th ed. Geneva, Switzerland: World Health Organization; 2010.



• **Fig. 19.20** Normal variation in sperm concentration (millions of spermatozoa per milliliter of ejaculate) in a healthy young man: results of frequent sampling over a period of 210 weeks. At several times during this period, sperm concentrations dropped below the normal range (15 million/mL). (From Matsumoto AM. The testis. In: Felig P, Frohman LA, eds. *Endocrinology and Metabolism,* 4th ed. New York, NY: McGraw-Hill; 2001:635–705.)

Sperm counts and concentrations exhibit extreme variability[9] (Fig. 19.20) for several reasons, including variations in sexual activity and abstinence, completeness of collection, recent illness (especially febrile illness) that may suppress spermatogenesis, and lifestyle factors such as frequent and prolonged hot tub use. Therefore, at least two or three seminal fluid analyses, separated by at least 1 to 2 weeks, should be performed to assess sperm production adequately. In addition, to assess motility, freshly collected semen (within 1 hour of ejaculation) should be used, necessitating collection at or near the laboratory in which the analysis is to be performed.

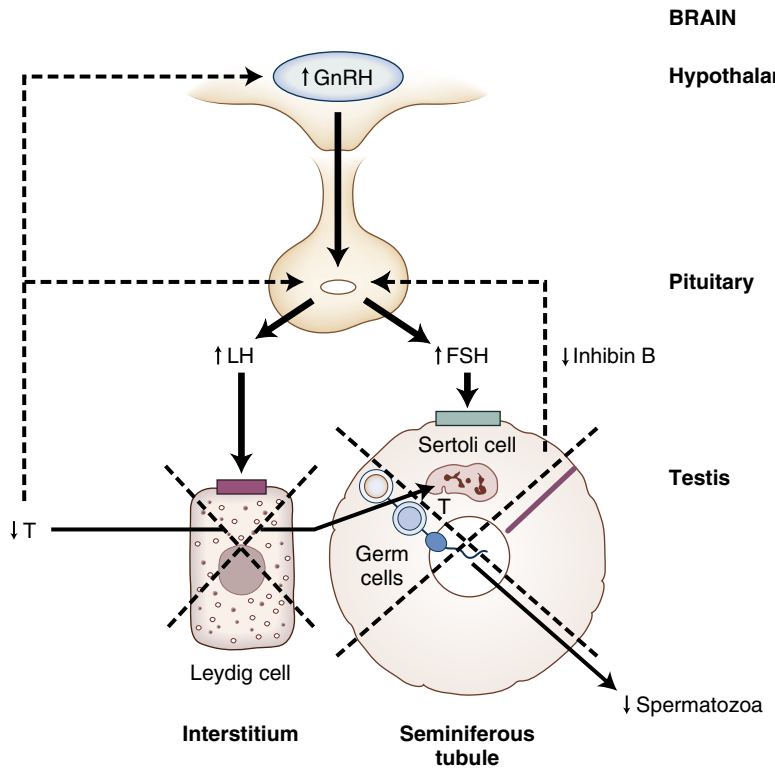
Gonadotropin Measurements

Androgen Deficiency and Impaired Sperm Production

The diagnosis of hypogonadism is confirmed in men with symptoms and signs consistent with androgen deficiency and in whom low testosterone concentrations are found on at least two occasions. Testosterone concentrations should not be measured shortly after an acute illness, medication use, or nutritional deficiency that could transiently lower testosterone. Furthermore, an accurate assay of free or bioavailable testosterone should be performed for men who have conditions that alter SHBG or a total testosterone level near the lower limit of normal. In men with confirmed hypogonadism, measurements of serum gonadotropins, LH, and FSH should be measured to distinguish primary from secondary hypogonadism[116] (see Fig. 19.19).

Men with primary hypogonadism caused by a disorder of the testis have low serum testosterone in association with elevated LH and FSH concentrations as a result of reduced negative feedback suppression of gonadotropin secretion by testosterone and inhibin B (FSH concentrations are higher than LH concentrations in men with primary hypogonadism) [9] (Fig. 19.21). In contrast, men with secondary hypogonadism caused by a disorder of the pituitary or the hypothalamus (or both) have low testosterone in association with low gonadotropin concentrations or inappropriately normal LH and FSH given the presence of low testosterone concentrations[9] (Fig. 19.22). In most local clinical laboratories, LH and FSH are measured by newer-generation nonradioactive immunoassays that have sufficient sensitivity to distinguish between normal and low concentrations. Although there are differences in reference ranges for LH and FSH among commercial gonadotropin assays, they are relatively minor, and a consensus reference range in healthy eugonadal young men for LH is 1.6 to 8.0 IU/L and FSH is 1.3 to 8.4 IU/L.[189] Reference ranges in which the upper limit is higher than these have included older men or men with unrecognized impairment of spermatogenesis.

Aging, some systemic illnesses (e.g., iron overload syndromes), and certain medications (e.g., glucocorticoids) may cause defects in both the testes and the hypothalamus or pituitary, resulting in combined primary and secondary hypogonadism. In most cases, a hormonal pattern consistent with either primary or secondary hypogonadism predominates. For example, men with hemochromatosis have defects in both the pituitary and the testes due to iron overload, but they usually have low testosterone and gonadotropin concentrations, a hormonal pattern consistent with secondary hypogonadism. Men with late-stage CKD have both testis and hypothalamic-pituitary dysfunction but usually have low testosterone and elevated LH and FSH concentrations, the latter mostly due to reduced clearance of gonadotropins by the kidney. However, in the presence of comorbid illness, nutritional deficiency, or certain medications, men with CKD may have suppression of gonadotropins and testosterone into the normal range and hormonal pattern consistent with secondary hypogonadism. Some men have more than one disorder influencing the gonadal axis, one affecting the testis and another affecting the hypothalamus or pituitary. This may result in a hormonal pattern that is predominantly consistent with either primary or secondary hypogonadism or in a combined pattern (e.g., very low testosterone concentrations with only slightly elevated or high-normal gonadotropin concentrations that are lower than expected given the presence of very low testosterone concentrations).



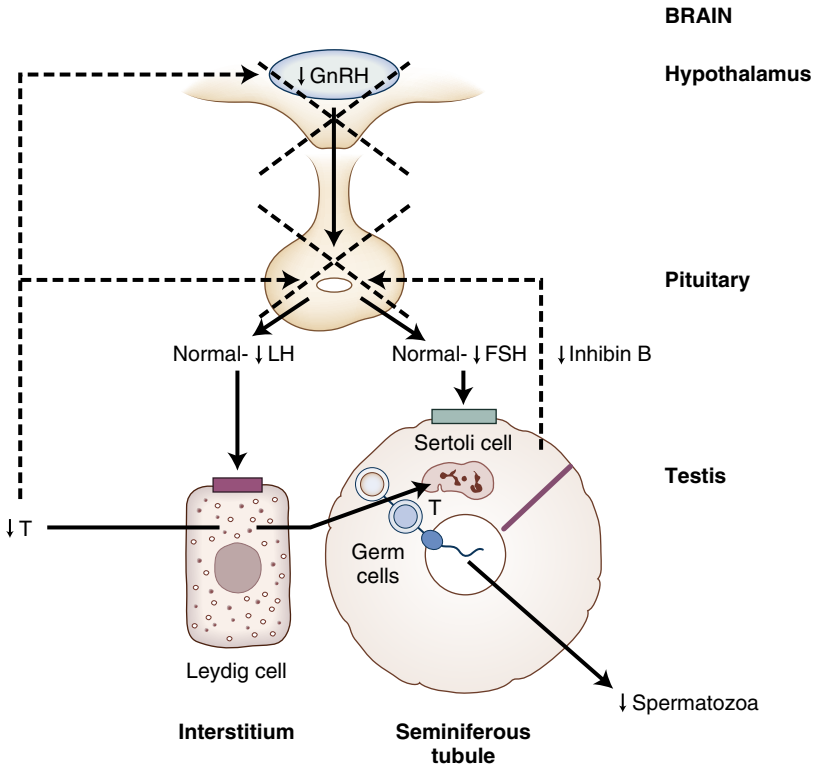
• **Fig. 19.21** Schematic diagram of alterations in the hypothalamic-pituitary-testicular axis with primary hypogonadism due to testicular disease, which results in both androgen deficiency and impairment of sperm production and elevated luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations (with FSH concentrations usually higher than LH concentrations) due to loss of negative feedback regulation of gonadotropins. *GnRH,* gonadotropin-releasing hormone; *T,* testosterone. (Modified from Matsumoto AM. The testis. In: Felig P, Frohman LA, eds. *Endocrinology and Metabolism.* 4th ed. New York, NY: McGraw-Hill; 2001:635–705.)

Distinguishing primary from secondary hypogonadism helps define the specific cause of hypogonadism and has important clinical and therapeutic implications.[9] Secondary hypogonadism may be caused by a destructive process in the pituitary or hypothalamus, such as a pituitary adenoma (i.e., organic causes). A large pituitary adenoma (macroadenoma) may cause space-occupying tumor mass effects such as headaches, visual field defects, hydrocephalus, or cerebrospinal fluid rhinorrhea, or it may result in impaired or excessive secretion of some anterior pituitary hormones, leading to clinical manifestations and therapeutic implications beyond the treatment of androgen deficiency alone. Secondary hypogonadism may be caused by disorders that are transient, such as an acute illness, certain medications (e.g., opioids, glucocorticoids), or malnutrition associated with illness (i.e., functional causes of gonadotropin suppression). In such cases, androgen deficiency may resolve with treatment of and recovery from the illness or malnutrition or discontinuation of the offending medication. Finally, in men with secondary hypogonadism who have gonadotropin deficiency but otherwise normal testes, gonadotropin or GnRH treatment may be used to stimulate spermatogenesis and androgen production and to restore fertility in men who wish to father children. In contrast, infertility in men with primary hypogonadism is not treatable with hormone therapy.

Isolated Impairment of Sperm Production or Function

Most men with isolated impairment of sperm production have low sperm counts or abnormalities in sperm motility or morphologic appearance (or both) but no clinical manifestations of androgen deficiency and normal concentrations of testosterone and gonadotropins. Most men with isolated impairment of sperm production or function are classified as having primary hypogonadism with an isolated defect in the seminiferous tubule compartment of the testes (Table 19.7); in such cases, there is no improvement in spermatogenesis with gonadotropin treatment like there is in secondary hypogonadism. Men with severe seminiferous tubule failure and azoospermia or severe oligozoospermia may demonstrate a selective elevation in FSH concentrations as a result in reduced negative feedback from inhibin B with normal LH concentrations[9] (Fig. 19.23). Occasionally, isolated impairment in sperm production is caused by gonadotropin deficiency (i.e., secondary hypogonadism) (Table 19.8); this may occur in men who are taking high doses of testosterone and in those who have androgen-secreting tumors; congenital adrenal hyperplasia; or, rarely, isolated FSH deficiency.

Men with nonfunctioning or gonadotropin-secreting pituitary macroadenomas often have secondary hypogonadism with clinical manifestations of androgen deficiency and low testosterone concentrations.[53] Many of these men secrete excessive amounts of



• **Fig. 19.22** Schematic diagram of alterations in the hypothalamic-pituitary-testicular axis with secondary hypogonadism due to hypothalamic or pituitary disease, which results in both androgen deficiency and impairment of sperm production and inappropriately normal or low luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations. *GnRH,* gonadotropin-releasing hormone; *T,* testosterone. (Modified from Matsumoto AM. The testis. In: Felig P, Frohman LA, eds. *Endocrinology and Metabolism,* 4th ed. New York, NY: McGraw-Hill; 2001:635–705.)

biologically inactive but immunoassay-detectable intact FSH, free α-, FSHβ-, and LHβ-subunits but rarely immunoassay-detectable intact LH. Therefore, a gonadotropin-secreting pituitary tumor should be suspected in a man who has clinical manifestations of androgen deficiency, low testosterone, and elevated FSH but normal or low LH (or, rarely, elevated LH but normal or low FSH), which is an atypical gonadotropin pattern for men with androgen deficiency.

Rarely, disorders of androgen action or androgen resistance manifest in adults (Table 19.9). These men usually present with clinical manifestations similar to those of men with mild androgen deficiency, usually with an almost normal male phenotype and frequently with varying degrees of hypospadias, cryptorchidism, scrotal abnormalities, or impairment in sperm production. Usually, both serum testosterone and gonadotropin concentrations are elevated (with LH concentrations higher than FSH concentrations) [9] (Fig. 19.24).

Further Evaluation

Testosterone measurements and assessment of sperm production, combined with measurement of gonadotropin concentrations, allow classification of the causes of male hypogonadism into primary or secondary hypogonadism and subclassification of the latter into disorders causing both androgen deficiency and impairment in sperm production and those causing isolated impairment of sperm production or function (see Tables 19.7 and 19.8).

Once hypogonadism has been classified as primary or secondary hypogonadism, further evaluation includes a history (including medication review), physical examination, and laboratory testing to identify a specific cause or causes of hypogonadism. In men with primary hypogonadism and suggestive clinical manifestations such as very small testes (e.g., <6 mL) and gynecomastia, low or low-normal testosterone, azoospermia, and markedly elevated gonadotropins, a karyotype may be obtained to confirm the diagnosis of Klinefelter syndrome.

In men with secondary hypogonadism, further evaluation may include measurements of serum prolactin (in almost all cases) to exclude hyperprolactinemia; iron saturation and ferritin to screen for hereditary hemochromatosis, especially in men with other manifestations of iron overload (e.g., liver failure, diabetes, and CHF) and in young men with unexplained selective gonadotropin deficiency; further testing to exclude excessive secretion or deficiency of anterior pituitary hormones; and magnetic resonance imaging (MRI) of the sella turcica to exclude a pituitary or hypothalamic tumor or infiltrative disease. Computed tomography (CT) of the sella turcica usually detects a pituitary macroadenoma but is less sensitive than sella MRI in detecting smaller tumors and infiltrative disease; CT is more sensitive in detecting parasellar bone destruction.[116]

It is not cost effective to perform sella MRI in all men with secondary hypogonadism. This modality should be reserved for men with secondary hypogonadism and the following: serum

TABLE 19.7 Causes of Primary Hypogonadism

Common Causes	Uncommon Causes
Androgen Deficiency and Impairment of Sperm Production	
Congenital or Developmental Disorders	
Klinefelter syndrome (XXY) and variants	Myotonic dystrophy Uncorrected cryptorchidism Noonan syndrome Bilateral congenital anorchia Polyglandular autoimmune syndrome Testosterone biosynthetic enzyme defects CAH (TART) Complex genetic syndromes Down syndrome LH receptor mutation
Acquired Disorders	
Bilateral surgical castration or trauma Drugs (spironolactone, ketoconazole, abiraterone, enzalutamide, alcohol, chemotherapy agents)[a] Ionizing radiation	Orchitis
Systemic Disorders	
Chronic liver disease (hepatic cirrhosis)[a,b]	Malignancy (lymphoma, testicular cancer)
Chronic kidney disease[a,b]	Sickle cell disease[b]
Aging[b]	Spinal cord injury Vasculitis (polyarteritis) Infiltrative disease (amyloidosis, leukemia)
Isolated Impairment of Sperm Production or Function	
Congenital or Developmental Disorders	
Cryptorchidism	Myotonic dystrophy
Varicocele	Sertoli cell–only syndrome
Y chromosome microdeletions	Primary ciliary dyskinesia Down syndrome FSH receptor mutation
Acquired Disorders	
Orchitis Ionizing radiation Chemotherapy agents Thermal trauma	Environmental toxins
Systemic Disorders	
Acute febrile illness Malignancy (testicular cancer, Hodgkin disease)[b] Idiopathic azoospermia or oligozoospermia	Spinal cord injury

[a]Functional causes that are potentially reversible with discontinuation of offending medication, or with liver or renal transplantation.

[b]Combined primary and secondary hypogonadism.

CAH, Congenital adrenal hyperplasia; *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone; *TART,* testicular adrenal rest tumor.

testosterone less than 150 ng/dL and low gonadotropin concentrations[218-220]; discordant LH and FSH concentrations (i.e., one gonadotropin markedly elevated but the other not); hyperprolactinemia (even mild); clinical and biochemical evidence of excessive secretion of other pituitary hormones (e.g., free α-subunit secretion, Cushing syndrome, acromegaly), panhypopituitarism, or diabetes insipidus; tumor mass effects (e.g., severe headache, new onset of blurry vision or visual impairment, visual field defects, or cerebrospinal fluid rhinorrhea); and no obvious functional cause of secondary hypogonadism (e.g., morbid obesity, long-acting opioid, or chronic high-dose glucocorticoid therapy).

In evaluating the etiology of secondary hypogonadism, it is important to consider potentially reversible/treatable causes of gonadotropin suppression that are more common than organic congenital or destructive causes of gonadotropin deficiency and might be managed by discontinuation of an offending medication (e.g., opioid pain medication), treatment of the underlying condition (e.g., obesity), or organ transplantation (e.g., renal transplantation).

In men who have severe androgen deficiency caused by primary or secondary hypogonadism or who have sustained a low-trauma or fragility fracture, dual-energy x-ray absorptiometry scanning to assess BMD should be performed to exclude osteopenia or osteoporosis.[116]

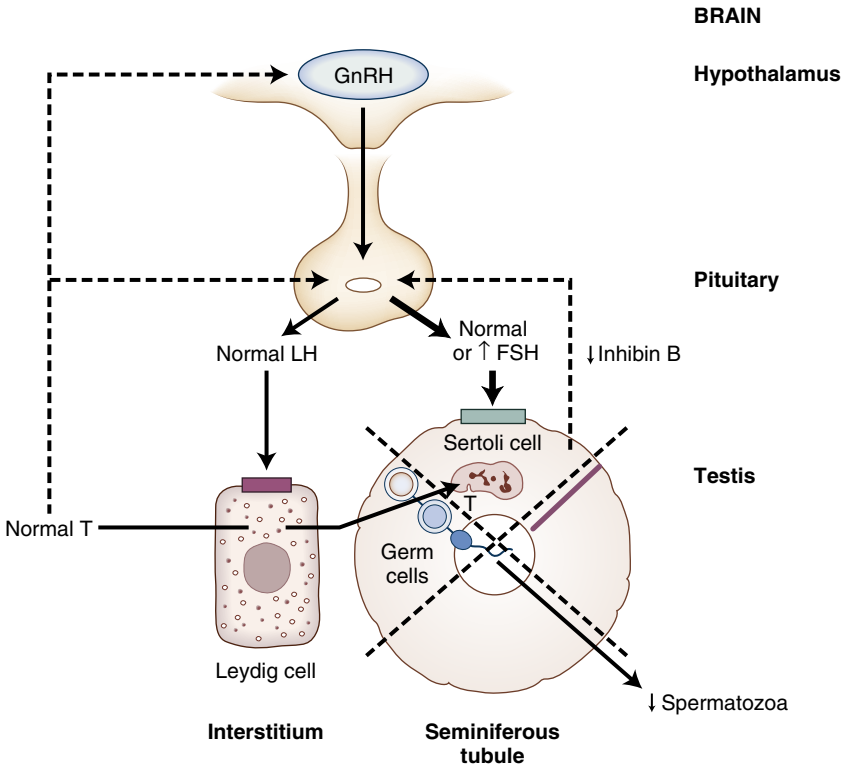
Causes of Primary Hypogonadism

Androgen Deficiency and Impairment in Sperm Production

Congenital or Developmental Disorders

Klinefelter Syndrome. Classically, Klinefelter syndrome is characterized by very small, firm testes; azoospermia and infertility; varying degrees of androgen deficiency and eunuchoidism; and uniformly elevated gonadotropin concentrations.[20,221,222] It is the most common sex chromosome abnormality and the most common cause of primary hypogonadism, resulting in androgen deficiency and impaired sperm production. It occurs prenatally and neonatally in 1 of every 500 to 700 males, and the prevalence in adults is 1 in 2500.[1,2] Because the syndrome does not cause premature death in boys, the low adult prevalence indicates that Klinefelter syndrome is often overlooked and underdiagnosed in men. Given the almost uniform finding of extremely small testes and other phenotypic abnormalities, it is surprising that about 75% of men with Klinefelter syndrome are never diagnosed. This might be due to failure to recognize mild phenotypes or failure to perform a testicular exam. The risk of having a child with Klinefelter syndrome increases with both maternal and paternal age.

The chromosomal abnormality in Klinefelter syndrome is the presence of one or more extra X chromosomes due to maternal meiotic nondisjunction (mostly in meiosis I) in approximately 50% of the cases or paternal meiotic nondisjunction in the remaining cases.[20,221,222] The principal karyotype in 90% of men with Klinefelter syndrome is 47,XXY. Most of the remaining 10% have mosaic Klinefelter syndrome (47,XXY/46,XY), in which there is a 47,XXY karyotype in some tissues or cells and a normal 46,XY karyotype in other tissues or cells. Mosaicism occurs as a result of postfertilization mitotic nondisjunction. Men with mosaic Klinefelter syndrome usually demonstrate a variable and less severe phenotype that depends on the specific tissues in which an extra X chromosome is present. Some men with mosaicism have a normal karyotype in the testis with intact spermatogenesis and fertility. Rarely, men with Klinefelter syndrome have more



• **Fig. 19.23** Schematic diagram of alterations in the hypothalamic-pituitary-testicular axis with an isolated seminiferous tubule defect that results in isolated impairment of sperm production or function. Follicle-stimulating hormone (FSH) concentrations may be normal or selectively elevated (if defect in spermatogenesis is severe) with normal testosterone and luteinizing hormone (LH) concentrations. *GnRH,* gonadotropin-releasing hormone; *T,* testosterone. (Modified from Matsumoto AM. The testis. In: Felig P, Frohman LA, eds. *Endocrinology and Metabolism.* 4th ed. New York, NY: McGraw-Hill; 2001:635–705.)

than one extra X chromosome (e.g., 48,XXXY, 49,XXXXY). Men with these variants manifest a more severe phenotype than is seen in classic Klinefelter syndrome.

Infants with Klinefelter syndrome may manifest micropenis, hypospadias, cryptorchidism, or developmental delay.[222,223] During childhood, boys with the syndrome commonly have small testes and reduced penile length relative to age-matched normal individuals and may manifest relatively tall stature, clinodactyly, hypertelorism, gynecomastia, elbow dysplasia, high-arched palate, hypotonia, language delay or learning and reading disabilities requiring therapy, and behavioral problems.[222,224] However, these manifestations may be mild and are often missed. Fewer than 10% of boys with Klinefelter syndrome (usually those with the most severe phenotype) are diagnosed before puberty. At puberty, the testes fail to increase in size and become firm due to a progressive loss of germ cells and seminiferous tubule hyalinization and fibrosis; Sertoli cell products, inhibin B, and AMH decline to very low or undetectable serum concentrations; circulating testosterone concentrations increase but to less than normal concentrations in some boys, resulting in varying degrees of eunuchoidism and gynecomastia; and FSH concentrations are disproportionately elevated relative to LH concentrations. Elevation of serum LH disproportionately to FSH is an indication of a disorder other than Klinefelter syndrome or any cause of primary hypogonadism that affects both Sertoli cell and Leydig cell dysfunction—for example, rare diseases such as disorders of testosterone biosynthesis or abnormalities of LH or its receptor should be considered.

In adults, the most prominent and consistent clinical feature of Klinefelter syndrome is very small testes, less than 4 mL in volume (<2.5 cm in length); this feature is easily detected on examination and should alert the clinician to the possibility of Klinefelter syndrome[8,225] (see Fig. 19.18). Men with this syndrome may present with a complaint of infertility and subsequently be found to have azoospermia and very small testes.[226] Other manifestations include varying degrees of androgen deficiency, eunuchoidism, and gynecomastia[221] (Fig. 19.25). In contrast to the classic long arms and legs of eunuchoidism seen in patients with androgen deficiency of prepubertal onset, Klinefelter syndrome results in a disproportionate increase in lower- compared with upper extremity long bone growth. Gynecomastia occurs in 50% to 80% of men with the syndrome and may be quite prominent and embarrassing. Learning and developmental disabilities occur in about 70% of men who are diagnosed with Klinefelter syndrome; the prevalence of cognitive impairment might be lower in men with undiagnosed Klinefelter syndrome. Character and personality disorders and behavioral problems occur commonly, possibly in part because of the psychosocial consequences of androgen deficiency and learning disabilities. Men with Klinefelter syndrome have intelligence quotient scores that are reduced by 10 to 15 points but not into the intellectual disability range. Taurodontism,

TABLE 19.8 Causes of Secondary Hypogonadism

Common Causes	Uncommon Causes
Androgen Deficiency and Impairment of Sperm Production	
Congenital or Developmental Disorders	
Constitutional delayed puberty	CHH due to genetic mutations
Hemochromatosis	CHH, idiopathic (IHH) Kallmann syndrome Congenital adrenal hypoplasia Isolated LH deficiency, LHβ mutations Complex genetic syndromes
Acquired Disorders	
Hyperprolactinemia[a] Opioids[a] Androgenic anabolic steroids, progestins, estrogen excess[a] GnRH agonist or antagonist[a]	Hypopituitarism
	Pituitary or hypothalamic tumor Surgical hypophysectomy, pituitary or cranial irradiation Vascular compromise, traumatic brain injury Granulomatous or infiltrative disease Infection Pituitary stalk disease Lymphocytic or autoimmune hypophysitis Acquired IHH
Systemic Disorders	
Glucocorticoid excess (Cushing syndrome)[a,b]	Chronic systemic illness[a,b]
Chronic organ failure[a,b]	Spinal cord injury
Chronic liver disease (hepatic cirrhosis), chronic kidney disease, chronic lung disease, chronic heart failure[a]	Transfusion-related iron overload (β-thalassemia)[a]
Chronic systemic illness[a,b]	Sickle cell disease
Type 2 diabetes mellitus[a] Malignancy[a] Rheumatic disease (rheumatoid arthritis)[a] HIV disease[a] Starvation,[b] malnutrition,[b] eating disorders, endurance exercise[a] Morbid obesity, obstructive sleep apnea[a] Acute and critical illness[a] Aging (comorbid illnesses associated with aging)[a,b]	Cystic fibrosis
Isolated Impairment of Sperm Production or Function	
Congenital or Developmental Disorders	
	Congenital adrenal hyperplasia (21-hydroxylase deficiency, 11β-hydroxylase deficiency) Isolated FSH deficiency, FSHβ mutations
Acquired Disorders	
Testosterone, androgenic anabolic steroids	Androgen- or hCG-secreting tumors
Malignancy (Hodgkin disease, testicular cancer)[b]	Hyperprolactinemia

[a]Functional causes that are potentially reversible or treatable with discontinuation of offending medication, treatment of underlying cause of gonadotropin suppression or organ transplantation.

[b]Combined primary and secondary hypogonadism.

CHH, Congenital hypogonadotropic hypogonadism; *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *hCG,* human chorionic gonadotropin; *HIV,* human immunodeficiency virus; *IHH,* idiopathic hypogonadotropic hypogonadism; *LH,* luteinizing hormone.

TABLE 19.9 Causes of Androgen Resistance

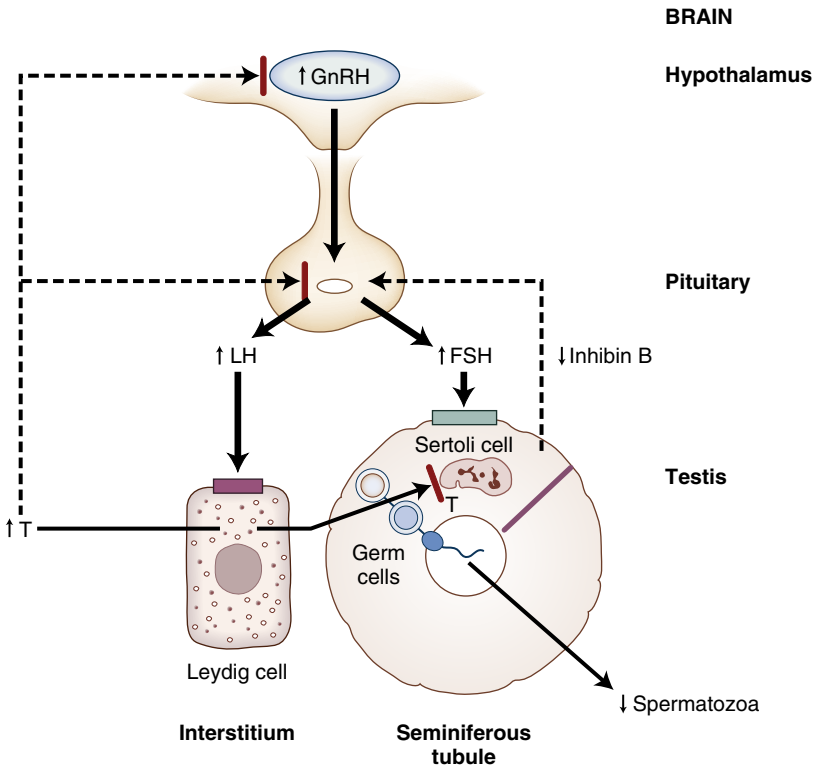
Common Causes	Uncommon Causes
Congenital or Developmental Disorders	
	Kennedy disease (spinal and bulbar muscular atrophy)
	Partial androgen insensitivity syndrome (PAIS; 40–60% with AR mutations)
	5α-Reductase type 2 deficiency (not a syndrome of androgen insensitivity, but it presents similarly to PAIS)
	Complete androgen insensitivity syndrome (female phenotype)
Acquired Disorders	
AR antagonists (bicalutamide, nilutamide)	Celiac disease
Drugs (spironolactone, cyproterone acetate)	

AR, Androgen receptor; *PAIS*, partial androgen insensitivity syndrome.

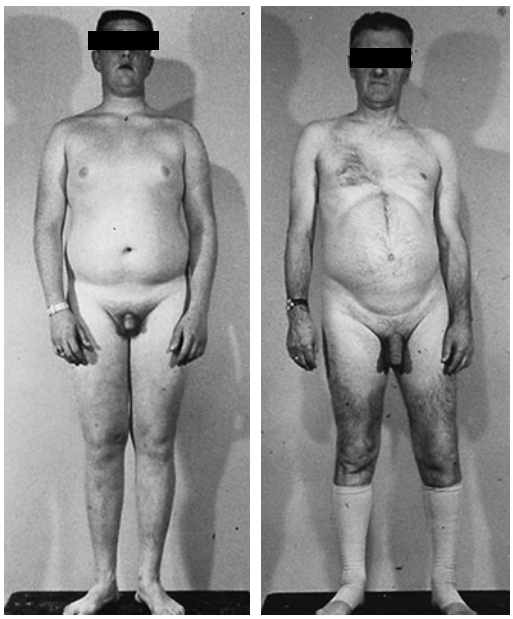
characterized by enlarged molar teeth resulting from enlargement and extension of the pulp chamber, is present in 40% of men with Klinefelter syndrome.

As described previously, the *AR* gene is located on the X chromosome, and the length of the highly polymorphic CAG repeat in exon 1 of the *AR* gene is inversely related to AR activity. In Klinefelter syndrome, the X chromosome carrying the *AR* gene with a short CAG repeat length (i.e., greater AR activity) undergoes inactivation preferentially.[139] Klinefelter syndrome patients with short CAG repeat lengths were found to have more stable relationships, higher educational concentrations, and greater responses to testosterone treatment. In contrast, men with long CAG repeat length (i.e., reduced AR activity) had longer arms and legs, smaller testes, a greater degree of gynecomastia, and lower BMD. Therefore, skewed inactivation of the X chromosome resulting in preferential activity of the long CAG repeat may contribute to phenotypic severity and variability of Klinefelter syndrome.

Most men with mosaic Klinefelter syndrome exhibit less severe clinical manifestations than those with the classic syndrome. Men with more than two extra X chromosomes have more severe manifestations and a higher incidence of intellectual disability and somatic abnormalities such as hypospadias, cryptorchidism, and radioulnar synostosis. Very rarely, some phenotypic males with a 46,XX karyotype exhibit typical clinical manifestations



• **Fig. 19.24** Schematic diagram of alterations in the hypothalamic-pituitary-testicular axis with androgen resistance due to impaired androgen action (e.g., androgen receptor mutation), which results in high testosterone (T) concentrations but reduced androgen action. This leads to manifestations of androgen deficiency, impaired sperm production, and elevated luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations (with LH concentrations that are higher than FSH concentrations) due to impaired androgen-mediated negative feedback regulation of gonadotropins. *GnRH,* gonadotropin-releasing hormone. (Modified from Matsumoto AM. The testis. In: Felig P, Frohman LA, eds. *Endocrinology and Metabolism.* 4th ed. New York, NY: McGraw-Hill; 2001:635–705.)

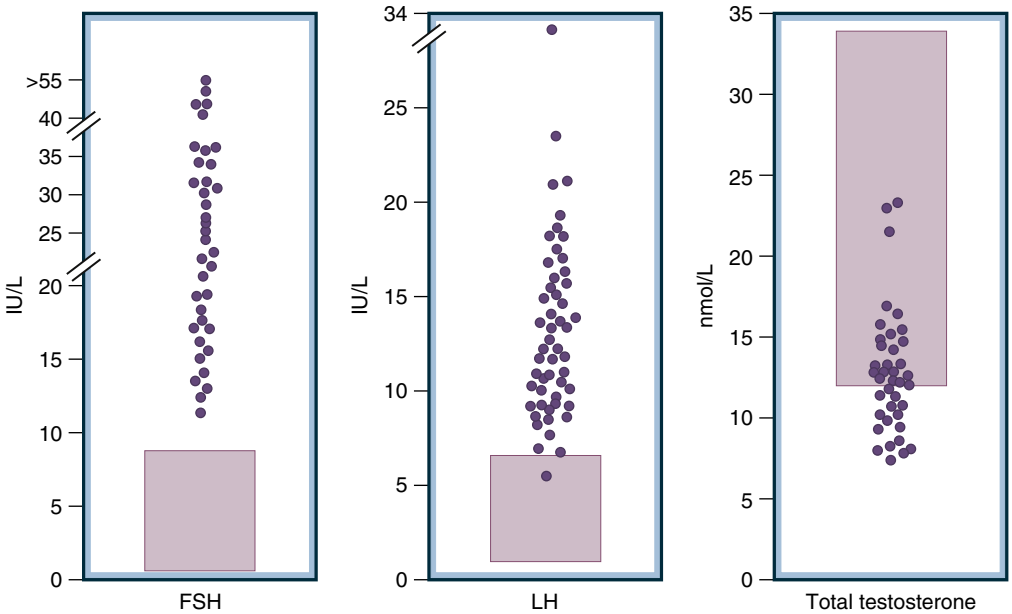


• **Fig. 19.25** Variability in the degree of androgen deficiency manifested in men with Klinefelter syndrome. The man on the left, who has classic 47,XXY Klinefelter syndrome, demonstrates androgen deficiency of prepubertal onset with eunuchoidal body proportions, small penis, sparse chest and pubic hair, poor muscle development, prepubertal fat distribution, and very small testes (2 mL bilaterally). The man on the right, who has mosaic 47,XXY/46,XY Klinefelter syndrome, demonstrates normal body proportions, penis size, and body hair but small testis size (8 mL bilaterally). (From Smyth CM, Bremner WJ. Klinefelter syndrome. *Arch Intern Med.* 1998;158:1309–1314.)

of Klinefelter syndrome but have shorter stature and a greater incidence of cryptorchidism, gynecomastia, and androgen deficiency.[227] In most of these cases, there has been a translocation of an SRY-containing segment of the Y chromosome onto an X chromosome.

In addition to infertility, variable androgen deficiency, and gynecomastia, patients with Klinefelter syndrome have an approximately 20- to 50-fold increased risk in breast cancer compared with normal men (although the absolute lifetime risk of <1% is low); such patients account for approximately 4% of all cases of male breast cancer.[228] Klinefelter syndrome is also associated with increased risk for mitral valve prolapse; lower extremity varicose veins, venous stasis ulcers, deep vein thrombosis, and pulmonary embolism; autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and Sjögren syndrome; other cancers such as extragonadal germ cell cancer and non-Hodgkin lymphoma; T2DM and the metabolic syndrome; and psychiatric illnesses such as depression and schizophrenia. There is a small reduction in life expectancy of approximately 5 to 6 years in men with Klinefelter syndrome, associated with a higher rate of death due to a variety of common causes.[222,229,230]

Azoospermia is present in more than 95% of men with classic Klinefelter syndrome and infertility in more than 99%. Serum total testosterone concentrations are usually low but may fall in the low-normal to mid-normal range in 40% to 50% of cases[20,221,231] (Fig. 19.26). Relative to normal men, some men with Klinefelter syndrome have relatively high serum estradiol concentrations, which probably contributes to the development of gynecomastia, and increased SHBG concentrations. Increased SHBG concentrations provide a partial explanation for normal total testosterone concentrations in the presence of low concentrations of free testosterone. Serum FSH concentrations are almost always elevated, and LH concentrations are usually elevated but may fall into the high-normal range in some men, consistent



• **Fig. 19.26** Serum concentrations of follicle-stimulating hormone (FSH, *left*), luteinizing hormone (LH, *middle*), and total testosterone (T, *right*) in men with Klinefelter syndrome (*dots*) compared with normal men (normal range depicted by *shaded boxes*). A substantial proportion of men with Klinefelter syndrome have total T concentrations within the normal range, but almost all have elevated LH and FSH concentrations. (From Smyth CM, Bremner WJ. Klinefelter syndrome. *Arch Intern Med.* 1998;158:1309–1314.)

with primary hypogonadism[20,221,231] (see Fig. 19.26). Symptoms of androgen deficiency in the presence of normal total and free testosterone and elevated gonadotropin concentrations occur in some men with Klinefelter syndrome, suggesting relative androgen deficiency or resistance.

The diagnosis of Klinefelter syndrome is confirmed by *karyotype analysis*, which is usually performed on cultured peripheral blood lymphocytes. Occasionally, karyotype analysis is performed on cultured skin fibroblasts and testis tissue if mosaicism is suspected. If a fetus is diagnosed on prenatal testing with 47,XXY Klinefelter syndrome, genetic counseling should be provided. Klinefelter syndrome has a generally good prognosis, but a minority of couples choose termination of a fetus with a prenatal diagnosis of Klinefelter syndrome.

Treatment of Klinefelter syndrome is directed at correction of androgen deficiency. Infants with micropenis may benefit from (systemic or penile topical) testosterone treatment. For boys with Klinefelter syndrome, early intervention with speech and reading therapy is important if speech delay and dyslexia are present. At puberty, testosterone treatment may be needed for adequate development of secondary sexual characteristics; peak bone mass and BMD; muscle mass and strength; and energy, motivation, mood, and behavior. Adults with Klinefelter syndrome who have clinical manifestations of androgen deficiency and consistently low concentrations of serum total or free testosterone (or both) should receive testosterone replacement therapy. In addition, men who have symptoms and signs of androgen deficiency but normal total and free testosterone concentrations should be offered a trial of testosterone treatment.

Although azoospermia and infertility are not generally reversible with medical therapy, TESE permits identification of the relatively few seminiferous tubules that contain active spermatogenesis and harvesting of sperm from 50% to 70% of men with Klinefelter syndrome for use in ICSI; this approach results in live birth rates of approximately 45% in specialized centers.[232] Because there is an increased risk of sex chromosome and autosomal aneuploidy, genetic counseling and prenatal or preimplantation testing should be provided to couples who undergo ICSI.

Chronic, nontender gynecomastia does not resolve with testosterone treatment and requires reduction mammoplasty. Psychological counseling for patients and spouses and participation in support groups may be extremely helpful for men with Klinefelter syndrome.

Myotonic Dystrophy. Myotonic dystrophy is an autosomal dominant, multisystem disorder that is characterized by progressive muscle weakness and wasting (particularly in the lower legs, hands, neck, and face) and results in physical disability; myotonia (involuntary sustained contraction of muscles); cataracts; cardiac conduction defects; respiratory insufficiency; dysphagia; testicular atrophy, impaired spermatogenesis, infertility, and androgen deficiency; premature frontal balding; and intellectual disability.[233] Features of the disease usually develop in young adults but can occur at any age, and their severity varies widely among affected individuals. Two types of myotonic dystrophies are caused by expansion of CTG trinucleotide repeats in two different genes. Myotonic dystrophy type 1 has more severe clinical features and is caused by CTG repeat expansion in the dystrophia myotonica protein kinase (*DMPK*) gene; it accounts for 98% of cases. Myotonic dystrophy type 2 is less severe and is caused by CTG repeat expansion in the CCHC-type zinc finger, nucleic acid–binding protein (*CNBP*) gene.

Primary hypogonadism occurs in approximately 80% of young to middle-age men with myotonic dystrophy.[234,235] Most of these men have isolated impairment of sperm production or function with testicular atrophy, oligozoospermia or azoospermia, moderate to severe testicular damage on testis biopsy, infertility, and disproportionately elevated FSH compared with LH concentrations. Approximately 20% to 40% of men with myotonic dystrophy have variable degrees of androgen deficiency with low testosterone concentrations and elevated LH and FSH; testosterone replacement therapy is appropriate for these men. High-dose testosterone therapy has been demonstrated to improve muscle mass but has not been shown to affect muscle strength.[236] Infertility due to myotonic dystrophy is not reversible with medical therapy, and patients who desire children need to pursue ART or other options; genetic counseling and preimplantation or prenatal testing should be provided, given the autosomal dominant nature of the disease.

Cryptorchidism. Cryptorchidism is the failure of one or both testes to descend normally from within the abdomen through the inguinal canal into the scrotum. It is the most common congenital disorder of the male genital tract in children, affecting 2% to 4% of full-term male infants.[237-239] It is more common in premature, low-birth-weight, and small-for-gestational-age infants. Spontaneous descent of the testis occurs during the first year in most infants (probably induced by the neonatal surge of gonadotropins and testosterone), so the prevalence of cryptorchidism in boys and adults is lower, approximately 0.3% to 1.0%. Because a patent processus vaginalis is usually also present, inguinal hernia is found in conjunction with cryptorchidism in 50% to 80% of cases. Both unilateral and bilateral cryptorchidism are associated with impaired sperm production, infertility, and an increased risk of testicular cancer.

In contrast to cryptorchidism, *ectopic testes* are located outside the normal path of testicular descent.[240] Ectopic testes may be located anywhere in the perineum or in the femoral or superficial inguinal regions. It is also important to distinguish cryptorchidism from *retractile testes* (pseudocryptorchidism). Retractile testes are located in the scrotum but withdraw into the inguinal canal or abdomen with minimal stimulation due to a hyperactive cremasteric reflex; retractile testes are not usually associated with impaired sperm production, infertility, or increased risk of testicular cancer. However, impaired spermatogenesis and fertility have been reported in men with bilateral retractile testes that are located high in the inguinal canal or, sometimes, in the abdomen.

Bilateral cryptorchidism may be associated with several disorders causing primary hypogonadism (including Klinefelter syndrome variants and Noonan syndrome), secondary hypogonadism (including CHH, Kallmann syndrome, and complex genetic disorders associated with multiple congenital anomalies or defects, e.g., Prader-Labhart-Willi syndrome or Laurence-Moon-Biedl syndrome), and androgen resistance syndromes (e.g., Reifenstein syndrome).[241] Cryptorchidism may also be caused by mutations of the Leydig cell product, INSL3, which controls growth of the gubernaculum or of its receptor (RXFP2) in up to 5% of cases.

Unilateral or bilateral cryptorchidism that is not related to known causes of hypogonadism or androgen resistance usually results in isolated impairment of sperm production associated with low sperm counts; normal testosterone concentrations; a selective elevation in FSH concentrations; and, occasionally, high LH concentrations.[237,242] Rarely, cryptorchidism causes Leydig cell failure and androgen deficiency (e.g., in adults with uncorrected bilateral cryptorchidism), producing low serum testosterone with high LH and FSH concentrations.[243,244] Azoospermia occurs in 50% to 60% and oligozoospermia in 75% to 100% of men with bilateral cryptorchidism; among men with unilateral cryptorchidism, these

figures respectively are 15% to 20% and 20% to 40%. This suggests that the function of both testes is compromised in unilateral cryptorchidism. An underlying developmental or environmental disorder affecting both testes (*testicular dysgenesis*) may contribute to impaired spermatogenesis in these individuals.[237,242] The formation of A_d spermatogonia from neonatal gonocytes is inhibited in undescended testes.[245] Uncommonly, normal testicular descent is impeded by an anatomic abnormality, such as a large external inguinal hernia. In this instance, both testes function normally, and orchiopexy before puberty is generally recommended to preserve spermatogenesis and fertility.

The risk of testicular cancer in an undescended testis is 2.5- to 8-fold greater than in a scrotal testis, and the risk remains higher even after the testis is surgically relocated into the scrotum, supporting the notion that cryptorchidism is a manifestation of a underlying testicular disorder (i.e., testicular dysgenesis).[237,241,242,246] Even though the incidence of testicular cancer is only 1 or 2 per 100,000 males, the lifetime risk of malignancy in a cryptorchid testis is substantial. The prevalence of testicular carcinoma in situ, which is thought to precede testicular cancer, is about 3% in the cryptorchid testis. Testicular cancer usually occurs in men from 20 to 40 years of age.

A careful physical examination should be performed to assess the location of testes within the scrotum or inguinal canal; the presence of an inguinal hernia, hydrocele, or other scrotal mass; the cremasteric reflex induced by stroking of the upper medial thigh; and penile size and the position of the urethral meatus. The examination should be performed with the patient standing, squatting, or supine with legs abducted. Scrotal examination may be difficult in a morbidly obese man with a large abdominal panniculus. Techniques that may be helpful in the detection of retractile testes include examination during a Valsalva maneuver, with pressure applied to the lower abdomen, or after scrotal warming with a warm towel to facilitate testicular descent. In addition, elicitation of the cremasteric reflex may cause a localized puckering of the scrotal skin if a retractile testis is present in the scrotum. Often, a low undescended testis may be confirmed by pushing with one hand on the lower abdomen, with firm strokes from the anterior superior iliac spine through the groin and to the pubis, toward and into the scrotum, and then grasping the testis with the other hand. After the testis has been held in the scrotum until the cremasteric muscle fatigues, it is released. If the testis then retracts, it is considered a retractile testis; a low cryptorchid testis returns to its undescended position after release. Absence of a palpable testis in the scrotum after repeated examinations may be a result of cryptorchidism, an extremely atrophic testis, or anorchia (absent testis). Up to 50% of men with a unilateral, nonpalpable testis in the scrotum have a severely atrophic or absent testis instead of cryptorchidism; in these instances, the contralateral testis may be relatively large (by about 2 mL). High-resolution ultrasonography (or MRI) usually localizes testes that are not palpable.

Treatment for persistent cryptorchidism should be started before puberty, when greater germ cell degeneration occurs.[237,242] The exact timing of treatment is controversial, but recent recommendations suggest that treatment should be instituted between 6 and 12 months or up to 24 months of age. Hormonal treatment with hCG in prepubertal boys is effective in stimulating descent of a cryptorchid testis in approximately 10% to 20% of cases. A trial of hCG therapy may be attempted in the hope of avoiding surgery. If hormonal treatment is unsuccessful or not attempted, an orchiopexy (surgical relocation and fixation of the testis into the scrotum with ligation of the hernia sac at the external or internal inguinal ring) should be performed to allow examination of the testes (e.g., as in monitoring for malignancy) and preservation of remaining testis function. Despite orchiopexy, spermatogenesis remains impaired and fertility rates are reduced, particularly with bilateral cryptorchidism (65% paternity rate after orchiopexy). If orchiopexy is performed before puberty, the risk of testicular cancer is reduced but is still increased two- to threefold. In patients with a history of unilateral cryptorchidism, the risk of malignancy in the contralateral testis is also increased. Because the risk of malignancy is two to six times higher in men who underwent orchiopexy after puberty and the fertility potential is poor, some clinicians recommend orchidectomy for men with cryptorchidism discovered after puberty. The majority of testis cancers found in persistently cryptorchid testes are seminomas, whereas those in cryptorchid testes after orchiopexy are mostly nonseminoma testicular cancers.

Noonan Syndrome. Noonan syndrome is an autosomal dominant or occasional sporadic genetic disorder that is characterized by short stature; unusual facial features (hypertelorism, downward-slanting eyes, ptosis, strabismus, low-set ears with thickened helices, high nasal bridge, micrognathia and triangular-shaped face, high-arched palate, low hairline, dental malocclusion); short, webbed neck; shield-like chest, pectus excavatum or carinatum, scoliosis, cubitus valgus, and joint laxity; intellectual disability; cardiac disease (pulmonary stenosis, hypertrophic cardiomyopathy); hepatosplenomegaly; lymphedema; and cryptorchidism.[247,248]

Because several these clinical features resemble those of females with Turner syndrome, Noonan syndrome was previously called *male Turner syndrome*. However, the karyotype in these men is normal. Noonan syndrome affects approximately 1 in 1000 to 2500 live births and is caused by mutations in genes in the Ras-MAPK signaling pathway.[247,248] Approximately 50% of men with Noonan syndrome have mutations in the protein tyrosine phosphatase nonreceptor type 11 (*PTPN11*) gene, and the remainder have mutations in the son of sevenless homolog 1 (*SOS1*), *RAF1*, or *KRAS* genes.

Men with Noonan syndrome may demonstrate primary hypogonadism characterized by androgen deficiency and impaired sperm production with elevated gonadotropin concentrations; they usually present with delayed puberty.[249] Cryptorchidism is present in more than 50% of men with the syndrome and contributes to the cause of hypogonadism.

Bilateral Congenital Anorchia. Congenital anorchia (also known as functional prepubertal castrate or vanishing testis syndrome) is a rare condition in which there is absence of one or both testes in a phenotypically and genotypically normal male.[250,251] Normal fetal testis function is needed for normal male internal and external genital differentiation and development during early gestation. The presence of otherwise normal male internal and external genitalia without müllerian duct–derived structures or descent of the spermatic cord structures (e.g., vas deferens, blood vessels) into the scrotum implies that normally functioning testes must have been present during the first 16 weeks of gestation and subsequently lost during fetal or neonatal life. The prevalence of bilateral congenital anorchia is 1 in 20,000, and that of unilateral congenital anorchia is 1 in 5000 males. The cause is not known but is probably heterogeneous. It is hypothesized that congenital anorchia may be caused by spermatic vascular compromise due to torsion or trauma during or after testicular descent.

Infants with bilateral anorchia present with micropenis in almost 50% of cases, supporting a prenatal origin of the

disorder.[250,251] Males with congenital anorchia usually present with prepubertal primary hypogonadism with delayed puberty and eunuchoidism (see Fig. 19.14), very low testosterone concentrations in the castrate range, and elevated gonadotropin concentrations. On examination, palpable testes are absent, but spermatic cords and epididymides are usually present. Normal testosterone and gonadotropin concentrations in pubertal or adult patients with absent testes exclude the diagnosis of congenital anorchia and should raise the possibility of bilateral cryptorchidism, which carries an increased risk for testicular malignancy.

An hCG stimulation test may be performed to distinguish congenital anorchia from bilateral cryptorchidism. In patients with congenital anorchia, serum testosterone concentrations do not increase in response to prolonged hCG administration (e.g., 1000–2000 IU three times weekly for 2 weeks), whereas most patients with bilateral cryptorchidism respond to hCG. However, lack of testosterone response to hCG administration for 6 weeks has been reported in men with bilateral cryptorchidism.[252] Serum AMH concentrations are usually undetectable in patients with congenital anorchia.[253] Measurements of AMH concentrations are more sensitive than those of testosterone concentrations but equally specific. If clinical examination and endocrine biochemical tests do not distinguish bilateral anorchia from cryptorchidism, then imaging studies (e.g., MRI) and laparoscopy or surgical abdominal exploration may be necessary to confirm the diagnosis.

Treatment of bilateral congenital anorchia consists of testosterone replacement to stimulate penile length in patients with micropenis and to induce and maintain sexual development in boys with delayed puberty and eunuchoidism. Implantation of testicular prostheses in the scrotum may be of psychological and cosmetic value.

Autoimmune Polyglandular Syndrome. Autoimmune polyglandular syndromes are characterized by a clustering of organ-specific autoimmune disorders that involve many endocrine and nonendocrine tissues and are associated with circulating autoantibodies to components of these tissues. Autoimmune polyglandular syndrome type 1, also called *autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy*, is a rare autosomal recessive disorder that is caused by a mutation in the autoimmune regulator (*AIRE*) gene.[254,255] Its main features are mucocutaneous candidiasis, hypoparathyroidism, primary adrenal insufficiency, and the presence of autoimmune disorders including primary hypogonadism. The prevalence of primary hypogonadism is much more common in females than in males. In females, hypogonadism is manifested as premature ovarian failure and is present in 35% to 70% of cases, whereas in males, it is present in 8% to 28% of cases and is manifested either by androgen deficiency and impairment in sperm production with elevated serum gonadotropin concentrations or isolated impairment in sperm production (azoospermia) and isolated elevation of FSH concentrations.

Autoimmune polyglandular syndrome type 2 is a common polygenic disorder associated with the genes for human leukocyte antigens DR3 and DR4.[254,255] It is characterized by autoimmune primary adrenal insufficiency, thyroid disease (Hashimoto or Graves disease), and T1DM in addition to other autoimmune disorders including primary hypogonadism (again, more common in females than in males). In autoimmune polyglandular syndrome type 2, primary hypogonadism is associated with circulating steroid-producing cell autoantibodies and with specific autoantibodies to P450 11A1 and P450 17A1 (cholesterol side-chain cleavage enzyme and 17α-hydroxylase/17,20-lyase, respectively).

Defects in Testosterone Biosynthetic Enzymes. Males with uncommon defects in 17,20-lyase/17α-hydroxylase, 17β-HSD/17-ketoreductase type 3, or 3β-HSD type 2, resulting from mutations in the *CYP17A1*, *HSD17B3*, and *HSD3B2* genes, respectively, usually present at birth as phenotypic females with partial virilization or with ambiguous genitalia. However, patients with incomplete defects in these enzymes occasionally present as phenotypic males with hypospadias, gynecomastia, and primary hypogonadism with androgen deficiency manifested by delayed puberty.

Because 17,20-lyase and 17α-hydroxylase activities reside in the same enzyme, mutations in *CYP17A1* usually cause deficiencies in both activities, leading to elevated concentrations of progesterone, corticosterone, and the aldosterone precursor 11-deoxycorticosterone.[256] Rarely, males exhibiting isolated 17,20-lyase deficiency with elevated 17-hydroxyprogesterone concentrations have been reported.[257] XY males with 17α-hydroxylase deficiency may have hypertension and hypokalemia due to excessive production of 11-deoxycorticosterone that has potent mineralocorticoid activity, as well as variable degrees of sex steroid hormone deficiency; more severe 17α-hydroxylase deficiency is characterized by a female phenotype due to lack of testosterone effects on the wolffian ducts. Patients with 17α-hydroxylase deficiency usually do not manifest adrenal insufficiency because of increased production of the cortisol precursor corticosterone that has glucocorticoid activity. Males with either partial, combined 17,20-lyase/17α-hydroxylase deficiency, or isolated 17,20-lyase deficiency have primary hypogonadism with low testosterone and elevated LH and FSH concentrations (LH > FSH); they require testosterone treatment at the time of puberty.

Patients with 17β-HSD/17-ketoreductase type 3 deficiency may have ambiguous genitalia and be raised as females; however, at puberty, their testosterone concentrations increase sufficiently to induce virilization, resulting in gender reassignment (similar to individuals with low serum DHT concentrations due to 5α-reductase deficiency).[258] Serum testosterone concentrations are low to normal, but androstenedione and gonadotropin concentrations are increased.

Incomplete deficiency of 3β-HSD type 2 is a rare disorder that may manifest in adolescents with mild ambiguous genitalia, delayed virilization, gynecomastia, low testosterone concentrations, and elevated LH and FSH concentrations.[259] Concentrations of pregnenolone, 17-hydroxypregnenolone, and DHEA are elevated. Spontaneous virilization and puberty due to direct effects of high concentrations of the weak androgen DHEA or conversion of DHEA to testosterone via 3β-HSD type 1 (or both) has been reported. A eugonadal male with partial 3β-HSD deficiency who presented with gynecomastia has also been reported.[260]

LH Receptor Mutations. Inactivating mutations of the LH receptor in XY males usually cause Leydig cell aplasia or hypoplasia. These patients usually present with DSD characterized by a female phenotype without breast development at puberty (Leydig cell aplasia) or genital ambiguity (hypoplasia) and cryptorchidism.[58] Rarely, partially inactivating LH receptor mutations result in a male phenotype with micropenis, hypospadias, delayed sexual development, undervirilization, low testosterone concentrations, impaired sperm production, and high serum LH concentrations. However, they usually have normal FSH concentrations. An individual with an LH receptor mutation was reported to exhibit normal concentrations of testosterone and spermatogenesis after hCG stimulation, suggesting that hCG action via the LH receptor may be dissociated from that of LH.[261]

Congenital Adrenal Hyperplasia. Adolescent and adult male patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency may develop *testicular adrenal rest tumors* (TARTs) that resemble Leydig cell tumors but do not contain intracytoplasmic Reinke crystalloids that are commonly found (approximately 40–50%) on histologic analysis in the latter.[262-264] TARTs may be large and easily palpable, or they may be detectable only on testicular ultrasonography. These tumors are thought to originate embryologically from aberrant adrenal tissue, and they are responsive to adrenocorticotropic hormone (ACTH). TARTs regress with supraphysiologic glucocorticoid therapy at a dosage that suppresses circulating ACTH concentrations, and they may grow with physiologic dosages of glucocorticoids.

21-Hydroxylase deficiency is an autosomal recessive condition caused by a mutation in the *CYP21A2* gene. It is the most common enzymatic defect causing congenital adrenal hyperplasia. 21-Hydroxylase deficiency produces an accumulation of steroid substrates (17-hydroxyprogesterone and progesterone) that results in excessive production of adrenal androgens (androstenedione, DHEA, and 11-hydroxyandrostenedione) that are subsequently converted to testosterone and 11-ketotestosterone. In addition, classic (severe) 21-hydroxylase deficiency causes reduced production of cortisol and aldosterone, resulting in deficiency of glucocorticoid and mineralocorticoid, respectively, and increased ACTH secretion (due to reduced cortisol negative feedback). Elevated ACTH secretion stimulates adrenal gland growth, resulting in adrenal hyperplasia. Glucocorticoid treatment lowers ACTH secretion, reduces excessive adrenal androgen production, treats clinical adrenal insufficiency, and prevents excessive adrenal hyperplasia.

Excessive adrenal androgen production due to untreated or inadequate glucocorticoid therapy for 21-hydroxylase deficiency suppresses gonadotropin secretion by sex steroid negative feedback regulation and causes secondary hypogonadism.[265] Because androgen concentrations are maintained by excessive adrenal production, men with 21-hydroxylase deficiency may not have clinical androgen deficiency. However, they usually have isolated impairment of sperm production due to suppression of circulating gonadotropin concentrations (due to the increased production of adrenal sex steroids and exogenous corticosteroid therapy). Glucocorticoid therapy reduces excessive adrenal androgen production and usually restores gonadotropin secretion and normalizes testis function, but supraphysiologic dosages of glucocorticoids may be required for these effects. Early glucocorticoid treatment may prevent or induce regression of adrenal rest tumors that may affect testicular function directly or cause mechanical obstruction of seminiferous tubules.[262-265]

If TARTs are present, lack of suppression of circulating ACTH stimulates their growth, often resulting in very large tumors that can cause irreversible testicular damage.[262-265] When testicular damage occurs, the patient will have irreversibly impaired sperm production due to loss of seminiferous tubules and may also have androgen deficiency due to loss of Leydig cells. With suppression of circulating ACTH with glucocorticoid treatment, TARTs may regress completely, but the damaged testes remain small and serum testosterone concentrations and sperm counts low. Although excessive amounts of glucocorticoid therapy will suppress ACTH production and TART growth, they also suppress serum gonadotropins, serum testosterone, and sperm production.

Down Syndrome. Down syndrome, or trisomy 21, is a chromosomal disorder in which all or part of an extra chromosome 21 is present.[266] It affects 1 of every 700 to 800 infants and is the most common cause of intellectual disability in children. Down syndrome is characterized by moderate to severe intellectual disability; spontaneous, warm, and cheerful personality; short stature; characteristic facial features (most notably round facies with microgenia, upward-slanting and almond-shaped eyes resulting from bilateral epicanthal folds, macroglossia, and flat nasal bridge); congenital heart defects; primary hypothyroidism; and defects affecting most other body systems. Males with Down syndrome usually manifest primary hypogonadism, most commonly characterized by isolated impairment in sperm production with normal or selective elevation of FSH concentrations. Histologically, they may manifest hypospermatogenesis (moderate to severe reduction in all germ cell types), maturation arrest, or Sertoli cell–only syndrome with loss of all germ cells. Less commonly, they demonstrate mild to moderate androgen deficiency with low or low-normal testosterone and elevated LH and FSH concentrations.

Complex Genetic Syndromes. Primary hypogonadism resulting in androgen deficiency and impaired sperm production or in isolated impairment in sperm production or function may occur as a manifestation of complex genetic syndromes, usually in association with several congenital anomalies or defects and distinct morphologic developmental manifestations.[267] Examples include the Alström, ataxia telangiectasia, Marinesco-Sjögren, Robinow, Rothmund-Thomson, Sohval-Soffer, Weinstein, Werner, and Wolfram syndromes.[268-277] Prader-Labhart-Willi syndrome (associated with cryptorchidism), Laurence-Moon-Bardet-Biedl syndrome, and Alström syndrome are disorders that are commonly associated with secondary hypogonadism, but primary hypogonadism may also occur in these disorders.[278-281]

Acquired Disorders

Bilateral Surgical Castration and Trauma. Bilateral surgical castration causes a rapid and profound decline in testosterone concentrations within hours, resulting in severe clinical manifestations of androgen deficiency, including hot flushes. Severe blunt trauma to the testes and associated vascular compromise may result in testicular atrophy and loss of testis function, including androgen deficiency and impaired spermatogenesis or isolated impairment in sperm production or function.

Drugs and Ionizing Radiation. Certain drugs that affect androgen production may cause androgen deficiency. Ketoconazole and abiraterone inhibit 17,20-lyase and 17α-hydroxylase activity at high doses (>400 mg daily and 1000 mg daily, respectively) and have been used, in conjunction with other agents, to lower both adrenal and testicular androgen production in the treatment of prostate cancer.[282-284] Spironolactone, a nonspecific aldosterone antagonist, acts mainly as a competitive AR antagonist and inhibits androgen action.[285] However, it also inhibits 17,20-lyase and 17α-hydroxylase activity and testosterone biosynthesis at high doses.[286,287] Enzalutamide is a competitive AR antagonist that also inhibits postreceptor AR action (translocation of AR into the nucleus, binding of AR cofactors, and DNA binding) that is used to treat castration-resistant prostate cancer.[288] Chronic excessive intake of alcohol may directly inhibit testosterone production, but it may also suppress gonadotropins; it may be associated with nutritional deficiency or chronic liver disease that may contribute to androgen deficiency and impairment in sperm production.[289,290]

In general, because spermatogenesis involves active cell replication, the germ cell compartment is much more sensitive to external or environmental influences (e.g., chemotherapy agents,

ionizing radiation) than are Leydig cells. Exposure to such agents often results in primary hypogonadism characterized by an isolated impairment in sperm production or function and elevated FSH concentrations. However, severe testis damage induced by these agents may cause Leydig cell dysfunction or damage, resulting in both androgen deficiency and impairment of sperm production, and elevated LH and FSH (FSH > LH) concentrations.

Combination chemotherapy regimens that include alkylating agents (e.g., cyclophosphamide, ifosfamide, procarbazine, busulfan, chlorambucil), such as those used for the treatment of Hodgkin and non-Hodgkin lymphoma and leukemia, or use of alkylating agents, such as cyclophosphamide, in the treatment of systemic rheumatic disorders are particularly toxic to the testes and may result in androgen deficiency in up to 20% of patients.[291,292] High-dose chemotherapy and total-body irradiation before bone marrow transplantation may also cause androgen deficiency in a substantial proportion of men. In contrast, men with testicular cancer who undergo combination chemotherapy that includes platinum drugs (together with unilateral orchidectomy and, often, radiation therapy) have a relatively low prevalence of usually mild androgen deficiency with slightly low to low-normal testosterone concentrations and elevated LH concentrations.[292]

Exposure of testes to ionizing irradiation commonly suppresses spermatogenesis in a dose-dependent manner, and doses greater than 600 to 800 cGy may compromise Leydig cell function and reduce testosterone production.[16,293,294]

Clinical practice guidelines for ongoing surveillance for testicular toxicity in childhood and young adult cancer survivors have been established by several groups.[295,296]

Orchitis. Viral infection of the testes may result in testicular atrophy; impaired sperm production; and, in severe cases, androgen deficiency. *Mumps orchitis* was common before the introduction of the measles/mumps/rubella vaccine in 1968, after which rates of mumps infection decreased profoundly.[297,298] Because some people are fearful about potential side effects of vaccinations, there has been a resurgence of mumps infections and orchitis in adolescents and young adults. Prepubertal mumps orchitis is very uncommon and is not associated with subsequent testicular dysfunction. Orchitis is the most common complication of mumps infection in pubertal boys and adults. It usually causes permanent seminiferous tubule damage; impaired spermatogenesis; and, in severe cases, Leydig cell failure and androgen deficiency.

Mumps infection usually manifests with headache, fever, and malaise followed by unilateral or bilateral parotid swelling due to parotiditis.[297,298] Testis pain and swelling due to orchitis occurs in about 10 days or up to 6 weeks after the onset of parotiditis and may be subclinical in up to 50% of cases. Epididymitis accompanies orchitis in 85% of cases. Mumps orchitis is usually unilateral but may be bilateral in 15% of cases in adolescents and in 30% of cases in young adults. Even if orchitis is clinically unilateral, degenerative changes may occur in the apparently unaffected testis. Germ cell sloughing occurs as a result of acute infection, inflammation, and ischemia resulting from pressure induced by swelling of the testes within the tunica albuginea. The acute phase is followed by seminiferous tubule fibrosis and then testicular atrophy (30–50% of cases) during the next several months, resulting in impaired spermatogenesis in 25% to 40% of cases. In severe cases, prednisone may be used to reduce inflammation and swelling associated with mumps orchitis, but it does not prevent testicular damage.

Untreated or undertreated HIV infection may cause gonadotropin suppression and secondary hypogonadism, particularly when associated with wasting and systemic illness. However, in 20% to 30% of cases, HIV infection causes primary hypogonadism characterized by low testosterone and elevated gonadotropin concentrations. The cause of primary hypogonadism is orchitis that is caused by HIV infection of the testis or occurs secondary to opportunistic infection (e.g., by cytomegalovirus [CMV], *Mycobacterium avium intracellulare*, *Toxoplasma gondii*) associated with the patient's immunocompromised state.[299,300]

Other causes of infectious orchitis usually associated with epididymitis include echovirus, arbovirus, or lymphocytic choriomeningitis infection; gonorrhea or *Chlamydia* infection in young adults and urinary pathogens such as *Escherichia coli* in older men; leprosy and tuberculosis; brucellosis, glanders, and syphilis; and parasitic infections such as filariasis and bilharziasis.[299,301]

Systemic Disorders

Low serum testosterone concentrations associated with symptoms and signs consistent with androgen deficiency are commonly associated with chronic diseases affecting the liver, kidney, heart, and lungs.[4,5] The underlying cause of both clinical and biochemical hypogonadism in these conditions is complex and multifactorial. The illness itself, associated complications, nutritional compromise, and medications used to treat the illness may contribute to or confound the clinical manifestations of androgen deficiency and also may suppress gonadotropin and testosterone production, thereby playing an etiologic role. Chronic systemic diseases usually affect both testicular and hypothalamic-pituitary function and cause combined primary and secondary hypogonadism. Clinically, however, measurements of gonadotropin concentrations usually suggest predominantly primary hypogonadism (i.e., elevated gonadotropins) or secondary hypogonadism (i.e., normal or low gonadotropins). The benefits and risks of testosterone therapy in patients with these systemic disorders have not been evaluated in long-term randomized, controlled outcome studies.

Chronic Liver Disease. In men with chronic liver disease of any cause (and particularly in those with hepatic cirrhosis or liver failure), sexual dysfunction, gynecomastia, and testicular atrophy resulting in impaired androgen and sperm production occur commonly, affecting 50% to 75% of these patients.[4,5,302-304] Total testosterone concentrations may be low but are often normal or high normal, because SHBG concentrations are increased substantially with cirrhosis and chronic active hepatitis. Therefore, measurements of free or bioavailable testosterone using accurate assay methods should be used to assess androgen deficiency. Free and bioavailable testosterone concentrations are usually low, and LH concentrations are usually elevated or in the high-normal range in patients with mild to moderate hepatic cirrhosis (Child-Pugh class A or B).

Estrogen (estrone and estradiol) concentrations are usually high due to increased production (e.g., induced by alcohol excess) and reduced clearance of adrenal androgens (e.g., androstenedione), which provide increased substrates for aromatization of androgens to estrogens. High estrogen concentrations contribute to the development of gynecomastia (high estrogen-to-androgen ratio), palmar erythema and spider angiomas, and increased prolactin concentrations. Treatment of ascites and edema with spironolactone may further lower testosterone concentrations and block androgen action, contributing to gynecomastia and other manifestations of androgen deficiency. Serum FSH and LH concentrations may be suppressed by high estrogen and prolactin concentrations and by the malnutrition that occurs commonly in men with hepatic cirrhosis and liver failure; these factors may

contribute a hormonal pattern more consistent with secondary hypogonadism in some men. Oligozoospermia or azoospermia associated with abnormalities in sperm motility and morphologic appearance occurs in approximately 30% to 50% of men with chronic liver disease.

Testosterone treatment of androgen deficiency is usually well tolerated but occasionally may worsen gynecomastia and rarely may increase edema and ascites by causing fluid retention. A preliminary double-blind, randomized, placebo-controlled 12-month trial in men with hepatic cirrhosis found that testosterone improved lean body and bone mass, reduced fat mass, and improved hemoglobin and hemoglobin A_{1c} concentrations.[305] Because immunosuppressive medications such as prednisone and cyclosporine are used to prevent rejection, liver transplantation only partially reverses hypogonadism associated with chronic liver disease.[302,306]

Chronic Kidney Disease. Late-stage CKD commonly causes combined primary and secondary hypogonadism, resulting in androgen deficiency and impairment in sperm production in 50% to 60% of these patients.[4,5,307] Serum testosterone concentrations are low, and LH and FSH concentrations are high, in large part because of markedly reduced renal clearance of gonadotropins and increased secretion resulting from reduced negative feedback. SHBG concentrations are usually not affected by CKD unless nephrotic syndrome is present, in which case concentrations of SHBG and, consequently, total testosterone may be low. In the latter situation, free or bioavailable testosterone measurements should be performed to assess androgen deficiency. Sperm production is impaired, and sperm motility and the percentage of sperm with normal morphologic appearance are reduced. The Leydig cell response to hCG administration is reduced, consistent with primary testicular dysfunction. The frequency and amplitude of pulsatile LH secretion are altered, suggesting an alteration in hypothalamic-pituitary function as well. Hyperprolactinemia, relative nutritional deficiency, uremia, the proinflammatory state, comorbid conditions that secondarily suppress gonadotropins, and zinc deficiency that affects testicular function may contribute to testicular dysfunction in men with CKD.

Randomized controlled trials that assess the long-term clinical benefits and risks of testosterone treatment have not been conducted.[308] Hemodialysis and peritoneal dialysis do not improve testosterone or sperm production.[307,309] Successful renal transplantation usually brings testosterone and sperm production to almost normal concentrations, although immunosuppression by rapamycin inhibitors (e.g., sirolimus) may impair testis function slightly.[307,310,311]

Aging. After age 40 years, there is a gradual and progressive decline in total testosterone concentrations (by approximately 1% per year) such that an increasing proportion of older men have low serum testosterone concentrations in the hypogonadal range.[312-314] Because SHBG concentrations increase with age, free and bioavailable testosterone concentrations decline even more rapidly (2–3% per year). Daily sperm production, sperm motility, percentage of sperm with normal morphologic forms, Sertoli cell number, and inhibin B concentrations also decline with aging.[144] Leydig cell number and testosterone production in response to stimulation by hCG are reduced, consistent with primary testicular dysfunction.[144] In older men, circadian variation in testosterone concentration is present but blunted.[64] Furthermore, pulsatile LH secretion is more irregular and disorderly and LH pulse amplitude is reduced in older men compared with those who are younger. Pulsatile GnRH administration normalizes pulsatile LH but not testosterone secretion, consistent with an impairment of hypothalamic GnRH secretion occurring in combination with a primary defect in testosterone production by the testis.[315,316]

Serum LH and FSH concentrations increase (by about 1–2% per year) with aging but do not usually rise above the normal range until very old age (>70 years).[312-314,317] Therefore, the most common hormonal profile associated with aging observed in middle-age to older men is low testosterone with normal LH and FSH concentrations, consistent with secondary hypogonadism; gonadotropin suppression is thought to be largely related to age-related comorbidities (e.g., obesity, illness, and medications). As men get older, gonadotropins continue to rise. A hormonal pattern of low testosterone and elevated LH and FSH concentrations, consistent with primary hypogonadism, is more prevalent in very old men, particularly after 70 years of age.

As men age, they may develop chronic organ failure or systemic illnesses, take an increasing number of medications, and develop nutritional deficiency or wasting syndromes that are associated with low testosterone concentrations.[318] It is likely that these comorbid conditions contribute to low testosterone and clinical hypogonadism associated with aging in men. Conversely, the age-related decline in testosterone concentrations may contribute to the susceptibility to or severity of clinical hypogonadism observed in these conditions.

In community-dwelling middle-age to older men, the prevalence of low testosterone increased from 12% among men in their 50s to 48% among men older than 80 years.[313] However, the prevalence of *clinical androgen deficiency* (i.e., symptoms and signs consistent with androgen deficiency and low testosterone concentrations) was 6% to 9% and increased with age, reaching 18% to 23% among men in their 70s.[196,197] When more stringent criteria for the diagnosis of androgen deficiency associated with aging were used (i.e., three sexual symptoms and a low testosterone level defined as late-onset hypogonadism), the prevalence was 2% and also increased with age, reaching 5% among men in their 70s.[198]

The decline in testosterone concentrations with aging is associated with alterations in body function that could be attributable to androgen deficiency.[144] These include a decline in muscle mass and strength associated with reduced physical function and performance; decreased BMD and increased risk of osteoporosis and fractures; increased body fat; reduction in sexual function and activity, including reduced libido and erectile dysfunction; decline in vitality, energy, mood, and cognitive function; and alterations in sleep quality. Similar changes occur in younger hypogonadal men and improve with testosterone treatment, raising the possibility that the decline in testosterone concentrations that occurs with aging may contribute to these age-associated changes in body function.

Previous relatively small, short-term (up to 3 years) studies of testosterone treatment in heterogeneous groups of older men with low or low-normal testosterone concentrations without regard to the presence of symptoms or signs of androgen deficiency have produced conflicting results. Most demonstrated beneficial effects of testosterone treatment on body composition, with increasing lean or muscle mass and decreasing fat mass, but less consistent effects on muscle strength and performance, BMD, sexual function, vitality, and cognitive function. The only adverse effect found in these studies was excessive erythrocytosis in some men.

The Testosterone Trials were seven coordinated, double-blind, placebo-controlled studies with allocation by minimization that evaluated the short-term effects of testosterone treatment for 1 year on sexual function, physical function, vitality, cognitive

function, bone density, anemia, and coronary artery plaque volume in a large number (788) of older men (average age of 72 years) with unequivocal hypogonadism (symptoms and signs of low libido, difficulty walking and slow walking speed, and/or low energy, as well as two morning testosterone concentrations of <275 ng/dL) for no apparent reason other than age. Compared with placebo, testosterone treatment for 1 year that raised testosterone concentrations to the mid-normal range for young men improved sexual function (sexual activity, libido, and erectile dysfunction), anemia and hemoglobin, volumetric BMD, and estimated bone strength; slightly improved walking distance, mood, and depressive symptoms; and did not improve multiple domains of cognitive function and vitality. In addition, testosterone treatment caused erythrocytosis (hemoglobin concentration >17.5 g/dL) and an increase in PSA concentration of more than 1.0 ng/mL in a small number of men and increased noncalcified coronary artery plaque volume on CT angiography. There was no difference in LUTS or cardiovascular or prostate adverse events in testosterone- and placebo-treated men (although the Testosterone Trials were underpowered for the latter outcomes).[162,319-323]

Previous studies of testosterone treatment in frail older men with low testosterone concentrations found beneficial effects on muscle strength and physical performance; there was an increase in self-reported cardiovascular adverse events in one small study but not in another similar study.[324,325] Meta-analyses of testosterone treatment trials[326-328] and pharmacoepidemiologic studies evaluating cardiovascular events associated with testosterone treatment[329-337] have also been conflicting. Meta-analyses of testosterone treatment trials, although underpowered, found no increased risk of prostate cancer,[338] and pharmacoepidemiology studies have been inconsistent, showing no or possibly increased risk of low-grade and reduced risk of high-grade, aggressive prostate cancer with testosterone treatment.[337,339-342]

Larger, long-term, randomized trials are needed to determine the balance of clinical benefits (e.g., related to frailty and fractures) and risks (particularly as related to prostate cancer and cardiovascular disease [CVD]) of testosterone treatment in elderly men. Until results from these outcome studies are available, testosterone treatment should be considered only for older men who have clinically significant manifestations of androgen deficiency (e.g., low libido and anemia) and unequivocally low serum testosterone concentrations, and only after a careful discussion of the uncertainty concerning the long-term benefits and risks of treatment.[116]

Other Systemic Disorders. Primary hypogonadism with low testosterone concentrations or elevated LH concentrations (or both) occurs in up to 20% of men with *malignancy* such as advanced Hodgkin disease or testicular cancer before gonadotoxic chemotherapy and radiation therapy.[343-345] Impairment of sperm production is found more frequently in about 30% to 50% of men with Hodgkin disease or testicular cancer before therapy. The mechanism of gonadal dysfunction before treatment is not clear.

Sickle cell disease is an autosomal recessive disorder caused by a point mutation in the β-globulin chain. It results in an abnormal hemoglobin (hemoglobin S) that polymerizes, leading to sickle-shape, rigid, and fragile red blood cells. The disease is characterized by recurrent episodes of painful, vaso-occlusive events in a variety of organs due to thrombosis, ischemia and infarction, and hemolysis. Sickle cell disease is a common disorder, affecting approximately 1 in 700 African-American infants. Sickle cell disease may cause primary hypogonadism characterized by low to low-normal testosterone concentrations, clinical manifestations consistent with androgen deficiency, testicular atrophy and impaired spermatogenesis, and elevated gonadotropin concentrations, possibly due to repeated testicular vaso-occlusive events and infarction.[346-348] Hydroxyurea therapy and possibly zinc deficiency may contribute to impaired spermatogenesis. Men with sickle cell disease may experience priapism due to penile vaso-occlusion, and this may be precipitated by restoration of libido with testosterone treatment of hypogonadism.

Within the first few months to 1 year after a *spinal cord injury*, testosterone concentrations and sperm production are suppressed, and gonadotropins are usually normal. However, in some men, LH or FSH concentrations or both may be elevated in the presence of low to low-normal testosterone, consistent with primary hypogonadism.[349] Some men demonstrate low testosterone and elevated gonadotropin concentrations chronically after spinal cord injury.[350-352] The latter condition may be caused by hyperprolactinemia associated with medications, as well as nutritional deficiency, obstructive sleep apnea, or comorbidities associated with chronic spinal cord injury. Testis biopsy has revealed impaired spermatogenesis in approximately 40% of men with spinal cord injury, but mature sperm that could be used for TESE and ICSI were present in almost 90%.[353]

Vasculitis (e.g., polyarteritis nodosa, granulomatosis with polyangiitis, Henoch-Schönlein purpura, Behçet disease) or *infiltrative disease* (e.g., systemic amyloidosis) involving both testes may cause testicular damage and may necessitate orchidectomy, resulting in both androgen deficiency and impaired sperm production.[354-356]

Isolated Impairment of Sperm Production or Function

Congenital or Developmental Disorders

As described previously, cryptorchidism, myotonic dystrophy, and Down syndrome most commonly manifest with primary hypogonadism characterized by isolated impairment in sperm production without androgen deficiency and selectively elevated FSH concentrations. In men with less severely impaired spermatogenesis, serum gonadotropin concentrations are normal, but it is most appropriate to classify these men as having primary hypogonadism with isolated impairment in sperm production, because gonadotropin treatment has not been demonstrated to improve fertility.

Varicocele

Varicocele is a dilatation of the pampiniform venous plexus surrounding the spermatic cord in the scrotum. It is caused by retrograde blood flow into the internal spermatic vein, which is usually caused by defective or absent valves in spermatic veins or, rarely, by obstruction of normal venous drainage by extrinsic or intrinsic venous compression (e.g., from a tumor). It usually occurs on the left side, and most cases are asymptomatic. A varicocele is present in 10% to 15% of men in the general population and more frequently in infertile men (up to 30–40%).[192,357]

The relationship of varicocele to impaired sperm production and infertility is unclear.[192,357] Approximately 50% of men who have a varicocele demonstrate normal semen analyses, and most with varicoceles are fertile. Men with a large varicocele and infertility usually exhibit low sperm counts with reduced motility and increased numbers of sperm with abnormal morphologic appearance (e.g., tapered or amorphous sperm heads), but these abnormalities are not specific for varicocele. Varicocele is painful in 2% to 10% of men with infertility. Testis size and serum concentrations of testosterone, LH, and FSH are usually normal, although

there may be an isolated elevation of serum FSH in men with decreased spermatogenesis. Testis biopsy in men with a varicocele and abnormal semen parameters reveals a spectrum of histopathologic findings, including hypospermatogenesis, maturation arrest, and Sertoli cell–only histology.

It is unclear whether varicocele ligation improves fertility in men who present with infertility. Controlled trials to investigate the efficacy of varicocele ligation have not demonstrated improved fertility. However, these trials were generally small, heterogeneous, and of poor quality. A small number of controlled trials of infertile men with palpable varicocele and at least one abnormal semen parameter suggested improvement in the spontaneous pregnancy rate with varicocele ligation. Some organizations have recommended surgical ligation for infertile men who have a large, palpable varicocele with an abnormal seminal fluid analysis.[192,357]

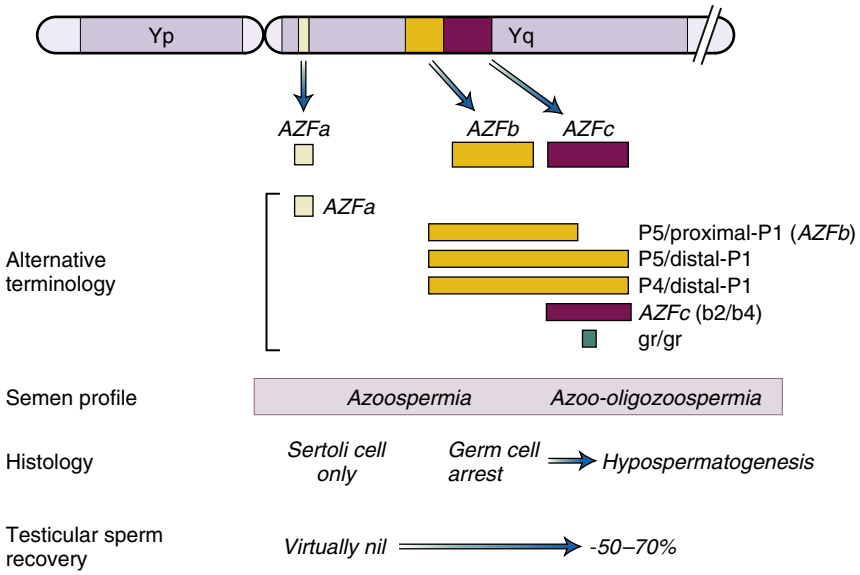
Y Chromosome Microdeletion. Yq chromosome (long arm of the Y chromosome) microdeletions are the most common genetic cause of impaired sperm production and male infertility. They are found in 5% to 10% of men with severe oligozoospermia and in 10% to 15% of men with azoospermia.[181] As mentioned earlier, microdeletions have been identified in three regions of the long arm of the Y chromosome[181,182,358] (Fig. 19.27).

Microdeletions in the *AZFa* region, which contains the *DDX3Y* and *USP9Y* (ubiquitin specific peptidase 9, Y-linked) genes, are usually associated with azoospermia and Sertoli cell–only histology. Microdeletions in the *AZFb* region, which contains multiple copies of the *RBMY* and *PRY* (PTPM13-like, Y-linked) genes, are usually associated with severe oligozoospermia and germ cell arrest at the pachytene primary spermatocyte stage and occasionally with hypospermatogenesis. Microdeletions in the *AZFc* region, which contains the *DAZ* (deleted in azoospermia) gene and is where the majority of Y chromosome microdeletions reside, are usually associated with germ cell arrest at the spermatid stage or with hypospermatogenesis with some mature spermatids present. *AZFc* microdeletions are found in approximately 12% of men with nonobstructive azoospermia and in 6% of men with severe oligozoospermia. Microdeletions in both the *AZFb* and the *AZFc* region are usually associated with azoospermia and Sertoli cell–only histology.

Yq microdeletion analysis should be considered for couples who are contemplating ICSI, because these microdeletions have been shown to be transmitted to male offspring with the ICSI procedure.[181] If ICSI is performed in men with Y chromosome microdeletions, genetic counseling and preimplantation or prenatal testing should be considered.

Sertoli Cell–Only Syndrome (Germ Cell Aplasia). Sertoli cell–only syndrome, or germ cell aplasia, is an uncommon histologic diagnosis in which the seminiferous tubules are completely devoid of germ cells and are lined only with Sertoli cells with little to no fibrosis or hyalinization.[359] Men with this disorder present with infertility, normal androgenization, moderately small testes (10 to 20 mL in volume), azoospermia, normal testosterone and LH concentrations, and selectively elevated FSH concentrations (indicating severe seminiferous tubule dysfunction).[360] Occasionally, LH concentrations are slightly high, and the testosterone response to hCG stimulation is reduced, suggesting mild Leydig cell dysfunction.

The cause of Sertoli cell–only syndrome is not known, but it is thought to result from congenital absence of germ cells due to a failure of gonocyte migration. In some families, however, germ cells were present before puberty but were subsequently lost during or after puberty. As described previously, a Sertoli cell–only histology may be associated with microdeletions in the long arm of the Y chromosome in the *AZF* regions.[361] Severe germ cell



• **Fig. 19.27** Schematic diagram of the short (Yp) and long (Yq) arms of the human Y chromosome. Deletions in regions of Yq, specifically in azoospermia factor (AZF) regions, are associated with severe defects in sperm production. Microdeletions in the *AZFa* region are usually associated with azoospermia and Sertoli cell–only histology. Microdeletions most commonly affect the *AZFc* region and may be associated with severe oligozoospermia or azoospermia. However, even if oligozoospermia or azoospermia is present, sperm for use in intracytoplasmic sperm injection are recoverable from half of the patients by testicular sperm extraction at biopsy. (From McLachlan RI, O'Bryan MK. Clinical review: state of the art for genetic testing of infertile men. *J Clin Endocrinol Metab.* 2010;95:1013–1024.)

damage and loss occurring with Klinefelter syndrome, mumps orchitis, cryptorchidism, ionizing radiation, or alkylating agents may result in seminiferous tubules lined only with Sertoli cells. However, in these cases of acquired Sertoli cell–only syndrome, there is usually extensive seminiferous tubule sclerosis or hyalinization, and the testes are usually smaller. Infertility is irreversible in congenital Sertoli cell–only syndrome, but it may be reversible with time in some cases of acquired Sertoli cell–only syndrome.

Primary Ciliary Dyskinesia (Immotile Cilia Syndrome). Primary ciliary dyskinesia, or immotile cilia syndrome, is a rare, heterogeneous, autosomal recessive genetic disorder of cilia. It is characterized primarily by recurrent respiratory infections (sinusitis and bronchitis) that lead to the development of bronchiectasis, caused by impaired mucociliary clearance due to dyskinesia of respiratory tract cilia, and to infertility caused by asthenozoospermia (nonmotile or poorly motile sperm) due to impaired sperm tail movement.[28,362] In half of the cases, primary ciliary dyskinesia is associated with situs inversus and is known as *Kartagener syndrome*. Some men exhibit abnormalities of sperm motility in the absence of respiratory tract involvement.

Patients with primary ciliary dyskinesia and impaired sperm motility demonstrate ultrastructural abnormalities of the axoneme, the microtubule cytoskeleton of the sperm flagellum, especially in the dynein arms (motor protein complexes). Almost all men with primary ciliary dyskinesia have mutations of the genes encoding the dynein axonemal heavy chain 5 (*DNAH5*), dynein intermediate chain 1 (*DNAI1*), or dynein axonemal heavy chain 11 (*DNAH11*). These men present with infertility and an isolated impairment in sperm motility with normal sperm counts and morphologic structure and normal testosterone and gonadotropin concentrations.

FSH Receptor Mutations. Rare inactivating FSH receptor mutations have been described in males.[58,86] In contrast to females with FSH receptor mutations, who have primary amenorrhea and infertility, men have a more variable presentation. Some demonstrate severe oligozoospermia, whereas others have moderate oligozoospermia or normal sperm concentrations with abnormal sperm morphologic appearance, and some have normal fertility. Serum testosterone concentrations are normal, FSH concentrations are elevated, and LH concentrations are slightly high to normal. It is possible that normal testosterone production within the testes contributes to the persistence of spermatogenesis and fertility in the absence of FSH action.

Acquired Disorders

Because seminiferous tubule function is more susceptible to damage than Leydig cell function, most men with primary hypogonadism due to chemotherapy agents, ionizing radiation, or orchitis caused by mumps or other infections manifest isolated impairment of sperm production without androgen deficiency.

Alkylating agents (e.g., cyclophosphamide, ifosfamide, procarbazine, busulfan, chlorambucil) used in combination chemotherapy regimens to treat lymphoma and leukemia almost uniformly cause azoospermia that recovers after 5 years in up to two thirds of men.[291,363-365] The effect of cyclophosphamide is dose dependent, and cumulative doses of cyclophosphamide of 10 g/m^2 for malignancy are likely to result in severe oligozoospermia or azoospermia that may be irreversible.[365] High-dose chemotherapy and total-body irradiation before bone marrow transplantation commonly cause irreversible germ cell damage associated with azoospermia or severe oligozoospermia and elevated FSH concentrations. Sperm production is suppressed initially in men with testicular cancer who undergo combination chemotherapy that includes platinum drugs, unilateral orchidectomy, and often radiation therapy.[292] However, sperm production recovers in 80% of men by 5 years. Before chemotherapy begins, cryopreservation of sperm for subsequent use in IUI, IVF, or ICSI should be offered to men who desire future fertility. Approaches aimed at suppressing gonadotropins with GnRH agonists or exogenous testosterone administration have not been uniformly effective in preventing germ cell damage by chemotherapy.

Methotrexate and sulfasalazine may cause oligozoospermia and low sperm motility and contribute to infertility.[366,367] The processes of human spermatogenesis, and particularly the spermatogonia, are very sensitive to the effects of exposure to ionizing radiation.[16,293,294] X-radiation doses as low as 15 cGy may suppress sperm production temporarily. The time required to recover spermatogenesis after x-irradiation is dose dependent. Recovery of sperm counts to baseline takes 9 to 18 months after exposure to 100 cGy or less and up to 5 years after 400 to 600 cGy. Although Leydig cell function is more resistant to ionizing radiation, x-irradiation of greater than 800 cGy may cause Leydig cell damage and androgen deficiency. As with chemotherapy, sperm banking before radiation therapy for later use in ART offers hope for subsequent fertility.

Prolonged and repeated *thermal trauma* (e.g., with excessive hot tub use) to the testes may transiently suppress sperm production.[368,369] In addition, many chemical agents used in industry and laboratories have been implicated as direct toxins to the testes. Examples of *environmental toxins* include carbon disulfide, a solvent used in rayon production; dibromochloropropane, an insecticide; lead; deuterium oxide; ethyl glycol; cadmium; fluoroacetamide; nitrofurans; dinitropyroles; diamines; and α-chlorohydrin.[370] Furthermore, it is postulated that environmental or xenobiotic agents such as phthalates act as antiandrogens or estrogens to alter reproductive function; these agents have been termed *endocrine disruptors*.[371] Such environmental agents have been implicated in causing the increased incidence of testicular dysgenesis syndrome (i.e., hypospadias, cryptorchidism, declining sperm counts, and testicular cancer).[372]

Systemic Disorders

An acute *febrile illness* may cause a temporary suppression of spermatogenesis.[373] In men with spinal cord injury resulting in tetraplegia or paraplegia, impaired sperm production may be caused in part by increased scrotal temperature due to loss of lumbar sympathetic innervations.[349-351,353] Men with malignancy, particularly Hodgkin lymphoma or testicular cancer, may have impaired spermatogenesis with azoospermia or oligozoospermia in 30% to 80% of cases before treatment (the former possibly related to B symptoms of fever, night sweats, and weight loss).[331,343,345,364]

In most men who present with infertility and an isolated impairment of sperm production, a cause cannot be identified. *Idiopathic oligozoospermia or azoospermia* occurs in 60% to 80% of cases (including men with varicocele).[178-180] If isolated impairment of spermatogenesis is severe in men with primary hypogonadism, serum FSH concentrations may be selectively elevated. As mentioned earlier, men with less severely impaired spermatogenesis and normal serum gonadotropin concentrations are still classified as having a disorder of primary hypogonadism, because gonadotropin treatment has not been demonstrated to improve fertility in such cases.

Causes of Secondary Hypogonadism

Androgen Deficiency and Impairment in Sperm Production

Congenital or Developmental Disorders

Constitutional Delay of Growth and Puberty. It is important to consider *constitutional delay of growth and puberty* (CDGP) in the differential diagnosis of secondary hypogonadism because it is a transient cause of secondary hypogonadism and is the most common cause of pubertal delay, usually associated with growth delay and short stature in boys.[146,147,374,375]

The initial endocrine event that precedes the phenotypic changes of puberty is activation of the CNS mechanisms that regulate GnRH production, which results in pulsatile LH followed by testosterone secretion, initially at night (see Fig. 19.5) and then throughout the day.[42] Under the influence of increasing testosterone concentrations, secondary sexual characteristics appear between 9 and 13 years of age.

The first physical sign of pubertal development is an increase of testis size to greater than 3 mL in volume and thinning of the scrotal skin that is followed by development of rugal folds and increased pigmentation.[146,147,149,374,375] Subsequently, penile length increases, and pubic hair develops over the next 1 to 2 years, followed by long bone growth (with peak height velocity occurring approximately 3 years later) and development of other secondary sexual characteristics, such as laryngeal enlargement and deepening of the voice. In boys, peak bone mass usually is not reached until the third decade. There is considerable variability in the onset and progression of puberty and the degree of virilization, and these variations are attributable in large part to an individual's genetic and ethnic background.

The absence of sexual maturation and testis size less than 3 mL at 14 years of age is diagnostic of delayed puberty.[146,147,149,374,375] Boys with delayed puberty often experience considerable psychosocial distress due to the lack of sexual and physical development that results in their being considered younger than their peer group and having difficulty competing in athletics. In addition to the lack of sexual development, both boys and their parents are usually also concerned about the boy's failure to undergo a growth spurt and his short stature.

CDGP, or transient secondary hypogonadism, is the cause of delayed puberty in approximately 65% of cases.[146,147,149,374,375] CDGP is usually clinically indistinguishable from permanent organic causes of secondary hypogonadism (e.g., due either to known genetic mutations or IHH) or acquired hypogonadotropic hypogonadism due to an identifiable cause (e.g., pituitary or hypothalamic disease). However, permanent causes of delay puberty occur in less than 10% of cases. Other causes of delayed sexual development and growth account for about 25% of cases, and they are usually clinically apparent on presentation. These causes include functional secondary hypogonadism (e.g., due to chronic systemic illness, hypothyroidism, medications) and primary hypogonadism (e.g., due to Klinefelter syndrome, mumps, chemotherapy, radiation therapy) or androgen resistance.

CDGP is a physiologic variant of normal puberty that is characterized by a slowing of the growth rate and of the timing and tempo of pubertal development.[146,147,149,374,375] Because there is an increased prevalence of CDGP in families with GnRH deficiency (CHH), CDGP could represent a mild variant of CHH in some cases.[376] However, a recent study found distinct genetic profiles in boys with CDGP and CHH.[377] No genes or genetic polymorphisms have been identified that determine the variations in the timing of normal puberty; however, a family history of delayed puberty or being a "late bloomer" is found in 80% of boys with CDGP.

Clinically, a boy with CGDP has congruent delays in growth, sexual development, and bone development. The height age (i.e., the age that corresponds to the child's height when plotted at the 50th percentile on a growth chart), stage of sexual development, and bone age are concordant and delayed compared with chronologic age.[146,147,149,374,375] Height velocity in boys with CGDP is slower than peers of the same chronologic age because puberty accelerates growth velocity.

In the absence of anosmia, hyposmia, or other morphologic abnormalities, CDGP cannot be distinguished clinically or biochemically from permanent CHH (see later discussion). If spontaneous puberty does not develop by 18 years of age in a normosmic patient, the diagnosis is usually CHH. It should be noted that reversal and relapse of hypogonadism after age 18 has been reported in a few older men with anosmic (Kallmann syndrome) or normosmic CHH.[378-380]

Eventually, boys with CDGP undergo normal growth and sexual development, although they do so several years after their peers.[146,147,149,374,375] Normal height is usually attained, but target mid-parental height may not be achieved. In addition, acquisition of peak bone mass may be compromised in some men. Boys with CDGP may experience severe emotional distress and social exclusion or isolation resulting from immature sexual development and short stature. Therefore, after exclusion of organic causes of delayed puberty, low-dose testosterone treatment is usually initiated in boys with CDGP at age 14 years, or sometimes sooner, to induce sexual maturation and growth that is more consistent with that of their peers. Testosterone is usually started at a low dose and gradually increased over several years; it is stopped intermittently to assess whether spontaneous puberty occurs (see later discussion).

Hereditary Hemochromatosis. Hereditary hemochromatosis is a common autosomal recessive disorder that is characterized by inappropriately high gastrointestinal iron absorption resulting in excessive iron storage in a number of tissues, most prominently those of the liver, pancreas, heart, joints, skin, testes, and pituitary gland.[381,382] In most cases, hereditary hemochromatosis is caused by mutations in the hemochromatosis gene (*HFE*), most commonly homozygous C282Y/C282Y mutations (70–85%) or compound heterozygote C282Y/H63D mutations (5–10%), or rarely, by other mutations in iron-regulating genes such as transferrin receptor 2 (*TFR2*), hepcidin antimicrobial peptide (*HAMP*), hemojuvelin (*HJV*), and solute carrier family 40A1 (*SLC40A1*). Homozygous C282Y mutations occur in approximately 1 of every 200 to 400 Caucasians of Northern European descent.

Regardless of the specific mutation causing hereditary hemochromatosis, iron overload results from insufficient hepatic production of hepcidin, a peptide hormone that degrades the iron-exporter protein, ferroportin; this causes unregulated iron absorption in the duodenum and iron overload in tissues.[381,382] Initially, iron overload causes an increase in transferrin iron saturation, followed by an increase in ferritin concentrations in most men with hemochromatosis. Therefore, the biochemical penetrance is high. Evaluation for hemochromatosis should be considered if iron saturation is greater than 45% in men. In the absence of inflammation or cancer, a serum ferritin level higher than 1000 µg/L is associated with greater risk of hepatic cirrhosis in patients with hemochromatosis, and a liver biopsy or MRI for hepatic iron content should be considered.

In contrast to biochemical abnormalities consistent with iron overload, the clinical penetrance of hereditary hemochromatosis is quite low (0.5–2.0%), and manifestations of iron overload (hepatic cirrhosis or carcinoma, diabetes mellitus, heart failure or arrhythmias, arthralgias or polychondritis, bronzing of skin, hypogonadism) are far less common.[381,382] This may be related to the importance of secondary insults to end organs (e.g., alcoholic liver damage) that contribute to clinical manifestations or to earlier diagnosis with increased awareness and screening. Clinical manifestations, when present, usually appear at between 40 and 60 years of age. Hypogonadism, changes in skin color, and arthralgias of the hands are usually the earliest clinical manifestations of iron overload.

Men with end-organ effects from hereditary hemochromatosis almost always have secondary hypogonadism resulting in androgen deficiency and impairment in sperm production due to iron overload in the pituitary gland that causes selective gonadotropin deficiency.[383] Serum testosterone concentrations and sperm counts are low, LH and FSH concentrations are usually low, and gonadotropin response to GnRH stimulation is absent or markedly attenuated. In the presence of cirrhosis caused by hemochromatosis, SHBG concentrations may be elevated; as a result, serum free testosterone concentrations may be low in the presence of normal total testosterone concentrations. Therefore, accurate and reliable assessment of free testosterone are needed to confirm biochemical androgen deficiency in men with hepatic cirrhosis due to hemochromatosis. Iron deposition in the pituitary may be detected by MRI. Iron overload also occurs in the testes and may occasionally cause a modest reduction in testosterone response to gonadotropin stimulation, resulting in combined primary and secondary hypogonadism. However, in most cases, gonadotropin treatment can stimulate normal testicular function, including spermatogenesis and fertility.

The prevalence of hypogonadism in iron overload has declined from between 10% and 100% in older reports to approximately 6% more recently due to earlier diagnosis and less severe iron overload. Hypogonadism is preventable and may be reversed with therapeutic phlebotomy early in the course of iron overload.

Congenital Hypogonadotropic Hypogonadism. CHH, either due to known mutations (see the following discussion) or idiopathic (referred to as IHH, previously known as hypogonadotropic eunuchoidism), is an uncommon, clinically heterogeneous group of disorders characterized by isolated gonadotropin deficiency of varying degree with otherwise normal pituitary function.[384,385] Most cases of CHH are likely due to genetic mutations, many of which remain to be identified. Males with CHH fail to undergo normal puberty, resulting in incomplete sexual maturation or eunuchoidism, androgen deficiency with very low testosterone (usually in the prepubertal range), low to low-normal LH and FSH concentrations, and impaired sperm production. Gonadotropin deficiency is caused by a defect in normal GnRH production or action, as evidenced by absent or abnormal patterns of pulsatile LH secretion[47] (see Fig. 19.7) and the ability of exogenous pulsatile GnRH treatment to restore normal gonadotropin secretion and testis function. Infants with CHH may have micropenis or cryptorchidism. Rarely, a man with normal virilization may develop CHH as an adult.[386]

In approximately 60% of cases, CHH is associated with anosmia or hyposmia and is known as Kallmann syndrome.[384] Developmental failure of olfactory bulbs (detectable on brain MRI) is responsible for the anosmia or hyposmia. The remaining 40% of men have normosmic CHH. In addition, some men with CHH exhibit other developmental defects, including synkinesia (mirror movements), unilateral renal agenesis, cleft lip/palate or high-arched palate, sensorineural hearing loss, digital skeletal abnormalities such as syndactyly or brachydactyly (fourth metacarpal), dental agenesis, eye movement abnormalities or color blindness (deuteranopia), and agenesis of the corpus callosum.

The prevalence of Kallmann syndrome is approximately 1 in 8000 to 10,000 men, and there is a marked male predominance (male-to-female ratio, 4:1–5:1).[387] X-linked recessive, autosomal dominant, and autosomal recessive modes of inheritance are observed, but many cases are sporadic. Family members of a man with Kallmann syndrome may have variable clinical expressions, such as normosmic CHH, isolated anosmia, or CDGP.[50,385,388] In approximately 30% to 40% of cases, Kallmann syndrome is caused by a known mutation of genes that play important roles in the migration of GnRH neurons from the olfactory placode to the hypothalamus and in normal development of the olfactory bulbs during fetal development. These include mutations of *ANOS1* (10–20%), *FGFR1/KAL2* (10%), *PROK2* (5%), and *PROKR2* (5%).[50,385,388] (*ANOS1* and *ANOS2* were formerly known as *KAL1* and *KAL2*). CHH without known gene mutation is sometimes called *idiopathic hypogonadotropic hypogonadism* (IHH).

The *ANOS1* gene is located on the X chromosome and encodes an extracellular adhesion glycoprotein known as anosmin 1.[50,385,388] Mutation or deletion of *ANOS1* results in failure of the normal migration of GnRH neurons from the olfactory placode to the hypothalamus, resulting in severe GnRH deficiency; it is the main cause of X-linked recessive Kallmann syndrome (type 1). The phenotype of Kallmann syndrome caused by *ANOS1* mutations is more severe and less variable than that of other known genetic defects. Synkinesia or dysdiadokinesia is present in 80% and unilateral renal agenesis in 30% of cases. The *FGFR1* gene encodes for a fibroblast growth factor receptor that also plays an important role in the migration of GnRH neurons during development.[50,385,388] Mutations in this gene cause a spectrum of phenotypes, from severe autosomal dominant Kallmann syndrome (type 2) and normosmic CHH to CDGP associated with cleft lip/palate (in 30% of cases), dental agenesis, and skeletal abnormalities such as brachydactyly and syndactyly. *PROK2* and *PROKR2* encode for a peptide and its G protein–coupled receptor that play important roles in normal GnRH neuronal migration and olfactory bulb development.[50,385,388] The clinical phenotypes in men with *PROK2* or *PROKR2* mutations are quite variable, ranging from severe Kallmann syndrome (type 4 or type 3, respectively) to normosmic CHH.

In approximately 30% of cases, normosmic CHH is caused by mutations in genes involved in hypothalamic-pituitary function (particularly during puberty). These include mutations in *GNRHR*, the GnRH receptor (10–20%); *KISS1R*, which encodes the receptor for kisspeptin 1/metastin, an important GnRH stimulatory neuropeptide particularly at the time of puberty (2–5%); *TAC3*, which encodes neurokinin B, another important GnRH stimulatory neuropeptide, and the gene for its receptor, *TAC3R*; *FGFR1/KAL2* (2–5%); *PROK2*; the genes for leptin (*LEP*) and its receptor (*LEPR*), which are associated with massive obesity; and, rarely, the GnRH gene, *GNRH1*.[50,385,388]

Hypogonadotropic hypogonadism is a component of complex genetic syndromes associated with specific dysmorphic features or combined hormonal defects. For example, the *CHARGE syndrome*, characterized by *C*oloboma of the eye or CNS anomalies, *H*eart anomalies, nasal choanal *A*tresia, growth *R*etardation, *G*enital defect (hypogonadism), and *E*ar anomalies (deafness, dysmorphic

ears, and hypoplasia of the semicircular canals), may be associated with Kallmann syndrome or normosmic CHH.[385,388-390] In approximately 60% of cases, CHARGE syndrome is caused by a mutation in the chromodomain helicase DNA-binding protein 7 gene (*CHD7*), which encodes for a chromatin remodeling protein. *CHD7* mutations have been found in approximately 3% to 4% of men with Kallmann syndrome or normosmic CHH, and it is hypothesized that CHH may be a mild variant of the CHARGE syndrome.

X-linked congenital adrenal hypoplasia, characterized by adrenal insufficiency due to adrenal hypoplasia and normosmic CHH, is caused by a mutation in the dose-sensitive sex reversal–adrenal hypoplasia critical region on the X chromosome protein 1 gene (*DAX1*), now known as *NR0B1* (nuclear receptor subfamily 0, group B, member 1), which encodes for an orphan nuclear receptor. Mutations of the SF-1 gene (*NR5A1*), which encodes another orphan nuclear receptor, the prohormone convertase 1 gene (*PC1*), which encodes an enzyme involved in post-translational processing of pituitary prohormones and neuropeptides, and genes for many pituitary transcription factors such as *HESX1* (septo-optic dysplasia or de Morsier syndrome), *LHX3*, *LHX4*, *POUF1*, and *PROP1* cause deficiencies in multiple anterior pituitary and other hormones in addition to gonadotropin deficiency. Mutations in the latter transcription factors may also be associated with specific dysmorphic features. Unlike men with CHH, those with multiple hormone defects usually fail to normalize gonadotropin and testicular function in response to chronic pulsatile GnRH administration.[50,385,388,391]

In the absence of anosmia, hyposmia, or features of Kallmann syndrome (e.g., synkinesia), it is not possible to confidently distinguish an individual who has normosmic CHH not associated with a complex genetic syndrome from someone who has CDGP.[146,147,374,375] In both conditions, there may be a family history of delayed puberty, CHH, or Kallmann syndrome; a history of cryptorchidism; clinical manifestations of delayed sexual maturation or eunuchoidism; and low serum testosterone and low to low-normal gonadotropin concentrations. In contrast to boys with CHH, who have normal height for their chronologic age, those with CDGP usually manifest some growth delay and short stature. Boys with CHH may have a history of micropenis and usually demonstrate pubertal delay beyond 19 years of age, although some individuals undergo spontaneous puberty after 20 years of age or reversal of CHH in adulthood; a subset of the latter may also subsequently relapse with CHH.[378-380] Currently, there is no diagnostic test that completely and reliably distinguishes isolated normosmic CHH from CDGP.

As discussed previously, after organic causes of delayed puberty (e.g., craniopharyngioma) have been excluded, low-dose testosterone therapy is usually initiated in boys with delayed puberty at about 14 years of age to induce sexual maturation and growth.[146,147,374,375] Testosterone is usually discontinued intermittently to assess whether spontaneous puberty occurs, as determined by an increase in testis size. Boys with CHH usually require continued testosterone treatment to achieve and maintain sexual maturation, whereas those with CDGP do not require further treatment after spontaneous secretion of gonadotropin and testosterone commences. Sustained reversal occurs after discontinuation of therapy in up to 10% of men with Kallmann syndrome or normosmic CHH who present initially with absent or partial sexual maturation.[378-380] Therefore, it is reasonable to discontinue treatment briefly to assess the reversibility of hypogonadotropic hypogonadism in all patients.

If fertility is desired, testosterone therapy is stopped and gonadotropin replacement therapy (or in some specialized centers, pulsatile GnRH) is initiated to stimulate sperm production. Previous treatment with testosterone might be expected to suppress endogenous gonadotropin production further; however, previous androgen therapy does not alter the subsequent overall response of spermatogenesis to gonadotropin therapy, although the response to gonadotropins might be slower.[392-394] Gonadotropin therapy is much more likely to stimulate spermatogenesis if there is some evidence of sexual maturation and larger testes at baseline and no history of cryptorchidism or another primary testicular disorder.[386,395] Even in the absence of clinical evidence of hypothalamic-pituitary-testicular disorder, 10% to 20% of men with CHH do not have an adequate gonadotropin or testicular response to chronic pulsatile GnRH therapy, suggesting underlying pituitary or testicular defects.[396]

Men with CHH have varying degrees of gonadotropin deficiency, as evidenced by a persistent but abnormal pattern of pulsatile LH secretion[47] (see Fig. 19.7). In some men with CHH, FSH secretion predominates relative to LH secretion, resulting in some germ cell maturation and spermatogenesis and a rare variant form of CHH known as *isolated LH deficiency*, or the "fertile eunuch syndrome."[397-399] This syndrome is characterized by androgen deficiency of prepubertal onset or eunuchoidism caused by LH deficiency but pubertal or almost adult-size testes in which advanced-stage spermatogenesis is present because of relatively preserved FSH secretion. However, spermatogenesis is usually not completely normal in these men, and they are not fertile, as the name of the syndrome might imply. Because there is only relative gonadotropin deficiency and some spermatogenesis is present, treatment with LH-like activity (hCG) stimulates Leydig cell testosterone production and ameliorates androgen deficiency, stimulating spermatogenesis sufficient for induction of fertility. Men with *isolated FSH deficiency* in the absence of an FSHβ mutation have been reported, but the degree and nature of the defect in pulsatile gonadotropin secretion have not been well documented.[400-402]

Men with inactivating *LHβ mutations* usually demonstrate a lack of pubertal development, impaired spermatogenesis or azoospermia, and infertility.[59,403,404] Recently, however, a man with an LHβ mutation resulting in a partially active LH molecule (as indicated by expression of steroidogenic enzymes in a few mature Leydig cells and low intratesticular testosterone concentrations) was reported to have complete and quantitatively normal spermatogenesis.[80] Complete spermatogenesis was achieved in the presence of very low LH and intratesticular testosterone concentrations and high serum FSH concentrations in this man. Men with inactivating *FSHβ mutations* generally have been found to have azoospermia with undetectable FSH, low or low-normal testosterone, and high LH concentrations.[80,82-85,405,406]

Secondary hypogonadism may be present in several *complex genetic syndromes*, such as Prader-Labhart-Willi, Laurence-Moon-Bardet-Biedl, Alström, Bjornstad, Börjeson-Forssman-Lehmann, Bosma, Chudley, Costello, Gordon-Holmes, Johnson-McMillin, Juberg-Marsidi, LEOPARD (multiple *L*entigines, *E*lectrocardiographic conduction abnormalities, *O*cular hypertelorism, *P*ulmonary stenosis, *A*bnormal genitalia, growth *R*etardation, and sensorineural *D*eafness), Martsof, Moebius-Poland, Roifman, Rud, and Woodhouse-Sakati syndromes.[267,280,407-422] Most of these syndromes are diagnosed by pediatricians and pediatric endocrinologists based on the clustering of specific dysmorphic features and congenital anomalies that are characteristic of the

syndrome.[267] Secondary hypogonadism in these disorders usually causes androgen deficiency of prepubertal onset. Many but not all of these syndromes are associated with CNS abnormalities or intellectual disability. Obesity may contribute to the cause of hypogonadism and may alert clinicians to the potential presence of a complex genetic syndrome. Some, such as the Prader-Labhart-Willi, Laurence-Moon-Bardet-Biedl, and Alström syndromes, have been reported to be associated with both primary and secondary hypogonadism.[270,278,279,281]

Acquired Disorders

Hyperprolactinemia. Hyperprolactinemia is a common cause of secondary hypogonadism. These patients have low testosterone concentrations and low to low-normal gonadotropin concentrations and present with sexual dysfunction (reduced libido and erectile dysfunction), infertility, and gynecomastia.[423,424] Because the male breast usually is not exposed to a relatively high estrogen and progestin milieu needed to induce ductal hyperplasia and glandular formation in women, high prolactin concentrations in men rarely result in galactorrhea. Hyperprolactinemia causes gonadotropin deficiency primarily by suppressing pulsatile hypothalamic GnRH secretion, as evidenced by reduced spontaneous LH pulse frequency and amplitude and by restoration of normal LH pulsatility and testosterone concentrations with dopamine agonist treatment or pulsatile GnRH administration.[425]

Common causes of hyperprolactinemia resulting in clinical secondary hypogonadism are a prolactin-secreting adenoma, pituitary stalk disease (e.g., stalk compression from a non–prolactin-secreting adenoma, traumatic stalk section); hypothalamic disease (e.g., hypothalamic tumors, granulomatous disease); and medications.[423,424]

In contrast to women who usually present with microadenomas, men with prolactin-secreting adenomas usually present with large macroadenomas because of a lack of overt symptoms or possibly a gender-specific difference in the biologic behavior of the tumor.[426,427] In approximately 10% of cases, there is excessive co-secretion of prolactin and GH. In men with prolactin-secreting macroadenomas, serum prolactin concentrations are usually higher than 250 ng/mL, and they can be higher than 1000 ng/mL with tumors larger than 2 cm in diameter. Some patients with very large prolactin-secreting macroadenomas have only modestly elevated serum prolactin concentrations, which is caused by an assay artifact from saturation of both the capture and detection antibodies used in two-site sandwich immunoassays.[428] This phenomenon is known as the pro-zone or "hook" effect and necessitates dilution of serum samples prior to assay. Measurement of prolactin after serial dilution should be considered for all patients with large pituitary tumors and modest hyperprolactinemia.

Diseases affecting the pituitary stalk and hypothalamic diseases may cause hyperprolactinemia because of disruption of the hypothalamic hypophyseal portal tract and transport of dopamine from the hypothalamus to the pituitary in the former condition or loss of hypothalamic dopamine-containing neurons in the latter. Suprasellar extension of a large, non–prolactin-secreting pituitary macroadenoma that compresses the pituitary stalk usually causes hyperprolactinemia with serum prolactin concentrations in the 20- to 250-ng/mL range, although higher concentrations are occasionally seen.[423,424]

Medications that cause hyperprolactinemia (prolactin concentration usually <100 ng/mL) interfere with hypothalamic dopamine production or action or affect the regulation of dopamine secretion by CNS neurotransmitters (e.g., serotonin).[429,430] The medications that most commonly cause hyperprolactinemia are dopamine D_2 receptor antagonists, such as typical antipsychotic drugs (phenothiazines, thioxanthenes, and butyrophenones), some atypical antipsychotic agents (e.g., risperidone, molindone), and gastrointestinal promotility agents (e.g., metoclopramide, domperidone). In contrast, newer atypical antipsychotic medications such as clozapine, olanzapine, quetiapine, ziprasidone, and aripiprazole much less commonly increase prolactin. Other medications that cause hyperprolactinemia less commonly include some tricyclic antidepressants (e.g., clomipramine), monoamine oxidase inhibitors (e.g., pargyline; clorgiline, which is rarely used), and antihypertensive agents (verapamil; α-methyldopa and reserpine, which are rarely used). Selective serotonin and serotonin/norepinephrine reuptake inhibitors in general have minimal to no effect on prolactin concentrations.

Serum prolactin concentrations may be elevated in patients with CKD, in proportion to the degree of renal impairment, because of both increased secretion and decreased clearance, and this may contribute to the hypogonadism associated with chronic renal failure. The mild hyperprolactinemia associated with primary hypothyroidism does not usually suppress gonadotropin secretion significantly or cause clinical secondary hypogonadism in men. However, if primary hypothyroidism is severe and long standing, it may cause slight enlargement of the pituitary gland, which may be confused with a pituitary adenoma.

Treatment is aimed initially at the underlying cause of hyperprolactinemia. In men with prolactin-secreting macroadenomas, treatment is initiated with a dopamine agonist medication such as bromocriptine or carbergoline.[423,424,426,431] Dopamine agonist therapy usually results in a reduction of serum prolactin, decreased tumor size, and improvement in visual field defects. Treatment with these agents may also improve sexual dysfunction, normalize testosterone concentrations, and improve semen quality. In men who remain persistently hypogonadal despite adequate dopamine agonist treatment, testosterone therapy may be initiated to treat manifestations of androgen deficiency. Testosterone is aromatized to estradiol, and this estrogen theoretically might increase prolactin concentrations and tumor growth by direct action on pituitary lactotrophs; this effect may also cause resistance to dopamine agonist therapy.[432] Therefore, monitoring of tumor size is required during testosterone replacement therapy.

In some men who do not respond to dopamine agonist treatment alone, gonadotropin or pulsatile GnRH therapy may be necessary to stimulate spermatogenesis sufficient to induce fertility. Surgery or radiation therapy may be needed for tumors that are resistant to dopamine agonists, and urgent surgery may be needed for pituitary apoplexy or rapidly progressive tumor mass effects such as visual loss. Medications that cause hyperprolactinemia may be stopped or switched to ones that do not elevate prolactin. For antipsychotic and antidepressant medications, such changes should be made in consultation with the patient's psychiatrist.[429] If discontinuing or switching drugs is not an option, testosterone treatment may be needed to treat androgen deficiency. Addition of a dopamine agonist while administration of an offending antipsychotic medication is continued should be done with extreme caution, because there is a risk of exacerbating psychosis.

Opioids. Use of opiates or opioid medications, particularly potent, long-acting narcotic analgesics such as methadone (>30 mg daily), controlled-release or intrathecal morphine sulfate or the transdermal fentanyl patch, and drugs of abuse such as heroin (diacetylmorphine) or time-released oxycodone (OxyContin), profoundly suppress gonadotropin secretion, resulting in severe androgen deficiency.[433-437]

Prolonged use of opioids causes symptomatic androgen deficiency, resulting in sexual dysfunction and long-term consequences such as loss of BMD and increased risk of osteoporosis; this is a common cause of secondary hypogonadism associated with androgen deficiency and impairment in sperm production. Although the benefits and risks of testosterone treatment have not been evaluated in randomized controlled studies,[438] testosterone treatment should be considered in cases of secondary hypogonadism due to chronic use of opioids. In contrast, short-term use of opioids or use of short-acting opioids (e.g., postoperatively) may cause only a transient suppression of gonadotropins and testosterone that does not require treatment.

Administration of opioid antagonists such as naloxone or naltrexone results in an increase in LH pulse frequency in normal men, suggesting that endogenous opioid neuronal systems within the hypothalamus exert a negative regulation on pulsatile GnRH secretion.[439] Therefore, exogenous administration of opioids most likely causes reduced gonadotropin secretion by suppressing hypothalamic GnRH secretion. The action of exogenous opioids on GnRH secretion is probably mediated by μ-opioid receptors. In this regard, the pure μ-opioid receptor agonist methadone more commonly and severely suppresses gonadotropins and testosterone than does buprenorphine, which is a partial μ-receptor, δ-receptor, and opioid receptor–like 1/nociceptin receptor agonist and κ-opioid receptor antagonist.[434,440] Both methadone and buprenorphine are used clinically for detoxification and maintenance treatment of opioid addiction.

In men taking chronic high-dose, long-acting opioids, serum testosterone, LH, and FSH are usually severely suppressed, and sperm production is impaired.[436,441] The most prominent abnormality demonstrated on seminal fluid analysis in men taking methadone is reduced sperm motility (asthenospermia), but abnormalities in sperm morphologic structure (teratospermia) and oligozoospermia are also seen.[367] Functional δ-, κ-, and μ-opioid receptors have been reported to be present on human spermatozoa.[442] Therefore, exogenous opioids may have a direct effect by slowing sperm motility, independent of their effects on the hypothalamic-pituitary-testicular axis.

Sex Steroids. Administration of sex steroids—androgens, progestins, or estrogens—suppresses gonadotropin secretion by negative feedback mechanisms at the hypothalamus or the pituitary gland or both; chronic administration may cause secondary hypogonadism, resulting in androgen deficiency and impaired sperm production.

Synthetic androgens (androgenic anabolic steroids) and testosterone are used by a significant number of teenage boys and men to increase muscle bulk and strength and enhance athletic performance or physical appearance. For these purposes, these androgens are used in extremely high doses in a variety of combinations and patterns for prolonged periods. The prevalence of anabolic steroid abuse ranges from approximately 1% to 6% in various populations, including high school and college students, weightlifters, body builders (who often have muscle dysphoria, an obsessive preoccupation with a muscular appearance), young recreational athletes, and competitive athletes.[131,443,444]

During chronic administration of high-dose androgenic anabolic steroids, serum concentrations of testosterone, LH, and FSH are very low, and sperm counts are usually suppressed to severe oligozoospermia or azoospermia.[131,443-445] Unless testosterone is being administered, serum testosterone concentrations are low because synthetic androgenic anabolic steroids do not cross-react in testosterone assays. Because of the androgenic effects of these anabolic steroids, individuals taking these agents may not complain of androgen deficiency symptoms.

After discontinuation of even prolonged anabolic steroid use, recovery of the hypothalamic-pituitary-testicular axis usually occurs within weeks to months. However, for unclear reasons, some men experience a protracted period of symptomatic hypogonadism that may last for several months to several years, particularly in older men.[131,443-446] It usually is not possible to know whether these men had underlying hypogonadism before taking anabolic steroids; therefore, if secondary hypogonadism is severe, an appropriate workup, which might include sellar imaging (if hypogonadism is severe), is usually needed. Prolonged secondary hypogonadism after androgenic anabolic steroid use often causes sexual dysfunction and depressed mood. Severe symptoms may lead to continued use of these agents and anabolic steroid dependence. Treatment with testosterone to relieve symptoms of androgen deficiency or with gonadotropins (hCG) to stimulate sperm production and induce fertility may be needed. Although poorly studied, off-label treatment with clomiphene citrate and aromatase inhibitors have also been reported to stimulate gonadotropin and testosterone secretion in these men.[447]

Chronic administration of high doses of progestins, such as megestrol acetate or depo-medroxyprogesterone acetate, or estrogens, such as diethylstilbestrol, also suppresses gonadotropins and testicular function, resulting in secondary hypogonadism. Megestrol acetate is used to stimulate appetite in wasting conditions such as cancer and HIV disease. At the doses used for this purpose, it causes severe symptomatic androgen deficiency and suppression of sperm production.[448] Weight gain induced by megestrol acetate is mostly fat rather than lean mass, in part because of the androgen deficiency that it causes.[449] Most importantly, megestrol acetate may cause symptomatic and potentially life-threatening secondary adrenal insufficiency from simultaneous suppression of cortisol production.[448] Both megestrol and depo-medroxyprogesterone acetate have been used to induce medical castration in patients with prostate cancer.[450] Medroxyprogesterone acetate has also been used to reduce libido in psychiatric conditions manifested by deviant sexual behavior (paraphilia), and it is used in combination with testosterone (to prevent androgen deficiency) for suppression of spermatogenesis in male contraceptive development trials.[105,451] Administration of estrogens (e.g., low-dose diethylstilbestrol for prostate cancer), exposure to estrogen-containing substances, or excessive estradiol production by estrogen-secreting tumors (e.g., Sertoli or Leydig cell tumors) suppresses gonadotropin and testosterone production and causes secondary hypogonadism, usually with prominent gynecomastia.[452-455]

GnRH Analogues. GnRH analogues, both agonists and antagonists, severely suppress endogenous gonadotropin and testosterone production (i.e., medical castration); they are used to treat androgen-dependent pathologic states such as locally advanced or metastatic prostate cancer and central precocious puberty.[202,456,457] Administration of GnRH agonists (e.g., leuprolide, goserelin) produces an initial stimulation of gonadotropin and testosterone secretion (known as a flare), which is followed in 1 to 2 weeks by GnRH receptor downregulation and marked suppression of gonadotropins and testosterone to castration concentrations.[202] The initial surge in testosterone concentrations has been associated with clinical flares in metastatic prostate cancer, and there have been reports of increased bladder outlet obstruction, bone pain, pathologic fracture, spinal cord compression, and death. However, these complications are very uncommon, and it is not clear that they are directly related to the initial increase in

testosterone concentrations. To prevent the potential complications associated with the testosterone flare, AR antagonists (e.g., bicalutamide) are usually coadministered with a GnRH agonist for men with metastatic prostate cancer.[458] In contrast to agonists, GnRH antagonists (e.g., degarelix) cause an immediate suppression of gonadotropin and testosterone secretion without a flare; whether clinically relevant outcomes differ between GnRH agonists and antagonists is not clear.[456,457]

Continuous administration of GnRH agonists in men with locally advanced or metastatic prostate cancer induces castration or near-castration testosterone concentrations and causes symptoms of severe androgen deficiency, including sexual dysfunction with reduced libido and reduced spontaneous erections, diminished energy and motivation, depressed mood and irritability, hot flushes and sleep disturbance, decreased memory and concentration, reduced in muscle mass and strength, increased fat mass and insulin resistance, decreased BMD resulting in osteopenia or osteoporosis, gynecomastia and loss of male hair pattern, and decreased hemoglobin and hematocrit, resulting in significant decline in quality of life.[201,202] As a result, increasingly, GnRH agonist therapy is administered intermittently in the treatment of advanced prostate cancer. However, in a substantial number of men who stop GnRH agonist therapy, testicular function remains suppressed, and testosterone concentrations persist within the castrate or hypogonadal range for prolonged periods (up to 1–3 years).[459-461] Risk factors for prolonged testicular suppression are longer duration of GnRH agonist therapy, older age (>70 years), and possibly low testosterone concentrations and hypogonadism that was present before treatment.

Some, but not all, large observational population-based studies have found that prolonged GnRH agonist therapy increases the risk of diabetes mellitus, coronary heart disease, myocardial infarction, sudden cardiac death, stroke, and fractures. Consequently, the US Food and Drug Administration (FDA) has recommended that the risk factors for these diseases should be assessed and the benefits and risks of GnRH agonist therapy weighed before it is used and that monitoring for these conditions should be continued during treatment.[202,462-465] A 2011 meta-analysis of randomized trials reported that androgen deprivation therapy of men with unfavorable-prognosis prostate cancer was not associated with an increased risk of cardiovascular mortality but was associated with an improved prostate cancer–specific mortality rate.[466] However, another meta-analysis of cohort and randomized trials found that androgen deprivation therapy was associated with an increase in nonfatal cardiovascular events and acute myocardial infarction.[467]

Hypopituitarism. A destructive or infiltrative lesion of the pituitary gland or hypothalamus commonly causes impaired pituitary hormone production (hypopituitarism) and gonadotropin deficiency, resulting in androgen deficiency and impairment in sperm production. The prevalence of hypopituitarism has been estimated to be approximately 1 in 2200.[468,469]

Hypopituitarism is most commonly caused by *pituitary adenomas* and their treatment (hypophysectomy or radiation therapy) or by *hypothalamic* or *parasellar tumors* such as craniopharyngioma, meningioma, optic glioma or astrocytoma, metastatic carcinoma (from the breast, lung, colon, or prostate), pinealoma, germinoma, chordoma, and ependymoma; together, these tumors account for approximately 90% of cases.[468,469]

Other conditions of the pituitary or hypothalamus (or both) that cause hypopituitarism include cranial radiation therapy (intracranial tumors, acute lymphoblastic leukemia prophylaxis, nasopharyngeal carcinoma, total-body irradiation), vascular compromise (traumatic brain injury,[470-472] infarction or pituitary apoplexy, subarachnoid hemorrhage, ischemic stroke, vascular malformation), granulomatous or infiltrative disease (sarcoidosis, histiocytosis X, granulomatosis with polyangiitis, hemochromatosis, transfusion-induced iron overload), infection (tuberculosis, fungal infections like aspergillosis or coccidioidomycosis, basilar meningitis, encephalitis, syphilis, Whipple disease), pituitary stalk disease (traumatic injury, e.g., basilar skull fracture or surgical pituitary stalk section, granulomatous disease, lymphocytic infundibuloneurohypophysitis, infection, tumor), lymphocytic hypophysitis (particularly lymphocytic infundibuloneurohypophysitis, which is more common in men, rather than lymphocytic adenohypophysitis, which is more common in women), and autoimmune hypophysitis (e.g., with cytotoxic T-lymphocyte–associated protein 4 inhibitors, e.g., ipilimumab therapy).[470-475] These conditions are discussed in Chapter 9.

Destructive or infiltrative lesions of the pituitary gland (e.g., nonfunctioning pituitary adenoma) usually result in a gradual, progressive loss of anterior pituitary function. In these instances, GH and gonadotropin (LH and FSH) deficiency (i.e., secondary hypogonadism) usually occur initially, followed by deficiencies of TSH (secondary hypothyroidism) and, eventually, ACTH (secondary adrenal insufficiency), resulting in *panhypopituitarism*.[468,469] However, there are many exceptions to this order of loss, depending on the specific location of the pituitary lesion and the nature of the underlying disease process. For example, lymphocytic hypophysitis usually causes ACTH and TSH deficiency without impairment of gonadotropin production, and ACTH deficiency is more common than TSH deficiency after radiation therapy involving the hypothalamic-pituitary axis. Anterior pituitary hormone loss is even less predictable in disease processes involving the hypothalamus, in part because of the more disperse anatomic arrangement in the hypothalamus of nuclei that produce releasing factors for pituitary hormones. Acute destructive processes such as pituitary apoplexy usually cause panhypopituitarism.

Diseases of the hypothalamus or high in the pituitary stalk may be associated with *diabetes insipidus*, which is caused by destruction or retrograde degeneration of neurons producing arginine vasopressin in the supraoptic or the paraventricular nuclei, respectively.[476-479] Processes involving only the pituitary gland do not cause diabetes insipidus.

Hypothalamic and pituitary stalk diseases may cause hyperprolactinemia due to loss of dopamine-containing neurons or interruption of the hypothalamic hypophyseal portal tract and transport of dopamine from the hypothalamus to the pituitary. Pituitary microadenomas or macroadenomas may produce prolactin, and suprasellar extension of nonsecretory pituitary macroadenomas or those secreting other hormones (e.g., GH) may cause hyperprolactinemia by interrupting the hypothalamic-hypophyseal portal system.

Prepubertal boys who have hypopituitarism resulting in gonadotropin deficiency present with delayed puberty and eunuchoidism, and men present with adult androgen deficiency and complaints of reduced libido and erectile dysfunction. However, in patients with secondary hypogonadism, clinicians must be alert to the possibility and clinical manifestations of *deficiencies of other pituitary hormones* (ACTH, TSH, GH, and arginine vasopressin); *excessive pituitary hormone production* by pituitary adenomas and resulting clinical syndromes, such as excessive prolactin production resulting in hyperprolactinemia, ACTH resulting in Cushing syndrome, GH resulting in acromegaly, gonadotropin and free α- and β-subunits (which usually do not result in a hormone excess

syndrome but rarely cause precocious puberty), or, rarely, TSH resulting in hyperthyroidism; and *tumor mass effects* such as headache, visual disturbance, and visual field defects (typically bilateral superior quadrantanopia or bitemporal hemianopia, but a unilateral effect and a variety of visual field defects may be present) and, uncommonly, cerebrospinal fluid rhinorrhea, cranial nerve palsies, temporal lobe epilepsy, and personality changes.[468,469,480,481] It is important to have a high index of suspicion for the presence of secondary adrenal insufficiency in patients with hypothalamic or pituitary disease, because it is a life-threatening and treatable condition that manifests with nonspecific symptoms and signs. In boys with hypopituitarism who present with a clinical picture of CDGP, GH deficiency may occur in conjunction with gonadotropin deficiency and may contribute to short stature and growth delay.

Usually in men with secondary hypogonadism due to hypopituitarism, the serum testosterone level and sperm count are very low, and LH and FSH concentrations are distinctly low or, less commonly, in the low-normal to slightly low range. Men with panhypopituitarism may have more severe testosterone deficiency than those with CHH.[482] The gonadotropin response to acute or chronic GnRH stimulation is not a clinically useful differential diagnostic test, because it does not reliably distinguish between pituitary and hypothalamic disease that cause gonadotropin deficiency in hypopituitarism. If hypopituitarism is suspected on the basis of the initial clinical and laboratory evaluation, further evaluation should include hypothalamic-pituitary imaging, preferably an MRI with gadolinium contrast enhancement, which can better define the presence and extent of hypothalamic and pituitary disease compared with a CT scan (although CT is less expensive and equally sensitive for identifying a pituitary macroadenoma or microcalcifications found frequently in craniopharyngioma, and it more accurately assesses parasellar bone destruction); formal visual field examination; and investigation of anterior pituitary hormone deficiency or excess.[468,469]

Treatment is aimed at the underlying cause of the hypopituitarism and treatment of pituitary hormone deficiency, including treatment of androgen deficiency secondary to gonadotropin deficiency with testosterone replacement therapy.[468,469] With transsphenoidal surgical treatment of pituitary adenomas, pituitary function is improved in approximately 50% of cases. Dopamine agonist treatment of prolactin-secreting pituitary adenomas improves pituitary function in 60% to 75% of cases. If fertility is desired, testosterone treatment is stopped and gonadotropin therapy is initiated, initially with hCG alone. In men with acquired gonadotropin deficiency, normal testicular volumes and no coexisting testicular disease, hCG treatment alone (without addition of FSH therapy) often stimulates spermatogenesis to concentrations sufficient to restore fertility.[79]

Systemic Disorders

Glucocorticoid Excess (Cushing Syndrome). Excessive concentrations of either exogenous or endogenous glucocorticoids (the latter due to pituitary Cushing disease, ectopic ACTH syndrome, or adrenal adenoma) is a common acquired cause of secondary hypogonadism, resulting in symptomatic androgen deficiency and impaired sperm production.[483-486] In contrast to those patients with adrenal adenoma, some men with glucocorticoid excess due to adrenal carcinoma secrete excessive amounts of androgens (and mineralocorticoids) and do not demonstrate manifestations of androgen deficiency.

Glucocorticoids act primarily to suppress gonadotropins via inhibition of hypothalamic GnRH secretion, but they may also have direct suppressive effects on testis function and therefore produce combined primary and secondary hypogonadism. However, high-dose immunosuppressive glucocorticoid therapy is most commonly associated with a hormone pattern characterized by low testosterone and low-normal gonadotropin concentrations, consistent with secondary hypogonadism. Occasionally, in men receiving glucocorticoid treatment, gonadotropins are high normal or slightly elevated, suggesting primary hypogonadism.

Although most commonly observed with high-dose glucocorticoid treatment, daily doses as low as 5.0 to 7.5 mg of prednisone may cause hypogonadism, particularly in older men. Because high doses of glucocorticoids may suppress SHBG concentrations, it is important to confirm the biochemical diagnosis of hypogonadism using an accurate measurement of free testosterone (i.e., calculated free testosterone or free testosterone by equilibrium dialysis). In preliminary studies of men receiving chronic glucocorticoid therapy, testosterone treatment was found to improve muscle mass, BMD, and quality of life.[487]

Chronic Organ Failure. Chronic organ failure, such as in hepatic cirrhosis, CKD, chronic lung disease, or CHF, is a common cause of symptomatic secondary hypogonadism.[4,5] As discussed previously, chronic systemic illness commonly affects the hypothalamic-pituitary-testicular axis at multiple sites and usually causes combined primary and secondary hypogonadism, but many disorders are associated with a hormone pattern characterized by low serum testosterone and low to low-normal gonadotropin concentrations, indicative of secondary hypogonadism.

The cause of clinical and biochemical hypogonadism in these cases is multifactorial and encompasses both the chronic disease itself and its associated conditions of malnutrition, wasting, a proinflammatory state (with elevated proinflammatory cytokines like IL2, IL6, and tumor necrosis factor α [TNFα]),[488] medication use (e.g., alcohol, opiates, glucocorticoids), chronic stress, and other comorbid illnesses. These associated factors play a large role in suppressing gonadotropin concentrations and contribute to the hormonal pattern of secondary hypogonadism associated with chronic organ failure. The degree to which these factors contribute to the clinical and biochemical manifestations of hypogonadism varies considerably among individuals. Furthermore, biochemical confirmation of low testosterone in patients with chronic organ failure or systemic illness may be confounded by alterations in SHBG. Therefore, accurate and reliable measurements of free testosterone are needed to establish biochemical androgen deficiency in the presence of chronic systemic illness.

Hepatic cirrhosis from any cause (e.g., alcoholic or nonalcoholic liver disease) is commonly associated with a hormone pattern that is consistent with primary hypogonadism (i.e., low free testosterone, high LH, and normal to high-normal FSH concentrations) in mild to moderate disease (Child-Pugh class A or B) and with secondary hypogonadism (i.e., low free testosterone and low-normal LH and FSH concentrations) in severe to end-stage liver disease (Child-Pugh class C).[303,304] SHBG concentrations increase progressively with the severity of cirrhosis, resulting in normal or high serum total testosterone concentrations despite low free testosterone concentrations and clinical manifestations of androgen deficiency. In men with cirrhosis, sperm production is commonly impaired, and sperm motility is reduced.

In alcoholic cirrhosis, serum estrone and estradiol concentrations are relatively high as a result of increased production of adrenal androgens (e.g., androstenedione) induced by alcohol and its metabolite, acetaldehyde; reduced clearance of these androgens by the liver; and subsequent aromatization of androstenedione to

estrone and its conversion to estradiol.[4,5] Relative hyperestrogenism is responsible for several of the clinical manifestations commonly observed in men with alcoholic cirrhosis compared with nonalcoholic cirrhosis, including gynecomastia, palmar erythema, plethora, spider angiomas, and loss of male body hair (reduced axillary and pubic hair and a female escutcheon). Men with severe alcoholic cirrhosis usually have atrophic testes (usually soft in consistency) due to direct toxic effects of alcohol.

In men with severe hepatic cirrhosis, pulsatile GnRH secretion and the pituitary response to GnRH are diminished, contributing to secondary gonadal failure.[489] Spironolactone, which is used to treat edema and ascites associated with portal hypertension, is an AR antagonist and an androgen biosynthesis inhibitor. Its use may contribute to symptoms of androgen deficiency, gynecomastia, and hypogonadism. Protein-calorie malnutrition, complications of cirrhosis such as infection, and continued alcohol abuse contribute to the clinical manifestations and cause of low testosterone in these chronically ill men. A preliminary 12-month double-blind, placebo-controlled study in men with cirrhosis and low testosterone concentrations found that testosterone treatment was safe and increased lean body and bone mass and hemoglobin concentrations and reduced fat mass.[305] Successful liver transplantation improves but does not normalize gonadal function, probably because of chronic immunosuppressive treatment with glucocorticoids and other agents.[490]

As described earlier, *CKD* is commonly associated with a hormone pattern of low serum testosterone and elevated gonadotropin concentrations resulting from reduced renal clearance, consistent with primary hypogonadism.[4,5,307,309,311] With increasing stage of CKD, there is progressive reduction in sperm concentration and motility and alterations in sperm morphology.[491] However, the amplitude of pulsatile LH secretion is reduced, suggesting impaired hypothalamic-pituitary function in men with CKD.[492] Gonadotropin secretion may also be suppressed by coexisting uremia, hyperprolactinemia, malnutrition, a proinflammatory state, comorbid conditions (e.g., diabetes), and obesity, and some men demonstrate a hormone pattern that is more consistent with secondary hypogonadism (i.e., with low testosterone and normal to high-normal gonadotropin concentrations). Randomized controlled trials to assess the long-term clinical benefits and risks of testosterone treatment in CKD patients who are high risk of cardiovascular events and mortality have not been performed.[308] Successful renal transplantation usually normalizes concentrations of testosterone and gonadotropins and sperm production, although spermatogenesis and Sertoli cell function recover more slowly and to a lesser extent.[307,309,493]

Men with *chronic lung disease*, especially chronic obstructive pulmonary disease (COPD), commonly have low serum testosterone concentrations.[494-496] The prevalence of biochemical hypogonadism depends on the population studied and varies from 12% in a community-based population to 38% in male veterans, the latter being a population with numerous comorbid conditions that lower testosterone concentrations. In the population of veterans, approximately 75% of men with COPD with low serum testosterone have low or low-normal gonadotropin concentrations, consistent with secondary hypogonadism, and the remainder have elevated gonadotropins, indicative of primary hypogonadism.[495] Coexisting factors that contribute to the clinical symptoms and biochemical diagnosis of hypogonadism in men with severe COPD include muscle wasting, inactivity, and deconditioning; malnutrition and cachexia; chronic stress and inflammation; medications (e.g., glucocorticoids); and hypoxia. Hypoxia suppresses gonadotropin and testosterone secretion independent of glucocorticoid therapy in men with COPD or idiopathic pulmonary fibrosis.[494-497] Preliminary studies have demonstrated an increase in lean mass with testosterone treatment in men with COPD and low testosterone concentrations but inconsistent improvements in muscle strength, including respiratory muscle function, and no effects on endurance or quality of life.

CHF is associated with biochemical androgen deficiency in approximately 25% to 30% of cases.[498-500] Men with CHF who have low serum testosterone usually have normal to low-normal gonadotropin concentrations, suggesting secondary hypogonadism. However, it is unclear whether men with CHF and low testosterone differ from those with normal testosterone in regard to symptoms and signs or response to testosterone therapy.[499] In limited initial clinical trials in men with CHF, testosterone treatment improved exercise tolerance, muscle strength, and oxygen capacity in men with either low or normal testosterone concentrations, suggesting a pharmacologic effect of testosterone independent of the presence of androgen deficiency. In a preliminary randomized controlled pilot study in men with CHF with reduced ejection fraction and low testosterone concentrations, testosterone treatment was safe but did not improve functional capacity, walking distance, left ventricular ejection fraction, brain natriuretic peptide concentrations, or quality of life.[501]

Chronic Systemic Illness. Several chronic systemic illnesses, such as T2DM, malignancy, rheumatic disease, and HIV disease, may also cause secondary hypogonadism characterized by low serum testosterone concentrations and low or low-normal gonadotropin concentrations.[4,5] As in men with chronic organ failure, the cause of clinical and biochemical hypogonadism is multifactorial due to the chronic illness itself and associated obesity (e.g., with diabetes mellitus) or malnutrition (e.g., with malignancy), wasting, proinflammatory state, medication use (e.g., opiates, glucocorticoids), chronic stress, or other comorbid illnesses. These factors suppress gonadotropin concentrations and contribute variably to the clinical and biochemical androgen deficiency seen in individuals with systemic illness. Because systemic illnesses and associated comorbid conditions and medications may alter SHBG concentrations, accurate measurements of free testosterone (i.e., calculated free testosterone or free testosterone by equilibrium dialysis) are needed to confirm biochemical androgen deficiency.

Low serum free testosterone and low or low-normal gonadotropin concentrations, consistent with secondary hypogonadism, occur in 30% to 50% of men with *T2DM*.[502,503] Low testosterone concentrations are associated with nonspecific clinical manifestations that may be caused by androgen deficiency, such as sexual dysfunction and reduced vitality. Insulin resistance and moderate obesity are commonly associated with T2DM. In patients with T2DM and moderate obesity, low total testosterone concentrations commonly result from reduced SHBG concentrations caused by insulin resistance and action on the liver. Therefore, it is important to confirm biochemical androgen deficiency in these men by using calculated free testosterone values or measurements of free testosterone by equilibrium dialysis.

Secondary hypogonadism and clinical manifestations of androgen deficiency may also be caused by comorbid conditions and complications associated with diabetes, such as obesity, atherosclerotic vascular disease, the proinflammatory state of diabetes, diabetic neuropathy, and CKD. A meta-analysis of seven placebo-controlled randomized trials of testosterone treatment in men with T2DM failed to improve glycemic control as assessed by hemoglobin A_{1c} and insulin resistance by homeostatic model assessment of

insulin resistance 2.[504] Therefore, testosterone treatment should only be considered only in those diabetic men with symptomatic androgen deficiency that has been confirmed by accurate free testosterone measurements. If symptoms do not improve with an adequate trial of testosterone therapy (e.g., 6 months), discontinuation of treatment should be considered, particularly in men who had borderline or slightly low testosterone concentrations before therapy.

Men with poorly controlled T1DM may have reduced serum testosterone and gonadotropin concentrations and reduced LH pulse amplitude and frequency, which are not present in those with well-controlled disease and in the absence of obesity.[505,506]

Men with *malignancy* commonly have secondary hypogonadism characterized by low serum testosterone concentrations and low or low-normal gonadotropin concentrations.[364,507,508] As mentioned previously, some men with malignancy present with low testosterone and elevated gonadotropin concentrations, consistent with primary hypogonadism. Malnutrition, wasting (cancer cachexia), systemic inflammation, medication use (e.g., opioid pain medications, glucocorticoids), chronic stress, and concomitant comorbid illnesses contribute to clinical and biochemical hypogonadism in men with cancer. Because SHBG may be reduced as a result of these associated conditions, accurate free testosterone measurements are needed to confirm androgen deficiency.

Primary or secondary hypogonadism may be present before systemic chemotherapy or radiation therapy, as well as after treatment. A low free testosterone concentration with normal or elevated gonadotropin concentrations was found in 40% to 60% of men with advanced malignancy (i.e., metastatic cancer) presenting with malnutrition and men with various stages of Hodgkin disease before chemotherapy.[344,345,364] Low concentrations of free testosterone and bioavailable testosterone were present, respectively, in approximately 78% and 66% of men with a variety of cancers, excluding those with androgen-dependent cancer (prostate, breast) or testicular cancer, most of whom received chemotherapy or radiation therapy or both.[364,507] Low testosterone was associated with reduced quality of life and sexual function.[508]

Men with *rheumatic diseases*, particularly the systemic autoimmune disorder rheumatoid arthritis, may manifest symptoms of sexual dysfunction (reduced libido and erectile dysfunction) and low serum free or bioavailable testosterone and gonadotropin concentrations in approximately 30% of cases.[509,510] Secondary hypogonadism in rheumatoid arthritis may be due in part to systemic inflammation (with elevated proinflammatory cytokines like IL2, IL6, and TNFα), complications such as rheumatoid lung, and treatment with glucocorticoids, but it may also occur early in the course of rheumatoid arthritis in the absence of complications and before glucocorticoid therapy.[509,510] In men with long-standing rheumatoid arthritis, low free testosterone concentrations do not normalize after marked suppression of inflammation induced by anti-TNF therapy.[511] Testosterone treatment may improve symptoms of androgen deficiency, but it does not reduce disease activity.[512]

Men with systemic lupus erythematosus may also demonstrate low free testosterone concentrations in conjunction with normal or low-normal gonadotropin concentrations, indicative of secondary hypogonadism or elevated gonadotropins, consistent with primary hypogonadism.[513-516] Factors that contribute to gonadotropin suppression and secondary hypogonadism include chronic systemic illness and inflammation, major organ involvement or organ failure (heart, lung, brain, kidney), and glucocorticoid therapy.[516] Factors that contribute to primary testicular dysfunction include systemic and local inflammation or vasculitis, organ failure, and treatment with cytotoxic agents such as cyclophosphamide.

In observational cohort studies, low testosterone concentrations are associated with rheumatic autoimmune disease, including rheumatoid arthritis and systemic lupus erythematosus.[517,518] It is possible that the hypogonadism contributes to the immunologic pathophysiology of rheumatic disorders.[519,520] Autoimmune diseases, particularly autoimmune rheumatic diseases (e.g., Sjögren syndrome, systemic lupus erythematosus, rheumatoid arthritis), thyroid disease (Hashimoto disease, Graves disease), and autoimmune neurologic diseases (e.g., myasthenia gravis, multiple sclerosis), occur more commonly in women than in men. Sex steroid hormones, primarily estrogens and androgens, modulate immune function by direct actions on immune cell function and may play a role in sex differences in autoimmunity and in the pathophysiology of autoimmune disorders.

Men with *HIV disease* commonly have hypogonadism characterized by symptomatic androgen deficiency and impaired sperm production with low free testosterone concentrations due to combined primary and secondary hypogonadism.[300,521] Hypogonadism occurred in up to 50% of men with HIV wasting before the advent of HAART. Although androgen deficiency is less common since the advent of HAART, it occurs in approximately 20% of HIV-infected men. In 75% to 90% of cases, low free testosterone is associated with low or low-normal gonadotropin concentrations, consistent with secondary hypogonadism.[522,523] In the remaining 10% to 25% of cases, gonadotropin concentrations are elevated, indicating primary hypogonadism.

As in other chronic systemic illnesses, the cause of hypogonadism in men with HIV disease is multifactorial.[4,5] In addition to HIV infection itself, gonadotropin suppression and secondary hypogonadism may be caused by malnutrition, wasting, and cachexia; opportunistic infections affecting hypothalamic-pituitary function (e.g., CMV, *T. gondii*); systemic inflammation (with elevated concentrations of cytokines like IL2, IL6, and TNFα); medications (e.g., opiates, glucocorticoids, megestrol acetate); ongoing substance abuse (e.g., alcohol); and acute and chronic illnesses. Conditions that may play a role in causing primary hypogonadism include opportunistic infections affecting the testes (CMV, *M. avium intracellulare, T. gondii*), malignancies involving the testes (Kaposi sarcoma, lymphoma), systemic inflammation, and medications (chemotherapy for secondary neoplasms, ketoconazole).[300,521,522,524]

Protein-calorie malnutrition may suppress SHBG concentrations, and advancing HIV infection is associated with elevated SHBG concentrations. Therefore, accurate measurements of free testosterone concentrations should be used to evaluate men with HIV disease for androgen deficiency.[523,525] Sperm production associated with abnormalities in sperm motility and morphologic structure and testicular atrophy may be present. It is important to recognize that HIV may be present in semen even when it is undetectable in plasma.[300]

In small clinical trials, treatment with testosterone or an androgenic anabolic steroid has been demonstrated to improve libido and sexual function, increase muscle mass and strength and BMD, decrease fat mass, improve mood, well-being, and quality of life, and increase hematocrit in HIV-infected men with low serum testosterone concentrations.[116]

Men with chronic *spinal cord injury* at any level resulting in tetraplegia or paraplegia may have secondary hypogonadism with low serum testosterone and low or normal gonadotropin

concentrations.[350,351] Less commonly, some men with chronic spinal cord injury demonstrate low testosterone and elevated gonadotropins concentrations (consistent with primary hypogonadism) or combined primary and secondary hypogonadism. In men with lower spinal cord injury causing paraplegia, there may be a transient suppression of testosterone concentrations within 4 months after the injury that resolves in most cases.[352,526] Gonadotropin suppression is caused in part by several conditions associated with spinal cord injury, such as acute and chronic trauma and stress associated with the injury and attendant complications; obstructive sleep apnea; obesity or nutritional compromise; hyperprolactinemia (usually associated with medications); and medication use (e.g., glucocorticoids, opioids, CNS-active drugs). The benefits and risks of testosterone treatment in patients with spinal cord injury are not clear.

Thalassemia major, or β-thalassemia, is an autosomal recessive disorder characterized by absent or severely deficient synthesis of β-globulin chains of hemoglobin resulting in severe anemia that requires lifelong blood transfusions. It is common in the Mediterranean region, in India, and in Southeast Asia. Chronic blood transfusions in patients with β-thalassemia cause *transfusion-related iron overload* in tissues and produce clinical manifestations similar to those that occur in patients with hereditary hemochromatosis. Transfusion-related iron overload may also occur in patients with sickle cell anemia, refractory aplastic anemia, or myelodysplastic syndrome.

Iron deposition in the testes and pituitary gland usually causes combined primary and secondary hypogonadism.[527-529] However, males with transfusion-related iron overload usually exhibit a hormonal pattern indicative of secondary hypogonadism, with low serum free testosterone and low to low-normal gonadotropin concentrations in most cases. Hypogonadism due to transfusion-related iron overload may manifest with androgen deficiency and impaired sperm production; affected boys usually have short stature and pubertal and growth delay.[530] Early iron chelation therapy with agents such as deferoxamine, deferasirox, or deferiprone may reverse hypogonadism and improve survival.[531] In men with long-standing β-thalassemia, chelation therapy does not reverse hypogonadism.[532]

Sickle cell disease may be associated with low serum testosterone and low to low-normal gonadotropin concentrations, indicative of secondary hypogonadism.[533-535] Gonadotropin suppression may be caused by transfusion-induced iron overload (although much less commonly than in men with β-thalassemia[348,536]), hypothalamic-pituitary microinfarctions, medications (e.g., opioids for chronic pain), systemic inflammation, nutritional deficiencies, and chronic systemic illness and stress secondary to repeated painful vaso-occlusive events. As discussed earlier, men with sickle cell disease may present with primary hypogonadism due to testicular microinfarctions caused by vaso-occlusive events or iron overload affecting the testes. Priapism, which may occur with vaso-occlusive events, has been reported in patients receiving testosterone therapy.[531,537] As in β-thalassemia, early iron chelation therapy may reverse hypogonadism.

Uncommonly, boys or men with *cystic fibrosis* have low serum testosterone and low to low-normal gonadotropin concentrations, a hormonal pattern consistent with secondary hypogonadism.[538] Chronic systemic illness and inflammation, malnutrition, and glucocorticoid use may contribute to gonadotropin suppression.

Nutritional Disorders or Endurance Exercise. Starvation, malnutrition, and eating disorders (anorexia nervosa) suppress gonadotropin and testosterone secretion, resulting in symptomatic secondary hypogonadism with androgen deficiency (usually manifested by reduced libido, sexual activity, and performance) and impaired sperm production; these effects are reversed with restoration of food/calorie intake and weight gain. Fasting for periods of 3 to 5 days suppresses gonadotropin and testosterone secretion and decreases LH pulse amplitude and frequency.[539-541] These changes are reversed completely by pulsatile GnRH administration or low-dose recombinant human methionyl leptin replacement, suggesting that short-term starvation suppresses leptin production, which in turn suppresses the hypothalamic GnRH pulse generator.[542-544] Severe protein-calorie malnutrition, often associated with other nutritional deficiencies, may cause severe suppression of testosterone and elevation of gonadotropin concentrations, indicative of primary hypogonadism.[540,545]

Chronic *endurance exercise* results in low serum testosterone, low to low-normal gonadotropin concentrations, and impaired sperm production and motility, consistent with secondary hypogonadism.[546-548] High-intensity endurance training and exercise, such as occurs in the military and with overtraining in athletes, is associated with relative calorie deprivation (resulting in energy expenditure that is greater than energy intake, i.e., a relative energy deficit) and intense stress that causes more severe suppression of gonadotropin and testosterone concentrations and symptoms of androgen deficiency than is seen with chronic lower-intensity endurance exercise. This condition has been referred to as relative energy deficit in sport by the International Olympic Committee.[539,546-549] Suppression of the hypothalamic-pituitary-testicular axis resolves with cessation of training and increased calorie intake.[546,547] Other than reduced spermatogenesis, the clinical consequences of androgen deficiency induced by endurance exercise are not clear.[550] In contrast to chronic endurance exercise, short-term endurance or resistance exercise in some men results in an acute and transient increase in testosterone concentrations that is modified by the intensity of exercise and prior training and is possibly related to hemoconcentration, reduced metabolic clearance, or transient increases in serum LH concentrations.[551]

Mild to moderate obesity results in reduced SHBG and total testosterone concentrations. Free testosterone is usually normal but may be reduced in association with low or low-normal gonadotropin concentrations in some men, particularly in those with comorbid conditions such as T2DM or obstructive sleep apnea.[552-555] In men with *morbid obesity* (body mass index [BMI] >40 kg/m^2) or massive obesity (BMI >45 kg/m^2), serum free testosterone is low, gonadotropin concentrations are low or low normal, and LH pulse amplitude (but not frequency) is reduced, indicative of secondary hypogonadism.[556] Morbidly and massively obese men often complain of reduced libido and sexual dysfunction, but these symptoms are confounded by obesity and comorbid conditions associated with morbid obesity, such as depression, diabetes, and obstructive sleep apnea. Gonadotropin suppression and androgen deficiency is likely due to proinflammatory cytokines and dysregulated leptin signaling, as well as obesity-related comorbidities.[555,557] Bariatric surgery and substantial weight loss increase serum gonadotropin and testosterone concentrations, but modest weight loss achieved by dieting is less effective in reversing obesity-related hypogonadism.[555,558-561] In a small, 56-week, randomized, double-blind, placebo-controlled trial in men with mild to moderate symptoms of androgen deficiency and low testosterone concentrations, testosterone treatment plus a very low energy diet improved symptoms and reduced fat mass while maintaining lean mass versus diet alone that resulted in reductions in both fat and lean body mass.[562,563]

Morbid obesity may be complicated by *obstructive sleep apnea* syndrome. Men with untreated or inadequately treated obstructive sleep apnea have low gonadotropin and testosterone concentrations in part related to but also independent of obesity and age.[553,564,565] Adequate treatment with continuous positive airway pressure (CPAP) may improve symptoms (e.g., erectile function) and reverse biochemical secondary hypogonadism in some but not all men. Treatment of hypogonadism with relatively high doses of testosterone (e.g., doses associated with the use of parenteral testosterone esters) may induce or worsen obstructive sleep apnea in men with predisposing conditions such as obesity and in older men.[553,566-570] Testosterone replacement therapy of obese men with obstructive sleep apnea that produce more physiologic testosterone concentrations has been demonstrated to improve sexual desire without inducing or worsening obstructive sleep apnea.[571]

Acute and Critical Illness. Acute and critical illnesses, including medical and surgical illnesses requiring hospital or intensive care unit admission (e.g., myocardial infarction, respiratory illness, sepsis, burns, surgery, polytrauma, stroke, traumatic brain injury, liver disease, fractures), suppress gonadotropin and testosterone secretion as a result of combined primary and secondary testicular dysfunction.[572-575] However, the predominant hormone pattern during acute or critical illness is low serum testosterone with low or low-normal gonadotropin concentrations, suggesting secondary hypogonadism. Spontaneous LH pulse amplitude is reduced, but pulse frequency is maintained, and pulsatile GnRH administration only partially corrects secondary hypogonadism, underscoring the presence of concomitant pituitary and testicular defects.[576] For unclear reasons, aromatization of testosterone to estradiol and serum concentrations of estradiol may be increased, sometimes markedly, in patients with acute or critical illness despite low testosterone concentrations.[577] Elevated estradiol concentrations are associated with fatality in critically ill and injured patients.[578-581] A caveat regarding these studies is that estradiol was measured by immunoassay, not by state-of-the-art mass spectrometry-based assays that are more accurate, sensitive, and specific than immunoassays for estradiol measurements in men.[582]

The severity and duration of testosterone suppression are related to the severity of the acute or critical illness, the presence of underlying chronic systemic illnesses, age, and the medications used (e.g., glucocorticoids, opioids).[572,573] Recovery of testosterone and gonadotropin concentrations may take several weeks to months, depending on the severity and duration of the acute illness, duration of subacute recovery and rehabilitation, complications including malnutrition, medications, and underlying chronic systemic illnesses or organ failure. In the presence of underlying chronic disease or organ failure, hypogonadism may persist long after recovery from the acute illness. For these reasons, evaluation for underlying hypogonadism should not be performed during acute or subacute illness and recovery. Assessment should be delayed for several months until recovery to the individual's baseline or near-baseline clinical condition has occurred.

Aging. As discussed earlier, aging is associated with a gradual and progressive decline in total and free testosterone concentrations; as a result, an increasing proportion of older men have low serum testosterone concentrations in the hypogonadal range.[312-314] The prevalence of clinical androgen deficiency is 6% to 9% and increases with age, reaching 18% to 23% among men in their 70s.[196,197] Serum gonadotropin concentrations increase with aging but do not usually rise above the normal range until very old age, usually beyond 70 years of age.[314] Therefore, the most common hormonal profile observed in clinically hypogonadal middle-aged to older men is low testosterone with normal LH and FSH concentrations, indicative of secondary hypogonadism. Pulsatile LH secretion is abnormal and is characterized by disorderly LH pulses of reduced amplitude; it is normalized by exogenous pulsatile GnRH administration, suggesting a defect in hypothalamic GnRH secretion.[316]

The presence of obesity, chronic systemic illness or organ failure, medications, or malnutrition or wasting syndromes that occur more frequently with increasing age may contribute to suppression of gonadotropin and testosterone production in middle-aged to older men. Conversely, the age-related decline in testosterone may also contribute to the susceptibility and severity of clinical hypogonadism that occur in these conditions.[200,318] Many of the comorbidities that contribute to low testosterone concentrations in older men are potentially reversible or treatable (functional hypogonadism, see later discussion). Therefore, management of underlying causes of functional causes of hypogonadism and symptoms androgen deficiency in older men should be utilized as an initial or adjunctive approach to testosterone replacement therapy.[583]

Previous relatively small, short-term studies of testosterone treatment in heterogeneous populations of older men have produced conflicting results, with most finding beneficial effects of testosterone treatment on body composition (increasing lean mass and decreasing fat mass) but less consistent effects on muscle strength and performance, BMD, sexual function, vitality, and cognitive function.

Recently, results of the Testosterone Trials, a coordinated set of seven double-blind, placebo-controlled studies that evaluated the short-term effects of testosterone treatment in 788 older men (average age of 72 years old) with unequivocal hypogonadism (symptoms and signs of low libido; difficulty walking and slow walking speed; and/or low energy; and average of two morning serum total testosterone concentrations <275 ng/dL) with no apparent cause of hypothalamic-pituitary-gonadal disease. Compared to placebo, testosterone treatment for one year that increased and maintained testosterone concentrations to the mid-normal range for young men significantly improved sexual function (sexual activity, libido and erectile dysfunction), anemia and hemoglobin, volumetric BMD and estimated bone strength; slightly improved walking distance, mood and depressive symptoms; and did not improve multiple domains of cognitive function and vitality. There has been controversy about the clinical importance of these findings. In addition, testosterone treatment caused erythrocytosis (hemoglobin concentration >17.5 g/dL) and an increase in PSA concentration >1.0 ng/mL in small number of men; and increased noncalcified coronary artery plaque volume on CT angiography. There was no difference in LUTS or cardiovascular or prostate adverse events in testosterone- and placebo-treated men (although the Testosterone Trials were underpowered for the latter outcomes).[162,319-323]

Larger, long-term, randomized trials are needed to determine the balance of clinical benefits and risks (particularly prostate cancer and cardiovascular risks) associated with testosterone treatment in older men. For now, testosterone treatment should be considered on an individual basis only for older men who have clinically significant symptoms and signs of androgen deficiency and unequivocally low serum testosterone concentrations, and only after a careful discussion of the uncertainty regarding the benefits and risks of treatment.[116] In addition, in older men, there should be consideration of treatment of underlying comorbidities and discontinuing medications that may cause functional hypogonadism.

Isolated Impairment of Sperm Production or Function

Congenital or Developmental Disorders

Congenital Adrenal Hyperplasia. If untreated or inadequately treated with glucocorticoids, congenital adrenal hyperplasia caused by deficiency of 21-hydroxlase or 11β-hydroxylase results in excessive secretion of adrenal androgen precursors (including androstenedione, DHEA, and 11-hydroxyandrostenedione) that are converted to testosterone and other androgens (e.g., 11-ketotestosterone). Elevated circulating androgen concentrations suppress gonadotropin secretion by negative feedback regulation, which in turn decreases endogenous testosterone secretion and sperm production, resulting in secondary hypogonadism. Excessive adrenal androgen production may offset testicular androgen deficiency, so secondary hypogonadism may be manifested by an isolated impairment of sperm production and function.[584]

Glucocorticoid therapy at dosages that suppresses serum ACTH concentrations to normal or low levels reduces excessive adrenal androgen production and may restore gonadotropin secretion and may normalize testis function, including spermatogenesis. As discussed previously, supraphysiologic glucocorticoid treatment is usually required to reduce adrenal androgen concentrations to normal, and the high dosages of glucocorticoids suppress the hypothalamic-pituitary-testicular axis and result in low gonadotropin, testosterone, and sperm concentrations in many men with congenital adrenal hyperplasia. In addition, some men with congenital adrenal hyperplasia continue to manifest impaired sperm production and function due to irreversible testicular damage caused by large TARTs.

Isolated FSH Deficiency and *FSHβ* Mutations. Rare cases of men with isolated FSH deficiency in the absence of *FSHβ* gene mutations have been reported; these patients had isolated impairment in sperm production characterized by azoospermia or severe oligozoospermia, and hypospermatogenesis or maturation arrest was found in the few who underwent testis biopsy.[400-402] These men had normal virilization, normal concentrations of testosterone and LH, low to undetectable serum FSH concentrations with poor or no response to GnRH administration, normal LH, and normal inhibin B and activin A concentrations when tested. In one man, administration of recombinant human FSH (rhFSH) alone resulted in a robust increase in sperm counts and induced fertility on two occasions.

Men with inactivating mutations of *FSHβ* have been found generally to have azoospermia with undetectable FSH, low or low-normal testosterone, and high LH concentrations.[82-85,405,406] In one man, rhFSH administration was demonstrated to increase testosterone concentrations, suggesting that FSH-stimulated Sertoli cells may enhance LH-induced Leydig cell production of testosterone via a paracrine mechanism.[585]

Acquired Disorders

Androgen Administration or Excess. Exogenous testosterone administration (in normal men or in men with partial hypogonadism)[586] or stimulation of endogenous testosterone production by hCG administration[587] or ectopic hCG-secreting tumors (e.g., testis cancer, lung cancer)[588] suppresses pituitary gonadotropin secretion by negative feedback regulation; this in turn suppresses spermatogenesis by the testes in the presence of normal or high serum testosterone concentrations (i.e., secondary hypogonadism with isolated impairment of sperm production).[589,590] Use of some androgenic anabolic steroids (e.g., nandrolone) may also produce low gonadotropin concentrations and isolated reduction in spermatogenesis while providing sufficient androgen activity to avoid clinical androgen deficiency; however, endogenous testosterone production is usually also suppressed by androgenic anabolic steroid use, resulting in low serum testosterone concentrations.

Discontinuation of androgen or hCG administration results in restoration of normal gonadotropin secretion and recovery of normal sperm production and testosterone production by the testis. Long-term anabolic steroid abuse in athletes has been reported to be associated with testicular atrophy and severe oligozoospermia or azoospermia that persists for several months to years after discontinuation of the performance-enhancing agents.[445] Suppressed sperm production induced by anabolic steroids may respond to treatment with hCG or with clomiphene citrate (off-label use).[447,591] Administration of testosterone in combination with progestins in normal men has been the main strategy used to suppress sperm production in recent hormonal male contraceptive development trials.[105]

Malignancy. Malignancies that occur commonly in men of reproductive age (e.g., testicular cancer, Hodgkin disease) manifest with impaired sperm production and function before chemotherapy or radiation therapy in 30% to 80% of cases.[343-345,364] Among men with cancer who provided semen samples for cryopreservation before treatment, approximately 64% had abnormal semen parameters, and 12% had no viable sperm.[592]

In population studies, testicular cancer is associated with infertility. This association may reflect abnormal testicular development, termed *testicular dysgenesis syndrome*, caused by exposure to environmental gonadotoxins or endocrine disruptors (e.g., estrogens) or by an underlying genetic predisposition.[343,364,593] Testicular dysgenesis syndrome is also associated with cryptorchidism and hypospadias, and the former is associated with an increased risk of testicular cancer and abnormal spermatogenesis. Ectopic hCG secretion and possibly increased scrotal temperature associated with cancer within the testis may also contribute to impaired spermatogenesis in men with testicular cancer.[593] Hodgkin disease and other lymphomas and leukemias may be associated with fever, weight loss, and systemic inflammation.[593] These cancers may also involve the testis. All of these factors may play a role in impairing sperm production. Men with these cancers who have systemic disease, symptoms, or inflammation may present with low-normal testosterone, suppressed gonadotropin concentrations, and abnormal semen analysis, consistent with secondary hypogonadism causing isolated impairment of sperm production or function.

Hyperprolactinemia. Men with severe hyperprolactinemia (e.g., prolactin concentrations >200 ng/mL) develop secondary hypogonadism causing androgen deficiency and impaired sperm production. Mild hyperprolactinemia may be associated with isolated impairment of sperm production.[594] In most of these cases, gonadotropin and testosterone concentrations are normal, and spermatogenesis is not improved with dopamine agonist treatment.[595] Therefore, hyperprolactinemia does not contribute to impairment in sperm production and probably is not clinically significant in most cases. In most instances, abnormal sperm production and function are caused by a primary testicular disorder, such as idiopathic oligozoospermia or azoospermia. Rarely, some men with moderate hyperprolactinemia (e.g., prolactin concentrations of 100–200 ng/mL) have low-normal testosterone and gonadotropin concentrations and isolated impairment of sperm production that respond to dopamine agonist treatment.[594]

Androgen Resistance Syndromes

Congenital Disorders

Congenital androgen resistance and insensitivity syndromes are usually caused by defects in androgen action due to mutations

in the *AR* gene or in the steroid 5α-reductase type 2 gene, *SRD5A2*.[125,136,596,597] Males with severe defects in androgen action present at birth either as phenotypic females, as occurs with *complete androgen insensitivity syndrome* (CAIS, previously known as testicular feminization syndrome), or as males with ambiguous genitalia and *46,XY DSD* (previously termed *male pseudohermaphroditism*).[597] Individuals with *partial androgen insensitivity syndrome* (PAIS) present with varying degrees of impaired androgen action and mildly to severely disordered male sexual development.

CAIS usually presents as a 46,XY phenotypic female with normal breast development, primary amenorrhea, and absence of body hair.[597] Severe androgen insensitivity results in absence of facial, axillary, and pubic hair; normal-appearing female external genitalia and distal two thirds of the vagina; and poorly developed or absent male internal genitalia (prostate, epididymides, seminal vesicles, and vasa deferentia) as a result of fetal androgen resistance. In adults with CAIS, female breast development is present due to conversion of normal to high concentrations of testosterone (which are secreted by the testes at puberty) to estradiol, which stimulates breast development. Because testes that are intra-abdominal or inguinal in location at birth secrete AMH normally during fetal development, female internal genitalia (proximal vagina, uterus, and fallopian tubes) are absent.

There is considerable variation in the presentation of individuals with PAIS.[597] Some of these males present at birth with ambiguous genitalia, whereas others present at puberty or in adulthood with mild genital abnormalities or relatively normal genital development. Clinical manifestations of androgen deficiency and disordered male sexual development range from severe undervirilization to near-normal virilization with infertility. In men with PAIS, manifestations that are indicative of disordered sexual development, such as microphallus, hypospadias, scrotal abnormalities (e.g., bifid scrotum), cryptorchidism, and gynecomastia, are common. Gynecomastia is present in almost all of these individuals.

As a result of the variability in clinical manifestations, PAIS encompasses many disorders, previously referred to as Reifenstein, Lubs, Rosewater, and Gilbert-Dreyfus syndromes. For example, Reifenstein syndrome is characterized by hypospadias, gynecomastia, undervirilization, a small prostate gland, cryptorchidism, and impaired spermatogenesis. However, even in the same family, different members may have different clinical manifestations. Some family members may not have hypospadias, and others may be normally virilized. Given this degree of variability in manifestations, the older eponyms are not clinically useful.[597]

Some men with PAIS have no evidence of disordered male sexual development and present with isolated impairment of sperm (idiopathic oligozoospermia or azoospermia), occasionally in association with gynecomastia, high to high-normal testosterone concentrations, and elevated LH concentrations. This disorder is referred to as minimal AIS.[136,596]

In CAIS and PAIS, serum testosterone concentrations are high or high normal with high serum LH concentrations, but FSH concentrations are usually normal. Most 46,XY individuals with CAIS have autosomal recessive mutations of the *AR* gene on the X chromosome that alter its primary sequence and structure (almost always resulting in CAIS) or its function, resulting in impaired androgen binding to AR, AR binding to DNA, or AR transactivation.[136,596] In men with PAIS, the correlation of *AR* genotype and clinical phenotype is relatively poor. In many men with PAIS or minimal AIS, no mutations in *AR* are identifiable. These men may have high CAG repeat length in the *AR* gene, misdiagnosis with unrecognized mutations of other genes such as *SRD5A2*, or mutations of coactivators or corepressors that regulate AR function.

An increase in the number of trinucleotide CAG repeats in the first exon of the *AR* gene results in expansion of the polyglutamine tract in the N-terminal domain of the AR.[139] The CAG repeat length is inversely correlated with AR function and action. Pathologic increase to more than 40 to 62 CAG repeats (normal range, 11–35) causes Kennedy disease (spinal and bulbar muscular atrophy), a rare neurodegenerative disease that is thought to be caused by neurotoxicity from intracellular aggregation of the abnormal AR and associated coregulator proteins (see earlier discussion).[137,138] Men with Kennedy disease have clinical manifestations of partial androgen resistance, including gynecomastia, sexual dysfunction, oligozoospermia or azoospermia, and infertility associated with high testosterone and high or normal gonadotropin concentrations. Higher CAG repeat numbers within the normal range have been reported to be associated with decreased virilization, impaired spermatogenesis and infertility, and gynecomastia in some studies but not others.[139]

5α-Reductase deficiency, caused by an autosomal recessive mutation in *SRD5A2*, is a rare condition similar to PAIS.[598] Affected individuals typically present with markedly ambiguous genitalia, usually characterized by a clitoris-like phallus, a severely bifid scrotum, an apparent vaginal opening with perineal and scrotal hypospadias (termed *pseudovaginal perineoscrotal hypospadias*), an atrophic prostate, and testes located in the inguinal canal or scrotum or sometimes intra-abdominally (cryptorchidism). In contrast to men with AISs, wolffian duct differentiation is unaffected, and patients with 5α-reductase deficiency have normal male internal genitalia (epididymides, seminal vesicles, ejaculatory ducts, and vasa deferentia). AMH secretion by the fetal testes causes regression of müllerian duct structures, so no female internal genitalia develop.

Because ambiguous genitalia resemble female more than male external genitalia, individuals with 5α-reductase deficiency are usually raised as females.[125] However, with the marked increase in testosterone production by the testes at puberty, partial virilization occurs (i.e., penile growth, rugation and pigmentation of the scrotum, increased muscle mass and height, deepening of the voice, increase in libido, and spontaneous erections), and some of these individuals take on a male gender role at puberty, depending on complex psychosocial factors and cultural background. Sebum production is normal in these men. Because normal androgen action in the skin and prostate requires conversion of testosterone to DHT by 5α-reductase, men with 5α-reductase deficiency do not develop a male body hair pattern, and the prostate gland remains nonpalpable. Prostate cancer and BPH have not been reported in these men.

Men with 5α-reductase deficiency have high-normal to high serum testosterone and normal to slightly elevated LH and FSH concentrations (LH > FSH). Serum DHT concentrations are low, and the serum testosterone/DHT ratio is variably elevated. Spermatogenesis is impaired (oligozoospermia or azoospermia) as a result of cryptorchidism, but normal sperm production has been reported in some men with descended testes.[599]

Individuals with CAIS are usually raised as females and undergo orchidectomy (particularly if the testes are intra-abdominal) and treatment with estrogen replacement; more recently, testosterone therapy for reduced sexual functioning has been shown to be more effective than estrogen.[597,600] In men with PAIS or 5α-reductase deficiency, virilization has been induced with high-dose testosterone treatment, which increases serum

testosterone concentrations to above the normal range and normalizes DHT concentrations.[597]

Acquired Disorders

AR antagonists (flutamide, bicalutamide, and nilutamide) induce androgen resistance and are used to treat androgen-dependent prostate cancer.[601] Drugs such as spironolactone, cyproterone acetate, marijuana, and H_2 receptor antagonists (specifically cimetidine) have AR antagonist activity.[602-606]

Men with *celiac disease* or gluten-sensitive enteropathy may experience manifestations of androgen deficiency, including reduced virilization, sexual dysfunction, impaired sperm production and function, and infertility. They may also demonstrate high to high-normal concentrations of serum total and free testosterone and high LH concentrations, indicative of androgen resistance.[607-610] Manifestations of androgen deficiency and biochemical androgen resistance may improve with dietary gluten restriction and improvement in small bowel atrophy in some men.[609] Malnutrition, nutritional deficiencies, chronic systemic illness, and hyperprolactinemia may occur in men with celiac disease and may contribute to their clinical manifestations. Serum DHT concentrations may be low despite high testosterone concentrations in men with celiac disease, suggesting that partial acquired 5α-reductase deficiency may also be present and may play a role in androgen resistance. The main source of circulating DHT is from conversion of testosterone to DHT by 5α-reductase type 1 in the skin and liver.[608] However, 5α-reductase is also present in the gastrointestinal tract, so it is possible that loss of enzyme activity in the small bowel with active celiac sprue contributes to low DHT concentrations.

Treatment of Androgen Deficiency

Functional Versus Organic Causes of Hypogonadism

Prior to initiating testosterone replacement therapy, it is important to consider whether the cause of hypogonadism is functional or organic.[200] *Organic hypogonadism* is caused by congenital/developmental, destructive, or infiltrative disorders of the hypothalamus, pituitary gland, or testes that result in *permanent* hypogonadism. Generally, organic hypogonadism presents with clinically unequivocal severe androgen deficiency (also referred to as classical hypogonadism). Most causes of primary hypogonadism and some causes of secondary hypogonadism are organic (see Tables 19.7 and 19.8). *Functional hypogonadism* is caused by nondestructive suppression of hypothalamic, pituitary, or, uncommonly, testis function that is *potentially reversible without medical or surgical therapy*. Drug-induced primary hypogonadism is a functional cause. Many causes of secondary and combined primary and secondary hypogonadism are due to functional gonadotropin suppression.

Management or treatment of functional causes of hypogonadism might improve or resolve clinical and biochemical androgen deficiency and should be considered before initiating testosterone replacement therapy. For example, functional hypogonadism caused by hyperprolactinemia may be treated by discontinuation of medications that cause hyperprolactinemia or dopamine agonist therapy; opioids, glucocorticoids, CNS-active medications, or progestins may be reversed by discontinuation of the offending drug; nutritional deficiency may be corrected by nutritional supplementation and weight gain; morbid obesity may be improved with diet-induced weight reduction or bariatric surgery; obstructive sleep apnea may be improved by CPAP therapy; T2DM may be improved by weight loss and reduction in insulin resistance; and alcohol abuse may be improved with treatment of alcohol dependence and abstinence. In many instances, however, functional causes of hypogonadism cannot be treated or managed in a reasonable time frame (e.g., opioid or glucocorticoid therapy for chronic comorbid illnesses), so testosterone treatment should be considered.

Testosterone Replacement Therapy

Therapeutic Goals and Management

The overall goal of testosterone replacement therapy is to correct or improve the clinical manifestations of androgen deficiency in men with primary or secondary hypogonadism. Because specific manifestations vary with the stage of sexual development, the specific goals of testosterone treatment vary depending on whether the patient is an adolescent or an adult.[6,116]

In boys with *androgen deficiency of prepubertal onset* and delayed puberty, the goals of testosterone treatment are the following[146,147,374,375]:

- To induce and maintain secondary sexual characteristics, including growth of the penis and scrotum, and a male body hair pattern
- To increase muscle mass and strength
- To stimulate BMD, acquisition of peak bone mass, and long bone growth without compromising adult height by inducing premature closure of epiphyses
- To stimulate libido and spontaneous erections
- To improve energy, mood, and motivation
- To induce laryngeal enlargement and deepening of the voice
- To increase red blood cell production into the normal adult male range.

Testosterone treatment also stimulates the growth of accessory sex glands (seminal vesicles and prostate), resulting in seminal fluid production and an increase in ejaculate volume, but it does not stimulate sperm production to a degree sufficient for induction of fertility. The most common cause of delayed puberty is not a pathologic condition but rather CDGP. Testosterone therapy in boys who present with delayed puberty utilizes low-dose testosterone to avoid premature epiphyseal closure and compromise of adult height, and treatment is given intermittently until spontaneous puberty occurs (see later discussion). If spontaneous puberty does not occur, the testosterone dose is increased gradually to adult concentrations.[146,147,374,375]

The goals of testosterone therapy in *adult hypogonadism* are the following[6,116]:

- To improve sexual function and activity by restoring libido and improving erectile function
- To increase muscle mass and strength, potentially improving physical function and performance
- To increase BMD, potentially reducing the risk of fractures
- To improve energy, vitality, mood, and motivation
- To increase hematocrit into the normal adult male range
- To restore male hair growth.

Recent-onset gynecomastia that is usually symptomatic may respond to testosterone treatment, but severe or long-standing gynecomastia requires surgical excision. Spermatogenesis requires relatively high intratesticular concentrations of testosterone that cannot be achieved by exogenous androgen administration. Therefore, testosterone replacement therapy does not stimulate sperm production or increase testis size, nor does it restore fertility. Treatment of infertility in hypogonadal men is usually possible only in those men with secondary hypogonadism and gonadotropin

deficiency; gonadotropin or GnRH therapy is used to induce spermatogenesis and fertility.[611]

The normal adult range of serum testosterone concentrations is broad and is usually based on results in healthy young men using blood samples drawn in the morning. In young men with androgen deficiency, testosterone replacement therapy produces beneficial clinical effects as serum testosterone concentrations are increased into this normal range. Serum testosterone concentrations decline gradually and progressively with age, but the physiologic significance of this age-related decline is unclear. Initial studies in older men with low serum testosterone concentrations demonstrated some clinical beneficial effects with testosterone treatment that increased testosterone concentrations into the normal young adult range.[322,612] Therefore, the goal of testosterone treatment of hypogonadism is to restore serum testosterone concentrations to within the normal adult range, irrespective of age.[116]

The dose-response effects of testosterone vary in different target organs and for different clinical outcomes.[613] For example, the action of testosterone on muscle mass demonstrates a continuous dose-response relationship. With testosterone administration, muscle mass increases when testosterone concentrations are increased from below normal to within the normal range, and it continues to increase as concentrations are raised from within to above the normal range. In contrast, the actions of testosterone on libido exhibit threshold dose-response characteristics: testosterone administration increases libido when serum testosterone concentrations are increased from low to low-normal concentrations but does not continue to stimulate libido further as serum testosterone is increased to normal or supraphysiologic concentrations.

In men with severe, long-standing androgen deficiency, testosterone replacement therapy induces profound alterations in sexuality, behavior, and physical appearance that may be upsetting to patients and their partners and may result in serious adjustment problems. To reduce the likelihood of problems, it is important to inform and counsel hypogonadal men and their partners regarding changes in body characteristics and behavior that are expected during testosterone replacement therapy. In some men with severe, long-standing hypogonadism, initiation of testosterone replacement with a low-dose regimen (e.g., testosterone enanthate or cypionate 100 mg every 2 weeks, testosterone patch 2 mg daily, or 1.62% testosterone gel 20.25 mg daily) for several months, followed by an increase to full testosterone replacement, may produce a more gradual symptomatic transition from hypogonadism to eugonadism and may result in fewer adjustment difficulties.[614]

Because the metabolic clearance rate of testosterone is reduced in older men with hypogonadism, therapeutic testosterone concentrations may be achieved with lower dosages of testosterone.[615] In some clinical situations, such as severe symptomatic BPH or the presence of numerous comorbid illnesses, full testosterone replacement may be ill advised. In these instances, low-dose testosterone supplementation may also be more prudent than full testosterone replacement therapy. Low dosages of testosterone may be sufficient to induce some beneficial effects while minimizing the potential worsening of LUTS (although this has not been demonstrated as an adverse effect of testosterone therapy).

The potential effectiveness of low-dose testosterone supplementation is suggested by studies of short-acting testosterone formulations (e.g., oral testosterone undecanoate and sublingual testosterone cyclodextrin) that produced anabolic effects despite serum testosterone concentrations that were not sustained within the normal range.[616,617] An analogy may be made to the use of hydrocortisone for glucocorticoid replacement therapy, in which the duration of biologic action of hydrocortisone on tissues is not reflected by its serum concentrations. Short-term administration of low-dose testosterone enanthate (50 mg/week IM) was found to increase muscle strength and power in some young men in whom hypogonadism was induced by concomitant GnRH agonist treatment.[618]

Hypogonadism due to gonadotropin deficiency may be caused by hypothalamic-pituitary disease that requires specific management in addition to testosterone replacement. Therefore, careful evaluation to determine the cause of secondary hypogonadism should be performed before testosterone treatment is started. For example, pituitary or hypothalamic tumors may cause mass effects such as visual field defects, or they may be associated with deficiency or excessive secretion of other pituitary hormones. These tumors may require surgery or radiation therapy, additional hormonal replacement, medical therapy, or some combination of these treatments to reduce excessive pituitary hormone secretion. In some cases, treatment of the underlying cause of secondary hypogonadism corrects the androgen deficiency (e.g., stopping a medication that causes hyperprolactinemia or gonadotropin deficiency). In men with gonadotropin deficiency and otherwise normal testes who are interested in fathering children, gonadotropin therapy may be used instead of testosterone replacement to stimulate sperm production, restore fertility, and correct androgen deficiency. Similarly, men with secondary hypogonadism due to hypothalamic disease may be treated with pulsatile GnRH to stimulate testosterone and sperm production and restore fertility.

A comprehensive clinical approach is important for optimal management of hypogonadism. It is important to consider causes other than androgen deficiency that might contribute to symptoms and signs, and to manage them appropriately. In hypogonadal men who complain primarily of sexual dysfunction, an underlying neurovascular disease or use of certain medications is usually the major cause of erectile dysfunction. In these men, testosterone treatment alone is insufficient to completely restore erections and permit satisfactory sexual intercourse. Additional treatment with a PDE5 inhibitor (sildenafil, vardenafil, or tadalafil),[161,619] intracavernosal or intraurethral alprostadil (MUSE), intracavernosal alprostadil/papaverine/phentolamine (tri-mix), penile vacuum device, or penile prosthesis might be needed for a satisfactory clinical outcome. In hypogonadal men who present with osteoporosis, it is critical to perform a thorough evaluation for other common causes of bone loss (e.g., vitamin D deficiency, alcohol abuse, smoking, medications, inactivity, primary hyperparathyroidism) and to treat them as well. It is also important to institute measures to prevent falls to reduce the risk of fractures.

Testosterone Formulations

Testosterone formulations that are used to treat male hypogonadism are summarized in Table 19.10.[6,116] In the United States, approved formulations include parenteral testosterone esters that are administered by long- and short-acting IM injection, transdermal testosterone patch and testosterone gels or solutions, a transbuccal testosterone tablet, and an intranasal testosterone gel.

Oral 17α-alkylated testosterone derivatives, such as methyltestosterone and fluoxymesterone, should not be used for testosterone replacement therapy.[6] It is difficult to achieve full androgen replacement with these oral formulations because they are weak androgens that have low bioavailability. They also have the potential for serious hepatotoxicity.[620] 17α-Alkylated androgens most commonly cause cholestasis that is reversible with discontinuation. More concerning is the potential for these agents to cause

TABLE 19.10 Treatment of Adult Male Hypogonadism

Formulation	Dosage	Advantages	Disadvantages
Treatment of Androgen Deficiency			
Formulations Available in the United States			
Parenteral Testosterone Esters			
Testosterone enanthate or cypionate, IM injections	*Adults:* 150–200 mg IM every 2 wk or 75–100 mg IM every wk 50 mg, 75 mg, or 100 mg SC by autoinjector *Prepubertal boys:* 50–100 mg monthly or 25–50 mg every 2 wk, increasing to 50–100 mg every 2 wk and then to adult replacement dosage over 2–4 yr or until spontaneous pubertal development occurs	Extensive clinical use Inexpensive with self-injection Some dose flexibility	IM injections, discomfort Symptomatic fluctuation of T concentrations (supraphysiologic after injection to low normal or low before next injection) Frequent IM injections to reduce fluctuations of T concentrations More erythrocytosis than with transdermal T
Testosterone undecanoate, IM injections	750 mg at 0 and 4 wk, then every 10 wk	Less frequent IM injections Maintenance of normal T concentrations for a longer duration No apparent fluctuations in symptoms	REMS: Slow, deep IM injection in clinic (no self-injection); 30-min observation for potential POME and anaphylaxis IM injections, discomfort Large-volume injection (3 mL) Self-injection not possible Rarely, cough immediately after injection Prolonged maintenance of T level after discontinuation if adverse effects develop
Transdermal Testosterone			
Testosterone patch (nonscrotal)	2 or 4 mg (one patch) or 6 mg (one 2-mg plus one 4-mg patch) applied daily over nonpressure areas	Low- to mid-normal physiologic T concentrations Mimics normal circadian variation when applied nightly No injections Less erythrocytosis than with parenteral T Rapid withdrawal of T replacement if adverse effects occur	Frequent skin irritation Low-normal T concentrations: two patches may be needed Skin adhesion poor with excessive sweating Daily application More expensive than parenteral T
Testosterone gels and solution	*1% T gel:* 5–10 g of gel (containing 50–100 mg of T) applied daily over shoulders or upper arms; available in foil sachets of 2.5 or 5.0 g (containing 25 or 50 mg of T, respectively), or a tube of 5 g (containing 50 mg of T) *1.62% T gel:* 20.25–81.00 mg (containing 20.25–81.00 mg of T) applied daily to shoulders or upper arms; available in 20.25- and 40.50-mg packets (containing 20.25 and 40.50 mg of T, respectively) or metered-dose pump delivering 12.5 mg per pump depression *2% gel:* 40–70 mg (containing 40–70 mg of T) applied daily to inner thighs; available in a metered-dose pump delivering 10 mg per pump depression *2% solution:* 30–120 mg applied to underarms; available in a metered-dose pump applicator delivering 30 mg per pump depression	Low- to high-normal steady-state physiologic T concentrations No injections Little skin irritation Dose flexibility Rapid withdrawal of T replacement if adverse effects occur For 1.62% or 2% gel: less gel amount in more concentrated formulations Less gel amount in more concentrated formulations For 2 % solution: absorption of solution not affected by deodorant or antiperspirant Absorption of solution not affected by deodorant or antiperspirant	Potential for contact transfer of T to women or children Daily application More expensive than parenteral T, especially with higher doses Moderately high DHT concentrations One formulation has a musk odor and another is associated with stickiness or skin dryness Slight skin irritation in some men Solution may drip under arms
Transbuccal testosterone	30-mg tablet applied between cheek and gum two times daily	Mid-normal steady-state physiologic T concentrations No injections, patch or gel application, or their associated disadvantages Rapid withdrawal of T replacement if adverse effects occur	Twice-daily application Gum irritation or inflammation Altered or bitter taste High learning curve for proper application; requires careful instruction or poor acceptability occurs Tablets may be difficult to remove or may fall off prematurely No dose flexibility Moderately high DHT concentrations More expensive than parenteral T

TABLE 19.10 Treatment of Adult Male Hypogonadism—cont'd

Formulation	Dosage	Advantages	Disadvantages
Treatment of Androgen Deficiency			
Testosterone nasal gel	11 mg (delivering 1.1 mg of T) three times daily (every 6–8 hours) for a total daily dose of 33 mg (delivering 3.3 mg of T) daily Available in a metered-dose pump that delivers 5.5 mg (delivering 0.55 mg of T) per pump depression	No injections No interaction with sympathomimetic nasal decongestants	Thrice-daily administration Learning curve for proper administration Fluctuation in T concentrations from lower- to upper-normal range after administration No nose blowing or sniffing for 1 hour after administration Discontinue with severe rhinitis Nasal irritation Not recommended with other intranasal drugs or chronic nasal conditions
Testosterone pellets	2–6 pellets (each 3.2 mm diameter × 9 mm in length pellet containing 75 mg of T, for a total of 150–450 mg of T delivered) implanted SC every 3–6 mo (usually 3–4 mo)	Maintenance of normal T concentrations for a longer duration	Requires surgical incision Extrusion, bleeding, and infection can occur uncommonly Large number of pellets Not easily removed; fibrosis may occur Lack of ability for rapid withdrawal of T replacement if adverse effects occur Infrequent use
Testosterone Formulations Available Outside the United States			
Oral testosterone undecanoate	40–80 mg PO with meals twice a day to three times daily	Oral administration is convenient for many	Twice- or thrice-daily administration Variable T concentrations and clinical responses Requires administration with meal High DHT concentrations
Testosterone-in-adhesive matrix patch	Two patches (delivering 4.8 mg of T per day) applied every 2 days	Low- to mid-normal physiologic T concentrations Duration 2 days No injections	Some skin irritation Two patches needed
Treatment to Initiate and Maintain Sperm Production in Men With Hypogonadotropic Hypogonadism			
Initially to Stimulate Testosterone and Potentially Sperm Production			
hCG	500–2000 IU given SC two to three times weekly to maintain serum T concentrations within the normal range for 6–12 mo	Effective in stimulating endogenous T production In men with acquired and some men with partial congenital hypogonadotropic hypogonadism, sperm production may be stimulated with hCG treatment alone SC injections easier than IM injections (smaller needle, injection not as deep) Less fluctuation in T concentrations compared with IM T ester injections No injection, patch, or buccal tablet	Injections two to three times weekly Expensive Higher doses needed in men with concomitant primary testicular disease (e.g., cryptorchidism) Breast tenderness or gynecomastia secondary to high estradiol production by testes May require dilution Occasional burning sensation with injection Ineffective in primary hypogonadism
Added to hCG to Stimulate Sperm Production			
FSH Human menopausal gonadotropin, human FSH, or recombinant human FSH	After 6–12 mo of hCG treatment alone resulting in normal T concentrations, add FSH 75–300 IU given SC three times weekly for an additional 6–12 mo or longer	Effective in stimulating sperm production in men with hypogonadotropic hypogonadism	Injections three times weekly Extremely expensive, prohibitive cost for most Breast tenderness or gynecomastia secondary to high estradiol production by testes May require dilution Occasional burning sensation with injection In men with concomitant primary testicular disease (e.g., cryptorchidism), stimulation of spermatogenesis is not likely
To Stimulate Testosterone and Sperm Production			
GnRH	5–25 ng/kg SC every 2 hr by programmable infusion pump for 6–12 mo	Effective in stimulating both endogenous T and sperm production	GnRH not readily available Requires pump use and management, usually in a specialized center Expensive Infrequently used except at certain sites Rarely, local irritation, infection

DHT, Dihydrotestosterone; FSH, follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *hCG,* human chorionic gonadotropin; *IM,* intramuscular; *PO,* orally; *POME,* potential pulmonary oil microembolism; *REMS,* Risk Evaluation and Mitigation Strategy; *SC,* subcutaneous; *T,* testosterone.

peliosis hepatis (blood-filled cysts in the liver) or benign or malignant hepatic tumors. 17α-Alkylated androgens also lower HDL cholesterol and raise LDL cholesterol, causing a proatherogenic lipid profile, and they are relatively expensive. Therefore, these oral androgens carry greater potential risks with few therapeutic benefits compared with other testosterone formulations, and they should not be used to treat male hypogonadism.

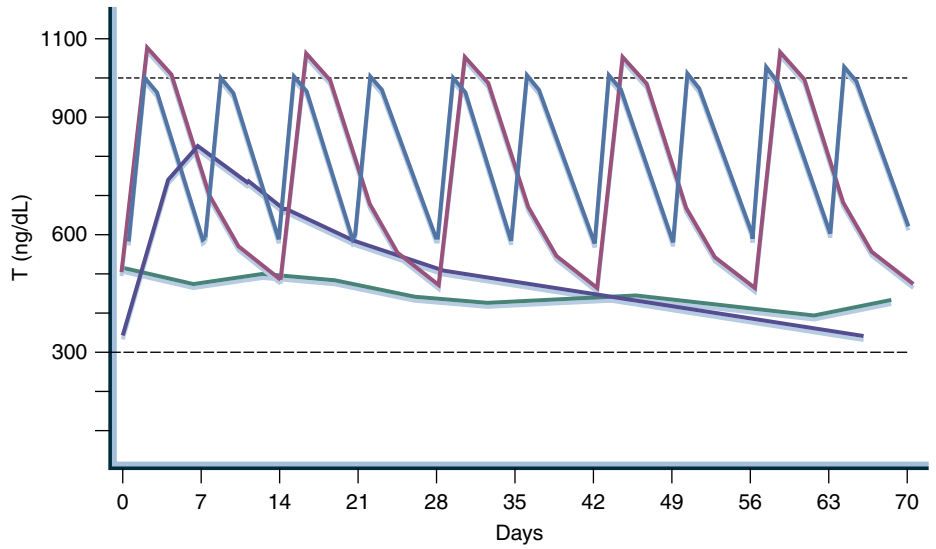
Parenteral Testosterone Esters. Relatively long-acting parenteral 17β-hydroxyl esters of testosterone, *testosterone enanthate* and *testosterone cypionate*, are administered by IM injection. These are effective, safe, and relatively practical and inexpensive preparations that have been used for testosterone replacement in hypogonadal men for decades. Transdermal testosterone gel formulations provide more physiologic testosterone concentrations and are now used more commonly than testosterone ester injections. However, testosterone esters are preferred over transdermal formulations by some hypogonadal men because they are the least expensive formulation available, require less frequent administration, and usually produce more consistent but higher average serum testosterone concentrations. Given proper instruction, most hypogonadal men (or a family member) can self-administer IM testosterone ester injections. Otherwise, testosterone injections need to be administered in a clinic setting.

Esterification of testosterone at the 17β-hydroxyl group increases its hydrophobicity and solubility within an oil vehicle (sesame oil for testosterone enanthate, cottonseed oil for testosterone cypionate). After IM injection, testosterone esters are released slowly from the oil vehicle within muscle and hydrolyzed rapidly to testosterone, which is released into the circulation, resulting in relatively high peak serum testosterone concentrations but an extended duration of release. Testosterone enanthate and testosterone cypionate have similar pharmacokinetic profiles, duration of action, and therapeutic efficacy, so they are considered therapeutically equivalent.[621,622]

In adults with hypogonadism, the usual starting dose of testosterone enanthate or cypionate is 150 mg to 200 mg via IM injection every 2 weeks. After IM administration of 200 mg of testosterone enanthate, serum testosterone concentrations usually rise above the normal range for 1 to 3 days and then decline gradually over 2 weeks to the lower end of the normal range, or sometimes to below-normal concentrations, before the next injection[623] (Fig. 19.28). The extreme rise and fall of serum testosterone concentrations may cause fluctuations in energy, mood, and libido that are disturbing to some men. Shortening the dosing interval to every 10 days and reducing the dose to 150 mg (i.e., 150-mg IM injection every 10 days) may alleviate symptoms associated with nadir testosterone concentrations occurring before the next injection. Alternatively, some patients prefer changing the dose of testosterone enanthate or cypionate to 75 to 100 mg IM every week to reduce swings in testosterone concentrations and associated symptoms (see Fig. 19.28). Administration of testosterone enanthate at doses of 300 mg IM every 3 weeks or 400 mg IM every 4 weeks produces extremely wide fluctuations in serum testosterone concentrations with markedly supraphysiologic concentrations for several days after an injection and concentrations below normal 3 weeks after an injection); these regimens are not recommended[623].

Because CDGP in which puberty eventually occurs spontaneously is clinically indistinguishable from delayed puberty caused by permanent hypogonadotropic hypogonadism (e.g., CHH),[146,147,374,375] testosterone treatment usually is not initiated in boys with androgen deficiency due to a cause of prepubertal onset until they are about 14 years of age (with a bone age of at least 10.5 years). Testosterone therapy is administered intermittently to allow determination of spontaneous puberty, if it occurs. Occasionally, testosterone therapy is started at a younger age if delayed genital development and growth are causing severe psychological distress in affected boys and their families.

In boys with androgen deficiency due to a cause of prepubertal onset, treatment is initiated with a very low dose of testosterone enanthate or cypionate (e.g., monthly IM injection of 50–100 mg or 25–50 mg every 2 weeks) to prevent premature closure of long bone epiphyses that would compromise adult height.[146,147,374,375] These low dosages of testosterone are sufficient to induce some



• **Fig. 19.28** Schematic diagram of serum testosterone (T) concentrations after chronic administration of T enanthate 200 mg intramuscularly every 2 weeks (red) or 100 mg every week (blue), T undecanoate 750 mg intramuscularly every 10 weeks (black), and T gel 1.62% transdermally daily (green). Dashed line denotes the reference range of serum T concentrations in adult men (300–1000 ng/dL).

virilization and long bone growth without interfering with the spontaneous puberty that occurs eventually in boys with CDGP. Testosterone treatment is continued for 3 to 6 months and then stopped for 3 to 6 months to assess whether spontaneous pubertal onset occurs. If there is indication that spontaneous puberty is occurring (e.g., testis size >8 mL), testosterone therapy is discontinued. If there is no evidence of spontaneous puberty, intermittent testosterone treatment is continued. The dose of testosterone enanthate or cypionate is increased gradually to 50 to 100 mg IM every 2 weeks and then to full adult replacement doses over the next several years to mimic the gradual increase in testosterone concentrations that occurs during spontaneous puberty.

At present, transdermal testosterone formulations are not approved for use in boys with delayed puberty. However, because they circumvent the need for IM injections, low-dose transdermal testosterone patches and gels would provide very useful alternatives for the treatment of androgen deficiency due to a cause of prepubertal onset in boys, but they are currently not approved for this indication.

A formulation of *testosterone undecanoate* in castor oil (*Aveed*, Endo Pharmaceuticals, Malvern, PA) was approved in 2014 for use in the United States for treatment of male hypogonadism. This formulation is administered by slow IM injection into the gluteus muscle at a dose of 750 mg in 3 mL of castor oil initially, followed by another injection of the same dose 4 weeks later and then every 10 weeks to produce and maintain serum testosterone concentrations within the normal range in most hypogonadal men.[624] Steady state is achieved after the third injection, and mean testosterone concentrations peak in the high-normal range at 7 days after injection and gradually decline over the next 10 weeks to mean nadir concentrations just above the lower limit of the normal range (see Fig. 19.28). Despite this decline in testosterone concentrations, fluctuations in or recurrence of symptoms of androgen deficiency have not been reported. Although some men experience discomfort with large-volume injections, they are generally tolerated well and have the advantage of fewer injections than shorter-acting testosterone ester formulations.

A different formulation of testosterone undecanoate in a castor oil vehicle (*Nebido*, Bayer Schering Pharma AG, Berlin, Germany) has been approved and is used in Europe and other countries for testosterone replacement therapy in hypogonadal men.[625] It is administered at a dose of 1000 mg in 4 mL IM, followed by another injection of the same dose 6 weeks later and then every 10 to 14 weeks.

Because of the large volume of drug administered and the need for proper injection technique, self-administration of IM testosterone undecanoate is not possible. Coughing may occur in a small number of men immediately after injection of testosterone undecanoate (this also occurs with shorter-acting testosterone ester injections). Although there is no direct evidence for the cause of coughing, it is conjectured to be related to pulmonary oil microembolism (POME) emanating from the large volume of castor oil vehicle that is injected into the muscle with this formulation. For these reasons, the FDA has required a risk evaluation and mitigation strategy for use of testosterone undecanoate (Aveed) in the United States that requires training of personnel and certification of the health care facility to ensure proper injection technique (slow IM injection) and adequate monitoring (for 30 minutes) and treatment capability for potential POME or anaphylaxis following injection.

In 2018, a testosterone enanthate formulation of 50 mg, 75 mg, or 100 mg (0.5-mL solution) in a prefilled, single-use, small-needle autoinjector for weekly SC injection was approved for use in testosterone replacement therapy of hypogonadal men (*Xyosted*, Antares Pharma, Ewing, NJ).[626,627]

Transdermal Testosterone. Transdermal testosterone formulations available for testosterone replacement therapy for male hypogonadism include an adhesive testosterone patch, two 1% testosterone gels, a 1.62% testosterone gel, a 2% testosterone gel, and a 2% testosterone solution (see Table 19.10). Transdermal delivery of testosterone is used in hypogonadal men who prefer this method or are unable to tolerate or self-administer IM injections of testosterone ester. Currently, testosterone gel is the most frequently used formulation for treatment of male hypogonadism in the United States.

In contrast to testosterone ester injections, which produce transient supraphysiologic testosterone concentrations, patch, gel, and solution formulations produce a more physiologic range of testosterone concentrations; use of the patch results in a circadian variation in testosterone concentrations, and the gel formulations usually produce relatively constant steady-state serum testosterone concentrations.

Testosterone stimulates red blood cell production, and testosterone replacement therapy may result in excessive erythrocytosis. In men with hypogonadism, excessive erythrocytosis occurs less commonly with testosterone patch therapy than with testosterone enanthate injections, suggesting that physiologic testosterone concentrations produced by transdermal testosterone therapy may be associated with fewer androgenic adverse effects.[628] Compared with testosterone ester injections, transdermal formulations have a short half-life in SC tissue and in the circulation; consequently, their discontinuation results in a rapid fall in serum testosterone concentrations and a shorter duration of action. Therefore, an advantage of transdermal testosterone is the ability to withdraw androgen replacement relatively rapidly if excessive erythrocytosis develops or if prostate cancer is detected.

Disadvantages of transdermal formulations include the requirement for daily application, greater expense compared with testosterone ester injections, skin irritation or rash with testosterone patches[629] (less common with testosterone gels and solution), and the potential with gel and solution formulations for transfer of testosterone to others through skin contact at the application site.

The first transdermal testosterone delivery system for treatment of male hypogonadism was a scrotal testosterone patch.[630] Treatment required daily application of a relatively large, nonadhesive patch to clean, dry, and preferably shaven scrotal skin and the use of brief-style underwear to hold them in place. These requirements were not acceptable to some hypogonadal men. In addition, in some men with congenital androgen deficiency, the scrotum was too small to accommodate even the smaller-size testosterone patch. Because of poor adherence to scrotal skin, thin adhesive strips were added as an option to this patch. Some men using this testosterone patch experienced skin irritation and itching. The scrotal testosterone patch produced serum DHT concentrations in the upper-normal range or above the normal range as a result of high 5α-reductase activity within scrotal skin. The nonscrotal testosterone patch and testosterone gels have supplanted the scrotal testosterone patch for testosterone replacement therapy, and the latter is no longer available in the United States.

A nonscrotal testosterone patch, *Androderm* (Allergan USA, Madison, NJ), is available for testosterone replacement therapy in male patients with hypogonadism.[631] This patch is composed of a central reservoir containing testosterone and permeation enhancers in an alcohol-based gel surrounded by an adhesive patch that is

applied to the skin of the back, abdomen, upper arms, or thighs, avoiding areas over a bony prominence. When applied at night, the testosterone patch produces serum testosterone concentrations that peak in the morning, mimicking the circadian variation of endogenous testosterone concentrations in normal men. Androderm patches are available in two sizes delivering 2 mg (32 cm^2) or 4 mg (39 cm^2) of testosterone daily. Long-term use of the testosterone patch usually maintains serum testosterone concentrations within the mid- to low-normal range and improves the clinical manifestations of androgen deficiency. Usually, to achieve consistent mid- to high-normal testosterone concentrations, application of two patches may necessary—one 2-mg patch plus one 4-mg patch or two 4-mg patches.[632]

The major limitation of Androderm is skin irritation or rash of varying severity; this side effect occurs in at least 30% to 60% of patients.[629] Mild to moderate erythema and irritation are almost always present, probably because of a skin reaction to the permeation-enhancing agent or adhesive. Uncommonly, severe contact dermatitis or burn-like skin reactions occur. Pretreatment of the skin under the reservoir of the patch with a topical corticosteroid such as triamcinolone acetonide 0.1% cream reduce the incidence and severity of skin irritation produced by testosterone patches.[633]

There are several testosterone gels on the market, and they are the most frequently used testosterone formulations for the treatment of male hypogonadism in the United States. The gels are dispensed into the palm of the hand and applied daily in the morning to clean, dry skin. The specified site of application varies by gel product, but none of them are applied on the face or scrotum. The alcohol-based gel dries rapidly after application, and testosterone is absorbed into the SC space, where it is released steadily over the remainder of the day, producing relatively steady-state testosterone concentrations. Residual testosterone remains on the surface of the skin of the hands and at the sites of application. Therefore, the hands should be washed with soap and water after application, the sites of gel application should be covered with clothing, and skin contact with the application sites by others (especially women and children) should be avoided for several hours or the site has been washed to prevent transfer of testosterone.[634] Because of reports of contact transfer of testosterone to children, these instructions and caution to avoid contact transfer are now in an FDA black box warning in the prescribing information for all transdermal testosterone formulations. Residual testosterone on the skin at application sites may be washed off (e.g., by showering or bathing), but washing the sites should be avoided for a minimum of 1 hour and ideally for 6 hours or more after application (to maximize testosterone absorption).

In contrast to testosterone patches, local skin irritation with testosterone gel formulations is relatively uncommon, occurring in fewer than 5% of men, and is probably related mostly to drying of the skin by the alcohol. Some men complain of stickiness of the skin after the alcohol-based gel dries. Testosterone gels produce serum DHT concentrations at the upper end or above the normal range as a consequence of 5α-reductase activity in the relatively large surface area of skin over which the gel is applied. A major limitation of the use of testosterone gel for testosterone replacement therapy is the high cost. Generic testosterone gel products have been approved and are less costly than brand products, but their availability has been variable, and they are not inexpensive.

The first gel developed, AndroGel 1% (Abbott, Abbott Park, IL) was available initially in foil sachets at two different doses: 2.5 g of gel (containing 25 mg of testosterone) or 5 g of gel (containing 50 mg of testosterone). (Because only about 10% of the medication is absorbed, these sachets deliver 2.5 or 5.0 mg of testosterone, respectively.) The starting dose of AndroGel is 5 g daily. Based on testosterone concentrations or clinical response, approximately 2 weeks after initiation of therapy, the dose may be increased to 7.5 g (i.e., one 2.5-g packet plus one 5.0-g packet) or to 10.0 g (two 5.0-g packets) daily or decreased to 2.5 g daily. AndroGel is now available in a more concentrated reformulation of 1.62% that is delivered by a metered-dose pump that delivers 12.5 mg of testosterone per actuation, or foil packets of 20.25 or 40.5 mg of testosterone. With titration, the dosage of AndroGel 1.62% ranges from 20.25 to 81 mg of testosterone.

Testim (Auxilium Pharmaceuticals, Chesterbrook, PA) is the other 1% hydroalcoholic testosterone gel that is available for treatment of male hypogonadism.[635] Like AndroGel, Testim is applied daily in the morning to intact, clean, dry skin over the shoulders and arms. In short-term, placebo-controlled trials, Testim maintained steady-state physiologic serum testosterone concentrations in hypogonadal men and improved the clinical manifestations of androgen deficiency.[636] After the initial application of Testim, serum testosterone concentrations are approximately 30% higher than those achieved after application of AndroGel. However, no direct comparison of steady-state testosterone concentrations with long-term use of the two testosterone gels is available.

Testim is packaged in a 5-g tube containing 50 mg of testosterone and delivering approximately 5 mg of testosterone (i.e., 10% absorption). The starting dose of Testim is 5 g daily. Based on testosterone concentrations or clinical response approximately 2 weeks after initiation of therapy, the dose may be increased to 10 g (two tubes) daily. In contrast to AndroGel, Testim is not available in a dose of 2.5 g or in a metered-dose dispenser, limiting dose adjustments with this formulation. Whereas AndroGel is odorless, Testim has a musk-like scent. Depending on the individual patient and his partners, this aroma might be pleasant or objectionable. Testim contains a skin emollient and is less drying to the skin than AndroGel.

Two additional transdermal formulations for the treatment of male hypogonadism have been approved in the United States. *Fortesta* (Endo Pharmaceuticals, Malvern, PA) is a 2% testosterone gel that is applied to the inner thighs at a daily dosage of 40 to 70 mg (delivering 4–7 mg of testosterone) daily by a metered-dose pump that delivers 10 mg of testosterone per pump depression (delivering 1 mg of testosterone).[637] *Axiron* (Eli Lilly, Indianapolis, IN) is a 2% testosterone solution that is applied to axillary skin at a dosage of 30 to 120 mg (delivering 3-12 mg of testosterone) daily using a metered-dose pump applicator that delivers 30 mg (delivering 3 mg of testosterone) per pump depression.[638] Both of these transdermal testosterone formulations are able to achieve and maintain relatively steady-state serum testosterone concentrations within the normal range in hypogonadal men. The advantages and disadvantages of these formulations are similar to those of AndroGel and Testim gels. There might be a higher risk of clinically important secondary transfer of testosterone to sexual partners with the use of Fortesta, and some men complain of dripping of the 2% Axiron testosterone solution from the axilla. In addition, some men might need to shave their axilla to apply Axiron; secondary transfer might be less likely to occur with axillary application, however.

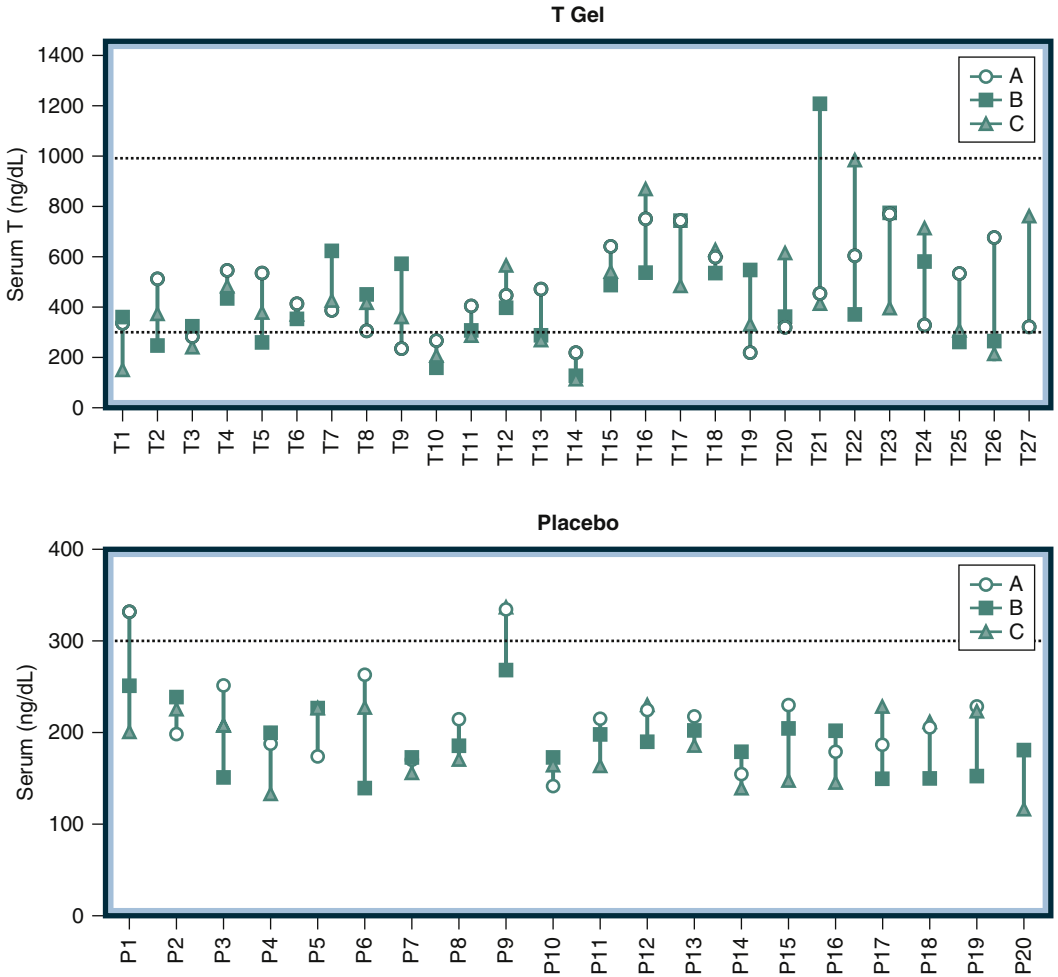
Long-term use of testosterone gel in hypogonadal men usually maintains steady-state physiologic serum testosterone concentrations and improves the clinical manifestations of androgen deficiency[208,639,640] (see Fig. 19.28). With all transdermal testosterone

formulations, serum testosterone concentrations may vary considerably both within and between individuals and from day to day, and therefore it is difficult to judge the adequacy of a particular dose in maintaining testosterone concentrations using single measurements of testosterone[641] (Fig. 19.29).

In contrast to FDA-approved transdermal testosterone formulations, testosterone concentrations contained in compounded testosterone gels and creams vary considerably and are inaccurate. In one study, AndroGel contained testosterone amounts that were consistent and accurate; there was little variation within and between batches of AndroGel. However, in this same study, only 30% to 50% of batches of compounded testosterone formulations from 10 different pharmacies contained testosterone amounts within 20% of the stated dose; one compounded formulation had virtually no testosterone.[642]

Transbuccal Testosterone. A transbuccal testosterone tablet, *Striant* (Columbia Laboratories, Livingston, NJ), is available for testosterone replacement therapy in hypogonadal men[643,644] (see Table 19.10). This formulation is a small mucoadhesive tablet that contains 30 mg of testosterone in an oil-water emulsion carrier vehicle. The tablet contains polycarbophil, which, after application, remains attached to buccal mucosa until epithelial cells turn over (approximately every 12–15 hours). The tablet is placed in the mouth between the inner cheek and gum, above the incisors, with the monoconvex side toward the gum and the flat side toward the cheek. After placement, the tablet softens and swells with hydration and becomes gelatinous and sticky, causing it to adhere to the gum. Testosterone is released at a controlled and sustained constant rate from the tablet through the buccal mucosa into the systemic circulation, circumventing first-pass hepatic metabolism.

Striant tablets are placed on the buccal mucosa twice daily, with one tablet applied in the morning and removed after 12 hours and another applied in the evening on the opposite side.[643,644] Use of Striant requires careful instruction to orient the tablet with the rounded side against the gum and to hold it firmly in place with a finger over the lip for approximately 30 seconds. If the tablet falls off or is dislodged, a new tablet should be applied and left in place until the next regularly scheduled dose. Swallowing of the tablet is not harmful. The buccal tablet is removed by gently sliding it downward toward the incisor to avoid scratching the gum.



• **Fig. 19.29** Large variability of serum testosterone (T) concentrations 2 hours after 16 ± 2 weeks of transdermal application of daily gel application at ambulatory clinic visits (*A*, open circles; *B*, closed squares) and an inpatient visit (*C*, shaded triangles) in participants in the Testosterone Trials who received T gel 1% (upper, participants T1–T27) and placebo gel (bottom panel, participants P1–P20). Within-subject variation is shown by a *shaded vertical line* between the highest and lowest T concentration for each man. The *dashed line* denotes the reference range of serum T concentrations in adult men (300–1000 ng/dL).

Application of a Striant tablet containing 30 mg of testosterone every 12 hours produces, on average, steady-state testosterone concentrations in the mid-normal range throughout the day.[643,644] Although a formal study has not been conducted, unrestricted intake of food and beverage (including alcohol), tooth brushing, mouth washing, and gum chewing did not appear to affect the absorption of testosterone in pharmacokinetic studies. Contact transfer of testosterone in saliva to others has not been reported to occur. Like the transdermal testosterone gels, Striant produces high-normal to high serum DHT concentrations, probably because of 5α-reductase activity in the buccal mucosa. In general, transbuccal testosterone tablets are tolerated well. In one study, approximately 10% to 15% of men developed gum or mouth irritation or inflammation, and 5% experienced an altered or bitter taste in the mouth.[643]

Like the transdermal testosterone formulations, Striant is relatively expensive compared with testosterone ester injections. Initially, patients are aware and bothered by the tablet between their cheek and gum, resulting in premature discontinuation of the formulation. However, with continued use, the unusual sensation and awareness of the buccal tablet diminish and become less bothersome. Twice-daily application of Striant is required to sustain physiologic testosterone concentrations, and this makes compliance challenging. Informing patients that awareness of the buccal tablet diminishes over time and linking application of the transbuccal tablet to a routine daily activity such as morning and evening tooth brushing may help improve and maintain compliance.

Testosterone Nasal Gel. A 1% testosterone nasal gel formulation, *Natesto* (Aytu Bioscience, Englewood, CO), has been approved by the FDA to treat men with hypogonadism.[645] This formulation is administered intranasally at a dosage of 11 mg testosterone in 1.1 g gel (delivering 1.1 mg of testosterone in each nostril) three times daily (every 6–8 hours) by a metered-dose pump that delivers 5.5 mg (delivering 0.55 mg of testosterone) per pump depression; the total daily dose is therefore 33 mg (delivering 3.3 mg of testosterone). It is advised that there should be no nose blowing or sniffing for 1 hour after administration, and treatment should be discontinued temporarily during episodes of severe rhinitis. Although there is no interaction with symphathomimetic nasal decongestants, potential interaction with other intranasally administered medications is not known, so use with other nasal drugs is not recommended. It is also not recommended for patients with chronic nasal conditions. When administered to hypogonadal men, mean serum testosterone concentrations peak in the high-normal range approximately 1 hour after administration and decline over 6 to 8 hours to concentrations at or slightly below normal.

Testosterone Pellets. SC testosterone pellets are used infrequently in the United States but more commonly in Australia and some European countries for testosterone replacement therapy in men with hypogonadism.[646,647] In the United States, *Testopel Pellets* (Auxilium Pharmaceuticals, Chesterbrook, PA) are available for testosterone replacement (see Table 19.10). These are cylindrical pellets, 3.2 mm in diameter by 8 to 9 mm in length, that contain 75 mg of testosterone. Testopel Pellets are recommended at doses that range from 150 to 450 mg testosterone (i.e., two to six 75-mg pellets) and are implanted every 3 to 4 months.[646] However, a recent report of implantation of 6 to 12 pellets (450–900 mg of testosterone) found that decline in serum testosterone concentrations to below normal occurred by 3 to 4 months after implantation regardless of BMI or the number of pellets implanted (from 6–9 or 10–12).[648]

Pharmacokinetic profiles for testosterone pellets depend on the specific pellet formulation.[647,649,650] In European studies using a different formulation, SC implantation of three to six 200-mg pellets (for a total of 600–1200 mg of testosterone) produced the almost zero-order, sustained release of testosterone and maintained steady-state physiologic serum testosterone concentrations for 4 to 6 months in hypogonadal men.[649] Testosterone pellets are implanted subcutaneously with the use of a trocar that is introduced through a small skin incision. This minor surgical procedure is repeated three to four times yearly to maintain normal serum testosterone concentrations.

Although spontaneous extrusion of pellets and local bleeding or infection may occur occasionally, these problems are uncommon in experienced hands. If adverse effects develop after implantation, a major concern is that removal of the testosterone pellets will be difficult, if not impossible. Therefore, the use of testosterone pellets is inappropriate for testosterone replacement in older patients who are predisposed to erythrocytosis and prostate disease during treatment.

Testosterone Formulations Available Outside the United States

Oral Testosterone Undecanoate. In many countries outside the United States, an oral 17β-hydroxyl ester of testosterone, testosterone undecanoate (*Andriol Testocaps*, Organon, Oss, Netherlands), is available for testosterone replacement therapy in hypogonadal men.[651,652] Testosterone undecanoate, which is formulated in a castor oil vehicle, is absorbed directly from the gastrointestinal tract into the lymphatic system and then into the systemic circulation, thereby avoiding first-pass hepatic accumulation and inactivation. Serum testosterone concentrations peak approximately 5 hours after administration of testosterone undecanoate and fall to pretreatment concentrations within 8 to 12 hours. For testosterone replacement therapy, it is administered at relatively high doses, 40 to 80 mg two to three times daily (total dose, 80–240 mg daily). The frequency of administration makes compliance difficult for many men. Absorption of testosterone undecanoate requires concomitant food ingestion, and serum testosterone concentrations and clinical responses are highly variable. Because of 5α-reductase activity in the gastrointestinal tract, serum DHT concentrations are often very high.

The use of castor oil and propylene glycol laurate instead of oleic acid, the vehicle used in the original formulation, permits storage at room temperature and extends the shelf life of Andriol Testocaps for up to 3 years while maintaining pharmacokinetic and pharmacodynamic characteristics similar to those of the original formulation. Testosterone concentrations fall quickly after discontinuation of testosterone undecanoate. Therefore, it may be particularly useful for testosterone replacement therapy in older men with clinically significant prostate disease and comorbid conditions, in whom rapid withdrawal of androgen action is desirable if adverse effects develop, and in those for whom only low-dose testosterone supplementation is needed.

Transdermal Testosterone Formulations. A *testosterone-in-adhesive matrix patch* (*Testopatch*, Pierre Fabre, Castres, France) is available in many countries in Europe for testosterone replacement therapy in patients with male hypogonadism.[654,655] This patch is composed of an adhesive matrix that contains testosterone (0.5 mg/cm^2) and excipients that comes in three sizes—30, 45, and

60 cm^2. Two 60-cm^2 patches (delivering approximately 4.8 mg of testosterone daily) are applied to the skin of the arms, trunk, or thighs every 2 days to maintain serum testosterone concentrations in the normal range in hypogonadal men. Skin irritation has been reported to occur in about 20% of patients using this patch.

Nontestosterone Therapies for Male Hypogonadism

Clomiphene and Aromatase Inhibitors. As noted previously, clomiphene and aromatase inhibitors (e.g., anastrozole) have been used off label to increase serum testosterone concentrations to enhance athletic performance or develop a muscular appearance. These drugs are not approved for use in male hypogonadism. Because their mechanism of action is to increase pituitary secretion of gonadotropins (thereby stimulating testicular production of testosterone), they will not be effective in men with primary hypogonadism (who already have high gonadotropin concentrations) and men with severe secondary hypogonadism who cannot produce more gonadotropins. In addition, safety and efficacy of these drugs have not established for the treatment of male hypogonadism.[116] Aromatase inhibitor therapy is associated with increased fat mass and decreased sexual function and is likely to decrease bone mass.[120-122]

Selective AR Modulators. There is considerable interest in the development of selective AR modulators (SARMs), which are nonsteroidal molecules that interact with AR and have differential effects on various androgen target organs.[656,657] The goal is to develop an orally active, nonsteroidal SARM that will maintain the beneficial anabolic and androgenic actions of testosterone on muscle, bone, sexual function, and mood but have reduced potential for adverse effects (e.g., on the prostate gland). These novel drugs are being developed primarily for use in muscle-wasting conditions such as age-related sarcopenia and cancer cachexia but not at present for treatment of male hypogonadism. The mechanisms by which SARMs act in a tissue-specific manner are unclear. It is possible that SARMs have less stimulatory effect on the prostate because they are not actively metabolized by 5α-reductase, or they may act by unique interactions with tissue-specific AR coactivator and corepressor molecules.

Nonsteroidal SARMs have been developed that have anabolic actions in muscle and bone but reduced stimulatory effects on the prostate gland in animals.[656,657] SARMs do not have intrinsic ER activity, and if they suppress endogenous gonadotropin, testosterone, and estradiol secretion, they will produce a state of relative estrogen deficiency. Therefore, studies evaluating the clinical benefits and risks of an SARM must consider potential adverse effects on target actions that are regulated by estradiol in men, such as effects on BMD, fat mass, sexual function, and possibly lipids (HDL cholesterol), cardiovascular function, and brain function. The importance of estrogens on bone, for example, was underscored by a recent long-term study of the potent, nonaromatizable androgen—DHT gel—administered to older men. Compared with placebo, DHT gel stimulated lean body mass and reduced fat mass but reduced BMD.[99]

Monitoring Clinical Response and Testosterone Concentrations

The clinical responses to testosterone replacement and serum testosterone concentrations are used to monitor the adequacy of testosterone therapy in androgen-deficient men[116] (Table 19.11). Symptoms and signs of androgen deficiency should be assessed before the initiation of testosterone treatment, 3 to 12 months after starting testosterone, and then yearly. By 3 to 6 months, most hypogonadal men experience improvements in libido, sexual function and activity, energy, vitality, motivation, and mood.[658] Increases in body hair growth, muscle mass and strength, and BMD occur over the subsequent months to years of testosterone therapy.

Serum testosterone concentrations are monitored to determine the adequacy of therapy and to avoid overreplacement or underreplacement. This is particularly important in men treated with the transdermal testosterone patch, gels, and solution, as the bioavailability of these formulations is highly variable among individuals; because of this variability, decisions regarding the adequacy of a given dosage of transdermal testosterone should not be made on a single testosterone measurement.[641] The goal of testosterone replacement therapy is to achieve average serum testosterone concentrations within the normal range.[116]

For testosterone ester injections, testosterone concentrations should be measured at 3 to 6 months of treatment, midway between two injections (e.g., 1 week after an injection if the injections are given every 2 weeks). Serum testosterone concentrations measured at the nadir of the injection interval (i.e., just before the next injection) may help to document an inadequate dosing interval. For the testosterone patch, testosterone concentrations should be measured after approximately 3 to 4 weeks of daily use, 8 to 10 hours after application of a patch on the previous evening. For testosterone gels and solution, testosterone concentrations should be measured after about 2 weeks of daily use, at any time after application of the gel. For buccal testosterone, serum testosterone should be measured 4 to 6 weeks after initiation of therapy, at any time after application of the buccal tablet, preferably in the morning.

Risks and Adverse Effects

Contraindications and Precautions. Testosterone treatment is contraindicated in men with metastatic prostate cancer or breast cancer.[116] The primary concern is that testosterone administration could stimulate the growth of these androgen- or estrogen-dependent malignancies. Testosterone therapy is particularly risky in men with metastatic prostate cancer, in whom rapid growth of metastatic tumors may worsen bone pain or cause spinal cord compression. In fact, the mainstay of treatment for metastatic prostate cancer is androgen-deprivation therapy to reduce endogenous testosterone production and action; this is achieved by GnRH agonist and AR antagonist treatment or by surgical orchidectomy.[659] The effect of testosterone replacement in hypogonadal men with localized prostate cancer is not known. However, in the absence of evidence, testosterone treatment in men with clinical evidence of active prostate cancer should be avoided.

The safety of testosterone therapy in hypogonadal men who have been surgically cured of organ-confined, low-grade prostate cancer (Gleason score <7 or 3+4, PSA <10 or 20 ng/mL, and clinical stage 1 or 2a) and have had clinically undetectable disease and an undetectable PSA level for several years also is not clear. Because these men would not have been treated with androgen-deprivation therapy, testosterone replacement to restore eugonadal concentrations of testosterone seems reasonable. In these patients, a careful discussion of the potential benefits and risks of testosterone replacement should be undertaken, and therapy should be initiated only after informed consent and with careful monitoring by DRE and measurement of PSA concentrations. It is prudent to avoid testosterone treatment in men with organ-confined, high-risk prostate cancer (e.g., Gleason score 8–10, PSA >20 ng/mL, and clinical stage ≥T3) despite undetectable PSA concentrations

TABLE 19.11 Monitoring During Testosterone Treatment

Parameter	Timing	Further Management
Measures of Efficacy		
Symptoms and signs of androgen deficiency	At baseline, after 3–12 mo, and then yearly	Continue testosterone treatment in men with clinical improvement and no adverse effects. Consider discontinuing testosterone treatment in men if no clinical improvement.
BMD	For men at high risk for fracture, BMD before treatment; for men with osteoporosis or minimal-trauma fracture, BMD after 1–2 yr	Institute appropriate treatment for men with osteoporosis, including calcium and vitamin D.
Serum testosterone	*Testosterone ester injection:* After 3–6 mo, measured midway between injections or at end of dosing interval (if androgen deficiency symptoms are present at that time) *Testosterone patch:* After 3–4 wk, at 8–10 hr after application *Testosterone gel:* After 2 wk, at any time after application *Buccal testosterone:* After 4–6 wk, at any time after application (preferably in the morning) *Testosterone pellets:* At end of dosing interval *Oral testosterone undecanoate:* After 1 wk, at 3–5 hr after oral dose *Testosterone undecanoate injection:* At end of dosing interval	Adjust dose or dosing interval to achieve serum testosterone concentrations in the mid-normal range.
Adverse Effects		
Hematocrit	At baseline, after 3–6 mo, and then yearly	If hematocrit is >54%, stop or reduce dosage of testosterone until hematocrit declines to normal and reinitiate testosterone at a lower dosage. Investigate for a hypoxic condition such as obstructive sleep apnea or chronic lung disease.
PSA level, with or without DRE (using shared decision making, i.e., if a patient desires prostate cancer screening after discussion of risks and benefits of PSA screening and monitoring), in men >50 yr (>40 if risk factors for prostate cancer)	At baseline, after 3–6 mo, and then according to accepted guidelines	Urologic evaluation with any of the following: • Confirmed PSA >4 ng/mL any time during testosterone treatment • PSA increase >1.4 ng/mL within 12 mo of testosterone treatment • Palpable abnormality (nodule or induration) on DRE • Worsening of lower urinary tract symptoms (e.g., IPSS score >19)
Obstructive sleep apnea (snoring, witnessed apnea, daytime somnolence, unexplained erythrocytosis, worsening hypertension or edema)	At baseline, after 3–12 mo, and then yearly	Evaluate for obstructive sleep apnea or adjustment of CPAP settings. Evaluate for other causes of hypoxia.
Formulation-specific adverse effects	At baseline, after 3–6 mo, and then yearly	Discontinue and switch to another formulation.
Testosterone ester injections	Discomfort, bleeding, or hematoma with IM injections Fluctuations in energy, mood, libido Allergy to oil vehicle (rare)	Reinstruct on the self-injection site technique. Consider shortening the injection interval if the nadir testosterone level is low.
Testosterone patch	Skin irritation Adhesion to skin	Coadministration of corticosteroid cream may reduce skin irritation.
Testosterone gel	Contact transfer to others Skin dryness at site of application	Reinstruct on washing hands and covering application area after gel dries or showering 4–6 hr after application, avoiding prolonged skin-to-skin contact of application site with women and children.
Buccal testosterone tablets	Gum irritation or inflammation Poor adhesion to gums Altered or bitter taste	Reinstruct on proper application and reassure to complete an adequate trial with the correct technique.
SC testosterone pellets	Pellet extrusion Implantation-site infection, bleeding, fibrosis	Reimplant pellets. Treat infection with appropriate drainage and antibiotics.

BMD, Bone mineral density; *CPAP,* continuous positive airway pressure; *DRE,* digital rectal examination; *IM,* intramuscular; *IPSS,* International Prostate Symptom Score; *PSA,* prostate-specific antigen.

after surgical or brachytherapy, as these individuals have high likelihood of recurrence and poor outcomes independent of testosterone therapy.

Before testosterone replacement therapy is started in men older than 50 years (>40 years if the man has risk factors for prostate cancer), prostate cancer screening should be offered after a shared decision-making discussion of the potential risks and benefits of prostate cancer screening (including detection and treatment of low-grade prostate cancer that might not benefit from treatment).[116] Minimal prostate cancer screening consists of measurement of serum PSA; there is controversy about the added value of a DRE as an initial screening test. Hypogonadal men with an abnormal DRE or a consistently elevated PSA value (e.g., >4 ng/mL in Caucasian men, >3 ng/mL in African-American men and men at high risk for prostate cancer) should have a urologic evaluation that may include a transrectal ultrasound study and biopsy of the prostate before starting testosterone. Older men, African-American men, men with an abnormal DRE, and men with a history of a first-degree relative with prostate cancer have an increased risk of prostate cancer; men with a previous negative prostate biopsy and men who are taking a 5α-reductase inhibitor have a reduced risk. A prostate cancer risk calculator, based on data from placebo-treated men in the Prostate Cancer Prevention Trial, is available to assess the risk of clinical (biopsy-detectable) and high-grade (Gleason score ≥7) prostate cancer according to ethnicity, age, PSA level, family history of prostate cancer, normal or abnormal DRE, results of prior prostate biopsy, and use of a 5α-reductase inhibitor. This calculator is available at http://myprostatecancerrisk.com/.[660,661] Although this tool has not been validated for men with untreated androgen deficiency (who usually have lower PSA concentrations than eugonadal men), the prostate cancer risk calculator may be helpful in assessing the risk of clinical or high-grade prostate cancer that may be present in a hypogonadal man before testosterone replacement is instituted.

Although breast cancer in men is rare, some disorders that cause androgen deficiency, such as Klinefelter syndrome, are associated with an increased risk of breast cancer.[662] Therefore, a careful breast examination for suspicious masses should be performed in hypogonadal men before starting testosterone treatment. The conversion of testosterone to estradiol may stimulate growth of ER-positive breast cancer.

Relative contraindications to testosterone replacement therapy include the following[116]:

- Presence of a PSA level greater than 4 ng/mL (or >3 ng/mL in individuals at high risk for prostate cancer, e.g., African-American men or those with a family history of prostate cancer), or prostate nodule or induration that has not been evaluated
- Baseline hematocrit in the high-normal range (e.g., hematocrit >48 at or near sea level or >50% at high altitudes), because further stimulation of erythropoiesis induced by testosterone therapy may result in erythrocytosis and, potentially, hyperviscosity and vascular complications, particularly in elderly men with underlying vascular disease
- Untreated obstructive sleep apnea, because higher-dose testosterone treatment may rarely worsen sleep-disordered breathing and its complications
- Severe edematous conditions (e.g., uncontrolled or poorly controlled CHF), because the fluid retention associated with testosterone therapy may worsen preexisting edema
- Severe LUTS due to BPH, such as in men with an IPSS greater than 19
- Desire for fertility in the near future
- Myocardial infarction or stroke within the past 6 months, because of an increased risk of a cardiovascular event independent of testosterone treatment, or thrombophilia or hypercoagulability, although rare, that predisposes men to venous thromboembolism (VTE) events.

Potential Adverse Effects and Monitoring. Monitoring for potential adverse effects of testosterone therapy is summarized in Table 19.11.[116]

Hematocrit. Testosterone replacement stimulates erythropoiesis in hypogonadal men, increasing the hemoglobin concentration and hematocrit from the female range into the normal adult male range.[663] Occasionally, testosterone treatment causes excessive erythrocytosis (e.g., hematocrit >54%) that may require temporary discontinuation of therapy or lowering of the testosterone dose until the hematocrit declines to normal; reinitiation of testosterone at a lower dose; or, uncommonly, therapeutic phlebotomy.[326] Excessive erythrocytosis occurs more commonly in older men[612] and in those treated with parenteral testosterone esters rather than transdermal testosterone patches, probably related to the supraphysiologic testosterone concentrations that are present for a few days after administration and the higher average testosterone concentrations produced by IM testosterone.[628] A greater erythropoietic response to testosterone is associated with stimulation of erythropoietin and suppression of ferritin and hepcidin (a liver-derived peptide that is the main negative regulator of iron bioavailability), possibly resetting the relationship between erythropoietin and hemoglobin and increasing the availability and use of iron for red blood cell production.[664,665]

Adverse effects of erythrocytosis induced by testosterone are poorly documented. However, there is concern that an excessive increase in red blood cell volume and blood viscosity could cause vascular complications such as thrombosis, especially in older men with underlying atherosclerotic CVD, resulting in stroke or myocardial infarction. Therefore hematocrit should be measured before testosterone therapy is initiated, 3 to 6 months after starting treatment, and then yearly.[116] If erythrocytosis develops during testosterone replacement therapy, testosterone should be stopped, and an evaluation should be performed to determine whether an underlying predisposing condition, such as hypoxia due to obstructive sleep apnea syndrome or chronic lung disease, is stimulating or contributing to erythrocytosis. Subsequently, testosterone may be restarted at a lower dosage.

CVD and VTE. The effects of testosterone replacement therapy in men with androgen deficiency on the risk of major cardiovascular events (MACE: myocardial infarction, stroke, and cardiovascular mortality) is not known. There are no adequately powered, large, long-term randomized controlled trials that have assessed the risk of MACE in hypogonadal men treated with testosterone. Pharmacoepidemiologic studies evaluating cardiovascular events associated with testosterone treatment[329-337] have been conflicting, with studies showing increased, no, or decreased risk of cardiovascular events associated with testosterone treatment. Meta-analyses of testosterone treatment trials[326-328,666-670] have largely (except for one study[327]) shown no increase in cardiovascular events associated with testosterone treatment; however, meta-analyses have been limited by heterogeneity of the study populations and testosterone treatment regimens; variability of study durations and reported outcomes including adverse events; and lack of sufficient sample size or prespecified, independent adjudication of MACE. Despite the lack of evidence, the FDA (but not the European Medicines Agency) required the addition of a warning regarding the potential

increased risk of cardiovascular events to the prescribing information for all testosterone formulations. A large, long-term, multicenter, randomized, placebo-controlled trial is currently being initiated to assess the risk of MACE in hypogonadal men with preexisting CVD or risk factors for CVD treated with testosterone.

A pharmacoepidemiologic and case-control study and a recent meta-analysis of testosterone treatment trials did not find an increased risk of VTE associated with testosterone treatment.[671-673] However, there were too few VTE events in randomized controlled trials of testosterone treatment to adequately assess risk. Case series have suggested that there is an increased risk of VTE events in men with a history of unprovoked VTE and thrombophilia who are treated with testosterone, even in the absence of erythrocytosis.[674] The FDA also required the addition of a warning regarding the possible risk of VTE to prescribing information for all testosterone formulations.

Prostate. Prostate size is reduced in men with androgen deficiency, and testosterone replacement therapy increases prostate volume to that of age-matched eugonadal men.[675] Testosterone therapy does not cause excessive prostate enlargement, and there is no evidence that it worsens LUTS, reduces urinary flow rate, causes urinary retention, or increases the need for invasive intervention for BPH (e.g., transurethral resection of the prostate). In the Testosterone Trials, testosterone versus placebo treatment for 1 year was not associated with an increase in IPSS score greater than 19 (severe LUTS).[162] However, long-term, controlled studies have not been performed to evaluate these outcomes in middle-age to older hypogonadal men, who are most at risk for development of clinically significant, symptomatic BPH. In hypogonadal men older than 40 years, LUTS may be monitored with the use of the IPSS or the AUA symptom score during testosterone treatment in accordance with accepted guidelines.[116] Concomitant therapy for LUTS (e.g., α-adrenergic receptor antagonists, 5α-reductase inhibitors, surgical bladder outlet procedures) should be considered in hypogonadal men with bothersome severe LUTS that affects quality of life.

There is no evidence that testosterone treatment causes prostate cancer. In middle-age to older men with androgen deficiency, there is concern that long-term testosterone therapy might stimulate previously unrecognized or clinically undetectable localized or metastatic prostate cancer or accelerate the growth of preexisting subclinical disease into clinically apparent prostate cancer. There is evidence that testosterone treatment initially stimulates the growth of metastatic prostate cancer, but its effect on progression of subclinical prostate cancer is not known. Meta-analyses of small, short-term, controlled studies (up to 3 years in duration) have not found an increased incidence of prostate cancer in older men treated with testosterone; however, these trials were underpowered to assess the risk of prostate cancer, particularly high-grade prostate cancer.[326,338] Pharmacoepidemiology studies have been inconsistent, showing no or possibly increased risk of low-grade prostate cancer (although this effect was confounded by ascertainment bias and greater prostate cancer screening in testosterone-treated men) and reduced risk of high-grade, aggressive prostate cancer with testosterone treatment.[337,339-342] Larger, longer-term, prospective randomized controlled trials are needed to determine whether testosterone therapy stimulates the growth and progression of subclinical prostate cancer into clinically evident and significant high-grade, aggressive disease.

Along with reduced prostate size, serum PSA concentrations in androgen-deficient men are decreased, and testosterone replacement increases PSA to concentrations observed in age-matched eugonadal men.[675] Because testosterone replacement is a potentially disease-modifying intervention that may alter the natural history of prostate cancer, prostate cancer screening and monitoring of PSA concentrations should be offered to patients older than 50 years (>40 years if the man has risk factors for prostate cancer) initiating testosterone treatment, after a discussion of risks and benefits of prostate cancer screening (i.e., shared decision making),[116] as PSA screening for prostate cancer has no effect or only modest effect on the mortality rate in the general population of men.[676-678]

In men older than 40 years who desire prostate cancer screening, PSA measurement (and DRE, depending on the skill of the practitioner and patient preference) should be performed before testosterone therapy is initiated, 3 to 12 months after starting treatment, and then in accordance with accepted guidelines. More intensive PSA monitoring should not be performed, because abnormal concentrations that would trigger a prostate biopsy are more likely to occur in older hypogonadal men on testosterone therapy, resulting in an increased likelihood of detecting localized, low-grade prostate cancer, for which the management is unclear.[679] The diagnosis of subclinical or localized low-grade prostate cancer does not affect overall mortality; however, the potential medical and surgical treatment, psychological, and socioeconomic consequences and morbidity of this diagnosis may be considerable.

Hypogonadal men receiving testosterone therapy who demonstrate a verified PSA level greater than 4 ng/mL or increase in PSA greater than 1.4 ng/mL within 12 months after starting therapy, an abnormal finding on DRE (nodule or induration), or an AUA symptom score or IPSS greater than 19 should undergo urologic evaluation.[116] Elevated PSA concentrations should be confirmed with a repeat measurement. If prostatitis or urinary tract infection that can markedly elevate PSA concentrations is suspected, PSA measurements should be repeated after appropriate antibiotic treatment.

Sleep Apnea. Testosterone treatment has been reported to induce or worsen obstructive sleep apnea, but the prevalence of clinically significant obstructive sleep apnea during testosterone replacement therapy is probably very low and may be dose related.[553,567,568] Short-term, high-dose testosterone treatment in older hypogonadal men significantly worsened obstructive sleep apnea, increased the duration of hypoxemia, and shortened total sleep time as assessed by polysomnography. In contrast, older men treated with a scrotal testosterone patch for 3 years did not demonstrate a significant worsening of sleep apnea as assessed by a portable device.[680] As discussed earlier, sleep apnea may conversely cause gonadotropin and testosterone suppression and secondary hypogonadism, probably because of the stress of oxygen desaturation and sleep disturbance.

Sleep apnea is associated with significant morbidity and mortality risks. Therefore, hypogonadal men, especially those at increased risk (e.g., obese men), should be monitored for symptoms of obstructive sleep apnea syndrome (e.g., loud snoring, apnea witnessed by a bed partner, daytime somnolence, unexplained erythrocytosis, worsening or recent onset of hypertension or edema) before starting testosterone therapy, after 3 to 12 months, and then yearly.[116] If symptoms suggest sleep apnea, a formal sleep study (polysomnography) should be performed. If obstructive sleep apnea is confirmed, appropriate treatment (e.g., CPAP) should be instituted before testosterone treatment is started or continued.

Reduced Sperm Production and Fertility. In men who have androgen deficiency and persistence of some sperm production

with either partial primary or secondary hypogonadism, testosterone treatment suppresses gonadotropin production by negative feedback regulation, which in turn suppresses spermatogenesis and may further impair fertility.[587] Suppression of sperm production is most important in men with secondary hypogonadism and normal testes who wish to father children. In these men, testosterone therapy should be discontinued, and gonadotropin therapy should be started, initially with hCG alone and then, if necessary, with combined hCG and FSH treatment to stimulate spermatogenesis.[79] In the absence of coexisting testicular disease (e.g., cryptorchidism), prior testosterone therapy does not impair—but might delay—the subsequent induction of sperm production with gonadotropins.[392-394]

Acne and Oily Skin. Boys with pubertal androgen deficiency who are receiving testosterone therapy to induce puberty and men with severe hypogonadism who are treated with full replacement doses of testosterone may develop acne and increased oiliness of the skin due to increased sebum production.[146,147,374,375] These conditions usually respond to local skin measures (e.g., benzoyl peroxide, retinoids) and antibiotics, reduction in testosterone dose, or both.

Gynecomastia. Occasionally, breast tenderness or gynecomastia develops or worsens during testosterone replacement therapy, particularly in boys who are receiving testosterone for induction of puberty, men with severe androgen deficiency who are treated with full replacement or high-dose testosterone, and hypogonadal men with predisposing conditions such as hepatic cirrhosis. Gynecomastia is commonly found in boys and men with androgen deficiency before the initiation of testosterone therapy. A careful breast examination should be performed before and again during testosterone replacement therapy to detect the presence or worsening of gynecomastia (breast tenderness or enlargement), or the rare occurrence of breast cancer.

Lipids. In hypogonadal men, testosterone replacement results in no change or only a slight decrease in HDL cholesterol and no change in total cholesterol or LDL cholesterol concentrations.[326,681] The reduction in HDL cholesterol is greater in men with more severe androgen deficiency and in those treated with supraphysiologic testosterone doses or with nonaromatizable, oral 17α-alkylated androgens.[620]

The clinical significance of HDL cholesterol reduction induced by testosterone in terms of cardiovascular risk is not known. Understanding of the effects of testosterone therapy on major cardiovascular outcomes (including myocardial infarction, stroke, cardiovascular mortality risk) will require larger, longer-term randomized controlled studies. Cardiovascular risk and lipid measurements should be evaluated as recommended by available practice guidelines; at present, more intensive monitoring in hypogonadal men receiving therapy is not justified.

Other Potential Adverse Effects. Frontal balding or androgenic alopecia may develop or worsen in genetically predisposed hypogonadal men during testosterone replacement therapy. Mild to moderate *weight gain* usually occurs during testosterone treatment because of the anabolic actions of testosterone on muscle mass and mild fluid retention. Testosterone therapy usually does not cause clinically significant edema, except occasionally in hypogonadal men with underlying edematous states such as CHF or hepatic cirrhosis.

Stimulation of excessive libido and erections by testosterone is rare and usually occurs in boys or young men with severe, long-standing androgen deficiency who are treated with full replacement or high-dose testosterone therapy. These symptoms usually resolve spontaneously or with a reduction in testosterone dose. Contrary to popular opinion, testosterone treatment does not cause pathologic aggressiveness, anger, or rage.[682,683] Instead, testosterone replacement therapy increases social aggressiveness, motivation, and initiative and reduces irritability and anger.

Profound behavioral and physical changes induced by testosterone treatment in men with severe, long-standing androgen deficiency may be upsetting to both patients and their partners. Therefore, changes that are expected to occur with testosterone replacement should be discussed with patients and their partners before and during treatment. Oral 17α-alkylated androgens may cause cholestasis or potentially serious hepatotoxicity.[620] However, liver toxicity does not occur with testosterone replacement therapy, and routine monitoring of liver enzymes is not necessary in hypogonadal men. IM injection of testosterone esters, particularly with large volume injections of testosterone undecanoate or rarely with testosterone enanthate and cypionate, has been reported to cause coughing that has been hypothesized to be due to POME, the significance of which is not known (see earlier discussion).

Formulation-Specific Adverse Effects. *Testosterone ester injections* may cause local discomfort, bleeding, or hematoma at the site of IM injections.[116] Instruction on proper injection technique minimizes the likelihood of these adverse effects. Fluctuations in energy, mood, and libido associated with the peak and nadir swings of testosterone concentrations after testosterone ester injections may be disturbing to some hypogonadal men and may require reduction of the dose injected and shortening of the injection interval or switching to transdermal testosterone. Rarely, an allergy may occur to the sesame oil (enanthate) or cottonseed oil (cypionate) vehicle used.

Testosterone patches frequently cause local skin erythema, irritation, itching, and contact dermatitis and occasionally lead to more severe reactions.[116] Use of a topical corticosteroid cream under the reservoir of the patch may reduce skin irritation and reactions, but often men prefer to switch to another testosterone formulation. Testosterone patches may adhere poorly to skin, particularly with excessive perspiration.

In contrast to testosterone patches, *testosterone gels* and *solution* cause little to no skin irritation. However, residual testosterone remains on the skin surface at the application sites, and there is a potential for transfer of testosterone to women and children who have prolonged intimate contact with these sites.[116] Precautions to avoid contact transfer of testosterone include washing the hands immediately after application of testosterone gel, covering the application site with clothing, washing off residual testosterone on skin by showering or bathing (4–6 hours after application), and avoiding prolonged skin-to-skin contact of the application site with women or children.

Buccal testosterone tablets may cause gum irritation, inflammation or recession, or an altered or bitter taste sensation, and they may adhere poorly to gums if not they are not properly applied.[116]

Nasal testosterone gel may cause nasal irritation, nasopharyngitis, rhinorrhea, epistaxis, and nasal scabbing.

SC testosterone pellets may uncommonly extrude spontaneously; rarely, there may be bleeding or infection at the site of implantation.[116]

Gonadotropin Therapy

Secondary hypogonadism manifests as prepubertal or adult androgen deficiency and impairment of sperm production due to gonadotropin deficiency. The primary goal of gonadotropin therapy in men with secondary hypogonadism is to initiate and

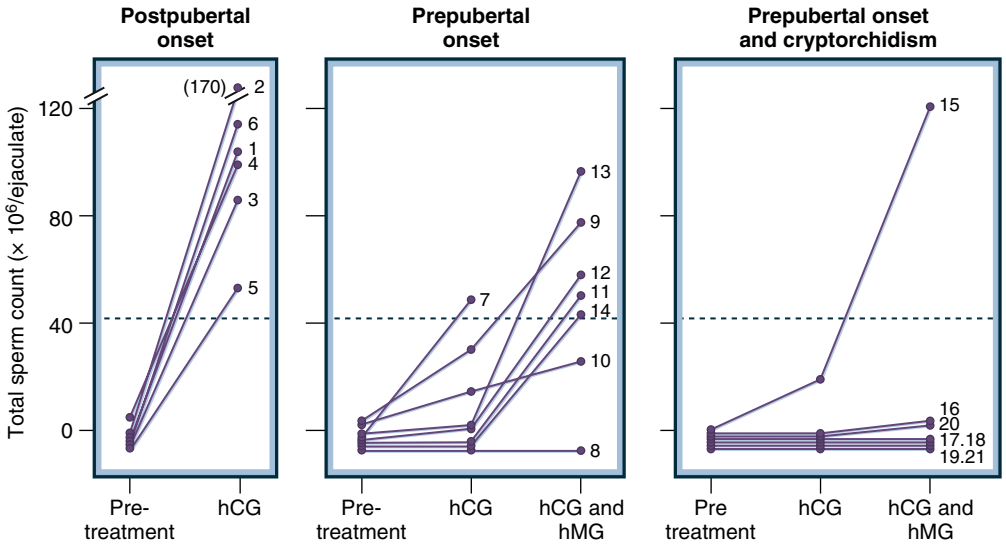
maintain spermatogenesis to establish and restore fertility.[614] Because gonadotropin therapy is more complex (requiring multiple injections weekly) and more expensive than testosterone replacement therapy, symptomatic androgen deficiency is usually treated with the latter. In patients with partial gonadotropin deficiency, testosterone treatment may suppress remaining gonadotropin secretion by negative feedback regulation. When fertility is desired and stimulation of sperm production is needed in a man with secondary hypogonadism, testosterone is discontinued and gonadotropin therapy is initiated. Previous testosterone treatment does not compromise subsequent stimulation of spermatogenesis by gonadotropins, although sperm production may be slower.[392-394]

The gonadotropin preparations most commonly used to treat secondary hypogonadism are purified urinary gonadotropins. Human recombinant gonadotropin preparations are now available and have much higher purity than urinary preparations, but they are more expensive. Because urinary gonadotropins are highly effective in treating gonadotropin deficiency, they remain the most commonly used preparations for treatment of infertility from secondary hypogonadism.

hCG is used to provide LH-like activity because its half-life is longer than that of LH. In contrast to LH, which would require pulsatile administration approximately every 2 hours, hCG is administered two to three times per week. Purified urinary preparations of hCG (derived from urine of pregnant women) that contain LH-like activity are used almost exclusively in gonadotropin therapy. FSH activity is usually provided by purified urinary *human menopausal gonadotropin* (hMG), which is derived from urine of menopausal women and contains both LH and FSH; by highly purified urinary *human FSH*; or by *rhFSH*. FSH preparations are administered three times weekly. hCG and FSH preparations may be administered either intramuscularly or subcutaneously.[684,685] Both are equally effective, but the latter is better tolerated and more easily self-administered.

In patients with prepubertal gonadotropin deficiency, initiation of sperm production requires treatment with both hCG and FSH[79] (Fig. 19.30). Gonadotropin therapy is initiated with administration of hCG alone at a dose of 500 to 2000 IU SC two to three times weekly to stimulate sufficient endogenous testosterone production to increase and maintain circulating testosterone concentrations in the normal range and to correct manifestations of androgen deficiency.[6] The dose of hCG is increased until serum testosterone concentrations are within the eugonadal range. Despite the more frequent injections, some men with secondary hypogonadism prefer hCG over testosterone treatment because costs are comparable (purified urinary hCG preparations compared with testosterone gels), SC injections are better tolerated than IM injections, testis size is generally larger, and spermatogenesis is increased. In addition, there is no risk of secondary transfer to intimate contacts, and some men prefer twice weekly SC injections to daily gel application.

Men with gonadotropin deficiency who also have primary testicular disease (e.g., cryptorchidism) or CHH may require larger doses of hCG (e.g., up to 3000–5000 IU two to three times weekly or occasionally much more). Men with secondary hypogonadism who have coexisting severe testicular damage may be completely unresponsive to hCG treatment (see Fig. 19.30). Because hCG stimulates Leydig cell aromatase activity within the testes, serum estradiol concentrations may increase disproportionately relative to testosterone concentrations, resulting in breast tenderness or gynecomastia more frequently than with testosterone replacement



• **Fig. 19.30** Total sperm count response to gonadotropin therapy with human chorionic gonadotropin (hCG) alone or in combination with human menopausal gonadotropin (hMG) in males with hypogonadotropic hypogonadism of postpubertal onset (*left*), prepubertal onset without cryptorchidism (*middle*), or prepubertal onset with cryptorchidism (*right*). Sperm production was induced by hCG alone in all men with postpubertal hypogonadotropic hypogonadism and in some with prepubertal onset who did not have cryptorchidism. Both hCG and hMG treatment was required to increase sperm production above the lower limit of normal (*dashed line*) in patients with prepubertal hypogonadotropic hypogonadism who did not have cryptorchidism. With one exception, men with prepubertal hypogonadotropic hypogonadism who also had cryptorchidism failed to respond to hCG alone or to combined hCG and hMG therapy. (From Finkel DM, Phillips JL, Snyder PJ. Stimulation of spermatogenesis by gonadotropins in men with hypogonadotropic hypogonadism. *N Engl J Med.* 1985;313:651–655.)

therapy. Some men may complain of burning at the site of SC injections of hCG.

hCG stimulates Leydig cell testosterone production, resulting in relatively high intratesticular testosterone concentrations that cause Sertoli cells to mature and sperm production to be initiated to varying degrees. In a small proportion of patients with partial gonadotropin deficiency of prepubertal onset and in almost all men with acquired postpubertal gonadotropin deficiency, treatment with hCG alone may stimulate sperm production[79] (see Fig. 19.30). Evidence for partial gonadotropin deficiency includes bilateral testicular volume larger than 8 mL, physical examination evidence of partial androgenization, low-normal serum gonadotropin concentrations, and low-normal serum inhibin B concentrations.[686-690] Most patients with severe CHH require FSH treatment in addition to hCG to stimulate complete spermatogenesis and induce fertility.[691-693]

If no sperm is present in the ejaculate after 6 to 12 months of treatment with hCG and serum testosterone concentrations in the eugonadal range, hMG or FSH at a dose of 75 IU three times weekly (increasing up to as much as 300 IU given subcutaneously three times weekly) is added, in combination with the same dose of hCG, for an additional 6 to 12 months or longer, until spermatogenesis is induced.

The induction of sperm production by gonadotropin therapy may take 12 to 24 months. Factors that limit the duration of gonadotropin treatment are impatience by patients and their partners and the expense of gonadotropins, particularly hMG or FSH preparations, which are very costly. Some couples may opt for alternative means to have children (e.g., adoption). In patients with CHH, sperm output is often low, possibly because of inadequate gonadotropin stimulation of Sertoli cell number and maturation during development.[694] Despite quantitatively low sperm production during gonadotropin therapy, fertility may be possible, sometimes at very low sperm counts (e.g., <1 million/mL).[686] Some patients who have very low sperm counts and remain infertile on gonadotropin treatment may elect to use ejaculated sperm for ICSI.

Once spermatogenesis has been initiated with combined hCG and FSH treatment in patients with prepubertal gonadotropin deficiency, sperm production may be maintained with hCG treatment alone.[611] In men with acquired postpubertal gonadotropin deficiency, reinitiation of spermatogenesis can usually be accomplished with hCG therapy alone.[79]

In men with secondary hypogonadism due to hypothalamic GnRH deficiency (e.g., CHH, Kallmann syndrome), pulsatile GnRH therapy may be used to stimulate production of endogenous gonadotropins (both LH and FSH) and testosterone and to initiate and maintain sperm production sufficient for fertility.[47] GnRH is administered subcutaneously with the use of a portable infusion pump that delivers small doses (pulses) of GnRH (e.g., GnRH 5–25 ng/kg SC every 1.5–2.0 hours, increasing to higher doses if needed); this treatment mimics a near-normal physiologic stimulus to the pituitary gland. In men with CHH, pulsatile GnRH treatment is successful in stimulating gonadotropin, testosterone, and sperm production in approximately 75% of cases; in the other 25% of cases, men fail to respond due to concomitant pituitary or testicular defects.[396] The effectiveness of pulsatile GnRH replacement in stimulating sperm production is comparable to that of gonadotropin therapy. Practically, however, the use of pulsatile GnRH therapy is limited to specialized centers because GnRH is not readily available, the infusion pump requires additional expertise and management, and therapy is expensive.

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The complete list of references is available online at ExpertConsult.com.

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20

Sexual Dysfunction in Men and Women

SHALENDER BHASIN AND ROSEMARY BASSON

CHAPTER OUTLINE

KEY POINTS

- In contrast to the earlier sexual response model depicting a linear progression of discrete phases, current research conceptualizes sexual response as a motivation/incentive-based cycle comprising phases of physiologic response. These phases of the cycle overlap, and their order is variable.
- In middle-age and older men, sexual dysfunction is often related to comorbid conditions, such as diabetes, coronary artery disease, or a hormonal problem.
- Penile erection results from biochemical and hemodynamic events that are associated with activation of central nervous system sites, cavernosal smooth muscle relaxation, increased blood flow into cavernosal sinuses, and venous occlusion.
- Corporal smooth muscle tone is regulated by transmembrane and intracellular calcium flux, which in turn is regulated by potassium channels; connexin43-derived gap junctions; and cholinergic, adrenergic, and nonadrenergic, noncholinergic mediators, including nitric oxide.
- Testosterone regulates sexual thoughts and desire, sexual arousal, attentiveness to erotic stimuli, and sleep-entrained erections. Testosterone deficiency is a treatable cause of hypoactive sexual desire in men. In randomized trials, testosterone replacement therapy has been shown to improve erectile function and satisfaction with intercourse.
- Selective phosphodiesterase 5 inhibitors are safe and effective and have emerged as first-line therapy for men with erectile dysfunction.
- Sexual response is understood to be incentive based; multiple reasons for sex motivate receptivity to sexual stimuli that can be appraised as sexually arousing.
- Physical and subjective arousal may diverge. Women complaining of low sexual arousal usually physically respond to sexual stimuli in a laboratory setting. In contrast, men with erectile dysfunction from endothelial or neurologic deficit—the most common cause of men's arousal complaints—typically still experience mental sexual arousal/excitement.
- Women's sexual dysfunctions do not link to androgen deficit.
- Psychological therapies predominate in the treatment of women's sexual dysfunctions with emerging evidence of benefit from mindfulness-based cognitive therapy.
- About 10% to 15% of women have dyspareunia from provoked vestibulodynia—a chronic pain disorder associated with central sensitization of the nervous system and occasionally precipitated by low-dose combined contraceptives.

Men and women rate sexual health as highly important to their quality of life.[1] Recognizing the importance of sexual function as a determinant of quality of life, the World Health Organization declared sexual health a fundamental right of men and women. Yet disorders of sexual function are highly prevalent in men and women.[2] Epidemiologic surveys, such as the National Survey of Sexual Attitudes and Lifestyle (Natsal-3), indicate that 42% of men and 51% of women experienced some problem with sexual response in the previous year, causing distress in the lives of 10% to 11%.[2] The 2015 systematic review by the International Consensus Committee on Sexual Medicine found that low sexual interest was the most prevalent sexual concern for

women and erectile dysfunction (ED) the most common form of sexual dysfunction in men.[3] Among women, the loss of pleasure and the loss of interest were the most commonly reported problems during the previous year, and their incidence was similar among younger and older women.[3] In contrast, middle-age and older men reported higher prevalence of sexual dysfunction than younger men; the prevalence of ED ranged from less than 20% among men younger than 50 years of age to 25% to 76% among men older than 70 years.[3]

Sexual response exemplifies the intricate blending of mind and body so that dysfunction is neither purely psychological nor entirely biologic, but psychological and biologic factors both contribute to sexual dysfunction to varying degrees. Recent advances in our understanding of the physiologic and biochemical mechanisms of penile erection and the development of mechanism-specific therapies for ED have greatly clarified the importance of vascular health in maintaining erectile function. The 1980s and 1990s witnessed remarkable progress in our understanding of the physicochemical mechanisms that lead to penile tumescence and rigidity. It was recognized that penile erections are the result of cavernosal smooth muscle relaxation and increased penile blood flow.[4–6] The appreciation of nitric oxide as a key vasodilator in the vascular smooth muscle was a pivotal discovery, recognized later by awarding of the Nobel Prize in Physiology or Medicine to Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad. The recognition that nitric oxide caused cavernosal smooth muscle relaxation by simulating guanylyl cyclase provided the foundation for the discovery of highly effective oral therapies for the treatment of ED.

The growing recognition that ED is commonly a manifestation of systemic disease[4,7–12] and the availability of easy-to-use therapeutic options, including oral and intraurethral drugs, have duly placed sexual disorders in men within the purview of the endocrinologist and the primary care provider. In middle-age and older men, but less so for women, sexual dysfunction is often related to comorbid medical conditions.[4,7–12] ED may signal asymptomatic coronary artery disease,[13,14] diabetes, or depression.[2] In women, sexual dysfunction is very strongly linked to mental health.[2,14–17] A similarly strong association was found between sexual dysfunction and relationship unhappiness in both men and women. The American Psychiatric Association's clinical definitions of sexual disorders published in the fifth edition of *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5) in 2013[18] attempted to quantify symptom duration and severity more precisely, address the overlap between sexual desire and arousal in women, and include the important entity of sexual motivation/interest. Also included in DSM-5 are previously ignored aspects of women's sexual response, such as mental sexual arousal/excitement, pleasure, ability to trigger arousal and desire from sexual stimuli, and physical genital and nongenital sexual sensations (Table 20.1).

This chapter describes the current conceptualization of sexual response in men and women, the underlying pathophysiologic mechanisms of sexual dysfunction, the sexual sequelae of various endocrine disorders, and clinical assessment and diagnosis of sexual dysfunction and its management. Management strategies for sexual dysfunction stemming from hormonal and nonhormonal factors are also outlined.

TABLE 20.1 Current Definitions of Female Sexual Disorders

Female Sexual Interest/Arousal Disorder

Lack of sexual interest/arousal for a minimum duration of 6 months as manifested by at least three of the following indicators:

1. Absent/reduced frequency or intensity of interest in sexual activity
2. Absent/reduced frequency or intensity of sexual/erotic thoughts or fantasies
3. Absence or reduced frequency of initiation of sexual activity and typically unreceptive to a partner's attempts to initiate
4. Absent/reduced frequency or intensity of sexual excitement/pleasure during sexual activity on all or almost all (approximately 75%) sexual encounters
5. Sexual interest/arousal is absent or infrequently elicited by any internal or external sexual/erotic cues (e.g., written, verbal, visual)
6. Absent/reduced frequency or intensity of genital or nongenital sensations during sexual activity on all or almost all (approximately 75%) sexual encounters

Female Orgasmic Disorder

At least one of the two following symptoms, which must have been present for a minimum duration of approximately 6 months and be experienced on all or almost all (approximately 75%) occasions of sexual activity:

1. Marked delay in, marked infrequency of, or absence of orgasm
2. Markedly reduced intensity of orgasmic sensation

Genitopelvic Pain/Penetration Disorder

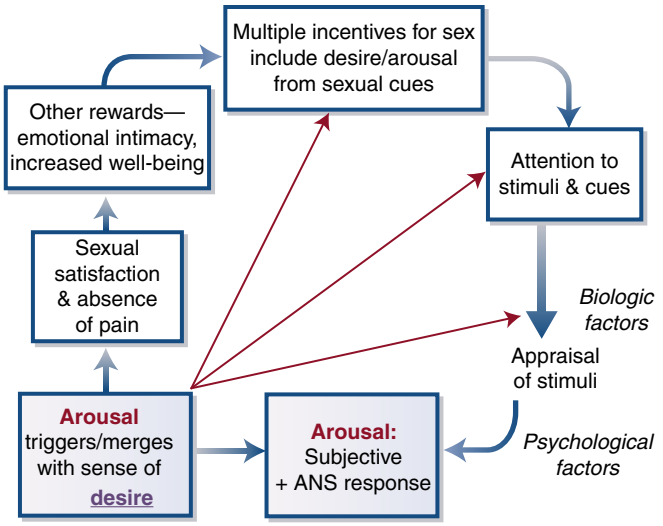
Persistent or recurrent difficulties for a minimum duration of approximately 6 months with one or more of the following:

1. Marked difficulty having vaginal intercourse/penetration
2. Marked vulvovaginal or pelvic pain during vaginal intercourse/penetration attempts
3. Marked fear or anxiety either about vulvovaginal or pelvic pain on vaginal penetration
4. Marked tensing or tightening of the pelvic floor muscles during attempted vaginal penetration

Human Sexual Response Cycle

The sexual response can be conceptualized as a motivation/incentive-based cycle comprising phases of physiologic response and subjective experience.[19–22] The phases of the cycle overlap, and their order is variable (Fig. 20.1). The motivations and incentives for sex are multiple and varied. A wish to both demonstrate and enhance emotional intimacy between partners is important for both men and women.[23] Depression is a major cause of reduced sexual motivation in otherwise healthy persons and in those with endocrine disease: comorbid depression has been identified as a major factor underlying sexual dysfunction in women with diabetes.[24–26] Endocrine disorders can markedly lessen sexual self-image, especially when associated with altered appearances, infertility, or the ability to be gainfully employed.[24,25,27,28]

Sexual desire—as in lust or drive triggered deliberately, accidently, even subliminally,[29] by sexual stimuli—is only one of many reasons people engage in sex and may or may not be sensed initially: desire can be triggered by the sexual excitement (i.e., the subjective sexual arousal) in response to sexual stimuli.[19,24,30,31] In both men and women, the relationship between desire and arousal is variable, complex, and often inseparable.[32–34] This overlap of phases is consistent with the neuroimaging data of sexual arousal, which have led to the concept that motivation is one facet of sexual arousal and desire is one component of motivation.[35] Many psychological and biologic factors influence the brain's appraisal and processing of sexual stimuli to enable or disable subsequent



• **Fig. 20.1** Circular human response cycle of vverlapping phases. Human sexual response is depicted as a motivation/incentive-based cycle of overlapping phases of variable order. A sense of desire may or may not be present initially: it can be triggered alongside the sexual arousal resulting from attending to sexual stimuli. Psychological and biologic factors influence the brain's appraisal of the sexual stimuli. Sexual arousal comprises subjective (pleasure/excitement/wanting more of the same) and physical (genital and nongenital) responses. The merged desire and arousal influence the ongoing attention to and appraisal of further sexual stimulation. The sexual and nonsexual outcomes influence present and future sexual motivation. ANS, autonomic nervous system. (Modified from Basson R. Human sexual response. *Handb Clin Neurol.* 2015;130:11–18.)

arousal.[16,23,24,26,30-32,34–42] The sexual and nonsexual outcomes influence future sexual motivation. The circle depicted in Fig. 20.1 may be partially or completely repeated several times during any given sexual encounter.[19] The sexual response cycle varies substantially among individuals and even within a person's own sexual life, and is influenced by multiple factors, including the stage of life cycle, age, relationship duration and happiness, and mental health[34] (see Fig. 20.1).

Even with sufficient sexual motivation and the presence of adequate stimuli, the arousal and pleasure may not occur if attention is not focused.[38] The attentional processes play a central role in facilitating the subjective and also the physiologic components of sexual arousal.[38] Sexual information is processed in the mind both automatically and consciously.[39] The sexual nature of the stimuli is processed by the limbic system, allowing genital congestion (observed to be rapid and involuntary in women and slower but still involuntary in men).[39] Conscious appraisal of sexual stimuli and contextual cues can lead to subjective arousal.[39-42] The latter may be further increased by awareness of the genital congestion of arousal, which is typically accurately registered and very relevant to men's experience.[19] For women, an important component of arousal is the change in perception of genital sensitivity such that touch is no longer neutral or even unpleasant but rather sexual and pleasurable. Subjective arousal is also cognitively appraised to evaluate whether sex is pleasurable and safe or whether it is shameful or likely to have a negative consequence.[39-42] These cognitive appraisals continually modify both the physiologic and subjective responses.[39,40]

Undue focus on nonerotic thoughts during sexual stimulation, generated possibly by anxiety, can be associated with sexual problems.[40] A study of men and women in long-term relationships found that women tended to report nonerotic thoughts about their body image and the consequences of sexual activity, whereas men were more likely to report nonerotic thoughts about problematic sexual performance.[41] A high frequency of nonerotic thoughts can be associated with increased likelihood of sexual dysfunction. Importantly, the more difficult it was to refocus back on an erotic thought, the more this predicted sexual difficulty occurred. This research is clearly relevant to patients with endocrine disease, which frequently has negative impact on sexual self-image and sexual function.[24,25]

The dual control theory for sexual appraisal by Bancroft and associates[43] envisions a balance between sexual activation and sexual inhibition in an individual's brain that determines whether sexual stimulation leads to arousal. In men, the inhibitions include the threat of performance failure and the threat of performance consequences; in women, relationship importance (reflecting the need for sex to be within a specific type of relationship), concerns about sexual function (worries and distractions about sexual function), and arousal contingency (the potential for arousal to be inhibited by contextual/situational factors) were inhibiting factors.[43]

Thus, the current conceptualization of men's and women's sexual responses is in marked contrast to an earlier model depicting a linear invariable progression of discrete phases—from desire to arousal to a plateau of high arousal, followed by orgasm/ejaculation and finally a phase of resolution.[44] Women's sexual dysfunction typically involves lessened arousal and desire and lessened frequency of orgasm, which is now embodied in DSM-5 as *sexual interest/arousal disorder* (SIAD)[45,46] (see Table 20.1). Although the focus in men has typically been on ED or premature ejaculation, they too may experience a more generalized sexual distress disorder affecting desire, erectile function, and ease of orgasm.[10,42]

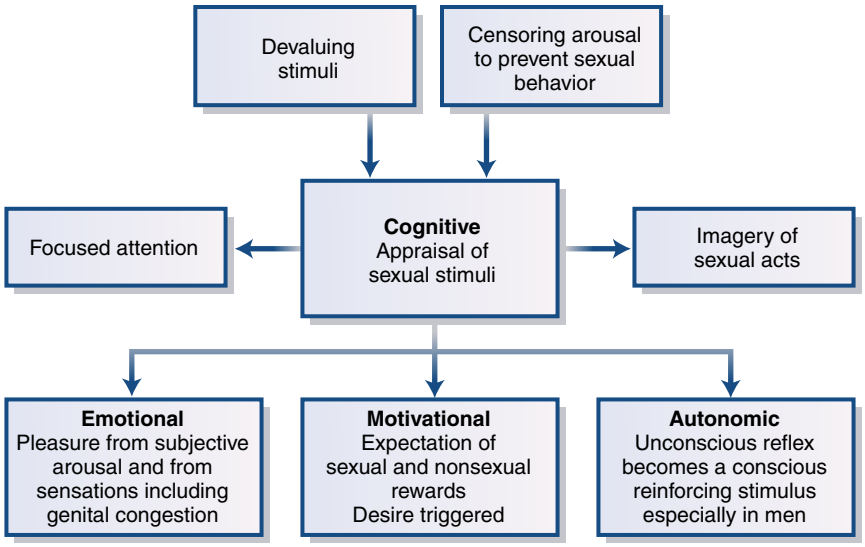
Physiologic Mechanisms of Human Sexual Response

Physiology of Desire and Arousal

Functional Brain Imaging of Sexual Arousal in Men and Women

Functional neuroimaging studies have clarified some of the neural correlates of sexual response.[47–53] These brain imaging studies during sexual stimulation—most of which were performed in healthy male heterosexual volunteers—has revealed engagement of a complex brain circuitry that involves activation of brain regions related to the different aspects of arousal and deactivation of other brain regions related to the inhibition of sexual arousal.[47–53]

In keeping with the current circular model of sexual response (depicting sexual incentives/motivations, information processing, overlap of arousal and desire, emphasis on both subjective and physiologic arousal, plus importance of reward), the model of sexual arousal emanating from the neuroimaging data comprises cognitive, motivational, emotional, and autonomic components[35] (Fig. 20.2). The cognitive component includes appraisal of potentially sexual stimuli, focused attention on those stimuli appraised as erotic, and imagery of actual sexual activity. The activations of the right lateral orbitofrontal cortex (OFC), of the right and the left inferior temporal cortices, of the superior parietal lobules, and of areas belonging to the neural network mediating motor imagery (inferior parietal lobules, left ventral premotor area, right and



• **Fig. 20.2** Brain areas activated during sexual arousal. Brain areas activated during sexual arousal allow (1) continued focus on sexual stimuli, imaging of sexual behavior, evaluation/censorship and limitation or prevention of actual behavior despite arousal (all constituting a cognitive component of arousal); (2) sexual feelings (an emotional component); (3) anticipation of reward (a motivational component); and (4) an autonomic/neuroendocrine response of physical sexual arousal. (Modified from Basson R, Weijmar Schultz W. Sexual sequelae of general medical disorders. *Lancet*. 2007;369[9559]:409–424.)

left supplementary motor areas, cerebellum) are considered to be the neural correlates of this cognitive component. The motivational component comprises the processes that direct behavior to a sexual goal, including the perceived urge to express overt sexual behavior. Thus, the motivational component is conceptualized as including the experience of sexual desire. Neural correlates are thought to involve the anterior cingulate cortex (ACC), claustrum, posterior parietal cortex, hypothalamus, substantia nigra, and ventral striatum. The emotional component is the brain activity underlying the pleasure from the mental excitement and the perception of genital and other physical responses. This pleasure comprises *liking* and *wanting*.[54] The left primary and secondary somatosensory cortices, the amygdalae, and the right posterior insula are conceived as neural correlates of this emotional component. The autonomic and neuroendocrine component includes the various responses (e.g., genital, cardiovascular, respiratory, changes in hormonal plasma levels) that allow preparedness for sexual activity: activations of the ACC, anterior insulae, putamens, and hypothalamus may contribute to this component.

The brain imaging studies during sexual arousal have highlighted three inhibitory components[35]:

1. Inhibition mediated by regions in the temporal lobes and the gyrus rectus of the OFC in the resting state. Patients with lesions in the gyrus rectus are noted to have excessive appetite for sexual and other pleasurable activities.[55] The Klüver-Bucy syndrome, a rare neurologic disorder associated with damage to both temporal lobes, is characterized by hypersexuality.[56] The inhibitory regions of the temporal lobes are distinct from those activated in response to visual sexual stimuli.
2. Inhibition of arousal once it has begun, to limit its expression because the circumstances are inappropriate, is mediated in the caudate nucleus and putamen. This is consistent with the case reports of hypersexuality associated with lesions in the head of the caudate nuclei.[57]
3. Activation of the left OFC is thought to block the ability of sexual stimuli to arouse. The brain regions that mediate inhibition of sexual arousal have been found to be activated during tasks that involve moral judgment, guilt, and shame.

In general, men typically show greater responsiveness to visual sexually arousing stimuli than do women.[58] Brain imaging in hypogonadal men before and after testosterone replacement therapy suggests that the left OFC might exert a testosterone-dependent inhibitory tonic control on sexual arousal and that this inhibitory control decreases on visual sexual stimulation.[53] In addition, the response of the right anterior insula to visual sexual stimulation appears to be related to circulating testosterone levels.[53]

Functional imaging during penile or clitoral stimulation to orgasm indicates that women show more activation in left frontoparietal regions, notably in the posterior parietal cortex and the supplementary motor area—regions associated with making a mental representation of another person's actions.[49] These findings may reflect gender differences in perspective and empathy. The brain responses during the orgasm(s) are similar in men and women, but men and women use different cerebral strategies to reach orgasm.[49]

The complexity and variability of these systems was reflected in a study of surgically menopausal women who were sexually active but were not receiving hormonal therapy.[48] When these surgically menopausal women viewed erotica during functional magnetic resonance imaging (fMRI), they failed to display the brain activation observed in premenopausal women or when they were treated with testosterone and estrogen. However, these women reported sexual arousal from the erotic videos regardless of the hormonal supplementation.[48]

Functional imaging studies in women with DSM-IV hypoactive sexual desire disorder (HSDD) show, unsurprisingly, reduced activity in sexual desire networks and increased activity in self-referential brain networks, areas involved in moral judgments, shyness, and visual analyses.[59] It is encouraging to note that even

structural changes associated with chronic pain can reverse with treatment, as these data suggest that both anatomic and functional changes associated with HSDD may be reversible with appropriate treatment.[52]

Neurotransmitters and Hormones Involved in Sexual Desire and Subjective Arousal

A variety of hormones and peptides are involved in the sexual response. The interplay among androgens and neurotransmitters is complex[60–70]: androgens influence neurotransmitter release, and neurotransmitters may modulate androgen receptor signaling.[58,62,64] The role of testosterone in desire and arousal is well documented in men but far less so in women.[63,64] Circulating levels of testosterone or androgen metabolites do not correlate with women's sexual function in large epidemiologic studies.[65–67,71] The radioimmunoassays used to measure testosterone concentrations in many epidemiologic studies were designed to measure the substantially higher levels of testosterone in men and lacked the sensitivity, precision, and accuracy in the low range prevalent in women. In studies that have used sensitive mass spectrometry–based assays to measure testosterone levels, no significant differences have been found in serum testosterone levels between women with low desire and low subjective arousal and healthy controls.[67,71,72] Additionally, Labrie and associates[68] proposed circulating levels of androgen metabolites—most notably androsterone glucuronide (ADT-G)—as markers of ovarian plus intracrine androgen activity. Circulating ADT-G levels decrease with age in women.[68] However, serum ADT-G levels have not been found to differ significantly between women with low desire and women without low desire[67,71] or to be associated with sexual dysfunction.[72]

ADT-G is viewed by many as a good marker of total androgenic activity. The C19 steroids 11β-hydroxyandrostenedione and testosterone can be converted to the androgenic steroid 11-ketotestosterone (11-KT) that can induce androgen receptor–mediated transactivation. 11-KT can be converted to 11-ketodihydrotestosterone, which can also activate androgen receptor signaling. The enzymes required for the production of 11-KT—P450 11B1 and HSD11B2—are expressed in the adrenal, peripheral tissues, and weakly in testes and the ovaries.[73] The circulating concentrations of 11-KT are similar to those of testosterone[73] in healthy women and have been reported to be increased in women with polycystic ovary syndrome and 21-hydroxylase deficiency.[74] Although 11-KT is known to serve as a major androgen in teleosts,[75] its precise role in androgenic disorders in women is incompletely understood.

Animal Models

In animal models, sex steroid hormones modulate sexual arousal by directing the synthesis of the enzymes and the receptors for neurotransmitters, such as dopamine, noradrenaline, melanocortins, and oxytocin.[60,61,69,70,76,77] Systems that act within the hypothalamus and limbic regions of the brain are involved in arousal, attention, and sexual behavior. It is thought that dopamine transmission in the medial preoptic area (MPOA) and the nucleus accumbens focuses the person's attention on sexual stimuli (the incentives or motivations for sexual activity). It is postulated that the behavioral pattern stimulated by these systems and the subjective feelings that accompany them constitute the phenomenon commonly referred to as *sexual desire* or *arousal*. The main part of this neural pathway includes the MPOA and its outputs to the ventral tegmental area. The latter contains dopaminergic cell bodies that project to various limbic and cortical regions, including the prefrontal cortex, the nucleus accumbens, the ACC, and the amygdala.

Brain pathways for sexual *inhibition* include opioid, endocannabinoid, and serotonin neural transmissions feeding back to various levels of the excitatory pathways.[61,62] It is thought that the behavioral pattern stimulated by the inhibitory pathways includes both refractoriness to sexual reward and satiety.

Exogenous opiates are sexually inhibiting independent of their inhibitory effect on luteinizing hormone (LH), gonadotropin-releasing hormone (GnRH), and testosterone.[76] Endogenous opioids modulate the feedback effects of sex steroids on the hypothalamus and pituitary.[76] β-Endorphin is synthesized in the anterior pituitary, the hypothalamus, and the nucleus of the tractus solitarius in the brainstem. The sexual inhibiting effects of opioids occur mainly through their action in the MPOA and the amygdala.[76] Low doses of opiates can have facilitating effects, possibly through actions in the ventral tegmental area to activate the mesolimbic dopamine system. Exogenous opiates can induce an intense feeling of pleasure, which has been likened to orgasm, followed by a state of relaxation and calm.[77]

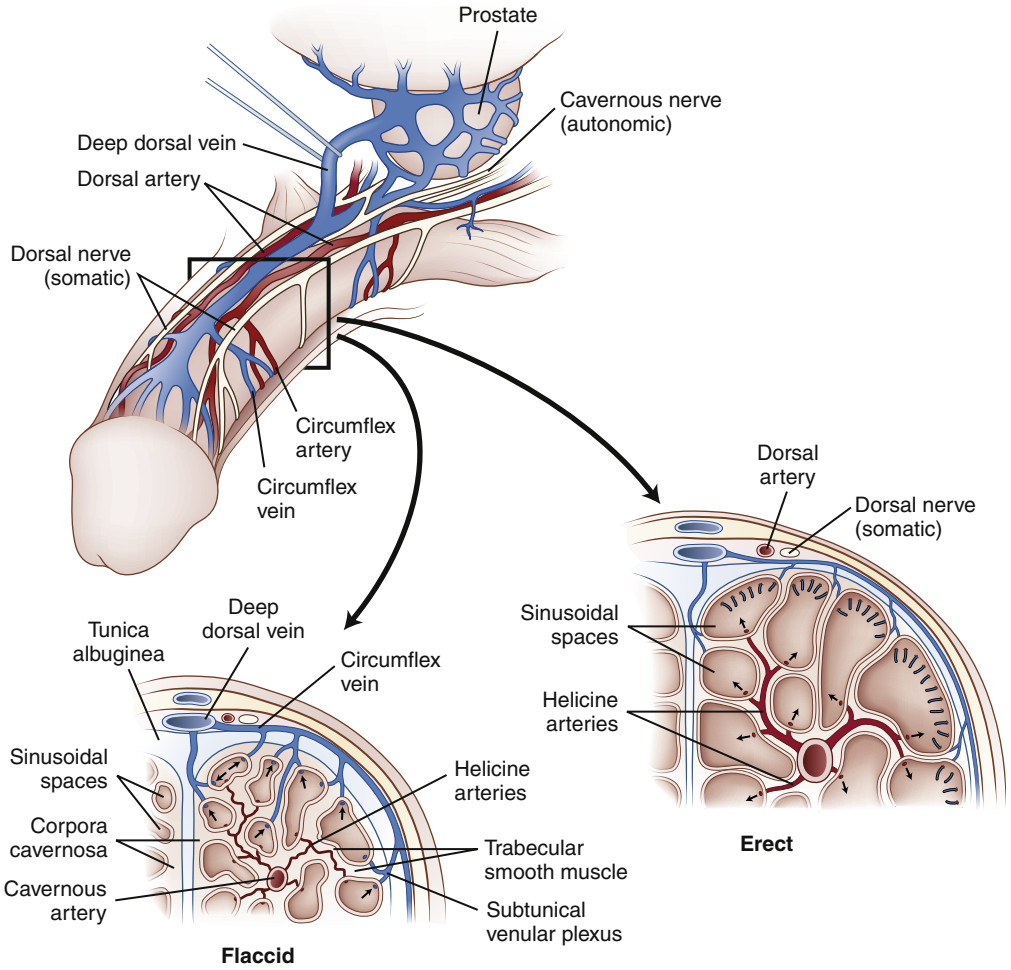
Melanocortins, derived from pro-opiomelanocortin, modulate sexual response through a specific receptor subtype, the melanocortin-4 receptor. Administration of melanocortin receptor agonists has been associated with an increase in spontaneous erections in healthy men and in men with ED, and with increased desire, but not genital responses, in women.[78,79]

Oxytocin levels increase close to orgasm. This hormone is known to be involved in pair bonding in some animal species, but its relevance in humans is less clear. The physiologic role of prolactin in the human sexual response remains uncertain.[80–82] Because a generalized reduction of dopamine activity in the hypothalamus results in increased prolactin secretion, it has been difficult to distinguish between the effects of raised prolactin itself and the possible effects of the reduced dopamine transmission. High levels of prolactin are associated with impaired sexual function in men and less clearly in women.[76,82]

The effects of the biologic factors are intertwined with those of the environmental and social factors. For instance, dopamine and progesterone, acting on their cognate receptors in the hypothalamus, can increase sexual behavior in oophorectomized, estrogenized female rats, and the presence of a male animal alongside the cage can cause an identical stimulation of the sexual behavior without the administration of either progesterone or dopamine.[83] In rodents, birds, and fish, complex neural networks enable the animal to assess the context of potential sexual activity and relate it to past experience and to the expectation of reward.[84]

Genital Sexual Congestion and Arousal

Men and women differ substantially with respect to the correlation between genital congestion and subjective sexual arousal (excitement). Whereas subjective arousal is typically concordant with genital congestion in men, there is highly variable but often poor correlation between subjective arousal and measures of genital congestion in women.[85,86] There are some exceptions in men: sleep-related erections are mostly dissociated from erotic dreams or from subjective sexual arousal.[87] In addition, psychophysiologic studies have found that men can get erections in response to films of assault or rape while experiencing no subjective arousal.[88] In contrast, a psychophysiologic study identified some 25% of men in a community sample with minimal penile response to an erotic video while their *subjective* arousal was similar to the remaining 75% of men with recorded penile congestion. There is preliminary evidence that mindfulness practice may increase concordance in association with improved sexual function.[89]



• **Fig. 20.3** Anatomy and mechanism of penile erection. Corpora cavernosa are made up of trabecular spaces that are surrounded by cavernosal smooth muscle. Helicine arteries provide the arterial supply to the cavernosal spaces. The dorsal nerve provides the sensory innervation to the penis. During erection, the relaxation of the trabecular smooth muscle and increased blood flow result in engorgement of the sinusoidal spaces in the corpora cavernosa. The expansion of the sinusoids compresses the venous return against the tunica albuginea resulting in entrapment of blood. This imparts rigidity to the tumescent penis. (Adapted from Lue TF. Erectile dysfunction. *N Engl J Med*. 2000;342[24]:1802–1813.)

In contrast to men's typically accurate assessment of their erections, women's assessment of their degree of genital congestion is less accurate. It is thought that genital congestion in women is a prompt, automatic reflex that occurs within seconds of an erotic stimulus; it may not be deemed at all sexually arousing by the woman, or it may even be deemed emotionally negative.[90] Viewing primates engaging in sexual activity subjectively arouses neither young men nor young women.[85] However, the young women viewing primate sex display marked genital congestion, as measured by vaginal photoplethysmography, whereas no genital response occurs in the men. Similarly, heterosexual women viewing lesbian women engaged in sexual activity report mostly low subjective arousal but show a prompt vasocongestive response; in contrast, heterosexual men viewing male same-sex activity show minimal genital or subjective response.[85]

Physiologic Mechanisms of Penile Erection

Penile Anatomy and Blood Flow

The erectile tissue of the penis consists of two dorsally positioned corpora cavernosa and a ventrally placed corpus spongiosum.[5,6,91,92] The erectile tissue of both the corpora cavernosa and corpus spongiosum is composed of numerous cavernous spaces separated by trabeculae.[5,6,91,92] These trabeculae are composed mainly of smooth muscle cells that are arranged in a syncytium. Endothelial cells cover the surfaces of the trabeculae.

The penile arterial blood supply is derived from pudendal arteries, which are branches of the internal iliac arteries (Fig. 20.3). The pudendal artery divides into cavernosal, dorsal penile, and bulbourethral arteries. The cavernosal arteries and their branches, the helicine arteries, provide blood flow to the corpora cavernosa.[5,6] Dilatation of the helicine arteries increases blood flow and pressure in the cavernosal sinuses.[5,6,91,92]

Penile Innveration

The neural input to the penis consists of sympathetic (T11-L2), parasympathetic (S2-S4), and somatic nerves[92] (Table 20.2). Sympathetic and parasympathetic fibers converge in the inferior hypogastric plexus, where the autonomic input to the penis is integrated and communicated to the penis through cavernosal nerves. In men the inferior hypogastric ganglionic plexus is located retroperitoneally near the rectum.[6,92]

TABLE 20.2 Innervation of the Penis

Types of Fibers	Location of Neurons in the Spinal Cord	Nerves Carrying the Fibers	General Function
Sympathetic	T10-L2	Prevertebral outflow through the hypogastric and cavernous nerves; additionally, paravertebral outflow through the parasympathetic ganglia, and pudendal or pelvic and cavernous nerves	Generally antierectile; sympathetic innervation plays an important role in regulating seminal emission
Parasympathetic	S2-S4	Cavernosal and pelvic nerves	Proerectile
Somatic	S2-S4	Pudendal nerve	Penile sensation, contraction of the striated muscles during ejaculation

Several brain regions, including the amygdala, MPOA, paraventricular nucleus of the hypothalamus, and periaqueductal gray matter, act coordinately to affect penile erections.[92] The MPOA of the hypothalamus serves as the integration site for the central nervous system control of erections; it receives sensory input from the amygdala and sends impulses to the paraventricular nuclei of the hypothalamus and the periaqueductal gray matter. Neurons in paraventricular nuclei project onto the thoracolumbar and sacral nuclei associated with erections.

The parasympathetic input to the penis is proerectile, and sympathetic input is mainly inhibitory.[92] The stimuli from the perineum and lower urinary tract are carried to the penis through the sacral reflex arc.[92]

Hemodynamic Changes During Penile Erection

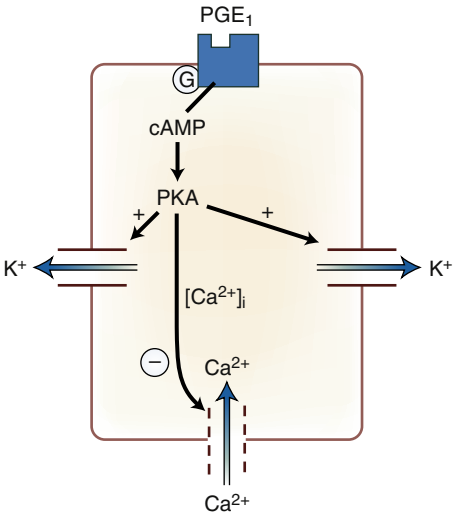
Penile erection results from a series of biochemical and hemodynamic events that are associated with activation of central nervous system sites involved in regulation of erections, relaxation of cavernosal smooth muscle, increased blood flow into the cavernosal sinuses, and venous occlusion resulting in penile engorgement and rigidity.[5,91] Normal penile erection requires coordinated involvement of intact central and peripheral nervous systems, corpora cavernosa and spongiosa, and normal arterial blood supply and venous drainage.[5,91]

As cavernosal smooth muscle relaxes and the blood flow to the penis increases, the increased pooling of blood in the cavernosal spaces results in penile engorgement[5,91] (see Fig. 20.3). The expanding corpora cavernosa compress the venules against the rigid tunica albuginea, restricting the venous outflow from the cavernosal spaces.[5,91] This facilitates entrapment of blood in the cavernosal sinuses, imparting rigidity to the erect penis.

Biochemical Regulation of Cavernosal Smooth Muscle Tone

The tone of the corporal smooth muscle cells determines the erectile state of the penis.[5,6,91] When the cavernosal smooth muscle cells are relaxed, the penis is engorged with blood and erect. When the cavernosal smooth muscle cells are contracted, there is predominance of sympathetic neural activity, and the penis is flaccid.[92]

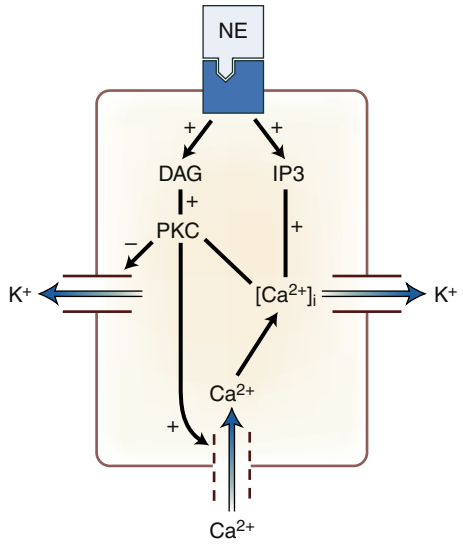
The smooth muscle tone in the corpora cavernosa is maintained by the release of stored intracellular calcium into the cytoplasm and influx of calcium through membrane channels.[93–96] The transmembrane influx of calcium in the cavernosal smooth muscle cells is mediated mostly by L-type voltage-dependent calcium channels, although T-type calcium channels are also expressed in cavernosal smooth muscle cells.[93–96] An increase in intracellular calcium activates myosin light chain kinase, resulting in phosphorylation of myosin light chain, actin-myosin interactions, and smooth muscle contraction.[96]



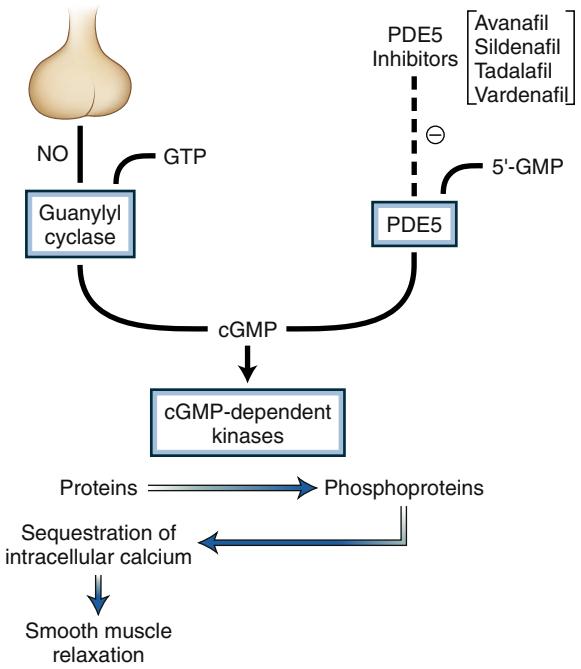
• **Fig. 20.4** Regulation of cavernosal smooth muscle contractility by PGE_1. The relaxation of the cavernosal smooth muscle is regulated by intracellular cAMP and cGMP. These intracellular second messengers by activation of specific protein kinases cause sequestration of intracellular calcium and closure of calcium channels, and opening of K^+ channels. This results in a net decrease in intracellular calcium causing smooth muscle relaxation. PGE_1 by binding to PGE_1 receptor increases the intracellular concentrations of cAMP, which activates PKA. PKA promotes the sequestration of intracellular calcium and inhibits calcium influx and stimulates K^+ channels. The nest result is a decrease in intracytoplasmic calcium and smooth muscle relaxation. Prostaglandin E_1 stimulates cAMP generation. cAMP, 3′,5′-cyclic adenosine monophosphate; *cGMP,* cyclic guanosine monophosphate; *PGE_1,* prostaglandin E_1; *PKA,* protein kinase A. (Adapted from Bhasin S, Benson GS. Male sexual function. In: De Kretser D, ed. *Knobil and Neill's Physiology of Reproduction*, 3rd ed. Boston, MA: Academic Press; 2006:1173–1194 and Lue TF. Erectile dysfunction. *N Engl J Med.* 2000;342[24]:1802–1813.)

The transmembrane and intracellular calcium flux in the cavernosal smooth muscle cells is regulated by several cellular processes that involve K^+ flux through potassium channels; connexin43-derived gap junctions; and several cholinergic, adrenergic, and nonadrenergic, noncholinergic mediators[93–101] (Figs. 20.4–20.6). The nonadrenergic, noncholinergic mediators include vasoactive intestinal peptide (VIP), calcitonin gene–related peptide (CGRP), and nitric oxide.[101]

Prostaglandin E_1 (PGE_1) binding to its cognate receptor results in generation of cyclic adenosine monophosphate



• **Fig. 20.5** Regulation of cavernosal smooth muscle contractility by norepinephrine. Norepinephrine (NE) that mediates adrenergic signals binds to adrenergic receptors and stimulates diacyl glycerol (DAG) and inositol-1,4,5-trisphosphate (IP_3). DAG stimulates protein kinase C (PKC), which along with IP_3 causes an increase in intracytoplasmic calcium and inhibition of K^+ channels. Increased intracellular calcium causes cavernosal smooth muscle contraction and loss of penile erection. (Adapted from Bhasin S, Benson GS. Male sexual function. In: De Kretser D, ed. *Knobil and Neill's Physiology of Reproduction*, 3rd ed. Boston, MA: Academic Press; 2006:1173–1194 and Lue TF. Erectile dysfunction. *N Engl J Med.* 2000;342[24]:1802–1813.)



• **Fig. 20.6** Regulation of cavernosal smooth muscle relaxation by nitric oxide (NO). cGMP regulates cavernosal smooth muscle relaxation by promoting sequestration of cytoplasmic calcium. NO is released from nonadrenergic, noncholinergic nerve endings and possibly from the endothelium. NO activates guanylyl cyclase that generates cGMP, which in turn activates cGMP-dependent kinases resulting in sequestration of intracellular calcium and smooth muscle relaxation. cGMP is degraded by cyclic nucleotide phosphodiesterases. Sildenafil, vardenafil, and tadalafil are selective inhibitors of PDE isoform 5 that is present in cavernosal smooth muscles. *cGMP,* cyclic guanosine monophosphate; *GTP,* guanosine triphosphate; *PDE5,* phosphodiesterase 5. (Adapted from Bhasin S, Benson GS. Male sexual function. In: De Kretser D, ed. *Knobil and Neill's Physiology of Reproduction*. 3rd ed. Boston, MA: Academic Press; 2006:1173–1194 and Lue TF. Erectile dysfunction. *N Engl J Med.* 2000;342[24]:1802–1813.)

(cAMP), which activates protein kinase A. Activated protein kinase A gates K^+ channels, resulting in K^+ efflux from the cell (see Fig. 20.4). The protein kinase A–mediated processes also result in a net decrease in intracellular calcium, favoring smooth muscle cell relaxation.

Adrenergic pathways, acting through norepinephrine and α_1-adrenergic receptors, activate phospholipase C, which generates diacyl glycerol and inositol trisphosphate (IP_3).[96] Diacyl glycerol activates protein kinase C, which inhibits K^+ channels and activates transmembrane calcium influx by activating L-type calcium channels[97,98] (see Fig. 20.5). IP_3 increases intracellular calcium by promoting the release of calcium from intracellular calcium stores.[97,98] The net increase in intracellular calcium promotes actin-myosin interactions, resulting in smooth muscle contraction and a flaccid penis.

Potassium Channels

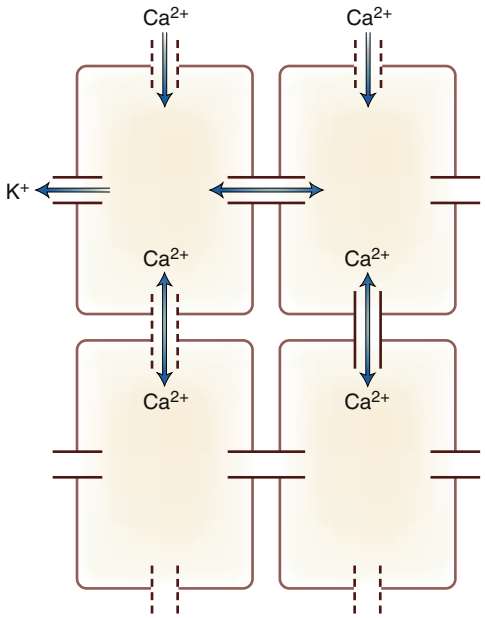
At least three types of potassium channels—adenosine triphosphate (ATP)-sensitive (K_{ATP}), voltage-gated (Kv), and calcium-sensitive K^+ channels (referred to as BK_{Ca} or maxi-K channels)—are expressed in the cavernosal smooth muscle cells.[99,100] Of these, the BK_{Ca} channels are the most important, as they account for 90% of K^+ efflux from the cavernosal smooth muscle cells. Agents that open BK_{Ca} channels have been shown to relax cavernosal smooth muscle cells in vitro.[100] Thus, the strategies that increase BK_{Ca} channel expression in vivo improve erectile capacity in diabetic and older rodents[100–102] and are being explored as therapy for ED. A phase I human gene therapy trial using this approach reported the feasibility of this approach, but there has not been a follow-up trial since then.[102] A phase 2a trial using a single intracavernosal injection of hMaxi K channel was initiated in 2016, but no data have been reported (NCT02713789).

Connexin43 Gap Junctions

The smooth muscle cells in the corpora cavernosa are connected by connexin43 gap junctions that allow the ions and some signaling molecules such as IP_3 to diffuse freely across smooth muscle cells[103] (Fig. 20.7). The ionic changes induced by a stimulus in one smooth muscle cell are communicated rapidly across other smooth muscle cells, resulting in coordinate regulation of the entire corpora cavernosa.[103] Thus, the corpora cavernosa can be viewed functionally as a syncytium of interconnected smooth muscle cells[103] (see Fig. 20.7).

Nitric Oxide

Nitric oxide, derived from the nerve terminals innervating the corpora cavernosa, the endothelial lining of penile arteries, and the cavernosal sinuses, is an important biochemical regulator of cavernosal smooth muscle relaxation. Nitric oxide also induces arterial dilatation.[104] The actions of nitric oxide on the cavernosal smooth muscle and the arterial blood flow are mediated through the activation of guanylyl cyclase, the production of cyclic guanosine monophosphate (cGMP), and the activation of cGMP-dependent protein kinase (also called *protein kinase G* [PKG]) (see Fig. 20.6). cGMP causes smooth muscle relaxation by lowering intracellular calcium. There is some evidence that nitric oxide inhibits Rho kinase–induced cavernosal smooth muscle sensitivity to calcium.[105]



• **Fig. 20.7** Interconnection of cavernosal smooth muscle cells in the penis. Connexin43-derived gap junctions connect adjacent corporal smooth muscle cells and allow flow of ions among interconnected smooth muscle cells. Therefore, alterations in action potential and K-channel activity in any myocyte affect the adjacent myocytes. (Modified from Melman A, Christ GJ. Integrative erectile biology: the effects of age and disease on gap junctions and ion channels and their potential value to the treatment of erectile dysfunction. *Urol Clin North Am*. 2001;28[2]:217–231, vii.)

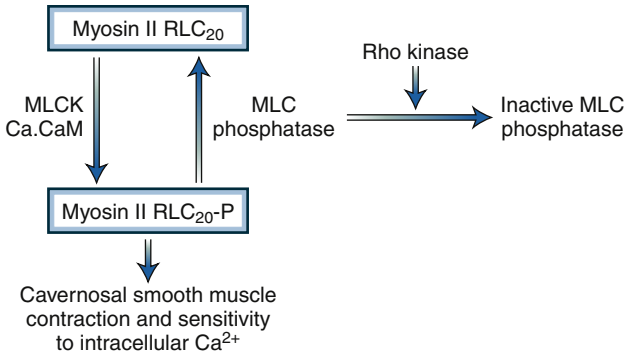
Cyclic Nucleotide Phosphodiesterases

Cyclic nucleotide phosphodiesterases hydrolyze cAMP and cGMP, thus reducing their concentrations within the cavernosal smooth muscle. Of the 13 or more isoforms of cyclic nucleotide phosphodiesterases that have been identified, isoforms 2, 3, 4, and 5 are expressed in the penis.[106–113] Only phosphodiesterase 5 (PDE5) is specific to the nitric oxide/cGMP pathway in the corpora cavernosa.[106–113] Hydrolysis of cGMP by this enzyme results in reversal of the smooth muscle relaxation and reversal of penile erection (see Fig. 20.6). Sildenafil, vardenafil, and tadalafil are potent and selective inhibitors of the activity of PDE5 that prevent breakdown of cGMP and thereby enhance penile erection[4,5] (see Fig. 20.6).

Regulation of Sensitivity to Intracellular Calcium by Rho A/Rho Kinase Signaling

Recently, considerable attention has focused on the role of Rho kinase in modulating the sensitivity of cavernosal smooth muscle to intracellular calcium.[114] A growing body of evidence suggests that sensitization to intracellular calcium is regulated by the balance between phosphorylation of the regulatory light chain of myosin II by a myosin light chain kinase and its dephosphorylation by a myosin light chain phosphatase[114–119] (Fig. 20.8). Phosphorylation of regulatory light chain of myosin II is necessary for activation of myosin II adenosine triphosphatases by actin, and its dephosphorylation prevents activation of myosin II adenosine triphosphatases.[114–119] The ratio of the activities of the kinase and the phosphatase is an important determinant of the contractile sensitivity of the cavernosal smooth muscle cell to intracellular calcium.[116]

Rho A is a guanosine triphosphatase (GTPase) of approximately 20 kDa that modulates Rho kinase activity, myosin light chain phosphorylation, and calcium sensitivity in smooth muscle cells.[114] The Rho A–GDP (guanosine diphosphate) complex is associated with a GDP dissociation inhibitor (RhoGDI) in its inactive state. Several



• **Fig. 20.8** The role of Rho A–Rho kinase in regulation of cavernosal smooth muscle sensitivity to intracellular Ca^{2+} is depicted. Sensitivity to calcium and smooth muscle contractility is regulated by the Rho A–Rho kinase system. The balance between phosphorylation of myosin regulatory light chain (Myosin II RLC_{20}) kinase and its dephosphorylation by a myosin light chain phosphatase is a major determinant of the smooth muscle sensitization to Ca^{2+}. By inhibiting the activity of myosin light chain (MLC) phosphatase, Rho kinase, the downstream effector of Rho A can regulate smooth muscle responsiveness to calcium. *Ca.CaM,* calcium-calmodulin; *MLCK,* myosin light-chain kinase. (Modified from Somlyo AP, Somlyo AV. Ca^{2+} sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev.* 2003;83[4]:1325–1358.)

intracellular signals can promote an exchange of GDP for GTP on Rho A through the mediation of guanine nucleotide exchange factors.[114–119] Rho A–GTP interacts with its downstream effector Rho kinase,[114–119] increasing the sensitivity of vascular smooth muscle to intracellular calcium by inhibiting the myosin light chain phosphatases. Although the Rho A/Rho kinase expression is not significantly different between young and older rats, the activity of Rho kinase is higher in older rats than in young rats[118]; the age-related increase in Rho kinase activity has been proposed as one possible mechanism to explain the age-related decrease in erectile capacity.[118] Inhibition of Rho kinase activity in experimental animals increases cavernosal smooth muscle relaxation and improves intracavernosal pressures and penile erections. Therefore, inhibitors of Rho A/Rho kinase signaling promise to provide attractive targets for the development of therapies for ED.[118,119]

Mechanisms of Ejaculation

The ejaculatory mechanisms consist of three processes: emission, ejection, and orgasm.[120–123] Although orgasm and seminal fluid ejection often occur contemporaneously, these two processes are regulated by separate mechanisms. Emission, the deposition of seminal fluid into the posterior urethra, is dependent on the integrity of the vasa deferentia, seminal vesicles, prostate gland, and the bladder neck.[122,123] Ejaculation refers to the ejection of seminal fluid containing sperm, and the secretions of seminal vesicles, prostate, and bulbourethral glands from the posterior urethra out through the urethral meatus, and is regulated primarily by the central nervous system activation of the sympathetic nervous system.[122] This emission is ejaculated out of the urethra by the contractions of the bulbospongiosus and levator ani muscles, the closure of the bladder sphincter due to sympathetic activation, and synchronized opening of the external urinary sphincter.[118] The sensation associated with the rhythmic contractions of these pelvic floor muscles is referred to as the *orgasm*.

The stria terminalis, the posteromedial amygdala, the subparafascicular thalamus, the MPOA of the hypothalamus, the periaqueductal gray matter in the midbrain region, and the

paragigantocellular nucleus in the pons integrate seminal fluid emission and ejection during copulatory behavior in animals[124,125] (see Table 20.2). The paragigantocellular nucleus in the pons through serotonergic pathways inhibits the lumbosacral motor nuclei that are involved in ejaculation[125,126]; the input from the MPOA to the paragigantocellular nucleus causes loss of this inhibition, resulting in ejaculation.[125,126] An ejaculation generator in the spinal cord integrates the central and peripheral sympathetic and parasympathetic signals to control ejaculation.[120,125] The parasympathetic fibers from the spinal ejaculation generator feed into the sacral parasympathetic nucleus and are carried from there through the pelvic nerve and the major parasympathetic ganglion into the seminal tract.[125] Sympathetic fibers are carried from the spinal ejaculation generator into the dorsal gray commissure and intermediolateral cell column and then through the lumbar sympathetic chain, pelvic nerve, superior hypogastric plexus, and major pelvic ganglion onto the seminal tract.[125]

Neural pathways that utilize serotonin (5-hydroxytryptamine [5HT]) and dopamine as neurotransmitters play an important role in regulating ejaculation.[122] Thus, administration of selective serotonin reuptake inhibitors (SSRIs) is being explored for the treatment of premature ejaculation.[124–127] At least 14 different serotonin receptor subtypes have been identified in different brain regions; 5HT1A somatodendritic receptors in the mesencephalic and medullary raphe nuclei reduce ejaculatory latency.[120] An improved understanding of the neurochemical mechanisms that regulate ejaculation may provide mechanism-specific targets for treatment of ejaculatory disorders.

TABLE 20.3 Domains of Sexual Function Regulated by Testosterone[a]

Domains of Sexual Function That Have Been Shown to Improve in Response to Testosterone Therapy of Androgen-Deficient Men

1. Sexual desire
2. Spontaneous sexual thoughts
3. Attentiveness to erotic stimuli
4. Frequency of nighttime and daytime erections
5. Duration, magnitude, and frequency of nocturnal penile erections
6. Overall sexual activity scores
7. Erectile function
8. Sexual satisfaction
9. Volume of ejaculate

Domains of Sexual Function That Have Not Been Shown to Improve in Response to Testosterone Therapy or for Which There Is Insufficient or Inconclusive Evidence

1. Erectile response to visual erotic stimulus
2. Erectile function in men who have normal or low-normal testosterone levels
3. Therapeutic response to selective phosphodiesterase inhibitors
4. Ejaculatory dysfunction
5. Orgasms

[a]Testosterone administration in androgen-deficient men improves overall sexual activity scores through its effects on specific domains of sexuality.

Modified from Bhasin S, Enzlin P, Coviello A, Basson R. Sexual dysfunction in men and women with endocrine disorders. *Lancet.* 2007;369(9561):597–611.

Role of Testosterone in Regulating Sexual Function in Men

Testosterone regulates many domains of sexual function in men and women.[27] Although androgen-deficient men can achieve penile erections in response to visual erotic stimuli, their overall sexual activity is decreased.[128] Spontaneous but not stimulus-bound erections are testosterone responsive[128] (Table 20.3). Testosterone promotes sexual thoughts and desire[53,128–134] and increases sexual arousal and attentiveness to erotic auditory and other stimuli.[129,130] Nocturnal erections, temporally related to peaks of nighttime testosterone secretion, are of lower amplitude and duration in androgen-deficient men, and testosterone therapy increases the frequency, fullness, and duration of nocturnal penile tumescence.[134,135] Maximum rigidity may require a threshold level of androgen activity.[136–140] Testosterone regulates nitric oxide synthase (NOS) in the cavernosal smooth muscle,[137,138] exerts trophic effects on cavernosal smooth muscle[139] and ischiocavernosus and bulbospongiosus muscles, and is necessary for the veno-occlusive response.[136–139] Androgen-deficient men show delayed orgasm and low ejaculatory volume.[27]

Testosterone therapy in androgen-deficient men improves overall sexual activity, sexual desire, erectile function,[141,142] spontaneous sexual thoughts, and attentiveness to erotic auditory stimuli; frequency of nighttime and daytime erections; duration, magnitude, and frequency of nocturnal penile erections; and the volume of ejaculate.[27,53,128–131,134-136,140,143–145] However, testosterone does not improve erectile response to visual erotic stimulus[128] or erectile function in men with ED who have normal testosterone levels.[140,143–147] Testosterone does not improve ejaculatory function in men with ejaculatory dysfunction and low testosterone levels.[148] In male rats, in whom endogenous testosterone production has been suppressed by administration of a GnRH antagonist, testosterone restores all measures of mating behavior at doses that are insufficient to restore prostate and seminal vesicle weights.[149,150] Similarly, in dose-response studies in humans rendered testosterone deficient by the administration of a GnRH agonist, sexual function is restored by testosterone doses that raise serum testosterone levels just above the lower limit of the normal male range[151]; further increase in serum testosterone levels does not further improve measures of sexual function.

Brain imaging studies suggest that processing of sexual stimuli may be altered in androgen-deficient men with decreased activation in those brain areas that are typically activated in eugonadal men and in androgen-deficient men after testosterone replacement in response to erotic stimuli.[53]

Acting on dopaminergic receptors in the MPOA of the hypothalamus, testosterone elicits reward-seeking behavior in male mammals.[61] This may be the basis for testosterone's motivational effects on mammalian sexual behavior.[61] The roles of cytochrome P450 19A1 (aromatase) and steroid 5α-reductase systems in mediating androgen effects on sexual function remain incompletely understood.[63] Studies suggest that 5α-reduction of testosterone to 5α-dihydrotestosterone is not essential for mediating testosterone's effects on desire or erectile function in eugonadal men.[152] Additional investigations, including those in men with mutations of the *CYP19A1* gene, suggest that aromatization to estradiol is important in mediating testosterone's effects on sexual desire.[153–155] Both testosterone and estradiol are required to maintain sexual function.

In surveys of community-dwelling middle-age and older men, low libido, poor morning erections, and ED are associated with low total and free testosterone levels.[156–158] However, androgen deficiency and ED are two independently distributed disorders that may coexist in middle-age and older men.[159–161] Selective

PDE5 inhibitors are highly effective first-line therapies for ED. However, one third of men with ED do not respond to PDE5 inhibitors; some of the men with ED who are PDE5 inhibitor nonresponders have low testosterone levels. Observations that testosterone stimulates penile NOS, increases penile blood flow, and has trophic effects on cavernosal smooth muscle and bulbospongiosus and ischiocavernosus muscles have led to speculation that testosterone therapy might improve erectile response to PDE5 inhibitors.[162–165] Spitzer and associates[166] evaluated whether the addition of testosterone to sildenafil improves erectile response in men with ED and low testosterone. In this randomized controlled trial (RCT), after optimization of sildenafil dose during an initial run-in period, subjects were assigned randomly to 14 weeks of daily testosterone or placebo gel. Sildenafil plus testosterone was not superior to sildenafil plus placebo in improving erectile function in men with ED and low testosterone.[166] In another placebo-controlled trial (TADTEST) of men with ED who were deemed tadalafil (Cialis) failures,[167] the primary analysis of all randomized subjects also did not show a significantly greater improvement in erectile function in the testosterone arm than in the placebo arm. However, in post hoc analysis, erectile function improved with the addition of testosterone in a subset of men with baseline testosterone of 10 nmol/L or less (300 ng/dL).[167] Thus, randomized trials have failed to support the hypothesis that addition of testosterone to PDE5 inhibitor improves erectile function in men with ED. Sildenafil alone raises testosterone levels, presumably because of its direct effects on Leydig cell steroidogenesis.[168] It is possible that testosterone may improve penile rigidity and overall satisfaction with sexual experience in men who are unequivocally hypogonadal.

Physiology of Physical Sexual Arousal in Women: Genital Congestion

Several physical changes occur in response to sexual stimuli with varying degrees of women's sexual excitement (i.e., their subjective sexual arousal), including genital swelling, increased vaginal lubrication, breast engorgement, and nipple erection; increased skin sensitivity to sexual stimulation; changes in heart rate, blood pressure, muscle tone, breathing, and temperature; and mottling of the skin with a sexual flush of vasodilatation over the chest and face.[169] These changes are reflexive, mediated by the autonomic nervous system. Within seconds, there is increased blood flow to the vagina; vasodilatation of the arterioles in the submucosal plexus increases transudation of interstitial fluid from the capillaries across the epithelium and into the vaginal lumen.[169] Simultaneously, there is relaxation of smooth muscle cells around the clitoral sinusoids, which promotes congestion.

The clitoris comprises the head, the shaft, the rami that extend along the pubic arch, and the periurethral tissue in front of the anterior vaginal wall, as well as the bulbar tissue that surrounds the anterior distal vagina and is contiguous with the periurethral tissue.[170] MRI studies have confirmed the presence of extensive clitoral tissue far beyond the visible portion of the clitoris.[170] As the clitoris becomes more swollen, it elevates to lie nearer the symphysis pubis. The vagina lengthens and dilates during arousal, elevating the uterus. The labia become swollen and darker red, and the lower third of the vagina swells.[170]

The autonomic nerves subserving the widespread genital congestion are at risk from gynecologic surgeries that sever the cardinal ligaments and the uterosacral ligaments to potentially injure Frankenhauser's nerve plexus and the uterovaginal nerve plexus.[171,172] Surgical procedures for hysterectomy that do not involve cutting or clamping the cardinal ligament, uterosacral ligament, or fascia and spare the pelvic nerve plexuses are less likely to impair sexual function.[171] Transvaginal tapes for urinary incontinence, especially transobturator tapes, may also compromise the autonomic nerves between the anterior vaginal wall and bladder.[172,173]

The correlation between genital congestion and subjective arousal in response to visual erotic stimuli is highly variable[49,82,83]; this is true in sexually healthy women and in women reporting a lack of desire or arousal or sexual pain. Women reporting chronic lack of arousal show prompt increases in vaginal congestion that are comparable to those in control women, but they report no subjective sexual excitement in response to the erotic stimulation. fMRI studies show that, unlike in men, activation of the areas organizing genital vasocongestion in women does not correlate with subjective excitement.[49,174]

The neurobiology of the genital vasocongestive response in women is complex and incompletely understood. Genital vasocongestion involves the release of nitric oxide and VIP from the sacral autonomic nerves.[175] Acetylcholine, which blocks noradrenergic vasoconstricting mechanisms and promotes nitric oxide release from the endothelium, is also released. There is communication between the nitric oxide–containing cavernosal nerve to the clitoris and the distal portion of the somatic dorsal nerve of the clitoris from the pudendal nerve. Pelvic sympathetic nerves release primarily vasoconstrictive norepinephrine and epinephrine, but some release acetylcholine, nitric oxide, and VIP. The provoked anxiety in the laboratory situation can *increase* the vasocongestive response of the genitalia to erotic stimulation in sexually healthy women.[87] The localization of NOS, cAMP, and cGMP-degrading PDE isoenzymes in human vaginal tissue is established, along with identification of cAMP and cGMP-binding proteins. The latter are co-localized with endothelial NOS (eNOS). Close proximity to VIP-positive nerves suggests that cAMP and cGMP work synergistically to control vaginal blood flow.[175] Neuropeptide Y (a vasoconstrictor), CGRP (possibly influencing capillary permeability and sensation), and substance P (a sensory transmitter) also innervate the vaginal microcirculation. The melanocortin-4 receptors and oxytocin also may be involved in clitoral and vaginal efferent pathways.[175]

Intermittency of the vaginal microcirculation due to contraction and relaxation of precapillary sphincters in response to hypoxia and the release of metabolites (CO_2, lactic acid, ATP) has been termed *vasomotion*. Vasomotion is present in the nonaroused state but decreases within seconds of a sexual stimulus, which increases arterial supply to recruit more capillaries and diminish vasomotion: vaginal vasocongestion follows. Slow oscillations in vaginal blood flow, independent of vaginal vasocongestion, have been shown to correlate with subjective arousal in healthy women and to be less marked in women with arousal disorder.[174]

Increased blood flow to the submucosal vaginal capillaries results in increased interstitial fluid production, which diffuses more quickly across the vaginal epithelial cells and onto the lumen: the lubrication fluid in the aroused state thereby contains less potassium and more sodium than in the nonaroused state. How important the contribution of permeability of the epithelial cells is to the process of lubrication is currently unclear. Relaxation of the vaginal wall smooth muscle that enables the vagina to move up into the pelvis is likely mediated by VIP.[174]

The clitoris is the most sexually sensitive area of the body. Immunohistologic studies have identified neurotransmitters

thought to be associated with sensation (substance P and CGRP) that are concentrated immediately under the epithelium of the glans clitoris. Nerve terminals in the glans clitoris known as *corpuscular receptors* are thought to be involved. They are mechanoreceptors, and their density is variable but can be up to 14 times greater than the density of similar receptors on the glans penis.[176] The physiology of nongenital physical changes and their correlation with subjective excitement remain poorly understood.

Physiology of Orgasm

Orgasm is largely a brain event typically triggered by genital stimulation; however, it can also be induced by stimulation of other parts of the body, including the breast and nipple; fantasy; sleep; certain medications; and, in women with spinal cord injury, by vibratory stimulation of the cervix. Vaginal stimulation involves all of the clitoral-urethral complex, including the clitoral rami, whereas direct stimulation of the clitoral shaft and glans does not involve the clitoral rami.[169] During vaginal intercourse, the penis distends the vagina and stretches the root of the clitoris. During penile thrusting, the anterior wall of the vagina is apposed against the root of the clitoris, perhaps explaining why some women can achieve orgasm by vaginal intercourse without simultaneous nonvaginal stimulation.[177] The root of the clitoris and the anterior wall of the vagina thus appear to be anatomically and functionally related.[178]

Orgasm is a subjective experience that is associated with ejaculation in healthy men and, in both men and women, with involuntary (reflexive) muscular contractions of the striated perineal muscles.[179] The characteristic fluctuations in the rectal pressure that occur during orgasm due to involuntary contractions of perineal muscles have been proposed as an objective measure that has a strong correspondence with the subjective experience of orgasm.[179]

Positron emission tomography (PET) studies during orgasm have shown largely similar brain activations and deactivations in men and women: activations mainly in the anterior lobe of the cerebellar vermis and deep cerebellar nuclei and deactivations in the left ventromedial cortex and OFC. The only gender difference in the PET findings during orgasm was the activation in the periaqueductal gray matter in men but not in women.[180,181] The lateral OFC is involved in urge suppression and behavioral release, whereas the medial parts encode hedonic experiences and become activated with increasing pleasure and deactivated with feelings of satiety. The medial OFC is part of the neuronal network that includes the amygdala, whose deactivation during orgasm is associated with a more carefree state of mind.[180] The variations in rectal pressure indicative of orgasm recorded using a rectal probe correlate with widespread blood flow changes in the prefrontal cortex measured using PET scanning.[181,182] The specific orgasm-related changes in the mid/anterior OFC during PET scanning suggest a role of the mid/anterior OFC in the experience of pleasure. Failed orgasm due to excessive behavioral suppression is associated with enhanced left lateral OFC activity. Researchers have speculated that the orgasm-related OFC dynamics may be related to the sense of loss of control during orgasm. Prefrontal, but not temporal, perfusion is negatively coupled to rectal pressure fluctuations associated with orgasm. Overall, the findings of reduced prefrontal metabolism during orgasm are in keeping with the critical role of the prefrontal cortex in behavioral and emotional control and with the experimental data relating exaggerated prefrontal activity with sexual dysfunction.

The role of oxytocin and prolactin in orgasm is unclear. Both hormone levels increase at the time of orgasm. PET scanning has confirmed increased pituitary blood flow in women, but not in men, at the moment of orgasm.[183] Both hormones can cause uterine and vaginal smooth muscle contraction, which may contribute to the sensations of orgasm.

Revised Definitions of Sexual Dysfunction in Men

In May 2013, DSM-5 provided an updated classification and definitions of male sexual disorders.[45] The salient differences of the new DSM-5 classification and definitions from DSM-IV are the following[46]:

1. DSM-5 includes only four male sexual disorders, as opposed to six in DSM-IV.[46] The four sexual disorders are the following:
   a. Male HSDD
   b. ED
   c. Premature ejaculation
   d. Delayed ejaculation
2. DSM-5 lists male HSDD as a separate entry.[45,46] Male orgasmic disorder has been renamed *delayed ejaculation*, and male ED has been changed to *ED.* Premature ejaculation remains unchanged.
3. Male dyspareunia, male sexual pain, sexual aversion disorder, and sexual dysfunction have been removed in DSM-5.[45,46]
4. Unlike DSM-IV, DSM-5 includes the requirement of experiencing the disorder 75% to 100% of the time to make a diagnosis of sexual disorder. DSM-5 also requires a minimum duration of approximately 6 months.[45,46]
5. DSM-5 requires that the sexual disorder must have caused significant distress. The DSM-IV requirement of "interpersonal difficulty" has been removed.[45,46]
6. DSM-5 added one new exclusion criterion: the disorder should not be better explained by a "nonsexual mental disorder or a consequence of severe relationship distress or other significant stressors."[45,46]

Male Hypoactive Sexual Desire Disorder

HSDD is the persistent or recurrent deficiency (or absence) of sexual fantasies and desire for sexual activity that causes marked distress and that is not better explained by another disorder, direct physiologic effects of a substance (medication), or general medical condition.[184–187] A diagnosis of HSDD is appropriate *only* if the person reports distress due to low sexual desire.[184-187] Low sexual desire is not necessarily pathologic, as low sexual desire may be an appropriate adaptation to relationship and health-related issues.[184–187]

Although DSM-5 limits a diagnosis of HSDD to causes other than medication, illness including androgen deficiency and depression, and relationship and addiction problems, in clinical practice the term *hypoactive sexual desire disorder* is often used for situations involving multiple factors, including androgen deficiency, prolactin excess, the use of medications (SSRIs, antiandrogens, GnRH analogues, antihypertensives, cancer chemotherapeutic agents, anticonvulsants), systemic illness, depression and other psychological problems such as low self-esteem, fear of emotional intimacy, fear of loss, long-term habit of sex alone with or without pornography, other causes of sexual dysfunction, or relationship problems. Thus, in practice, androgen deficiency is an important,

treatable cause of low desire in men and should be excluded by measuring serum total and free testosterone levels.[184–187]

The incidence and prevalence of HSDD in the general population are unknown. In studies of referred patient populations, the prevalence has been estimated to be 5% in men.[7,186–189] Prevalence increases with age.[186,187,189] HSDD often coexists with other sexual disorders, such as ED, and may develop as a consequence of other sexual disorders.[186,187,189]

Appropriate evaluation and treatment of HSDD are important because evaluation may lead to detection of treatable disease, including androgen deficiency, prolactin excess, or depression. Furthermore, low sexual desire may impede or reduce the effectiveness of treatments for other sexual dysfunctions.

Erectile Dysfunction

ED, previously referred to as *impotence* or *male ED*, is the inability to attain or maintain an erection or to achieve penile rigidity sufficient for satisfactory sexual intercourse.[5,6,45,46] DSM-5 requires that the inability to attain or maintain an erection should occur in 75% to 100% of encounters over a period of at least 6 months.[45,46] *Sexual dysfunction* is a more general term that also includes libidinal, orgasmic, and ejaculatory dysfunction, in addition to the inability to attain or maintain penile erection. Epidemiologic surveys,[188–198] including the Massachusetts Male Aging Study (MMAS)[190] and the National Health and Social Life Survey (NHSLS),[188] revealed a surprisingly high prevalence of ED. ED significantly affects quality of life of both the affected individual and his partner. In one study, ED had a negative impact on the sexual life of female partners, specifically on their sexual satisfaction and sexual drive.[191]

Prevalence and Incidence

The best data on the prevalence of ED in men have emerged from two cross-sectional studies that have used population-based sampling techniques, namely the MMAS[190,192,195,196] and the NHSLS.[7,188] The MMAS was a cross-sectional and longitudinal, community-based epidemiologic survey in which 1709 men, 40 to 70 years of age, residing in the greater Boston area, were surveyed between 1987 and 1989.[190] This survey revealed that 52% of men between the ages of 40 and 70 years were affected by ED of some degree; 17.2% of surveyed men reported minimal ED, 25.2% moderate ED, and 9.6% complete ED.[190,195,196] The NHSLS was a national probability survey of English-speaking Americans, 18 to 59 years of age, living in the United States.[7,188] This survey also revealed a high prevalence of ED in men; the prevalence of ED increased with increasing age.[7,188] These two studies and several other studies are in agreement that ED is a common problem worldwide.[7,188–197] In the U.S. civilian population, the prevalence of ED in men 20 to 39 years of age has been estimated to be 5.1%, and almost three times as high (14.8%) in men age 40 to 59 years of age. Men suffering from other medical problems, such as hypertension, diabetes, cardiovascular disease (CVD), and end-stage renal disease, have a significantly higher prevalence of ED than healthy men.[190]

There is a paucity of longitudinal data on the incidence of ED in men. In the MMAS, the crude incidence of ED in white men in the Boston area was found to be 25.9 cases per 1000 person-years.[192] The incidence rates increased from 12.4 cases per 1000 person-years for men 40 to 49 years of age to 29.8 cases per 1000 person-years for men 50 to 59 years of age and 46.4 per 1000 person-years for men 60 to 69 years of age.[192] In another study, incidence rates were derived from a survey of men seen at a preventive medicine clinic.[7] This study found the incidence rates of ED to be less than 3 cases per 1000 person-years among men less than 45 years of age and 52 cases per 1000 person-years among men 65 years of age or older. These studies suggest that 600,000 to 700,000 men in the United States develop ED each year.[195,196]

Risk Factors for Erectile Dysfunction

The risk factors for ED include age, diabetes mellitus, hypertension, smoking, medication use, depression, dyslipidemia, and CVD.[4–6,190,198–208] Advancing age is an important risk factor for ED in men[4–6,188,190]: less than 10% of men younger than 40 years and more than 50% of men older than 70 years have ED. In both the MMAS and the NHSLS, the prevalence of ED increased with each decade of life.[188,190]

Among the chronic diseases associated with ED, diabetes mellitus is the most important risk factor. In the MMAS, the age-adjusted risk of complete ED was three times higher in men with history of treated diabetes mellitus than in those without history of diabetes mellitus.[190,198] Fifty percent of men with diabetes mellitus will experience ED sometime during the course of their illness. In the MMAS, treated heart disease, treated hypertension, and hyperlipidemia were associated with a significantly increased risk of ED. Among men with treated heart disease and hypertension, the probability of ED was more than two times greater for smokers than for nonsmokers.[4–6,188,190] Smoking also increases the risk of ED in men taking medications for CVD. Cardiovascular disorders, including hypertension, stroke, coronary artery disease, and peripheral vascular disease, are all associated with increased risk of ED. Physical activity is associated with reduced risk of ED.[207]

Several reviews have emphasized the relationship of prescription medications and the occurrence of ED. In the MMAS, the use of antihypertensives, cardiac medications, and oral hypoglycemic drugs was associated with an increased risk of ED.[190] Thiazide diuretics and psychotropic drugs used in the treatment of depression may be the most common drugs associated with ED, simply because of the high prevalence of their use. However, a variety of drugs, including almost all antihypertensives, digoxin, histamine-2 receptor antagonists, anticholinergics, cytotoxic agents, and androgen antagonists, have been implicated in the pathophysiology of ED.[190]

Erectile Dysfunction as a Marker of Cardiovascular Disease

CVD and ED share common risk factors, such as diabetes mellitus, obesity, hypertension, smoking, and dyslipidemia.[199–207] ED precedes the symptoms of coronary artery disease by 2 to 3 years and cardiovascular events such as myocardial infarction or stroke by 3 to 5 years.[199–207] ED in men is associated with increased risk of death, particularly fatality due to CVD.[201] The presence of ED is a good predictor of subsequent coronary artery disease, especially in younger men, independent of traditional coronary risk factors, although it does not enhance the predictive ability of models that include traditional risk factors, likely reflecting the common pathophysiologic mechanisms of ED and coronary artery disease.[202] Men reporting ED are 1.3 to 1.6 times more likely to experience a cardiovascular event within 10 years than men without ED.[199–207]

Lower Urinary Tract Symptoms and ED

Epidemiologic surveys have reported a strong association of lower urinary tract symptoms (LUTS) with ED[208–214] even after adjusting for age and other risk factors. The Cologne Male Survey and

the Multinational Survey of the Aging Male revealed that the presence and severity of LUTS is an independent predictor of ED independent of age.[209] LUTS and age are stronger predictors of ED than all other risk factors, including diabetes, dyslipidemia, and hypertension. Considering that LUTS and ED are two common conditions in middle-age and older men, it is possible that this association reflects the coexistence of two highly prevalent conditions. However, there is growing evidence that the two conditions may be mechanistically linked, as the biochemical mechanisms that regulate bladder detrusor and cavernosal smooth muscle function share many similarities.[214,215] K^+ channels, especially calcium-sensitive K^+ channels (BK_{Ca} channels), Rho A/Rho kinase signaling, L-type calcium channels, and gap junctions are important mediators of both detrusor and cavernosal smooth muscle contractility and relaxation.[214,215] Increased myocyte contractility that characterizes both bladder detrusor dysfunction and ED may be mechanistically related to increased Rho kinase activity, impairments of K^+ channel function,[215] α-adrenergic receptor imbalance, and endothelial dysfunction. Additional proposed hypotheses include increased sympathetic activity and autonomic dysfunction, and alterations in nitric oxide generation or PKG activity in the detrusor and cavernosal smooth muscles.[214–216] Some therapies for LUTS, such as some types of surgical procedures and steroid 5α-reductase inhibitors, may worsen sexual dysfunction. α-Adrenergic blockers used in the treatment of LUTS can cause ejaculatory problems. Some PDE5 inhibitors have been approved for the treatment of LUTS.[215–218]

Ejaculatory Disorders

Ejaculatory disorders include premature ejaculation, delayed ejaculation, retrograde ejaculation, anejaculation, and painful ejaculation.[120–123] Recent surveys have highlighted the high prevalence and clinical importance of ejaculatory disorders.[10,120–123,219,220] Although the availability of oral PDE5 inhibitors has increased awareness of ED, ejaculatory disorders are at least as prevalent and may be even more prevalent than ED.[10,220]

Premature ejaculation, a common form of ejaculatory dysfunction in men, can be lifelong or acquired later in life, and it negatively affects interpersonal relationships, sexual satisfaction, and quality of life.[221,222] An ad hoc committee of the International Society for Sexual Medicine defined premature ejaculation as follows[222]:

> *a male sexual dysfunction characterized by (i) ejaculation that always or nearly always occurs prior to or within about 1 minute of vaginal penetration from the first sexual experience (lifelong PE [premature ejaculation]) or a clinically significant and bothersome reduction in latency time, often to about 3 minutes or less (acquired PE); (ii) the inability to delay ejaculation on all or nearly all vaginal penetrations; and (iii) negative personal consequences, such as distress, bother, frustration, and/or the avoidance of sexual intimacy.*

This definition of premature ejaculation is based on a multinational survey of men, 18 years or older, in stable heterosexual relationships, which found the median intravaginal ejaculation latency time (IELT) to be 5.6 minutes (range, 0.55–44.1 minutes)[223]; in this survey, 2.5% of men had an IELT of less than 1 minute and 6% of less than 2 minutes. Most men seeking treatment for lifelong premature ejaculation ejaculate within 1 minute after penetration, and nearly all ejaculate within 2 minutes.[224] Compared with patients with lifelong premature ejaculation, the men with acquired premature ejaculation are older and have a longer IELT.[222] The acquired premature ejaculation is often also associated with ED and other comorbid conditions, such as diabetes and CVD.

Some men who seek treatment for premature ejaculation do not meet the preceding definition established by the International Society for Sexual Medicine but express distress due to their perception of lack of control over the timing of ejaculation and the IELT to be shorter than they wish. Waldinger[225] proposed the terms *subjective premature ejaculation* and *variable premature ejaculation* to describe these men whose IELT is either consistently or inconsistently greater than 5 minutes but who are preoccupied with a perceived short ejaculatory latency or the lack of control over the timing of ejaculation. Specific duration criteria for defining premature ejaculation among men engaged in nonvaginal sexual activities have not been established.

The prevalence of premature ejaculation is not known; previous estimates of prevalence of 20% to 25% based on older definitions are likely inaccurate. Although there are no epidemiologic surveys based on the International Society for Sexual Medicine criteria or the DSM-5 definition of premature ejaculation, the experts have inferred from the data on the distribution of IELT that the prevalence is unlikely to be greater than 4%.[221,226,227]

The neurobiologic mechanisms of premature ejaculation are not well understood. 5HT (serotonin), oxytocin, and dopamine are involved as neurotransmitters in the central nervous system pathways that control ejaculation.[228,229] In preclinical models, serotonin delays ejaculation, and dopamine and oxytocin appear to stimulate ejaculation; these data have led to speculation that men with lower ejaculatory thresholds may either have deficient serotonin neurotransmission or 5HT 2C receptor hyposensitivity. The serotonin hypothesis is supported by data showing that SSRIs and the tricyclic antidepressant clomipramine increase ejaculatory control and delay ejaculation in men with premature ejaculation. The earlier theories that premature ejaculation is largely due to psychological factors such as anxiety or sequelae of early life sexual experiences have not been supported by recent data from clinical trials of pharmacologic agents. Genetic studies of the polymorphisms in the serotonin transporter protein (5HTTLPR) gene, oxytocin, serotonin, and vasopressin genes, and tandem repeats of the dopamine transporter gene (*DAT1*) have not yielded consistent association with premature ejaculation.[230–234]

Delayed Ejaculation

DSM-5 defines delayed ejaculation as a marked involuntary delay in ejaculation or absence of ejaculation on almost all or all occasions of partnered sexual activity, persisting for at least 6 months and causing distress to the individual.[235] A timing-based definition of delayed ejaculation has not been established. Because the median intravaginal ejaculatory latency in a multinational population-based survey was 5.4 minutes[223] and the 97.5th percentile value was 23 minutes, some experts have suggested that intravaginal ejaculatory latency greater than 25 or 30 minutes should be considered delayed ejaculation.[236]

Mild to moderate delay in ejaculation occurs with advancing age; older men with comorbid conditions are at a higher risk of delayed ejaculation.[237,238] Epidemiologic surveys have consistently found LUTS to be strongly associated with delayed ejaculation, even after adjusting for age.[208,238–240] Ejaculatory disorders are common after surgical treatment of benign prostatic hyperplasia including transurethral resection of the prostate.[241] Several drugs—SSRIs, highly selective α1-adrenergic blockers, steroid 5α-reductase inhibitors—can cause delayed ejaculation. The use of highly selective α1-adrenergic blockers such as tamsulosin and

silodosin can lead to marked delay or inability to ejaculate; ejaculatory dysfunction is less common with nonselective α-adrenergic blockers such as alfuzosin, doxazosin, and terazosin. The use of steroid 5α-reductase inhibitors such as finasteride and dutasteride is associated with increased risk of ejaculatory disorder. SSRIs can prolong intravaginal ejaculatory latency and cause delayed ejaculation in some middle-age and older men.[242] Excessive exposure to pornography and sexual abuse may reduce sexual desire, impair arousal, and lead to ejaculatory dysfunction.[243] Serum testosterone levels have not been consistently associated with delayed ejaculation.[244]

Retrograde Ejaculation

Retrograde ejaculation is the failure of the semen to be ejected out through the urethral meatus; instead, the semen is propelled backward into the urinary bladder.[120–123] Retrograde ejaculation can be the result of autonomic neuropathy associated with diabetes mellitus; sympathectomy; therapy with adrenergic antagonists, some types of antihypertensives, antipsychotics, or antidepressants; bladder neck incompetence; or urethral obstruction. Retrograde ejaculation due to diabetes-associated autonomic neuropathy is the second most prevalent ejaculatory disorder.[120–123] Following transurethral resection of the prostate, the bladder neck closure mechanism may be damaged. Patients remain continent because of a second, more distal, continence mechanism that is present in the region of the membranous urethra; however, many patients who have undergone transurethral resection of the prostate experience retrograde ejaculation. Ejaculatory disorders can lead to infertility among men.[120–123]

Current Definitions of Sexual Disorders in Women

The current definitions for female sexual disorders in DSM-5 are shown in Table 20.1.[45,46] A disorder is diagnosed only if there is clinically significant distress or impairment; additionally, the sexual dysfunction should not be more attributable to a nonsexual psychiatric disorder, to the effects of a substance (e.g., a drug of abuse, a medication), to a medical condition, or to relationship distress, partner violence, or other significant stressors.[45,46] Disorders are identified as early onset (lifelong) versus late onset (acquired).

Sexual Interest/Arousal Disorder

The DSM-5 definition addressing problematic desire merges sexual interest (motivation) with arousal and deemphasizes the need to have initial anticipatory desire.[245,246] Increasing evidence suggests that desire ahead of and at the outset of sexual engagement, although welcomed probably by both partners, is not mandatory for a woman's sexual enjoyment and satisfaction.[21,247–249] It is the inability to trigger desire and arousal during sexual engagement (as well as an absence of desire initially) that constitutes the disorder. Empirical support for the concept that arousal may precede desire and the two then coexist is now strong and includes data from older and younger women.[21,24,248] Therefore, merging of sexual arousal and desire difficulties into one disorder appears logical. However, validated questionnaires used to assess sexual function are based on models of sexual response in which desire is assumed necessary at the outset of engagement. This is now acknowledged as a serious limitation to research,[250] and the prevalence of what is currently understood to be *disorder* is not known.[37,249–257] Studies that simply report "low desire and distress" indicate prevalence of about 10%, not increasing with age.[37,255] Early research suggests that some two thirds of women diagnosed with HSDD would merit a diagnosis of SIAD as well. This research also indicated that women with both diagnoses have more severe and more numerous symptoms.[258]

Although, according to DSM-5, the SIAD diagnosis excludes situations where relationship issues, mood, medications, or illness are strong contributors to sexual dysfunction, in clinical practice most patients seeking help have risk factors including negative feelings for the partner and mood disorders.[252] Depression, either currently or in the past and even in the absence of a clinical diagnosis of depression, depressive and anxious thoughts and low self-esteem are found significantly more commonly in women with SIAD than in women without SIAD.[17,37,255]

Female Orgasmic Disorder

The prevalence of women's orgasmic disorder is not known, because many studies include women with low arousal who rarely reach orgasm.[14,37,255] Risk factors include anxiety about the partner's presence, fear of being vulnerable, fear of not being in control, and fear of intimacy.[14] These factors often stem from childhood (nonsexual) experiences.

Genitopelvic Pain/Penetration Disorder

The merging of former terms *vaginismus* and *dyspareunia* in DSM-5 has some merit. According to DSM-IV, the term *vaginismus* described (phobic) avoidance, involuntary pelvic muscle contraction, and anticipation of, fear of, or the experience of pain in the absence of structural or other abnormalities on examination. Some women who reported typical phobic avoidance of penetration received an initial diagnosis of vaginismus; however, in many such women, a physical examination had to be deferred until therapy enabled a thorough pelvic examination to be performed. However, in some of these women, a subsequent careful introital examination enabled by successful therapy could reveal allodynia of the vestibule. Thus, the initial diagnosis of vaginismus based on history would need to be changed to provoked vestibulodynia (PVD) after introital examination.

Reported prevalence of sexual pain varies between 20% and 35%.[259–263] PVD, which is the most common form of pain with penetration, affects some 16% of mostly premenopausal women, many of whom have had pain consistently from first attempts at penetration.[253] Risk factors for PVD include some personality traits—perfectionism, reward dependency, fear of negative evaluation—as well as harm avoidance, hypervigilance for pain, higher levels of trait anxiety, and shyness.[253] In a small subset, vaginal infections, such as vaginal candidiasis, appear to precipitate and maintain the condition.

In DSM-5, vaginismus is now included within genitopelvic pain/penetration disorder (GPPPD).[45] Risk factors for GPPPD include depression, anxiety, social phobia, somatization, and hostility. Some studies identify increased catastrophic thinking in women with vaginismus compared with women without pain or women with other forms of pain: moreover, women with vaginismus show greater disgust propensity.[253] There is no evidence that vaginismus is related to religious orthodoxy, negative sexual upbringing, or concerns about sexual orientation. Typically, there is extreme fear of vaginal entry, fear that harm will come from

something the size of a penis entering the vagina, and fear of damage by vaginal delivery.

Persistent Genital Arousal Disorder

Not included in DSM-5 but clinically an increasingly common and poorly understood entity is persistent genital arousal disorder, which involves spontaneous, intrusive, and unwanted genital arousal (e.g., tingling, throbbing, pulsating) in the absence of sexual interest and desire. The awareness of subjective arousal is typically, but not invariably, unpleasant. The arousal is unrelieved by one or more orgasms, and the feelings of arousal may persist for hours or days.[254] Persistent genital arousal disorder can be associated with a broad range of symptoms, of which the severity can vary from mild (and perhaps pleasant) to intrusive and distressing, and which can interfere with life. Prevalence is unknown.

Sexual Dysfunction in the Context of Endocrine Disease

We will focus on sexual sequelae of endocrine disease and its treatment, but in any given person, nonendocrine factors may be more important. These factors include psychological, relational, contextual, cultural, and nonendocrine medical influences—especially depression, hypertension, neurologic disease, and LUTS.[14] For patients with chronic disease, the disease itself, its treatment, and its psychological effects, plus interpersonal, personal, and contextual issues, affect sexual response.[14]

In healthy women, factors such as attitudes toward sex, feelings for the partner, past sexual experiences, duration of relationship, and mental and emotional health have been shown to more strongly modulate desire and arousability than do biologic factors.[14] Contrary to gender stereotypes, recent analysis of the 1035 sexually active adults who participated in the NHSLS in 1992 showed that men's physical sexual pleasure was more closely linked to relational factors than was the case for women.[37,188,191] Similarly, in a recent international study of midlife and older couples, men rated the importance of sex for closeness and intimacy to their partner more highly than did their female partners.[37] Qualitative research also suggests that both men and women note that positive self-esteem and feeling attractive enhance desire and arousal.[37,191] Sexual context is also important for both men and women.[191]

Endocrine Disorders and Sexual Dysfunction in Men

Androgen Deficiency Syndromes

Androgen deficiency in men is a syndrome characterized by a constellation of signs and symptoms associated with consistently and unequivocally low testosterone levels due to disorders of the testes, pituitary, or hypothalamus.[27,63,264] Androgen deficiency can occur either because of primary testicular dysfunction or as a result of disorders affecting the hypothalamus or the pituitary.[63] Common causes of primary testicular dysfunction include Klinefelter syndrome, uncorrected cryptorchidism, radiation and cancer chemotherapy, Yq microdeletions, human immunodeficiency virus (HIV) infection, orchitis, trauma, and torsion.[63] Secondary testicular dysfunction can result from systemic illness; excessive exercise; recreational drugs, especially opiates, anabolic steroids, marijuana, cocaine, and alcohol; pituitary and suprasellar tumors; hemochromatosis; hyperprolactinemia; and infiltrative disorders. Exclusion of these secondary causes of hypogonadism may then justify a diagnosis of idiopathic hypogonadotropic hypogonadism, which is a heterogeneous group of disorders characterized by disordered GnRH secretion.[63] Opioid use has emerged as an important cause of testosterone deficiency and sexual dysfunction. Similarly, in many men's health clinics, a substantial proportion of men receiving testosterone replacement therapy are former anabolic steroid users. The testosterone levels required to maintain sexual function in men are close to the lower limit of the normal male range.[65,144,155,158,265,266] Therefore, some men with pituitary tumors may remain asymptomatic until their tumor has grown substantially and testosterone levels have declined to a level below this threshold.

Androgen deficiency is an important treatable cause of HSDD in men. Therefore, men diagnosed with HSDD should be evaluated for androgen deficiency by measurement of testosterone levels using a reliable assay, preferably in an early morning fasting blood sample.[63] Although ED and androgen deficiency in men are separate disorders with distinct pathophysiologic mechanisms, the two can coexist in the same patient. Testosterone levels should be measured in men presenting with any form of sexual dysfunction because androgen deficiency is treatable; furthermore, androgen deficiency may be a manifestation of another underlying disease, such as a pituitary tumor, which may require additional evaluation and disease-specific intervention.

Diabetes and Sexual Dysfunction in Men

Men with diabetes mellitus are at increased risk of ED, retrograde ejaculation, and low testosterone levels. Peyronie disease is an important comorbid condition in older diabetic men with ED.[267–274] Men with diabetes have significantly lower scores for sexual desire, activity, arousal, and satisfaction,[268–274] in part due to the medical and psychological factors associated with diabetes, such as the variations in glycemic control, reduced energy, and altered self-image. Diabetes also is associated with increased risk of low testosterone levels.[63,275–280] In population studies, sex hormone–binding globulin (SHBG) and total testosterone are more strongly associated with diabetes risk than free testosterone levels; these data suggest that the observed association of testosterone with diabetes risk may be related to factors, such as obesity and inflammation, that alter SHBG.[279,280]

The prevalence of ED in men with diabetes increases with age and has been reported to be as high as 75% in some studies. ED in men with type 2 diabetes, even without other risk factors for coronary artery disease, may signal silent cardiac ischemia.[280–284] Among men with diabetes, those with ED are more likely to be older and smokers, and to have a longer duration of diabetes, poor metabolic control, untreated hypertension, neuropathy, microalbuminuria and macroalbuminuria, retinopathy, CVD, diuretic treatment, low testosterone levels, and psychological vulnerability.[270–273] The risk of ED generally increases with chronic elevation of hemoglobin A_{1c}.[270] Increased physical activity and consumption of small amounts of alcohol have been found to be associated with lower risk of ED.

Endothelial and smooth muscle dysfunction, autonomic neuropathy, and psychological and interpersonal issues contribute to sexual dysfunction in men with diabetes.[283,284] Endothelial dysfunction is evident in both penile blood vessels and nongenital vascular beds.[212] eNOS is reduced, possibly due to overexpression of arginase or lack of nicotinamide adenine dinucleotide phosphate (NADPH), which is an essential cofactor for NOS.[284–289] Additionally, accumulation of oxygen free radicals, including

those from advanced glycosylation end products, quench nitric oxide and attenuate the action of K^+ channels.[288,289] Men with long duration of diabetes may not be able to generate sufficient nitric oxide and cGMP within the cavernosal smooth muscle; thus, they may become unresponsive to PDE5 inhibitors. The reduction in NADPH is also associated with increased diacylglycerol and protein kinase C, and, consequently, increased smooth muscle contractility.[286] An increased activation of the Rho A/Rho kinase pathway may increase the sensitivity of cavernosal smooth muscle to calcium.[286] Autonomic neuropathy affecting the pelvic nerves may lead to both ED and ejaculatory dysfunction.[287]

Retrograde ejaculation and partial ejaculatory incompetence affect up to one third of men with diabetes.[290] Autonomic neuropathy in diabetes may be associated with dysfunction of the internal sphincter so that all or a part of the seminal fluid is propelled into the bladder.[287] Partial ejaculatory incompetence refers to the condition in which ejaculatory emission remains intact but the expulsion phase is inhibited; consequently, the semen trickles out of the penis and the experience of orgasm is altered in quality. The ejaculatory problems may be a cause of infertility.

Sexual Dysfunction Associated With Therapies for Benign Prostatic Hypertrophy

Benign prostatic hyperplasia is frequently associated with LUTS and sexual dysfunction.[208–214] Some α_1-adrenergic receptor blockers, such as tamsulosin, are associated with ejaculatory dysfunction.[291,292] Treatment of men with LUTS with 5α-reductase inhibitors has been associated with increased risk of ejaculatory disorder, ED, and decreased libido.[293,294]

Several surveys have reported the development of sexual symptoms, including loss of libido, ED, depressive symptoms and even suicidality, and cognitive symptoms in a subset of young men who have taken finasteride for alopecia.[295–298] These symptoms have been reported to persist even after discontinuation of finasteride, and this condition has been referred to as postfinasteride syndrome. Our recent investigations of men who developed sexual symptoms after using finasteride and whose symptoms persisted even after discontinuation of finasteride have revealed that these men do not have evidence of androgen deficiency, decreased peripheral androgen action, or persistent peripheral inhibition of SRD5A.[299]

Symptomatic finasteride users revealed depressed mood and fMRI findings consistent with those observed in men with depression.[299] The causal role of finasteride and the pathophysiology of these symptoms remain to be established.[299,300]

Hyperprolactinemia and Sexual Dysfunction

Hyperprolactinemic men often present with decreased libido or ED; 75% of men with macroprolactinomas and 50% of men with microprolactinomas report reduced desire or ED, and almost all have subnormal nocturnal penile erections.[301–305] Hyperprolactinemia affects 1% to 5% of men presenting with ED[302]; a fraction of these men have prolactin-secreting pituitary adenomas. Prolactin lowers testosterone levels through its inhibitory effects on GnRH secretion and on the pituitary gonadotrope response to GnRH. Most, but not all, men with sexual dysfunction and hyperprolactinemia have low testosterone levels.[301,302] Whether and how hyperprolactinemia directly affects erectile function through target organ effects is not well understood. Erectile function generally improves in hyperprolactinemic men following treatment with dopamine agonists.[304,305]

Sexual Dysfunction in Patients With Thyroid Disease

Hypothyroidism has been associated with increased risk of hypoactive sexual desire and ED.[306–310] The exact prevalence of sexual dysfunction in men with hypothyroidism is unknown. Free testosterone levels are lower in hypothyroid men than in control subjects and become normal after thyroxine replacement.[306–310] Serum LH and follicle-stimulating hormone levels are typically not elevated in men with primary hypothyroidism.[309] Hyperprolactinemia is noted in a small proportion of hypothyroid men.[309]

Free testosterone levels are typically normal in men with hyperthyroidism, but SHBG and estradiol levels are elevated, resulting in a high estradiol-to-testosterone ratio and gynecomastia in some hyperthyroid men.[308] Hyperthyroidism has been observed in a small fraction of men with ED.[310]

Sexual Dysfunction in Men With Metabolic Syndrome

Men with metabolic syndrome have a higher prevalence of ED than men without metabolic syndrome.[311–314] The risk of ED is correlated with the number of identified components of metabolic syndrome.[310–314]

Endocrine Disorders and Sexual Dysfunction in Women

Thyroid Disease in Women

Both hyper- and hypothyroid states have been found to be risk factors for sexual dysfunction, which mostly remits with return to a euthyroid state.[315–317] Studies are few and small, and the sexual dysfunction was assessed in these studies using neither modern psychometrically robust instruments nor the DSM-5 classification of sexual disorders. Comorbid depression is associated with sexual dysfunction in the context of thyroid disease.[27,315] There is some evidence that thyroid autoimmunity lessens sexual desire independent of altered thyroid status: euthyroid women with Hashimoto thyroiditis may report persistent loss of desire.[315–317] One research group found that women with nodular goiter have significantly more sexual dysfunction than control subjects. This group also had the highest body mass index.[317] It is not clear whether the sexual dysfunction in patients with thyroid disease is related to the underlying thyroid disease or to comorbid conditions or body image problems.

Hyperprolactinemia in Women

Hyperprolactinemia can be associated with increased risk of menstrual irregularities, amenorrhea, hypoestrogenemia, and galactorrhea.[79,318] Some studies find that women with hyperprolactinemia report greater overall dissatisfaction with sexual function and lower scores for sexual desire, arousal, lubrication, and orgasm than women with normal prolactin levels. The lower scores for sexual function and desire have also been found in women with hyperprolactinemia who have regular menses.[318] However, normal menstruation, younger age, and smaller size of prolactinoma are more likely to be associated with normal sexual function.[14] Prolactin inhibits GnRH pulses, attenuates gonadotropin response to GnRH, and is associated with reduced ovarian secretion of estradiol and androgens. Sexual outcomes in hyperprolactinemic women after treatment with dopamine agonists have not been well studied.

Diabetes in Women

A recent meta-analysis[319] including 3168 women with diabetes and 2823 control subjects from 26 studies confirmed that sexual

dysfunction is more frequent in women with diabetes than in age-matched controls. Compared with women without diabetes, the risk of sexual dysfunction was 2.27 and 2.49 times higher in women with type 1 and type 2 diabetes, respectively.[319] However, postmenopausal women with any form of diabetes did not demonstrate an increased risk of sexual dysfunction.

Sexual dysfunction in women with diabetes is complex, and the interrelationships between body mass index, fat distribution, diabetic complications, insulin resistance, inflammation, CVD, relationship satisfaction, and depression remain incompletely understood.[14,320,321] The increased prevalence of sexual dysfunction and lower Female Sexual Function Index (FSFI) score in women with diabetes may be related to body weight. This association would be in keeping with other studies showing an increased prevalence of sexual dysfunction in obese women[320–322] and in women with metabolic syndrome.[323,324] Unlike the situation in men with diabetes, sexual dysfunction has not been associated with the duration of diabetes or the presence of diabetic complications in the majority of studies. Most studies in women with type 2 diabetes are small, but one larger study of 600 women with type 2 diabetes confirmed depression and marital status to be independent risk factors for sexual dysfunction.[325]

Women's sexual response and satisfaction may be compromised by diabetes-associated changes in their well-being, mood, self-image, weight gain, recurrent vaginitis from candidiasis, or infertility.[319,320,322,325–335] In addition, genital sexual response may be compromised by autonomic neuropathy, endothelial dysfunction, and microvascular disease. Some, but not all, studies show increased prevalence of dyspareunia, orgasmic difficulties, and sexual dissatisfaction.[318] In women with type 1 diabetes, sexual dysfunction is mostly correlated to psychological factors including depression, anxiety, and marital status.[322,326,327,329] A large prospective study of 625 women with type 1 diabetes confirmed depression as a major predictor of sexual dysfunction.[333] Type 1 diabetes also may be associated with loss of genital sexual sensitivity and subsequent loss of orgasm.[336] Young women with diabetes on multiple insulin dose regimens had impaired arousal and lubrication, whereas those using an insulin pump had sexual function comparable to healthy age-matched women.[337]

Autonomic and somatic neuropathy may contribute to loss of genital sexual sensitivity. When there is less engorgement of the vascular sinusoidal tissue comprising the shaft, head, rami, and bulbs of the clitoris, massaging the structures during sexual stimulation may fail to elicit typical sexual sensations, compromise arousal, and limit the experience of orgasm.[338,339]

In animal studies, diabetes has been shown to impair vaginal smooth muscle relaxation responses to the neurotransmitters, particularly VIP and nitric oxide.[338] These studies also report decreased clitoral and vaginal blood flow to nerve stimulation, diffuse fibrosis of the clitoral and vaginal tissues, and reduced muscular layer and epithelial thickness in vaginal tissue. Endothelial dysfunction and reduced clitoral blood flow have been documented in women with diabetes.[339]

Most studies have not found increased prevalence of dyspareunia in women with diabetes. However, they are at higher risk of recurrent candidiasis, which may contribute to dyspareunia.

Metabolic Syndrome in Women

Metabolic syndrome has been shown to have a deleterious effect on women's sexuality, independent of diabetes and obesity.[14,27,340,341] This negative effect seems to be more prevalent in premenopausal women than in postmenopausal women.[14,27,340,341]

Polycystic Ovary Syndrome

Some studies have suggested that women with polycystic ovary syndrome may be less sexually satisfied and may regard themselves as less attractive than control subjects.[342–345] The presumption is that obesity and androgen-related symptoms may contribute to poor body image, which may increase the risk of sexual dysfunction.[27,320] However, it is not clear whether polycystic ovary syndrome per se, as opposed to obesity, is a risk factor for sexual dysfunction.[345,346] There is no evidence of heightened sexual desire from the higher androgen levels.

Congenital Adrenal Hyperplasia

Whereas the classic form almost always presents at birth with adrenal insufficiency and virilization in girls, nonclassic forms of congenital adrenal hyperplasia (NC-CAH) may present with signs of hyperandrogenism in childhood or adulthood, depending on the severity of the 21-hydroxylase enzyme deficiency.[347] The presenting features of 21-hydroxylase enzyme deficiency may include amenorrhea, anovulation, hirsutism, or oligomenorrhea with infertility. A recent study found women with NC-CAH had a lower total FSFI score, with lower sexual arousal, lubrication, sexual satisfaction, and more dyspareunia, plus higher depressive symptom scores compared with well-matched controls.[347] Women with classic and NC-CAH may show gender-atypical behavior[348]; in one study, male-typical role-playing in childhood correlated with reduced satisfaction with the female gender role and reduced heterosexual interest in adulthood.[349] Disturbed body image, repeated genital examinations, and genital surgery may also affect sexual function in women with classic congenital adrenal hyperplasia.[348] Decreased sexual frequency, sexual satisfaction, and sensuality, high rates of sexual avoidance, and difficulty with vaginal penetration and orgasm, regardless of whether they have undergone prior surgery, have been reported in women with classic and NC-CAH. Caring for these women requires careful individualized treatment of both androgen excess and psychosexual counseling.[350]

Pituitary Disease in Women

There is limited research on sexual function in women who have deficiencies of various pituitary hormones. Women with pituitary disease and gonadotropin deficiency may suffer from amenorrhea or menstrual irregularity or problems with sexual function, including decreased sexual desire and problems with lubrication or orgasm.[351] Although women with hypopituitarism have lower estradiol and testosterone levels than healthy menstruating women, the short- and long-term effects of replacing testosterone in women with hypopituitarism who are receiving estrogen replacement have not been well studied.[351,352] In one randomized trial of 51 women, testosterone therapy in women who were receiving estrogen therapy was associated with some benefit in sexual function and mood, compared with placebo, but with a higher frequency of androgenic side effects than placebo.[353] The effects of replacing dehydroepiandrosterone (DHEA) on sexual function and mood in women with hypopituitarism are also poorly understood.[351]

Adrenal Insufficiency in Women

In addition to deficiency of cortisol and aldosterone, women with adrenal insufficiency also have low levels of testosterone and DHEA.[27,354–363] Adrenal insufficiency in women has been associated with low health-related quality of life.[359] However, a 2010 larger study comparing 174 women with Addison disease with

740 age-matched healthy control subjects and with 234 women who had received a risk-reducing bilateral salpingo-oophorectomy (BSO) demonstrated that despite subnormal levels of androgens and androgen metabolites, the women with Addison disease reported higher sexual pleasure and less discomfort with intercourse than the normative control women.[360] Clinical trials of DHEA replacement in women with adrenal insufficiency have been small and mostly negative.[27,354–358,361,363] An earlier small trial in women with primary or secondary adrenal insufficiency reported greater improvements in sexual interest and satisfaction and in mood for women receiving DHEA compared with placebo[354]; however, four subsequent studies did not find significant improvements in sexual function.[355–358] In 2009, a meta-analysis of the 10 notably small studies concluded that DHEA therapy in adrenal insufficiency may result in small improvements in health-related quality of life and depression, but it had no effects on anxiety or sexual well-being.[363] Thus, there are insufficient data to support the routine use of DHEA in women with adrenal insufficiency. Unfortunately, given that the typical multiple factors underlying women's sexual dysfunctions and small studies with limited assessments of sexual function may not be helpful in evaluating benefit from absent hormones. In addition to being a prohormone for the intracellular production of testosterone and estrogen, DHEA is now known to have multiple direct actions, including the modulation of various receptors and synaptic transmission in the brain, where its concentration can be six times higher than in serum.[364,365]

Natural Menopause

A majority of women who discontinue previously needed postmenopausal estrogen supplementation develop signs of vulvovaginal atrophy, now termed *genitourinary syndrome of menopause* (GSM), which is a risk factor for sexual dysfunction.[259–263,366,367] However, symptoms from GSM may remit spontaneously within 1 year; risk factors for more severe symptoms are diabetes, younger age, and low body mass index.[263,366,367] The traditional notion that maintaining sexual activity will prevent symptomatic GSM has been refuted.[367] Subjective symptoms and objective signs of GSM correlate poorly.[368] Epidemiologic studies have not shown an increase in the prevalence of dyspareunia with age.[259–261] Clearly, not all postmenopausal women develop sexual symptoms of estrogen deficiency: of 1525 women followed from age 47 to 54 years, most did not experience sexual dysfunction.[262] It is likely that multiple factors contribute to sexual symptoms, including variations in the intracrine production of estrogen from adrenal precursors, the number and sensitivity of estrogen receptors, and the degree of sexual arousal or excitement at the time of vulval stimulation and vaginal entry.[369–371] Psychological factors rather than estrogen levels were shown to moderate symptoms when GSM is present.[371]

Most studies report a decrease in sexual desire with advancing age[372] that is not easily explained by hormonal deficiency alone. Adaptive changes occur in the brain in response to the reductions in circulating levels of sex hormones associated with age and menopause.[373,374] Sex hormones are produced locally within the brain: in women, steroidogenic enzymes and sex-steroid receptors in the brain are upregulated in response to decreased circulating levels of sex hormones.[373,374] We do not know whether there is biologic adaptation to reduced amounts of circulating sex hormones. In studies of age, menopausal status, and sexual function, the postmenopausal state has generally been negatively associated with desire, mainly among women who experienced low emotional intimacy with their partners. Similarly, the negative association between age and sexual desire was particularly pronounced in women experiencing little intimacy.[375]

Surgical Menopause

Surgical menopause is a state of both androgen and estrogen depletion of sudden onset and has often been viewed as a risk factor for sexual dysfunction. However, most women undergoing bilateral BSO for benign clinical indications do not develop sexual dysfunction. Three prospective studies found that women choosing BSO plus hysterectomy for benign indications did not develop sexual dysfunction over the next 1 to 3 years.[376–378]

A national survey of 2207 American women confirmed an increased prevalence of distress about low sexual desire in women with a recent BSO.[372] Thus, in women undergoing nonelective surgery, the thematic context of bilateral oophorectomy may impair sexual desire and function. For example, women who are treated for malignant disease or those who desire to preserve their fertility may experience greater distress about low sexual desire after BSO than those who undergo BSO for benign conditions. In the same survey, both older and younger women with a relatively recent BSO reported low sexual desire, per se, as often as age-matched subjects with intact ovaries.[372] Despite their continued hormonal deficit, women older than 45 years who underwent oophorectomy before menopause had fewer complaints of low desire than women of similar age with intact ovaries.[372]

A recent study of 1352 women showed no difference in the report of sexual ideation, sexual function, or sexual problems between women who have had and women who have not had bilateral oophorectomy.[379] Having thoughts about sex is less likely to be affected by contextual details including the sexual relationship than is sexual function or motivation for partnered sex.

The women who carry a *BRCA1* or *BRCA2* mutation and undergo BSO to lessen the risk of breast, ovarian, or fallopian tube cancer mostly remain satisfied with their decision for surgery.[380] However, after risk-reducing surgery, women reported significantly less sexual pleasure, more discomfort, and less frequent sex compared with controls.[381]

Aging-Associated Decline in Sex Hormone Precursors in Women

From the middle 30s to the early 60s, a woman's adrenal production of precursor hormones—DHEA, androstenedione, and DHEA sulfate—declines by 70%.[66,68] However, the trajectories of decline in these precursor steroids vary among women.[66–68] The relationship of the age-related decline in these circulating precursors to sexual function remains poorly understood. On the population level, variation in circulating levels of sex steroids and their precursors is related to variation in steroidogenic enzyme activities, such as 3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, 17,20-lyase, and aromatase, and to the variation in the plasma clearance of these hormones and precursors. Labrie and associates[68,369,382] have proposed that the androgen metabolites, most notably ADT-G, may serve as useful markers of ovarian and tissue production and activity of androgens in women. How the decline in total androgen activity with age may relate to sexual function has yet to be studied.

Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators are a class of ligands that bind estrogen receptor subtypes and induce a unique profile of

tissue-specific gene expression. Accordingly, each selective estrogen receptor modulator may also be associated with a unique set of clinical responses. Ospemifene has estrogen antagonist action on breast and endometrium. Ospemifene, but not raloxifene or tamoxifen, can ameliorate the genital sexual symptoms of lack of estrogen.[383] Limited research suggests raloxifene and tamoxifen are not associated with sexual adverse effects.

Hormonal Contraceptives

The estrogen in combined systemic contraceptives increases SHBG and thus decreases available free testosterone. The decrease in sexual desire and subjective arousability in some women receiving oral contraceptives has been attributed to the decrease in free testosterone levels. However, to date, low desire has not been associated with testosterone levels, even when mass spectrometry methods are used.[66,67] Dyspareunia is an occasional consequence of very low estrogen formulations. Hormonal contraceptives exert multiple psychological and biologic actions, some of which may positively affect sexuality, for example, by reducing anxiety about unwanted pregnancy and diminishing dysmenorrhea.[384]

Androgen Insensitivity Syndrome

Women with complete androgen insensitivity syndrome have a female phenotype with full breast development but variable shallow vagina, which may require surgical intervention or progressive dilatation. Small cross-sectional studies indicate that women with complete androgen insensitivity are capable of a healthy sexual response with orgasms of self-stimulation and of intercourse to be possible despite complete androgen insensitivity.[385–389] However, these women are often confronted with complex psychosocial issues related to the mismatch between their genetic sex and their gender role, the timing of diagnosis and timing of disclosure to the woman, and infertility. Reduced sexual confidence, poor self-esteem, and depression are noted in these studies.

Assessment of Sexual Dysfunction

Assessment of sexual function is an important part of the evaluation of patients with endocrine diseases. Open-ended, nonjudgmental questions such as "Many men with diabetes notice changes in their erections or ejaculation—are you having any difficulties?" can facilitate further discussion of sexual problems. When sexual problems are identified, sensitive and respectful inquiry into their nature and the sexual context is necessary. Evaluating both partners together, as well as individually, can often uncover problems that may not be apparent in individual interviews.

Evaluation of Men With Sexual Dysfunction

There are four important considerations in the evaluation of men with HSDD. First, an important initial step in the evaluation is an interview of the couple to determine whether the patient primarily has ED or a sexual desire problem. Second, ascertain whether the couple has a relationship problem. Establish whether self-stimulation continues despite lack of desire for partnered sex. With the easy accessibility of Internet sites, sex alone, possibly on a frequent basis, may allow sexual expression despite relationship difficulties. Third, general health evaluation is necessary to exclude systemic illness, depression, and medication use. Serum total and free testosterone levels should be measured to exclude androgen deficiency because androgen deficiency is an important treatable cause of HSDD.

The diagnostic workup of men with ED should start with an evaluation of general health[5,6,390–396] (Tables 20.4 and 20.5). The presence of diabetes mellitus, coronary artery disease, peripheral vascular disease, hypertension, chronic kidney disease, stroke, spinal cord or back injury, a neurologic disorder, depression, and dementia should be verified. Information about use of recreational drugs such as alcohol, opioids, marijuana, cocaine, and tobacco, prescription medications, particularly antihypertensives, antiandrogens, antidepressants, antipsychotic drugs, and opioid analgesics, and nonprescription over-the-counter supplements is important because almost one fourth of all cases of impotence can be attributed to medications. A detailed sexual history should elicit the nature of relationships, partner expectations, situational erectile failure, performance anxiety, and marital discord. It is important to distinguish between inability to achieve erection, changes in sexual desire, failure to achieve orgasm and ejaculation, and dissatisfaction with the sexual relationship, as the etiologic factors vary with the type of sexual disorder.

A directed physical examination should focus on secondary sex characteristics, the presence or absence of breast enlargement and testicular volume; evaluation of femoral and pedal pulses; neurologic examination to determine the presence of motor weakness, perineal sensation, anal sphincter tone, and bulbocavernosus reflex; and examination of the penis to evaluate any unusual curvature, palpable plaques, or superficial lesions.[390–395]

The initial diagnostic workup in most men presenting with ED usually includes the measurement of hemoglobin, blood glucose and hemoglobin A_{1C}, blood urea nitrogen and creatinine, plasma lipids, and serum total and free testosterone levels. Further evaluation using more invasive diagnostic testing is limited to those men who do not respond to an empiric trial of oral PDE5 inhibitors; these patients should be referred to a specialist for detailed urological evaluation.

Self-reporting questionnaires are useful because many men with ED do not voluntarily disclose their sexual complaints to their health care provider.[390–393] The International Index of Erectile Function, for example, is a multidimensional scale consisting of 15 questions that address relevant domains of male sexual function, including sexual desire, intercourse satisfaction, orgasmic function, and overall satisfaction[390]; a short form is also available.[393]

The diagnosis of androgen deficiency should be made only in men with consistent symptoms and signs and unequivocally low early morning serum testosterone levels that are consistently below the lower limit of the normal range for healthy young men.[6,27,65,264] Because of the diurnal, pulsatile, and interday variations in circulating testosterone levels, and the known suppressive effects of food and glucose intake, total testosterone concentrations should be measured in a fasting state on two separate mornings. Serum testosterone levels should be measured using a reliable assay, such as liquid chromatography tandem mass spectrometry (LC-MS/MS) assays, which have become widely available from many commercial and research laboratories, and which offer a higher level of accuracy and precision than immunoassays, especially at the low levels present in hypogonadal men. Ideally, testosterone levels should be measured using an assay that has been certified by an accuracy-based

TABLE 20.4 Assessment of a Patient With Sexual Dysfunction

Assessment Questions	Comments
Questions Asked of One or Both Partners	
1. Sexual problems and reason for presenting at this time	Ask patients to describe sexual problems in their own words; clarify further with direct questions, giving options rather than leading questions, support and encouragement, acknowledgement of embarrassment, and reassurance that sexual problems are common.
2. Duration, consistency, and priority if more than one problem is present	Are problems present in all situations? Which problem is most troubling?
Context of sexual problems	Emotional intimacy between partners, activity or behavior just before sexual activity, privacy, sexual communication, time of day and fatigue level, birth control (adequacy, type), risk of STIs, usefulness of sexual stimulation, sexual knowledge.
3. Rest of each partner's sexual response, other than the given problem area	Both currently and before the onset of the sexual problems.
4. Reaction of each partner	How each has reacted emotionally, sexually, and behaviorally.
5. Previous help	Compliance with recommendations and effectiveness.
Questions Asked of Each Partner When Seen Alone[a]	
1. Partner's own assessment of the situation	Sometimes it is easier to disclose symptom severity (e.g., total lack of desire) in the partner's absence.
2. Sexual response with self-stimulation	Also inquire about sexual thoughts and fantasies.
3. Past sexual experiences	Positive and negative aspects.
4. Developmental history	Relationships to others in the home while growing up; losses, traumas, to whom (if anyone) was the patient close; was he or she shown physical affection, love, respect?
5. Past or current sexual, emotional, and physical abuse	Explain that abuse questions are routine and do not necessarily imply causation of the problems; it is helpful to ask whether the patient ever felt hurt or threatened in the relationship and, if so, whether he or she wishes to give more information.
6. Physical health, especially conditions leading to debility and fatigue, difficulty with mobility (e.g., in caressing a partner, performing self-stimulation), and difficulties with self-image (e.g., from obesity, Cushing syndrome, hypogonadism)	Specifically, ask about medications with known sexual side effects, including SSRIs, SNRIs, β-blockers, narcotics, antiandrogens, and GnRH agonists.
7. Evaluation of mood	A significant correlation of sexual function and mood (including anxiety and depression) warrants routine screening for mood disorder using either a questionnaire (e.g., Beck Inventory) or semistructured series of questions.

[a]Items 3 through 5 of the single-patient interview may sometimes be omitted (e.g., for a recent problem after decades of healthy sexual function).

STIs, Sexually transmitted infections; *SSRIs,* selective serotonin reuptake inhibitors; *SNRIs,* selective serotonin norepinephrine reuptake inhibitors; *GnRH,* gonadotropin-releasing hormone

Adapted from Basson R. Clinical Practice. Sexual desire and arousal disorders in women. *N Engl J Med.* 2006;354(14):1497–1506.

standardization or quality control program (e.g., the Hormone Standardization Program for Testosterone from the Centers for Disease Control and Prevention (CDC).[264] The advent of LC-MS/MS, the availability of a testosterone calibrator from the National Institute of Standards and Technologies, and the institution of the Hormone Standardization Program for Testosterone have greatly improved the accuracy of testosterone assays and reduced interlaboratory variability among CDC-certified laboratories.[264,397–400]

The reference ranges for testosterone in men reported by various laboratories vary substantially because of assay differences, calibrator differences, and differences in the reference populations used to generate ranges. Recently, under the auspices of the Endocrine Society and the Partnership for the Accurate Testing of Hormones, we have published harmonized reference range based on data from community-dwelling men from four large cohorts in the United States and Europe.[401] The harmonized reference range for total testosterone in healthy, nonobese young men (age 19–39 years) was 264 to 916 ng/dL (9.2–31.8 nmol/L).[401] This reference range can be applied to all CDC-certified total testosterone assays.

Circulating testosterone is bound with high affinity to SHBG and with lower affinity to albumin, orosomucoid, and cortisol-binding globulin; only 2 to 4% of circulating testosterone is unbound or free.[402] The alterations in SHBG concentrations can alter the total testosterone concentrations; therefore, in conditions that alter SHBG concentrations, such as obesity, diabetes, aging, liver disease, hypo- and hyperthyroidism, and intake of many medications, determination of free testosterone levels is necessary to assess androgen status.[402] Free testosterone concentrations can be measured using the equilibrium dialysis assays or calculated from total testosterone, SHBG, and albumin concentrations.[402] The direct tracer analogue free testosterone assays are used in many hospital laboratories, but they are inaccurate and should not be used.

Zakharov and colleagues[403] have shown that testosterone's binding to SHBG is a dynamic multistep process that includes heterogeneity in circulating isoforms of SHBG dimer, an allosteric interaction between the two binding sites on SHBG such that the binding affinities of the two binding sites on SHBG are not equivalent, and convergence to an energetically favored bound state in

TABLE 20.5 Directed Diagnostic Evaluation of ED

History

Ascertain Psychosexual History

Nature of sexual dysfunction: whether the primary problem is decreased desire, erectile dysfunction, premature or delayed ejaculation, or difficulty in achieving orgasms
Strength of marital relationship and marital discord
Depression
Stress
Sexual performance anxiety
Knowledge and beliefs about sexuality

Ascertain Risk Factors

Presence of diabetes mellitus, hypertension, hyperlipidemia, coronary artery disease, end-stage renal disease, and peripheral vascular disease
History of spinal cord injury, stroke, Alzheimer disease, or other neurologic disorder
Prostate or pelvic surgery
Pelvic injury
Medications such as antihypertensives, antidepressants, antipsychotics, opioid analgesics, and inhibitors of androgen production or action
Use of recreational drugs such as alcohol, cocaine, opiates, and tobacco

Ascertain Factors That Might Affect Choice of Therapy and the Patient's Response to It

Coexisting coronary artery disease and its symptoms and severity
Exercise tolerance
Use of nitrates or nitrate donors
Use of α-adrenergic blockers
Use of vasodilators for hypertension or congestive heart failure
Use of foods (e.g., cranberry juice) or drugs (e.g., erythromycin, protease inhibitors, ketoconazole, and itraconazole) that might affect metabolism of PDE5 inhibitors

Physical Examination

Ascertain signs of androgen deficiency, such as loss of body hair, eunuchoidal proportions, small testicular volume, or breast enlargement
Genital and perineal sensation to evaluate neurologic deficit from spinal cord lesion, previous stroke, peripheral neuropathy, or other neurologic disorder
Blood pressure and postural change in blood pressure
Evaluate femoral and pedal pulses and evidence of lower extremity ischemia
Penile examination to exclude Peyronie disease or other penile deformities

Basic Laboratory Evaluation That Should Be Performed in All Men With ED

Fasting blood glucose
Plasma lipids
Serum total and free testosterone level using reliable assays

ED, Erectile dysfunction; *PDE5,* phosphodiesterase 5.

which both sites are occupied.[403] The free testosterone levels computed using this dynamic multistep binding with allostery match closely the values measured directly by equilibrium dialysis.[403] These studies also showed that the published law of mass action equations based on a linear model of testosterone's binding to SHBG, in which one molecule of SHBG binds one molecule of testosterone with a single binding affinity constant, are erroneous.[403]

In men found to be androgen deficient, measurement of LH levels helps distinguish between testicular (LH elevated) and hypothalamic-pituitary (LH low or inappropriately normal) defects.[65] Men with hypogonadotropic hypogonadism may require measurement of serum prolactin, serum iron, and total iron-binding capacity; evaluation of other pituitary hormones; and a pituitary MRI.

There is considerable debate about the usefulness and cost-effectiveness of hormonal evaluation and the extent to which androgen deficiency should be investigated in middle-age and older men presenting with ED. In cross-sectional surveys,[65] only a small fraction of men with ED and low testosterone levels have been found to have space-occupying lesions of the hypothalamic-pituitary region.[301,302] The diagnostic yield of pituitary imaging to exclude pituitary tumor can be improved by limiting these procedures to men whose total testosterone level is less than 150 ng/dL or who have panhypopituitarism, persistent hyperprolactinemia, or symptoms of tumor mass.[301,302]

Between 8% and 10% of men with ED have low testosterone levels; the prevalence of androgen deficiency increases with advancing age.[158,404–406] The prevalence of low testosterone levels is not significantly different among men who present with ED and in an age-matched population.[159] These data are consistent with the proposal that ED and androgen deficiency are two common but independently distributed disorders.[159] However, it is important to exclude androgen deficiency in this patient population. Androgen deficiency is a correctable cause of sexual dysfunction, and some men with ED and low testosterone levels will respond to testosterone replacement. Androgen deficiency may be a manifestation of serious systemic disease and may have additional deleterious effects on the individual's health; for instance, androgen deficiency might contribute to osteoporosis and loss of muscle mass and function.

If the history, physical examination, and laboratory tests do not identify medical problems needing further workup, then a cost-effective approach is to prescribe a trial of oral PDE5 inhibitor, provided there are no contraindications (e.g., nitrate use).

Tests that evaluate the integrity of penile vasculature and blood flow[407,408] are not needed in most patients with ED, are reserved for patients in whom the results of these tests would alter the management or prognosis, and should be performed only by those with considerable experience with their use. The penile-brachial blood pressure index is a simple and specific, but not a sensitive, index of vascular insufficiency. It is rarely used today in the evaluation of ED.

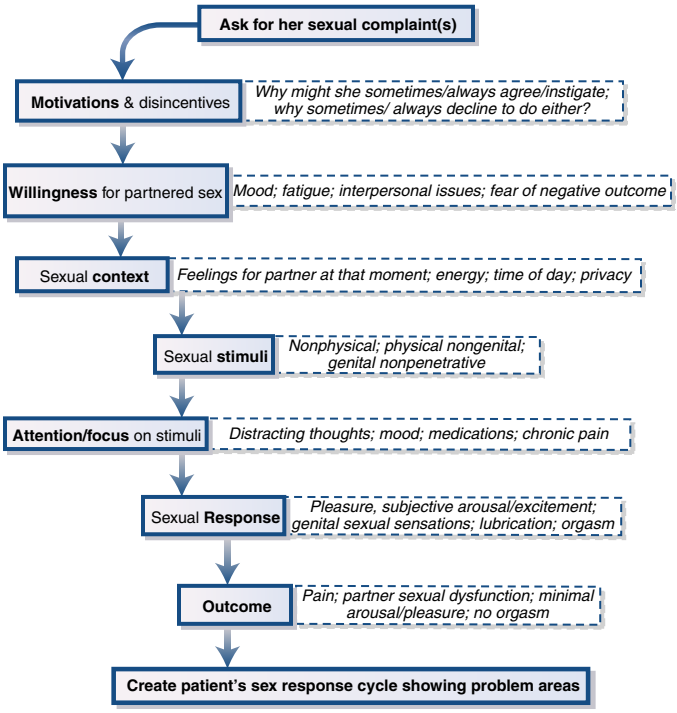
Intracavernosal injection of a vasoactive agent such as PGE_1 can be useful as a diagnostic and a potential therapeutic modality. This procedure can reveal whether the patient will respond to this therapeutic modality and can facilitate patient education about the procedure and its potential side effects. Failure to respond to intracavernosal injection can raise the suspicion of vascular insufficiency or a venous leak that might need further evaluation and treatment.

Most men with ED do not need duplex color sonography, cavernosography, or pelvic angiography.[5,6,394,395,407] For instance, angiography could be useful in a young man with arterial insufficiency associated with pelvic trauma. Similarly, suspicion of congenital or traumatic venous leak in a young man presenting with ED would justify a cavernosography. In each instance, confirmation of the vascular lesion might lead to consideration of surgery. Duplex ultrasonography can provide a noninvasive evaluation of vascular function.[407]

TABLE 20.6 General Evaluation of Women Presenting With Sexual Dysfunction

Item	Comments
General health and past medical history[a]	
Medications[a]	Current and past medications
Aspects of chronic disease relevant to sexual life	Pain, fatigue, continence, self-image, mobility
Mood[a]	Depression, depressive symptoms, current and past
Relationship[a]	Type(s), satisfaction
Past sexual experiences	Positive, negative, coercive, abusive
Partner's sexual function[a]	
Previous therapy	Details and compliance
Motivation to address problems	Willingness to prioritize addressing sexual life now

[a]Amenable to questionnaire format.



• **Fig. 20.9** Focused evaluation of women presenting with sexual complaints.

Nocturnal penile tumescence testing is rarely needed for patients being evaluated for ED and is recommended only for a limited number of patients with a high clinical suspicion of psychogenic ED or situational problems, to document preoperatively poor penile rigidity, or for medical-legal reasons. Although recording of nocturnal penile tumescence in a sleep laboratory for successive nights can help to differentiate organic from psychogenic impotence, this test is expensive and labor intensive. The introduction of portable RigiScan (GOTOP Medical, St. Paul, MN) devices in 1985 has provided clinicians with a reliable means of continuously monitoring penile tumescence and rigidity at home.[408] It is a multicomponent device that the patient wears at bedtime for two to three nights. It has two wire gauge loops that are placed around the base and tip of the penis that record changes in penile circumference and rigidity. Data are stored and downloaded via a software program that allows for interpretation. For most cases, a careful history of nighttime or early morning erections provides a reasonable correlation with nocturnal penile tumescence and RigiScan studies.[408]

Evaluation of Women With Sexual Dysfunction

Sexual dysfunction is diagnosed by clinical interview and, when necessary, physical examination. After obtaining details of general and mental health, the sexual complaints are clarified (Table 20.6 and Fig. 20.9).

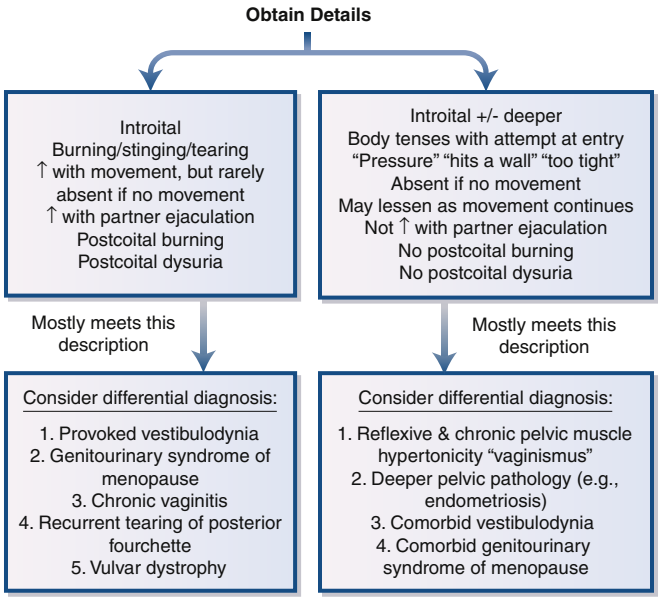
Sexual function questionnaires can be used to monitor treatment response but should not be used for establishing a diagnosis of sexual dysfunction in women.[409–411] One such instrument is the FSFI,[409,410] which was designed to monitor treatment response and not to diagnose sexual dysfunction in women who have not been clinically assessed. Moreover, the FSFI is based on the DSM-IV criteria of sexual disorders, which are grounded in the conceptualization of female sexual response beginning with conscious desire leading to a phase of arousal, then orgasm, and then resolution—these phases being in set order, discrete, and necessary for normal function. The limitations of the FSFI include the lack of recognition of triggered desire and the normality of beginning an experience that is sexually neutral; the possibility of sexual satisfaction without orgasm; a focus on partnered sex, which limits its use in women, who currently do not have a sexual partner;[410] and the limited period of assessment (the last 4 weeks), which may increase the risk of erroneous results during transient periods of stress or the partner's absence.[412] Although the FSFI has been used in many studies of women's sexual function, including those of women with endocrine disease, more contemporary instruments, such as the 17-question Natsal-SF, developed for the Natsal-3,[411] better reflect the importance of sexual satisfaction in contrast to many previous instruments. This attribute of the Natsal-SF instrument is particularly important, because patients may report satisfaction despite dysfunction, and dissatisfaction can occur in the context of a functional response[413]: women's satisfaction may or may not include orgasms.[246] A recent study confirms strong links between sexual satisfaction and sexual motivation.[414]

Physical Examination

Physical examination, including pelvic and genital examination, is part of routine care and can be reassuring to the patient by confirming normal anatomy and overall health. Unless dyspareunia is involved, it is not often that physical examination identifies the cause of sexual dysfunction. For some women with a history of coercive or abusive sexual experience, or previous traumatic medical experience, such examination may cause extreme anxiety and, unless sex is painful, may not be necessary. The reason for an examination—usually the presence of dyspareunia or loss of genital sexual sensitivity—and an explanation of what will and will not be done should be provided before the examination begins (Table 20.7 and Fig. 20.10, Parts 1 & 2 Assessment of GPPPD algorithm). If the woman prefers to invite her partner to be present, then a careful examination can be highly educational for both partners. In women with GPPPD with a marked component of vaginismus, the vaginal examination should be delayed until psychological therapy renders the examination possible and informative for both the patient (and partner if present) and the clinician.

TABLE 20.7 Evaluation of Genitopelvic Penetration Disorder—Part (2): the Detailed Genital Examination

Diagnoses (May Be Multiple)	Findings on Examination
Anatomic variations	Congenital (e.g., hypoplasia), past surgery or genital mutilation
Provoked vestibulodynia	No visual abnormalities on examination; Q-tip test to confirm allodynia of introital edge
Reflexive and chronic hypertonic pelvic muscles "vaginismus"	Palpation of pelvic muscles and their insertion into ischial spines confirms high tone, inability to do reverse Kegel maneuver
Genitourinary syndrome of menopause	Pallor, dryness, thinning, loss of turgor and elasticity and rugae, possible stenosis
Chronic vaginitis	Erythema, discharge, confirm by culture
Recurrent tearing of posterior fourchette	Taut fourchette, white line scars of past tears
Vulvar dystrophy (e.g., lichen sclerosus)	Abnormal mucosa typical of dystrophy
Deep pelvic pathology (e.g., endometriosis), bladder pathology	Tenderness, nodules in cul-de-sac, tender adnexae, pain with cervical movement or with bladder or uterine compression



• **Fig. 20.10** Evaluation of genitopelvic penetration disorder—part (1): the detailed history.

Laboratory Testing

The laboratory testing plays a small role in women's sexual evaluation. Estrogen activity is best evaluated by history and examination. The commercially available estradiol radioimmunoassays lack the sensitivity and precision required to measure the low concentrations present in the older woman; in addition, these assays do not measure estrone, the major estrogen after menopause. As discussed earlier, serum testosterone levels do not correlate with sexual function, even when LC-MS/MS assays are used.[66,67] The circulating testosterone levels may not reflect intracrine production, metabolism, or activity of androgens. Measurement of testosterone metabolites has been proposed as a marker of intracrine plus gonadal production of testosterone,[68] but the circulating levels of these metabolites have been shown to be similar in women with and without sexual dysfunction.[67,71,72] The clinical usefulness of androgen metabolites remains to be demonstrated. Currently available evidence does not support the usefulness of measuring serum testosterone concentrations in women with low sexual desire.[415] Fasting glucose, hemoglobin A_{1c}, prolactin, and thyrotropin should be measured if clinically indicated by the general health evaluation.

Management of Sexual Dysfunction in Men

Treatment of Hypoactive Sexual Desire in Men

There is a need to focus on the couple when the patient has a sexual partner. Treating the sexual dysfunction in the male partner improves the female partner's sexual function and satisfaction. Comorbid depression should be treated and relationship difficulties addressed. The efficacy of cognitive and behavioral therapies has not been evaluated systematically in men with HSDD. Testosterone therapy should be considered in men with HSDD who have androgen deficiency, even though there are no randomized trials of testosterone in men with HSDD.

Much of the information about the effects of testosterone on sexual desire has emerged from open-label trials of testosterone in hypogonadal men and a small number of randomized placebo-controlled trials of testosterone in hypogonadal men.[65,131,141,142,416–420] Most testosterone trials recruited men based on the presence of low testosterone levels alone, whereas a small number of recent trials required men to have low testosterone levels and one or more symptoms of testosterone deficiency.[131,141,142,146,416–420] Testosterone therapy in these trials has been associated with significant improvements in overall sexual activity, sexual desire, attention to erotic cues, and the duration and frequency of nocturnal penile erections.[65,131,416–419]

The Testosterone Trials were a set of several coordinated placebo-controlled trials, funded primarily by the National Institute on Aging, to determine the efficacy of testosterone in improving sexual function, physical function, and vitality in community-dwelling older men, 65 years or older, with low libido, mobility limitation, and/or fatigue, and an unequivocally low fasting early morning total testosterone level.[141] The eligible participants were required to have an average of two morning total testosterone levels less than 275 ng/dL measured using a CDC-certified LC-MS/MS assay.[421] Low libido was defined as the Derogatis Interview for Sexual Functioning for Men sexual desire domain score of less than 20. The eligible participants were assigned using the procedure of minimization to receive either 1% transdermal testosterone gel or placebo gel daily for 1 year. The testosterone dose was adjusted to achieve and maintain on-treatment testosterone levels between 400 and 800 ng/dL. The primary outcome was overall sexual activity. Assignment to the testosterone arm was associated with greater improvements in overall sexual activity, sexual desire, and erectile function than placebo.[141,142] Testosterone treatment improved most categories of sexual activity. The overall improvements in sexual desire and erectile function were modest but were

deemed clinically meaningful.[422] A carefully performed meta-analysis of randomized testosterone trials in hypogonadal men, who met the Endocrine Society's definition of testosterone deficiency, confirmed that compared with placebo administration, testosterone treatment is associated with greater improvements in sexual activity, sexual desire, erectile function, and satisfaction with sexual experience.[423]

Several points should be considered when initiating testosterone replacement therapy. First, testosterone treatment has been shown to improve some but not all domains of sexual function; testosterone improves sexual desire and erections but has not been shown to improve ejaculatory disorder. Second, testosterone treatment has been shown to be efficacious in improving sexual function only in men with unequivocally low testosterone levels and low sexual desire; it has not been shown to improve sexual function in men who have normal or low-normal testosterone levels. Third, there is enormous variability in on-treatment testosterone levels, especially with transdermal testosterone gels; a substantial fraction of hypogonadal men started on the initial dose of a transdermal testosterone gel will not raise their on-treatment levels into the target therapeutic range, and 5% to 10% may have supraphysiologic levels due to substantial variations in transdermal absorption and plasma clearance of testosterone. Unsurprisingly, nearly half of testosterone-treated men discontinue testosterone treatment after 3 months, and only about 25% are still on testosterone treatment after 12 months. Fourth, some men with HSDD may have relationship problems, which are not likely to be corrected by testosterone therapy. Finally, the long-term safety and efficacy of testosterone therapy has not been established.

Treatment of Erectile Dysfunction

The current practice employs a stepwise approach that first utilizes minimally invasive therapies that are easy to use and have fewer adverse effects and progresses to more invasive therapies that may require injections or surgical intervention after the first-line choices have been exhausted (Fig. 20.11). The physician should discuss the risks, benefits, and alternatives of all therapies with the couple. The selection of the therapeutic modality should be based on the underlying cause, patient preference, the nature and strength of the relationship with his sexual partner, and the absence or presence of underlying CVD and other comorbid conditions.[5,6,394,395] All patients with ED can benefit from psychosexual counseling.[5,6,394,395,424–428]

In the execution of good medical practice, treatment of all associated medical disorders should be optimized. In men with diabetes mellitus, glycemic control should be optimized, although improving glycemic control may not improve sexual function. In men with hypertension, control of blood pressure should be optimized and, if possible, the therapeutic regimen may be modified to remove antihypertensive drugs that impair sexual function. This strategy is not always feasible because almost all antihypertensive agents have been associated with sexual dysfunction; the frequency of this adverse event is less with converting-enzyme inhibitors and angiotensin-receptor blockers than with other agents.

First-Line Therapies

Psychosexual Counseling

The goals of psychosexual therapy are to reduce performance anxiety, develop the patient's sexual skills and knowledge, modify negative sexual attitudes, and improve communication between partners.[424] Counseling can be beneficial in both psychogenic and organic causes of sexual dysfunction[424–430] (Table 20.8).

An individual's focus on sexual performance rather than erotic stimulation is a major factor in the pathophysiology of psychogenic ED[424,425]; this behavior is referred to as *spectatoring*. Many experts recommend a *sensate focus* treatment approach, in which the couple avoids intercourse and engages in nongenital, nondemanding, pleasure-seeking exercises to reduce performance anxiety.[424]

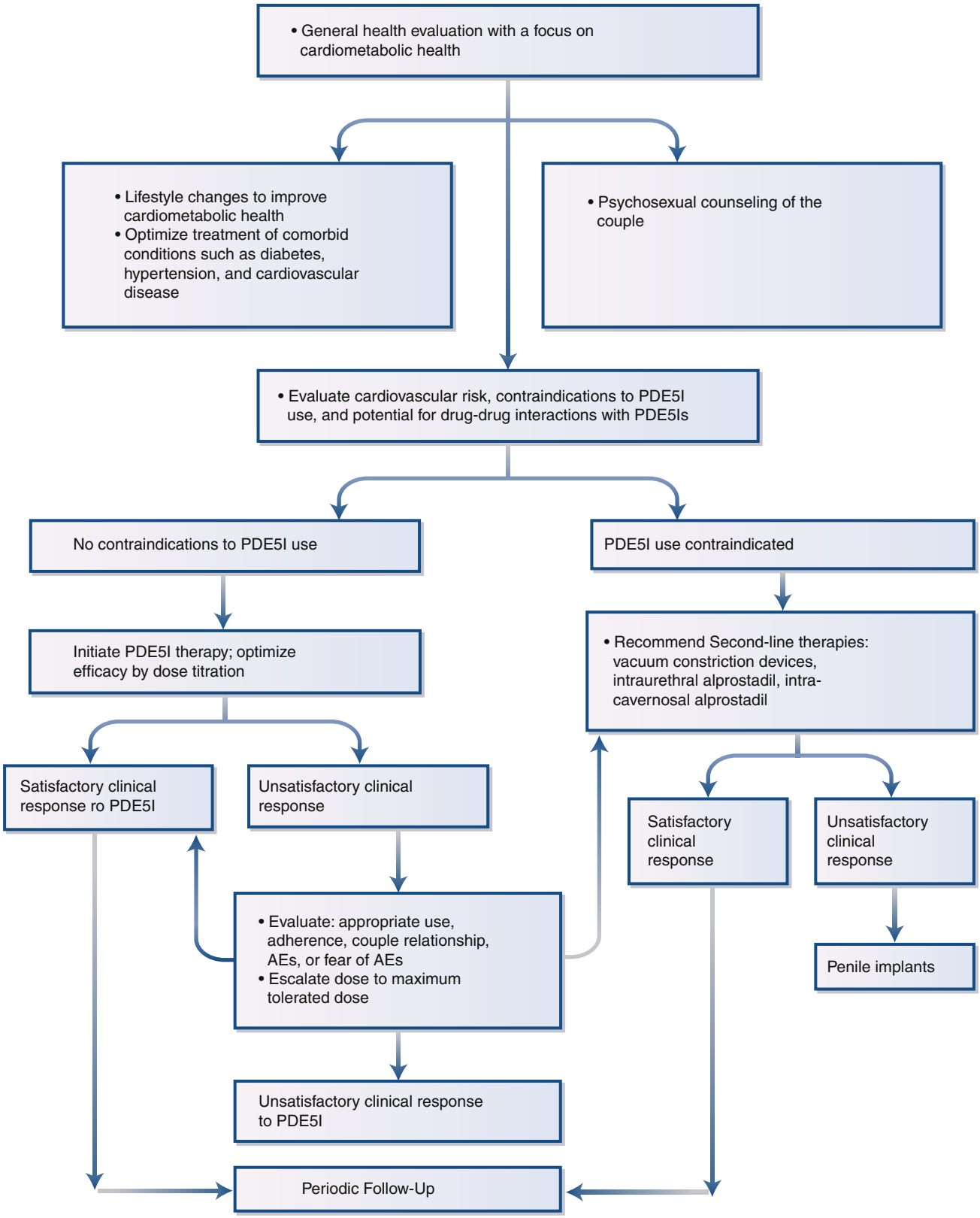
Involving the partner in the counseling process helps to dispel misperceptions about the problem, decreases stress, enhances intimacy and the ability to talk about sex, and increases the chances of successful outcome.[424] Counseling sessions are also helpful in uncovering conflicts in relationships, psychiatric problems, alcohol and drug abuse, and sometimes significant misperceptions about sex. As many men and women may harbor misinformation and unrealistic expectations about sexual performance and age-related changes in sexual function, cognitive restructuring techniques are helpful in correcting sexual myths and beliefs.[424] There is a paucity of outcome data on the effectiveness of this psychobehavioral therapy, but meta-analyses have reported benefit from group psychotherapy administered in conjunction with PDE5 inhibitors.[427]

Selective Phosphodiesterase 5 Inhibitors

Selective PDE5 inhibitors are safe and effective and have become widely accepted as first-line therapy for patients with ED, except in men for whom these drugs are contraindicated[4-6,106,108,394,395,430–432] (Tables 20.9 and 20.10). Selective PDE5 inhibitors are contraindicated in men using nitrates on a regular basis, in those with severe heart disease in whom sexual activity may not be safe, and in those with nonarteritic anterior ischemic optic neuropathy.[5,6,106,108,394,395,431,432]

Mechanisms of Action. Four classes of enzymes—NOS, adenylyl cyclase, guanylyl cyclase, and PDEs—play important roles in regulating the intracavernosal concentrations of cAMP and cGMP. Three isoforms of NOS catalyze the formation of nitric oxide from arginine using NADPH and oxygen as substrates: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). Nitric oxide activates soluble guanylyl cyclase to stimulate the production of cGMP from GTP, which then activates cGMP-dependent protein kinases to induce myosin phosphatase, inositol 1,4,5-trisphosphate receptor 1–associated protein, and some cation channels resulting in smooth muscle relaxation. PDEs hydrolyze cGMP, thus reducing its concentrations within the cavernosal smooth muscle.[107,108,111–113,433–436] Although PDE isoforms 2, 3, 4, and 5 are expressed in the penis, only PDE5 is specific to the nitric oxide–cGMP pathway in the corpora cavernosa.[436] PDE inhibitors sildenafil, vardenafil, tadalafil, avanafil, and udenafil are relatively selective inhibitors of PDE5.[107,108,111–113,433–459] These drugs block the hydrolysis of cGMP, thus promoting cavernosal smooth muscle relaxation. The action of these drugs requires an intact nitric oxide response, as well as constitutive synthesis of cGMP by the smooth muscle cells of the corpora cavernosa. By selectively inhibiting cGMP catabolism in the cavernosal smooth muscle cells, PDE5 inhibitors restore the natural erectile response to sexual stimulation but do not produce an erection in the absence of sexual stimulation.

Clinical Pharmacology. Although the four currently available PDE inhibitors have some structural similarities, they differ in their selectivity and pharmacokinetics (see Table 20.9). The



• **Fig. 20.11** An algorithmic approach to the treatment of men with erectile dysfunction. *AE,* adverse event; *PDE5I,* phosphodiesterase 5 inhibitor.

TABLE 20.8 Goals of Psychosexual Therapy in Men With Sexual Dysfunction

- Reduce performance anxiety; train the couple to avoid "spectatoring" and be "sensate focused"
- Identify relationship problems and improve partner communication and intimacy
- Modify sexual attitudes and beliefs
- Improve couple's sexual skills

Data from from Rosen RC. Psychogenic erectile dysfunction: classification and management. *Urol Clin North Am.* 2001;28(2):269–278.

common adverse effects of the available PDE5 inhibitors—headache, visual problems, flush, and myalgias—are related to nonselective inhibition of PDE isoforms 6 and 11 in other organ systems[108] (see Table 20.9). The selectivity of a PDE5 inhibitor is the ratio of its inhibitory potency for PDE isoforms other than type 5 relative to its inhibitory potency for PDE isoform 5.[108] For PDE6, tadalafil is the most selective and sildenafil is the least selective; for PDE11, vardenafil is the most selective and tadalafil is the least selective.[108] The retinal side effects of sildenafil are related to inhibition of PDE6 in the retina, whereas muscle aches experienced by a small fraction of men using tadalafil are related to inhibition of PDE11 in the skeletal muscle.[108]

Pharmacokinetics. After oral administration of sildenafil, peak plasma concentrations are achieved within 30 to 120 minutes, after which plasma concentrations decline, with a half-life of 4 hours[107,433–442] (see Table 20.9). Vardenafil achieves peak concentrations within 0.7 to 0.9 hour and has a half-life of 4 to 5 hours. The peak concentrations of tadalafil are achieved at 2 hours, and its half-life of 16.9 hours in young men is significantly longer than the half-lives of sildenafil and vardenafil. The half-life of tadalafil is even longer in older men (21.6 hours) than in young men (16.9 hours).[107,433–436] Because of the relatively short half-lives of vardenafil and sildenafil, these drugs should be taken 1 to 4 hours before the planned intercourse; in contrast, tadalafil, because of its longer half-life, does not have to be taken on demand, although it can be.[107,433–436] The second-generation PDE5 inhibitors avanafil and udenafil have a more rapid onset of action than the first-generation PDE5 inhibitors sildenafil, vardenafil, and tadalafil.[437–440]

Food, particularly a high-fat meal and large amounts of alcohol, can delay and decrease the absorption of sildenafil.[441,442] However, early pharmacokinetic studies have not reported changes in maximum serum concentrations or absorption rates of vardenafil or tadalafil due to food or moderate alcohol ingestion.[436]

Efficacy. Orally active, selective PDE5 inhibitors—sildenafil, vardenafil, avanafil, udenafil, and tadalafil—have been shown to be effective and safe in randomized clinical trials of men with ED.[4–6,439–465] In men treated with oral, selective PDE5 inhibitors, the rates of successful intercourse vary from 50% to 65%, and rates of improved erections vary from 70% to 75%.[439–455] Selective PDE5 inhibitors are effective in men of all ethnic groups and ages[439–465] who have ED due to a multitude of causes, although response rates vary in different patient subgroups.[439–465]

Introduced to the U.S. market in March 1998, sildenafil citrate (Viagra; Pfizer, New York, NY) was the first effective oral agent for the treatment of ED.[466] The efficacy of sildenafil has been demonstrated in men with organic, psychogenic, or mixed ED in multiple RCTs[443–450,466] and confirmed by meta-analyses of randomized trials.[446–448] In these trials, patients receiving sildenafil experienced greater increments in the number of successful attempts per month, penile rigidity, frequency of vaginal penetration, and maintenance of erection than those receiving placebo.[446–448] Increasing doses of sildenafil were associated with higher mean scores for the frequency of penetration and maintenance of erections after sexual penetration. The mean scores for orgasms, intercourse satisfaction, and overall satisfaction were also significantly higher in the sildenafil group than in the placebo group.[446–448] Sildenafil also is an effective treatment for ED in men with diabetes mellitus.[449,450] A meta-analysis of randomized clinical trials of sildenafil confirmed its efficacy in improving erectile function in men with diabetes mellitus.[450]

In the vardenafil efficacy trials, 5-, 10-, and 20-mg doses of vardenafil were all superior to placebo in improving erectile function domain scores; the improvements in erectile function scores were dose related.[451–457] Vardenafil improved rates of vaginal penetration, penile rigidity, intercourse success, and satisfaction with sexual experience in men with ED from diverse causes.[451–457]

Similarly, in randomized clinical trials, 2.5-, 5-, 10-, and 20-mg doses of tadalafil were each superior to placebo in improving erectile function scores.[458–462] The beneficial effects of tadalafil were dose related.[458–462]

Two new PDE5 inhibitors have been introduced recently in clinical practice. Avanafil has a very rapid onset of action because of its rapid absorption, which allows it to reach maximum circulating concentration in about 30 to 45 minutes.[437–439] Therefore, a majority of patients taking avanafil are able to engage in sexual activity within 15 minutes.[437–439] Udenafil also has a relatively rapid onset of action, with a time to maximum serum concentrations of 1.0 to 1.5 hours. It has been approved in Korea, Russia, and the Philippines, but not in the United States.[440]

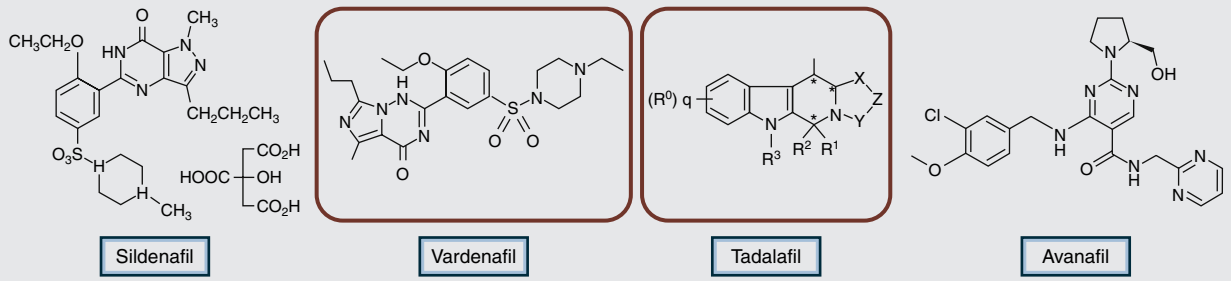
PDE5 inhibitors are effective in men with ED due to a variety of causes, including spinal cord injury and radical prostatectomy.[449,450] In general, baseline sexual function correlates positively with response to PDE5 inhibitors, and patients with diabetes mellitus or previous prostate surgery respond less well than patients with psychogenic or vasculogenic ED.[449,450] Because there is no baseline characteristic that predicts the likelihood of failure to respond to sildenafil therapy, a therapeutic trial of PDE5 inhibitors should be tried in all patients, except in those in whom it is contraindicated.[432]

Adverse Effects. In clinical trials, the adverse effects that have been reported with greater frequency in men treated with PDE5 inhibitors than in those treated with placebo include headaches, flushing, rhinitis, dyspepsia, muscle aches, and visual disturbances[108,432,463–465] (Table 20.11). The occurrence of headache, flushing, and rhinitis, a direct consequence of nonselective PDE5 inhibition in other organ systems, is related to the administered dose. These drugs do not affect semen characteristics.[467,468] No cases of priapism were noted in the pivotal clinical trials.

Several cases of nonarteritic anterior ischemic optic neuropathy have been reported after oral PDE5 inhibitor use.[469,470] This condition is characterized by the sudden onset of monocular visual loss due to acute ischemia of the anterior portion of the optic nerve in the absence of demonstrable arteritis. This may progress to partial or complete infarction of the optic nerve head, resulting in permanent visual loss or visual field cuts.[469,470] Although a cause-and-effect relationship with PDE5 inhibitor use has not been established, patients with history of sudden visual loss should not be treated with PDE5 inhibitors without ophthalmologic evaluation.

TABLE 20.9 Clinical Pharmacology of Selective PDE5 Inhibitors[a]

Feature	Sildenafil	Vardenafil	Tadalafil	Avanafil
Commercial name	Viagra	Levitra	Cialis	Stendra, Spedra
T_{max}	0.5–2.0 h	0.7–0.9 h	2 h	30–45 min
T½	3–4 h	4–5 h	16.9 h (young) 21.6 h (old)	5 h
Onset of erection (min)	30–60	15–45	20–30 min	15 min
Muscle selectivity (ratio of PDE6 IC_{50}/PDE5 IC_{50})	11 (most selective)	25	187 (least selective)	>100-fold
Retinal selectivity (ratio of PDE11/PDE5 IC_{50}), higher number indicates greater PDE5 selectivity	780	1160 (most selective)	5 (least selective)	>10,000-fold
Effect of food and alcohol	C_{max} decreased	Minimal change	No change	Absorption delayed
Protein binding	96%	94%	94%	98–99%
Bioavailability	41%	Not available	15%	Not available



[a]Comparative pharmacokinetic data are provided on the four oral selective PDE5 inhibitors that have been approved by the U.S. Food and Drug Administration. Additional PDE5 inhibitors such as udenafil and mirodenafil have been approved for use in some other countries. Selectivity refers to the ratio of the IC_{50} for a PDE isoform other than PDE5 to the IC_{50} for PDE5. A higher number implies greater selectivity. Sildenafil is more selective than tadalafil for PDE5 relative to PDE11, but it is less selective than tadalafil for PDE6 relative to PDE5.

C_{max}, Maximum plasma concentration; *IC_{50}*, 50% inhibitory concentration; *PDE*, phosphodiesterase; *T ½*, half-life; *T_{max}*, time to peak concentration.

Data from Haning H, Niewohner U, Bischoff E. Phosphodiesterase type 5 (PDE5) inhibitors. *Prog Med Chem.* 2003; 41:249–306; Bischoff E. Potency, selectivity, and consequences of nonselectivity of PDE inhibition. *Int J Impot Res.* 2004;16(Suppl 1):S11–S14; and Saenz de Tejada I, Angulo J, Cuevas P, et al. The phosphodiesterase inhibitory selectivity and the in vitro and in vivo potency of the new PDE5 inhibitor vardenafil. *Int J Impot Res.* 2001;13:282–290.

The U.S. Food and Drug Administration (FDA) has noted several reports of sudden hearing loss with and without vestibular symptoms, such as tinnitus, vertigo, or dizziness, in temporal relationship to administration of sildenafil, vardenafil, and tadalafil in postmarketing surveillance. Hearing loss was also reported in a few patients in clinical trials of these drugs.[471,472] Hearing loss has been noted in patients using sildenafil for the treatment of pulmonary arterial hypertension. Although a causal relationship has not been established, the temporal relationship between the use of PDE5 inhibitors and the onset of sudden hearing loss prompted the FDA to recommend changes in the product labeling for the drug class. A few observational studies have reported an association of PDE5 inhibitor use with an increased risk of melanoma and basal cell carcinoma; however, due to the lack of association between melanoma risk and the number of prescriptions filled, a causal relation has not been established.[473–475]

Cardiovascular and Hemodynamic Effects. In postmarketing surveillance of adverse events associated with sildenafil use, several instances of myocardial infarction and sudden death were reported in men using sildenafil in temporal relation to the ingestion of the drug[476]; many of these deaths occurred in individuals who also were taking nitrates. Because most men presenting with ED also have high prevalence of cardiovascular risk factors, it is unclear whether these events were causally related to the ingestion of sildenafil, underlying heart disease, or both.[476] In controlled studies,[477–480] oral administration of 100 mg of sildenafil to men with severe coronary artery disease produced only a small decrease in systemic blood pressure and no significant changes in cardiac output, heart rate, coronary blood flow, and coronary artery diameter. In a separate pooled analysis of five randomized, placebo-controlled trials of vardenafil,[479] the overall frequency of cardiovascular events was similar in both vardenafil- and placebo-treated men. However, vardenafil treatment was associated with a mild reduction in blood pressure (4.6 mm Hg decrease in systolic blood pressure) and a small increase in heart rate (two beats per minute).[480] This led the American Heart Association and other experts to conclude that preexistent coronary artery disease by itself does not constitute a contraindication for the use of PDE5 inhibitors[459,480–485] (Table 20.12).

Drug-Drug Interactions. Sildenafil is metabolized mostly by the CYP2C9 and the CYP3A4 pathways. Cimetidine and erythromycin, inhibitors of CYP3A4, increase the plasma concentrations of sildenafil. HIV protease inhibitors may also alter the activity of the CYP3A4 pathway and affect the clearance of sildenafil.[486] Conversely, sildenafil is an inhibitor of the CYP2C9 metabolic pathway, and its administration could potentially affect

TABLE 20.10 Guidelines for Optimizing the Use of Selective PDE5 Inhibitors

1. Men with ED are at increased risk for cardiometabolic disorders. Baseline evaluation should include general health evaluation, as well as screening for diabetes and cardiovascular risk. Patients should be counseled for lifestyle modification to improve overall health and reducing cardiometabolic risk.
2. All men with ED can benefit from psychosexual counseling.
3. PDE5 inhibitors are the first-line therapy for ED. The men who are prescribed PDE5 inhibitors should be counseled on their appropriate use guided by their clinical pharmacology.
4. Due to the substantial variation in metabolism of PDE5 inhibitors among men, the dose should be titrated to the maximum tolerated dose to maximize efficacy while avoiding dose-related adverse effects.
5. The metabolism of PDE5 inhibitors may be affected by drug-drug interactions with other drugs and foods that affect the CYP3A4 pathway. These interactions should be considered in dose selection.
6. PDE5 inhibitors may augment the vasodilator effects of nitrates. Therefore, PDE5 inhibitors should not be used in men taking daily doses of nitrates.
7. PDE5 inhibitors may produce hypotension when taken together with other vasodilator drugs, such as α-adrenergic blockers or antihypertensive medications. These interactions should be considered when prescribing PDE5 inhibitors in men who are using α-adrenergic blockers or other vasodilators.
8. In human immunodeficiency virus–infected men, consider potential drug-drug interactions between selective PDE5 inhibitors and antiretroviral drugs and antimicrobial agents.
9. Some men who do not respond to an on-demand PDE5 inhibitor may respond to the use of daily tadalafil, which is a U.S. Food and Drug Administration–approved regimen.

ED, Erectile dysfunction; *PDE5,* phosphodiesterase 5.

Data from Burnett AL, Nehra A, Breau RH, et al. Erectile dysfunction: AUA guideline. *J Urol.* 2018;200(3):633–641.

TABLE 20.11 Adverse Effects of Selective Phosphodiesterase Inhibitors

Common Adverse Effects

1. Headache
2. Flushing
3. Dyspepsia
4. Nasal congestion
5. Dizziness, lightheadedness
6. Back pain[a]
7. Myalgia[a]

Uncommon Adverse Effects

1. Abnormal vision[a]
2. Hearing loss
3. Nonarteritic anterior optic neuropathy

[a]These adverse effects are related to nonselective inhibition of phosphodiesterase isoforms in other tissues. Headache, flushing, and nasal congestion are related to the drug's mechanism of vasodilator action.

Data from Wespes E, Rammal A, Garbar C. Sildenafil no-responders: hemodynamic and morphometric studies. *Eur Urol.* 2005;48:136–139; Brock GB, McMahon CG, Chen KK, et al. Efficacy and safety of tadalafil for the treatment of erectile dysfunction: results of integrated analyses. *J Urol.* 2002;168:1332–1336; Morales A, Gingell C, Collins M, et al. Clinical safety of oral sildenafil citrate (Viagra) in the treatment of erectile dysfunction. *Int J Impot Res.* 1998;10:69–73; Katz EG, Tan RB, Rittenberg D, Hellstrom WJ. Avanafil for erectile dysfunction in elderly and younger adults: differential pharmacology and clinical utility. *Ther Clin Risk Manag.* 2014;10:701–711.

the metabolism of drugs metabolized by this system, such as warfarin and tolbutamide. Combined administration of sildenafil and ritonavir results in significantly higher plasma levels of sildenafil than sildenafil given alone.[486] There are similar interactions with other drugs, including saquinavir and itraconazole. Therefore, the doses of PDE5 inhibitors should be reduced appropriately in men taking protease inhibitors or erythromycin.

Grapefruit juice can alter oral drug pharmacokinetics by different mechanisms. Grapefruit juice given in normal amounts (e.g., 200–300 mL) or as whole fresh fruit segments can irreversibly inactivate intestinal CYP3A4, thus reducing presystemic metabolism and increasing oral bioavailability of PDE5 inhibitors.[487] Although the magnitude of this problem in clinical practice is unknown, it seems prudent to warn men who are contemplating the use of PDE5 inhibitors not to ingest more than a small amount of grapefruit juice.

The most serious interactions of PDE5 inhibitors are with the nitrates. The vasodilator effects of nitrates are augmented by PDE5 inhibitors; this also applies to inhaled forms of nitrates such as amyl nitrate or nitrites that are sold under the street name "poppers." Concomitant administration of the two vasodilator drugs can cause a potentially fatal decrease in blood pressure.[216–218]

PDE inhibitors should be used carefully in men taking α-adrenergic blockers. In men with congestive heart failure or those receiving vasodilator drugs, or those who are using complex regimens of antihypertensive drugs, blood pressure should be monitored after initial administration of PDE5 inhibitors.[216–218] Several trials have demonstrated the safety of administering PDE5 inhibitors in combination with α-adrenergic blockers in men with ED and LUTS.[216–218]

Therapeutic Regimens.[488] Men with ED are at increased risk for cardiometabolic disorders (see Table 20.10). Baseline evaluation should include general health evaluation and screening for diabetes and cardiovascular risk. Patients should be counseled for lifestyle modification to improve overall health and reducing cardiometabolic risk. All men with ED can benefit from psychosexual counseling. PDE5 inhibitors are the first-line therapy for ED. The men who are prescribed PDE5 inhibitors should be counseled on their appropriate use guided by their clinical pharmacology. Due to the substantial variation in metabolism of PDE5 inhibitors among men, the dose should be titrated to the maximum tolerated dose to maximize efficacy while avoiding dose-related adverse effects.

In most men with ED, sildenafil is started at an initial dose of 50 mg. If this dose is not effective yet does not produce any adverse effects, the dose should be titrated up to 100 mg or the maximum tolerated dose.[106,431,432,489,490] The dose adjustment should be guided by the therapeutic response to therapy and occurrence of adverse effects. Vardenafil should be started at an initial dose of 10 mg; the dose should either be increased to 20 mg or the maximal tolerated dose or decreased[490] to 5 mg depending on the clinical response and the occurrence of adverse effects. Tadalafil is started at an initial dose of 10 mg, with further adjustment of dose based on effectiveness and side effects. Tadalafil need not be taken more frequently than once every 48 hours. Avanafil should be started at an initial dose of 50 or 100 mg with further adjustment up to 200 mg. In men taking protease inhibitors (particularly ritonavir and indinavir), erythromycin, ketoconazole, itraconazole, or large amounts of grapefruit juice, the doses of PDE5 inhibitors should be reduced.

TABLE 20.12 Guidelines for the Use of Selective Phosphodiesterase Inhibitors in Men With Cardiovascular Disease

1. Do not administer selective PDE5 inhibitors to men taking long- or short-acting nitrate drugs on a regular basis.
2. If the patient has stable coronary artery disease, is not taking long-acting nitrates, and uses short-acting nitrates only infrequently, the use of selective PDE5 inhibitor should be guided by consideration of risks.
3. Do not administer selective PDE5 inhibitors within 24 hours of the ingestion of any form of nitrate.
4. Advise men about the risks of the potential interaction between selective PDE5 inhibitors and nitrates, nitrate donors, and α-adrenergic blockers. Concurrent use of nitrates, nitrate donors, or α-adrenergic blockers could result in hypotension that could be serious.
5. In men with preexisting coronary artery disease, assess the risk of inducing cardiac ischemia during sexual activity before prescribing PDE5 inhibitors. This assessment may include a stress test.
6. In men who are taking vasodilators and diuretics for the treatment of hypertension or congestive heart failure, consider the potential risk of inducing hypotension because of potential interaction between PDE5 inhibitors and vasodilators, especially in patients with low blood volume.

PDE5, Phosphodiesterase isoform 5.

Data from Cheitlin MD, Hutter AM Jr, Brindis RG, et al. Use of sildenafil (Viagra) in patients with cardiovascular disease: Technology and Practice Executive Committee [published erratum appears in *Circulation.* 1999;100(23):2389]. *Circulation.* 1999;99(1):168-177; Lue TF, Giuliano F, Montorsi F, et al. Summary of recommendations on sexual dysfunctions in men. *J Sex Med.* 2004;1:6-23.

Sildenafil and vardenafil are taken at least 1 to 2 hours before sexual intercourse and not more than once in any 24-hour period; because of its longer half-life, tadalafil need not be taken immediately before intercourse. Avanafil is taken 30 minutes before sexual intercourse.

Based on the results of the randomized clinical trials,[468,491,492] the FDA has approved 2.5 or 5 mg of tadalafil once daily for the treatment of ED. In the pivotal trials, men using 2.5 or 5 mg of tadalafil once daily experienced greater improvements in erectile function compared with those taking a placebo.[468,491,492] Subsequent open-label extension studies indicated that once-daily 5-mg tadalafil for up to 2 years was effective in maintaining improvements in erectile function.[468,491,492] The adverse events associated with once-daily administration of tadalafil included headaches, indigestion, back pain, muscle aches, nasal congestion, and flushing and were similar to those observed with on-demand tadalafil ingestion.[468,491,492] Once-daily regimen of tadalafil had no significant effect on semen or reproductive hormone levels.[468]

Use of PDE5 Inhibitors in Men With Coronary Artery Disease.[485] Excellent therapeutic guidelines have been published on this topic by expert panels from several societies.[106,431,432,481,490] To minimize the risk of hypotension and adverse cardiovascular events during the use of PDE5 inhibitors, the American Heart Association/American College of Cardiology has published a list of recommendations (see Table 20.12), which should be followed rigorously.[481]

Before prescribing PDE5 inhibitors, cardiovascular risk factors should be assessed. If the patient has hypertension or symptomatic coronary artery disease, the treatment of those clinical disorders should be addressed first.[485] The use of nitrates must be ascertained, because PDE5 inhibitors are contraindicated in individuals taking any form of nitrates regularly. PDE5 inhibitors should not be used within 24 hours of the use of nitrates or nitrate donors.[5,6,484,485]

Sexual activity can induce coronary ischemia in men with preexisting coronary artery disease[482]; therefore, men contemplating use of ED therapies should undergo assessment of their exercise tolerance. One practical way to assess exercise tolerance is to have the patient climb one or two flights of stairs. If the patient can safely climb one or two flights of stairs without angina or excessive shortness of breath, he can likely engage in sexual intercourse with a stable partner without inducing similar symptoms. Exercise testing may be indicated in some men with significant heart disease to assess the risk of inducing cardiac ischemia during sexual activity.[481–483] Selective PDE5 inhibitors have been shown not to impair the ability of patients with stable coronary artery disease to engage in exercise at levels equivalent to that attained during sexual intercourse.[481–483] Similarly, each of the three PDE5 inhibitors has been shown not to have significant adverse effects on hemodynamics and cardiac events in carefully selected men with ED who did not have any contraindication for the use of PDE5 inhibitors.[459,479–485] None of the PDE5 inhibitors adversely affects total exercise time or time to ischemia during exercise testing in men with stable angina.[459,479–485] In one recent analysis, treatment with PDE5 inhibitors for ED after a first myocardial infarction was associated with a reduced mortality and hospitalization for heart failure.[493] Another cohort study also reported reduced rates of myocardial infarction or other CVD events during the first 3 years after initiation of treatment with a PDE5 inhibitor for ED.[494]

Treatment of Patients Who Do Not Respond to PDE5 Inhibitors. Although oral PDE5 inhibitor therapy has revolutionized the management of ED, not all men respond to this treatment. The cumulative probability of intercourse success with sildenafil citrate increases with the number of attempts, reaching a maximum after eight attempts.[495] Based largely on these data,[495] the failure to respond to PDE inhibitor therapy has been defined as the failure to achieve satisfactory response even after eight attempts of either the highest approved dose (e.g., 100–mg sildenafil) or the highest tolerable dose of PDE5 inhibitor, whichever is lower. Many factors may contribute to apparent treatment failure, including failure to take the medication as recommended, suboptimal dose, dose-limiting adverse effects, absence of sexual stimulation, psychological issues, partner and relationship issues, incorrect diagnosis, and patient-specific pathophysiologic factors.[495–497] In clinical trials of PDE5 inhibitors, treatment failures were reported predominantly in men who had diabetes mellitus, non–nerve-sparing radical prostatectomy, cavernosal nerve damage, venous leak, and high disease severity.[495–497] In an evaluation of cavernosal smooth muscle biopsies in sildenafil nonresponders, Wespes and coworkers[497] found severe vascular lesions, as well as cavernosal smooth muscle atrophy and fibrosis, to be the underlying pathologic processes.

Patients may not take the medication appropriately because of inadequate instructions, failure to understand the instructions, adverse effects, or fear of adverse effects.[495–497] Oral PDE5 inhibitors are taken optimally 1 to 2 hours before planned intercourse. The medication is unlikely to be effective if it is taken immediately before intercourse; a high-fat meal and large amounts of alcohol may further affect the maximal serum concentrations of sildenafil citrate. Similarly, patients may not take the appropriate dose because of side effects or fear of side effects. The men who have been misdiagnosed as having ED and whose primary sexual

disorder is unresponsive to PDE5 inhibitors may be incorrectly deemed treatment failures. For instance, men with HSDD, Peyronie disease, or orgasmic or ejaculatory disorder would not be expected to respond to PDE5 inhibitors. The anxiety associated with resumption of sexual activity and unresolved relationship and partner issues can attenuate response to treatment. The sexual partner may not be willing or able to engage in sexual activity because of relationship issues, sexual disorder, or real or perceived health issues. Because erotic stimulation is necessary for local production of nitric oxide, which then stimulates the production of cGMP, PDE5 inhibitors do not induce spontaneous erections and may not be effective in inducing erections in the absence of sexual stimulation.

Patients who report lack of satisfactory response to initial administration of PDE5 inhibitors should be asked about the time of drug administration, the dose taken, and adverse effects experienced. Psychological and partner issues should be evaluated. The dose of PDE5 inhibitor should be increased gradually as tolerated. Some men who do not optimally respond to on-demand PDE5 inhibitors may respond to daily low-dose tadalafil (2.5 or 5 mg), which is an FDA-approved regimen.[498–500] Should the patient not respond to maximal tolerable doses of PDE5 inhibitors, the PDE5 inhibitors can be combined with vacuum devices or intraurethral therapy. Second-line therapies, such as intracavernosal injections, should be pursued. The men who are unresponsive to oral PDE5 inhibitors and second-line therapies may find penile implant an acceptable alternative.[495]

Cost-Effectiveness of Phosphodiesterase 5 Inhibitor Use for Erectile Disorder. Several studies have evaluated the economic cost of treating ED in men in managed care health plans.[501–505] One simulation estimated sildenafil citrate cost to be approximately $11,000 per quality-adjusted life year.[503] This amount is less than that for many other accepted treatments for medical disorders that cost $50,000 to $100,000 per quality-adjusted life year; thus, the cost-effectiveness of PDE5 inhibitor therapy compares favorably with other accepted medical therapies. Other analyses have concluded that PDE5 inhibitors and vacuum constriction devices are the most cost effective of all available therapeutic options.[501–505] Several recent analyses have shown that the financial burden imposed by patients with ED on managed care plans is surprisingly small.[501–505] In one such cost-utility analysis, the monthly cost of providing ED-related treatment services in a health plan with 100,000 members amounted to less than $0.10 per member.[503] Thus, the failure of many insurance companies to cover the cost of PDE5 inhibitor therapy is not informed by cost-utility analyses.

Second-Line Therapies

Vacuum Devices for Inducing Erection

The vacuum devices typically consist of an acrylic cylinder, a vacuum pump, and an elastic constriction band.[506–508] The cylinder fits over the penis and is connected to a vacuum pump. The negative pressure created by the vacuum within the cylinder draws blood into the penis, producing an erection. An elastic band slipped around the base of the penis traps the blood in the penis, maintaining an erection as long as the rubber band is retained. The constriction band should not be left in place for more than 30 minutes. In addition, only vacuum devices with a pressure-limiting mechanism should be recommended to prevent injury due to high vacuum.

Limited data on the efficacy of vacuum devices from open-label trials indicate that these devices are safe, relatively inexpensive, and moderately effective.[506–508] They can impair ejaculation, resulting in entrapment of semen. Some couples dislike the lack of spontaneity engendered by the use of these devices. Partner cooperation is important for successful use of these devices.[508]

Intraurethral Therapies

An intraurethral system for delivery of alprostadil called *MUSE* (medicated urethral system for erection; VIVUS, Menlo Park, CA) was released in 1997. Alprostadil is a stable, synthetic form of PGE_1, which stimulates generation of cAMP and activation of protein kinase A. Activated protein kinase A stimulates K^+ channels, resulting in K^+ efflux from the cell. In addition, protein kinase A–mediated processes result in a net decrease in intracellular calcium, favoring smooth muscle cell relaxation.

Alprostadil, when applied into the urethra, is absorbed through the urethral mucosa into the corpus cavernosum. In comparison to intracavernosal injection of PGE_1, intraurethral alprostadil is easier to administer and has a lower frequency of adverse effects, particularly penile fibrosis.

Alprostadil is available in 125-, 250-, 500-, and 1000-µg strengths. Typically, the initial alprostadil dose of 250 or 500 µg is applied in the clinician's office to observe changes in blood pressure or urethral bleeding secondary to misapplication of the device into the urethra.

Initial randomized, placebo-controlled studies reported 40% to 60% success rates, defined as having at least one successful sexual intercourse during a 3-month study period.[509–511] In clinical practice, only about a third of men using intraurethral alprostadil will respond.[512]

Common side effects of intraurethral alprostadil are penile pain and urethral burning in up to 30% of patients[509–512]; its use also may cause dizziness, hypotension, and syncope in some users. Intraurethral alprostadil can cause mild burning or itching in the vagina of the sexual partner. Intraurethral alprostadil should not be used by men whose partners are pregnant or planning to get pregnant.

Intracavernosal Injection of Vasoactive Agents

The use of intracavernosal injections of vasoactive agents has been a cornerstone of the medical management of ED since the early 1980s (Table 20.13). Patients can be taught to inject a vasoactive agent into their corpora cavernosa using a 27- or 30-gauge needle prior to the planned intercourse. Erections occur typically 15 minutes after intracorporal injection and last 45 to 90 minutes. Although intracavernosal injection therapy is highly effective,[513–520] it is associated with higher complication rates than oral therapy and should be used only by practitioners who are experienced in the use of this therapy and who can provide emergency medical support to their patients in the event of a serious adverse event, such as priapism.

Although several different agents—PGE_1, papaverine, and phentolamine—have been used alone or in combination,[513–521] only intracavernosal PGE_1 has been approved for clinical use. The long-term data on the efficacy and safety of intracavernosal therapy are sparse.

Several formulations of alprostadil (PGE_1) are commercially available (Caverject, Pharmacia, Stockholm, Sweden; Prostin VR, Pharmacia; Edex, Schwarz Pharma, Milwaukee, WI). PGE_1 binds to PGE_1 receptors on the cavernosal smooth muscle cells, stimulates adenylyl cyclase, increases the concentrations of cAMP, and is a powerful smooth muscle relaxant. The usual dose is 5 to 20 µg, and response to therapy is dose related and should be titrated.[513–519]

In one placebo-controlled efficacy trial, the intracavernosal alprostadil injection resulted in satisfactory sexual performance

TABLE 20.13 Guidelines for Intracavernosal Therapy

1. Do not prescribe intracavernosal therapy to men who have psychiatric disorders, hypercoagulable states, or sickle cell disease; those who are receiving anticoagulant therapy; or those who are unable to comprehend the risks or take appropriate action should complications occur.
2. Designate a physician or a urologist to be available to handle emergencies related to complications of intracavernosal injections, such as prolonged erection and priapism.
3. Instruct the patient in the injection technique, the risks of intracavernosal therapy, and the steps to be taken in the event of prolonged erection or priapism.
4. Administer the first injection in the office and observe the blood pressure and heart rate response. This provides an excellent opportunity for educating the patient, observing adverse effects, and determining whether the patient will respond to intracavernosal therapy.
5. Start with a low dose of alprostadil, and titrate the dose based on the erectile response and the duration of erection. Adjust the dose of alprostadil to achieve an erection that is sufficient for sexual intercourse but that does not last more than 30 minutes.
6. If the erection does not abate in 30 minutes, the patient should be instructed to take a tablet of pseudoephedrine or terbutaline, or an intracavernosal injection of phenylephrine. If this is not effective, the patient should call the designated physician or the urologist, and go to the emergency room.

after more than 90% of administrations, and approximately 85% of men and their partners reported satisfactory sexual activity.[513] Intracavernosal alprostadil is more effective than intraurethral alprostadil.[517]

The common adverse effects of intracavernosal therapy include penile pain, hematoma, formation of corporal nodules, penile fibrosis, and prolonged erections.[513–520] Despite the effectiveness of this approach in producing rigid erections, many patients do not relish injecting a needle into their penis; therefore, it is not surprising that long-term dropout rates are high. Intracavernosal injections should not be used in patients with sickle cell disease, multiple myeloma or leukemia, hypercoagulable disorder, anatomic deformation of the penis, or penile implants.

Intracavernosal injections of papaverine, phentolamine, forskolin, and VIP have also been used, although these agents are not approved by the FDA.[521] Papaverine, derived originally from the poppy seed, is a nonspecific PDE inhibitor, which increases both intracellular cAMP and cGMP. It has a greater propensity to induce priapism and fibrosis with long-term use, and efficacy and long-term safety data from randomized, placebo-controlled trials are lacking. Therefore, there is insufficient information to evaluate its efficacy and safety.

Phentolamine is a competitive α_1- and α_2-adrenergic antagonist that contributes to smooth muscle relaxation. As a single agent it is minimally efficacious, but it has been used to potentiate the effects of papaverine, VIP, and PGE_1.[521] Randomized clinical trial data on its efficacy and safety are lacking. Therefore, there is insufficient information to evaluate its efficacy and safety.

Various combination of these drugs are available as "bi-mix" or tri-mix" depending on the number of drugs included in the formulation. Tri-mix, a combination of papaverine, phentolamine, and PGE_1, obtained from compounding pharmacies, is often used in urologic practices even though it is not an FDA-approved drug.

Priapism is a serious concern with the use of intracavernosal injection therapy. In patients who develop a prolonged or painful erection with PGE_1, either 5 mg of brethane or 60 mg of pseudoephedrine, administered orally, may be of benefit. If priapism persists longer than 4 hours, patients should be instructed to seek medical care in which aspiration alone or with the injection of an α-adrenergic agent is used to induce detumescence. If this fails, surgical therapy may be needed to reverse a prolonged erection; otherwise, anoxic damage to the cavernosal smooth muscle cells and fibrosis can occur.

Third-Line Therapies

Penile Prosthesis

The penile prostheses are invasive and costly, but they can be an effective method for restoring erectile function for patients with advanced organic disease who are unresponsive to other medical therapies, have significant structural disorders of the penis (e.g., Peyronie disease), or have suffered corporal loss from cancer or traumatic injury.[522–524]

Penile implants are paired supports that are placed in each of the two erectile bodies. There are two basic types of penile implants: (1) hydraulic (fluid filled), referred to as inflatable prostheses, and (2) malleable, semirigid rods, which are bendable but always remain firm in the penis.[522–524] Penile prostheses come in a variety of lengths and girths. Implantation surgery usually takes less than an hour and in most cases can be done as an outpatient procedure under general or regional anesthesia.

Infection, perforation of the corpora during device placement, extrusion or migration of the device, and mechanical malfunction are the most common problems with penile prostheses. With recent improvements in materials and design, the chance of mechanical malfunction has decreased to 5% to 10% in the first 10 years.[522–524] Infection occurs in 1% to 3% of cases, but infection rates can be higher in revision surgery, especially in men with diabetes mellitus.

The total cost of penile prosthesis implantation varies from $3,000 to $20,000, depending on the type of device used and the community in which the procedure is performed. There are no randomized efficacy trials, but retrospective analyses have reported that greater than 80% of patients and 70% of partners are pleased with their prosthesis.[522–525]

Testosterone Replacement in Androgen-Deficient Men Presenting With Erectile Dysfunction

Randomized trials have shown that testosterone replacement therapy improves erectile function, sexual desire, overall sexual activity, and satisfaction with intercourse in hypogonadal men who have low sexual desire; meta-analyses of randomized trials have confirmed these findings. Testosterone treatment does not improve sexual function in men with ED who have normal testosterone levels.[27,65,143,144,160] It is not known whether testosterone replacement improves sexual function in men with ED and borderline serum testosterone levels. Not all men with ED and low testosterone levels will experience improvements in their libido and overall sexual activity with testosterone replacement therapy;[27,65,143,144] the response to testosterone therapy even in this group of men is variable because of the coexistence of other disorders, such as diabetes mellitus, hypertension, CVD, and psychogenic factors.[27,143,144,160]

ED in middle-age and older men is a multifactorial disorder, often associated with other comorbid conditions such as diabetes mellitus, hypertension, CVD, medications, peripheral vascular disease, psychogenic factors, and chronic kidney disease.

Therefore, it is not surprising that testosterone treatment alone may not improve sexual function in all men with androgen deficiency. Testosterone induces NOS activity,[137,138] has trophic effects on cavernosal smooth muscle and ischiocavernosus and bulbospongiosus muscles,[139] increases penile blood flow,[163] and is essential for achieving venous occlusion.[136] These observations have led to speculation that testosterone might improve response to PDE5 inhibitors; however, as discussed earlier, data from randomized trials have not shown the superiority of testosterone over placebo in improving erectile function in men with ED who have low testosterone levels and in whom the PDE5 inhibitor dose has been optimized.[166] However, it is possible that in men with ED who have testosterone deficiency, correcting testosterone deficiency first by testosterone replacement therapy may augment subsequent response to PDE5 inhibitors. In addition, testosterone replacement therapy in hypogonadal men with ED may improve libido and overall satisfaction with sexual experience, and may have other beneficial effects in correcting anemia, as well as improving bone mineral density and bone strength.

Therapies With Either Unproven Efficacy or Limited Efficacy Data

There are insufficient efficacy data to support the use of trazodone[526] or yohimbine[527] in men with ED. The literature on the effectiveness of herbal therapies is difficult to interpret because of lack of consistency in product formulations and potencies, contamination of herbal products with PDE5 inhibitors, poor trial design, and paucity of randomized clinical trial data.[528–532] One randomized trial of Korean red ginseng reported this product to be effective in the treatment of ED[529]; these data need further confirmation. Icariin is a flavonoid, derived from several species of plants, whose extracts have been known in herbal medicine to produce aphrodisiac effects and enhance erectile function.[532] Dipyridamole also inhibits PDE5 and can augment the effects of nitric oxide. 4-Methylpiperazine and pyrazolopyrimidine, components of the lichen *Xanthoparmelia scabrosa*, have also been claimed to inhibit PDE5.[531] The use of these or other herbal therapies is not recommended.[531] Apomorphine also functions as a dopamine agonist and acts centrally to initiate erection; its main adverse effects are nausea and dizziness.

Gene Therapy and Erectile Dysfunction

The goal of gene therapy for ED is to introduce novel genetic material into the cavernosal smooth muscle cells to restore normal cellular function and produce a therapeutic effect.[533–535] Gene therapy has been proposed as a treatment option for diseases that have a vascular origin, such as arteriosclerosis, congestive heart failure, and pulmonary hypertension.[533–535] ED may be particularly amenable to gene therapy because of the easily accessible external location of the penis,[533–535] which permits direct injection into the corpora cavernosa. A tourniquet placed around the base of the penis limits entry of the injected material into the systemic circulation. This is a distinct advantage of the gene therapy of penile diseases over gene therapy for other systemic diseases, because introduction of the genetic material into the systemic circulation can potentially induce adverse systemic effects due to insertion of the material into an incorrect organ or vascular bed. Additionally, in the penis, only a small number of cells need to be transfected, because the interconnection of smooth muscle cells in the corpus cavernosum by gap junctions allows second messenger molecules and ions to be transferred to other interconnected smooth muscle cells.[533–535] The low turnover rate of the vascular smooth muscle cells of the penis allows the desired gene to be expressed for long periods of time.

The strategies of gene therapy for ED treatment have focused on the molecules that regulate corporal smooth muscle relaxation or neovascularization[533–535] (Table 20.14). Several candidate genes have been explored, including the maxi-K^+ channel, penile-inducible NOS, eNOS, VIP, CGRP, vascular endothelial growth factor (VEGF), the brain neurotrophic factor angiopoietin 1, neurturin (a member of the glial cell line–derived neurotrophic factor family), superoxide dismutase, IGF1, PKG1α, and Rho A/Rho kinase[533–543] (see Table 20.14). Several vectors have been used to transfer exogenous genes, including adenoviruses, adeno-associated viruses, retroviruses, sindbis viruses, replication-deficient retroviruses, liposomes, naked DNA, and gold nanoparticles.[533–549]

Garban and associates[536] first demonstrated that gene therapy can be performed in the penis by utilizing naked complementary DNA (cDNA) encoding the penile-inducible *NOS* gene leading to physiologic benefit in the aging rat. Christ and colleagues[537] injected hSlo cDNA (which encodes the human smooth muscle maxi-K^+ channel) into the rat corpora cavernosa and demonstrated increased gap junction formation and enhanced erectile responses to nerve stimulation in the aged rat. Adenoviral constructs encoding the eNOS and CGRP cDNAs were shown to reverse age-related ED in rats.[538,539] In these studies, both eNOS and CGRP expression were sustained for at least 1 month in the corpora cavernosa of the rat penis. Five days after transfection with the AdCMVeNOS or AdRSVeNOS viruses, aged rats had significant increases in erectile function as determined by response to cavernosal nerve stimulation and pharmacologic injection of endothelium-dependent vasodilator acetylcholine and PDE5 inhibitors.[538,539] In one study, intracavernosal injection of adeno-associated virus construct carrying the brain-derived neurotrophic factor gene improved erectile function after cavernosal nerve injury.[543] This neurotrophic factor purportedly restored neuronal NOS in the major pelvic ganglion, thus enhancing the recovery of erectile function after bilateral cavernous nerve injury.[543] In other studies, intracavernosal VEGF injection and adeno-associated virus-mediated VEGF gene therapy were each shown to reverse venogenic ED in rats.[540,541] These other preclinical studies using gene therapy targets such as CGRP, superoxide dismutase, and Rho A/Rho kinase provided proof of concept that in vivo gene transfer can be accomplished technically. The translation of these preclinical data into human trials has been slow and unsuccessful so far.

Ion Channel Innovations Inc. has completed a phase I trial of slow K^+ channel gene therapy in men with ED.[99,545,546] In this trial, hMaxi-K, a "naked" DNA plasmid carrying the human cDNA encoding for the gene for the α-subunit of the human smooth muscle maxi-K^+ channel, was injected directly into the penises of 11 men with ED. Patients who received the highest dose of hMaxi-K experienced significant improvements in their erectile function that was sustained for the 24-week duration of the trial. This trial demonstrated the feasibility and safety of injecting naked DNA into the human penis.[545,546] A trial of hMaxi-K in patients with overactive bladders is ongoing. Phase I gene therapy trials using VEGF and hepatocyte growth factor have been conducted in patients with peripheral vascular disease and chronic limb ischemia; these trials have reported low frequencies of serious adverse effects. However, phase II studies have not confirmed

TABLE 20.14 Physiologic Targets for Gene Therapy

Gene Target	Vector and Mechanism	Reference
Nitric oxide isoforms	Increase eNOS, nNOS, and iNOS activity in the cavernosal smooth muscle	Champion 1999; Bivalacqua 2000, 2003, 2005; Gonzalez-Cadavid 2004; Kendirci 2005; Wessels 2006
PIN	Antisense and short hairpin RNA constructs targeting PIN	Magee et al., 2007
Maxi-K^+ channel	Transfer of maxi-K^+ channels using a plasmid vector that carries the *hSlo* gene encoding the α-subunit of the maxi-K^+ channel; first human trial demonstrated the safety and feasibility of gene therapy in humans	Christ et al., 2002, 2004, 2004; Melman 2003, 2005, 2006, 2007, 2008; So et al., 2007
PKG1α	Replication-deficient recombinant adenoviruses carrying PKG1α	Bivalacqua et al., 2007
VEGF	Transfer of VEGF cDNA into rat corpora cavernosa to promote neovascularization	Rogers et al., 2003; Buchardt et al., 2005; Dall'Era et al., 2008
Angiopoietin 1	Adenovirus-mediated transfer of human angiopoietin 1	Ryu et al., 2006; Jin et al., 2010
BDNF	Transfer of BDNF using adeno-associated virus	Rogers et al., 2005; Gholani et al., 2003
Neurotrophin 3 gene	Transfer of neurotrophin 3 gene using HSV vector	Bennett et al., 2005
Neurturin	Neurturin, a member of glial cell line–derived neurotrophic factor family	Kato et al., 2009
VIP	Transfection of corpora cavernosa of streptozotocin-treated diabetic rats using pcDNA3 carrying VIP cDNA	Shen et al., 2005
CGRP	Adenoviral transfer of CGRP in aged rats	Bivalacqua et al., 2001; Deng 2004
Superoxide dismutase	Adenoviral-mediated gene transfer of extracellular superoxide dismutase injected into the corpora cavernosa	Bivalacqua 2003; Brown 2006; Lund 2007
IGF1	Adenoviral-mediated gene transfer of IGF1	Pu et al., 2007

BDNF, Brain-derived neurotrophic factor; *cDNA,* complementary DNA; *CGRP,* calcitonin gene–related peptide; *HSV,* human syncytial virus; *IGF1,* insulin-like growth factor 1; *NOS,* nitric oxide synthase (epithelial [e], inducible [i], or neuronal [n] isoforms); *PIN,* protein inhibitor of NOS; *PKG,* protein kinase G; *VEGF,* vascular endothelial growth factor; *VIP,* vasoactive intestinal protein.

Data from Melman A, Davies K. Gene therapy for erectile dysfunction: what is the future? *Curr Urol Rep.* 2010;11(6):421–426; Harraz A, Shindel AW, Lue TF. Emerging gene and stem cell therapies for the treatment of erectile dysfunction. *Nat Rev Urol.* 2010;7(3):143–152; Strong TD, Gebska MA, Burnett AL, et al. Endothelium-specific gene and stem cell-based therapy for erectile dysfunction. *Asian J Androl.* 2008;10(1):14–22; Deng W, Bivalacqua TJ, Hellstrom WJ, Kadowitz PJ. Gene and stem cell therapy for erectile dysfunction. *Int J Impot Res.* 2005;17(Suppl 1):57–63.

efficacy. Thus, the early therapeutic promise of gene therapy has yet to be realized. Successful gene therapy may require introduction of multiple gene products using vectors with higher efficiency of transfection of a larger number of target cells and more prolonged action than can be realized with the current generation of vectors. Additional concerns about long-term safety, sustained gene expression, and efficiency of gene delivery and expression remain to be fully addressed.

Potential of Stem Cell Therapy for Erectile Dysfunction

The past decade has seen considerable interest in the transplantation of stem cells derived from bone marrow, adipose tissue, or skeletal muscle into the corpora cavernosa.[550–558] However, even when injected within the corpora cavernosa, the stem cells escape rapidly from the penis and hone into the bone marrow.[550–558] The mechanism of the reported improvements in intracavernosal pressure after stem cell injection into the corpora cavernosa remains unclear.[553] The safety and efficacy of stem cell therapy in humans has yet to be demonstrated. It remains to be demonstrated whether transplanted human mesenchymal stem cells (hMSCs) can differentiate into functional cavernosal smooth muscle cells and restore erectile capacity in men with ED. In addition, long-term outcomes including the tumorigenic potential of these transplanted progenitor cells are unknown.

hMSCs may also be attractive gene delivery vehicles because these cells can replicate in vitro as well as in vivo, thus potentially providing a large pool of cells.[554,555] Initial studies have demonstrated that rat mesenchymal stem cells, expanded and transfected ex vivo and implanted into the corpora cavernosa, are capable of expressing the gene product of interest.[550–555] Stem cell therapy using stem cells carrying angiogenic or neurotrophic genes or proteins is also being explored. Although several animal studies have reported improved erectile function with hMSC transplantation, few studies have shown evidence of long-term stem cell survival in the cavernosal smooth muscle or evidence of differentiation of the transplanted stem cells into endothelial cells or cavernosal smooth muscle cells.[550–558]

Management of Retrograde Ejaculation

Case reports have claimed benefit from methoxamine, imipramine, midodrine, and ephedrine; however, randomized clinical trial data are lacking.[559–561] Induction of fertility in men with retrograde ejaculation may require retrieval of sperm from the urinary bladder after sexual stimulation or electrostimulation of the prostatic nerve plexus per rectum plus assisted reproductive techniques, such as intrauterine insemination or in vitro fertilization with or without intracytoplasmic sperm injection using the retrieved sperm.[562–565]

Management of Sexual Dysfunction in Women

Psychotherapeutic methods are the mainstay of management of female sexual dysfunction (Fig. 20.12). A systematic review of literature on the psychogenic aspects of sexual dysfunction by the 2016 International Consultation on Sexual Medicine committees found strong literature support for the routine investigation of personal, interpersonal, and contextual factors in women.[566] This type of assessment facilitates treatments such as cognitive behavioral therapy (CBT), mindfulness-based cognitive therapy (MBCT), and sex therapy approaches.

Management of Low Desire and Arousal in Women

In the management of SIAD, the circular model of sexual response (see Fig. 20.1) can serve as a useful basis for discussing which areas are problematic with one, or preferably both, partners (Table 20.15; see Fig. 20.1. When insufficient emotional intimacy is identified, the normality of low interest to be sexual can be clarified, and referral for couple counseling may be indicated. When the lack of sexual context and stimuli are contributing factors, simply emphasizing the requirement of appropriate environment and sufficiently erotic stimuli is usually sufficient, but referral to a sex therapist may be appropriate. Nonsexual distractions, fears about sexual outcome, self-monitoring of sexual response, anxiety, low self-image, and depression can interrupt the mental appraisal of stimuli. These issues can be explained and addressed. The main modalities of treatment for SIAD are cognitive therapy, sex therapy, and psychoeducation (see Table 20.15).

Psychoeducation

Psychoeducation includes providing information while simultaneously soliciting the woman's thoughts and feelings, which are then addressed and processed. It also includes dispelling widespread myths about sexuality in women (e.g., the absence of desire preceding sexual activity denotes sexual dysfunction). Bibliotherapy with self-help reading material or videos can be helpful. For some women, information on anatomy and physiology is necessary.

Cognitive Behavioral Therapy

CBT can assist the woman to recognize, challenge, and, in time, change her negative and often catastrophic self-view imposed by underlying illness, endocrine problems, or infertility. Some of the exaggerated or catastrophic thoughts amenable to cognitive therapy include "sex is only for healthy women," "I am no longer fertile, so I am no longer sexually attractive," and "if intercourse is painful, then no one will want me." Identifying these biased/maladaptive thoughts is the first step: CBT then attempts to practice changing such thoughts. A growing body of data has provided empiric support for the benefit of targeting cognitions and emotions during sex to increase physical and subjective arousal.[39,567–570]

Mindfulness-Based Cognitive Therapy

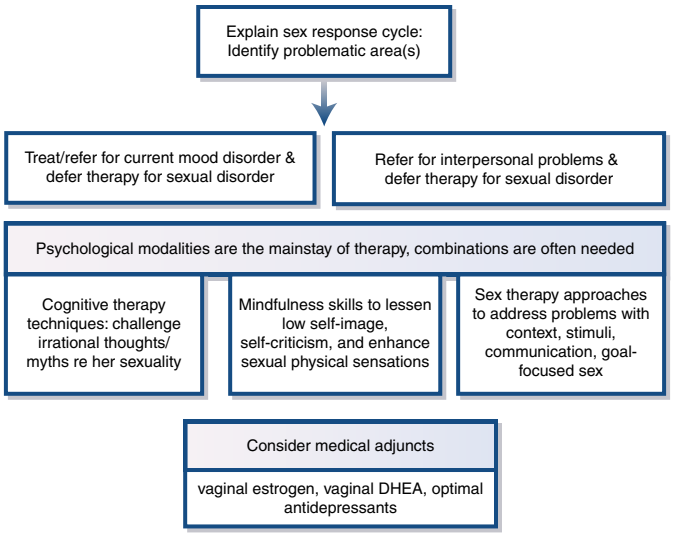
Mindfulness has been shown to be of benefit for women with sexual dysfunction,[568,569,571] who are medically healthy, women with pelvic cancer,[570] and in those with PVD.[571] Mindfulness is an Eastern practice of meditation and relatively new to Western medicine: the learned skill is to be fully present and accepting of all that is sensed in that moment. Attention is enhanced by the gradual ability to identify thoughts arising in that moment, be they future- or past oriented, and not to engage in them but ultimately view these negative or positive thoughts more distantly, as if they are just sensations not dissimilar to physical sensations. The practice of mindfulness focuses the attention on and acceptance of sexual sensations rather than on self-monitoring. Functional brain imaging performed before and after mindfulness training supports the clinical finding that such training lessens self-referencing of sensations and emotions, including pain and anxiety.[573–575] Resources for mindfulness practice in the community and through the internet can be offered.

Recently, an adapted form of CBT blended with mindfulness was defined—the treatment is called *mindfulness-based cognitive therapy*, or MBCT. Regular mindfulness practice is an integral component of MBCT. As with CBT, the skill of detecting maladaptive thoughts, including those that are critical or evaluative, is learned but with simple observation of their presence and an acknowledgment that they are just mental events, not necessarily the truth; there is no focus here to change thoughts. MBCT has been used to treat anxiety disorders and depression and has been adapted to treat arousal and desire disorders and chronic pain of PVD.[568,569,572,576]

Detailed treatment manuals for CBT and MBCT for clinicians with separate versions for patients have been developed; these manuals provide sufficiently detailed information for non–mental health professionals and non–sexual medicine specialists to provide CBT and MBCT to small groups of women. This small group format is consistently rated as beneficial.[577]

Sex Therapy

Sex therapy usually focuses on sensate focus exercises whereby each partner takes turns giving and receiving sensual and, later on, sexual touches, caresses, and kisses. Initially, genital areas and breasts are off-limits. The idea of any goal or expectation



• **Fig. 20.12** Algorithmic approach to the management of female sexual dysfunction. Sexual dysfunction in women is strongly linked to mental health. Therefore, evaluation of mental health and relationship issues is an important component of the evaluation of women with sexual dysfunction. Psychological therapies are the mainstay of treatment of women's sexual disorders with growing evidence of benefit from mindfulness-based cognitive therapy. Topical estrogens or vaginal dehydroepiandrosterone (DHEA) may be useful in a subset of women with menopausal urogenital symptoms.

TABLE 20.15 Management of Sexual Interest and Arousal Disorder in Women

Problematic Phase of SRC	Details of Phase Problem	Management Options
Sexual context problematic	Interpersonal	Explain usual need for emotional closeness +/– counseling referral
	Environmental	Explain usual need for privacy, personal preferences regarding surroundings
Sexual stimuli insufficient	Nonphysical, physical nongenital, genital nonpenetrative	Give information, ↑ sexual communication, consider sex therapy or referral
	Deterrents (e.g., partner's hygiene, alcohol use)	Information, consider couple counseling referral
Processing of stimuli problematic	Depression/dysthymia	Refer to mental health
	Fatigue-chronic illness, poor sleep	Non–goal-oriented sex, address sleep
	Too late in day	Explanation
	Chronic pain	Time pain meds, +MBCT for pain Rx
	Medications: opioids, SSRIs, SNRIs	Consider vortioxetine, bupropion, mirtazapine, vilazodone
↓ Genital sensations	Sensations muted or fleeting	Mindfulness (e.g., body scan)
	Onset with menopause	Vaginal DHEA, vaginal estrogen
↓ Mental sexual arousal	Physical arousal too similar to feelings of anxiety	MBCT, mental health referral
	Distractions, "busy mind"	Mindfulness practice

DHEA, Dehydroepiandrosterone; *MBCT,* mindfulness-based cognitive therapy; *SNRIs,* serotonin and norepinephrine reuptake inhibitors; *SSRIs,* selective serotonin reuptake inhibitors; *SRC,* sexual response cycle.

is put aside. Together, the couple and the clinician decide as to when breasts and genital areas are to be included. Ultimately, the act of intercourse (or vaginal penetration with a dildo) may be included—but not as the focus.

Outcome of Psychological Treatments for Women's Sexual Dysfunctions

Data are limited regarding the long-term effects of psychological treatments for women's sexual dysfunction. Systematic review and meta-analysis of controlled clinical trials for female and male sexual dysfunction published before 2009[578] concluded that psychosocial interventions for sexual dysfunction were efficacious. A more recent meta-analysis of the 11 reported trials of MBCT for women's sexual dysfunction concluded that all aspects of sexual function and subjective sexual well-being exhibited significant improvement during mindfulness-based therapy, with moderate effect sizes.[579]

For women with sexual dysfunction associated with the hormonal changes arising from breast cancer treatment, a systematic review suggests that the most effective interventions are couple-based psychoeducational interventions that include an element of sex therapy.[580] The American Society of Clinical Oncology 2018 guideline recommends psychosexual counseling along with addressing estrogen deficiency–related dyspareunia.[581]

Management of Women's Orgasmic Disorder

Management is guided by the details of the difficulties reaching a high level of arousal and being free to let the orgasm occur (Table 20.16).

Although CBT and mindfulness training have been used in clinical practice to treat women with orgasmic disorder, outcome research is sparse. A 2013 meta-analysis found evidence of benefit with CBT and mindfulness training in symptom severity and sexual satisfaction for women with orgasmic dysfunction.[578] To date, there is no pharmacologic treatment for orgasmic disorder. In one trial with highly selective inclusion criteria, sildenafil was reported to improve orgasm dysfunction associated with SSRI use.[582] In a small trial, transdermal testosterone did not improve sexual function compared with placebo as measured by the Sabbatsberg Sexual Self-Rating Scale as the primary outcome.[583] The strategies to treat antidepressant-associated orgasmic disorder include the use of medications that are less likely to impair sexual function, such as vortioxetine, vilazodone, mirtazapine, and bupropion.[584]

Management of Genitopelvic Pain/Penetration Disorder (Dyspareunia and Vaginismus)

The most common type of dyspareunia is PVD, which affects about 12% to 18% of women.[585] PVD and the other common type of dyspareunia—that stemming from vulvovaginal atrophy—are typically associated with pelvic muscle hypertonicity and reflexive involuntary contractions when penetration is anticipated. DSM-5 therefore merges the previous terms of *dyspareunia* and *vaginismus*. The use of transperineal ultrasound for pelvic floor muscle morphometry in women with PVD has confirmed abnormalities both at rest (e.g., small levator hiatus area, small anorectal angle suggesting increased tone) and with maximal contraction. These findings are thought to be consistent with weakness and poor control of pelvic muscle.[586] Pelvic floor physiotherapy is frequently a component of sexual pain management (see Table 20.7 and Fig. 20.10)

Management of Provoked Vestibulodynia

There is no definite association between PVD and endocrine status, but recent investigation of the presence of polymorphism in the guanine triphosphate cyclohydrolase gene (*GCH1*) is of interest. Specific single nucleotide polymorphisms in the *GCH1* gene are associated with reduced pain sensitivity. Although no correlation between PVD and the pain-protective *GCH1* polymorphism was found, patients with PVD using oral contraceptives and carrying a specified *GCH1* polymorphism had higher pain

TABLE 20.16 Management of Orgasmic Disorder in Women

Etiology	Management
Insufficient arousal	Manage as for sexual interest/arousal disorder
Antidepressant induced	Consider switch to vortioxetine, bupropion, mirtazapine, vilazodone
Clitoris "too sensitive"	Information on deeper clitoral bulbs, non-genital stimuli, G spot Vibrator to vulva away from clitoris
General need to be in control	Mental health referral, use of vibrator may bypass this difficulty
Orgasmic alone but not with partners	Encourage couple sexual communication; consider psychology referral regarding fear of intimacy and fear of vulnerability

sensitivity.[587] This finding is in keeping with the clinical experience that some women with PVD benefit from discontinuing oral contraceptives.[588]

Randomized trials of oral and topical medications for PVD including tricyclics, anticonvulsants, lidocaine, fluconazole, cromolyn, and nifedipine gave similar results in analgesic benefit compared with placebo.[589] Investigational botulinum toxin was statistically inferior to placebo in reduction of sexual distress.[589] Because medical treatments for PVD have been unsatisfactory, an interdisciplinary biopsychosexual approach is currently encouraged.[590,591]

Although rarely chosen by women, surgical vestibulectomy may be of benefit in a small subset of women; however, there are numerous exclusions for vestibulectomy,[592] and benefit is obtained mostly in women with acquired as opposed to lifelong histories. Other common negative prognostic factors for successful vestibulectomy include comorbid muscle tightening, widespread allodynia of the introital margin, involvement of the Skene duct openings, unwillingness to have sex therapy if offered, and comorbid depression and anxiety. Negative prognostic factors for all treatment modalities include disgust and contamination sensitivity, erotophobia (the tendency to respond with negative effects to sexual cues), and coexistence of depression and anxiety.[593]

Personality traits of women with PVD, including negative self-evaluation and fear of negative evaluation by others, predispose to self-labeling as sexually substandard or even sexually inadequate. The stress model of pain posits that sexual stress not only contributes to the chronic pelvic muscle hypertonicity but also maintains a heightened reactivity of the pain circuitry from top-down modulation afforded by neuroplasticity.[589,593–595] The cause of the sensitization within the nervous system has not been established with certainty, but internal stress appears to be a likely cause. Women with PVD report higher levels of premorbid depression and anxiety, as well as perfectionism, reward dependency, fear of negative evaluation, increased prevalence of type D personality, self-dislike, harm avoidance, hypervigilance for pain, and shyness compared with women without PVD.[596–600]

Given that mood disorder is so commonly comorbid with PVD, management needs to address both pain and depression/anxiety. There is evidence of sustained benefit from CBT.[601] Catastrophic thinking, amenable to CBT approaches, is particularly common in women with PVD.[602] The lack of self-acceptance apparent in women with PVD, considered to be a pain-maintaining stress, is potentially amenable to MBCT given that a key component of MBCT is acceptance. A brief mindfulness-based group intervention was more efficacious in reducing both cotton swab–induced vestibular pain and psychological measures of pain in comparison to a waitlist control.[572,577] In a study of women with PVD, group MBCT was recently compared with group CBT, finding similar benefit in all secondary endpoints, but improvements in reported pain with vaginal penetration after MBCT were greater than those reported after CBT.

Management of Phobic Reflex Pelvic Muscle Contractions Component of Genitopelvic Pain/Penetration Disorder (Vaginismus)

Heightened pelvic muscle tone, often along with muscle tension elsewhere, may be the only physical findings in women reporting dyspareunia or painful but unsuccessful attempts at penetration. Often guided by pelvic floor physiotherapists, management involves progressive desensitization and progressive vaginal accommodation using a variety of relaxation techniques and vaginal inserts.[603] The term *dilators* is preferably avoided, because women fear that their therapy is going to (painfully) stretch the vagina. In-clinic physical therapy has been associated with better outcomes than traditional guidance for insert therapy at home.[604] Psychotherapies, including mindfulness and CBT, are often used to reduce anxiety.[605]

Testosterone Therapy for Women With Sexual Dysfunction

As discussed earlier in this chapter, testosterone deficiency has not been demonstrated in women diagnosed with sexual dysfunction, either by measurement of blood levels or by measurement of androgen metabolites. Several randomized trials of testosterone therapy have been conducted, mostly in postmenopausal women. These testosterone trials were conducted largely in women distressed by reduced sexual desire since their menopause. The eligibility criteria for these trials did not meet the DSM-IV diagnostic criteria for HSDD, or for the newly coined SIAD of DSM-5.

The first series of randomized trials showed a statistically significant improvement in the numbers of satisfactory sexual encounters (SSEs) in women randomized to testosterone: on average, SSEs increased from two to three per month to five per month in women on active drug and to four per month in women receiving placebo. Testosterone was given transdermally in the form of a patch with a nominal testosterone delivery of 300 μg/day. Doses of either 150 or 450 μg/day were not effective.[606] Serum testosterone and dihydrotestosterone concentrations exceeded the upper limits of the target serum concentrations of these hormones in a significant number of women receiving testosterone.[607] Women on an active drug reported further improvements in arousal, pleasure, orgasm, self-image, and responsiveness to a statistically significantly greater extent than did women receiving placebo.

These testosterone trials focused mostly on surgically menopausal women, but one testosterone patch study included naturally menopausal women[608] with comparable results. Two testosterone studies recruited naturally and surgically menopausal women who were not receiving estrogen therapy. One of these studies reported a significant increase in SSEs in the naturally menopausal women from the active drug but not in the smaller subgroup of surgically menopausal women.[609] Only 464 of the 814 participants completed treatment, with similar distribution of high discontinuation rates in all three arms. A second study of 272 naturally

menopausal women, of whom a total of 73% of the participants were not receiving systemic estrogen therapy, showed a significant increase in SSEs.[610]

On the basis of these studies, all by the same sponsor, the transdermal testosterone patch was approved in Europe, but not in North America or elsewhere, for the treatment of surgically menopausal women with persistently distressing low sexual desire despite adequate systemic estrogen therapy. Although approved, the patch is no longer available in Europe because of low sales.

In contrast to the previous studies, two large phase III RCTs by a different sponsor of 1172 postmenopausal women, approximately half of whom received systemic estrogen, showed *no* benefit of transdermal testosterone in the form of a gel over placebo.[611] Full study details are not available because these two studies have not been published. The entry criterion of distressingly reduced sexual desire after menopause was similar to the previous randomized trials; endpoints were the numbers of SSEs per month and the level of sexual desire as assessed using a daily diary.

A dose-response study of menopausal women with past hysterectomy with or without bilateral oophorectomy and with low testosterone levels (free testosterone <3.5 pg/mL) found that some aspects of sexual function improved compared with placebo, but only in those receiving the 25-mg weekly intramuscular testosterone dose and not the 12.5-mg or lower doses. All treatment doses increased circulating testosterone concentrations above those of regularly cycling women—the 25-mg dose leading to 10 and 20 times the physiologic levels of total and free testosterone, respectively.[612] Even at these doses, sexual receptivity, pleasure, orgasm, or problems with sexual function did not improve.

There is very little information available on the effects of testosterone in premenopausal women. One study of 261 women who experienced loss of their former sexual satisfaction reported minimal benefit from testosterone.[613]

A systematic review concluded that currently available evidence does not support the measurement of serum testosterone levels in the evaluation of women with low libido.[415]

Testosterone Plus a Phosphodiesterase Inhibitor

Studies have begun to evaluate the efficacy of a pharmacologic dose of testosterone (0.5 mg sublingually) to improve attentiveness for erotic cues in women with low desire.[614] The testosterone was combined with sildenafil, a PDE5 inhibitor, to facilitate genital congestion. In those women who at baseline already showed high levels of subconscious attention bias for erotic cues (as measured by a masked version of the emotional Stroop task), this drug combination had no effect, and in fact, testosterone alone *reduced* attention to erotic cues. However, the women with lower arousability or sensitivity to erotic cues at baseline showed increased physiologic genital congestion and increased awareness of the genital sensations and of sexual desire when they subsequently viewed an erotic video. Recent dose-finding studies found that 0.5 mg of sublingual testosterone, but not 0.25 mg, plus 50 mg of sildenafil increased SSEs compared with baseline and compared with either drug alone. For women with higher arousability but presumed to have more inhibiting thoughts, 0.5 mg of sublingual testosterone plus 10 mg of buspirone increased SSEs compared with baseline and compared with either drug alone.[615] The safety of intermittent use of, albeit temporary, markedly supraphysiologic testosterone therapy is totally unknown.

Limitations of Trials of Testosterone Therapy in Women

A major limitation of testosterone trials to date is the targeted population. Studies have recruited women with decreased desire since menopause, most of whom retained the ability to be aroused and sexually satisfied on at least some (on average 50%) occasions. Thus, an absence of sexual desire between sexual encounters has been the focus. However, research confirms this to be well within the range of normal female sexual experience. The majority of 3250 multiethnic middle-age women in the SWAN cohort indicated that although moderately or extremely sexually satisfied, they never or very infrequently felt desire.[616] In an online survey of 3687 younger women, 1865 were assessed to be without evidence of sexual dysfunction, specifically confirming their easy sexual arousal—close to one third of this group rarely or never began a sexual experience with a sense of sexual desire.[617] As noted earlier in this chapter, an incentives/motivations model of human sexual response is now considered to more accurately reflect sexual experience, desire for sex per se being just one of many reasons or incentives for sex. When absent at the beginning of a sexual encounter, desire can be triggered along with arousal after effective stimulation.

Women able to have satisfactory sexual experiences 50% of the time are thought unlikely to have a biologic cause of dysfunction to merit any hormonal therapy.[618–620] The trials did show improvements in desire and response domains using validated sexual questionnaires; however, increasing the degree and frequency of pleasure and arousal currently experienced by study subjects does not necessarily imply that improvements would be observed in women with a consistent absence of pleasure and arousal.[618]

There has been criticism of the use of statistical significance alone to evaluate the difference between the powerful placebo effects and active drug treatments in the area of women's sexual dysfunction—especially that of low desire.[621,622] It is suggested that effects might be better reported in terms of the percentage of participants no longer meeting current criteria for sexual dysfunction.[621] As noted, the women in the testosterone trials were not recruited on the basis of a clinical diagnosis of sexual dysfunction but based on confirmation of low desire after menopause that caused distress.

Risks of Testosterone Therapy

Long-term safety data are lacking; published safety data from trials of up to 12 months' duration are reassuring.[622] There are theoretical reasons to consider exogenous testosterone as either a risk factor or a protective factor for breast cancer; high endogenous testosterone may be associated with an increased risk.[351,620] A high endogenous testosterone-to-estrogen ratio can increase the risk of metabolic syndrome and CVD.[623] However, some data suggest that low SHBG may be related to the risk of diabetes, metabolic syndrome, and CVD.[624] In the Melbourne Women's Midlife Health Project, weight gain and free androgen index, but not total testosterone, were strong predictors of CVD risk.[625] Similar results were observed in a 9-year follow-up of the SWAN cohort.[626] In this study, the free androgen index was positively and SHBG was negatively associated with the development of obesity. Weight gain preceded changes in the free androgen index and SHBG. The expert panel of the Endocrine Society noted that the association between the free androgen index, CVD risk factors, and the metabolic syndrome phenotype appears to be more driven by obesity and low SHBG rather than testosterone.[351] In a very recent prospective study of postmenopausal women, higher levels of testosterone were associated with an increased incidence of CVD and coronary artery disease, whereas higher estradiol levels were associated with a lower incidence of coronary artery disease.[627]

In most randomized trials, testosterone therapy has been administered in the background of concurrent estrogen therapy.[351] However, the present advice is to only initiate estrogen therapy

close to menopause, noting the increase in CVD when initiated after 10 years' post menopause.[628] This risk not only limits use of combination therapy but, theoretically, also testosterone alone given its aromatization to estrogen.

The Endocrine Society task force noted (1) the limited safety data (median follow-up, 4 months; range, 6 weeks to 2 years) and (2) the efficacy data focus on sexually responsive women without the common comorbid conditions including depression or antidepressant treatment. The task force requested a meta-analysis of transdermal testosterone RCTs; the gel studies, however, were excluded because they are only published in abstract form.

Needed Research in the Area of Testosterone Supplementation

Further research is needed in women with low sexual interest/incentives and low arousal (and typically few orgasms) to reflect the prevalent clinical situation and to merit a diagnosis of SIAD. It is of note, however, that in the study of 125 women with and 125 women without HSDD, when no group differences in androgen activity were found, 55% of the women with HSDD also met criteria for SIAD.[629]

Women diagnosed with SIAD and in remission from depression but taking antidepressants and women who, despite treatment, still score in the depressive range again reflect the clinical situation. Given that depression typically blunts sexual response, it has been an exclusion factor in clinical trials, as has the use of antidepressant therapy, but the reality is that mood disorder and its treatment commonly accompany complaints of low sexual desire.[629–631] Not only is depression the factor most robustly linked to low desire in otherwise healthy women, but also depression frequently determines the presence of sexual dysfunction even when other medical conditions including diabetes are comorbid.[14]

Oral Dehydroepiandrosterone for Sexual Dysfunction in Healthy Women

Small trials of DHEA have been conducted in older healthy women. A 2014 systematic review and meta-analysis to evaluate the benefits and risks of systemic DHEA therapy for postmenopausal women[632] included 15 randomized trials that were generally considered at high risk of bias and were of short duration. Statistically, DHEA use was marginally significant for desire, and there were no other significant improvements to outcome. The quality of evidence was considered low to moderate for benefit and very low for long-term harm. A more inclusive systematic review (38 studies but with 8 having fewer than 50 participants) found benefit in 11 studies, noting that the main benefit was for postmenopausal women.[633] Importantly, recruitment has not focused on women clearly diagnosed with sexual disorder with or without relatively low serum DHEA.[634,635] Recent research revealed benefits from 50 mg of DHEA daily to reduced genital sexual sensitivity attributed to oral contraceptives.[636]

Local Dehydroepiandrosterone Therapy for Sexual Dysfunction in Healthy Women

Local vaginal DHEA therapy has recently been approved for postmenopausal women with GSM. Treatment was found to improve vaginal symptoms of dryness and dyspareunia and all domains of sexual function. Moreover, all steroids, measured by mass spectrometry methods, remained in the postmenopausal range. Specifically, ADT-G remained constant. This delivery of precursor hormones to the target tissue may allow strictly local estrogen and androgen actions and may be a preferable choice for women in whom any systemic estrogen therapy is undesirable, such as those receiving aromatase inhibitors for breast cancer, who can develop severe vulvovaginal atrophy. Rodent work suggests that local DHEA's beneficial effect on genital sexual sensitivity might stem from its potent stimulatory effect on vaginal nerve fiber density.[637] The increase in desire is interesting given lack of systemic increase in testosterone and minor increase in serum DHEA. Restored feedback from augmented genital response may be relevant. However, a caveat is that trial participants were recruited on the basis of symptoms from GSM, not on the diagnosis of SIAD. When part or all of the sexual dysfunction stems from loss of reward due to loss of genital +/– nongenital sexual sensations, vaginal DHEA can be tried after explaining that this use of an approved medication is off-label (see Table 20.15).

Estrogen Therapy for Women With Sexual Dysfunction

Local vaginal therapy is recommended for dyspareunia associated with GSM. Low doses of estrogen can be supplied by a Silastic vaginal ring, vaginal cream, or a mucoadhesive vaginal tablet with similar benefit and low systemic absorption. Use of estradiol, 10 μg twice weekly, and the Estring (Pfizer, New York, NY) (a Silastic ring containing estradiol, placed high in the vaginal vault) results in serum levels of 4.6 and 8.0 pg/mL, respectively. Progesterone is usually considered unnecessary for endometrial protection. Smaller doses of these formulations of estrogen are being investigated (e.g., 10 μg rather than 100 μg estradiol cream, 0.03 mg rather than 0.2 mg estriol vaginal pessaries) or have already been approved (e.g., 10-μg rather than 25-μg estradiol vaginal tablets). When local estrogen does not ameliorate GSM-associated dyspareunia, comorbid PVD may be present.[638]

Of concern is that women using aromatase inhibitors and vaginal estrogen may show a small increase in serum estradiol levels.[639] Small increases in estradiol are reported from use of both the estradiol ring and the former 25-μg vaginal tablet, but not from a new 4-μg vaginal capsule and only on day 1 from a 10-μg capsule.[640] A combination of 0.03 mg of estriol and lactobacilli was found to benefit GSM symptoms without systemic absorption.[641] In another recent study of women receiving aromatase inhibitors, both 5 mg of vaginal testosterone three times a week or an estradiol-releasing ring showed benefit to signs of atrophy, sexual interest, and sexual dysfunction.[642] A small number of patients in the testosterone arm sustained estradiol elevations and high treatment-induced testosterone levels. Systemic absorption of testosterone could increase serum estrogen through aromatization. Previously, using a lower dose of testosterone (150–300 μg daily), no significant estradiol elevation was reported at 4 weeks.[643] Vaginal DHEA is now being trialed in breast cancer patients, given the lack of systemic uptake of estrogen or testosterone. The American Society of Clinical Oncology recommendations include offering vaginal DHEA to women on aromatase inhibitors who have current or past breast cancer and have not responded to previous treatment.[581] A study of 300 μg of vaginal testosterone nightly showed benefit to dyspareunia without estrogen effect on maturation index or reduction of visible signs of GSM and without increase in serum testosterone or estrogen. The benefit is presumed to be from vasodilatation alone.

Of particular relevance to women with past breast cancer is a 2013 report of a hyaluronic acid vaginal gel improving dyspareunia in 85% of women, comparable to women receiving vaginal estriol.[644]

Vaginal Lubricants and Moisturizers

Nonhormonal preparations are often a first choice for women with and without histories of estrogen-sensitive tumors. Other causes of reduced lubrication include hypothalamic hypogonadism, radiation, non–nerve-sparing surgeries for pelvic cancer, and medications such as ultralow-dose combined contraceptives, GnRH agonists, antihistamines, and anticholinergics, as well as breastfeeding or lack of any sexual arousal. Lubricants reduce friction and are applied just before intercourse. Moisturizers also reduce friction but are used two or three times weekly to maintain moisture over the long term. Hyaluronic acid can be used alone or in combination with aloe vera and tea tree oil. There is some evidence that hyaluronic acid can benefit the urinary symptoms of GSM—such symptoms can lessen sexual motivation. Many preparations are marketed, but many clinicians recommend coconut oil, which is usually nonirritating, longer lasting, inexpensive, and thought to be bacteriostatic and fungistatic; however, it is not compatible with latex condoms. Water-based lubricants can dry out quickly. They may contain glycerin (to increase moisture retention), which may promote yeast infections or sexually transmitted infections such as herpes and HIV, and possibly promote chronic bacterial vaginosis. Parabens may be added to deter microbial growth but may cause irritation and increase the risk of HIV or herpes transmission, and these additives do have ultraweak estrogenic activity. Replens (Church and Dwight, Ewing, NJ), which contains a patented bioadhesive that attaches to vaginal cells and preserves moisture, has received more study than most products and was shown to increase elasticity, lower pH, and benefit dryness and dyspareunia. Silicone products are suitable for sensitive skin, do not alter vaginal pH, and are longer lasting, but they are expensive and must be washed off with soap and water—and are also extremely flammable. Lubricants that are iso-osmolar so as to preserve sperm motility are recommended when conception is wanted.

Systemic Estrogen

When systemic estrogen is needed for other menopausal symptoms, it is sometimes necessary to give additional local estrogen for dyspareunia. In contrast, for some women, ultralow-dose (0.014 mg/day) systemic transdermal estradiol may be sufficient for all menopausal symptoms, including dyspareunia.[646] If systemic supplementation improves insomnia and dyspareunia, then sexual motivation would logically be expected to increase, but this has not been vigorously studied. No significant differences were found between estrogen and placebo groups in reported sexual satisfaction in the Women's Health Initiative trial.[647] However, sexual dysfunction was not a primary focus of that trial, women with marked menopausal symptoms were excluded, and the instruments used to assess sexual function were substandard. The Kronos Early Estrogen Prevention Study results suggest that the transdermal, rather than oral, route may be more likely to benefit sexual function, but again, the participants were not recruited on the basis of sexual dysfunction.[648]

Ospemifene—the only approved estrogen receptor modulator for GSM—is another alternative to estrogen. Its estrogenic effects on vulvar and vaginal tissues restore the maturation index, vaginal pH, and dyspareunia.[649] However, its theoretical safety on breast tissue is not established.

Approved But Not Recommended Medication for the Former DSM-IV Hypoactive Sexual Desire Disorder

Flibanserin—a 5HT1A agonist and a 5HT2A antagonist studied originally for potential benefit to depression—was trialed by different pharmaceutical companies and received limited FDA approval for the treatment of HSDD. This approval is despite benefit being similar to placebo and serious risk of harm.[650] There are strict contraindications to alcohol and to medications that inhibit CYP3A4, including oral contraceptives and fluconazole. Safety concerns include hypotension, syncope, somnolence, fatigue, and potential carcinogenicity. Overall risks of sedation or hypotension-related events were 28.6% with flibanserin versus 9.4% with placebo. Two recent meta-analyses have been based on both published and unpublished RCTs.[651] Meta-analysis showed that flibanserin led to a mean increase of 0.49 satisfying sexual events per month.[652] There was an increase of 0.3 (range, 1.2–6.0) on the desire subscale of the validated questionnaire, which was considered not to differ from placebo. Recruited women reported two to three rewarding sexual experiences each month at baseline (i.e., the participants did not have SIAD). Due both to safety concerns and to the short duration of studies and questionable accuracy of 4-week recall along with marginal efficacy, approval had been denied and additional safety studies recommended. Surprisingly, despite lack of additional efficacy data, but with data ruling out driving impairment and assessing alcohol's enhancement of side effects in 23 men and 2 women, on June 4, 2015, an FDA advisory committee voted 18 to 6 to accept approval of flibanserin, although with reservations. Fifteen members endorsing its approval declared their reluctance to do so. A recent review of scientific knowledge on flibanserin to date seriously questioned its use, noting the tenuous risk-benefit profile along with prohibitive prescribing restrictions and doubtful presence of sexual disorder in trialed women.[653]

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The complete list of referneces is available online at ExpertConsult.com.

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21

Transgender Endocrinology

STEPHEN M. ROSENTHAL AND VIN TANGPRICHA

CHAPTER OUTLINE

KEY POINTS

- Transgender people express a gender identity that differs from the gender implied by the sex assigned (recorded) at birth.
- A number of biological, environmental, and cultural factors likely influence one's gender identity.
- Youth with gender dysphoria should undergo an evaluation by a qualified mental health/gender specialist to confirm the diagnosis and to screen for other coexisting mental health concerns.
- Youth deemed eligible to initiate hormone therapy generally start with gonadotropin-releasing hormone agonists at Tanner stage 2 to halt puberty and at the appropriate time start gender-affirming hormone therapy of the desired gender.
- Transfeminine adults with confirmed gender dysphoria will start on a regimen of estrogen and androgen-lowering medications.
- Transmasculine adults with confirmed gender dysphoria will start on testosterone therapy.
- Only limited data currently exist regarding long-term outcomes of pubertal suppression and of gender-affirming hormone use in transgender youth.
- The long-term risks of gender-affirming hormone therapy are relatively few when hormone levels remain in the physiologic range; however, monitoring of potential adverse events is still recommended until long-term risks can be better ascertained.

Introduction

During the last decade there has been increasing public awareness of individuals whose gender identity is not aligned with their physical sex characteristics. Concurrently there has been an increasing number of gender nonconforming/transgender individuals seeking medical services to enable development of physical sex characteristics consistent with their affirmed gender identity. This chapter will review relevant terminology/definitions, diagnosis and prevalence of gender dysphoria (GD), mental health comorbidities, studies that shed light on the biological determinants of gender identity, current treatment models, barriers to care, and priorities for research.

Definitions and Diagnosis of Gender Dysphoria

Terminology and definitions used in this chapter are summarized in Table 21.1.

Prevalence of Gender Nonconforming Identity

Based on state-level population-based surveys, a 2017 report from the Williams Institute of the University of California–Los Angeles School of Law indicated that 0.6% of US adults and 0.7% of youth age 13 to 17 years identify as transgender.[1] A recent international review reports transgender prevalence estimates ranging from 0.5% to 1.3% of birth-assigned males and from 0.4% to 1.2% of birth-assigned females, representing an estimate of 25 million transgender people worldwide.[2] Of note, there has been a striking inversion in the sex ratio of adolescents with GD, with a predominance of birth-assigned females in recent years.[3]

Biological Determinants of Gender Identity

Compelling data have emerged supporting the concept that gender identity is not simply a psychosocial construct; rather, it reflects a complex interplay of biological, environmental, and

TABLE 21.1 Definitions From the Endocrine Society 2017 Guidelines

Term	Definition
Biological sex, biological male or female	These terms refer to physical aspects of maleness and femaleness. As these may not be in line with each other (e.g., a person with XY chromosomes may have female-appearing genitalia), the terms *biological sex* and *biological male* or *female* are imprecise and should be avoided.
Cisgender	This means "not transgender." An alternative way to describe individuals who are not transgender is *nontransgender people*.
Gender-affirming (hormone) treatment	See "gender reassignment."
Gender dysphoria	This is the distress and unease experienced if gender identity and designated gender are not completely congruent. In 2013, the American Psychiatric Association released the fifth edition of *Diagnostic and Statistical Manual of Mental Disorders* (DSM-V), which replaced *gender identity disorder* with *gender dysphoria* and changed the criteria for diagnosis.
Gender expression	This refers to external manifestations of gender, expressed through one's name, pronouns, clothing, haircut, behavior, voice, or body characteristics. Typically, transgender people seek to make their gender expression align with their gender identity, rather than their designated gender.
Gender identity/experienced gender	This refers to one's internal, deeply held sense of gender. For transgender people, their gender identity does not match their sex designated at birth. Most people have a gender identity of man or woman (or boy or girl). For some people, their gender identity does not fit neatly into one of those two choices. Unlike gender expression (to come), gender identity is not visible to others.
Gender identity disorder	This is the term used for gender dysphoria/gender incongruence in previous versions of DSM (see "gender dysphoria"). The International Statistical Classification of Diseases and Related Health Problems, Tenth Edition (ICD-10) still uses the term for diagnosing child diagnoses, but the upcoming ICD-11 has proposed using *gender incongruence of childhood*.
Gender incongruence	This is an umbrella term used when the gender identity and/or gender expression differs from what is typically associated with the designated gender. Gender incongruence is also the proposed name of the gender identity–related diagnoses in ICD-11. Not all individuals with gender incongruence have gender dysphoria or seek treatment.
Gender variance	See "gender incongruence."
Gender reassignment	This refers to the treatment procedure for those who want to adapt their bodies to the experienced gender by means of hormones and/or surgery. This is also called *gender-confirming* or *gender-affirming treatment*.
Gender-reassignment surgery (gender-confirming/gender-affirming surgery)	These terms refer only to the surgical part of gender-confirming/gender-affirming treatment.
Gender role	This refers to behaviors, attitudes, and personality traits that a society (in a given culture and historic period) designates as masculine or feminine and/or that society associates with or considers typical of the social role of men or women.
Sex designated at birth	This refers to sex assigned at birth, usually based on genital anatomy.
Sex	This refers to attributes that characterize biological maleness or femaleness. The best-known attributes include the sex-determining genes, the sex chromosomes, the H-Y antigen, the gonads, sex hormones, internal and external genitalia, and secondary sex characteristics.
Sexual orientation	This term describes an individual's enduring physical and emotional attraction to another person. Gender identity and sexual orientation are not the same. Irrespective of their gender identity, transgender people may be attracted to women (gynephilic), attracted to men (androphilic), bisexual, asexual, or queer.
Transgender	This is an umbrella term for people whose gender identity and/or gender expression differs from what is typically associated with their sex designated at birth. Not all transgender individuals seek treatment.
Transgender male (also: transman, female-to-male, transgender male)	This refers to individuals assigned female at birth but who identify and live as men.
Transgender woman (also: transwoman, male-to-female, transgender female)	This refers to individuals assigned male at birth but who identify and live as women.
Transition	This refers to the process during which transgender persons change their physical, social, and/or legal characteristics consistent with their affirmed gender identity. Prepubertal children may choose to transition socially.
Transsexual	This is an older term that originated in the medical and psychologic communities to refer to individuals who have permanently transitioned through medical interventions or desired to do so.

From Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2017;102:3869–3903; by permission of the Endocrine Society.

cultural factors. Studies that shed light on biological determinants of gender identity are derived principally from three biomedical disciplines: genetics, endocrinology, and neuroscience, highlights of which are summarized in this chapter. The intent in focusing on biological underpinnings of gender identity is not to pathologize or to identify a mechanism that can be "fixed." Instead, the purpose is to underscore biological variation in gender identity that may in turn destigmatize gender-variant individuals and decrease health disparities for gender minorities.

With respect to genetics, a recent study notes heritability estimates for gender identity in the range of 30% to 60%.[4] A study in twins supporting a role for genetic factors in gender identity outcome demonstrated a 39.1% concordance for what was previously called *gender identity disorder* (based on the *Diagnostic and Statistical Manual of Mental Disorders* [Fourth Edition] [DSM-IV] criteria) in 23 monozygotic female and male twin pairs, but no concordance in 21 same-sex dizygotic female and male twin pairs or in 7 opposite-sex twin pairs.[5] A number of investigators have sought to identify polymorphisms in specific candidate genes (e.g., the androgen receptor, estrogen receptor, and aromatase) that may be more prevalent in transgender individuals in comparison to a control population of nontransgender individuals; however, such findings have been inconsistent and lacking strong statistical significance.[6–9]

With respect to endocrine considerations, it should be noted that most transgender individuals do not have a disorder/difference of sex development (DSD). However, studies in individuals with DSDs have informed our understanding of the role that hormones (prenatal/early neonatal androgens, in particular) may play in gender identity development. For example, in studies of 46,XX individuals raised female with virilizing congenital adrenal hyperplasia (CAH) caused by mutations in the *CYP21A2* gene, there was a greater degree of female-to-male transgenderism in comparison to what would be expected based on prevalence estimates in a control population.[10,11] In a meta-analysis of 250 adults with this condition raised female, while nearly 95% accepted a female gender identity, 5.2% reported either a male gender identity or gender dysphoria.[11] This rate is higher than what would be expected for a transgender prevalence of 0.6% to 0.7% in recent population estimates.[1] A relationship between severity of disease and gender identity outcome was demonstrated in a study of adult 46,XX individuals with classic 21-hydroxylase deficiency: Three of the 42 patients (7.1%) with the salt-wasting form had gender dysphoria; no gender dysphoria was reported in the less severely affected individuals.[10] A study in 46,XX youth with the same condition found that of 43 individuals with either salt-wasting or simple virilizing CAH, 12.8% demonstrated cross-gender identification that was considered to be independent of gender role behavior.[12] In contrast, no cross-gender identification was noted in a nearly equivalent number of 46,XY individuals with virilizing CAH due to mutations in the *CYP21A2* gene or in a nearly equivalent number of controls (age-matched relatives).[12] A role for prenatal/early natal androgens in gender identity development is also supported by studies in a variety of other hormonal and nonhormonal DSDs, as recently reviewed.[13]

With respect to brain and gender identity, some studies in transgender individuals carried out before treatment with gender-affirming cross-sex hormones have demonstrated that some sexually dimorphic structures (both gray and white matter) are more closely aligned with a person's gender identity than with their physical sex characteristics.[14,15] In addition, some functional studies (e.g., analysis of hypothalamic blood flow in response to smelling odorous compounds or brain imaging studies carried out during mental rotation tasks) have demonstrated that patterns typically observed to be sexually dimorphic were more closely aligned with gender identity than with physical sex characteristics, even before cross-sex hormone treatment in both transgender adults and adolescents.[16–18]

Care of Transgender Youth

Gender dysphoria that either worsens or emerges with onset of physical puberty implies a high likelihood of a transgender identity during adulthood.[19] Given the complexity of gender dysphoria—and of adolescence itself—it is essential that gender dysphoric youth undergo a thorough evaluation by a qualified mental health/gender specialist. A psychodiagnostic evaluation is important not only to determine the presence or absence of gender dysphoria but also to assess for the presence of other mental health concerns that may co-occur. While the majority of gender dysphoric children and adolescents do not have an underlying severe psychiatric illness, it is important to recognize that there is an association of gender dysphoria and autism spectrum disorder.[19–21]

Based on pioneering work from the Netherlands, care of transgender youth has been primarily informed by clinical practice guidelines (CPGs) from the Endocrine Society (ES) and cosponsoring organizations and by standards of care (SOCs) from the World Professional Association for Transgender Health (WPATH).[22,23] These documents endorse the use of pubertal blockers in GD adolescents using gonadotropin-releasing hormone (GnRH) agonists at Tanner stage 2 of pubertal development (testicular volume ≥4 mL for assigned males at birth; initial stage of breast budding for assigned females at birth). Considered fully reversible treatments, GnRH agonists, by pausing puberty in this clinical setting, provide additional time for gender-identity exploration without the pressure of continued pubertal progression and prevent irreversible development of secondary sex characteristics associated with puberty that are not aligned with the person's gender identity. Such undesired physical changes include a prominent Adam's apple, lowered voice, male bone configuration, and potentially tall stature in assigned males at birth; conversely, breast development, female body habitus, and potentially short stature in assigned females at birth.[22] A recommended baseline and follow-up protocol for physical examination and laboratory monitoring during pubertal suppression is outlined in Table 21.2. While GnRH agonists are the preferred option for pubertal suppression, this treatment is costly and often inaccessible. Other options for pubertal suppression include depot and oral progestins.[22,24]

Adolescents who continue to meet the criteria for gender incongruence and gender dysphoria may request phenotypic transition with sex steroids. While age-specific recommendations for initiation of sex steroids in gender dysphoric adolescents are not delineated in the WPATH SOCs, the most recent version of the ES CPGs recommends initiating treatment "using a gradually increasing dose schedule after a multidisciplinary team of medical and mental health professionals has confirmed the persistence of gender dysphoria/gender incongruence and sufficient mental health capacity to give informed consent, which most individuals have by age 16 years."[22] Table 21.3 provides a protocol for induction of puberty in such adolescents; Table 21.4 provides recommendations for baseline and follow-up physical examination and laboratory evaluation during induction of puberty.

An important change in the current ES guidelines is the acknowledgment that there may be compelling reasons to start

TABLE 21.2 Endocrine Society Guidelines for Baseline and Follow-Up Protocol for Physical Examination and Laboratory Monitoring in Transgender Children During Pubertal Suppression With a GnRH Agonist

Every 3–6 Months

Anthropometry: height, weight, sitting height, blood pressure, Tanner stages

Every 6–12 Months

Laboratory: LH, FSH, E2/T, 25(OH)D

Every 1–2 Years

Bone density using DXA

Bone age on X-ray of the left hand (if clinically indicated)

25(OH)D, 25-Hydroxyvitamin D; *DXA,* dual-photon absorptiometry; *E2,* estradiol; *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone; *T,* testosterone.

From Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2017;102:3869–3903.

TABLE 21.3 Endocrine Society Guidelines for Induction of Puberty in the Affirmed Gender

Induction of female puberty with oral 17β-estradiol, increasing the dose every 6 months:
- 5 μg/kg/day
- 10 μg/kg/day
- 15 μg/kg/day
- 20 μg/kg/day
- Adult dose = 2–6 mg/day

In postpubertal transgender female adolescents, the dose of 17β-estradiol can be increased more rapidly:
- 1 mg/day for 6 months
- 2 mg/day

Induction of female puberty with transdermal 17β-estradiol, increasing the dose every 6 months (new patch is placed every 3.5 days):
- 6.25–12.5 μg/24 hours (cut 25-μg patch into quarters, then halves)
- 25 μg/24 hours
- 37.5 μg/24 hours
- Adult dose = 50–200 μg/24 hours

For alternatives once at adult dose, see Table 21.5.

Adjust maintenance dose to mimic physiologic estradiol levels (see Table 21.6).

Induction of male puberty with testosterone esters increasing the dose every 6 months (intramuscularly or subcutaneously):
- 25 mg/m^2/2 weeks (or alternatively, half this dose weekly, or double the dose every 4 weeks)
- 50 mg/m^2/2 weeks
- 75 mg/m^2/2 weeks
- 100 mg/m^2/2 weeks
- Adult dose = 100–200 mg every 2 weeks

In postpubertal transgender male adolescents, the dose of testosterone esters can be increased more rapidly:
- 75 mg/2 weeks for 6 months
- 125 mg/2 weeks

For alternatives once at adult dose, see Table 21.5.

Adjust maintenance dose to mimic physiologic testosterone levels (see Table 21.7).

From Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2017;102:3869–3903; adapted from Hembree WC, Cohen-Kettenis P, Delemarre-van de Waal HA, et al. Endocrine treatment of transsexual persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2009;94:3132–3154.

TABLE 21.4 Endocrine Society Guidelines for Baseline and Follow-Up Physical Examination and Laboratory Monitoring in Transgender Children During Induction of Puberty

Every 3–6 Months

Anthropometry: height, weight, sitting height, blood pressure, Tanner stages

Every 6–12 Months

In transgender males: hemoglobin/hematocrit, lipids, testosterone, 25-hydroxyvitamin D

In transgender females: prolactin, estradiol, 25(OH)D

Every 1–2 Years

Bone mineral density (BMD) using dual-energy X-ray absorptiometry (DXA)

Bone age on X-ray of the left hand (if clinically indicated)

BMD should be monitored into adulthood (until the age of 25–30 years or until peak bone mass has been reached).

For recommendations on monitoring once pubertal induction has been completed, see Tables 21.5 and 21.6.

25(OH)D, 25-Hydroxyvitamin D.

From Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2017;102:3869–3903; adapted from Hembree WC, Cohen-Kettenis P, Delemarre-van de Waal HA, et al. Endocrine treatment of transsexual persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2009;94:3132–3154.

sex hormone treatment before the age of 16 years in some adolescents with gender incongruence and gender dysphoria.[22] Delaying sex hormone treatment until age 16 years in an adolescent whose puberty was blocked at Tanner stage 2 could not only be detrimental to bone health, but also keeping someone suspended in an early pubertal state until this age could isolate the individual further from age-matched peers, with potentially negative consequences for emotional well-being.[22]

When puberty is initiated with gradually increasing doses of sex steroids, the initial levels will not be sufficient to suppress endogenous sex steroids. In transgender females, it is therefore recommended that GnRH analog treatment (or an alternative antiandrogen) be continued until gonadectomy.[22] Since some transgender young adults may opt not to have gonadectomy, long-term studies are necessary to examine the potential risks of prolonged GnRH analog treatment. In transgender male adolescents, GnRH analog treatment can typically be discontinued once adult levels of testosterone have been reached.[22] If uterine bleeding occurs, a progestin can be added.[22,25]

Some transgender adolescents first come to medical attention when they are already in the later stages of puberty or are postpubertal. Transgender males can be treated with testosterone alone; transgender females are treated with a combination of estrogen and an agent that blocks testosterone secretion and/or action using protocols similar to those used in transgender adults (see later discussion on hormone therapy in transgender adults).

With respect to estrogen treatment, 17β-estradiol (transdermal, oral, or parenteral) is preferred to conjugated (e.g., Premarin) or synthetic estrogens (e.g., ethinyl estradiol) since conjugated and synthetic estrogen levels cannot be monitored in the serum, and ethinyl estradiol is associated with an increased risk for venous thromboembolic disease and death from cardiovascular causes in adult studies (see later discussion on hormone therapy in transgender adults).

Some transgender adolescents seek surgical procedures to bring their bodies into closer alignment with their gender identity. The

current ES guidelines recommend that gender-affirming genital surgery involving gonadectomy and/or hysterectomy not take place until the patient has reached at least 18 years of age or the age of legal majority in his or her country.[22] With respect to breast surgery/mastectomy for transgender males, the current ES CPG notes that some adolescents may consider this procedure before age 18 years, though there is insufficient evidence to recommend a specific age requirement. Rather, the ES CPG suggests that clinicians determine timing of this procedure based on the physical and mental health status of the individual patient.[22]

Outcomes of Current Treatment Models for Transgender Youth and Potential Adverse Effects

Mental Health Outcomes

Only limited outcomes data are currently available to support the ES guidelines and WPATH SOCs for care of transgender youth. A study from the Netherlands evaluated the mental health in 55 transgender adolescents/young adults at three time points: before the start of GnRH agonist treatment (average age 13.6 years), at the start of cross-sex hormone treatment (average age 16.7 years), and 1 year after gender-reassignment surgery (average age 20.7 years). Following the completion of this three-stage approach to care, gender dysphoria was resolved, general psychologic functioning steadily improved, and a sense of well-being based on standardized survey results was noted to be equal to or greater than that seen in age-matched controls from the general population.[26] None of the study participants regretted treatment.[26]

Potential Adverse Effects of Pubertal Blockers in Transgender Youth

There are potential adverse effects of pubertal suppression with GnRH agonists in transgender youth. The primary risks include impaired bone mineral density (BMD), compromised fertility, and unclear effects on brain development, body mass index (BMI), and body composition, as recently reviewed.[27]

Skeletal Health

With respect to skeletal health, a 6-year longitudinal study assessed BMD in 34 transgender adolescents (15 transgender females, 19 transgender males) at the start of GnRH agonist treatment, at the start of cross-sex hormone treatment, and at age 22 years, following gonadectomy at a minimum age of 18 years (at which time GnRH agonist was stopped and cross-sex hormone treatment was continued). During the 6-year study period, there was a significant decrease in lumbar spine areal BMD Z-scores (relative to natal sex) in the transgender females, with a similar trend in the transgender males, suggesting either a delay in attainment of peak bone mass or an attenuation of peak bone mass itself.[28] The authors acknowledged potential study limitations, including a relatively small number of participants, use of relatively low doses of cross-sex hormones during the initial period of that phase of treatment, and a lack of information regarding vitamin D levels, dietary calcium intake, and weight-bearing exercise, all of which can influence BMD.[28] Particularly during GnRH agonist treatment, it would seem important to ensure adequate intake of calcium and vitamin D to encourage weight-bearing exercise and to routinely monitor levels of 25(OH)D.[24]

A separate study investigated bone turnover markers as well as BMD in transgender adolescents who underwent GnRH agonist treatment followed by cross-sex hormone therapy (the latter at about the age of 16 years, similar to previous reports). At baseline, bone mineral apparent density (BMAD) in the lumbar spine was lower in young (bone age <15 years) transgender female adolescents versus young (bone age <14 years) transgender male adolescents.[29] Bone turnover markers and BMAD Z-scores decreased following GnRH agonist treatment in the younger transgender adolescents, while an increase in BMAD was seen after 24 months of cross-sex hormone treatment in the younger transgender adolescents (bone age as previously noted) and older transgender adolescents (bone age ≥15 years in transgender females and ≥14 years in transgender males).[29]

A 22-year follow-up study of the first described gender dysphoric adolescent, treated initially with GnRH agonist and subsequently with cross-sex hormones, found that BMD was in the normal range for both sexes when evaluated at 35 years of age.[30]

Fertility

It is essential that an informed consent process and a discussion about implications for fertility precede any treatment of transgender youth with pubertal blockers and/or gender-affirming sex hormones. Transgender adolescents may wish to preserve fertility, which may be otherwise compromised if puberty is suppressed at an early stage and the patient subsequently pursues physical transition with gender-affirming sex hormones. In vitro maturation of human germ cells has not yet been achieved, though promising studies have been carried out in mice.[31] Late pubertal and fully pubertal adolescents have the option of cryopreservation of mature sperm or eggs.

Brain

Few studies have thus far investigated potential adverse effects of GnRH agonists on the brain in transgender adolescents. A cross-sectional study in gender dysphoric adolescents treated with GnRH agonist, in comparison to untreated gender dysphoric adolescents, showed no significant compromise of executive functioning, a developmental milestone thought to reflect prefrontal brain activation and to be typically achieved during puberty.[32] A 28-month longitudinal study in a single pubertal transgender adolescent undergoing GnRH agonist treatment reported lack of significant variation in brain white matter fractional anisotropy, thought to be a measure of brain maturation, and a nine-point drop in operational memory testing after 22 months of pubertal suppression.[33] Further longitudinal studies are needed to determine the impact of GnRH agonist treatment on brain development and function in transgender adolescents.

Body Mass Index and Body Composition

The impact of GnRH agonist treatment of transgender adolescents on BMI and body composition has also been studied. While variable results have been reported with respect to BMI,[28,34] a decrease in lean body mass percentage and an increase in fat percentage were reported after 1 year of treatment with GnRH agonist in both transgender male and female adolescents.[35] In an earlier study, 1 of 27 adolescents treated with GnRH agonist developed significant weight gain, though BMI was above the 85th percentile before treatment.[35]

Potential Adverse Effects of Gender-Affirming Sex Hormones in Transgender Adolescents

Few studies have thus far evaluated potential adverse effects of gender-affirming sex hormones in transgender adolescents. A study from the Netherlands in 28 transgender female adolescents treated primarily with gradually increasing doses of 17β-estradiol

for 1 to 3 years described no change in blood pressure, BMI standard deviation score, lean body mass percentage, or fat percentage (despite the presence of a desired female pattern of fat distribution). No abnormalities were seen with creatinine or with liver enzymes, and there was no change in hematocrit or hemoglobin A1c. One individual developed hyperprolactinemia, though this was seen in the context of high-dose ethinyl estradiol treatment to limit statural growth.[36]

A study from the United States assessed potential adverse effects of gender-affirming sex hormones in 116 transgender adolescents and young adults (44 transgender females and 72 transgender males treated with 17β-estradiol or testosterone, respectively). Following 17β-estradiol treatment, no abnormalities were reported in blood pressure, BMI, hemoglobin/hematocrit, lipids, renal and liver function studies, and prolactin. Following testosterone treatment, there was an increase in BMI and hemoglobin/hematocrit (3 of 44 individuals developed supraphysiologic hematocrit levels) and a decrease in high-density lipoprotein (HDL) cholesterol. No abnormalities were reported in blood pressure, renal and liver function studies, and hemoglobin A1c.[37]

Care of Transgender Adults

Because adults are able to more clearly articulate their gender identity and most adults have a gender identity that is fixed, any physician with the knowledge and expertise to diagnose gender dysphoria can manage and prescribe gender-affirming hormone therapy in adults with gender incongruence/gender dysphoria (GI/GD). Endocrinologists and other physicians with expertise should evaluate individuals with GI/GD for eligibility and readiness for hormone therapy and should serve as a liaison between mental health providers and surgeons. Endocrinologists should follow updated national/international guidelines on how to initiate and monitor hormone therapy in GI/GD individuals and serve as a resource for medical professionals in the community. In some instances, co-management with an endocrinologist along with another hormone prescriber may be necessary if there are other coexisting mental health or medical comorbidities.

The initial encounter with a transgender adult should include a history of the duration and severity of the gender dysphoria and a careful review of chronic medical issues that can be exacerbated by hormone therapy. An assessment of the individual's family and social support structure is important to determine if the timing of hormone therapy is appropriate (especially if not currently living in the affirmed gender role) or if additional social support is needed prior to starting hormone therapy. It is important to discuss any history of depression and to assess suicide since transgender individuals are at increased risk.[38,39] The prescription of an antidepressant medication or urgent referral to a mental health provider may be necessary. Finally, the endocrinologist should carefully review the letter provided from the mental health provider if the individual has already been receiving counseling. It is not a requirement to have a mental health referral in adults as stated in the ES guidelines[22]; however, in cases where the severity and duration of gender dysphoria are not quite clear and where there may be other mental health concerns that impact the gender transition, a mental health referral may be helpful.

Follow-up visits with the endocrinologist should focus on the response of the gender-affirming hormone therapy on the individual's gender dysphoria and mood. The endocrinologist should continue to assess the individual's social support structure and environment. The endocrinologist should also assist in any legal matters that arise, such as gender or name change, and referral to surgical specialists for gender-affirming surgery when the timing is appropriate.

Hormone therapy protocols have been developed to mimic the sex steroid (testosterone and estradiol) concentrations in the expected reference range for males and females. Many centers around the world have utilized different preparations and delivery methods of sex steroid hormones along with adjunctive treatments to achieve this goal. Over time, these protocols have been refined and adopted by other clinics around the world. These protocols involve careful titration of hormone doses according to the blood concentrations of sex steroid hormones until a steady-state concentration is achieved. The ES guidelines recommend a number of different hormone regimens that can be used for transgender

TABLE 21.5 Endocrine Society Recommendations for Hormone Therapy in Transgender Adults

TRANSGENDER FEMALES[a]	
Estrogen	
Oral	
Estradiol	2.0–6.0 mg/day
Transdermal	
Estradiol transdermal patch (new patch placed every 3–5 days)	0.025–0.2 mg/day
Parenteral	
Estradiol valerate or cypionate	5–30 mg IM every 2 weeks 2–10 mg IM every week
Antiandrogens	
Spironolactone	100–300 mg/day
Cyproterone acetate[b]	25–50 mg/day
GnRH Agonist	3.75 mg SQ (SC) monthly 11.25 mg SQ (SC) 3-monthly
TRANSGENDER MALES	
Testosterone	
Parenteral testosterone	
Testosterone enanthate or cypionate	100–200 mg SQ (intramuscularly) every 2 weeks or SQ (subcutaneously) 50% per week
Testosterone undecanoate[c]	1000 mg every 12 weeks
Transdermal testosterone	
Testosterone gel 1.6%[d]	50–100 mg/day
Testosterone transdermal patch	2.5–7.5 mg/day

[a]Estrogens used with or without antiandrogens or GnRH agonist.

[b]Not available in the United States.

[c]1000 mg initially followed by an injection at 6 weeks then at 12-week intervals.

[d]Avoid cutaneous transfer to other individuals.

From Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2017;102:3869–3903.

TABLE 21.6 Endocrine Society Recommendations for Physical Examination and Laboratory Monitoring in Transgender Women

1. Evaluate patient every 3 months in the first year and then one to two times per year to monitor for appropriate signs of feminization and for the development of adverse reactions.
2. Measure serum testosterone and estradiol every 3 months.
   a. Serum testosterone levels should be <50 ng/dL.
   b. Serum estradiol should not exceed the peak physiologic range: 100–200 pg/mL.
3. For individuals on spironolactone, serum electrolytes, particularly potassium, should be monitored every 3 months in the first year and annually thereafter.
4. Routine cancer screening is recommended, as in nontransgender individuals (all tissues present).
5. Consider bone mineral density testing at baseline. In individuals at low risk, screening for osteoporosis should be conducted at age 60 years or in those who are not compliant with hormone therapy.

From Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2017;102:3869–3903.

adults (Table 21.5) and a protocol for monitoring for potential adverse events (Table 21.6). In general, when hormone therapy is taken under medical supervision, the risks of adverse events are low likely due to the careful attention not to exceed supraphysiologic concentrations.[40] However, it is important to note that self-treatment with hormone therapy still remains common in countries where hormones can be obtained without prescription, particularly in the developing world.

Transfeminine Hormone Therapy

Estrogen

Several formations of estrogen have been used in the past, including synthetic estrogens (e.g., diethylstilbestrol, ethinyl estradiol), conjugated estrogens (Premarin), or estradiol. Estrogens can be given by several routes of administration, including orally, transdermally, or intramuscularly/subcutaneously (see Table 21.5). Studies have demonstrated that conjugated estrogens and synthetic estrogens are associated with increased risks of thromboembolism and thus are no longer recommended for use by the ES guidelines.[22] Another issue with conjugated estrogens and synthetic estrogens is their inability for physicians to measure these drugs in blood and/or estimate equivalent estradiol levels, which impairs efforts to avoid supraphysiologic exposure to estrogen.

The most commonly prescribed and common route of administration of estrogen is oral estradiol due to its convenience, cost, and relatively good safety profile. The dose of oral estradiol is titrated to a serum estradiol range of 200 to 300 pg/mL.[41] Liang et al[42] reported that oral estradiol doses in the range of 2 to 7.5 mg daily in combination with spironolactone will result in serum estradiol concentrations between 50 and 200 pg/mL in transwomen followed in the United States for over 3 years. In transwomen followed in the European Network for the Investigation of Gender Incongruence (ENIGI), oral estrogen is prescribed to those under age 40 at 4 mg daily in combination with cyproterone acetate; estradiol levels reach 100 to 200 pg/mL.[43]

Transdermal estrogen, presumed to be less stimulatory of prothrombotic proteins, is preferred in transgender women over the age of 40 due to the increased risk of thromboembolic disease.[44] In the Netherlands, all transgender women are switched to transdermal estrogen after age 40.[45] In an academic endocrinology practice in Austria, 162 transwomen were prescribed primarily transdermal 17β-estradiol. They found no cases of thromboembolism after a mean follow-up time of 64 months.[46] However, in 676 transgender women prescribed oral estradiol followed in the United States, only one person developed a thromboembolism after a mean follow-up time of 1.9 years.[47] Therefore it is unclear at this point if oral or transdermal formulations are equally as safe for use in transgender women. There have been no head-to-head studies that have studied these two methods of administration.

Testosterone-Lowering Agents

Most published transfeminine hormone regimens involve the use of a testosterone-lowering agent in combination with estrogen therapy (see Table 21.5). Testosterone-lowering agents allow for estrogen therapy to induce secondary sex characteristics unopposed from the actions of testosterone, and thus a lower dose of estrogen is required. There are three commonly prescribed testosterone-lowering agents, spironolactone, cyproterone acetate, and GnRH agonists.

Spironolactone is most commonly used in the United States for its widespread availability and ease of administration. It is taken orally once or twice a day in doses ranging from 100 to 300 mg total daily (see Table 21.5). Spironolactone was developed for its antagonism of the mineralocorticoid receptor and as a potassium-sparing diuretic. Less is known about the precise mechanism by which spironolactone lowers testosterone, but it is widely appreciated in cisgender men to cause gynecomastia likely due to antagonism of the androgen receptors, increased clearance of testosterone, increased conversion of testosterone to estradiol, and direct inhibition of testosterone production.[48,49] Largely retrospective studies based in the United States on the effectiveness of spironolactone to lower testosterone concentrations in transgender women report mixed results.[42,50] Unfortunately, no prospective randomized controlled trial has been conducted to examine estrogen regimens with or without spironolactone.

In Europe and parts of Asia, cyproterone acetate has been commonly used as the testosterone-lowering agent. Cyproterone acetate is taken orally, between 25 and 50 mg daily. Cyproterone has progestin-like activity and lowers testosterone by lowering gonadotropin levels. A recent study in transgender girls found that cyproterone acetate monotherapy resulted in lower gonadotropins and lower total and free testosterone, which were associated with favorable physical changes, including less facial hair and increased breast growth.[51] However, there have been some recent concerns associated with cyproterone acetate that are not seen with spironolactone, including increased incidence of hyperprolactinemia and meningiomas.[52–54]

In the United Kingdom, GnRH agonists are primarily used to lower testosterone in transgender women.[55] These medications are highly effective in lowering levels of gonadotropins and lowering testosterone to nearly undetectable levels. Some of the limitations of GnRH agonists are the high cost of therapy and the lack of long-term data with this approach.

5α-Reductase Inhibitors

5α-Reductase inhibitors such as finasteride or dutasteride have not been routinely recommended for use in transgender women

since they do not lower serum concentrations of testosterone and they have a higher cost. In addition, there have been concerns regarding an increased risk of sexual dysfunction and depression as reported in cisgender men.[56–58] Since there is an increased rate of depression and suicide in the transgender population,[38,39] these medications should not be used as antiandrogens in first-line therapies. However, 5α-reductase inhibitors may have a role in transgender women who have androgenetic alopecia and who do not achieve an adequate response to spironolactone.[59]

Progesterone

Many transgender women request progesterone as part of their gender-affirming hormone regimen despite the lack of any high-quality data to support its use. Several websites and forums have suggested that progesterone may enhance breast development and areolar darkening based on anecdotal reports. A population-based survey of more than 100 transgender women living in New York City revealed that 17% of transgender women were taking progesterone.[60] The Endocrine Society guidelines do not make a recommendation for the use of progesterone as part of a gender-affirming hormone regimen. The Guidelines for the Primary and Gender-Affirming Care of Transgender and Gender Nonbinary People by the University of California–San Francisco (UCSF) Center of Excellence for Transgender Health mention the use of progesterone as a treatment option, with the caveat that there are limited high-quality data supporting its effectiveness.[61] Concerns raised about the use of progesterone include data from cisgender postmenopausal women indicating increased risk of stroke when progesterone is taken in combination with conjugated estrogens.[62] There currently is insufficient evidence to support the use of progesterone for transgender women. Physicians and other clinicians should discuss the known risks of progesterone with transgender women who ask for progesterone therapy and make a shared decision of whether a trial of progesterone is indicated.

Transmasculine Hormone Therapy

The main hormone used for transgender men for gender-affirming therapy is testosterone. Testosterone esters (cypionate or enanthate) can be given intramuscularly every 1 to 2 weeks or subcutaneously weekly[63] (see Table 21.5). In a small retrospective study of 22 transgender men who received both intramuscular and subcutaneous testosterone at some point during their gender transition, all preferred testosterone administration subcutaneously.[63] Testosterone undecanoate is a longer-acting testosterone that can be given by intramuscular injection every 12 weeks and is also well tolerated among transgender men as reported by a prospective study of 17 transgender men in Europe.[64] Transdermal preparations of testosterone as gels or patches are also an option for treatment of transgender men. In Europe, but not in the United States, testosterone is available orally as testosterone undecanoate, which is becoming less commonly prescribed because of its short half-life, which requires dosing three to four times daily.

The goal of the testosterone therapy is to reach serum testosterone concentrations in the range expected for cisgender men and to induce male secondary sex characteristics, including increased body and facial hair, deeper voice, increased muscle mass, and cessation of menses (see Table 21.6). Testosterone will also reduce (but not completely eliminate) the gender dysphoria, as the physical changes will better align with the patient's gender identity.

TABLE 21.7 Endocrine Society Recommendations for Physical Examination and Laboratory Monitoring in Transgender Men

1. Evaluate patient every 3 months in the first year and then one to two times per year to monitor for appropriate signs of virilization and for development of adverse reactions.
2. Measure serum testosterone every 3 months until levels are in the normal physiologic male range[a]:
   a. For testosterone enanthate/cypionate injections, the testosterone level should be measured midway between injections. The target level is 400–700 ng/dL to 400 ng/dL. Alternatively, measure peak and trough levels to ensure levels remain in the normal male range.
   b. For parenteral testosterone undecanoate, testosterone should be measured just before the following injection. If the level is <400 ng/dL, adjust dosing interval.
   c. For transdermal testosterone, the testosterone level can be measured no sooner than after 1 week of daily application (at least 2 hours after application).
3. Measure hematocrit or hemoglobin at baseline and every 3 months for the first year and then one to two times a year. Monitor weight, blood pressure, and lipids at regular intervals.
4. Screening for osteoporosis should be conducted in those who stop testosterone treatment, are not compliant with hormone therapy, or who develop risks for bone loss.
5. If cervical tissue is present, monitor as recommended by the American College of Obstetricians and Gynecologists.
6. Ovariectomy can be considered after completion of hormone transition.
7. Conduct subareolar and periareolar annual breast examinations if mastectomy is performed. If mastectomy is not performed, then consider mammograms as recommended by the American Cancer Society.

[a]Adapted from Lapauw B, Taes Y, Simoens S, et al. Body composition, volumetric and areal bone parameters in male-to-female transsexual persons. *Bone*. 2008;43:1016–1021; Ott J, Kaufmann U, Bentz EK, et al. Incidence of thrombophilia and venous thrombosis in transsexuals under cross-sex hormone therapy. *Fertil Steril*. 2010;93:1267–1272.

From Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2017;102:3869–3903.

Potential Adverse Effects of Gender-Affirming Sex Hormones in Transgender Adults

In general, gender-affirming hormone therapy is safe when prescribed under medical supervision with monitoring of circulating hormone levels to ensure that these values remain in the physiologic range (Table 21.7; see also Table 21.6).[40] However, there have not been any head-to-head studies on which hormone preparations are the safest to use in this population, and increased risks of several conditions associated with gender-affirming hormone therapy have been identified and should be considered.

Potential Risks Associated With Transfeminine Hormone Therapy

Venous Thrombosis and Pulmonary Embolism

Thromboembolism is the most well-studied and most serious complication resulting from transfeminine hormone therapy, primarily from estrogen. Earliest reports found a 45-fold increase in the expected incidence of venous thrombosis and/or pulmonary embolism in Dutch transgender women taking primarily ethinyl

estradiol and cyproterone acetate.[44] After changing the route from oral to transdermal estrogen in transgender women above the age of 40, the risks of thromboembolism declined to 20-fold.[45] A more recent review of 10 cohort studies based in Europe and Canada found a low incidence of thromboembolism in transgender women on oral estradiol in the range of 2 to 4 mg daily or transdermal estradiol 0.1 to 0.2 mg/day.[65] A cohort study from Austria found no thromboembolic events in 162 transgender women followed for a mean of 5 years exclusively treated with transdermal estradiol, cyproterone acetate, and finasteride, suggesting improved safety with transdermal formulations of estrogen. In a systematic review of 23 studies, representing 3231 subjects, venous thromboembolism was found in 56 subjects and had a prevalence between 0% and 5% in the individual studies.[66] The study subjects ranged between 19 and 44 years of age and were followed between 3 months and 41 years. In a European cohort of 214 transgender women, the cases of venous thromboembolism before and after gender-affirming hormone therapy increased from 9.2 cases to 60.7 cases per 1000.[66] In a large cohort based in the United States of more than 7000 transgender women followed in a managed health care system, the rates of venous thromboembolism increased starting after 5 years following the initiation of estrogen therapy compared to a reference group of cisgender men and women.[67]

Estrogen is likely the cause for the increased incidence of thromboembolism given its known mechanism for promoting prothrombotic factors and given evidence from clinical trials of cisgender women on estrogen therapy. However, there are other factors that increase the risk of thromboembolism in transgender women, including the formulation of estrogen, the route of administration, postoperative state (especially following gender-affirming surgery), and age.[65] The ES guidelines recommend the use of estrogen compounds that can be measured in blood and against synthetic or conjugated estrogens, which make monitoring blood levels difficult.[22] Transgender women should hold their estrogen therapy for a period of time prior to gender-affirming surgery, typically 2 to 3 weeks.[65]

Myocardial Infarction and Cerebrovascular Accidents

Most cohort studies in transgender women show low rates of myocardial infarction (MI) or cerebrovascular accidents (CVA). In the Maraka et al systematic review,[66] they found only 14 MIs and 8 CVAs among 3231 subjects. However, in the European ENIGI cohort,[67] the cases of MI and CVA found in transgender women initiating on gender-affirming hormone therapy increased from 4.7 to 18.7 and 23.4 per 1000, respectively. In a cohort of over 7000 transgender women followed in the United States, the risks of CVA appear to increase after 7 years following the initiation of hormone therapy, while in contrast, there does not appear to be an increase in MI compared to cisgender women and men. The ES guidelines recommend that clinicians evaluate for risk of cardiovascular disease, but there are no current recommendations on prevention or treatment in transgender women who have risk factors.[22]

Hypertriglyceridemia

In a systematic review of 29 studies, gender-affirming hormone therapy with estrogen in transgender women was associated with increased levels of triglycerides by 31.9 mg/dL (95% CI, 3.9–59.9) at 24 months with no significant increase in low-density lipoprotein (LDL) or HDL cholesterol for up to 24 months. In transfeminine youth, no changes in triglycerides but an increase in HDL cholesterol were seen after 24 months.[68] In another study of transfeminine youth, there were no changes seen in lipids after treatment for up to 35 months.[37] It is not known whether the changes in triglycerides seen in adult transgender women are clinically meaningful and/or result in an increased risk of cardiovascular disease in the long term.

Hyperprolactinemia

Early reports of prolactinomas occurring in transgender women and animal studies suggesting that estrogen may induce the growth of lactotroph cells prompted the Endocrine Society to recommend screening for hyperprolactinemia in transgender women.[69,70] However, in the majority of these cases, the transgender women were also taking the antiandrogen cyproterone acetate. Defreyne et al demonstrated that prolactin levels declined significantly after discontinuation of cyproterone acetate therapy following orchiectomy.[53] Furthermore, in a study conducted in Canada, transgender women who were prescribed cyproterone acetate had much higher increases in prolactin compared to transgender women prescribed spironolactone as the antiandrogen drug.[71] These studies suggest that cyproterone has a more stimulatory impact on prolactin secretion than estrogen. In fact, a recent study in the United States, where transgender women were treated with estrogen and spironolactone, demonstrated no significant rise in prolactin concentrations.[54]

Osteoporosis

Adequate estrogen therapy is protective against bone loss in transgender women. In an early study of 20 transgender women, lower luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations (markers of estrogen adequacy) were associated with a lower rate of decline in bone mineral density at the lumbar spine.[72] In a cohort of 231 transgender women, 1 year of gender-affirming hormone therapy resulted in a significant increase in BMD at the lumbar spine and hip.[73] A recent systematic review of 13 studies representing 392 transgender women found that gender-affirming hormone therapy was associated with increased lumbar BMD at 12 and 24 months.[74] However, despite the positive effects of gender-affirming hormone therapy on bone density, transgender women have been reported to have lower expected BMD prior to the initiation of hormone therapy.[75] Therefore BMD screening may be indicated in those transgender women who have risk factors for osteoporosis as recommended by the Endocrine Society.[22]

Breast Cancer

Transgender women have been reported to develop breast cancer in a few case series, raising the concern that gender-affirming hormone therapy may be associated with increased risk of breast cancer.[76] Data from the North American Association of Central Cancer Registries (NAACCR) report increased proportional incidence ratios (PIR) of breast cancer compared to cisgender men (PIR 20.7: 95% CI, 15.0–27.9) but lower PIR of breast cancer compared to cisgender women (PIR 0.2: 95% CI, 0.1–0.2). Similarly, in a large cohort of US transgender women followed at Kaiser Permanente, the risk of breast cancer was increased compared to cisgender men but the same as compared to cisgender women.[77]

Liver Dysfunction

Early reports from Dutch transgender women found elevations in liver enzymes in 10% (88 of 816) of the cohort initiated on gender-affirming hormone therapy. However, a third of these cases

were related to hepatitis B infection and alcohol, and another quarter were only transient elevations lasting less than 6 months. The Endocrine Society initially recommended routine measurement of liver enzymes in transgender women, but subsequent reports showed that most changes in liver function due to gender-affirming hormone therapy were transient and did not result in any permanent liver abnormalities. Thus the recommendation for routine monitoring of liver enzymes was removed in the 2017 guidelines update.[22,37,78,79]

Potential Risks Associated With Transmasculine Hormone Therapy

Erythrocytosis

Gender-affirming therapy with testosterone in transgender men is commonly associated with increases in red blood cell mass and hematocrit due to stimulation of erythropoietin.[80] Several cohort studies of transgender men report significant increases in hemoglobin and hematocrit, but very few studies have demonstrated serious consequences from these increases.[81–83] The ES guidelines recommend maintaining hematocrit below 55% to avoid adverse events rising from increased red cell mass.[22] Some clinics have advocated reduction of red cell mass with phlebotomy, but there are no trials examining this approach in terms of improving safety.[50]

Hyperlipidemia

A recent systematic review found that testosterone therapy in transgender men results in lowered HDL cholesterol, higher LDL cholesterol, and higher triglycerides.[30] In transgender boys, testosterone therapy also results in lowered HDL cholesterol and higher triglycerides over a period of 2 years.[34] However, there are insufficient data to determine if this adverse pattern of lipids results in long-term cardiovascular events. Data from a cohort of transmasculine adults indicate that the risks of MI are not increased.[67] Routine pharmacologic therapy to improve lipid parameters is not indicated given the available evidence in low-risk individuals.

Uterine and Cervical Cancer

The risk of cancers of the reproductive tract in transgender men appears to be comparable to the risk of cisgender women; therefore, the ES guidelines recommend screening based on remaining organs as recommended for the general population. Among transmasculine adults receiving care in the Kaiser Permanente system in the United States, the risk of cervical cancer was not increased compared to matched cisgender women.[77] There were no cases of uterine cancer reported.

Barriers to Care and Priorities for Research for Transgender Youth and Adults

While there have been significant advances in our understanding and management of transgender youth and adults, many questions remain. There are limited efficacy and safety data regarding current treatment models, with minimal available data regarding the use of GnRH agonists in gender-nonconforming youth younger than 12 years of age, of gender-affirming sex hormones in adolescents younger than 16 years of age, and long-term risks (>10 years) on cardiovascular disease and cancer. Questions also arise regarding the dose and choice of hormone therapy in elderly transgender adults. Furthermore, GnRH agonists and gender-affirming sex hormones are off-label for GI/GD youth and adults, can be expensive (GnRH agonists and transdermal preparations of hormones, in particular), and are often denied by insurance companies. In addition, while new clinical programs for the care of gender-incongruent youth have emerged in many parts of the world in recent years, there are still relatively few such programs, and patients are often required to travel long distances to receive care. Gender-affirming surgical procedures are only performed at a few centers and are often not a covered benefit under insurance. The success and long-term complication rates of these procedures are not known. Finally, formal training for medical providers is not universally available, and there is often prejudice and misunderstanding. Further training and further prospective studies focused on long-term safety and efficacy are needed to optimize health care for transgender youth and adults.

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The complete list of references is available online at ExpertConsult.com.

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22

Endocrine Changes in Pregnancy

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CHAPTER OUTLINE

KEY POINTS

- The impact of pregnancy on the endocrine system is profound and begins with very early production of human chorionic gonadotropin from the trophoblast occurring at implantation.
- Changes in maternal metabolism during pregnancy prioritize fetal growth, including hyperinsulinemia, insulin resistance, increased plasma lipids, and more efficient plasma amino acid transport.
- Increased maternal adipose stores account for a small fraction of maternal weight gain during pregnancy.
- Pharmacokinetics and pharmacodynamics of many medications change during pregnancy due to increased glomerular filtration rates and blood volume.
- The cardiac demand of pregnancy may cause hemodynamic destabilization in women with preexisting heart disease.
- Placental sex is the same as fetal sex, and sex differences in placental responses to exposures translate into sex differences in the developmental origins of adult disease.
- Universal transplacental bidirectional trafficking of maternal, placental, and fetal cells and deoxyribonucleic acid (DNA) occurs. Detecting cell-free fetal DNA in maternal circulation enables noninvasive screening for fetal aneuploidy and other conditions. Persistent postpartum microchimerism has been linked to autoimmune endocrinopathies in later life in both mother and offspring.

Placental Development

Normal placentation requires a coordinated series of events beginning with fertilization. The fertilization rate following unprotected regular intercourse during a single menstrual cycle is 25% to 30%. However, in approximately one-third of conceptions there is either failure of implantation or clinical or subclinical spontaneous abortion.[1]

For the first 5 days, preimplantation development takes place within the fallopian tube. During this period, the zygote undergoes cleavage division and, at least through the eight-cell stage, the blastomeres remain totipotential. In the 16-cell stage, differentiation of the innermost cells into the *inner cell mass* and the surrounding cells into the *trophectoderm* occurs. The inner cell mass develops into the fetus, and the trophectoderm gives rise to the placenta and membranes. On approximately day 5 or 6 after fertilization, the blastocyst enters the uterus, but implantation does not occur for another 1 to 2 days. Implantation occurs after the zona pellucida disappears from around the embryo.[2]

Recent developments in embryo culture during in vitro fertilization (IVF) have allowed embryos to mature to the blastocyst stage, which in turn allows biopsy of the trophectoderm. The trophectoderm cells are then tested for euploidy or aneuploidy and, if a suitable probe is available, single gene disorders.[3]

Implantation is a complex process that involves apposition of the microvilli present on the trophectoderm cells with pinocytes (fused microvilli) on the endometrial cells, followed by removal of fluid between the cells through pinocytosis by the endometrial cells, a process stimulated by progesterone.[4] Progesterone synthesis by the corpus luteum is stimulated and sustained during this time and for the first 6 to 7 weeks of pregnancy by secretion of human chorionic gonadotropin (hCG) by the trophoblast cells. The hCG is first detected in the maternal serum 6 to 9 days after conception.[5] Embryo attachment is enhanced through the expression of a variety of adhesion molecules, including mucins, integrins, and trophinin, a trophoblast-specific cell membrane adhesion protein, as well as cytokines, growth factors, and a variety of transcription factors encoded by homeobox genes.[4,6]

After the trophoblast attaches to the endometrium during the "window of implantation" 6 to 10 days postovulation, the embryo invades the endometrium through a complex process involving matrix metalloproteinases and differentiation of the trophectoderm into *cytotrophoblasts* or *syncytiotrophoblasts*. The syncytiotrophoblasts are multinucleated cells formed by the fusion of cytotrophoblasts. The cytotrophoblasts form a column of cells that invade the endometrium, form anchoring villi, and enter the maternal vasculature, eventually replacing the endothelial layer of the endometrial and myometrial spiral arterioles with a layer of cytotrophoblasts (vascular trophoblasts).[7] This process converts the high-resistance, low-capacity uterine vessels into low-resistance, high-volume vessels, essential for growth of the placenta and fetus.[8] At the site of implantation, the endometrial cells undergo decidualization, enlarging and increasing their metabolic activity with enhanced production of tissue inhibitors or metalloproteinase, extracellular matrix proteins, cytokines, and growth factors that modulate the extent of trophoblast invasion and influence trophoblast function.[4,5,9]

The trophoblast cells secrete several angiogenic proteins, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF), which stimulate blood vessel development within the villi.[10] The syncytiotrophoblasts form an outer layer of cells in the chorionic villi, between the cytotrophoblast cells and the maternal blood space on the exterior surface. Only three tissues separate fetal blood from maternal blood: (1) the endothelium of the fetal vessels in the villi, (2) connective tissue, and (3) the trophoblasts; this form of placentation is referred to as *hemochorial*. Thus in addition to hCG secretion, which is responsible for maintaining early pregnancy, progesterone secretion, required for continuation of pregnancy after the luteal-placental shift, and the synthesis and secretion of other hormones and growth factors (Table 22.1), the syncytiotrophoblasts provide the major site for transportation of oxygen and nutrients to and removal of waste from the fetus.

Substances are transferred across the placenta through transcellular pathways that include carrier-mediated transport (e.g., immunoglobulin G through the Fcγ receptor) and simple extracellular diffusion. The degree of transplacental passage of a hormone from the mother to the fetus through diffusion depends on (1) the rate of placental blood flow; (2) the maternal concentration of the free or readily dissociable hormone; and (3) the molecular mass, lipid solubility, charge, and degree of placental metabolic degradation of the hormone. Maternal-to-fetal transfer occurs for hormones smaller than 700 Da, but the placenta is not permeable to hormones larger than 1200 Da.[11]

The trophoblast also anchors the placenta and fetus to the uterus and helps to protect the fetus, which contains paternal antigens, from rejection by the maternal immune system. This immunologic protection may be mediated by high concentrations of progesterone at the trophoblast-maternal interface and the expression by the trophoblasts of a histocompatibility complex antigen, human leukocyte antigen G (HLA-G), which exhibits reduced polymorphism in comparison with other major HLA antigens.[12] The mass of the trophoblast increases logarithmically during the first trimester, followed by a more gradual increase throughout the remainder of pregnancy. Trophoblastic mass closely correlates with maternal serum concentrations of human placental lactogen (hPL) and pregnancy-specific β_1-glycoprotein throughout pregnancy and with hCG during the first trimester but not during the subsequent trimesters.[13]

TABLE 22.1 Placental Products

Hypothalamic Analogs
Gonadotropin-releasing hormones
Corticotropin-releasing hormone
Urocortin
Somatostatin
Growth hormone–releasing hormone
Ghrelin
Thyrotropin-releasing hormone
Dopamine
Neuropeptide Y
Enkephalin
Pituitary Analogs
Chorionic gonadotropin
Placental lactogen
Chorionic corticotropin
β-endorphin
α-Melanocyte-stimulating hormone
Placental variant growth hormone
Oxytocin
Steroid Hormones
Estrogens (estriol, estradiol, estetrol)
Pregnenolone
Progesterone
Other
Activins
Inhibins
Follistatin
Relaxin
Calcitonin
Leptin
Parathyroid hormone–related protein
Erythropoietin
Renin
Interleukins
Nitric oxide
Transforming growth factor β
Tumor necrosis factor α
Epidermal growth factor
Insulin-like growth factor type 1
Insulin-like growth factor type 2
Insulin-like growth factor binding protein 1
Colony-stimulating factor 1
Basic fibroblast growth factor
Corticotropin-releasing hormone-binding protein
Platelet-derived growth factor
Vascular endothelial growth factor
Endothelin 1
Anandamide (endocannabinoid)
Hepatocyte growth factor
Oncomodulin

Sex Differences in the Placenta

There is now a substantial body of literature describing physiologic differences between men and women and how these differences impact numerous acute and chronic conditions and diseases. Some of these sex differences, such as differences in life

span and differential risk for and outcomes related to chronic conditions such as cardiovascular and cerebrovascular disease, have been recognized for decades. Ongoing investigations have revealed a burgeoning list, suggesting that sex differences are the norm rather than the exception. There are sex differences in frequency and manner of presentation as well as in pathophysiology and pharmacology. Examples of diseases and conditions that demonstrate sex differences include systemic lupus erythematosus, thyroid disease, osteoporosis, diabetes mellitus, Alzheimer disease, and schizophrenia. Differences may be strictly biologic (sex) or due to sociocultural disparities (gender) interacting with biologic predilections.[14] For example, women are at an increased risk of systemic lupus erythematosus, thyroid disease, and osteoporosis; men are at increased risk of cardiovascular disease and schizophrenia. There are also sex differences in responses to acute insults such as trauma, sepsis, and surgery, with women often responding more favorably than men.[14–19]

Two important determinants of pathophysiology of the conditions and diseases are sex and age. We now recognize that sex differences begin during fetal and early neonatal life.[20–25] Thus sex differences interact with aging from conception. The epidemiology surrounding the impact of these very early events is often referred to as the *developmental origins of health and disease* (DOHAD) or *fetal origins of adult disease* (FOAD). Sex differences in the fundamental processes mediating embryogenesis and intrauterine fetal development are just beginning to be understood. Insults during intrauterine life such as natural disasters, death of a family member, and severe maternal anxiety or depression are associated with an increased risk of neurodevelopmental disorders (depression, anxiety, schizophrenia, and autism) in the offspring.[26] A classic epidemiologic example is the famine produced by the invasion of the Netherlands in 1940.[27] Women in their second trimester during this time had male but not female offspring with an increased risk of schizophrenia as adults. In most cases female offspring are more resilient and male offspring more susceptible to the negative effects of gestational insults. Recent data suggest that sex differences in developmental risk are mediated by sex differences in the placental response to common exposures and stressors. It is often not appreciated that because the placenta derives from the same cell mass as the embryo that the placenta has a sex. However, the placenta does not have a gender because gender refers to sociocultural constructs and behaviors related to sex-related expectations and stereotypes.

Sex chromosomes and gonadal steroids are thought to play a large role in mediating sex differences in placental and fetal responses to exposures.[28] Total and free testosterone levels are higher in male fetuses as expected; however, so are levels of the female sex hormone estradiol.[29] However, testosterone is a more potent inhibitor of cytokine production in explants from female compared to male placentas, suggesting female fetuses may be more sensitive to the effects of testosterone than male fetuses.[28] Indeed, sex-specific hormone action may be the norm and not just confined to sex steroids.

In addition to global sex differences described earlier, evidence is accumulating that epigenetic modifications also play a role in gating differential sensitivity between the sexes to common exposures. At baseline, female placentas demonstrate increased expression of immune-regulating genes (*JAK1, IL2RB, Clusterin, LTBP, CXCL1,* and *ILR1L1*), while in males, placental genes reported to mediate graft versus host disease and inflammation (*HLA-DQB1, HLA-DOA1, HCP5, NOS1, FSTL3*, among others) are more highly expressed.[30] Overall the sex differences in physiology appear to produce a less reactive female and more reactive male placental phenotype. This would better equip female placentas to buffer the embryo from insults during critical periods of development. The protection provided by a female placenta is in keeping with the long-known improved mortality and morbidity due to prematurity in female compared to male neonates.[31–33]

Although sex differences in gene expression profiles that drive placental structure and function are now well described, epigenetic changes also play a role. Studies using the established mouse model of early prenatal stress have identified DNA methyltransferases (DNMT)[34] and O-linked N-acetylglucosamine transferase (OGT)[35,36] as top candidates for primary roles in the epigenetic changes. A major function of OGT is to stabilize enhancer of zeste homolog 2 (EZH2), a histone H3K27 methyltransferase that leads to global repression of transcription. As OGT is X-linked and escapes X inactivation in the placenta, levels are twice as high in the female placenta.[35,36] The lower levels of OGT in males would lead to a globally reactive phenotype in the placenta. More targeted regulation of expression may be mediated through DNMT1, which, in conjunction with the methyl-binding protein MeCP2, results in DNA methylation and repression of specific genes. DNMT1 is more highly expressed at baseline in the female placenta and is further increased with maternal stress in female but not in male placentas.[34] Again this would further contribute to a reactive male placental phenotype that could leave male fetuses at greater risk of complications from maternal stress.

Maternal Adaptations to Pregnancy

To some extent, every maternal organ system is altered in pregnancy. The impact of pregnancy on the endocrine system is profound and begins very early with the production of hCG from the trophoblast at implantation. The effects on many other organ systems are more gradual and may not appear until later in pregnancy. While the vast majority of these changes are hormonally mediated, some organ systems are also affected by the anatomic alterations caused by the enlarging uterus or the physiologic increase in maternal blood volume. It is critically important for the clinician to understand these changes when caring for pregnant women, as many physical exam findings, laboratory values, and imaging findings that would be viewed as abnormal and of great concern outside of pregnancy can be normal and reassuring in pregnant women.

Physiologic Adaptations

During pregnancy some amount of weight gain is expected, with overweight and obese women expected to gain less than normal or underweight women. Excessive gestational weight gain has been associated with a number of adverse neonatal[37,38] and maternal outcomes.[39–43] Given this association and the increasing prevalence of obesity among women of childbearing age, the Institute of Medicine (IOM) recommended an updated set of guidelines regarding weight gain in pregnancy based on the woman's prepregnancy body mass index[44] (Table 22.2).

In recent years many experts and medical professional organizations with a stake in the obesity epidemic have proposed further subdividing obesity (body mass index [BMI] >30) into class I (BMI 30–34), class II (BMI 35–39), and class III (BMI ≥40).[45] The IOM guidelines did not further refine their recommendations for weight gain based on level of obesity, citing a lack of evidence regarding proper weight gain in women with very high BMI. While this more specific classification system is helpful for

TABLE 22.2 Weight Gain During Pregnancy

	TOTAL WEIGHT GAIN		RATES OF WEIGHT GAIN SECOND AND THIRD TRIMESTERS	
Pre-pregnancy BMI	Range in kg	Range in lb	Mean (range) in kg/week	Mean (range) in lb/week
Underweight (<18.5 kg/m^2)	12.5–18	28–40	0.51 (0.44–0.58)	1 (1–1.3)
Normal weight (18.5–24.9 kg/m^2)	11.5–16	25–35	0.42 (0.35–0.50)	1 (0.8–1)
Overweight (25–29.9 kg/m^2)	7–11.5	15–25	0.28 (0.23–0.33)	0.6 (0.5–0.7)
Obese (≥30 kg/m^2)	5–9	11–20	0.22 (0.17–0.27)	0.5 (0.4–0.6)

consistency in categorizing patients for epidemiologic or clinical studies, it is not clinically meaningful as the impact of obesity on maternal health has a much more linear relationship than the abrupt classification system would suggest. As with the BMI classification schema proposed by the IOM, the subclassification of obesity also groups large numbers of patients into the high end of the scale (i.e., class III). Such patients are often encountered in current clinical practice as the prevalence of women of childbearing age with BMI over 40 is approximately 7.5% overall and as high as 15% among certain ethnic groups.[46]

It is important to remember that only a small portion of the recommended weight gain is due to an increase in maternal adipose stores. For example, in a normal weight woman with a weight gain of 12.5 kg, 9.25 kg are due to factors other than maternal adipose stores (fetus accounts for about 3.4 kg, placenta for 0.65 kg, amniotic fluid for 0.8 kg, uterus for 1 kg, breasts for 0.4 kg, blood for 1.5 kg, extravascular fluid for 1.5 kg).[14] Thus only 3.25 kg is due to the increase in maternal adipose stores. If one considers the weight gain goal for overweight and obese women (5–9 kg) and assumes that most will be expected to gain 9.25 kg of nonadipose weight during pregnancy, there should be a negligible increase and potentially a decrease in adipose stores in these women if IOM guidelines are followed.

The volume of the uterine cavity increases from 10 mL in the nonpregnant state to an average of 5 L at term, and blood flow through the uteroplacental circulation reaches 450 to 650 mL/minute, approximately a 10-fold increase.[47] To maintain appropriate perfusion of the mother and the fetal-placental unit, systemic blood volume increases throughout pregnancy and is 40% to 45% higher at term than in the nonpregnant state. The plasma volume increases by about 45% to 50% as a result of aldosterone-stimulated sodium and water retention. The red cell mass increases approximately 20% because of increased production, resulting from a twofold to threefold increase in erythropoietin secretion. The net effect is a physiologic decrease in the hematocrit by about 15% at term.[47]

The increase in uterine blood flow, while essential to maintain the fetus and placenta during pregnancy, carries a risk of severe hemorrhage surrounding delivery. Laceration of a vessel during a vaginal delivery can lead to heavy bleeding due to the high rate of blood flow to the uterus, cervix, and vagina. Bleeding can be life threatening if it tracks into the retroperitoneum where a large amount of blood can accumulate before symptoms prompt an evaluation and confirmation of the diagnosis. Damage to a branch of the uterine artery during a cesarean section can lead to bleeding rapid enough to require intraoperative blood transfusion to replace ongoing blood loss. For these reasons, elective operations are typically avoided in the third trimester. Fortunately, there are also maternal adaptations that lessen the potential impact of these and more typical amounts of blood loss. The physiologic drop in hematocrit during pregnancy leads to less red cell mass lost per a given volume of blood. This allows women to maintain oxygen-carrying capacity after volumes of blood loss that would lead to advanced stages of shock in nonpregnant individuals.[48]

The renal blood flow and glomerular filtration rate (GFR) increase rapidly and peak during the second trimester, and a 50% increase in creatinine clearance results in a reduction in the serum creatinine level. Atrial natriuretic peptide (ANP) levels increase during pregnancy and may be in part responsible for increased renal blood flow, GFR, 24-hour urine volume, and natriuresis.[49] An alteration in the osmotic thresholds for the release of vasopressin and activation of the hypothalamic thirst centers, possibly caused by an extragonadal effect of hCG, lead to an approximately 4% reduction in serum osmolality (~10 mOsm/kg).[50]

It is important to consider the increase in GFR and blood volume when treating women with medications that are cleared in the kidney. Many medications, both prescription and over the counter, are cleared in this way, including aminoglycosides, β-lactams, antiviral agents, antifungal agents, histamine (H_1 and H_2) blockers, among others. Some of these medications require dose adjustments due to increased GFR of pregnancy. Adjustments in dose are particularly relevant for antiepileptic drugs, the plasma levels of which can become subtherapeutic if not monitored and steadily increased over the course of pregnancy. Although all the reasons for the increased drug requirement in pregnancy are unclear, it is likely that the increase in the volume of distribution is a contributing factor.[51] This is also true of low-molecular-weight heparins, a preferred anticoagulant in pregnancy. Although only a small portion of the drug is cleared in the kidney, the increased GFR of pregnancy is thought to contribute to the much larger doses required in pregnant women to maintain effectiveness.[52]

Several hemodynamic changes are induced by the low-resistance, high-capacity uteroplacental vasculature, which, in many respects, acts like an arteriovenous shunt. The large quantities of estrogens, progesterone, prostaglandins, and angiotensin present during pregnancy are thought to mediate these changes. Other changes include an increase in the heart rate by 10 to 15 beats/minute, a 30% to 50% increase in cardiac output, resulting from increased stroke volume in early pregnancy and heart rate during the third trimester, a reduction in diastolic blood pressure of 10 to 15 mm Hg with little or no change in systolic pressure, and an approximately 20% reduction in peripheral vascular resistance.[47]

While these increases in cardiac demand are easily met by most pregnant women, for some, particularly those with preexisting

coronary artery or structural heart disease, the added strain can threaten the health of both mother and baby. Although women with a history of myocardial infarction (MI) with normal left ventricular function tolerate pregnancy well, women with poor hemodynamics with significant left ventricular injury are discouraged from attempting pregnancy as maternal morbidity and mortality are markedly increased.[53] In women with structural heart disease, particularly Eisenmenger syndrome, the increase in cardiac output and blood volume can overwhelm an already impaired heart leading to arrhythmia, congestive heart failure, pregnancy loss, and maternal death.[54]

In pregnancy the pulmonary vascular resistance is reduced by about one-third, and pulmonary tidal volume increases by about 30%. The latter, which results in a respiratory alkalosis that is compensated by increased bicarbonate excretion by the kidneys, increases in minute ventilatory volume by 30% to 40%. There are no changes in respiratory rate, maximum breathing capacity, or forced or timed vital capacity. However, there is an approximately 40% reduction in the expiratory reserve because of the elevation of the diaphragm by the enlarged uterus.[47]

While these changes do not increase the risk of pregnant women contracting respiratory infections such as influenza and bacterial pneumonia, their morbidity and mortality are notably increased during pregnancy. This was most recently noted in the 2009 H1N1 pandemic, in which pregnant women accounted for 5% of the deaths but made up only 1% of the population.[55] Pregnant women were also more likely to be hospitalized with pneumonia,[56] but they did not appear to be at an increased risk of infection compared to the general population.[57]

Gastrointestinal tract function is altered during pregnancy. Gastric emptying time is unchanged until labor, when it becomes prolonged. Reduced lower esophageal sphincter tone and displacement of the abdominal contents by the pregnant uterus result in a marked increase in gastroesophageal reflux. Motility of the intestine is also reduced, contributing to nausea and vomiting early in pregnancy and constipation that is common later in pregnancy. Decreased motility of the gallbladder leads to an increased gallbladder volume and reduced emptying of bile after meals, producing a more lithogenic bile and increasing cholelithiasis risk during pregnancy.[47]

Metabolic Adaptations

Numerous changes in maternal metabolism occur in pregnancy to ensure that the fetus has a consistent supply of metabolic fuel during in utero development. These changes include hyperinsulinemia, insulin resistance, increased plasma lipids, and more efficient plasma amino acid transport.[58] As a result of these alterations, maternal energy requirements are met predominately by lipolysis, allowing glucose and other carbohydrates to supply the energy needs of the fetus. This shift in maternal fuel utilization can be conceptualized as an accelerated starvation. Although carbohydrates are readily available in the maternal circulation, insulin resistance reduces entry of glucose into maternal cells, thereby limiting her use of carbohydrates for energy. To compensate, maternal cells turn toward lipid metabolism for energy-producing levels of ketones similar to that seen after prolonged fasting.[59] The increased maternal low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) that occur in pregnancy[60,61] are also thought to be the main cholesterol supply for steroid hormone production in the placenta, as the placenta has very low hydroxymethylglutaryl–coenzyme A (HMG-CoA) reductase activity and thus limited ability to produce cholesterol from acetate in situ.[62]

The underlying causes of the shift in metabolic fuels during pregnancy are largely understood, but significant gaps in knowledge persist. It is clear that the increasing levels of hPL and human placental growth hormone (hPGH) reduce insulin receptors and glucose transport.[63] However, to what extent glucagon and cortisol, which demonstrate strong diabetogenic effects outside of pregnancy, play a role in this process has not been thoroughly investigated.

Maternal Endocrine Alterations

Pituitary Gland

The anterior pituitary gland enlarges by an average of 36% during pregnancy, primarily because of a 10-fold increase in lactotroph size and number. This enlargement increases the height and convexity of the pituitary that is observed on magnetic resonance imaging (MRI). Somatotroph and gonadotroph numbers are reduced, and there are no changes in corticotroph or thyrotroph numbers.[64] The size of the posterior pituitary gland diminishes during pregnancy.[65]

The marked increase in estrogen levels during pregnancy enhances prolactin synthesis and secretion, and maternal serum prolactin levels increase in parallel with the enlargement of the lactotrophs. At term, the mean serum prolactin concentration is 207 ng/mL (range, 35–600 ng/mL), in contrast to a mean of 10 ng/mL in nonpregnant, premenopausal women.[66] Prolactin also is present in the amniotic fluid and appears to be primarily of decidual origin because the decidua actively synthesizes prolactin. Amniotic fluid prolactin levels are 10 to 100 times higher than in the maternal circulation in early pregnancy. Serum prolactin levels return to the baseline of nonpregnancy approximately 7 days after delivery in the absence of breastfeeding. With breastfeeding, the basal prolactin levels remain elevated for several months but gradually decrease; however, with suckling, there is a brisk rise in prolactin levels within 30 minutes.[64]

Although rare, pituitary tumors do occur in women of childbearing age. While most are diagnosed prior to pregnancy, some are diagnosed for the first time in pregnancy. The most common type of tumor is a prolactinoma, which (as the name implies) is due to an overgrowth of lactotrophs. Prolactinomas, and other pituitary tumors, are typically classified based on size, with those less than 10 mm in diameter designated *microadenomas* and those greater than 10 mm in diameter designated *macroadenomas*. The increase in pituitary size during pregnancy can lead to complications in women with prolactinomas. In a study of 352 women with untreated microadenomas, 2.3% experienced visual disturbances, 4.8% experienced headache, and 0.6% experienced diabetes insipidus. The rates of complications were much higher in 144 women with macroadenomas, with 15.3% experiencing visual disturbances, 15.3% experiencing headache, and 2.14% experiencing diabetes insipidus.[67] In cases of symptomatic tumor expansion in pregnancy, the dopamine agonist bromocriptine can be safely used and is successful in reducing tumor size and symptoms in the majority of cases.[68] Cabergoline, a more selective dopamine 2 receptor agonist and the preferred treatment outside of pregnancy, also appears safe, but there is less experience with this drug in pregnancy. Transsphenoidal resection of the pituitary is occasionally needed if rapid tumor growth occurs or symptoms persist despite bromocriptine treatment.

Growth hormone (GH) levels in maternal serum remain unchanged throughout pregnancy, although the source of immunoreactive GH during gestation changes due to placental

production of the hormone. Relaxin, secreted by the corpus luteum of pregnancy, and estrogens stimulate GH secretion during early pregnancy.[69]

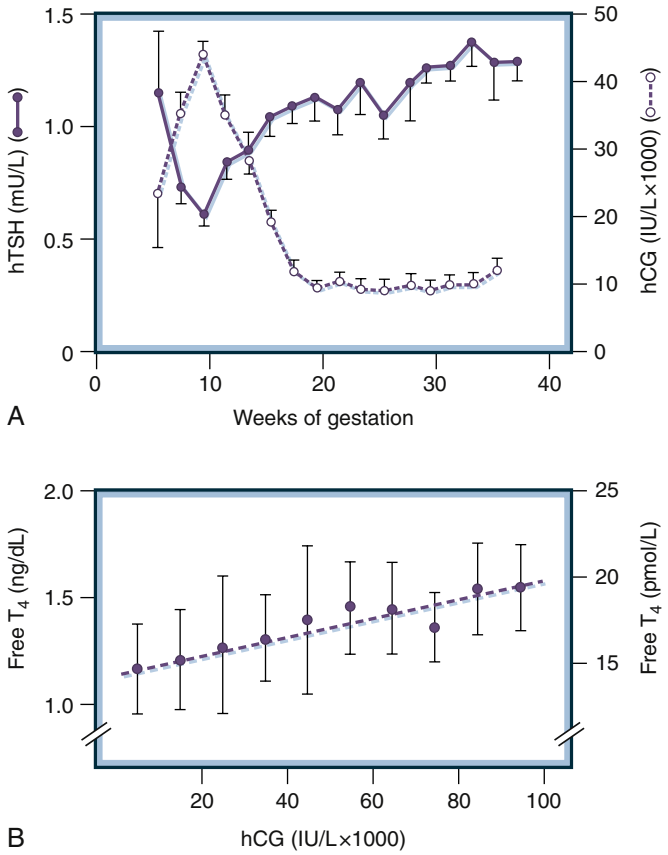
Pituitary GH is known as GH1 or hGH-N; its messenger ribonucleic acid (mRNA) and GH1 secretion decrease after week 25 of pregnancy, and beginning in the fourth month of gestation, the placental syncytiotrophoblasts secrete a variant of GH (called GH2 or hGH-V) in a nonpulsatile pattern. In concert with the different sources of GH during the first and second halves of pregnancy, the GH response to provocative stimuli differs in each half. Insulin hypoglycemia or arginine infusion results in an enhanced GH response during the first half of gestation, and during the second half the response is decreased compared with the response in nonpregnant women.[64]

Maternal serum concentrations of insulin-like growth factor type 1 (IGF1) are elevated during the second half of pregnancy, probably through the combined effect of the placental GH variant and hPL, which is evolutionarily related to GH and prolactin. hPL has somatotropic biologic activity, and its serum concentration increases throughout pregnancy, paralleling that of IGF1.[70] It is likely that the suppression of GH1 synthesis and secretion is caused by the high IGF1 concentrations, which in late pregnancy are five times higher than those in nonpregnant women.[71]

Although the placenta synthesizes and secretes biologically active gonadotropin-releasing hormone (GnRH), pituitary gonadotropin production decreases throughout pregnancy, as indicated by a marked reduction in gonadotropin immunoreactivity in the gonadotrophs beginning at 10 weeks of gestation and by a reduction in serum levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[64] Suppression is probably mediated through the elevated blood levels of ovarian and placental sex steroid hormones and by placental production of inhibin. Suppression is incomplete because administration of exogenous GnRH leads to release of gonadotropins, although the response is blunted compared with that of nonpregnant women and does not return to normal until a month after birth.[71]

The mean concentrations of human thyroid-stimulating hormone (thyrotropin or hTSH) during the first trimester are significantly lower than in the second and third trimesters or in the nonpregnant state.[72] Much of this early decrease may be accounted for by the intrinsic thyrotropic activity of hCG. The nadir in TSH in maternal serum corresponds to the peak concentration of hCG at 10 to 12 weeks after the last menstrual period, demonstrating a reciprocal relationship between the rising hCG levels and falling hTSH concentrations[72,73] (Fig. 22.1A). Higher hCG levels are associated with higher T4 levels (see Fig. 22.1B). Despite the lower mean hTSH during early pregnancy, the hTSH response to exogenous thyrotropin-releasing hormone (TRH) is normal.[64]

Maternal adrenocorticotropic hormone (ACTH, or corticotropin) levels rise during pregnancy, increasing fourfold over concentrations in the nonpregnant state between 7 and 10 weeks of gestation. There is a further gradual rise to weeks 33 to 37, when a mean 5-fold increase over prepregnancy values is found, followed by a 50% drop just before parturition and a marked 15-fold increase during the stress of delivery.[74] The ACTH concentration returns to the prepregnancy level within 24 hours of delivery. The pituitary gland and placenta are sources of circulating ACTH during pregnancy, and exogenous corticotropin-releasing hormone (CRH) stimulates the release of ACTH from both tissues in a dose-dependent manner.[47] Biologically active CRH is synthesized and secreted by the placenta and, to a lesser extent, by the decidual and fetal membranes, but unlike the inhibitory



• **Fig. 22.1** (A) Concentrations of serum thyrotropin (hTSH, *filled circles*) and human chorionic gonadotropin (hCG, *open circles*) throughout pregnancy. Between 8 and 14 weeks of gestation, there is a significant negative correlation between the individual hTSH and hCG levels (p <0.001). Each point represents the mean (± standard error). (B) Linear regression of maternal serum free thyroxine (T_4) and hCG concentrations during the first half of gestation (p <0.001). (From Glinoer D, de Nayer P, Bourdoux P, et al. Regulation of maternal thyroid during pregnancy. *J Clin Endocrinol Metab.* 1990;71:276–287.)

effect on pituitary CRH, glucocorticoids stimulate the expression of placental CRH.[75]

The relationship between CRH and ACTH is altered during pregnancy. Although biologically active CRH would be expected to stimulate ACTH production, the qualitative patterns of CRH production, which show an exponential rise during the sixth month of gestation, and ACTH secretion, which demonstrates a more gradual rise during pregnancy, are quite different. The lack of a significant correlation between maternal plasma CRH and ACTH during pregnancy suggests that factors such as the elevated levels of free cortisol in the maternal serum may modulate the response to CRH. The circadian rhythm and the ability to respond to stress are maintained throughout pregnancy; however, the ACTH response to exogenous CRH during the third trimester is blunted, whereas the responsiveness to vasopressin is maintained, suggesting that the elevation of CRH in the maternal serum downregulates the responsiveness to CRH.[74]

Arginine vasopressin (AVP) concentrations in the maternal serum are similar to those in nonpregnant women. During pregnancy, however, there is increased synthesis of AVP, which is offset by the increased metabolic clearance of the hormone through destruction by a trophoblast-derived cysteine aminopeptidase (i.e., vasopressinase), which rises throughout pregnancy in parallel

with the increase in trophoblastic mass.[76,77] The osmolar set-point for thirst is reduced, and the release of AVP is related to the 10-mOsm/kg average decrease in plasma osmolality during pregnancy, possibly reflecting an extragonadotropic effect of hCG.[48] Taking into account the reduced set-point, the AVP response to dehydration and water loading is normal.

Oxytocin levels progressively increase in the maternal blood and parallel the increase in maternal serum levels of estradiol and progesterone. The levels increase further with cervical dilation and vaginal distention during labor and delivery, stimulating contraction of the uterine smooth muscles and enhancing fetal expulsion.[78] Uterine oxytocin receptors also increase throughout pregnancy, resulting in a 100-fold increase in oxytocin binding at term in the myometrium.[79]

Thyroid Gland

The thyroid gland enlarges by an average of 18% during pregnancy.[72] Enlargement is associated with an increase in the size of the follicles with increased amounts of colloid and enhanced blood volume. This enlargement may be a response to the thyrotropic effect of hCG and asialo hCG, which may account for some of the increase in serum thyroglobulin concentrations observed during pregnancy. Although clinically contraindicated, experimental evidence suggests that enhanced iodine (^{131}I) uptake by the maternal thyroid gland reflects the combined effects of hCG stimulation and reduction of the blood levels of iodide by increased renal iodide clearance.[72]

Rising estrogen concentrations during pregnancy induce increased hepatic synthesis of thyroxine-binding globulin (TBG) and enhanced sialylation of TBG, which decreases its metabolic clearance rate.[73] The results are a twofold increase in TBG and increased total thyroxine (T_4) and triiodothyronine (T_3) levels in maternal serum throughout pregnancy,[73] whereas for most of the gestation, the free T_4 and free T_3 concentrations remain normal. There are no significant changes in the levels of thyroxine-binding prealbumin, but albumin levels are decreased because of the increase in vascular volume.

While hyperthyroidism is rather rare, hypothyroidism is relatively common in women of childbearing age. When managing a pregnancy complicated by hypothyroidism, it is important to note that the pregnant woman is the source of T_4 and T_3 for the fetus throughout the first trimester. As a result, the maternal thyroid hormone replacement requirements increase as early as the fifth week of gestation. Although requirements often plateau in the second trimester, they continue to increase well into the third trimester in many patients. Increased doses of thyroid hormone are needed in 50% to 85% of women, and the total dose may increase as much as 50%.[80,81] Thus in women with preexisting hypothyroidism, it is necessary to check thyroid function at the first prenatal visit to determine if additional thyroid hormone replacement is necessary. Alternatively, the dose can be empirically increased when pregnancy is confirmed prior to any laboratory assessment of thyroid function.[82] Intermittent monitoring throughout pregnancy is warranted every 4 to 8 weeks to make certain replacement remains adequate as gestation progresses. Routine screening for undiagnosed hypothyroidism in pregnancy is controversial and is not currently endorsed by the American Thyroid Association (ATA), American College of Obstetricians and Gynecologists (ACOG), or the Endocrine Society.[83–85] Instead, a targeted screening approach has been adopted based on risk factors such as family or personal history of thyroid disease, prior head or neck radiation, morbid obesity, or age greater than 30 years.

Parathyroid Glands

During pregnancy, approximately 30 g of calcium is transferred from the maternal compartment to the fetus, with most of the transfer occurring during the last trimester. Maternal total serum calcium levels decrease during pregnancy, with a nadir at 28 to 32 weeks related to the decrease in albumin levels that accompanies the increase in vascular volume. However, the albumin-adjusted total calcium and the ionized calcium concentrations actually rise slightly above this level in the nonpregnant state.[86] The urinary calcium excretion rate increases in parallel with the increased GFR, and intestinal calcium absorption undergoes a twofold increase.[86]

Although some studies have suggested that parathyroid hormone (PTH) levels increase during pregnancy, measurements of intact PTH levels by two-site immunometric assays indicate that they are within the normal, nonpregnancy range throughout pregnancy. In contrast, the circulating concentrations of PTH-related protein (PTHrp) increase throughout pregnancy.[87] Many normal tissues produce this protein, and the source of the elevated levels during pregnancy is unclear, although the two most likely sites are the mammary tissue and the placenta.[87] This protein is probably involved in placental and mammary gland calcium transport.

The serum levels of 25-hydroxyvitamin D are unchanged during pregnancy, but the estrogen-induced rise in vitamin D–binding globulin results in a twofold increase in 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) concentrations in maternal serum.[86] There is also a rise in the biologically active free fraction of $1,25(OH)_2D$, which may reflect both increased maternal renal 1α-hydroxylase activity and the synthesis and secretion of $1,25(OH)_2D$ by the placenta.[86] This increase in the active metabolite of vitamin D may be responsible in part for the enhanced intestinal calcium absorption.

Although hyperparathyroidism and hypoparathyroidism are uncommon, they both can lead to serious complications in pregnancy. Pregnancy is somewhat protective in women with hyperparathyroidism because calcium uptake by the fetus helps lower maternal calcium levels. Although women with hypercalcemia in pregnancy often have mild to moderate symptoms of nausea, vomiting, pain, and renal colic,[88] some experience more serious complications such as nephrolithiasis, pancreatitis, hypertension, bone disease, and hypercalcemic crisis.[89] A crisis can be difficult to treat as many of the agents used in nonpregnant patients, such as bisphosphonates and plicamycin, are avoided because of concern for the fetus. Calcitonin can potentially be used as it does not cross the placenta; however, its benefits and safety in pregnancy have not been sufficiently investigated.[90]

Hypoparathyroidism typically poses fewer serious risks, but it can lead to both maternal and fetal fractures if sufficiently severe.[91,92] These complications can generally be prevented with sufficient oral calcium and vitamin D supplementation. As mentioned earlier, a significant amount of maternal calcium is consumed by the fetus during gestation. This often requires a steady increase in calcium (1–1.5 mg of elemental calcium per day) and vitamin D (up to 50,000–100,000 units/day or more) throughout pregnancy to maintain normal maternal serum calcium homeostasis.[93]

Pancreas

Hyperplasia and hypertrophy of the beta cells in the islets of Langerhans are probably the result of stimulation by estrogen and progesterone.[94] During early pregnancy, the glucose requirements of the fetus lead to enhanced transport of glucose across the placenta by facilitated diffusion, and maternal fasting hypoglycemia may be present. Although basal insulin levels may be normal, there is

hypersecretion of insulin in response to a meal. Because the half-life of insulin is not altered during pregnancy,[95] this increase represents an increase in synthesis and secretion. The results are enhanced glycogen storage and decreased hepatic glucose production.

As pregnancy progresses, the levels of hPL rise, as do the levels of glucocorticoids, leading to the insulin resistance found during the last half of pregnancy.[96] Thus, in late pregnancy, glucose ingestion results in higher and more sustained levels of glucose and insulin and a greater degree of glucagon suppression than in the nonpregnant state.

Adrenal Glands

As a result of the hyperestrogenemia of pregnancy, hepatic production of cortisol-binding globulin is increased. The increased production results in a doubling of the maternal serum levels of cortisol-binding globulin, which in turn results in decreased metabolic clearance of cortisol and a threefold rise in total plasma cortisol by week 26, when the levels reach a plateau until they rise at the onset of labor.[74,97] The rate of cortisol production and the plasma free cortisol concentrations are also increased.[92] The enhanced cortisol production is due to an increase in the maternal plasma ACTH concentrations and hyperresponsiveness of the adrenal cortex to ACTH stimulation during pregnancy.[74] Cortisol secretion follows that of ACTH, and the diurnal rhythm is maintained during pregnancy.[97] Despite the elevated free cortisol levels, pregnant women do not develop the stigma of glucocorticoid excess, possibly because of the antiglucocorticoid activities of the elevated concentrations of progesterone.

Levels of androstenedione and testosterone, whether they are of adrenal or ovarian origin, are elevated because of the estrogen-induced increase in hepatic synthesis of sex hormone–binding globulin. However, the free androgen levels remain normal or low. The adrenal production rates of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are increased twofold, but the maternal serum concentration of DHEAS is reduced to one-third to one-half the nonpregnancy levels because of the enhanced 16-hydroxylation and placental utilization of 16-hydroxydehydroepiandrosterone sulfate in estrogen formation. Adrenal medullary function remains normal throughout pregnancy. Thus 24-hour urine catecholamine and plasma epinephrine and norepinephrine levels are similar to concentrations in the nonpregnant state.[98]

Fortunately, hypercortisolism is rare in pregnancy as the disorder often leads to menstrual disturbance and infertility. While outside of pregnancy hypercortisolism is most commonly caused by an ACTH-producing pituitary[99] adenoma (so-called Cushing disease), when encountered in pregnancy it is more likely due to primary adrenal hyperplasia (so-called Cushing syndrome). This discrepancy may be due to the lower degree of menstrual disturbance in patients with primary adrenal hyperplasia.[100] The high levels of cortisol in both conditions can lead to hypertension, diabetes, preeclampsia, and even maternal death. Cushing syndrome in pregnancy it also associated with a 43% risk of preterm delivery and a 6% risk of stillbirth.[74]

Renin-Angiotensin System

Plasma renin substrate levels are increased as a consequence of the effects of estrogen on the liver. Renin levels are also increased, and increased renin activity results in increased levels of angiotensin II, which leads to an 8-fold to 10-fold increase in aldosterone production and serum aldosterone levels.[74] The aldosterone levels peak in midpregnancy and are maintained until delivery.

Despite their baseline elevations, the various components of the renin-angiotensin-aldosterone system demonstrate normal responses to positional changes, sodium restriction, and sodium loading. The elevated aldosterone levels do not lead to an increase in serum sodium, a decrease in serum potassium, or an increase in blood pressure, which again may reflect the high progesterone concentrations, which are capable of displacing aldosterone from its renal receptors. Another mineralocorticoid, 11-deoxycorticosterone, shows a 6-fold to 10-fold increase in concentration at term.[45] Elevated levels of this hormone are due to estrogen-induced extraglandular 21-hydroxylation of progesterone produced by the placenta.[101]

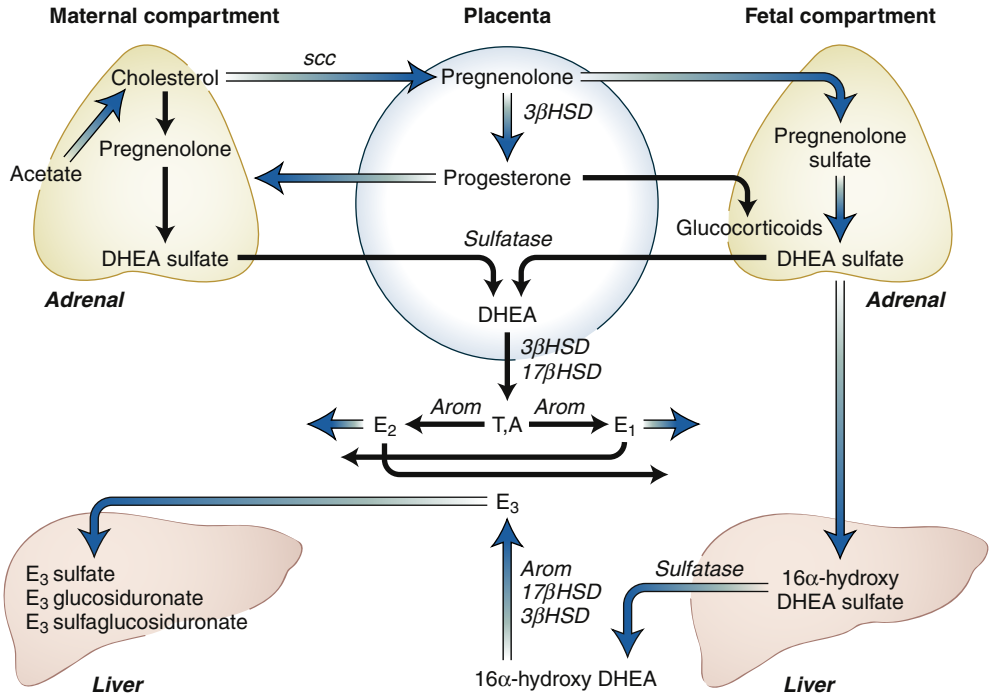
The prevalence of hypertension in women of childbearing age has steadily increased over time and is now a common problem encountered by obstetric providers. Outside of pregnancy, angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) are widely used in the treatment of hypertension, particularly in people with diabetes, as they have been shown to slow the progression of diabetic nephropathy.[102] ACE inhibitors have been linked to oligohydramnios, fetal renal dysplasia, and fetal calvarial hypoplasia.[103] As a result, this group of medications is avoided in pregnancy. Although ARBs have not been shown to have the same adverse fetal effects, they are also typically avoided in pregnancy.[104] Patients on these medications should be switched to alternative agents prior to or in the first trimester of pregnancy. Methyldopa is the best studied antihypertensive medication in pregnancy and is safe for use at any point in gestation.[105] Although less well studied, the calcium channel blocker nifedipine appears to be safe for the fetus and is often used in pregnancy.[106] Beta blockers are often used, but these medications have been associated with an increased risk of fetal growth restriction[107]

Placental Hormone Production

Sex Steroid Production From the Maternal-Fetal-Placental Unit

Sex steroid production in the adult ovary has been classically described as a "two-cell process," wherein the theca cells convert cholesterol to progesterone, testosterone, and other androgens but are then unable to produce estrogen as the theca cells lack the crucial enzyme aromatase. This enzymatic obstacle is overcome by the nearby granulosa cells, which have an abundance of aromatase and take up androgens and rapidly convert them to estrogens.[108] A similar strategy using multiple locations and cell types is also utilized in the massively increased sex steroid machinery of pregnancy. In this system, both the maternal and fetal adrenal glands interact with the placenta to produce very large amounts of progesterone and estrogens. This dependence has led to the concept of the *maternal-fetal-placental unit*.[109] These interactions are outlined in Fig. 22.2.[110,111]

The placenta, like the theca cell of the ovary, has the enzymes necessary to produce progesterone. However, the placenta has very low levels of HMG-CoA activity, so it utilizes cholesterol from the maternal circulation as a substrate for progesterone production.[112] Cholesterol is delivered to the placenta by VLDL, LDL, and HDL, as receptors for all these lipoproteins are present in the syncytiotrophoblast. Cholesterol is converted to pregnenolone by the enzyme CYP11A1,[109,113] which is in turn converted



• **Fig. 22.2** Steroidogenesis in the maternal-fetal-placental unit. *AROM,* aromatase-enzyme complex; *DHEA,* dehydroepiandrosterone; *HSD,* hydroxysteroid dehydrogenase; *SCC,* cholesterol side-chain cleavage enzyme.

to progesterone by 3β-hydroxysteroid dehydrogenase (3βHSD). The placenta also lacks the enzyme 17α-hydroxylase and cannot convert progesterone to androgens even though it has sufficient aromatase activity to convert androgens into estrogens.[114] Thus progesterone is not further modified in the placenta, and approximately 90% is released into the maternal circulation. However, placental progesterone is also an important substrate for production of glucocorticoids and mineralocorticoids in the fetal adrenal glands.[109,113]

The trophoblast lacks 17α-hydroxylase and 17,20-lyase (CYP17) activities and therefore cannot directly convert progesterone to estrogen. Pregnenolone produced in the placenta enters the fetal compartment, where it is taken up by the fetal zone of the adrenal cortex, which also synthesizes pregnenolone from fetal LDL cholesterol. Pregnenolone is conjugated with sulfate by fetal steroid sulfotransferase in the fetal liver and adrenals to form pregnenolone sulfate and is converted in the fetal adrenals to 17α-hydroxypregnenolone sulfate and then DHEAS by 17α-hydroxylase and 17,20-lyase (CYP17) activities.[115]

The DHEAS enters the fetal circulation and undergoes hydroxylation in the fetal liver to form 16α-hydroxy-DHEAS, which is converted to 16α-DHEA in the placenta through the action of placental sulfatase. Further metabolism in the trophoblast by 3βHSD1, 17βHSD, and aromatase (CYP19) leads to the generation of estriol, which is quantitatively the major estrogen in the maternal circulation during pregnancy. The maternal liver actively conjugates estriol with glucosiduronate and sulfate, which are excreted into the urine. Approximately 90% of the estriol present in the maternal serum and urine is derived from fetal precursors, and therefore measurement of estriol levels in serum or urine serves as an index of fetal well-being.[115]

DHEAS from both the fetus and mother is also taken up by the placenta and converted to estradiol by the actions of sulfatase, 3βHSD1, 17βHSD, and aromatase or to estrone by sulfatase, 3βHSD1, and aromatase. An estrogen unique to pregnancy, estetrol, is generated by 15α-hydroxylation of 16α-DHEAS in the fetal adrenal followed by enzymatic conversion by placental sulfatase, 3βHSD1, 17βHSD, and aromatase.[115]

Protein Hormones

Human Chorionic Gonadotropin

Chemistry

Human chorionic gonadotropin is a glycoprotein composed of two dissimilar subunits, α and β, which are noncovalently linked through hydrophobic bonding. This molecule shares structural homology with the other glycoprotein hormones, human luteinizing hormone (hLH), hFSH, and hTSH. These hormones have α-subunits that contain the same sequence of 92 amino acids and differ only in their carbohydrate composition; the β-subunits differ in both amino acid and carbohydrate structure and are responsible for the biologic and immunologic specificity of the heterodimeric (intact) hormones. The 22,200-Da β-subunit of hCG is composed of 145 amino acids. Approximately 80% of the first 115 amino acids are homologous to those in the β-subunit of hLH. hCG has an additional 24 amino acids on its carboxyl-terminal end that enhance its biologic activity.

Both subunits of hCG contain two oligosaccharide chains attached to asparagine residues through N-glycosidic linkages, and the β-subunit contains in addition four O-serine–linked oligosaccharide units in the carboxyl-terminal peptide. The carbohydrate composition of hCG contains microheterogeneity and affects hormone clearance and biologic activity. The tertiary structure of hCG is determined by the carbohydrate composition and multiple disulfide bonds within each subunit. The β-subunit contains five disulfide bonds; the β-subunit has six. In each of the subunits, three of the disulfide bonds form a cysteine knot, similar to that found in PDGFβ and transforming growth factor β (TGFβ).[116]

Biosynthesis

The single α-subunit gene, located on chromosome 6, is actively expressed in both the cytotrophoblast and syncytiotrophoblast. In contrast, the β-subunit is encoded by a cluster of six genes located on chromosome 19 in proximity to the hLHβ gene. Three of the hCGβ genes are actively transcribed during pregnancy, primarily in the syncytiotrophoblast, which synthesizes and secretes free subunits and intact hCG. After synthesis of the protein core, each subunit is glycosylated, undergoes further post-translational modification through trimming of the carbohydrate, then combines to form intact hCG.[116]

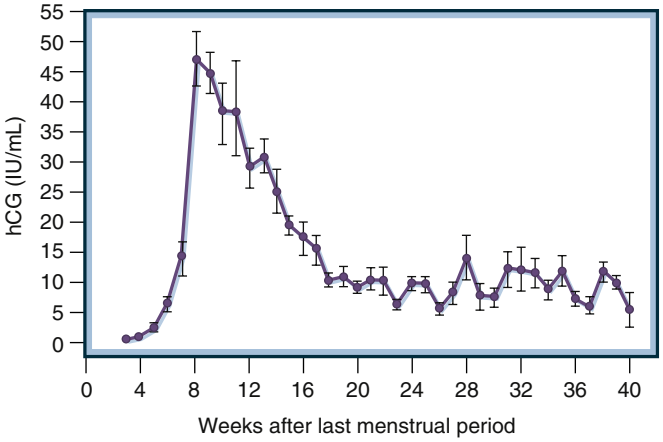
Secretion of hCG differs from that of many of the other placental proteins, whose secretory pattern parallels that of the trophoblastic mass. hCG is first detected in maternal serum 6 to 9 days after conception.[5] The levels rise in a logarithmic fashion, peaking 8 to 10 weeks after the last menstrual period, followed by a decline to a nadir at 18 weeks, with subsequent levels remaining constant until delivery[117] (Fig. 22.3). The placenta also secretes free subunits. During the first 13 weeks of pregnancy, relatively more β-subunit is synthesized than α-subunit, and throughout the remainder of pregnancy the opposite occurs.[118] In addition, a hyperglycosylated form of α-subunit *(big α)* that is unable to combine with free α-subunit is secreted into the maternal serum.

The physiologic factors that regulate hCG secretion in vivo are unknown. Much of the data concerning factors that stimulate or inhibit hCG synthesis and secretion have been derived from in vitro studies and are difficult to extrapolate to the in vivo situation. There is strong circumstantial evidence that GnRH, synthesized in both the cytotrophoblast and syncytiotrophoblast, may be an important factor in hCG secretion. This peptide is identical to hypothalamic GnRH and stimulates placental hCG production both in vitro and in vivo, whereas GnRH antagonists decrease basal hCG secretion.[116,119,120]

Immunohistochemical staining for GnRH in placental tissue is highest at 8 weeks of gestation and lower afterward,[121] roughly paralleling the pattern of hCG production, as do the circulating levels of GnRH measured in maternal serum.[120] In addition, the placenta contains GnRH receptors.[122] Placental GnRH release is stimulated by cyclic adenosine monophosphate (cAMP), prostaglandin E_2 (PGE_2), prostaglandin F_2 (PGF_2), epinephrine, epidermal growth factor, insulin, and vasoactive intestinal peptide (VIP), factors also noted to increase hCG secretion in vitro.[116,119,123]

Two other peptides synthesized by the cytotrophoblast, activin and inhibin, also modulate GnRH and hCG secretion; activin increases both, and inhibin inhibits the action of GnRH on the syncytiotrophoblast.[119] Increases in hCG production have also been found after trophoblast exposure to FGF, calcium, glucocorticoids, and phorbol esters.[119] Decreased production occurs with TGFβ, follistatin, and progesterone.[119] The decidua may also influence hCG production through paracrine mechanisms.[9] Decidual interleukin-1 stimulates hCG secretion in cultured trophoblasts,[124] while decidual prolactin and an 8-kDa to 10-kDa decidual protein inhibit hCG production.[125]

Finally, hCG may autoregulate its own production to some extent. hCG receptors are present on the surface of trophoblastic cells, and the addition of hCG to placental cells in culture stimulates cAMP production as well as proliferation and differentiation of the cytotrophoblasts into syncytiotrophoblasts.[116] Both hCG mRNA and hCG production are stimulated by analogues of cAMP or agents that activate adenylate cyclase, probably through a protein kinase.[116,119] Thus the net effect of an increase in syncytiotrophoblast mass and cAMP would be an enhancement of hCG secretion.



• **Fig. 22.3** Mean (± standard error) levels of maternal serum human chorionic gonadotropin (hCG) throughout normal pregnancy. (From Braunstein GD, Rasor J, Danzer H, et al. Serum human chorionic gonadotropin levels throughout normal pregnancy. *Am J Obstet Gynecol.* 1976;126:678–681.)

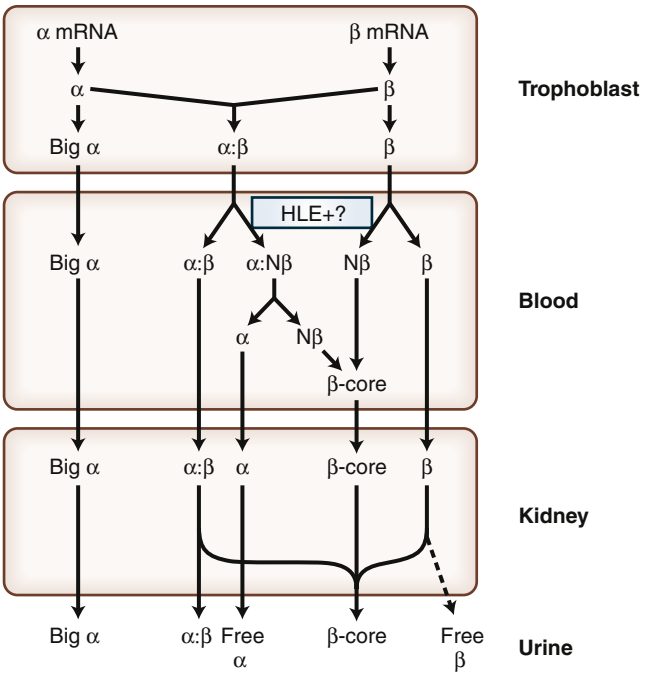
The placenta is not the only site of hCG synthesis. Immunoreactive hCG has been found by immunocytochemistry or by immunoassay of extracts of a wide variety of normal tissues, including the spermatozoa, testes, endometrium, kidney, liver, colon, gastric tissue, lung, spleen, heart, fibroblast, brain, and pituitary gland,[126] and the hormone has been shown to be synthesized in some fetal tissues.[116] The pituitary gland appears to be the major source of hCG or an hCG-like material present in nonpregnant individuals. Immunoactive and bioactive hCG have been partially purified from pituitary glands; the material is secreted in vitro by fetal pituitary cells and is shown by immunocytochemistry to be present in gonadotroph-type cells that do not contain hLH or hFSH.[126,127]

Immunoreactive hCG has been measured in sera from normal, nonpregnant individuals, with the highest concentrations found in postmenopausal women.[127,128] In postmenopausal women, this material is secreted in a pulsatile fashion in parallel with hLH pulses, and during the normal menstrual cycle, the immunoreactive hCG shows a midcycle peak concomitant with the hLH peak.[129] In both men and postmenopausal women, GnRH stimulates secretion of the hormone, whereas its secretion is inhibited by oral contraceptives in women and by a GnRH agonist in agonadal men.[128,130]

Both gestational and nongestational trophoblastic tumors secrete hCG and its free subunits. The sources of hCG secretion in nongestational trophoblastic neoplasms are the syncytiotrophoblastic cells and in seminomas are the trophoblastic giant cells.[130] In many instances, the tumors produce incomplete forms of hCG or its subunits, and differences in carbohydrate content from the hCG in pregnancy have been especially apparent. A wide variety of nontrophoblastic tumors also secrete hCG, although the predominant moiety appears to be the free β-subunit of hCG.[130,131]

Metabolism

After it is secreted, hCG exhibits a biexponential clearance from the circulation with a fast half-time ($t_{1/2}$) of 6 hours and a slow $t_{1/2}$ of close to 36 hours. In contrast, the free β-subunit has a 41-minute fast $t_{1/2}$ and a slow $t_{1/2}$ of 4 hours, and the free α-subunit has a 13-minute fast $t_{1/2}$ and a 76-minute slow $t_{1/2}$.[132] Approximately 22% of the intact hormone appears in the urine unchanged; the rest undergoes metabolic degradation (Fig. 22.4). One of the early steps is proteolytic cleavage ("nicking") of the β-subunit at



• **Fig. 22.4** Proposed pathways for metabolism of human chorionic gonadotropin (hCG). *α:β,* intact hCG; *α:Nβ,* hCG with nicked β-subunit; *Big α,* hyperglycosylated form of the α-subunit; *CTP fragment,* carboxy-terminal fragment; *HLE,* human leukocyte elastase; *mRNA,* messenger RNA; *Nβ,* free nicked β-subunit. (From Braunstein GD. Physiologic functions of human chorionic gonadotropin during pregnancy. In: Mochizuki M, Hussa R, eds. *Placental Protein Hormones.* Amsterdam: Elsevier Science; 1988:33.)

Val44-Leu45 and Gly47-Val48. Human leukocyte elastase, present in macrophages and leukocytes, appears to be responsible for some of the nicking of the α-subunit.[132]

Nicked hCG is unstable and dissociates into free α-subunit and nicked free β-subunit. The latter is further metabolized, primarily in the kidney, to produce the β-core fragment, which is composed of the β-subunit amino acids 6 to 40 disulfide bridged to amino acids 55 to 92, trimmed of a portion of carbohydrate, and has a molecular mass of 10,479 Da.[133] This fragment is the major form of immunoreactive hCG present in the urine in pregnancy. In normal pregnancy, the urine also contains variable quantities of the hyperglycosylated form of α-subunit, free α-subunit, free β-subunit, nicked hCG, nicked free β-subunit, carboxyl-terminal fragments of the β-subunit, and fragments of the α-subunit.[132]

Physiologic Functions

Most, if not all, of the physiologic functions of hCG occur after interaction of the hormone with the hLH-hCG receptor. The receptor gene is located on chromosome 2 and encodes for a G protein–coupled receptor with seven hydrophobic transmembrane domains and a large extracellular amino terminus that binds to hCG (and hLH). The receptor is part of a superfamily of receptors, including those for hFSH, hTSH, AVP, VIP, PTH, and receptors for a variety of biogenic amines and neurotransmitters.[116] The hCG-receptor interaction results in increased cAMP production and, in some tissues, increased phosphoinositide turnover.[132]

Because of the close structural homology of the hLH-hCG receptor with the other glycoprotein hormone receptors, hCG may interact with the hTSH and hFSH receptors and thus has weak intrinsic hTSH and hFSH biologic activity. As previously noted, the hTSH-like activity of hCG is clinically manifested during normal pregnancy by the reciprocal decrease in maternal hTSH at the time of the hCG peak between 8 and 12 weeks after the last menstrual period. It is especially important in patients with hydatidiform moles and other forms of trophoblastic disease, in which hCG levels may exceed 100,000 IU/L and result in clinical thyrotoxicosis[72,73] (see Fig. 22.1).

One of the major functions of hCG during pregnancy is the "rescue" of the corpus luteum during the conception cycle.[134] During a menstrual cycle without conception, progesterone concentrations in the serum increase for the first 6 to 7 days of the luteal phase, followed by a plateau of 3 to 4 days and then a decrease, resulting in the shedding of the endometrial lining. After conception and implantation, the corpus luteum continues to secrete progesterone and 17-hydroxyprogesterone for another 4 to 6 weeks. The maternal serum progesterone and 17-hydroxyprogesterone concentrations then decrease, indicating a marked diminution in corpus luteum function.[135] The fall in 17-hydroxyprogesterone concentrations continues, but the drop in progesterone levels is only transient. This marks the transition from dependence on ovarian progesterone production to placental progesterone secretion (the luteal-placental shift). As previously noted, luteectomy during the first 50 days after the last menstrual period is associated with a decline in progesterone levels and expulsion of the products of conception. After a therapeutic abortion, progesterone levels also drop rapidly.

Thus the fetal-placental unit is responsible for the signal to maintain the corpus luteum. The data supporting the notion that hCG is that physiologic signal include the following:

- The presence of hLH-hCG receptors on the corpus luteum
- The early production of hCG by the implanting trophoblast
- The dose-dependent increase in cAMP, progesterone, and estradiol from luteal cells cultured in vitro after exposure to hCG
- The parallel rise of progesterone and hCG in early pregnancy
- The enhanced progesterone secretion and prolongation of the menstrual cycle in nonpregnant women given exogenous hCG during their luteal phase

The inability of hCG to prolong the life of the corpus luteum of pregnancy beyond the sixth to eighth week of pregnancy appears to be due to homologous desensitization of the adenylate cyclase system and the inhibitory effects of high estrogen levels on progesterone synthesis through inhibition of 3βHSD1 and $\Delta^{5\text{-}4}$-isomerase in the corpus luteum.

Another physiologic role for hCG is in the differentiation of fetal male genitalia through stimulation of the hLH-hCG receptors on the fetal testicular Leydig cells during the period when differentiation of wolffian duct structures and development of the external genitalia occur. The maximum testosterone production per unit weight of the testes coincides with the maximum binding of ^{125}I-labeled hCG to the fetal testicular receptors at 10 to 12 weeks of development, and fetal Leydig cells produce cAMP and testosterone in vitro after exposure to hCG. The hCG concentrations in fetal serum parallel the fetal testicular testosterone levels at a time when the amount of fetal pituitary hLH is not sufficient to stimulate testosterone production.[136]

There are several other possible actions of hCG during normal pregnancy. In vitro, hCG stimulates the differentiation of cytotrophoblast to syncytiotrophoblast and hence may play an important

paracrine role in regulating syncytiotrophoblast mass and production of trophoblast hormones.[125,137] Additional data supporting this autoregulatory effect of hCG include the in vitro stimulation of placental synthesis of cAMP, activation of glycogen phosphorylase, and incorporation of radiolabeled galactose and leucine into placental proteins upon exposure to hCG.[136] hCG stimulates the secretion of VEGF from the cytotrophoblast, which may be important for placental angiogenesis.[116] Vasodilation of myometrial blood vessels mediated by hCG binding to vascular hCG receptors may enhance uterine blood flow in early pregnancy.[116] The fetal zone of the adrenal releases DHEAS in response to hCG exposure in vitro, and therefore hCG may have adrenocorticotropic activities in concert with fetal pituitary ACTH and placental ACTH.[136]

It has also been suggested that hCG plays a role in the immunosuppression that occurs during pregnancy. Many early studies on this topic were hampered by the use of impure preparations of hCG or the presence of preservatives such as phenol that may alter the end-points of the test systems used to define immunosuppression. In addition, the immunosuppressive effects may be due to gonadal steroid secretion in response to the hCG in the in vivo models used in some of the studies.[138] Relaxin secretion from the corpus luteum is stimulated by hCG both in vivo and in vitro.[116]

Finally, the decrease in osmotic threshold for thirst and AVP release during pregnancy is clearly related to hCG.[77] Whether this decrease is due to a direct effect of hCG or an indirect effect through stimulation of gonadal steroids or interaction with hLH-hCG receptors present in vascular smooth muscle is unclear.

Gestational Trophoblastic Disease

Gestational trophoblastic disease (GTD) includes complete and partial hydatidiform moles, choriocarcinoma, and placental-site trophoblastic tumor.[139] Complete molar pregnancy is the most common variety, occurring in 1 to 2 in 1000 pregnancies. Patients usually present with vaginal bleeding, a uterus that is larger than expected for the duration of pregnancy, anemia, and excessive vomiting. Pathologically, trophoblast hyperplasia, marked edema of the chorionic villi, and absence of fetal tissues are observed. In contrast, partial moles demonstrate focal trophoblast hyperplasia and villous swelling and often have fetal tissues with congenital malformations. Approximately 20% of patients with complete moles develop persistent trophoblastic disease, whereas only 2% to 4% of patients develop persistent disease after partial molar pregnancy. Persistent trophoblastic disease also can occur after a normal term pregnancy and in pregnancies that end in spontaneous or induced abortion.

Choriocarcinoma is the most aggressive malignant form of persistent trophoblastic disease and may involve complications from local uterine disease, such as bleeding and rupture of the uterus, or from the effects of metastases, especially those involving the liver, lungs, and brain. The least common form of GTD is placental-site trophoblastic tumor, which is derived from the intermediate trophoblast and is often associated with vaginal bleeding and amenorrhea.[139]

All of these neoplasms secrete hCG, free β-subunit, and often additional forms of these molecules. With the exception of placental-site trophoblastic tumor, which secretes relatively low amounts of hCG, the serum and urine concentrations of hCG roughly parallel the tumor burden and also provide prognostic information. Thus hCG measurements in concert with clinical and radiologic findings, especially vaginal ultrasonography findings, are useful for making the diagnosis of GTD. On rare occasions, false-positive, low level hCG results may be found in some women who have heterophilic antibodies and other interfering substances in their sera. This may lead to a misdiagnosis of GTD. Since these substances are not excreted in the urine, a urine pregnancy test will be negative in the presence of such "phantom hCG."[139]

Hydatidiform moles are initially treated with uterine dilatation and evacuation with or without adjunctive single-agent chemotherapy with methotrexate or actinomycin D. Approximately 90% of patients with low-risk, persistent trophoblastic disease are cured by single-agent chemotherapy; 75% of patients with high-risk, metastatic disease are cured by multiagent chemotherapy, including etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine. Serial hCG measurements are invaluable for monitoring as they accurately reflect the effect of therapy on the tumor.[139]

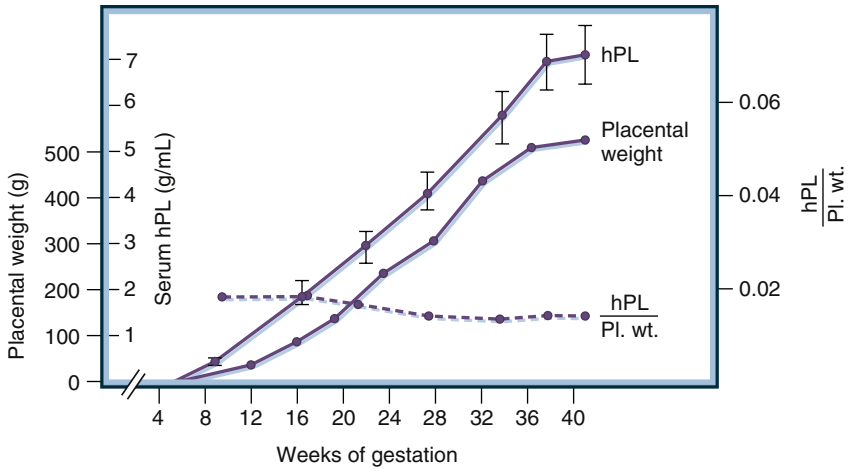
Human Placental Lactogen

Also called chorionic somatomammotropin, hPL is a single-chain, nonglycosylated polypeptide composed of 191 amino acid residues and two disulfide bridges, with a molecular mass of 21,600 Da.[70] It is closely related chemically and biologically to both GH (85% amino acid homology) and prolactin (13% amino acid homology). The hGH-hPL gene cluster is located on the long arm of chromosome 17 and consists of five genes—one coding for pituitary hGH (hGH-N), one for placental hGH (hGH-V), and three for placental hPL (hPL-L, hPL-A, and hPL-B, of which only hPL-A and hPL-B are transcribed).[140]

hPL is synthesized and secreted by the syncytiotrophoblast and is detected in maternal serum between 20 and 40 days of gestation. The maternal serum levels rise rapidly and peak at 34 weeks, followed by a plateau[70] (Fig. 22.5). Both the serum concentrations and placental hPL mRNA concentrations are closely correlated with placental weight and syncytiotrophoblastic mass.[141] The maternal serum concentrations at term average between 6 and 7 μg/mL; at that time, on the basis of the 9-minute to 15-minute $t_{1/2}$ of disappearance from the circulation, the placental production rate of hPL is in excess of 1 g/day. The fetal serum levels are 1/50 to 1/100 of the maternal levels.[140]

The physiologic in vivo regulation of hPL synthesis and secretion, other than the constitutive production related to placental mass, is unknown. Several studies have examined the possible role of nutrients in hPL secretion in pregnant women. Neither acute hyperglycemia nor hypoglycemia appeared to alter the hPL concentrations, although prolonged glucose infusions decreased and prolonged fasting increased the concentrations.[70,118] Arginine infusions, dexamethasone administration, and changes in plasma free fatty acid levels did not affect the maternal hPL concentrations.[142,143] Glucose, estrogens, glucocorticoids, prostaglandins, epinephrine, oxytocin, TRH, GnRH, and L-dopa have been examined in in vitro systems and found to be without consistent effects.[144–147]

Angiotensin II, IGF1, phospholipase A_2, arachidonic acid, and epidermal growth factor stimulated hPL release in vitro.[119,148] Epidermal growth factor probably enhances production through promotion of cytotrophoblast-to-syncytiotrophoblast differentiation.[148] Apolipoprotein AI also stimulated hPL synthesis and release through cAMP-dependent and arachidonic acid–dependent pathways.[70,149,150] Because changes in the maternal plasma apolipoprotein AI concentrations parallel those of hPL during pregnancy, it is likely that this apoprotein, alone and as part of circulating HDL, is important in the secretion of hPL.[150]



• **Fig. 22.5** Placental weight (Pl. wt.), maternal serum concentrations of human placental lactogen *(hPL),* and the ratio of hPL to Pl. wt. during pregnancy. (From Selenkow HA, Saxena BN, Dana CL. Measurement and pathophysiologic significance of human placental lactogen. In: Pecile A, Finzi C, eds. *The Feto-Placental Unit.* Amsterdam: Excerpta Medica; 1969:340.)

hPL has a number of biologic activities that are qualitatively similar to those of hGH and prolactin and can bind to both the hGH and prolactin receptors.[151] In various bioassay systems, hPL had weak somatotropic and lactogenic effects.[151,152] It appears to be a major regulator of IGF1 production, and during pregnancy hPL concentrations are correlated with those of IGF1.[70,151] HPL also affects the metabolism of maternal nutrients. It stimulates pancreatic islet insulin secretion, both directly and after carbohydrate administration,[151] and is a diabetogenic factor during pregnancy through its promotion of insulin resistance. It enhances lipolysis, leading to a rise in free fatty acids, which may in part be responsible for the insulin resistance.[151]

The various biologic activities of hPL have led to the hypothesis that the role of hPL during pregnancy is to provide the fetus with a constant supply of glucose and amino acids.[151] The hPL-stimulated lipolysis allows the mother to utilize free fatty acids for energy during fasting, allowing glucose, amino acids, and ketone bodies to cross the placenta for use by the fetus. In addition, hPL has actions in the fetus, promoting amino acid uptake by muscle and stimulating protein production, IGF1 production, and glycogen synthesis.[152]

Despite the proposed importance of hPL in maternal and fetal metabolic homeostasis during pregnancy, its absence does not appear to impair pregnancy. Deficient or absent hPL production related to gene defects has been described in several women who experienced normal pregnancies and delivered normal infants.[153]

Placental Growth Hormone

Placental GH, hGH-V, is synthesized and secreted by the syncytiotrophoblast.[70] Alternate splicing of the hGH-V gene results in two nonglycosylated isoforms with molecular masses of 22 and 26 kDa.[70,140] The 22-kDa variant may also be glycosylated and circulate as a 26-kDa protein.[140] HGH-V is detected in the maternal plasma from 10 weeks of gestation and peaks during the third trimester[70,133,154] (Fig. 22.6).

HGH-V has somatotropic activity and stimulates IGF1 production, and the increase in IGF1 concentrations may in turn be responsible for the suppression of maternal pituitary hGH secretion[152] (see Fig. 22.6). Unlike pituitary hGH, hGH-V is not secreted in a pulsatile fashion, nor is it released from the trophoblast by growth hormone–releasing hormone (GHRH), but it is inhibited by glucose. It has been estimated that at term, 85% of the GH biologic activity in maternal serum is due to hGH-V, 12% to hPL, and only 3% to pituitary hGH.[155] Within 48 hours of delivery, pituitary hGH secretion returns to normal.

Human Chorionic Corticotropin

The syncytiotrophoblast synthesizes an ACTH-like peptide, human chorionic corticotropin (hCC), as well as several pro-opiomelanocortin–derived peptides, including β-lipotropin, β-endorphin, and α-melanocyte–stimulating hormone.[74] The maternal serum concentrations of ACTH increase as pregnancy progresses, and the elevation of free cortisol levels during pregnancy may be related in part to both placental hCC and pituitary ACTH production.[74]

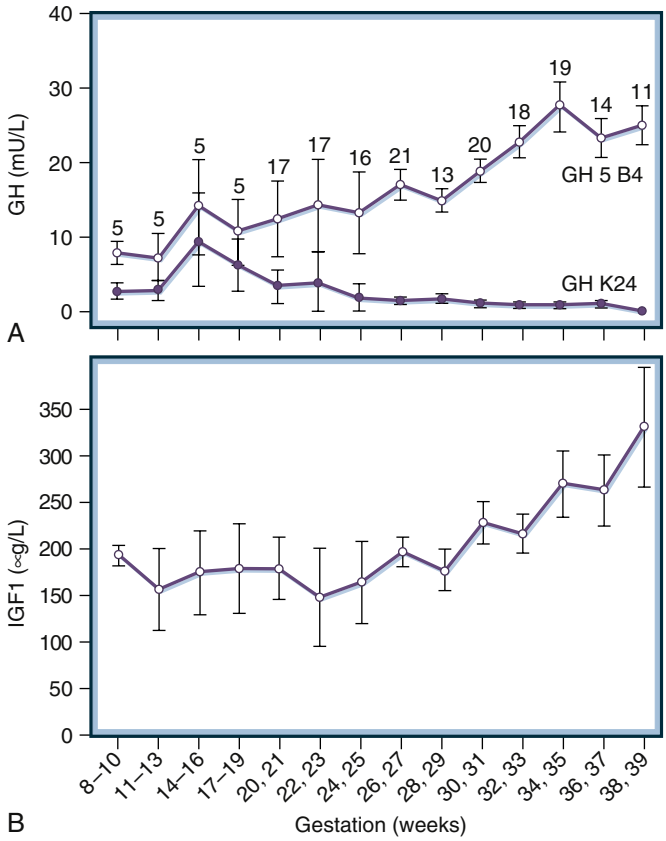
hCC secretion is stimulated by CRH, which is probably the most important factor regulating the local production of the peptide through paracrine or autocrine mechanisms, or both, because it is also produced by both the cytotrophoblast and the syncytiotrophoblast. Unlike the situation with the pituitary, glucocorticoids and oxytocin also stimulate hCC release from placental cultures.[75] Indeed, the resistance of maternal plasma ACTH concentrations to suppression after glucocorticoid administration may reflect the placental hCC contribution to the total pool of circulating immunoreactive ACTH.[74]

Hypothalamic Peptides

Gonadotropin-Releasing Hormone

Both the cytotrophoblast and the syncytiotrophoblast synthesize and secrete GnRH, which has the same chemical structure and biologic activity as hypothalamic GnRH.[119,121] Although the GnRH mRNA levels in the placenta are similar throughout gestation, the highest concentrations of the peptide in the placenta and serum are found during the first trimester and correlate with the mass of the cytotrophoblast and peak hCG concentrations.[121,156]

In vitro, GnRH production by placental explants or purified trophoblasts is stimulated by prostaglandins, epinephrine, activin, insulin, epidermal growth factor, VIP, estradiol, and estriol, and secretion is reduced by inhibin, progesterone, and κ-opiate and μ-opiate agonists.[155,157] The syncytiotrophoblast contains low-affinity GnRH receptors, whose concentrations parallel the hCG secretory pattern.[122]



• **Fig. 22.6** Mean (± standard error) of plasma human growth hormone (GH) (A) and insulin-like growth factor type 1 (IGF1) (B) levels throughout pregnancy. The number of individual assays of GH and IGF1 at each gestational stage is indicated in (A) at the top of the vertical bars. *GH 5 B4,* placental GH (GH2); *GH K24,* pituitary GH (GH1). (From Mirlesse V, Frankenne F, Alsat E, et al. Placental growth hormone levels in normal pregnancy and in pregnancies with intrauterine growth retardation. *Pediatr Res.* 1993;34:439–442.)

Because GnRH stimulates hCG secretion by placental explants and purified trophoblast cells in vitro, with the response of early to midtrimester placentas being greater than that of term trophoblast, it is reasonable to conclude that GnRH is an important autocrine or paracrine regulator of hCG secretion.[155] The hCG-stimulatory effect of GnRH can be blocked by administration of a GnRH antagonist.[158] Because GnRH stimulates metalloproteinases in cytotrophoblasts, the peptide may be important during implantation.[159]

Corticotropin-Releasing Hormone

Both the cytotrophoblast and the syncytiotrophoblast synthesize and secrete a 41–amino acid peptide that is identical to hypothalamic CRH.[119,160] CRH mRNA is first detected in trophoblast at 7 weeks of gestation. The levels remain low during the first 30 weeks of pregnancy but rise 20-fold during the final 5 weeks, a pattern that is parallel to the rise of CRH content in the placenta and concentrations in maternal plasma.[102] In maternal plasma, CRH circulates bound to a 37-kDa protein that is synthesized by the placenta, liver, and brain and that reduces the biologic activity of the CRH.[75,160]

In vitro, placental CRF production is stimulated by prostaglandins (E_2 and $F_{2\alpha}$), norepinephrine, acetylcholine, oxytocin, neuropeptide Y, AVP, angiotensin II, and interleukin-1. Glucocorticoids have been shown to increase both CRH mRNA and peptide, whereas in the hypothalamus suppression is found. CRH secretion is reduced by progesterone and nitric oxide donors. The placenta contains CRH binding sites, and the addition of CRH to cultured placental cells results in a dose-dependent increase in hCC, β-endorphin, and α-melanocyte–stimulating hormone secretion.[74,119,160] Thus it is likely that CRH has an autocrine or paracrine effect in the placenta.

Whether CRH has a physiologic effect on the maternal pituitary secretion of ACTH is unclear; the circulating CRH may be biologically inactive because of the binding protein. However, just before parturition, the binding protein concentration decreases by approximately 50% and the CRH levels rise.[74,160] At this time, CRH stimulates the synthesis and release of prostaglandins from the decidua, amnion, and chorion, which enhances cervical ripening.[161] The myometrium contains CRH receptors, and CRH may increase myometrial contractility.[102] Thus CRH may have a role in initiating and promoting parturition. CRH may also stimulate the fetal pituitary production of ACTH, which in turn may lead to increased fetal adrenal DHEA production and ultimately estriol synthesis by the fetoplacental unit.[160] In addition to CRH, the syncytiotrophoblast and fetal membranes secrete urocortin 1, which in vitro stimulates placental ACTH, PGE_2, and activin secretion through the CRH receptor.[162]

Endocrinology of Pregnancy and Parturition

Roles of Estrogens and Progesterone

Although it is an oversimplification, viewing the maintenance of pregnancy versus the initiation of labor as a balance between the effects of progesterone and estrogen, it is helpful to conceptualize this very complicated process. Progesterone has long been known to be essential for pregnancy; in fact its name is a contraction of the term *progestational steroid hormone*. It is essential from the very beginning of pregnancy as interruption of its production prior to 7 weeks of pregnancy leads to pregnancy loss.[163] Later in pregnancy is it thought to maintain uterine quiescence by limiting the production of prostaglandins and the expression of genes such as ion channels, oxytocin receptors, prostaglandin receptors, and ion channels involved in the contraction machinery of the uterus.[164,165] Progesterone's role in maintaining uterine quiescence is also illustrated in the benefits of progesterone supplementation to prevent preterm birth and is now the standard of care in women at high risk for preterm birth.[166]

Estrogen clearly has a role in the endocrinology of pregnancy, but it appears less important than progesterone both during pregnancy and labor. During pregnancy estrogen enhances the uptake of LDL in the syncytiotrophoblast to aid in steroid production, increases uterine blood flow to allow for adequate gas exchange and nutrient transport across the placenta, and causes hypertrophy of mammary tissue to prepare the breasts for lactation.[109]

In many animal models it has been clearly demonstrated that a decline in progesterone concentration, either spontaneous or induced, is sufficient to initiate labor.[167] However, in women there is no clear spontaneous drop in progesterone levels in the weeks leading up to labor. While this may seem contradictory at first, there is mounting evidence that functional progesterone withdrawal does occur in humans despite a constant level of the hormone throughout late gestation. The progesterone receptor (PR) has two distinct isoforms, PR-A and PR-B. PR-B is thought

to mediate most of progesterone's effects of uterine quiescence by activating progesterone responsive genes and suppressing estrogen receptor (ER) production. PR-A, on the other hand, predominantly acts as a repressor or PR-B. The onset of labor is associated with an increase in the relative levels of PR-A compared to PR-B in the myometrium, suggesting a functional decrease in progesterone activity occurs in humans as it does in other mammals.[168–172]

This functional decrease occurs in concert with an increase in ER expression, which in turn produces a contractile uterine phenotype in preparation for labor, including an increase in myometrial gap junctions and prostaglandin production. The nonessential role of estrogen in pregnancy and labor is demonstrated in pregnancies complicated by placental sulfatase deficiency. These pregnancies generally continue to term, despite the very low estrogen production throughout pregnancy. Although there is a delay in the onset of labor and the uterus is relatively refractory to prostaglandins and oxytocin, labor does ultimately occur spontaneously and inductions of labor, although difficult, can be successfully carried out.[173]

Role of Prostaglandins

There is overwhelming evidence for the role of prostaglandins as mediators of labor.[164,165] Their production is carefully compartmentalized within the uterus, with PGE_2 confined to the fetal membranes, $PGF_{2\alpha}$ in the decidua, and PGI_2 in the myometrium. Although structurally very similar, the different prostaglandin species can have opposing effects, adding to the complexity of how prostaglandins regulate uterine activity. While the PGE_2 produced by the fetal membranes and PGI_2 produced by the myometrium inhibit uterine activity, the $PGF_{2\alpha}$ produced by the decidua is a potent uterotonic,[174] an action that is exploited in the treatment of postpartum hemorrhage. $PGF_{2\alpha}$ production is suppressed in pregnancy with prostaglandin levels in the gravid uterus being lower than those found in the nongravid uterus at any time during the menstrual cycle.[175,176] Later in pregnancy, and particularly at term with the onset of labor, $PGF_{2\alpha}$ levels of prostaglandins increase in maternal serum and amniotic fluid.[174]

The physiology of prostaglandins in the labor process is extensively exploited in modern obstetrics. Procontractile prostaglandins, such as PGE_1, are commonly used for cervical ripening and induction of labor.[177] Postpartum hemorrhage due to uterine atony is very effectively treated by administration of PGE_1 or $PGF_{2\alpha}$.[178] Furthermore, inhibitors of prostaglandin synthesis, such as indomethacin, are among the most potent tocolytic agents used in the treatment of preterm labor.[179]

Role of Oxytocin

Oxytocin is a polypeptide hormone produced in the hypothalamus and stored in the posterior pituitary gland. It is one of the first endogenous compounds discovered to play a role in human labor, and it is one of the few medications to receive US Food and Drug Administration (FDA) approval for specific use in pregnancy. It has long been known to produce uterine contractions, and it is frequently used for induction of labor and to treat postpartum hemorrhage due to uterine atony.[178] Oxytocin mediates its effects by binding to G protein–coupled receptors that are present throughout the uterus. The receptors are preferentially distributed in the uterine fundus, with lower levels found in the lower uterine segment and cervix, corresponding to the increased contractility of the fundus compared to the lower uterine segment.[180]

Although the levels of oxytocin do not detectably increase as pregnancy progresses until late in the second stage of labor,[180] receptor concentrations increase up to 100-fold in the first trimester and up to 300-fold in the third trimester of pregnancy.[180] The increase in receptors, mediated predominantly by estrogen, leads to the increased sensitivity of the myometrium to oxytocin in the second trimester.[180]

Use of Placental Hormones in Genetic Screening and Pregnancy Outcomes

Modern biochemical screening for Down syndrome, other aneuploidies, and less common genetic disorders rely heavily on assessing hormones and other proteins produced by the placenta. For example, maternal levels of hCG and pregnancy-associated plasma protein-A (PAPP-A) are combined with a measurement of fetal nuchal translucency in a first-trimester screening. In general, hCG levels are higher in pregnancies affected by Down syndrome and lower in pregnancies affected by trisomy 18 than euploid pregnancies. PAPP-A levels are lower in pregnancies affected by the common aneuploidies (i.e., Down, trisomy 13, trisomy 18) than in euploid pregnancies.[181,182] For screening in the second trimester, hCG is combined with estriol and inhibin-A—all of which are produced by the placenta—and alpha-fetoprotein (AFP)—which is predominantly produced by the fetus. Estriol is lower in pregnancies affected by Down syndrome and other trisomies, whereas levels of inhibin-A are higher in pregnancies affected by Down syndrome compared to euploid pregnancies.[183] Although not designed to do so, the hormone levels assayed in these genetic screening tests can provide some clues to other genetic conditions and even help predict future pregnancy complications. For example, very low levels of estriol are seen in pregnancies complicated by placental sulfatase[184] deficiency and Smith-Lemli-Opitz syndrome,[185] a disorder in which an enzymatic defect in the cholesterol synthesis pathway leads to a disturbance of all steroid hormones, including estriol. These analyte patterns are summarized in Table 22.3.

It is now well established that certain abnormal maternal serum analytes used in genetic screening are associated with pregnancy complications later in pregnancy in euploid nonanomalous fetuses. The association is strongest for elevated maternal serum AFP (MSAFP) and inhibin-A, which are both associated with an increased risk of fetal growth disturbance and intrauterine fetal death. Elevated inhibin-A is also associated with an increased risk of preterm birth.[184,186,187] Once discovered, these pregnancies are often monitored by serial fetal ultrasounds to assess for growth disturbance and ongoing documentation of fetal well-being if abnormal growth is found. Although the association is less well established, an abnormally low PAPP-A in a euploid pregnancy is associated with growth restriction and stillbirth, preterm birth, and preeclampsia.[188] Both low first trimester PAPP-A and increased second trimester inhibin-A have been associated with an increased risk of preeclampsia. The correlations between abnormal serum analytes and pregnancy complications are outlined in Table 22.4.

Noninvasive Prenatal Testing and Microchimerism

Transplacental bidirectional trafficking of maternal, placental, and fetal cells occurs universally in pregnancy, beginning around 6 to 7 weeks of gestation and peaking at term. Both fetal and maternal

TABLE 22.3 Maternal Serum Analyte Patterns for Genetic Disorders

Genetic Disorder	PAPP-A	hCG	AFP	uE3	Inh A
Down syndrome	↓	↑	↓	↓	↑
Trisomy 18	↓	↓	↓	↓	—
Trisomy 13	↓	↓	—	—	—
Smith-Lemli-Opitz syndrome	—	—	↓	↓	—
Turner syndrome (45,XO) without hydrops	↓	↓	↓	↓	—
Turner syndrome (45,XO) with hydrops	↓	↑	↓	↓	↑
Placental sulfatase deficiency	—	—	—	↓	—

AFP, Alpha-fetoprotein; *hCG,* human chorionic gonadotropin; *InhA,* inhibin-A; *PAPP-A,* placenta-associated plasma protein-A; *uE3,* unconjugated estriol; ↓ = decreased; ↑ = increased.

TABLE 22.4 Maternal Serum Analyte Levels and Pregnancy Outcomes

	Preeclampsia	Growth Restriction	Fetal Demise	Preterm Birth
PAPP-A (<0.42 MoM)	↑	↑	↑	↑
Free hCG (<0.021 MoM)	—	↑	↑	—
AFP (>2 MoM)	—	↑	↑	↑
hCG (>2 MoM)	↑	—	↑	—
uE3 (<0.5 MoM)	—	↑	↑	
Inh A (>2 MoM)	↑	↑	↑	↑

AFP, Alpha-fetoprotein; *hCG,* human chorionic gonadotropin; *InhA,* inhibin-A; *MoM,* multiples of the median; *PAPP-A,* placenta-associated plasma protein-A; *uE3,* unconjugated estriol; ↓ = decreased; ↑ = increased.

cells persist indefinitely in the host.[189] In addition to fetal cells, the maternal circulation also contains fetal cell free DNA. The cell free DNA from maternal circulation can be sequenced and used to screen for fetal aneuploidy. This approach is termed *noninvasive prenatal testing* (NIPT). The technique is generally thought to be highly sensitive and specific for fetal aneuploidy, but it also suffers from the same pitfalls as trophectoderm biopsy in that mosaicism may be difficult to detect and, when detected, to interpret. Nonetheless, the availability of NIPT has resulted in a marked increase in its use in high-risk patient populations defined as pregnant women older than 35 years and those with a prior abnormal NIPT screen, abnormal ultrasound, and family history of aneuploidy. The superior accuracy of NIPT compared with conventional screening methods has led to significant decreases in the number of invasive diagnostic procedures, in addition to a concomitant decrease in the number of procedure-related fetal losses.[190] The introduction of NIPT significantly decreased the use of screening using placental hormone patterns in high-risk but not in low-risk populations.[191] While NIPT is more accurate, it is also more expensive. Furthermore, invasive fetal karyotyping is still recommended to confirm abnormal screens regardless of screening method.[192] Improving screening technologies and decreasing costs are likely to propel the use of NIPT, but wise application will also require improved patient and provider appreciation of its limitations.[193]

More intriguing than the utilization of fetal cell free DNA in maternal circulation for prenatal screening, however, is the putative contribution of persistent maternal and fetal microchimerism to the fetal origins of adult disease and long-term postpartum maternal health. Indeed, microchimerism apparently persists indefinitely, as fetal cells have been found in maternal circulation decades after birth and can be transmitted from grandmother to grandchild.[194] While both maternal and fetal microchimerism have been implicated as causes of autoimmune diseases, including endocrine disorders such as autoimmune thyroid disorders and type 1 diabetes,[195] microchimerism may also provide benefits, including enhanced tissue repair or rejuvenation and slowed aging via transmission of stem cells. Microchimerism has been hypothesized to protect the host from the development of certain types of cancer.[195] Thus acute and persistent maternal and fetal microchimerism can be adverse, neutral, or beneficial, depending on factors such as HLA match or mismatch, persistence, microchimeric cell mass, and other factors as yet to be determined. The "discovery" of persistent microchimerism years after parturition adds a new dimension to our understanding of the endocrine changes and consequences associated with pregnancy.

The "Fourth Trimester" and the Parental Brain

The physiologic and homeostatic endocrine and metabolic adaptations of pregnancy profoundly impact all maternal and fetal tissues, including the maternal brain. While delivery is a relatively abrupt transition and represents the greatest risk for

maternal and neonatal mortality and morbidity, a new set of endocrine, metabolic, psychologic, and sociocultural challenges follows delivery. Indeed, pregnancy permanently imprints both mother and child and determines the future health of both, both acutely and chronically. Acutely, following delivery, lactation and infant sleeping and feeding patterns present an immediate and enormous set of challenges that elicits maternal endocrine, metabolic, and psychologic adaptations that differ remarkably from those of pregnancy. Placental hormone levels fall acutely and the neuroendocrine axis responds. The catch-phrase for the adaptations related to immediate adjustment following delivery is the "fourth trimester." Short shrift has been given to the term and the many endocrine and other adaptations that follow. What we do know is that adverse pregnancy outcomes such as gestational diabetes and preeclampsia not only heighten the risk for maternal cardiovascular health[195–200] but also pose sociocultural adjustments that are only just beginning to be identified. Not all women are equally able to meet the endocrine and metabolic challenges of normal, much less complicated, pregnancy and delivery. While the hormones of pregnancy prepare the maternal brain for "nesting" and facilitate lactation and care of her infant, we know far less about the endocrine and metabolic impact of pregnancy and parenthood on the longer term health of women and men. Common knowledge suggests that pregnancy poses differential challenges to each sex or, in the case of same-sex liaisons, to the gestational and nongestational parent. The psychologic challenges of parenting are often termed "parental brain."[201] Postpartum maladaptation may result in syndromal postpartum psychiatric disorders, and this burden seems to fall exclusively to the gestational parent.[202] Our current view is that the abrupt withdrawal from the hormonal milieu of pregnancy that is associated with parturition creates an endocrine challenge that overwhelms the endocrine, metabolic, and sociocultural adaptation of vulnerable individuals. Further, we are beginning to recognize that pregnancies complicated by gestational diabetes, hypertension, and other maternal and fetal medical conditions heighten the risk for future maternal disease. Particularly, we now know that gestational diabetes predisposes to maternal diabetes after gestation and that gestational hypertension is a harbinger of later maternal cardiovascular disease. Psychologic sequelae of pregnancy such as postpartum depression also appear to heighten the risk not only of later syndromal psychiatric conditions but also endocrine and cardiovascular conditions. A team approach is needed at both scientific and clinical levels if we are to ensure both individual and population health. In particular, women should be queried by health care providers about medical and psychiatric complications during and after pregnancy so that risk assessment, screening, surveillance, and interventions are adjusted appropriately.[203]

Summary

The endocrine, metabolic, psychologic, and sociocultural changes associated with pregnancy and parturition represent a coordinated and cooperative set of homeostatic responses that facilitate maternal and fetal health. Normal pregnancy represents an enormous challenge, and complications of pregnancy such as preeclampsia and gestational diabetes predispose the mother and her offspring to endocrine, metabolic, cardiovascular, immune, and other conditions after pregnancy. A better understanding of the physiology and pathophysiology of pregnancy and its aftermath is required for appropriate medical and societal care.

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The complete list of references is available online at ExpertConsult.com.

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23

Endocrinology of Fetal Development

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CHAPTER OUTLINE

KEY POINTS

- The placental-fetal endocrine environment is created by a spectrum of placental hormones and growth factors and a variety of fetal endocrine adaptations to the intrauterine environment.
- Disordered thyroid, parathyroid, pituitary, pancreas, and gonadal development can be associated with clinical endocrine phenotypes.
- The fetal adrenal cortex, the para-aortic chromaffin system, and the intermediate lobe of the pituitary are prominent fetal endocrine glands.
- Fetal adrenocorticotropic hormone is required for adrenal cortex steroidogenesis. Paradoxically, adrenal steroidogenesis leads to production of mostly inactive steroids such as pregnenolone and dehydroepiandrosterone.
- Some adrenal steroids are converted by the fetal adrenal gland and liver to provide substrates for placental estrone and estradiol production.
- The anterior and posterior pituitary lobes develop from oral ectoderm and ventral diencephalon. Pituitary hormone secretion starts at 8 to 10 weeks' gestation.
- Thyroid hormone synthesis starts at 11 weeks' gestation. Circulating T_4 increases to maximal levels after 35 weeks' gestation, but maturation of hypothalamic-pituitary control and response of thyroid gland to thyroid-stimulating hormone develops in the third trimester.
- In the presence of *SRY*, the sex-determining region of the Y chromosome, male gonadal differentiation starts at 7 weeks of gestation. Development of Leydig cells leads to an increase in fetal testosterone production from week 10 and stimulates differentiation of the primitive mesonephric ducts into bilateral ductus deferens, epididymides, seminal vesicles, and ejaculatory ducts. Dihydrotestosterone stimulates male differentiation of the urogenital sinus and external genitalia.
- Active calcium transport across the placenta occurs in the third trimester to maintain fetal calcium concentrations and is dependent on parathyroid hormone–related protein.
- Calcium-sensing receptor and fibroblast growth factor 23 are required for normal neonatal calcium and phosphate metabolism.
- Growth of the fetus involves complex interactions between environmental, epigenetic, and genetic factors. Insulin-like growth factors are important for fetal growth and are regulated mainly by transplacentally derived nutritional substrate. Hormones most important for postnatal growth, including T_4, growth hormone, and gonadal steroids, have a limited role in fetal growth.
- Fetal hormonal systems are programmed to maintain anabolism with minimal perturbation. Production of catabolic and thermogenic hormones is limited, and effects of hormones altering metabolic substrate supply and distribution pathways are muted.
- Transition to extrauterine life involves the adrenal cortex and autonomic nervous system in the immediate postnatal period. Longer term transition to an intermittent nutrient supply and transient substrate deficiency requires maturation of secretory control mechanisms for the parathyroid hormone–calcitonin system and the endocrine pancreas.

The unfolding of our understanding of mammalian pregnancy and fetal development represents one of the dramatic chapters of scientific progress during the past half-century. Human pregnancy necessitates an ensemble of autocrine, paracrine, and endocrine networks that coordinate maternal-placental-fetal communication. The evolution of a pregnancy is accompanied by dramatic changes in the hormonal milieu. This is a time of potential vulnerability but tremendous plasticity and involves complex genetic, cellular, and hormonal interactions that serve to create a unique, protective intrauterine environment for the developing fetus (Table 23.1).[1] An orchestra of signaling molecules, transcription factors, and epigenetic events regulates embryo implantation, placentation, and ultimately the maintenance of pregnancy until the onset of parturition with transition of the fetus to extrauterine life. It is becoming increasingly clear that the intrauterine environment not only enables fetal development; it also has a significant impact on the lifelong health of an individual, as well as exerting multigenerational effects. This chapter reviews our current understanding of the transplacental passage of hormones, fetal endocrine development and hormone production, adaptations of the fetal endocrine systems to extrauterine life, and programming of developing fetal endocrine systems.

TABLE 23.1 Features of the Fetal Endocrine Environment

Placental Hormone Production
Estrogens
Progesterone
Neuropeptides
Growth factors

Neutralization of Hormone Actions
Growth hormone
Cortisol
Thyroxine
Catecholamines

Unique Fetal Endocrine Systems
Fetal adrenal cortex
Para-aortic chromaffin system
Intermediate lobe of the pituitary

Prominent Fetal Hormones or Metabolites
Vasotocin
Calcitonin
Cortisone
Reverse triiodothyronine (rT_3)
Sulfated iodothyronines
Ectopic neuropeptides

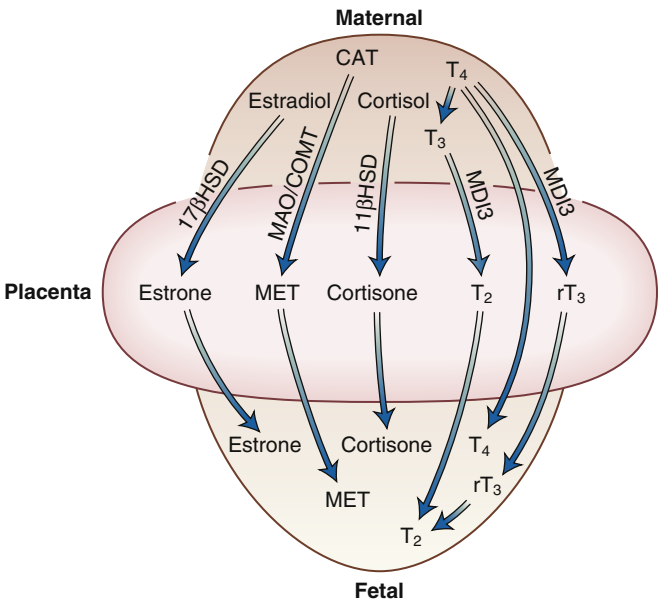
Fetal Endocrine System Adaptations
Adrenal-placental interactions
Testicular control of male phenotypic differentiation
Developmentally regulated growth factor control of fetal growth
Neuropeptides and fetal water metabolism
Parathyroid glands and placental calcium transport
Catecholamine and vasopressin responses to hypoxia
Cortisol programming for extrauterine exposure
Catecholamine and cortisol control of extrauterine adaptation
Perinatal hormonal programming

Placental Transfer of Hormones

The placenta forms the materno-fetal interface, delivering nutrients and oxygen to the fetus and acting as a selective barrier. The placenta demonstrates remarkable capacity to adapt to adverse environments and lessen their impact on the fetus. Placental transfer of hormones decreases with increasing molecular weight, and those larger than 0.7 to 1.2 kDa have little or no access to the fetal compartment.[2] Maternal hormones therefore play a very limited role in the fetal endocrine milieu. Hormones that do cross the placenta may be metabolized en route (Fig. 23.1), including steroid hormones (cortisol), thyroid hormones (T_3, T_4), estradiol, and catecholamines.[3–6]

The concentration of maternal cortisol is almost 10-fold higher than that in the fetus. Placental cells contain an active 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) that catalyzes conversion of maternal cortisol to inactive cortisone.[7] Synthetic glucocorticoids, such as dexamethasone or betamethasone, can bypass this protective mechanism resulting in exposure of the fetus to steroid hormones. While this is used acutely for its beneficial effects on fetal lung maturation in cases of threatened preterm delivery, chronic use may produce adverse effects on blood pressure, blood glucose, and memory, as demonstrated in rodent models,[8–10] as well as impacting negatively on placental and fetal growth.[11] Clinical use of single or multiple courses of glucocorticoid treatment in the obstetric management of threatened preterm delivery continues, but treatment for other proposed indications lacking robust evidence (e.g., use in pregnancy to reduce virilization of a fetus with congenital adrenal hyperplasia [CAH]) should take place only in a research setting with careful auditing.[12,13]

Estrogens modulate many intrauterine processes throughout gestation, and the balance between estrogens and progesterone in



• **Fig. 23.1** Placental neutralization of biologic activity of hormones during maternal-fetal transfer. The neutralizing enzymes, 17β-hydroxysteroid dehydrogenase (17βHSD) and 11βHSD, are shown (see text for details). *CAT,* catecholamines; *COMT,* catechol *O*-methyltransferase; *MAO,* monoamine oxidase; *MDI3,* type 3 iodothyronine monodeiodinase; *MET,* metamachines; *rT_3,* 3,3′5′ (reverse) triiodothyronine; *T_2,* diiodothyronine; *T_3,* 3,5,3′-triiodothyronine; *T_4,* thyroxine.

utero is thought to be critical to the maintenance of pregnancy, fetal maturation, and the onset of parturition. The placenta produces vast amounts of estrogens in the form of estradiol, estrone, estriol, and estetrol. The estrogen products released from the placenta depend on the nature of the substrate available. Estradiol is the primary estrogen circulating at term. In addition, significant levels of estriol and estetrol are also found in the maternal circulation, and they increase particularly late in gestation. These hydroxylated forms of estrogen are produced in the placenta, using substrates from the combined efforts of the fetal adrenal gland and liver. Though the primary site of estrogen biosynthesis is the placenta, the placenta lacks the cytochrome P450 enzyme CYP17 and accordingly is unable to synthesize estrogens de novo.[14] Placental estrogen biosynthesis relies on a supply of C_{19} androgens, mainly dehydroepiandrosterone (DHEA) and its sulfoconjugate, DHEA sulfate (DHEAS), derived principally from the fetal and maternal adrenal cortex.[14–16] By term, estradiol and estrone concentrations are 100-fold higher than those of nonpregnant women, and estriol concentrations are 1000-fold higher.[16] The developing fetus is protected from excessive estrogen exposure by conversion of active estradiol to inactive estrone by placental 17β hydroxysteroid dehydrogenase.[5]

During the first trimester, transplacental passage of thyroid hormones from the maternal circulation to the fetus is important for neurodevelopment. Placental tissue contains an iodothyronine inner ring monodeiodinase, which deiodinates most of the thyroxine (T_4) to inactive reverse triiodothyronine (rT_3) and converts active 3,5,3′-triiodothyronine (T_3) to inactive diiodothyronine.[6,17] Inappropriately high concentrations of iodothyronines are associated with fetal loss,[18] and the quantity and complement of thyroid hormones passing across the placenta are therefore carefully regulated. There is, however, some transplacental passage of T_4 to the fetus in early pregnancy,[19–21] and epidemiologic data report impairment to intellectual function and behavior in infants born to mothers with mild untreated or subclinical hypothyroidism,[22] although this may also be due to placental passage of thyroid peroxidase (TPO) antibodies.[22]

Catecholamine-degrading enzymes in placental tissue include monoamine oxidase and catechol O-methyltransferase, and both metanephrine and dihydroxymandelic acid metabolites of catecholamines are present in placental homogenates (see Fig. 23.1).[23]

Ectopic Fetal Hormone Production

A number of hormones appear to have a much broader tissue distribution in the fetus, although their functional roles in utero are uncertain, and indeed ectopic hormone production is not always associated with endocrine manifestations.

Human chorionic gonadotropin (hCG), a heterodimeric glycoprotein hormone produced in abundance by the placenta, is preferentially secreted into maternal circulation. hCG and luteinizing hormone (LH) (a structural and functional homolog of hCG, produced by the anterior pituitary gland) bind to the same transmembrane glycoprotein hCG/LH receptor; however, hCG binds with higher affinity. Maternal serum hCG concentrations exponentially increase and reach a peak by about the end of the first trimester, followed by a rapid decline.[24] The fetal circulation also contains low concentrations of hCG; it has been shown that fetal kidney, liver, and testes from human fetuses 16 to 20 weeks old produce immunoreactive and bioactive hCG in vitro.[25,26] hCG is also found in fetal ovary, kidney, lung, adrenal, thymus, spleen, and muscle[27]; hCG/LH receptors have been demonstrated in fetal kidney, liver, pancreas, lung, small and large intestines, and adrenals.[28] Although the role of hCG in the fetus is largely unknown, it is able to stimulate production of DHEA[29] and additionally stimulates testosterone production by Leydig cells, which is essential for masculinization of fetal tissues.[30] hCG signaling in other tissues may be important for their growth and differentiation. The presence of fetal nongonadal hCG/LH receptors suggests mediation of the pleiotropic actions of hCG in the growing human fetus.[31]

Ectopic production of adrenocorticotropic hormone (ACTH), derived presumably from a pituitary-independent pro-opiomelanocortin (POMC) parent molecule, has been shown with ACTH-like immunoreactivity present in relatively high concentrations in neonatal rat pancreas and kidney.[25] POMC is also secreted by the neuroendocrine cells of fetal lung. POMC is known to alter adrenal sensitivity to ACTH; this interplay between fetal pituitary, lung, and adrenal may regulate adrenal response to stress or the onset of parturition.[32] The fetal lung neuroendocrine cells synthesize other hormones in addition to POMC, including vasoactive intestinal peptide (VIP) and serotonin, although the function of these is unknown.[32]

Hypothalamic neuropeptides (thyrotropin-releasing hormone [TRH], corticotropin-releasing hormone [CRH], growth hormone–releasing hormone [GHRH]) are present in a variety of adult tissues, particularly pancreas and gut, as well as in fetal gut and tissues derived from it.[33–35] In neonatal rat pancreatic and gastrointestinal tract tissues, high concentrations of TRH and somatostatin immunoreactivity have been reported, in contrast to low hypothalamic concentrations.[36,37] Furthermore, pancreatectomy, rather than encephalectomy, results in a significant reduction of circulating TRH concentrations in the neonatal rat. In the sheep fetus, thyroid hormones modulate pancreatic and gut TRH concentrations.[38] TRH and somatostatin are also present in the human neonatal pancreas and detectable in the circulation, with both hormones derived mostly from extrahypothalamic sources.[39–42] These data suggest a role for extrahypothalamic TRH in the control of fetal pituitary thyroid hormone secretion before the near-term maturation of hypothalamic TRH. The role of extraneural somatostatin in the fetus remains uncertain.

Ghrelin is a hormone with potent GH-releasing activity, produced by rat and human gastric endocrine cells and by the pituitary, hypothalamus, and placenta. Ghrelin protein has also been identified in the endocrine cells of the fetal lung in decreasing amounts from embryonic to late fetal periods, and its expression was maintained in neonates.[43] Fetal lung is therefore an additional source of circulating ghrelin, whose functions at the respiratory tract level remains to be clarified.[43,44]

CRH is a peptide hormone released by the hypothalamus; however, human placenta, fetal membranes, and decidua also express CRH that is identical to hypothalamic CRH, and from the second trimester the placenta is the major source of CRH secretion. The circulating concentrations of CRH increase 1000-fold as pregnancy progresses,[45] reaching values of 0.5 to 1 nmol/L at term; normal values in nonpregnant women are lower than 0.01 nmol/L. In the last 12 weeks of gestation, CRH plasma concentrations rise considerably, peaking during labor and then falling precipitously after delivery.[46,47] Umbilical cord blood levels and amniotic fluid levels of CRH are similarly increased in late gestation.[48] Fetal CRH concentrations are lower than those in maternal circulation (50 vs. 1000 pmol) but are still quite substantial compared with those in men and nonpregnant women. Unlike

hypothalamic CRH, placental CRH gene expression and production can be stimulated by glucocorticoids.[49] This positive feed-forward system is a unique feature of placental CRH and indicates a distinct role in pregnancy. The fetal adrenal and pituitary express CRH receptors.[50,51] In vitro experiments using cultured primary adrenal cells suggest that in vivo placental CRH stimulates pituitary ACTH secretion, driving fetal HPA activation and directly stimulating adrenal steroid production, as well as affecting fetal adrenal responsiveness to ACTH, thereby indirectly promoting steroidogenesis.[51–53] It appears that during most of pregnancy CRH-binding protein (CRH-BP) binds most of the circulating CRH in the fetal and maternal compartment, which likely serves to tightly control the activity of placental CRH.[54] At the end of pregnancy, there is increased bioavailability of CRH due to a fall in levels of its binding protein, which results in an exponential increase in maternal CRH concentrations from 35 weeks of gestation to term.[45] The steep rise and peak in CRH has been proposed as an initiator of parturition by forming a feed-forward loop that leads to increased production of adrenal androgens and hence placental estrogen.[45]

Fetal Endocrine Systems

The fetus has a unique hormonal network that combines not only its own maturing endocrine systems but also that of the corpus luteum, placenta, and maternal hormones. Fetal endocrine organs develop from early in the first trimester in a carefully coordinated manner, and their abnormal development can impact on a wide range of other systems in the body.

Pituitary

Human Hypothalamic-Pituitary Development

The fetal forebrain is identifiable by 3 weeks' gestation and the diencephalon and telencephalon by 5 weeks; Rathke pouch, the buccal precursor of the anterior pituitary gland, separates from the primitive pharyngeal stomodeum by 5 weeks' gestation.[55–57] The neural components (hypothalamus, pituitary stalk, and posterior pituitary) are largely developed by 7 weeks of gestation, and the bony floor of the sella turcica is also present by that time, separating the adenohypophysis from the primitive gut.

Hypothalamic neurons containing the neuropeptides somatostatin (SS), growth hormone–releasing hormone, thyrotropin-releasing hormone, and gonadotropin-releasing hormone (GnRH) are present in hypothalamic tissue by 10 to 14 weeks of gestation. Interconnecting fiber tracts are demonstrable by 15 to 18 weeks. Maturation of the pituitary portal vascular system continues through 30 to 35 weeks when the system becomes functional with portal vascular extension into the hypothalamus.

The definitive Rathke pouch comprises proliferative progenitors that gradually relocate ventrally. A proliferative zone containing SRY (sex-determining region Y-box 2 *[SOX2]*–expressing) progenitors is maintained in the mouse embryo in a periluminal area and persists in the adult.[58–60] This can give rise to all cell types within the anterior pituitary. Specialized anterior pituitary cell types (lactotropes, somatotropes, corticotropes, thyrotropes, and gonadotropes) are present between 7 and 16 weeks.[57] The cellular differentiation of the five distinct hormone-producing cell types in the anterior pituitary is a highly regulated and coordinated process; however, the final differentiation is still unclear. Secretory granules are present within anterior pituitary cells by 10 to 12 weeks and all pituitary hormones can be identified by immunoassay between 10 and 17 weeks.[57,61] Thus the anatomy and biosynthetic mechanisms that make up the hypothalamic-pituitary neuroendocrine transducer appear functional by 12 to 17 weeks of gestation.

Anterior Pituitary and Target Organs

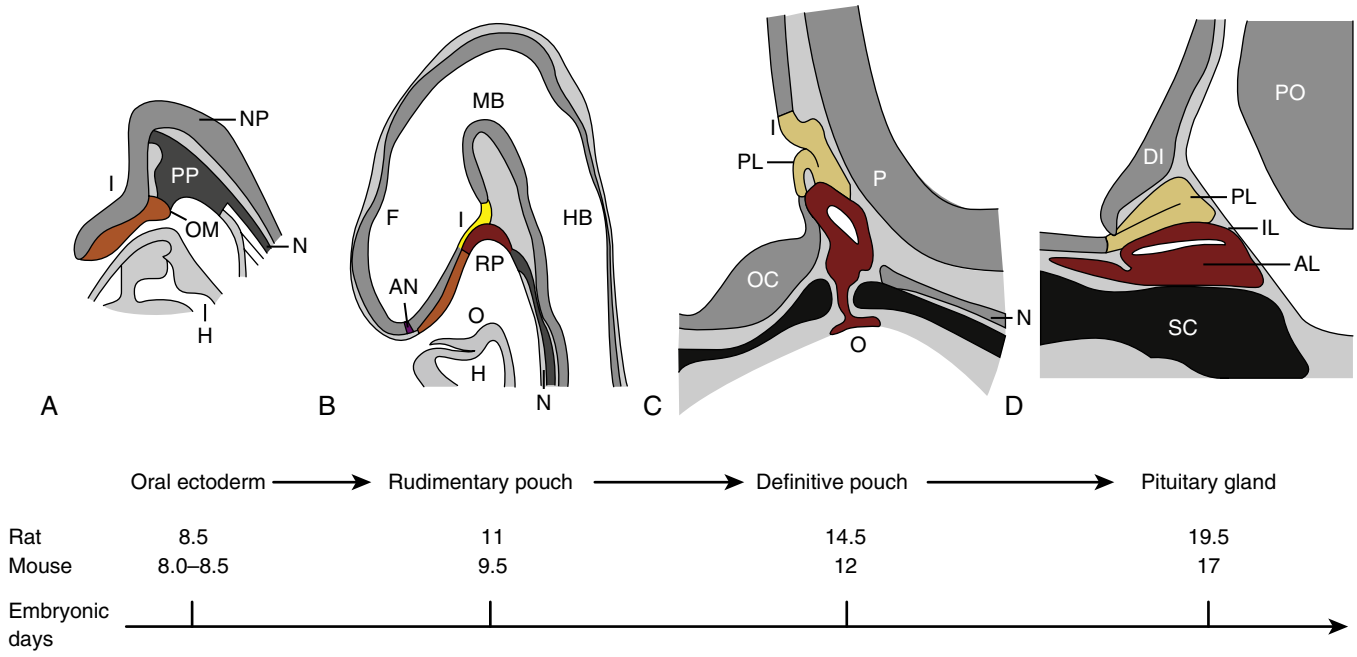
Two distinct components of the pituitary become evident during embryogenesis: the adenohypophysis (anterior and intermediate lobes) and neurohypophysis (posterior lobe).[62–65] The three lobes of the mature pituitary gland have a dual embryonic origin: The anterior and intermediate lobes are derived from oral ectoderm; the posterior pituitary originates from the infundibulum, a specific region of the developing central nervous system (CNS) that forms in the midline of the ventral diencephalon. Much information has been derived from the mouse as a model organism for pituitary development in mammals, but fate map studies have shown that these processes are similar in all vertebrate species studied, including zebrafish, amphibians, chicks, and rodents.[66–69] In the mouse, the first signs of pituitary development occur at 7.5 days postconception (dpc) with the development of the hypophyseal placode, a thickening of the ectoderm in the midline of the anterior neural ridge (Fig. 23.2). At approximately 9 dpc the placode invaginates dorsally to form a rudimentary Rathke pouch, the primordium of the anterior and intermediate lobes. By 10.5 dpc, the overlying neuroectoderm evaginates to form the infundibulum from which the posterior pituitary and pituitary stalk will derive, which then comes into direct contact with Rathke pouch. The juxtaposition of Rathke pouch and the diencephalon is maintained throughout the early stages of pituitary organogenesis. This close relationship is required for tissue interactions between neural and oral ectoderm, which are critical for the initial stages of pituitary specification. The iterative nature of the inductive interactions required for pituitary morphogenesis makes it very sensitive to both loss-of-function and gain-of-function mutations.[70]

Intermediate Pituitary Lobe

The intermediate lobe of the pituitary gland is prominent in both the human and the sheep fetus. Intermediate lobe cells begin to disappear near term and are virtually absent in the adult human pituitary, although the intermediate lobe in the adult of some lower species is anatomically and functionally distinct.[71] The major secretory products of the intermediate lobe are α-melanocytic-stimulating hormone (α-MSH) and β-endorphin, both derived from cleavage of the POMC molecule.[72] Cleavage of POMC in the anterior lobe results predominantly in corticotropin and β-lipotropin formation. In rhesus monkeys and humans, the fetal pituitary contains high concentrations of compounds resembling α-MSH and corticotropin-like intermediate lobe peptide (CLIP).[73] In the human fetus, α-MSH levels decrease with increasing fetal age.[74] The circulating concentrations of β-endorphin and β-lipotropin are high in the fetal lamb, and the ratio of β-endorphin to β-lipotropin increases during hypoxic stimulation of the anterior pituitary.[75] Because hypoxia provokes corticotropin release and β-lipotropin production from the anterior pituitary, these data have been interpreted to suggest that basal β-endorphin levels in the fetus originate in the intermediate lobe. α-MSH and CLIP may play a role in fetal adrenal activation, and α-MSH may play a role in fetal growth.[76,77] The processing of pituitary POMC in the human fetus by the end of the second trimester is similar to that in the adult, but the role of these intermediate lobe peptides in the fetus remains obscure.[78]

Posterior Pituitary

The posterior lobe comprises axonal projections of neurons, which traverse the pituitary stalk and median eminence at the base of the



• **Fig. 23.2** Schematic representation of the developmental processes implicated in pituitary development. Mouse pituitary development in sagittal section. Stages of development are indicated in dpc. *AL,* anterior lobe; *AN,* anterior neural pore; *DI,* diencephalon; *F,* forebrain; *H,* heart; *HB,* hindbrain; *I,* infundibulum; *IL,* intermediate lobe; *MB,* midbrain; *N,* notochord; *NP,* neural plate; *O,* oral cavity; *OC,* optic chiasma; *OM,* oral membrane; *P,* pontine flexure; *PL,* posterior lobe; *PO,* pons; *PP,* prechordal plate; *RP,* Rathke pouch; *SC,* sphenoid cartilage. (Based on Sheng HZ, Westphal H. Early steps in pituitary organogenesis. *Trends Genet*. 1999;15:236–240. As adapted in Gevers EF, Fisher DA, Dattani MT. Fetal and neonatal endocrinology. In Jameson JL, De Groot LJ, eds. *Endocrinology: Adult and Pediatric.* 7th ed. Philadelphia: Elsevier; 2016.)

hypothalamus. The neurons originate from hypothalamic magnocellular bodies termed the supraoptic, suprachiasmatic, and paraventricular nuclei. The former two release arginine vasopressin and the latter releases oxytocin.[79]

The fetal neurohypophysis is well developed by 10 to 12 weeks of gestation and contains both arginine vasopressin (AVP) (also called antidiuretic hormone, ADH) and oxytocin (OT).[80,81] In addition, arginine vasotocin (AVT), the parent neurohypophyseal hormone in submammalian vertebrates, is present in the fetal pituitary and pineal glands and in adult pineal glands from several mammalian species, including humans.[82] AVT is present in the pituitary during fetal life from 11 to 19 weeks, is secreted by cultured human fetal pineal cells during the second trimester, and disappears in the neonatal period.[81,82] In adult mammals, instillation of AVT into cerebrospinal fluid inhibits gonadotropin and corticotropin release, stimulates prolactin (PRL) release by the anterior pituitary, and induces sleep; however, the physiologic importance of these effects remains unclear. The role of AVT in the fetal pineal gland is unknown. Recent work shows a role for oxytocin in the development of the neurovascular interface in the posterior pituitary.[83] By 40 weeks, the concentrations of AVP and OT approximate 20% of those in adults. Fetal pituitary oxytocin concentration, detectable by 11 to 15 weeks, exceeds AVP concentration by 19 weeks. The AVP-oxytocin ratio falls progressively thereafter.

In the fetal sheep, the baseline fetal plasma AVP concentrations are similar to maternal levels after midgestation. During the last trimester of gestation, fetal hypothalamic and pituitary responsiveness to both volume and osmolar stimuli for AVP secretion are well developed, and AVP exerts antidiuretic effects on the fetal kidney.[80,81] Baseline plasma levels of AVT in fetal sheep during the last trimester approximate values for AVP and OT.[82] Presumably this AVT is derived from the posterior pituitary, but the stimuli for AVT secretion in the fetus are not defined. The neurohypophyseal peptides are synthesized as large precursor molecules (neurophysins) and processed to bioactive amidated peptides.[84] Enzymatic processing involves progressive cleavage of carboxyl terminal–extended peptides, sequentially producing (for OT) OT-glycine-lysine-arginine (OTGKR), OTGK, OTG, and OT. Similar progressive processing yields AVP-glycine and AVP from the AVP neurophysin. Enzymatic processing of neurophysins matures progressively in the fetus so that early in gestation fetal plasma contains relatively large concentrations of the extended peptides.[84] For OT, the ratio of OT-extended peptides to OT in fetal sheep serum is approximately 35:1 early in gestation and 3:1 late in gestation.[84]

In the fetus, AVP appears to function as a stress-responsive hormone. Perhaps the major potential stress for the fetus is hypoxia, and the response of AVP to hypoxia is increased compared with the maternal response and with fetal AVP responses to osmolar stimuli.[81,85–87] Plasma AVP concentrations in human cord blood are elevated in association with intrauterine bradycardia and meconium passage.[86] The vasopressor action of AVP may be important in the maintenance of fetal circulatory homeostasis during hemorrhage and hypoxia; AVP has a limited effect on fetoplacental blood flow.[81,88] Fetal hypoxia is also a major stimulus for catecholamine release. There is little information on interactions between AVP and catecholamines during fetal hypoxia, but both fetal hypoxia and AVP stimulate anterior pituitary function.[88] A role for AVP as a corticotropin-releasing hormone is established in the adult, and the ovine fetal pituitary responds separately and synergistically to AVP and CRH early in the third trimester.[89] The role of AVP in controlling fetal corticotropin release seems to decrease

with gestational age. It is not known whether AVT functions as a corticotropin-releasing hormone in the fetus.

OT receptors have been demonstrated in human fetal membranes at term, and AVP receptors have been found in renal medullary membranes of newborn sheep.[82,90,91] Both AVP and AVT evoke antidiuretic actions in the sheep fetus during the last third of gestation, and both hormones act to conserve water for the fetus by inhibiting fluid loss into amniotic fluid through the lungs and kidneys.[81,82] Water channel receptors (aquaporins 1, 2, and 3) are present in the human fetal and newborn kidney, and the ability of the newborn infant to regulate free water clearance in response to volume and osmolar stimuli has been demonstrated.[92] Whether AVT exerts its effects through AVP receptors or separate fetal AVT receptors is not clear. Maximal concentrating capacity by the fetal kidney is limited to about 600 mmol/L. This limitation is not related to inadequate AVP stimulation but to inherent immaturity of the renal tubules.

Lack of AVP is associated with diabetes insipidus (DI) and with a failure to retain water in the body, leading to polyuria and polydipsia. Although most cases of DI are from acquired causes such as pituitary germinoma, craniopharyngioma, or Langerhans cell histiocytosis, the condition may rarely be due to mutations in the AVP-neurophysin gene or to other congenital causes such as septo-optic dysplasia or holoprosencephaly. Mutations in *WFS1* are associated with autosomal recessive Wolfram syndrome that includes diabetes insipidus, diabetes mellitus, optic atrophy, and sensorineural hearing loss.[93]

Hypothalamus and Pituitary Stalk

The anatomy of the developed hypothalamus is well understood. It extends from the anteriorly located optic chiasm to the posteriorly located mammillary body and is organized into distinct rostral-to-caudal regions: preoptic, anterior, tuberal, and mammillary. The organ is subdivided into three medial-to-lateral regions: periventricular, medial, and lateral.[94] Contained within the medial region are the preoptic nucleus, the anterior hypothalamus, the dorsomedial nucleus, the ventromedial nucleus, and the mammillary nuclei. The lateral zone comprises the preoptic and hypothalamic areas.[94]

As Rathke pouch invaginates, part of the ventral diencephalon evaginates ventrally to form the infundibulum and, later, the posterior pituitary lobe and pituitary stalk. The pituitary stalk acts as a physical connection between the pituitary gland and brain and contains the hypophyseal (hypothalamic-pituitary) portal system as well as the neuronal connections traversing across the hypothalamic median eminence. These neurons originate from the supraoptic, suprachiasmatic, and paraventricular nuclei, which are large hypothalamic magnocellular bodies located within the periventricular region of the hypothalamus.[94] Within the median eminence itself at the base of the hypothalamus is the capillary bed into which the widely dispersed hypothalamic parvocellular neurons secrete hypophysiotrophic hormones. These stimulate the release of the seven anterior/intermediate pituitary lobe hormones via the hypophyseal portal system. The parvocellular neurons also secrete oxytocin and arginine vasopressin, although at much lower concentrations than the magnocellular neurons, with the parvocellular-derived arginine vasopressin acting synergistically with corticotropin-releasing hormone in regulating ACTH release. It is therefore evident that it is the hypothalamus acting through the pituitary gland that is the central mediator of growth, reproduction, and homeostasis.[55]

Deciphering hypothalamic development during embryogenesis has proved difficult, perhaps due to its anatomic complexity and diverse collection of cell groups and neuronal subtypes. There is a dearth of data defining the genetics and the signaling and marker molecules involved in their delineation and identification.[95,96] A dual novel role for fibroblast growth factor (FGF)3/FGF10 was identified in innervation and vascularization of the prospective vertebrate hypothalamic region, acting as chemoattractive cues to the neurohypothalamic neurons.[97] Little is known about the molecular mechanisms governing the formation of the hypothalamus, and genetic expression studies within the hypothalamus have knock-on effects on multiple neuronal subtypes and downstream physiologic processes. Studies are slowly elucidating hypothalamic development, and this will in time elaborate the processes involved.

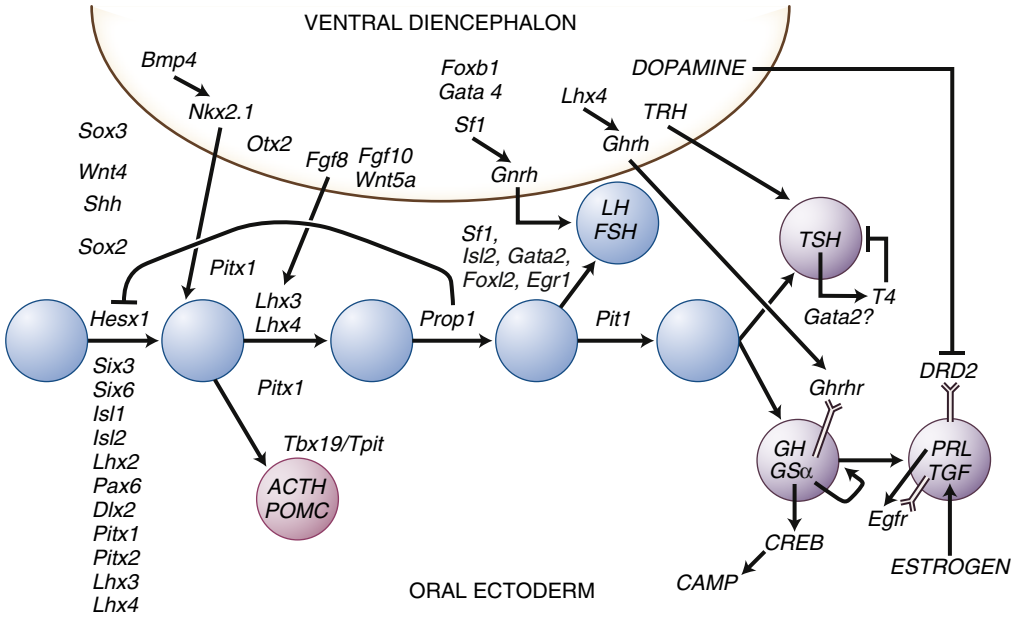
Genes Involved in Pituitary Disease

Complex genetic interactions dictate normal pituitary development.[55] A cascade of signaling molecules and transcription factors plays a crucial role in organ commitment, cell proliferation, cell patterning, and terminal differentiation. The final product is a culmination of this coordinated process (Fig. 23.3).

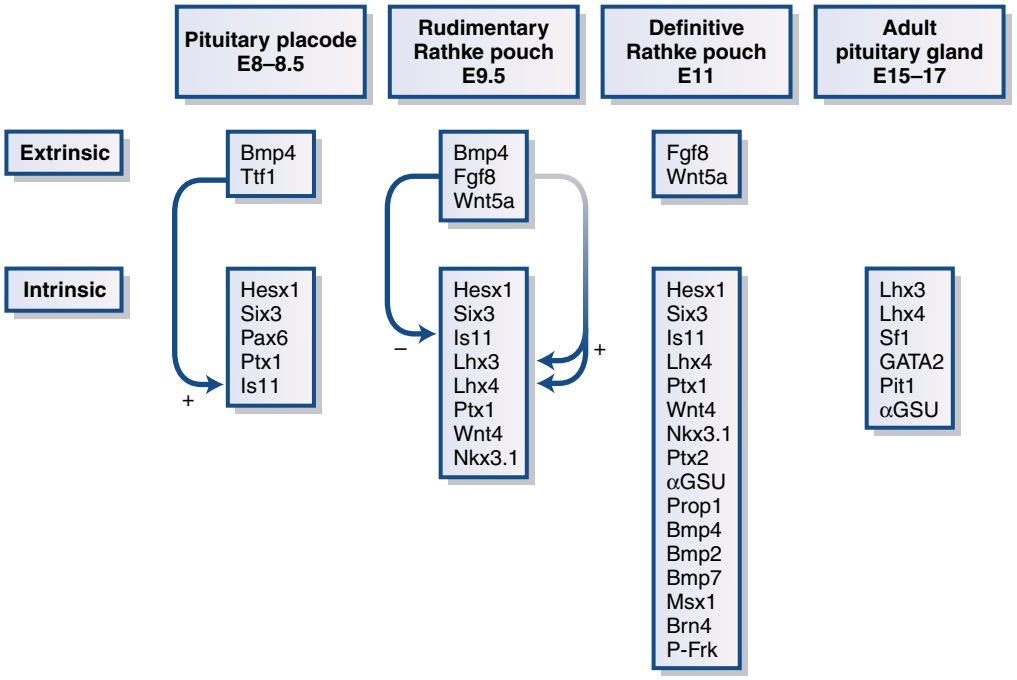
Initially, cells within the primordium of the pituitary gland are competent to differentiate into all cell types. After expression of the earliest markers of pituitary gland development, such as homeobox gene expressed in embryonic stem (ES) cells *(Hesx1),* further signaling pathways are established from within the gland and ventral diencephalon that direct these cells toward terminal differentiation into mature hormone-secreting cell types. Signaling molecules and transcription factors are expressed sequentially at critical periods of pituitary development, and expression of many of these factors is subsequently attenuated (Fig. 23.4). Genes expressed early are implicated in organ commitment but are also implicated in repression and activation of downstream target genes that have specific roles in directing the cells toward a particular fate.

Spontaneous or artificially induced mutations in the mouse have led to significant insights into human pituitary disease, and identification of mutations associated with human pituitary disease have in turn been invaluable in defining the genetic cascade responsible for the development of this embryologic tissue. Mutations involved specifically in human hypothalamic-pituitary disease are listed in Table 23.2 and briefly discussed in this section.

Extrinsic molecules within the ventral diencephalon and surrounding structures, such as bone morphogenetic proteins 2 and 4 (BMP2, BMP4), FGF8, sonic hedgehog (SHH), wingless (Wnt4), thyroid transcription factor 1 (TTF1; also called Nkx2-1), and molecules involved in Notch signaling, play critical roles in early organogenesis.[55,56,97] Recent studies in the mouse have shown that a close interaction between oral ectoderm and neural ectoderm is critical for initial development of the pituitary gland. Rathke pouch develops in a two-step process that requires at least two sequential inductive signals from the diencephalon. First, induction and formation of the rudimentary pouch are dependent upon BMP4; second, FGF8 activates two key regulatory genes, LIM homeobox 3 *(LHX3)* and LIM homeobox 4 *(LHX4)*, that are essential for subsequent development of the rudimentary pouch into a definitive pouch. BMP4 and FGF8 are present only in the diencephalon, not in Rathke pouch. Murine mutations within the gene encoding *TTF1/Nkx2-1* (also called thyroid-specific enhancer binding protein), which is expressed only in the presumptive ventral diencephalon, can cause severe defects in the development of the diencephalon and the anterior pituitary



• **Fig. 23.3** Schematic representation of the developmental cascade of genes implicated in human pituitary development with particular reference to pituitary cell differentiation.



• **Fig. 23.4** Transcription factors and signaling molecules involved in anterior pituitary development.

gland. Conditional deletion of *RBPJ*, which encodes the major mediator of the Notch pathway, leads to conversion of the late (pituitary-specific transcription factor 1 [Pit1]) lineage into the early (corticotroph) lineage. Notch signaling is required for maintaining expression of prophet of Pit1 *(PROP1)*, which is required for generation of the Pit1 lineage. Attenuation of Notch signaling is necessary for terminal differentiation in Pit1 cells and for maturation and proliferation of the GH-producing somatotrophs.[98]

Mutations of the sonic hedgehog *(SHH)* signaling pathway *(SHH, TGIF, ZIC2, PTCH1, GLI2)* and the transcription factors sine oculis–related homeobox 3 *(SIX3)*, teratocarcinoma-derived growth factor 1 *(TDGF1)*, and *FOXH1/FAST1* have been identified in patients with holoprosencephaly, with and without hypothalamic-pituitary defects.[99–103] Mammalian *SIX6* and *SIX3* are vertebrate homologues of Drosophila optix, and expression of these are restricted to the developing eye and brain. *SIX6* knockout mice have hypoplastic pituitaries, may be blind, and fail to progress through the estrous cycle with resultant infertility. *SIX3* and *SIX6* are transcription factors of importance for pituitary lineage specifications, in particular gonadotropic cell lines.[104]

Mutations in *FGF8* have also been identified in association with both holoprosencephaly associated with diabetes insipidus and Kallmann syndrome (KS).[62,105] Mutations in *HESX1* have been identified in patients with septo-optic dysplasia (SOD; a

TABLE 23.2 Comparison of Murine and Human Hypothalamic-Pituitary Development Phenotypes

Gene	Protein	Murine Loss-of-Function Phenotype	Human Phenotype	Inheritance (Murine and Human)
HESX1	HESX1	Anophthalmia or microphthalmia, agenesis of corpus callosum, absence of septum pellucidum, pituitary dysgenesis, or aplasia	Variable: SOD, CPHD, IGHD with EPP Anterior pituitary hypoplastic or absent Posterior pituitary ectopic or eutopic Frequency of mutations: <1%	Dominant or recessive in humans, recessive in mouse
OTX2	OTX2	Lack of forebrain and midbrain, olfactory placode, optic placodes	Anophthalmia, APH, ectopic posterior pituitary, absent infundibulum Frequency of mutations: 2%–3% of anophthalmia/microphthalmia cases	Heterozygous: haploinsufficiency/dominant negative
SOX2	SOX2	Homozygous null mutants: embryonic lethal Heterozygous mice and further dose reduction: poor growth, reduced fertility, CNS abnormalities, anophthalmia; pituitary hypoplasia with reduction in all cell types	Hypogonadotropic hypogonadism; APH, abnormal hippocampi, bilateral anophthalmia/microphthalmia, abnormal corpus callosum, learning difficulties, esophageal atresia, sensorineural hearing loss, hypothalamic hamartoma Frequency of mutations: 3%	De novo haploinsufficiency in humans, heterozygous mutation associated with haploinsufficiency in mouse
SOX3	SOX3	Poor growth, weakness, craniofacial abnormalities, ACC, hypothalamic and infundibular abnormalities	IGHD and mental retardation, hypopituitarism; APH, infundibular hypoplasia, EPP, midline abnormalities Frequency of mutations: 6% (duplications), 1.5% (mutations)	X-linked recessive in both mice and humans
GLI2	GLI2	N/A	Holoprosencephaly, hypopituitarism, craniofacial abnormalities, polydactyly, single nares, single central incisor, partial ACC Frequency of mutations: 1.5%	Haploinsufficiency in humans
FGF8	FGF8	Holoprosencephaly, reduction of vasopressin and oxytocin, reduced GnRH neurons	Holoprosencephaly, hypogonadotrophic hypogonadism, ACTH and TSH deficiencies, diabetes insipidus	AR in both human and mouse; AD in some cases
LHX3	LHX3	Hypoplasia of Rathke pouch	GH, TSH, gonadotropin deficiency with pituitary hypoplasia ACTH insufficiency variable Short, rigid cervical spine Variable sensorineural hearing loss Frequency of mutations: 1.3%	Recessive in both
LHX4	LHX4	Mild hypoplasia of anterior pituitary	GH, TSH, cortisol, and gonadotropin deficiency, persistent craniopharyngeal canal and abnormal cerebellar tonsils; APH, ectopic/eutopic posterior pituitary, absent infundibulum Frequency of mutations: 1.2%	Recessive in mouse, recessive/dominant or recessive in humans
PROP1	PROP1	Hypoplasia of anterior pituitary with reduced somatotropes, lactotropes, thyrotropes, corticotropes, and gonadotropes	GH, TSH, PRL, and gonadotropin deficiency Evolving ACTH deficiency Enlarged pituitary with later involution Frequency of mutations: 1.1% sporadic cases, 29.5% familial cases	Recessive in both
POU1F1	POU1F1 (PIT1)	Anterior pituitary hypoplasia with reduced somatotropes, lactotropes, and thyrotropes	Variable anterior pituitary hypoplasia with GH, TSH, and PRL deficiencies Frequency of mutations: 3.8% sporadic cases, 18% familial cases	Recessive in mouse, dominant/recessive in humans
ARNT2	ARNT2	Anterior pituitary hypoplasia, TRH, somatostatin, oxytocin and CRH deficiencies, reduced vasopressin neurons	TSH, GH, ACTH deficiencies, DI, small anterior pituitary, vesicoureteric reflux, renal impairment, visual impairment, neonatal seizures with progressive microcephaly	Autosomal recessive
PNPLA6	PNPLA6	N/A	Oliver-McFarlane syndrome; trichomegaly, congenital hypopituitarism and retinal degeneration with choroidal atrophy; hypogonadotrophic hypogonadism	Autosomal recessive
TCF7L1	TCFL1	Forebrain, eye and pituitary deficits	Septo-optic dysplasia with variable hypopituitarism	Autosomal dominant

ACC, Agenesis of corpus callosum; *ACTH,* adrenocorticotropic hormone; *APH,* anterior pituitary hypoplasia; *AR,* androgen receptor; *CNS,* central nervous system; *CPHD,* combined pituitary hormone deficiencies; *EPP,* ectopic posterior pituitary; *GH,* growth hormone; *IGHD,* isolated growth hormone deficiency; *N/A,* not applicable; *PRL,* prolactin; *SOD,* septo-optic dysplasia; *TSH,* thyroid-stimulating hormone.

combination of pituitary, eye, and midline forebrain defects), combined pituitary hormone deficiencies (CPHDs), and isolated growth hormone deficiency (IGHD).[55,106] Mutations in *SOX2* and *OTX2* have been described in association with severe eye defects, hypogonadotropic hypogonadism, and variable hypopituitarism.[107–111] Mutations and genomic duplications in *SOX3* have been identified in patients with hypopituitarism with and without learning defects.[112]

Mutations in the gene encoding the LIM homeodomain transcription factor *LHX3* have been identified in patients with hypopituitarism, neck abnormalities, and sensorineural deafness, whereas mutations in *LHX4* have been identified in patients with hypopituitarism, which can be lethal, and cerebellar abnormalities.[113–115] Mutations in genes expressed later in pituitary development, such as *PROP1* and *POU1F1* (previously known as *PIT1*), are associated with more specific pituitary phenotypes (variable GH, thyroid-stimulating hormone [TSH], ACTH, PRL, and gonadotropin deficiencies and often a large anterior pituitary that later involutes with *PROP1* mutations; GH, TSH, and PRL deficiencies with *POU1F1* mutations), in keeping with a role for these genes in cellular proliferation and differentiation and hormone secretion.[55,116–118] Mutations in the T-box transcription factor *TBX19/TPIT* have been described in patients with early-onset isolated ACTH deficiency.[119] Mutations in *TSHβ* have been associated with central hypothyroidism.[120] More recently, mutations and deletions of *IGSF1* have been associated with TSH and variable GH and PRL deficiencies associated with macro-orchidism. Immunoglobulin superfamily, member 1 (IGSF1) is a glycoprotein that is expressed in the pituitary and testis. The function of the gene remains unclear but it may be involved in TRH signaling.[121,122] Central hypothyroidism may be more prevalent than previously thought, affecting up to 1 in 16,000 neonates in the Netherlands where screening is via a combination of T_4 and TSH.

Rare recessive biallelic inactivating mutations in *TRHR*, namely p.S115-T117del and p.A118T, have been reported in three affected individuals from two unrelated pedigrees with central congenital hypothyroidism (CCH), with absent TSH and prolactin responses to exogenous TRH.[123,124] More recently, the p.P81R missense mutation described in isolated CCH highlights the importance of the second transmembrane helix in mediating TRH receptor activation via hormone binding, making it the first deleterious missense *TRHR* defect that gives rise to CCH.[125] In addition, a recently identified novel homozygous mutation, p.I131T, that decreases TRH affinity was identified in an overweight patient with CCH and normal stature.[126]

The X-linked transducin β-like protein 1 (*TBL1X*) gene is a component of the thyroid hormone receptor–corepressor complex, mutations that have been previously associated with sensorineural hearing loss.[127,128] In a recent study, six mutations in unrelated pedigrees with isolated CCH have been identified.[129] Like *IGSF1*, *TBL1X* is associated with an X-linked form of TSH deficiency.

PCSK11 mutations result in ACTH deficiency, hypoglycemia, hypogonadotrophic hypogonadism, obesity, GH deficiency, and DI. In a study of 13 children with proprotein convertase 1/3 (PC1/3) deficiency, an autosomal recessive disorder caused by rare mutations in the proprotein convertase subtilisin/kexin type 1 *(PCSK1)* gene, obesity and severe malabsorptive diarrhea were associated with a number of endocrine abnormalities. PC1/3 is an endoprotease that processes many prohormones expressed in endocrine and neuronal cells. Failure of enteroendocrine cells to produce functional hormones resulted in generalized malabsorption. Additional endocrine abnormalities that developed with disease progression included central DI, GH deficiency, primary male hypogonadism, adrenal insufficiency secondary to ACTH deficiency, and hypothyroidism.[130]

The hypothalamic-pituitary-gonadal axis is the key regulator of sex development and reproduction, processes that are initiated through the decapeptide GnRH. GnRH is produced and released after the successful migration during embryogenesis of GnRH neurons from the olfactory placode, across the cribriform plate, to the hypothalamic arcuate nucleus, where they are detectable from about 9 weeks of gestation. These neurons are then projected into the hypothalamic median eminence. It has recently been reported that the number of GnRH neurons in the brain at the end of the first trimester is fivefold higher (~10,000) than previously described in the hypothalamus.[131] New insights from three-dimensional (3D) imaging include the presence of GnRH neurons in nonhypothalamic areas, raising the possibility of nonreproductive roles for GnRH.[131]

A functional connection with respect to GnRH is detected by 16 to 20 weeks' gestation. This hormone is released in a pulsatile fashion and binds to its receptors on pituitary gonadotrophs, which in turn respond by synthesizing and releasing the gonadotropins LH and FSH. These bind to their cognate receptors in the gonads, where they stimulate the production of sex steroids, such as androgens or estrogens, and stimulate gametogenesis. The sex steroids then regulate gonadotropin secretion via negative feedback at the level of the hypothalamus or pituitary. Congenital disorders of gonadotropin secretion include normosmic isolated GnRH deficiency causing hypogonadotropic hypogonadism and KS, a disorder characterized by hypogonadotropic hypogonadism, anosmia/hyposmia, cleft lip/plate, sensorineural hearing loss, dental anomalies, synkinesia, and renal abnormalities.

Mutations in a number of genes have been reported to date in Kallmann syndrome, including *KAL1, CCDC141, FEZF1, IL17RD, SEMA3A*, *SEMA3E,* and *SOX10.*[105,132–134] Pathogenic variants in *CHD7, FGF8, FGF17, FGFR1, DUSP6, SPRY4,* and *FLRT3, HS6ST1*, *NSMF (NELF), PROK2, PROKR2,* and *WDR11* cause both KS and normosmic hypogonadotropic hypogonadism.[134,135] Variants in encoding prokineticin receptor 2 and prokineticin 2 (*PROKR2* and *PROK2*, respectively) have been identified in approximately 9% of patients with Kallmann syndrome.[136] Prokineticins are secreted cysteine-rich proteins that possess diverse biologic activities, including effects on neuronal survival, gastrointestinal smooth muscle contraction, circadian locomotor rhythm, and appetite regulation.[136] Prokineticins PROK1 and PROK2 act through their G protein–coupled receptors PROKR1 and PROKR2, which are expressed in the olfactory bulbs. PROK2 functions as a chemoattractant for neuronal progenitors, which follow a rostral migratory stream. *Prokr2*$^{-/-}$ mice have reduced LH in the pituitary, small gonads, and abnormal olfactory bulb formation, but *Prokr2* seems to be dispensable for normal pituitary formation.[84,85] Variations in *PROKR2* have also been associated with hypopituitarism, pituitary stalk interruption syndrome, and septo-optic dysplasia but are unlikely to be causative in isolation and likely only contribute to the phenotype, including KS, in combination with other genetic mutations or environmental factors.

Heterozygous pathogenic variants or microdeletions in *CHD7* can also cause CHARGE syndrome, characterized by *c*oloboma, *h*eart abnormalities, choanal *a*tresia, *r*etardation of growth and development, *g*enital hypoplasia, and *e*ar abnormalities.[137] The

genital abnormalities in CHARGE syndrome are caused by hypogonadotropic hypogonadism and are frequently accompanied by olfactory defects and cleft lip/palate.[138]

Mutations in a number of genes have also been identified in association with normosmic hypogonadotropic hypogonadism, and these mutations include genes encoding GnRH1 and its receptor GnRHR, kisspeptin *(KISS1)* and its receptor KISS1R, and neurokinin B (encoded by tachykinin 3 *[TAC3]*) and its receptor TACR3.[134,139–142]

Recently, mutations in genes such as *RNF216*, *OTUD4, STUB1,* and *PNPLA6* have been found in syndromic forms of GnRH deficiency that have associated features such as ataxia and dementia, as part of Gordon Holmes syndrome *(RNF216*, *OTUD4*, *STUB1)* and Boucher-Neuhäuser syndrome *(PNPLA6).*[143–145] *RNF216*, *OTUD4,* and *STUB1* are involved in protein ubiquitination; *PNPLA6* encodes an enzyme involved in the production of the neurotransmitter acetylcholine.

Other genes implicated in syndromic forms of hypothalamic-pituitary disease include *GLI3*, a component of the SHH signaling pathway, haploinsufficiency of which results in Pallister-Hall syndrome associated with polydactyly, hypothalamic disorganization, hypothalamic hamartoma, and hypopituitarism.[146,147] *PITX2* mutation has been identified as one cause of Axenfeld-Rieger syndrome, including ocular, dental, and hypothalamic abnormalities.[148] Pitx2-null mutant mice manifest pituitary hypoplasia and decreased *GHRH receptor (GHRH-R), GH, FSH, LH,* and *TSH* gene expression.[148]

Mutations in *ARNT2* are associated with severe pituitary insufficiency, including GH, TSH, and ACTH deficiencies, and diabetes insipidus with progressive microcephaly, seizures, severe visual impairment, severe learning difficulties, and abnormalities of the renal and urinary tracts.[149] Aryl hydrocarbon receptor nucleus translocator 2 (ARNT2) is a basic helix-loop-helix transcription factor critical for normal development of the paraventricular and supraoptic nuclei.

Deficient anterior pituitary with variable immune deficiency (DAVID) syndrome is a rare disorder comprising central ACTH deficiency and common variable immune deficiency (which can be mild, absent, or develop at a later stage), and a small anterior pituitary has been described in this condition. The syndrome is caused by mutations in *NFKB2*.[150]

Rare isolated pituitary hormone deficiencies have been associated with mutations in the respective hypothalamic-releasing hormones or the releasing hormone receptors (e.g., familial GH deficiency due to *GHRH-R* mutations), TSH deficiency due to *TRHR* mutations, and gonadotrophin deficiency due to *GNRHR* mutations.[123,140,151]

Far fewer mutations have been identified to account for abnormalities of the posterior pituitary and stalk. Pituitary stalk interruption syndrome comprises a thin or absent pituitary stalk, an ectopic posterior pituitary, and small or absent anterior pituitary, usually accompanied by pituitary hormone deficiencies. Roundabout *(robo)* genes, first identified in Drosophila, encode Robo proteins that belong to the immunoglobulin superfamily of protein secreted by midline glial cells and play a role in axon guidance. Whole exome sequencing recently identified *ROBO1* variants in 25 patients with pituitary stalk interruption syndrome (PSIS); however, functional studies were not performed to support causality.[152]

However, no genetic etiology has been identified in most cases of congenital hypopituitarism, which suggests a role for other unidentified genes or environmental or epigenetic factors.

Growth Hormone and Prolactin

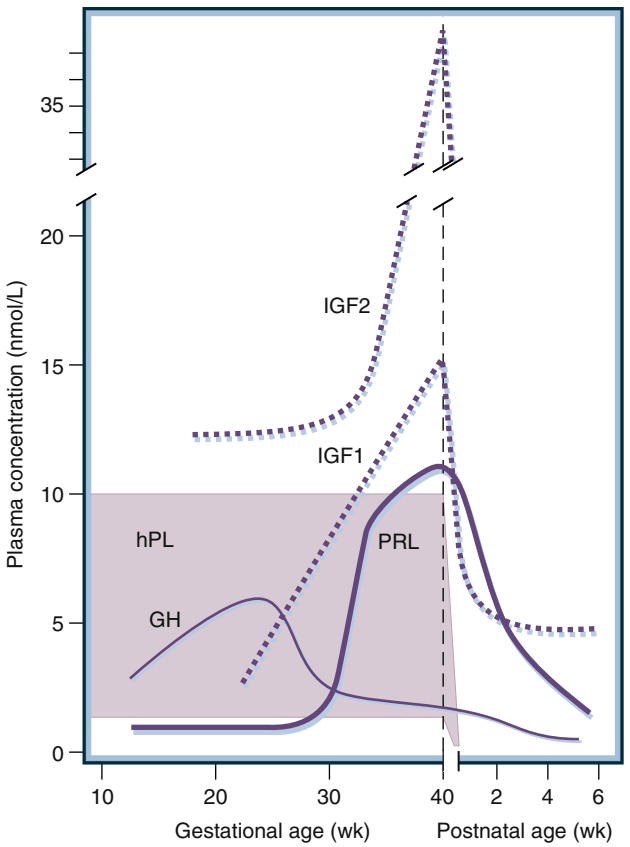
The fetal pituitary gland can synthesize and secrete GH by 8 to 10 weeks' gestation. Pituitary GH content increases from about 1 nmol (20 ng) at 10 weeks to 45 nmol (1000 ng) at 16 weeks. Fetal plasma GH concentrations in cord blood samples are in the range of 1 to 4 nmol/L during the first trimester and increase to a mean peak of approximately 6 nmol/L in midgestation. Plasma GH concentrations fall progressively during the second half of gestation, to a mean value of 1.5 nmol/L at term.[57] The responses of plasma GH to somatostatin and GHRH and to insulin and arginine are mature at term in human infants.[57,153] The high plasma GH concentrations after development of the pituitary portal vascular system at midgestation may reflect unrestrained secretion.[57] Whatever the mechanisms, control of GH secretion matures progressively during the last half of gestation and the early weeks of postnatal life, so that mature responses to sleep, glucose, and l-dopa are present by 3 months of age. GH secretion is already pulsatile soon after birth in humans,[154] but trough concentrations are still higher than in later life so that random GH sampling can be used to detect GH deficiency in the neonatal period, which is not possible at a later age.[155]

The ontogenesis of fetal plasma PRL differs significantly from that of GH; concentrations are low until 25 to 30 weeks' gestation and increase to a mean peak value of approximately 11 nmol/L at term (Fig. 23.5).57 Pituitary PRL content increases progressively from 12 to 15 weeks. PRL release increases in response to TRH and decreases in response to dopamine. Brain and hypothalamic control of PRL matures late in gestation and during the first months of extrauterine life.[57,153] The marked increase in fetal plasma PRL concentration in the last trimester parallels the increase in fetal plasma estrogen concentrations, although it lags by several weeks.[57,153]

Postnatally, GH acts through receptors in liver and other tissues to stimulate production of IGF1 and, to a lesser degree, IGF2. Prenatally, GH receptor mRNA levels and receptor binding are low in fetal liver, although receptor messenger ribonucleic acid (mRNA) is present in other fetal tissues.[57] The growth of anencephalic fetuses is almost normal, suggesting that factors other than GH stimulate fetal IGF production. Nutrition plays an important role.[156,157] PRL receptors are present in most fetal tissues during the first trimester of gestation, and it is likely that lactogenic hormones have a significant role in organ and tissue development early in gestation.[61,157]

Human chromosome 17q22-24 contains a growth hormone/placental lactogen (GH/PL) gene cluster containing five related genes: *GH-N* encodes pituitary growth hormone; *GH-V* encodes placental growth hormone; and *hPL-A, hPL-B,* and *hPL-L* encode placental lactogens (also called chorionic somatomammotropins [CS]). The major circulating placental lactogens derive from the *hPL-A* and *hPL-B* genes. *GH-V* differs in 13 of the 191 amino acids of *GH-N* and is produced in the syncytiotrophoblast.[158] It rises sharply after midgestation to a peak at 34 to 37 weeks, and within 1 hour after delivery of the placenta it disappears from the circulation.[159] Placental *GH-V* is secreted into the maternal circulation and reduces insulin sensitivity in the mother and so spares glucose and other nutrients for transplacental delivery and fetal growth.

Human placental lactogen (hPL) is structurally homologous to GH but functionally closer to prolactin and is secreted directly into both fetal and maternal circulations. hPL is first detected in the mother at 6 weeks of gestation to reach a peak of 5000 to 7000 ng/mL at 32 to 35 weeks; fetal hPL concentrations, however,

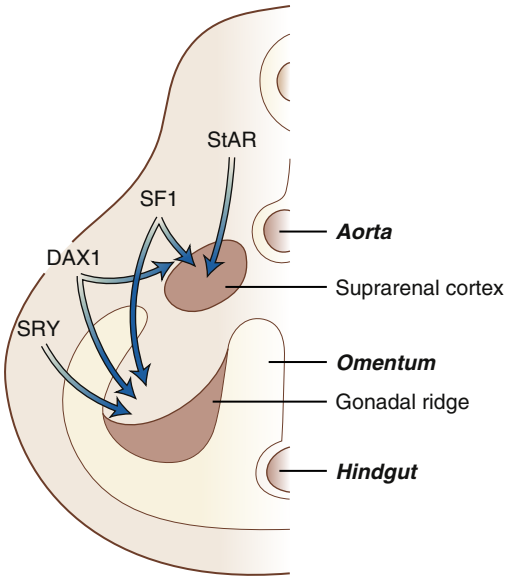


• **Fig. 23.5** Patterns of change of fetal plasma human placental lactogen (hPL), growth hormone (GH), prolactin (PRL), insulin-like growth factor 1 (IGF1), and insulin-like growth factor 2 (IGF2) during gestation and in the neonatal period. The *shaded area* indicates the range of fetal plasma hPL concentrations. (Data from Bennett A, Wilson DM, Liu R, et al. Levels of insulin-like growth factors I and II in human cord blood. *J Clin Endocrinol Metab*. 1983;57:609–612; Kaplan SL, Grumback MM, Aubert ML. The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus: maturation of central nervous system regulation of anterior pituitary function. *Recent Prog Horm Res*. 1976;32:161–243; Bala RM, Lopatka J, Leung A, et al. Serum immunoreactive somatomedin levels in normal adults, pregnant women at term, children at various ages and children with constitutionally delayed growth. *J Clin Endocrinol Metab*. 1981;52:508–512.)

approximate to 20 to 50 ng/mL at term. hPL concentrations increase with placental mass and are higher in twin than singleton pregnancies. Lactogens affect insulin production, hypothalamic gene expression, and leptin action in the mother and so maintain metabolic homeostasis while providing the substrates for nutrition for the fetus and newborn infant. Studies in rodents suggest that maternal hyperphagia is mediated by progesterone and prolactin.[158] At least in rodents, evidence suggests that prolactin and placental lactogen signaling through the PRL receptor is essential for the increase in β-cell mass.[160]

Adrenal

An appreciation of human adrenal development is essential to appropriately understand adrenal physiology as well as the pathologic basis of many congenital adrenal disorders. Furthermore, understanding the regulation of adrenal development sheds light on the emerging critical role the fetal adrenal plays as a component of the fetoplacental unit during pregnancy. The uniqueness of the human fetal adrenal was largely overlooked until 1911



• **Fig. 23.6** Hemi cross section of a 5-week human embryo showing the locations of the adrenal primordia (suprarenal cortices) and gonadal ridges. Steroidogenic factor 1 *(SF1)* is involved in testicular and ovarian development. *SRY* is the single critical regulator of testicular embryogenesis. Inactivation of the *DAX1* gene leads to adrenal hypoplasia. The steroidogenic acute regulatory protein (StAR) is the rate-limiting factor for adrenal steroidogenesis (see text for details).

when Starkel and Węgrzynowski, two Polish medical students, described the fetal zone (FZ) for the first time as an immature cortex, which undergoes involution in the first years of life.[161] At the same time, Elliott and Armour published the article, "The Development of the Cortex in the Human Suprarenal Gland and Its Condition in Hemicephaly," which described the unique FZ.[162] The transient FZ is not present in most mammals and appears to be unique to humans and a few higher primates.

Embryology

The adrenal appears as the bipotential adrenogonadal primordium (AGP) at 28 to 30 dpc in humans[163] due to expression of the transcription factor, steroidogenic factor 1 (SF1, NR5A1), a nuclear receptor essential for adrenal development and steroidogenesis (Fig. 23.6). The adrenal cortex is derived from a thickening of the intermediate mesoderm, known as the gonadal ridge, in contrast to the adrenal medulla, which is derived from the ectoderm.

The AGP cells give rise to the steroidogenic cells of the adrenal gland and the gonad. The gonadal cells migrate caudally. Those cells that are more medial, expressing the highest levels of SF1, migrate retroperitoneally to the upper pole of the mesonephros to form the adrenal primordium at 33 dpc. At about 48 dpc, sympathetic neural crest cells begin migrating to the area where the adrenal primordium (AP) is developing. These cells coalesce and differentiate into the catecholamine-producing chromaffin cells of the adrenal medulla after birth. Following neural crest invasion, the AP becomes encapsulated, resulting in the formation of a distinct organ just above the developing kidney. By 50 to 52 dpc, there are two distinct zones within the cortex: The larger inner FZ contains eosinophilic cells with high levels of expression of steroidogenic enzymes; the smaller outer definitive zone (DZ) is composed of tightly packed cells with much lower levels of steroidogenic enzyme expression. A third cortical zone, the transitional zone (TZ), becomes identifiable from about 14 weeks

postconception (wpc). The TZ lies between the DZ and FZ, containing cells with histologic appearances of both.[14]

The fetal adrenal undergoes tremendous growth as pregnancy progresses largely due to an increase in size of the FZ, which accounts for 80% to 90% of the gland's mass by midgestation.[164,165] The fetal adrenal itself forms 0.4% of body weight at term, weighing 3 to 5 g with a relative size 10-fold to 20-fold that of the adult adrenal.[14] Growth is paralleled by functional development, specifically steroidogenesis for production of androgens from the FZ, with brief periods of increased cortisol secretion. These steroids maintain intrauterine homeostasis and prepare the fetus for life ex-utero. FZ cells robustly express cytochrome P450 17 alpha (P450c17α), a bifunctional enzyme with both 17 hydroxylase and 17,20 lyase activities that converts pregnenolone to DHEA. DHEA is sulfated to DHEAS, which is subsequently aromatized by the placenta to estrogens.[14]

Immediately after birth the FZ rapidly involutes and remodels by a process involving apoptosis of cells in its inner region with a concomitant decrease in adrenal androgen secretion.[163] The weight of the adrenal gland drops by 50% within the first 2 weeks after birth,[166] and the FZ is absent by 6 months of age in most cases. Whether the timing of fetal adrenal involution is determined by gestation or by birth is controversial. FZ androgen production has been reported to persist in infants born prematurely[167]; however, a more recent sonographic study demonstrated a similar pattern of adrenal involution within the first 2 weeks after birth in all neonates examined, regardless of their gestational age at birth.[14]

Cells with zona reticularis morphology are detectable in human adrenal cortex from around 3 years of age until a continuous ZR forms at around 6 years of age and adrenal androgen synthesis recommences, a stage referred to as adrenarche.[168] The DZ and TZ give rise, respectively, to the zona glomerulosa and zona fasciculata of the developing adult adrenal cortex.

Transcriptional Regulation of Adrenal Development

Insight into the genetic regulation of adrenal development has been gleaned from human and animal models of disordered development. The spatiotemporal expression of key genes is critical. Genes involved in the formation of the intermediate mesoderm and urogenital ridge affect kidney, adrenal, and gonadal development; those affecting AGP development affect both adrenal and gonadal development, and there are also those that specifically affect AP development.

The earliest stages of adrenal development appear to be regulated by a number of transcription factors (e.g., ODD1, SALL1, FOXD1/FOXD2, WT1, SF1, DAX1), coregulators (e.g., CITED2), signaling molecules (e.g., SHH/GLI3, WNT3/WNT4/WNT11), matrix proteins (e.g., SPARC), and regulators of telomerase activity (e.g., ACD). The transcription factors most significant for early development of the AGP have emerged as *SF1* and *DAX1* (dosage-sensitive sex reversal, adrenal hypoplasia congenita [AHC], X-chromosome factor, NR0B1).[169–171] As well as expression in the adrenal cortex, these genes are also expressed in the gonads, hypothalamus, and pituitary tissues. In the absence of *SF1* expression, the adrenal gland does not form.[163] *Sf1*-knockout mutant mice manifest adrenal and gonadal agenesis, gonadotropin deficiency, and absence of the hypothalamic ventromedial nucleus.[170] Severe disruption of *SF1* in humans can cause adrenal dysfunction, although most pathogenic variants in *SF1* in humans cause impaired testicular development and Leydig cell dysfunction rather than adrenal insufficiency. Heterozygous loss-of-function mutations in *SF1* in humans result in 46,XY and 46,XX DSD, complete gonadal dysgenesis, and primary adrenal failure.[170] No cases of homozygous *SF1* mutations have been reported to date. Current data indicate that, while Wilms tumor 1 (WT1) regulates *Sf1* expression in the AGP, Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2 (CITED2) expression in the AGP is necessary for proper differentiation of the AP.[163]

SF1 upregulates DAX1, which is itself a negative regulator of SF1 transcriptional activity and hence steroidogenesis. It is unclear how disruption of SF1 and its negative regulator can cause similar defects; however, DAX1 can function as a coactivator for SF1 transcriptional activity in steroidogenic cells when expressed at high levels. Inactivating *Dax1*/*DAX1* mutations in mice and humans are associated with gonadotropin deficiency and adrenal hypoplasia.[170] Knockdown of *Dax1* results in premature differentiation of mouse adrenocortical progenitor cells, and adrenal failure occurs as the reservoir of stem/progenitor cell pluripotency is depleted. Pathogenic variants or deletions of *DAX1* in humans are well established as the cause of X-linked adrenal hypoplasia congenita (AHC).[172]

Transcription factors involved in the early formation of the rodent urogenital ridge include *Odd1, Wt1, Sall1, Pbx4, Wnt4, FOxD1,* and *FoxD2*. *Wt1* knockout mice have renal and gonadal anomalies and lack adrenal glands, and in humans, germline *WT1* mutations cause defects in gonad and kidney formation.[171] *Pbx1* knockout mice die in utero with multiple organ defects that include adrenal agenesis and impaired testis development. *Wnt4*-null mutant mice, in contrast, have impaired kidney development from 15 dpc, masculinization of XX females, müllerian duct agenesis, and abnormal adrenal glands with reduced steroidogenic enzyme expression. A homozygous missense mutation in *WNT4* in humans was shown to be associated with renal agenesis, gonadal defects, and adrenal hypoplasia from 19 weeks' gestation. *Sall1*-null mutants have severe kidney dysgenesis or agenesis and hypoplastic adrenal glands at birth. Heterozygous mutations in *SALL1* result in Townes-Brocks syndrome, which comprises renal and genital abnormalities. The murine model that phenocopies the deletion seen in Townes-Brocks syndrome shows absent adrenal glands and kidneys and hypoplastic gonads at 16 dpc.

Signaling Pathways in Adrenal Development

Sonic hedgehog, a member of the vertebrate hedgehog (Hh) family of secreted ligands, performs numerous crucial roles during embryonic development and is required in the adult for tissue maintenance, differentiation, and the regulation of stem cell populations. SHH signaling is required for normal adrenal development, at a later stage than SF1 and DAX1, and is expressed in the AP in cells just beneath the capsule.[173–175] It has long been thought that undifferentiated, pluripotent stem cells exist in the adrenal cortex to maintain homeostasis in the adult. The precise origin of adrenocortical cells is debatable, and their origin remains ambiguous. Studies focused on a downstream activator of the hedgehog pathway, glioma-associated oncogene homolog 1 (zinc finger protein) (Gli1), provide evidence that the adrenal capsular cells also give rise to the DZ. Gli1-expressing cells are specifically located in the adrenal capsule and do not express SF1. In vitro this subpopulation of cells is capable of giving rise to SF1-expressing differentiated adrenocortical cells during embryonic development.[174] Shh expression marks cortical progenitors,[173] and Shh-expressing cells give rise to all steroidogenic cells in cortical zones. Homozygous deletion of Shh in murine embryos is lethal; however, analysis of their adrenal gland at 14.5 and 16.5 dpc indicates that the adrenal primordium forms but is much smaller than in the wild type.[174,175]

Several lines of evidence suggest that β-catenin plays an important role in adrenal zonation and maintenance. Activation of the β-catenin pathway is restricted to the ZG,[176] and ectopic expression leads to the activation of ZG markers in ZF cells.[87] Members of the R-spondin *(Rspo)* gene family have recently been identified to be expressed from E12.5 onward within mesenchymal cells surrounding the forming adrenal.[177] R-spondins are signaling molecules that positively regulate the β-catenin signaling pathway. This study showed capsular RSPO3 signals to the underlying steroidogenic compartment to induce β-catenin signaling and imprint glomerulosa cell fate. SHH in turn signals back to recruit capsular cells to form the adrenal cortex, at least during development. This study therefore identifies the adrenal capsule as a crucial signaling center that is continuously required for proper zonation.

FGF signaling controls early developmental processes such as anterior/posterior patterning and organogenesis.[178] FGFs are a large family of secreted glycoproteins that bind to four signaling FGF receptors, FGFR1 to FGFR4. FGF signaling interacts with SF1 and SHH signaling.[179] *Fgfr2* and *Fgfr4* are expressed in the developing adrenal cortex. Embryos with a global *Fgfr2IIIb* deletion have hypoplastic adrenal glands,[179] and deletion of both isoforms of *Fgfr2* from steroidogenic tissue recapitulates this phenotype and causes male-to-female sex reversal, implying that *Fgfr2* is not necessary for AGP formation but is required for the subsequent growth and development of the adrenal gland.[180] Epidermal growth factor (EGF) stimulates proliferation of both the fetal and definitive zones. The fetal adrenal expresses high levels of IGF2 mRNA and protein, which are responsive to ACTH.[15] IGF2 augments ACTH-stimulated expression of steroidogenic enzymes and stimulates steroid hormone production in fetal adrenal cortical cells.

Cyclin-dependent kinase inhibitor 1 C *(CDKN1C, P57KIP2)* is a paternally imprinted gene located on chromosome 11p.15, which encodes the CDKN1C protein, an inhibitor of cell cycle progression. Variations in *CDKN1C* or its genomic imprinting can lead to adrenal pathology.[171] Loss of function of *CDKN1C* results in Beckwith-Wiedemann syndrome (BWS), an overgrowth syndrome with increased susceptibility to adrenal carcinoma. IMAGe (intrauterine growth restriction, metaphyseal dysplasia, AHC, and genital anomalies) syndrome is a rare multisystem disorder[181] that mirrors the features of BWS and is caused by gain-of-function mutations in *CDKN1C*. Most affected individuals are born small and develop skeletal abnormalities. Males present with genital malformations, including micropenis and undescended testes and urethral anomalies, but the most clinically important component of the syndrome is adrenal insufficiency that causes salt wasting, hypoglycemia, and shock due to loss of both mineralocorticoid and glucocorticoid synthesis and that can be life threatening shortly after birth.[181]

Fetal Adrenal Steroidogenesis

The fetal adrenal expresses five steroidogenic apoenzymes as in the adult gland: CYP17A1 (P450c17, 17-hydroxylase/17-20-lyase), CYP21A2 (P450c21, 21-hydroxylase), CYP11A1 (P450scc, side-chain cleavage), CYP11B1/CYP11B2 (P450c11/aldosterone synthase). The fifth enzyme, expressed by the smooth endoplasmic reticulum, exhibits both 3βHSD and Δ^4, Δ^5-isomerase activities.[14] The spatiotemporal expression of these results in differences in zonal activity of these enzymes, and transcription of steroidogenic genes is tightly regulated.[14] Steroid production from the fetal adrenal has important roles in the maintenance of intrauterine homeostasis and in the maturation of the fetus in preparation for postnatal adaptation to extrauterine life. Vast quantities of cholesterol are required as a precursor for steroid production; they are obtained through low-density lipoprotein receptors on the cell surface as well as intracellular synthesis of cholesterol from acetate. As in the adult adrenal, cholesterol use for steroids is tightly controlled by the expression of steroidogenic acute regulatory (StAR) protein, which regulates transport of cholesterol to the inner mitochondrial membrane, where CYP11A converts it to pregnenolone. Sphingosine-1-phosphate lyase (SGPL1) is an endoplasmic reticulum enzyme, ubiquitously expressed in tissues. This enzyme mediates irreversible cleavage of the lipid signaling molecule sphingosine-1-phosphate. Several recent studies report mutations in SGPL1 resulting in primary adrenal insufficiency and steroid-resistant nephrotic syndrome.[182,183]

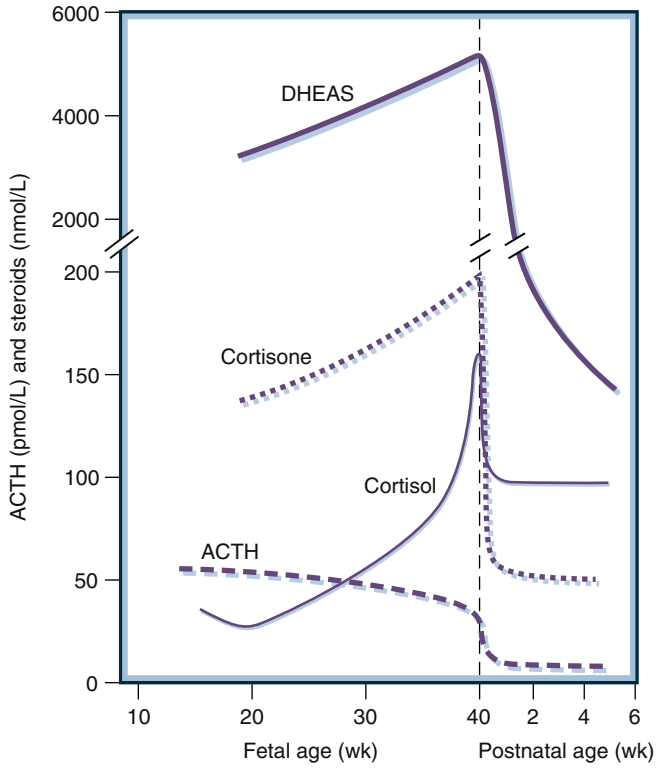
During most of gestation, the fetal adrenal lacks 3βHSD2, preventing cortisol and aldosterone synthesis and directing steroid production toward DHEA production. High expression of SULT2A1 in the FZ accounts for sulfation of most of the Δ^5 steroids produced, including DHEA and pregnenolone. DHEA is sulfated in the FZ to DHEAS, which is then converted by placental aromatase to estrogens.[14]

The TZ produces cortisol, with an early peak from 8 to 9 wpc coinciding with transient expression of 3βHSD2.[184] The hypothalamic-pituitary-adrenal (HPA) axis is sensitive to glucocorticoid-mediated feedback at this time; 46,XX fetuses with steroidogenic defects (e.g., in CYP21 or CYP11) lack cortisol and have an elevated ACTH drive that results in excess production of fetal androgens at a time when the genital and scrotal folds are sensitive to androgen exposure, resulting in virilization of female genitalia.[184] Cortisol can retard fetal and placental growth and so is converted to biologically inactive cortisone by the enzyme 11βHSD2 in placental and fetal tissues. In this way the fetus is protected from high concentrations of cortisol in utero. Concentrations of circulating cortisone in the fetus at midgestation are fourfold to fivefold higher than cortisol concentrations (Fig. 23.7).

The pattern of enzyme maturation in the fetal adrenal suggests that cortisol production does not occur de novo from cholesterol until 30 weeks' gestation, but some production using progesterone as precursor probably occurs earlier.[15] During the third trimester, expression/activity of 11βHSD2 drops and selected fetal tissues, including liver and lung, express 11βHSD1 so that glucocorticoids are available to the fetus to promote the maturation of fetal organs, including the lung and brain. This increase in fetal cortisol production has an important role for extrauterine survival (see "Transition to Extrauterine Life"). The number of glucocorticoid receptors in the sheep fetal hypothalamus increases at term, at the time of increasing glucocorticoid concentrations, suggesting that some process in the fetus allows the normal autoregulation of glucocorticoid receptors to be overridden at term.[185] Mice lacking GR function manifest enlarged and disorganized adrenal cortices, adrenal medullary atrophy, lung hypoplasia, and defective gluconeogenesis. They appear normal at birth but are not viable.

Postnatally, biologically active cortisol predominates over cortisone in the circulation. Healthy term infants have initial pulsatility of cortisol secretion and can develop a circadian rhythm from as early as 1 month after birth, although there is considerable individual variability. There is, however, no consensus about the definition of a "normal" cortisol concentration in preterm infants.

Antenatal steroids for a threatened preterm delivery have clear beneficial effects, with a reduction in mortality and morbidity, but there are consequences of such early high steroid exposure, and long-term effects are unknown. The HPA axis is highly susceptible



• **Fig. 23.7** Patterns of change of fetal plasma adrenocorticotropic hormone *(ACTH),* cortisol, cortisone, and dehydroepiandrosterone sulfate *(DHEAS)* during gestation and in the neonatal period. The trend of average values is shown for each hormone in nanomoles per liter. Notice the broken scale for DHEAS. (Data from Geller DH, Miller WL. Molecular development of the adrenal gland. In: Pescovitz OH, Eugster EA, eds. *Pediatric Endocrinology*. Philadelphia: Lippincott Williams & Wilkins; 2004:548–567; Winters AJ, Oliver C, Colston C, et al. Plasma ACTH levels in the human fetus and neonate as related to age and parturition. *J Clin Endocrinol Metab*. 1974;39:269–273; Murphy BEP. Human fetal serum cortisol levels related to gestational age: evidence of a midgestational fall and a steep late gestational rise, independent of sex or mode of delivery. *Am J Obstet Gynecol*. 1982;144:276–282; Beitins IZ, Bayard F, Ances FIG, et al. The metabolic clearance rate, blood production, interconversion and transplacental passage of cortisol and cortisone in pregnancy near term. *Pediatr Res*. 1973;7:509–513.)

to programming during development. Unlike the endogenous glucocorticoids that are inactivated by 11βHSD2 in the placenta, synthetic glucocorticoids readily cross the placenta. In the fetal sheep, hypothalamic and pituitary glucocorticoid receptors are present at midgestation, and corticotropin suppressibility can be demonstrated by the midpoint of the third trimester of gestation.[185]

Longitudinal follow-up of the offspring of women treated with betamethasone for threatened preterm birth has shown that individuals exhibit insulin resistance 30 years after treatment, particularly women. Previous antenatal treatment of the fetus of a mother whose previous child had CAH by administering dexamethasone orally to the mother at 8 to 12 wpc was found to reduce fetal androgens and hence the virilization of female fetuses, but due to paucity of high-quality evidence in terms of efficacy and safety this should be regarded as a highly controversial experimental treatment.[13] The constant dexamethasone dose used can result in cortisol concentrations that exceed physiologic fetal concentrations by around 60-fold. To date, a number of side effects and adverse associations have been reported, including neurologic abnormalities in the offspring.[13]

Use of steroids postnatally to prevent or treat chronic lung disease in preterm infants facilitates earlier extubation and reduces the risk of chronic lung disease, but it is associated with hyperglycemia, hypertension, gastrointestinal bleeding and perforation, hypertrophic cardiomyopathy, growth failure, and cerebral palsy and thus cannot be recommended.[186] Trials involving more physiologic doses of hydrocortisone are currently underway, but all systemic and inhaled steroids are associated with adrenal suppression in neonates.

Early low-dose hydrocortisone treatment has been reported to improve survival in very preterm infants without bronchopulmonary dysplasia. In an analysis of secondary outcomes of the PREMILOC (Early Low-Dose Hydrocortisone to Improve Survival Without Bronchopulmonary Dysplasia in Extremely Preterm Infants) randomized clinical trial of extremely preterm infants, early low-dose hydrocortisone was not associated with a statistically significant difference in neurodevelopment at 2 years of age.[187] Further randomized studies are needed to provide definitive assessment of the neurodevelopmental safety of hydrocortisone in extremely preterm infants.

The human fetal adrenal gland is capable of aldosterone secretion near term with the development of the zona glomerulosa, and fetal plasma aldosterone concentrations in infants who are born by caesarean section are threefold or fourfold higher than maternal concentrations.[188] Vaginal delivery and maternal salt restriction increase concentrations in both mother and infant. The increased aldosterone concentrations in the fetus are a result of increased fetal adrenal secretion and persist during the first year of extrauterine life. There is poor correlation between plasma renin activity (PRA) and aldosterone concentrations in cord blood.[189] Mineralocorticoid receptors (MRs) are present in fetal tissues from 12 to 16 weeks' gestation.[190] MR immunoreactivity is detectable in fetal kidney, skin, hair follicles, trachea and bronchioles, esophagus, stomach, small intestine, colon, and pancreatic exocrine ducts. The role of MRs in these fetal tissues remains unclear. MR knockout mice appear normal at birth but demonstrate defects in mineralocorticoid and renin-angiotensin system functions in the postnatal period.[191]

Angiotensin II concentrations in the sheep fetus are similar to maternal values, and blockade of fetal production with angiotensin-converting enzyme (ACE) inhibitors decreases the fetal glomerular filtration rate.[192] Both subtypes of angiotensin receptors, AT_1 and AT_2, are detectable in various tissues, including kidney, early in fetal development.[191] Hormonal factors modulate fetal renal AT gene expression in sheep: Angiotensin II suppresses both AT_1 and AT_2, and cortisol increases AT_1 gene expression in kidney and lungs.[193] The role of the fetal renin-angiotensin system is not clear; rather than modulating renal sodium excretion through aldosterone, it may maintain renal excretion of salt and water into amniotic fluid to prevent oligohydramnios.[192] The mechanism for the high aldosterone concentrations in the fetal and neonatal periods remains unclear.

Aldosterone affects renal sodium excretion in the fetal sheep and in premature infants.[189] Manifestations of mineralocorticoid deficiency in the newborn term infant can occur because of aldosterone deficiency or competition for binding to renal MRs by other steroids such as 17-hydroxyprogesterone. Relatively reduced glomerular filtration in the newborn limits sodium loss initially, but by 1 week of age, aldosterone deficiency produces the characteristic manifestations of hyponatremia, hyperkalemia, and volume depletion.

Hormonal Regulation of Adrenal Development

The hormonal regulation of adrenal development is not understood. A major stimulus to fetal adrenal function is fetal pituitary ACTH, a 39–amino acid peptide secreted from the anterior pituitary gland under control of CRH. ACTH binds to the transmembrane receptor melanocortin 2 receptor 2 (MC2R), specifically in adrenocortical cells, and exerts its effect through downstream cyclic adenosine monophosphate (cAMP) and Ras/MEK/ERK signaling pathways. The growth-promoting effects of ACTH are mediated in part through the stimulation of locally produced growth factors such as insulin-like growth factor 2 (IGF2) and fibroblast growth factor β (FGFβ).[163] During development, as the outer DZ emerges, ACTH participates in the regulation of steroidogenesis, cell differentiation, and cell growth.[163] While adrenocortical growth and differentiation are independent of ACTH during the first trimester of human pregnancy, ACTH begins to play an essential role in the morphologic and functional development of the adrenal gland after 15 weeks' gestation.[194]

In vivo ACTH stimulates steroid production by activating StAR and increasing delivery of substrate cholesterol to P450scc.[188] In vitro ACTH directly stimulates DHEAS and cortisol production, and in vivo excess ACTH is clearly involved in driving the high androgen concentrations seen in fetuses affected with CAH. The HPA axis clearly functions early in fetal life, but anencephalic fetuses, which lack pituitary ACTH, have adrenals that contain a fairly normal complement of steroidogenic enzymes and retain their capacity for steroidogenesis.[171] Furthermore, growth and rising steroid production by the fetal adrenal during pregnancy are not paralleled by increasing concentrations of fetal plasma ACTH. A possible explanation for this paradox is that there may be gestational age–dependent alterations in responsiveness of the fetal adrenal tissue to ACTH. Thus it appears that both ACTH-dependent and ACTH-independent mechanisms regulate fetal adrenal steroidogenesis.

The presence of all CRH peptides and their receptors in the adrenal suggests that the CRH system can function locally within human and rodent adrenals. An important source of CRH is the human placenta, which releases CRH into the fetal circulation. As gestation advances, CRH concentration increases. The remarkable increase in placental CRH production at the end of gestation has been suggested to contribute to the parturition process by forming a feed-forward loop that leads to increased production of cortisol and DHEA/DHEAS in human fetal adrenals. Studies have shown that CRH stimulates cortisol production in primary cultures of fetal adrenocortical cells by elevating the mRNA levels of steroidogenic acute regulatory protein and other steroidogenic enzymes, including *3βHSD2, CYP21A2,* and *CYP11B1*. In addition, CRH enhances the adrenal response to ACTH, further driving the production of cortisol and DHEA/DHEAS.[51] *CRH* gene knockout in mice leads to neonatal death due to pulmonary hypoplasia, suggesting that CRH-stimulated glucocorticoid production is essential for adrenergic chromaffin and normal lung development. Circulating CRH concentrations are elevated in the fetus, largely from extrahypothalamic and placental sources. The circulating concentrations of CRH increase 1000-fold as pregnancy progresses[45] and reach values of 0.5 to 1 nmol/L at term; normal values in nonpregnant women are lower than 0.01 nmol/L. At the end of pregnancy, there is increased bioavailability of CRH due to a fall in levels of its binding protein. This results in an exponential increase in maternal CRH concentrations from 35 weeks' gestation to term.[45,195] Postpartum, CRH normalizes to nonpregnant levels within 24 hours of delivery in keeping with the fact that the placenta is the primary source.[45,195] This placental CRH is bioactive, and concentrations correlate with maternal cortisol concentrations, suggesting that this circulating placental CRH plays a role in stimulating maternal corticotropin release. Midgestation fetal plasma corticotropin concentrations average about 55 pmol/L (250 pg/mL), levels that maximally stimulate fetal adrenal steroidogenesis, and concentrations are higher throughout gestation than in postnatal life, although they fall near term (see Fig. 23.7).[25,188]

The Fetal-Placental Unit

The paradox of human fetal adrenal function is that steroidogenesis is programmed by the steroidogenic enzyme expression pattern (e.g., relative 3βHSD deficiency) to produce inactive products (including DHEA and pregnenolone and their sulfate conjugates).[188] Much of the DHEA is converted to 16-hydroxy-DHEAS by the fetal adrenal and fetal liver. As previously discussed, this is designed to provide a substrate for placental estrone and estradiol production: 16-hydroxy-DHEA undergoes metabolism to estriol in the placenta. Fetal DHEAS production and maternal estriol concentrations increase progressively to term; DHEAS production approximates to 200 mg/day near term.[25] In pregnant baboons with placental estrogen production suppressed by administration of an aromatase inhibitor, the volume of the FZ of the fetal adrenal increased markedly.[196] This effect was reversed by administration of inhibitor plus estrogen, suggesting that estrogen selectively suppresses FZ growth and development during the second half of primate pregnancy. It is proposed that this represents a feedback system to regulate secretion of fetal adrenal DHEA, thereby maintaining normal fetal-placental function and development.[196]

Adrenal Insufficiency

Adrenal insufficiency is a rare condition that may occur secondary to ACTH deficiency or is primary resulting from adrenal failure. The mature ACTH peptide is cleaved from the larger precursor molecule, POMC, together with other small peptides such as β-endorphin and α-MSH and β-MSH. Defects in ACTH synthesis, processing, or release can lead to secondary adrenal hypoplasia resulting in neonatal hypoglycemia, prolonged jaundice, or collapse. Given that mineralocorticoid secretion is largely independent of ACTH secretion, it is preserved, and salt loss is therefore unusual. Nevertheless, mineralocorticoid deficiency can be an issue in some patients with ACTH insensitivity. Low serum concentrations of ACTH, absence of hyperpigmentation, and presence of associated features such as pale skin, red hair, diarrhea, and obesity (*POMC*/*PC1* mutations) are important diagnostic clues.

The presence of multiple pituitary hormone deficiencies (e.g., GH, ACTH, TSH, gonadotropins, vasopressin, and PRL) may point to a diagnosis of multiple pituitary hormone deficiency, often in association with structural abnormalities of the pituitary gland, eye abnormalities, and forebrain abnormalities (septo-optic dysplasia). Signs such as those of congenital hypothyroidism, hypoglycemia, congenital hypogonadotropic hypogonadism (micropenis, undescended testes), and severe postnatal growth failure may be suggestive of the diagnosis. A number of single-gene defects have now been associated with congenital hypopituitarism (e.g., mutations in *HESX1*, *SOX3*, *OTX2*, *GLI2*, *ARNT2*, *LHX3*, *LHX4,* and *PROP1*).[55,149,197] Occasionally ACTH insufficiency may not be present at the time of diagnosis but may develop progressively with time.

Recessive mutations in the T-box factor *TPIT (TBX19)* have been identified in patients with severe, early-onset isolated ACTH deficiency with profound hypoglycemia, prolonged jaundice, and sudden neonatal death.[118] *TPIT* is required for the specification, maturation, and maintenance of both precorticotroph and premelanotroph populations and for the suppression of gonadotroph fate. It is also required to activate the expression of POMC in conjunction with the transcription factor PTX1. Murine transgenesis resulted in ACTH and glucocorticoid deficiencies, adrenal hypoplasia, and pigmentation defects in mice deleted for *TPit.*[118] Sixty-five percent of patients with severe congenital isolated ACTH deficiency are found to have *TBX19* mutations, but mutations are not found in partial or later-onset ACTH deficiency.[198]

ACTH resistance can occur in a number of well-defined entities, such as defects in the ACTH receptor (MC2R), familial glucocorticoid deficiency (FGD) type 1 (FGD1), in MC2R accessory protein (MRAP, FGD2), or as part of the triple-A syndrome (alacrimia, achalasia, addisonism; also known as Allgrove syndrome, caused by defects in ALADIN/*AAAS*). These disorders are characterized by isolated glucocorticoid deficiency, hyperpigmentation, and markedly elevated concentrations of ACTH.[199,200] Nevertheless, approximately 15% of individuals with triple-A syndrome have evidence of mineralocorticoid insufficiency, those with the most severe loss of function manifesting hyponatremia on presentation. Recently, mutations in *NNT* (nicotinamide nucleotide transhydrogenase), *GPX1* (glutathione peroxidase 1), and *MCM4* (mini-chromosome maintenance-deficient-4 homologue) have been found as a cause of FGD.[201–203] Mutations in *MCM4* were found in an Irish Traveller community and result in late-onset, less severe glucocorticoid deficiency, short stature, and natural killer cell deficiency due to increased chromosomal breakage. Mutations in *GPX1* and *NNT*, involved in detoxification of reactive oxygen species, can also result in FGD.[204]

Primary adrenal failure may be caused by congenital adrenal hypoplasia (or AHC).[205] This results in severe salt-losing primary adrenal failure in early infancy or childhood, although milder, delayed-onset forms of the condition exist. The most common form of the condition is X-linked. Patients have mutations in the nuclear receptor DAX1 *(NROB1)*; in addition to adrenal failure, the males suffer from hypogonadotropic hypogonadism. Rarely, patients present with isolated mineralocorticoid deficiency with normal cortisol concentrations; however, glucocorticoid deficiency usually develops later. *DAX1* is expressed in ES cells, steroidogenic tissues (gonads and adrenals), the ventromedial hypothalamus (VMH), and pituitary gonadotrophs. It acts as a transcriptional repressor of other nuclear receptor pathways but is also involved in maintenance of pluripotency of stem cells.[206]

Heterozygous and homozygous mutations in SF1 have been associated with adrenal failure in 46,XY phenotypic females, as well as in at least one 46,XX girl, although the latter phenotype is rare.[205] Mutations in SF1 have also been associated with gonadal dysgenesis in 46,XY individuals in the absence of adrenal insufficiency.[207] Additionally, SF1 mutations have been associated with primary ovarian failure, but this is rare.[208,209]

Various forms of congenital adrenal hyperplasia may be associated with variable adrenal failure (e.g., mutations in *CYP11A1*, *StAR*, *HSD3B2*, *CYP17*, *CYP21A2*, *CYP11B1*) with varying degrees of genital ambiguity. The enzyme P450c11 (aldosterone synthase), which is found in the ZG, has 11β-hydroxylase, 18-hydroxylase, and 18-methyloxidase activities and catalyzes all the reactions needed to convert 11-deoxycorticosterone (DOC) to aldosterone. Mutations in the gene encoding the enzyme are associated with isolated mineralocorticoid deficiency. Functional mineralocorticoid deficiency with severe salt loss resulting in hyponatremia and hyperkalemia may also arise as a result of mutations in the *MR* or in the gene encoding the epithelial sodium channel (ENaC).

Primary adrenal insufficiency due to adrenal hypoplasia can be classified into nonsyndromic and syndromic (triple-A syndrome, IMAGe syndrome, Irish Travellers syndrome) types, and up to 30% of patients have no cause identified. Whole exome sequencing of patients with syndromic primary adrenal insufficiency and a striking phenotype of a multisystem disorder, including myelodysplasia, infection, growth restriction, adrenal hypoplasia, genital anomalies, and enteropathy, revealed mutations in the *SAMD9* gene (MIRAGE syndrome).[210,211] A diagnosis of adrenal insufficiency must be prompt in view of the potential life-threatening consequences of glucocorticoid and mineralocorticoid deficiencies to the infant.

Thyroid Development

Embryology

The thyroid gland, one of the first endocrine organs to develop, is derived from contributions of two anlagen: By 22 dpc a midline thickening of the pharyngeal floor (median anlage) forms as the precursor of the T_4-producing follicular cells. Paired ultimobranchial bodies originating from the fourth pharyngobranchial pouches (lateral anlagen) give rise to the parafollicular calcitonin-secreting (C) cells.[21]

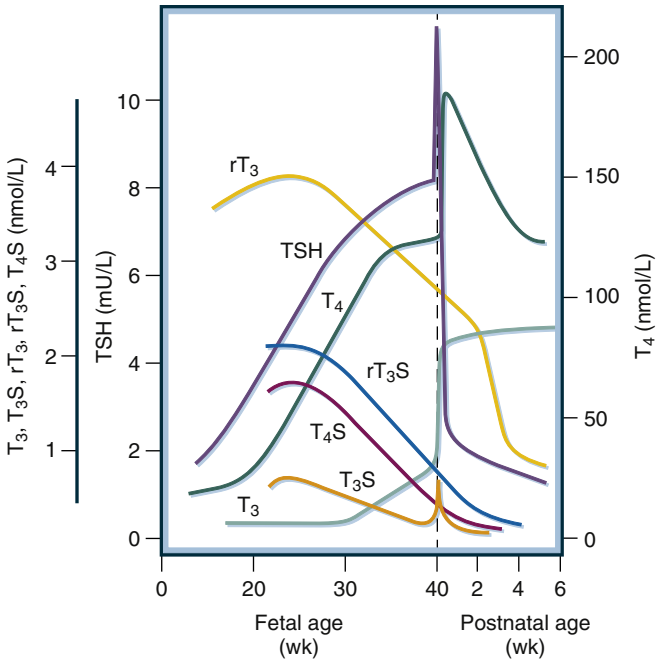
The thyroid precursor cells migrate caudally between 28 and 48 dpc, proliferate, and expand laterally, a process known as lobulation. The lateral and median anlage fuse at around 44 dpc, and during descent the developing thyroid retains an attachment to the pharynx by an epithelial stalk known as the thyroglossal duct.[212] By 37 dpc this structure that connects the median anlage with the floor of the buccal cavity (a point later known as the foramen cecum on the developing tongue) has usually disappeared and the only remnant of the duct is the foramen cecum itself.[212] An ectopic thyroid and a persistent thyroglossal duct or cyst may occur because of abnormal thyroid descent.

By 51 dpc, the gland comprises two lateral lobes with a connecting isthmus, and it reaches its final position below the thyroid cartilage by 9 wpc. At 10 wpc, the fetal thyroid gland weighs about 80 mg and at term 1 to 1.5 g. Terminal differentiation begins at around 60 dpc, only after migration is complete. Differentiation is evidenced by expression of the genes encoding the TSH receptor *(TSHR)*, the sodium-iodide symporter *(NIS)*, thyroglobulin *(Tg)*, and thyroperoxidase *(TPO)*, and results in formation of follicles and functional capacity.[213] Production of thyroid hormones is stimulated by TSH and requires an adequate supply of iodine. By 70 dpc colloid is visible histologically and the thyroid gland expresses *Slc5a5*, encoding the 13-transmembrane domain glycoprotein NIS, enabling uptake of iodine and synthesis of thyroid hormones. Thyroid hormones can be detected in the thyroid from 9 wpc and in fetal blood from 10 wpc.[214–217]

Thyroid Hormone Biosynthesis

Pituitary and plasma thyrotropin (TSH) concentrations begin to increase during the second trimester in the human fetus, at about the time that pituitary portal vascular continuity develops (Fig. 23.8).

In the rat, expression of the *Tshr* gene is significantly upregulated on fetal day 17, and this is accompanied by significant



• **Fig. 23.8** Patterns of change of fetal plasma thyroid-stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3), reverse T_3 (rT_3), and iodothyronine sulfates (T_4S, rT_3S, and T_3S) during gestation and in the neonatal period. The patterns for T_4S and rT_3S are based on limited 30-week data. (Data from Roti E. Regulation of thyroid stimulating hormone [TSH] secretion in the fetus and neonate. *J Endocrinol Invest*. 1988;11:145–158; Fisher DA, Klein AH. Thyroid development and disorders of thyroid function in the newborn. *N Engl J Med*. 1981;304:702–712; Santini F, Chiovato L, Ghirri P, et al. Serum iodothyronines in the human fetus and the newborn: evidence for an important role of placenta in fetal thyroid hormone homeostasis. *J Clin Endocrinol Metab*. 1999;84:493–498.)

growth and rapid development in terms of structure and function. The expression of *Tg* and *TPO* is increased, and thyroid follicles are seen with thyroid hormonogenesis, suggesting that the TSHR has an important role to play in these events. Murine mutation of the *Tshr* gene is associated with the *hyt/hyt* phenotype, which exhibits severe hypothyroidism and a hypoplastic but normally located thyroid gland with a poorly developed follicular structure. In humans, a similar phenotype is observed in babies of mothers with potent TSHR-blocking antibodies and in babies with severe loss-of-function mutations in *TSHR*.

Although onset of fetal thyroid hormone synthesis starts at the end of the first trimester, levels are low and necessitate a high dependency on maternal thyroid hormones during pregnancy. Overt maternal hypothyroidism has been shown to be associated adversely with pregnancy outcomes, including miscarriage and premature delivery, as well as adverse perinatal and neurocognitive outcomes in offspring. The effects of subclinical maternal hypothyroidism or hypothyroxinemia are less clear. Using a zebrafish model, the ontogeny of the hypothalamic-pituitary-thyroid axis and its negative-feedback regulation by thyroid hormones has been characterized. Embryonic exposure to excess thyroid hormone during a developmental time window resulted in long-term dysregulation of pituitary function by apoptosis of developing thyrotrope cells.[218] These findings are relevant in the context of maternal hyperthyroidism due to maternal Graves or overreplacement of pregnant hypothyroid women in the first trimester with levothyroxine.

Plasma thyrotropin concentrations increase progressively during the last half of gestation. Plasma concentrations of T_4-binding globulin and total T_4 increase progressively, from low levels at 14 to 16 wpc to maximal levels at term. Free T_4 concentrations increase with the increase in T_4 production. The increases in plasma TSH and T_4 concentrations during the third trimester reflect progressive maturation of hypothalamic pituitary control and of thyroid gland responsiveness to TSH. Pituitary TSH secretion is responsive to hypothyroxinemia and to TRH early in the third trimester.[215] The period of parallel increases in fetal TSH and free T_4 concentrations during the latter half of gestation is followed by sequential surges of TSH and free T_4 in the early neonatal period and a final slow equilibration of the TSH/free T_4 ratio to adult values during infancy and childhood.[219–222] This sequence includes coordinated maturation of hypothalamic TRH secretion, pituitary TRH sensitivity, thyrotropin negative-feedback control, and thyroid follicular cell responsiveness to TSH. Fetal serum TRH concentrations are higher than in maternal blood, which is the consequence of extrahypothalamic (placenta and pancreas) TRH production and decreased TRH degradation in fetal serum. Functionally, the fetus progresses from a state of both primary (thyroidal) and tertiary (hypothalamic) hypothyroidism at midgestation, through a state of mild tertiary hypothyroidism during the final weeks in utero, to a fully mature hypothalamic-pituitary-thyroid axis by 2 months after birth.

The adult thyroid follicular cell can modify iodine transport or uptake with changes in dietary iodine intake, independent of variations in serum TSH concentrations.[223,224] Before 36 to 40 weeks' gestation, the thyroid gland lacks this autoregulatory mechanism and is susceptible to iodine-induced inhibition of thyroid hormone synthesis.[224,225] The fetal thyroid follicular cell, when exposed to high circulating levels of iodide, is unable to reduce iodide trapping and prevent the high intracellular iodide concentrations that produce the blockade of hormone synthesis referred to as the *Wolff-Chaikoff effect*. Failure of the immature thyroid to exhibit autoregulation is probably due to failure of downregulation of thyroid cell membrane NIS units, which may be related to the absence or reduced iodination of an 8-kDa to 10-kDa protein in the thyroid follicular cell.[223,224] In addition to maturation of autoregulation, thyroidal responsiveness to TSH increases during the last trimester.[21]

The metabolism of thyroid hormones occurs through a progressive series of monodeiodinations.[219,226] Three deiodinase enzymes act to remove an iodine atom from the outer (phenolic) ring or the inner (tyrosyl) ring of the tetraiodothyronine (T_4) molecule thus, respectively, activating or inactivating the hormone. The deiodinases are encoded by separate genes and share sequence homology. Most of the circulating biologically active T_3 in adults is derived by outer-ring monodeiodination of T_4 in liver and other nonthyroidal tissues; biologically inactive rT_3 derives from inner-ring deiodination of T_4 in peripheral tissues. The type I enzyme (D1), an outer-ring monodeiodinase, is a high–Michaelis constant (K_m) enzyme inhibited by propylthiouracil (PTU) and stimulated by thyroid hormone. It deiodinates T_4 to T_3 and rT_3 to T_2. D1 also has inner-ring deiodinase activity, converting T_3 to T_2. Activity of D1 is low throughout gestation. The type 2 outer-ring monodeiodinase (D2) is a low-K_m enzyme that is insensitive to PTU and inhibited by thyroid hormone. It deiodinates T_4 to T_3 and rT_3 to T_2, and it is highly expressed in brain and pituitary. Type 3 monodeiodinase (D3) inactivates T_4 and T_3 via inner-ring deiodination of T_4 to rT_3 and of T_3 to T_2; it is highly expressed in fetal tissues and placenta. D1 is largely responsible

TABLE 23.3 Deiodinase Expression in Human and Rodent Tissues

Tissue	D1	D2	D3
Brain	X	X	X
Pituitary	X	X	
Thyroid	X	X[a]	
Liver	X		X[b]
Kidney	X		
Ovary	X		X
Ear		X[a]	
Heart		X[a]	
Muscle		X[b]	
Skin		X	X
Testes		X	X
Uterus		X	X
Brown fat		X	

[a]Expressed in human only.
[b]Expressed only in fetus.
Modified from St. Germain DL, Hernandez A, Schneider MJ, et al. Insights into the role of deiodinases from studies of genetically modified animals. *Thyroid.* 2005;15:905–916.

for the production of T_3 that escapes from the cells, especially liver and kidney, into the circulation; D2 is responsible for the production of local tissue T_3. Inactive rT_3 also diffuses out of most tissues to appear in plasma.

Iodothyronine deiodinases belong to a family of selenoproteins. Selenium is an essential trace element required for the biosynthesis of selenoproteins. Selenocysteine insertion sequence (SECIS) binding protein 2 (SBP2) represents a key factor for the insertion of selenocysteine into selenoproteins. Recently, mutations in the *SBP2* gene have been described that lead to a multisystem disorder with deficiencies of multiple selenoproteins, resulting in growth retardation, myopathy and skin photosensitivity, nonketotic hypoglycemia, colitis, and infertility.[227,228] Due to abnormal D2 activity, T_3 is low, T_4 and rT_3 are increased, and TSH is slightly elevated. As selenoproteins also serve as antioxidants, tissue damage may be related to increased concentrations of reactive oxygen species (ROS).[228,229]

The distribution of the deiodinases has been characterized in rodent and human tissues (Table 23.3).[230] D2 is detectable by midgestation and plays an important role in supplying T_3 to developing brain tissue, regulating thermogenesis in brown adipose tissue in the neonatal period, and regulating pituitary TSH secretion. D3 activity is present in placenta, liver, and perhaps fetal skin, accounting for the higher concentrations of rT_3 in the fetus and limiting the metabolic effects of thyroid hormones during much of fetal life. There is little conversion of T_4 to circulating T_3 via D1 deiodination until midgestation in the human fetus; plasma T_3 concentrations are low (<0.2 nmol/L or <15 ng/dL) until 30 weeks' gestation, after which the mean value increases to 0.7 nmol/L (50 ng/dL) at term (see Fig. 23.8).[231] On the other hand, fetal brain T_3 concentrations are 60% to 80% of those in the adult by fetal age 20 to 26 weeks owing to D2 activity. In the presence of fetal hypothyroidism, D2 increases while D3 decreases in an effort to maintain near-normal brain T_3 concentrations.

Sulfation is active in fetal tissues, and the predominant thyroid hormone metabolites in the fetus are iodothyronine sulfates.[219,232,233] High levels of phenolsulfotransferases (SULT) have been characterized in fetal liver, lung, and brain by midgestation. SULT activities decrease rapidly in the neonatal period.[233] In the last third of gestation in fetal sheep, the mean plasma production rates for T_4 and metabolites (in micrograms per kilogram of body weight per day) are as follows: T_4, 40; T_4 sulfate (T_4S), 10; rT_3, 5; rT_3S, 12; T_3, 2; and T_3S, 2. All metabolites are biologically inactive except T_3 and perhaps T_3S, so 90% of the T_4 metabolites in the fetus are biologically inactive.[232] The sulfated metabolites accumulate in fetal serum as a result of the low D1 activity in fetal tissues and because the sulfated iodothyronines are not substrates for D3.[223,234] The production rate of T_3 increases progressively between 30 weeks' gestation and term because of maturation of D1 activity in the liver and other tissues and because of decreasing D3 activity in placenta.[21,233] In fetal sheep, hepatic D1 activity increases progressively during the last trimester.[235]

It has long been assumed that thyroid hormones passively diffuse into cells. However, several classes of cell membrane iodothyronine transporters have been described, questioning this hypothesis.[236–238] These transporters belong to different families of organic anion, amino acid, and monocarboxylate solute carriers, including the organic anion transporting polypeptide (OATP) family and the solute carrier family 21 (SLC21).[236–238] The significance of these transporters is not yet clear, but mutation of the human monocarboxylate transporter 8 (MCT8), a member of the SLC21 family shown to be a specific thyroid hormone transporter present in developing brain, leads to a syndrome of combined thyroid dysfunction and psychomotor retardation (X-linked Allan-Herndon-Dudley syndrome).[239,240] *Mct8* expression in neonatal mice has been localized to neurons in the olfactory bulb, cerebral cortex, hippocampus, and amygdala. Presumably, all thyroid hormone–sensitive cell populations express iodothyronine membrane transporters. A cell surface T_4 receptor has been characterized as an $\alpha_V\beta_3$ integrin that serves as the initiation site for T_4-induced activation of the mitogen-activated protein kinase (MAPK) pathway for angiogenesis and perhaps actin polymerization and neuronal migration.[241] The ontogenesis and significance of these cell surface receptors and membrane transporters in fetal development remain to be further defined. Recent studies have suggested that MCT8 is also required for normal thyroid hormone secretion, in addition to its role as a thyroid hormone transporter.[242] Brain damage in human fetuses is already induced in utero.[276] Treatment options for patients with MCT8 deficiency are limited. PTU blocks thyroid hormone production and inhibits conversion of T_4 to T_3. PTU, in combination with levothyroxine, has shown limited metabolic benefits but no effect on neurologic features. To date, two thyromimetics, diiodothyropropionic acid (DITPA) and triiodothyroacetic acid (TRIAC), are under investigation.[277,278] DITPA has been used in a small number of patients from age 9 months; it has been shown that DITPA crosses the placenta and reaches neuronal target cells in mice, inducing a T_3-like effect.[277,278] DITPA may therefore be a future candidate for in utero treatment of MCT8 deficiency.

Thyroid Hormone Action

Classic thyroid hormone actions are mediated via functional thyroid hormone nuclear receptors (TRs), members of the steroid/retinoid/vitamin D family of nuclear transcription factors. Two genes code for the receptors: *THRA* on chromosome 17 encodes

TABLE 23.4 Predominant Thyroid Hormone Receptor Subtype Functions in Developing Mice

Brain	Thermogenesis
TRα1, TRβ1	TRα1, TRβ1, TRβ2
Pituitary TSH secretion	Inner ear
	TRα1, TRβ2
	TRβ2, TRα1
Pituitary GH secretion	Retina
	TRβ2
	TRα1
Bone maturation	Intestine
TRα1	TRα1
Liver	Heart
TRβ1	TRα1

GH, Growth hormone; *TR,* thyroid hormone receptor; *TSH,* thyroid-stimulating hormone.
Modified from Yen P. Genomic and nongenomic actions of thyroid hormones. In: Braverman LE, Utiger RD, eds. *The Thyroid.* 9th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2005:135–150; Flamant F, Samarut J. Thyroid hormone receptors: lessons from knockout and knockin mutant mice. *Trends Endocrinol Metab.* 2005;14:85–90; Ortiga-Carvalho TM, Sidhaye AR, Wondisford FE. Thyroid hormone receptors and resistance to thyroid hormone disorders. *Nat Rev Endocrinol.* 2014;10:582–591.

TRα, and *THRB* on chromosome 3 encodes TRβ.[243] These genes code for four classic receptor isoforms (TRα1, TRα2, TRβ1, and TRβ2), three of which bind thyroid hormones (T_3/T_4 affinity 10:1) and bind to DNA to effect gene transcription. The TRα2 isoform does not bind thyroid hormone but binds to DNA and can inhibit binding of other TRs. The TRs exist as monomers, homodimers, and heterodimers with other nuclear receptor family members such as retinoid X (RXR). Other TR transcripts, including TRΔα1 and TRΔα2, have been characterized; they do not bind DNA or T_3 but can inhibit TR and retinoid receptor activities.[244]

The TRs are expressed developmentally and differentially in various fetal and adult tissues. TRα proteins are present in most tissues. TRβ1 is expressed in liver, kidney, and lung and in developing brain, cochlea, and pituitary. TRβ2 expression is restricted largely to the pituitary gland, retina, and cochlea.[21,243] The receptors function redundantly, as indicated by knockout studies in mice, but predominant effects of one or another TR have been characterized (Table 23.4). Knockout of both the TRα and TRβ genes in mice is not lethal but results in elevated TSH concentrations, deafness, bradycardia, and decreased postnatal growth with delayed bone maturation.[21,243] Lethality occurs because of improper intestinal development associated with persistent TRΔα isoforms in TRα or combined TRα and TRβ knockout mice.[244] In the fetal rat brain, TRα1 mRNA and receptor binding are detectable by 12 to 14 days of gestation (term is 21 days), increasing to maximal levels at birth. The TRβ1 isoform is detected at birth and increases approximately 40-fold in the early postnatal period.[20,21] In human fetal brain, TRα1 and TRβ1 isoforms and receptor binding are present by 8 to 10 weeks' gestation; TRα1 transcripts and receptor occupancy increase 8-fold to 10-fold by 16 to 18 weeks.[21,245,246] Liver, heart, and lung receptor binding can be identified by 13 to 18 weeks.[21,246,247]

Heterozygous missense mutations in *THRA,* coding for the TRα1, have recently been described in association with short stature, developmental delay, and chronic constipation. The mutations act in a dominant negative fashion. The clinical phenotype is in line with the TRα1 being the predominant subtype receptor in bone, the gastrointestinal tract, cardiac and skeletal muscle, and the CNS.[248,249]

Ontogeny of Thyroid Hormone Secretion

The role of maternal thyroid hormone during fetal development remains controversial. The high placental concentration of D3 inactivates most of the thyroid hormone presented from the maternal circulation. The iodide released in this manner is used for fetal thyroid hormone synthesis. Despite the limited maternal-fetal placental transfer of T_4 and the predominant production of inactive thyroid hormone metabolites in the human fetus, significant levels of free T_4 are present in fetal fluids, from placental transfer early in gestation and from fetal thyroid production during later gestation.[20,21] Early in gestation, placental transfer is the only source of T_4 in fetal fluids and is essential for normal fetal neurodevelopment. T_4 is detectable in human coelomic fluid at levels of 0.5 to 2 nmol/L between 6 and 11 weeks' gestation, before the onset of fetal thyroid function.[19] Low concentrations of T_4 are detectable in the fetal brain at about 10 weeks of gestation. Significant placental transfer continues to term, when serum T_4 levels in the athyroid fetus range from 30 to 70 nmol/L (2.3–5.4 μg/dL).[250] Isotopic equilibrium studies with pregnant rats at term suggest that 15% to 20% of the T_4 in fetal tissues is of maternal origin.[251] As indicated, thyroid hormones cross the placenta early in gestation, supplying the low levels of free T_4 that are essential for brain development between 12 and 20 weeks, before the onset of fetal thyroid hormone production.[20] Most thyroid hormone in the fetal compartment is inactivated to sulfated and deiodinated analogues until the perinatal period.[219,232] This neutralization of active circulating thyroid hormone maintains the low T_3 metabolic state, facilitating fetal growth and programmed tissue maturation.

Thyroid hormone–programmed development of selective fetal tissues requires the interaction of local tissue D1, D2, thyroid receptors, receptor coactivators, and thyroid-responsive genes. In most responsive tissues, the timing of maturation events is controlled by the state of the thyroid receptors acting as a molecular switch.[244,252] In the absence of T_3, the unliganded receptor (aporeceptor) recruits corepressors, repressing gene transcription. Non–T_3-binding receptors also can repress transcription by inhibiting receptor DNA binding. Local tissue maturation events are initiated by the coincident availability of T_3, liganded T_3 receptor, T_3-mediated receptor exchange of corepressor with coactivators for creation of an active holoreceptor, and activation of responsive gene transcription.

These programming events have been investigated in studies of transgenic mice, including brain, liver, heart, intestine, and bone tissues; thermogenesis; and spleen erythropoiesis.[239,240,252–256] The timing of these events in the mouse ranges from early midbrain neuronal development at gestational day 15; through perinatal activation of hepatic enzymes, cardiac ion channels, and spleen erythropoiesis; to postnatal brain, intestinal, and bone maturation and thermogenesis. Parturition occurs at a gestational age equivalent to human midgestation. In hypothyroid mice, repressive effects of aporeceptors have been shown to delay tissue maturation in brain, bone, intestine, spleen, and heart.[240] The increase in circulating T_3 levels associated with parturition in mice and humans

normally triggers development of tissue functions essential to postnatal metabolism and homeostasis (e.g., hepatic, intestinal, and cardiac functions and brown fat thermogenesis). Thyroid hormone–stimulated maturation of vision and hearing appear to be triggered by the local expression of D2, mediating local T_3 production, postnatally in the mouse and probably toward the end of the second midtrimester in the human fetus.

In humans, T_3-mediated maturation of fetal tissues, including liver, heart, brown adipose tissue, and bone, allows them to become thyroid hormone responsive during late gestation and in the perinatal period. Paracrine actions of thyroid hormone are critical for normal fetal development (e.g., in the cochlea, where D2 is expressed in connective tissue immediately adjacent to the sensory epithelium, and in spiral ganglion, where thyroid hormone receptors are located). This implies that D2-containing cells in the connective tissue take up T_4 from the circulation, convert it to T_3, and then release D3 to adjacent responsive cells. Similarly, in the brain, D2 is expressed predominantly in glial cells, whereas TRs are expressed in adjacent neurons and oligodendrocytes. In other areas of the brain, such as the pituitary gland, hippocampus, and caudate nucleus, there is coexpression of D2 and TRs. On the other hand, D3 is coexpressed with TRs in neurons, thereby protecting sensitive tissues from the effects of excess thyroid hormone.

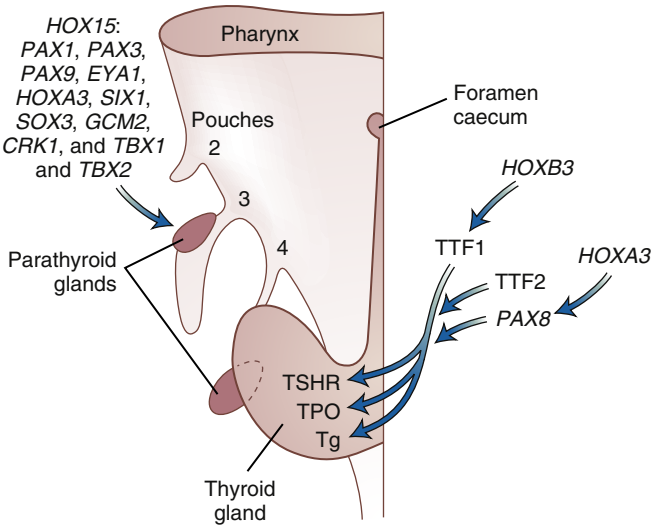
The actions of thyroid hormones and their developmental regulation in the brain are complex. Functionally, thyroid hormones are critical for the establishment of neural circuits during a critical window of brain development. They provide inductive cues for the differentiation and maturation of a number of processes such as neurogenesis and neural cell migration (occurring between 5 and 24 weeks of gestation), neuronal differentiation, dendritic and axonal growth, synaptogenesis, gliogenesis (late fetal period to 6 months postpartum), myelination (second trimester to 24 months postpartum), and neurotransmitter enzyme synthesis. TRs are found in highest concentration in developing neurons and in multiple areas of the fetal brain, including the cerebrum, the cerebellum, and the auditory and visual cortices. The hormones bind to receptors and stimulate a number of genes such as myelin, neurotropins and their receptors, cytoskeletal components, transcription factors, extracellular matrix proteins and adhesion molecules, intracellular signaling molecules, and mitochondrial and cerebellar genes.

Thyroid hormone is also important for normal bone growth. T_3 regulates endochondral ossification and controls chondrocyte differentiation in the growth plate both in vivo and in vitro. TRs are expressed on osteoblasts and growth plate chondrocytes, and T_3 target genes have been identified in bone. T_3 stimulates skull suture closure in vivo.[257]

In the perinatal period, thyroid hormone stimulates the transcription of thermogenin (also known as uncoupling protein 1 [UCP1]), a protein that uncouples nucleotide phosphorylation and the storage of energy as adenosine triphosphate (ATP), actions that are important for nonshivering thermogenesis by brown adipose tissue.

Genetic Regulation of Thyroid Development

At least five developmental genes are involved in thyroid and parathyroid gland embryogenesis. These include the genes for thyroid transcription factors *Pax8* (paired box 8), *Nkx2-1* (NK2 homeobox 1, previously known as TTF1), *Hhex* (hemopoetically expressed homeobox), *Foxe1* (forkhead box E1, previously known as TTF2), and *Nkx2-5* (NK2 homeobox 5) (Fig. 23.9).



• **Fig. 23.9** Homeobox genes that program development of the thyroid and parathyroid glands. *HEX* is involved early in the integrated cascade that programs thyroid gland embryogenesis. *HOXB3* and *HOXA3* may be responsible for activation of thyroid transcription factors TTF1 and TTF2, respectively, during early embryogenesis. *PAX8* is essential in the cascade. These factors are also involved in thyroid follicular cell function, promoting thyroglobulin (Tg), thyroid peroxidase (TPO), and thyroid-stimulating hormone receptor (TSHR) gene transcription. *HOX15* gene knockout in mice causes parathyroid gland aplasia. See text for details.

As well as mediating the formation of the thyroid bud, these transcription factors drive functional differentiation, regulate the expression of genes involved in thyroid hormone biosynthesis, and play a role in the maintenance of the mature thyroid gland (see Chapter 11). Mutations in *NKX2-1, PAX8,* and *FOXE1* are all associated with thyroid dysgenesis in humans.[215,217,258–260]

Putative Contributing Mechanisms to Thyroid Morphogenesis

Animal studies in mouse and zebrafish suggest that extrinsic factors such as permissive signals from blood vessels provide guidance cues in thyroid morphogenesis.[261] Genes known to contribute to thyroid morphogenesis in animal models include the Notch ligand *JAG1,* the thyroid and cardiac transcription factor *Nkx2-5,* and *NTN1,* which is implicated in zebrafish aortic arch artery formation and thyroid morphogenesis.[262–265] Other autonomous factors that may play a role include *Tbx,*[266] a member of the T-box family of transcription factors and the prime candidate gene missing in DiGeorge syndrome. In mice, *Tbx1* is expressed in both the pharyngeal endoderm and subpharyngeal mesoderm but only its mesodermal activity promotes the generation of *Nkx2-1*+ progenitors in the thyroid placode.[267] The effect of *Tbx1* on the embryonic thyroid is mediated by *Fgf8* that is also produced in the mesoderm.[267] It is known that signaling molecules such as those in the Shh pathway are also implicated in murine thyroid development.[261] In late organogenesis, *Shh* appears to have an important role in the symmetric bilocation of the thyroid; it also suppresses the ectopic expression of thyroid follicular cells.[268]

Thyroid Function in Preterm Infants

Compared to infants born at term, neonatal free T_4 increments are reduced in premature infants born at 31 to 34 weeks of gestation, attenuated at 28 to 30 weeks, and absent in infants 23 to 27 weeks.[269] This reflects hypothalamic-pituitary-thyroid system

immaturity inversely related to gestational age.[220] Age-specific changes in thyroid function in children with small for gestational age (SGA) and normal birth weight from birth to adulthood are available.[270] Premature birth interrupts the changes in thyroid hormone metabolism that occur over the third trimester resulting in low T_4 and T_3 concentrations that increase in proportion to increasing gestational age at delivery in preterm infants.[269] The thyroid hormone concentrations appear to be even lower than those expected in utero[271]; the reasons for this are multifactorial, including loss of maternal fT_4 in association with an immature TSH-T_4 axis, low iodide stores, and reduced deiodinase 1 (DIO1) activity.

A natural postnatal nadir in thyroxine concentration occurs at 7 days of life with a subsequent rise thereafter.[220,269,272] Several patterns of thyroid dysfunction can occur in preterm neonates[226,269,273]:

1. Transient hypothyroxinemia of prematurity—identified by a low free T_4 and normal TSH and seen in 50% of preterms born less than 28 weeks—does not require treatment. Maturation of the hypothalamic pituitary axis is likely to be delayed in prematurity and, although the postnatal TSH surge is seen at all gestational ages, it appears to be attenuated in the most premature infants.
2. Primary hypothyroidism—very low birth weight (VLBW) infants have a higher risk of primary hypothyroidism and can exhibit a pattern of delayed TSH rise, although in a considerable number of these infants the hypothyroidism is transient. Thyroid hormone treatment should be initiated and continue at least until thyroid hormone–dependent brain maturation has completed at approximately 3 years of age. At this stage thyroid function can be reassessed.
3. Hypothyroidism due to iodine excess—preterm infants are at risk from iodine-containing antiseptics and contrast agents.
4. Hypothyroidism due to iodine deficiency—preterm infants are at risk of iodine deficiency as they have low iodine stores (usually established in the third trimester), and enteral and parenteral nutrition contain little iodine.
5. Thyroid dysfunction due to nonthyroidal illness—often with low T_4, T_3, and TSH. Treatment is not indicated, but repeat testing every 1 to 2 weeks is recommended.

Congenital Hypothyroidism

Classic signs of congenital hypothyroidism (jaundice, lethargy, feeding difficulties, macroglossia, myxedema, hypothermia, growth retardation and progressive developmental delay, and intelligence quotient [IQ] deterioration) appear during the initial critical weeks and months of extrauterine life as maternal T_4 becomes unavailable and the non-CNS tissues become responsive to thyroid hormone.[21,219] Rarely, hypothyroidism is associated with respiratory distress in the neonatal period. The period of brain dependency for thyroid hormone extends up to 3 years of age, and most countries have a rigorous screening program to ensure early diagnosis and treatment.[280] Most cases of permanent primary congenital hypothyroidism are due to thyroid dysgenesis. Mutations in known genes account for less than 5% of patients with congenital hypothyroidism and thyroid dysgenesis. Carré and colleagues recently described the impact of *BOREALIN* mutations as a genetic cause of thyroid dysgenesis in humans. *BOREALIN* expression in human embryonic thyrocytes was shown before and after the onset of thyroid hormone synthesis, and *BOREALIN* is involved in the adhesion and migration of the thyrocytes, in keeping with a phenotype of thyroid ectopy.[281]

Lower neonatal screening thresholds have increased the incidence of congenital hypothyroidism with a normally located gland in situ, and recent studies reveal that a significant proportion of patients have multiple gene mutations in more than one thyroid-specific gene, supporting the hypothesis of an oligogenic etiology of congenital hypothyroidism.[282] Known causative genes in congenital hypothyroidism with gland in situ include *TG, TPO, DUOX2, DUOXA2, SLC5A5, SLC26A4, IYD,* and *TSHR,* and the molecular spectrum is increasing.

In congenital hypothyroidism there is an increased net flux of maternal thyroid hormone to the fetus, resulting in cord T_4 concentrations that are 25% to 50% of normal. There is increasing evidence of maternal-fetal T_4 transfer in the first half of pregnancy, when fetal thyroid hormone levels are low.[283] The transplacental passage of thyroid hormone, in conjunction with adjustments in brain deiodinase activity, has a critical role in minimizing the adverse effects of fetal hypothyroidism and helps explain the normal or near-normal outcome of hypothyroid fetuses (provided that prompt and adequate treatment of hypothyroidism ensues postnatally) as well as the relatively normal clinical appearance of the majority of babies with congenital hypothyroidism at birth. On the other hand, in the presence of both maternal and fetal hypothyroidism—such as that observed in the presence of potent TSHR-blocking antibodies, maternal and fetal POU1F1 deficiency, and severe iodine deficiency—there is severe neurocognitive impairment despite early and adequate commencement of thyroid replacement. Importantly, the presence of maternal hypothyroxinemia or inadequately controlled hypothyroidism is also associated with significant neurocognitive deficit in the offspring that is not reversible by early postnatal therapy.[284] Table 23.5 shows mechanisms resulting in congenital hypothyroidism due to abnormal thyroid development, dyshormonogenesis, abnormal thyroid hormone transport or action, and genes involved in these mechanisms.[285] International guidelines for treatment of congenital hypothyroidism are available.[286]

Gonadal Development

Sex determination is the result of a series of molecular events that direct the undifferentiated bipotential gonad to become a testis or ovary. Sex differentiation takes place once sex determination has occurred via factors produced by the gonads that determine the development of the phenotypic sex. The work of Alfred Jost, elucidating the pathophysiology of an experiment of nature, the freemartin calf, was seminal in the field of fetal sex development.[287]

The freemartin phenotype appears in a dizygotic twin pregnancy, where a genetically female fetus is masculinized in the presence of a male twin. The phenomenon of freemartinism is a disorder of sex development, explained by the endocrine effects of testosterone and antimüllerian factor from the male calf on the genital development of the female, made possible by the placental vascular anastomoses in cattle resulting in conjoined circulations of the twin calves. Subsequent advances in uncovering the genetic control and the pathophysiology of sex development have revealed that there is potential for disruption of the sex differentiation process at different stages, and clinical phenotype will depend on the nature of the disruption

Embryology

The mammalian gonad originates from the intermediate mesoderm, from which the bipotential urogenital ridge differentiates. Several genes (including those encoding SF1, WT1, EMX2,

TABLE 23.5 Mechanisms Involved in Congenital Hypothyroidism

Thyroid Dysgenesis (1 in 4500)

Isolated thyroid aplasia, hemiagenesis, hypoplasia, or ectopy
- Transcription factor defect *(PAX8)*
- Unknown[a]

Associated with other developmental abnormalities
- Transcription factor defect (TTF1, FOXE1 [TTF2], NKX2-5, SHH, Tbx1)

Inborn Errors of Thyroid Hormonogenesis (1 in 35,000)

- Abnormal iodide uptake via Na-I transporter *(NIS, SLC5A5)*
- Abnormal concentration of iodine
- Abnormal organification of iodine
- Abnormal iodination of thyroglobulin catalyzed by thyroid peroxidase *(TPO)*
- Abnormal H_2O_2 generation *(THOX, DUOX2, DUOXA2)*
- Efflux of iodide into colloid via apical anion channel (Pendred syndrome, *SLC26A4)*
- Defective thyroglobulin synthesis or transport
- Abnormal iodotyrosine deiodinase *(DEHAL1)*
- Abnormal thyroid hormone transport in the brain *(MCT8)*
- Abnormal selenium incorporation *(SECISBP2)*

Secondary and Tertiary Hypothyroidism (1 in 50,000–100,000)

Hypothalamic abnormality
- Isolated TRH deficiency
- Multiple hypothalamic hormone deficiencies
- Isolated hypothalamic defect
- Associated with other midline facial/brain dysmorphic features (e.g., *SOD*, cleft lip/palate)

Pituitary abnormality
- Isolated TSH deficiency *(IGSF1)*
- TRH resistance
- Abnormal TSHβ molecule
- Multiple pituitary hormone deficiencies
- Posterior pituitary eutopic (transcription factor defect, e.g., *POU1F1, PROP1, LHX3)*
- Posterior pituitary ectopic (idiopathic, transcription factor defect, e.g., *HESX1, SOX3, LHX4, OTX2)*

- TSH resistance
- TSH receptor gene mutation *(TSHR)*
- Postreceptor defect?
- Thyroid hormone resistance (1 in 100,000)
- $G\alpha_s$ gene mutation *(GNAS)*

[a]Most common.

CBX2, and PBX1) are required for formation of the bipotential gonadal ridge.[288] The gonad is derived from two tissue anlagen, the primordial germ cells of the yolk sac wall and the somatic, stromal cells that migrate from the primitive mesonephros.[289,290]

By 2 to 3 wpc, the germ cells have begun their migration from the yolk sac, and the gonadal ridge has appeared as a derivative of the mesonephros. The germ cells are incorporated into the developing urogonadal ridge during the fourth week postconception in the human embryo as a thickening of the mesothermic mesonephros covered by coelomic epithelium. The primitive gonad is composed of a surface epithelium, primitive gonadal cords continuous with the epithelium, and a dense cellular mass referred to as the adrenogonadal primordium, which includes the steroidogenic cell precursors.[290] As this primordium grows, the cells delaminate from the coelomic epithelium and invade the underlying mesenchyme, with the cells adjacent to the mesonephros migrating dorsolaterally to form the gonadal primordium.

Until the appearance of testicular cords made up of pre-Sertoli cells at 6 wpc, the fetal testis and ovary are indistinguishable. The development of a specific sex phenotype requires the action of networks of transcription factors and a complex signaling cascade that regulates differentiation of the bipotential gonad into a testis or ovary from 6 wpc.

Embryogenesis of the gonads is programmed by genes encoding the male sexual determinant SRY, as well as SF1, SOX9, and DAX1.[291,292] SRY, thought to have evolved from SOX3, is a critical regulator of male gonadal differentiation. The X chromosomal *SOX3* gene is not normally expressed in the developing gonad. Therefore, when ectopically expressed, it might substitute for *SRY* in driving testicular development. Loss-of-function mutations of *SOX3* do not affect sex determination, but *SOX3* overexpression in murine XX gonads leads to testis differentiation; in humans, *SOX3* duplication or rearrangements of the *SOX3* regulatory region have been found in 46,XX individuals with dysgenetic testes.[293]

SF1 is required for testicular and ovarian development and mediates müllerian-inhibiting hormone (i.e., antimüllerian hormone [AMH]) gene expression and gonadotropin production. Both SF1 and DAX1 are required for normal gonadal development.

By 7 wpc, in the XY gonad, *SRY* is expressed in pre-Sertoli cells resulting in the upregulation of *SOX9* expression that is further augmented by the synergistic action of SRY and SF1, leading to the initiation of definitive Sertoli cell differentiation. The transcriptional activation by *SRY* and *SF1* is mediated by the binding of these two proteins to the testis-specific enhancer of *SOX9* core (TESCO) region, which lies approximately 13 kb upstream of *SOX9.* Genomic rearrangements (such as duplication and triplication) affecting *SOX9* regulatory elements, located significantly further upstream (500 kb) than the TESCO enhancer region, have been found in patients with 46,XX sex reversal.[288]

Once SOX9 levels reach a critical threshold, several positive regulatory loops are initiated, including autoregulation of *SOX9* expression and formation of feed-forward loops via FGF9 or PGD2 signaling. During testicular development, *SOX9* functions by regulating the production of AMH from Sertoli cells and possibly by repressing genes involved in ovarian development such as *WNT4* and *FOXL2.* The DMRT1 transcription factor may also be involved in this process.[288]

WT1, the Wilms tumor suppressor gene, is also expressed in the bipotential gonadal ridge during its differentiation from the intermediate mesoderm and is critical for normal male sexual differentiation. Located at 11p13, it is expressed in both the primitive kidney and the genital ridge. WT1 isoforms associate and synergize with SF1 to promote expression of AMH. *WT1* missense mutations associated with 46XY disorders of sexual development (DSD) in Denys-Drash syndrome fail to synergize with SF1.[294] In addition, WT1 binds to and activates the SRY promoter.

The expression of SRY is transcriptionally regulated by WT1, SF1, GATA-4 and its cofactor zinc finger protein FOG2 (also called ZFPM2), and chromobox protein homologue 2 (CBX2).[295] In the presence of SRY, male gonadal differentiation begins with organization of the gonadal blastema into interstitium and germ cell–containing testicular cords. The primitive cords lose their connections with the epithelium, primitive Sertoli cells and spermatogonia become visible within the cords, and the epithelium differentiates to form the tunica albuginea.[296] Sertoli cells induce the development of fetal Leydig cells via a hedgehog-signaling pathway, which produces androgens and INSL3, a member of the insulin-like family, at 8 to 9 wpc.[297] INSL3 is required for

testicular descent.[288] Rare mutations have been described in cryptorchidism.[298,299] Testosterone and AMH cause regression of müllerian structures and differentiation of the wolffian duct into the epididymis, vas deferens, and seminal vesicles. In 46,XY males, testosterone is converted to 5-dihydrotestosterone (DHT) by the enzyme 5α reductase, resulting in the development of male external genitalia. The activity of DHT is mediated by the nuclear transcription factor, androgen receptor (AR), which has high affinity for DHT.

The fetal testes grow from approximately 20 mg at 12 wpc to 800 mg at birth; at 5 to 6 months they descend into the inguinal canal in association with the epididymis and the vas deferens.[296] The gonad, adrenal, and kidney all initially develop in close proximity, and as the testes descend they may carry rests of adrenocortical cells with them. These adrenal rests may become hyperplastic with resulting testicular enlargement if subjected to prolonged ACTH stimulation (e.g., in patients with poorly controlled congenital adrenal hyperplasia).

SF1 mutations result in a number of phenotypes related to gonadal development and function. A recurrent specific heterozygous mutation of SF1 accessory DNA-binding region was recently reported in XX individuals with varying degrees of testis development.[300] One individual, raised as female, was found to have a 46,XY karyotype and partial testicular dysgenesis. These findings highlight how a specific variant in a developmental transcription factor can switch organ fate from ovary to testis in mammals, which represents the first missense mutation causing isolated nonsyndromic 46,XX ovotesticular DSD in humans.

In human females, differentiation of ovaries begins during week 5 of conception, in the absence of *SRY*. In the XX gonad, the absence of SRY results in the inability of *SOX9* expression to reach a critical threshold, and together with the expression of factors such as RSPO1/WNT4 signaling, FST and FOXL2 lead to formation of the ovary, at least in part through suppression of the activity of "testis" genes. In the 46,XX female, the absence of androgens leads to the development of female genitalia; the wolffian duct regresses and the müllerian duct is maintained and forms the oviduct, uterus, cervix, and upper part of the vagina.[288] The gonadal blastema differentiates into interstitium and medullary cords containing the primitive germ cells, referred to as *oogonia*. The cords degenerate and cortical layers of surface epithelium, containing individual small oogonia, appear. By 9 to 10 wpc, clusters of dividing oogonia are surrounded by cord cells within the cortex; the medulla at this time consists largely of connective tissue.[301] At 10 wpc, primitive granulosa cells begin to replicate, and many of the large oogonia in the deepest layers of the cortex enter their first meiotic division.

Primordial follicles are first observed at about 16 wpc, and the number increases rapidly thereafter.[302] However, the number of oocytes progressively declines from a peak of 3 to 6 million at 5 months of gestation to approximately 2 million at term.[25,302] Germ cell proliferation and apoptosis occur simultaneously. Proliferating oocytes cluster, but the clusters break down with the development of follicles because only those oocytes enfolded by developing granulosa cells as primordial follicles survive.[25,302] By 5 months of gestation and during the seventh month, stroma-derived thecal cells develop around the primordial follicles as they mature to primary follicles. This process continues after birth progressing toward the superficial layers.

Each fetal ovary weighs about 15 mg at 12 wpc and 300 to 350 mg at term.[301] The number of surviving primary follicles at birth correlates with the duration of subsequent postpubertal ovulation. Interstitial cells with characteristics of steroid-producing cells are present after 12 weeks and, during the third trimester, theca cells with steroidogenic capacity surround the developing follicles.[25] Significant aromatase activity also is present but few if any steroids are produced by the ovary during development.[25,301]

The specific genetic mechanisms dictating ovarian development are being unraveled; some of the most powerful regulators include the WNT/FZD/β-catenin, FOXO/FOXL2, and TGFβ/SMAD pathways.[295,303] *FOXL2*, encoding a forkhead transcription factor, is required for ovarian development.[304] DMRT1, expressed in testis, prevents expression of FOXL2 and hence female programming in the postnatal testis.[305] In the XX gonad, *RSPO1, WNT4, CTNNB1, FOXL2,* and *FST* are also expressed in a female-specific manner to promote ovarian development and repress testicular development. In humans and mice, R-spondin-1 (encoded by *RSPO1*) augments β-catenin signaling, possibly via WNT4.[306] Homozygous mutations in *RSPO1* result in a syndrome with palmoplantar hyperkeratosis and 46,XX DSD with sex reversal and dysgenetic testes or ovotestes.[307] Aberrations of *WNT4* cause Mayer-Rokitansky syndrome or SERKAL syndrome (*s*ex *r*eversal, dysgenetic *k*idneys, *a*drenals, and *l*ung).[302,308]

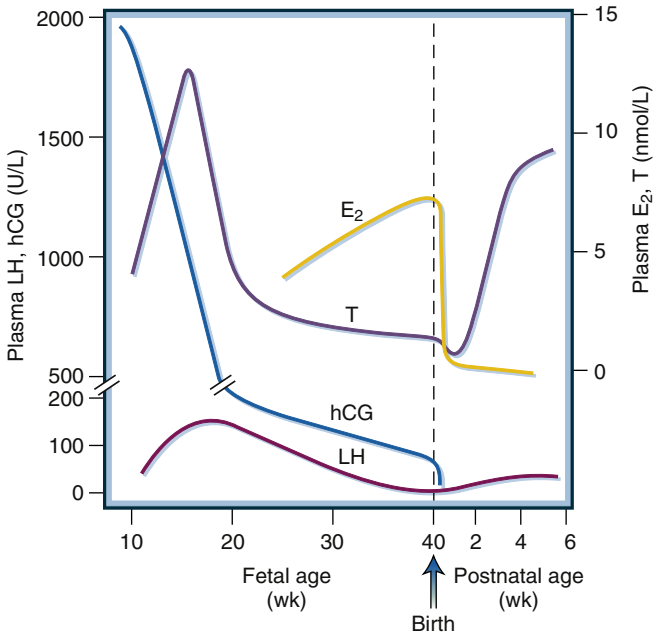
Fetal Sex Steroid Production

In the male fetus, the development of Leydig cells leads to an increase in fetal testosterone production between 8 and 18 wpc (Fig. 23.10).[296] Androgen receptors appear in the epithelium during development at 7 to 10 wpc[308] with no difference in expression between sexes.

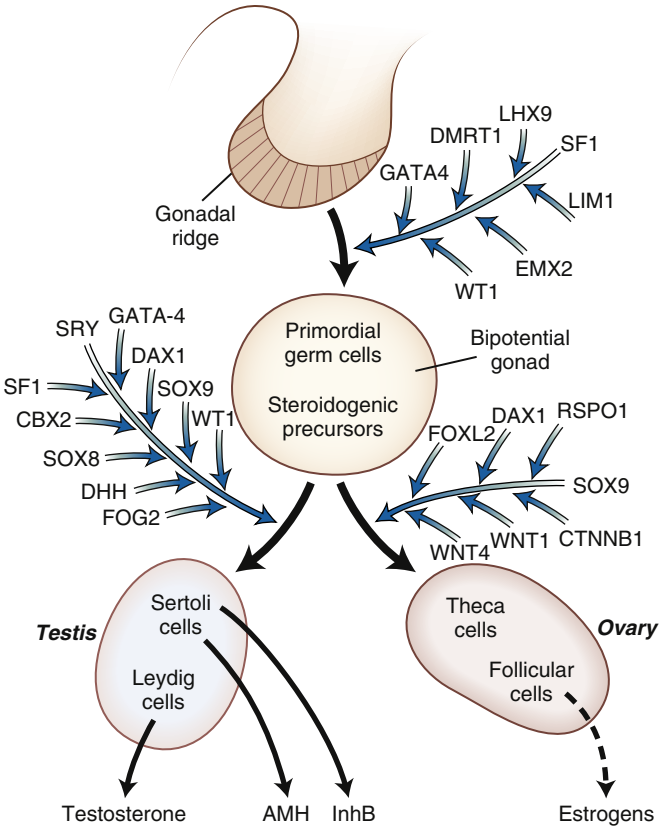
In vitro studies in rat tissue have shown that hCG binding to fetal testis cells does not downregulate LH receptors. Fetal LH may contribute to fetal Leydig cell function, but quantitatively hCG is the predominant gonadotropin. Testosterone itself, acting through the androgen receptor, stimulates differentiation of the primitive mesonephric ducts into bilateral vas deferens, epididymides, seminal vesicles, and ejaculatory ducts. DHT stimulates male differentiation of the urogenital sinus and external genitalia, including differentiation of the prostate, growth of the genital tubercle to form a phallus, and fusion of the urogenital folds to form the penile urethra. DHT mediates the action of testosterone in the wolffian ducts.

The fetal testis also produces AMH, which causes dedifferentiation of the müllerian duct system in the male fetus.[309,310] AMH is a glycoprotein with a monomer that has a molecular size of approximately 72 kDa and multimer sizes ranging from 145 to 235 kDa. It is a member of the transforming growth factor β (TGFβ) family. It is produced by testicular Sertoli cells and reaches the müllerian ducts largely by diffusion; duct regression in vitro requires an exposure to AMH of 24 to 36 hours. AMH is synthesized early in gestation, with production peaking at the time of müllerian duct regression; biosynthesis continues throughout gestation and decreases after birth. *AMH* gene expression is activated by the *SRY* and *SF1* genes.[309] AMH also has autocrine and paracrine effects on testicular steroidogenic function during fetal life.[310] Male phenotypic differentiation is mediated by testicular testosterone and AMH and occurs between 6 and 12 wpc.

In the female fetus, the müllerian duct system differentiates in the absence of AMH, the mesonephric ducts fail to develop in the absence of testosterone, and the undifferentiated urogenital sinus and external genitalia mature into female structures. Mutation of the *AMH* gene results in a persistent müllerian duct syndrome in the XY fetus.[309]



• **Fig. 23.10** Patterns of change of plasma concentrations of human chorionic gonadotropin (hCG), luteinizing hormone (LH), testosterone (T), and estradiol (E_2) in a male fetus during gestation and in the neonatal period. (Data from Mann DR, Gould KG, Collins DC, et al. Blockade of neonatal activation of the pituitary-testicular axis: effect on peripubertal luteinizing hormone and testosterone secretion and on testicular development in male monkeys. *J Clin Endocrinol Metab*. 1989;68;600–607; Reyes FI, Boroditsky RS, Winter JS, et al. Studies on human sexual development: II. Fetal and maternal serum gonadotropin and sex steroid concentrations. *J Clin Endocrinol and Metab*. 1974;38:612–617; Kaplan SL, Grumbach MM, Aubert ML. The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus: maturation of central nervous system regulation of anterior pituitary function. *Recent Prog Horm Res*. 1976;32:161–243; Winter JS, Faiman C, Hobson WC, et al. Pituitary-gonadal relations in infancy: I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab*. 1975;40:545–551; Forest MG, Cathiard AM. Pattern of plasma testosterone and Delta[4]-androstenedione in normal newborns; evidence for testicular activity at birth. *J Clin Endocrinol Metab*. 1975;41:977–980.)



• **Fig. 23.11** Summary of molecular and cellular events of gonadal differentiation. *AMH,* antimüllerian hormone or müllerian inhibiting substance; *DHH,* desert hedgehog; *InhB,* inhibin B. See text for details. (Molecular cascades developed from Harley VR, Clarkson MJ, Argentaro A. The molecular action and regulation of the testis-determining factors, SRY [sex-determining region of the Y chromosome] and SOX9 [SRY-related high-mobility group (HMG) box 9]). *Endocr Rev*. 2003;24,466–487; Park SY, Jameson JL. Minireview: transcriptional regulation of gonadal development and differentiation. *Endocrinology*. 2005;146:1035–1042.)

Estrogen effects are mediated by cognate receptors, members of the large family of steroid and thyroid hormone, vitamin D, and retinoid receptors.[311,312] Two receptors, ERα (encoded by *ESR1* on chromosome 6) and ERβ (encoded by *ESR2* on chromosome 14), have been identified, with 96% and 58% homology in the DNA-binding and ligand-binding domains, respectively. Expression profiles of mRNAs of both receptors have been characterized in the human fetus at 16 to 23 weeks. One or both receptor mRNAs are present in most tissues. The ERβ message is predominant, particularly in testis, ovary, spleen, thymus, adrenal, brain, kidney, and skin. The ERα message is prominent in the uterus, with relatively low levels in most other tissues.[311,312]

The significance of ERs in fetal development is unclear. Knockout of the *ERα* gene in mice does not impair fetal development of any tissue, but adult females are infertile with hypoplastic uteri and polycystic ovaries while adult males manifest decreased fertility.[312] ERβ knockout mice develop normally, and female adults are fertile with normal sexual behavior; adult males reproduce normally but have prostate and bladder hyperplasia.[311] It is known that estrogens regulate DHEA production in the baboon and human fetal adrenal.[311]

Knockout of both *ERα (ESR1)* and *ERβ (ESR2)* genes has little impact on fetal development, but after birth, the uterus, fallopian tubes, vagina, and cervix in females are hypoplastic and unresponsive to estrogen.[312] In humans, *ESR1* mutations in males are associated with tall stature, osteoporosis, and insulin insensitivity.[313]

Androgens and estrogens are both involved in the structural development of the rat brain.[314] Gonadal hormones also control gonadotropin production in the brain that results in cyclic ovarian function and normal function of the testes.[315,316] Testosterone administration to neonatal female rats produces permanent inhibition of cyclic hypothalamic control through local aromatization to estradiol and ER binding. In primates and humans, estrogens seem to be more effective in this regard, but there is no evidence for permanent programming in the primate nor does there appear to be major tissue biochemical differences between the sexes in utero to account for sexual dimorphic behavioral or gonadotropic programming.[315] The mechanisms for these effects are not yet clear in the primate and human fetus.

A current view of the pathways for genes programming gonadal differentiation is shown in Fig. 23.11. The full menu of downstream gene targets remains to be defined, but the net result is the highly organized pattern of gonadal development and phenotypic sexual differentiation. Fetal pituitary gonadotropins

are not required for gonadal development or sexual differentiation; *LH or FSH receptor* knockout mice are born phenotypically normal.[317]

Disorders of Sex Development

The increasing knowledge about sex determination and differentiation and society's increasing fluidity about the nature of sexual identity and gender roles have led to a reappraisal of former management practice in DSD. Optimal care for infants with DSD requires an experienced multidisciplinary team that transitions into adulthood, and there is a need for greater data sharing and formal research and clinical expertise networks at several levels, as highlighted by the Chicago Consensus in 2005. Human mutations in several genes programming gonadal differentiation have been described, and it is likely that future innovation in "omic" technologies (e.g., transcriptomics) will lead to improved understanding of the underlying abnormality as well as explain the variability in the phenotype.

Fetal Autonomic Nervous System

The activity of the autonomic nervous system may be regarded as a landmark brain function reflecting overall ability to regulate the fetal central nervous system. The interplay between the sympathetic and parasympathetic nervous systems is effected via the cerebral cortex, the medulla oblongata, the sympathetic ganglia, and the vagus nerve. There is an increasing realization that neural function in the fetus serves specialized needs particular to development and that the onset of maturity represents the loss of these unique patterns of neural activity. Nowhere is this more evident than in the sympathetic nervous system and its endocrine counterpart, the adrenal medulla.

Embryology

As early as 4 to 5 wpc the primordia of the sympathetic trunk ganglia are visible in the human fetus. The sympathoadrenal (SA) cell lineage is a derivative of the neuroectodermal crest (NC), which gives rise to sympathetic neurons and neuroendocrine chromaffin cells of the adrenal medulla and extra-adrenal chromaffin cells. Signals that are important for specification of these two types of cells are largely unknown. The SA progenitor cells that migrate in a dorsolateral direction form numerous extramedullary sympathetic paraganglia,[318] which are scattered throughout the abdominal and pelvic sympathetic plexuses.[319] Each of the paraganglia may reach a maximal diameter of 2 to 3 mm by 28 wpc; the largest of these, the organs of Zuckerkandl near the origin of the inferior mesenteric arteries, enlarge to 10 to 15 mm in length at term. After birth, the paraganglia gradually atrophy and disappear by 3 years of age.

The SA cells that migrate ventrally through the anterior sclerotome to reach the dorsal aorta[320] give rise to tyrosine hydroxylase-expressing, catecholaminergic neuronal progenitor cells in response to bone morphogenetic protein cues from cells of the wall of the dorsal aorta and the surrounding mesenchyme.[321] Some cells then migrate from the dorsal aorta along nerves and blood vessels to enter the adrenal primordium at the cranial end from 6 wpc[322,323] and acquire the phenotype of chromaffin cells. It was thought that these were SA cells that lost expression of neuronal genes, but more recently it has been shown that at least some of the presumptive chromaffin cell population enter the adrenal primordium still expressing neural crest markers and then gain tyrosine hydroxylase and dopamine β-hydroxylase expression but not neuronal markers.[324] A variety of transcription factors have been identified in the development of the SA cell lineage, including PHOX2B (paired-like homeobox 2b), MASH1 (mammalian achaete-scute homolog 1), PHOX2A (paired-like homeobox 2a), and HAND2 (heart and neural crest derivatives-expressed protein 2).[318] The *PHOX2B* gene is pivotal in the development of the autonomic nervous system. Phox2b$^{-/-}$ mice die in utero since autonomic nervous system neurons either fail to develop or degenerate. Mutations in human *PHOX2B* cause a rare syndrome of dysautonomia (congenital central hypoventilation syndrome) and a predisposition to neuroblastoma.[325]

Nerve growth factor (NGF) is required for the survival of developing sympathetic neurons. In the rodent, NGF antiserum injected into neonatal rats resulted in degeneration of immature chromaffin cells, sympathetic cells, and pheochromoblasts.[326] It is unclear whether NGF and other growth factors are involved in maturation of the human fetal autonomic nervous system.

Functional Development of the Sympathoadrenal System

Chromaffin cells are initially scattered within the adrenal cortex and are mostly noradrenergic. At around 10 wpc, small islets of chromaffin cells express phenylethanolamine *N*-methyltransferase (PNMT), the enzyme required to convert noradrenaline into adrenaline, to become adrenergic.[327] With increasing gestational age, there is progressive growth of the adrenal medullae, increasing catecholamine content and progressive maturation of medullary functional capacity. Histologically the adrenal medullae are somewhat immature at birth, but by 1 year they resemble the adult glands.

Catecholamines are present in the para-aortic chromaffin tissue by 8 to 13 wpc, and concentrations increase until term. The predominant catecholamine is norepinephrine (NE), presumably because of low activity of PNMT in para-aortic chromaffin tissue. In contrast, the adrenal medulla has high levels of PNMT activity. This enzyme catalyzes the methylation of NE to epinephrine and is activated by the high levels of cortisol that diffuse into the adrenal medulla from the adrenal cortex.[319,328]

How the fetus withstands an in utero environment of relative hypoxia has been a vibrant area of research for over a century. The ovine model provides a comparative system for physiologic and developmental research in the fetus that is applicable to human medicine. Basal plasma epinephrine, NE, and dopamine concentrations decrease during the last trimester in sheep[329,330] due to maturation of clearance mechanisms.[330] This model has been used to study fetal adaptive responses to chronic oxygen and nutrient deprivation during placental insufficiency. The fetal sheep responds to hypoxia with increased catecholamine concentrations.[331] This response is also present in human fetuses during the third trimester of gestation.[332] Central and adrenal enkephalins are also involved in fetal autonomic nervous system function; pretreatment with naloxone potentiates and methadone inhibits the catecholamine response to hypoxia.[319,333]

Catecholamines are critical for fetal cardiovascular function and fetal survival. Gene knockout studies in mice, targeting either tyrosine hydroxylase or dopamine β-hydroxylase, produced fetal catecholamine deficiency and midgestation fetal death in 90% of the mutant embryos.[334,335] In addition, fetal catecholamines are the major stress hormones in the fetus.[328,332] The fetal adrenal and the para-aortic chromaffin masses discharge large amounts of catecholamines directly into the circulation in response to fetal hypoxia.[328] Moreover, the defense against fetal hypoxia involves catecholamine actions mediated

through cardiac α-receptors that are unique to immature animals. α-Adrenergic receptors predominate in immature cardiac tissue and gradually decline in number as β-adrenergic receptors increase with maturation. Chromaffin tissue in the fetus is also innervated by opiate receptors and contains relatively large amounts of opiate peptides that appear to be co-secreted with the catecholamines.[328] The extent to which these peptides or pituitary endorphins are involved in modulating fetal catecholamine secretion remains unclear.

The human neonate responds to parturition with an increase in plasma epinephrine and NE concentrations, and these responses are augmented by hypoxia and acidosis.[331,334,335] In the newborn, catecholamine secretion also increases after cold exposure and hypoglycemia.[328,332] Labor-induced catecholamine release may provide an important mechanism underlying the neonate's process of adapting to extrauterine conditions and the recruitment of vital postnatal adaptations.

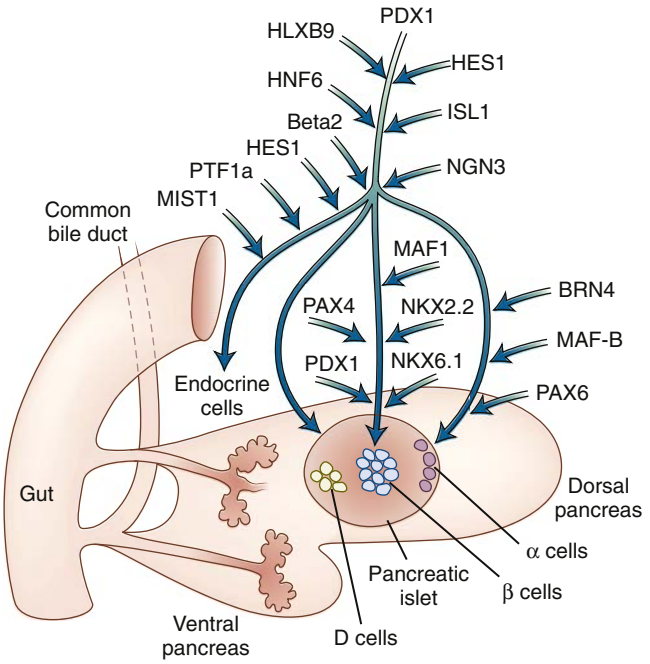
Endocrine Pancreas: Insulin and Glucagon

Embryology

Pancreatic development represents a fascinating process in which two morphologically distinct tissue types must derive from one simple epithelium. These two tissue types, exocrine (including acinar cells, centroacinar cells, and ducts) and endocrine (alpha cells secrete glucagon, beta cells secrete insulin, delta cells secrete somatostatin, and PP or gamma cells secrete pancreatic polypeptide), serve disparate functions. Pancreatic development is a multistep process defined by three major periods (primary and secondary transition and the postnatal period).

Embryogenesis of the pancreas is mediated by a series of homeobox genes and transcription factors that program pancreatic budding from the gut tube, development of branching ducts and undifferentiated epithelium, differentiation of exocrine and endocrine cell lineages that originate from endodermal tissue, and organization of the endocrine cells into islets of Langerhans.[336] Members of the EGF family of growth factors, laminin, and perhaps other growth factors, including the IGFs, also contribute to pancreatic growth and differentiation.[337,338]

Cell lineage determination during pancreatic development involves regulation by many transcription factors. The process has been well studied in the mouse and begins at day 8 of the 21-day gestation, extending to 2 to 3 weeks after birth (Fig. 23.12). An outgrowth of dorsal and ventral pancreatic buds from the dorsal foregut endoderm occurs at around embryonic day 8.5 (e8.5).[339] These cells express pancreatic and duodenal homeobox 1 (Pdx1) without which the pancreas is unable to form.[339] This phenotype in mice is mimicked in humans with homozygous loss of *PDX1* that is responsible for pancreatic agenesis. Pdx1 expression is quickly followed by expression of the basic helix-loop-helix protein pancreas-specific transcription factor 1a (Ptf1a), necessary for further development of both the endocrine and exocrine pancreas in mice as well as humans, forkhead box O1 (FOXO1), NK2 homeobox 2 (NKX2.2), and NK6 homeobox 1 and 2 (NKX6.1 and NKX6.2, respectively).[339,340] While regulatory factor X 6 (Rfx6) is also expressed during these early time points in mice, it appears only to be required for endocrine pancreatic development, a phenotype shared in humans with mutations in *RFX6*.[339] Motor neuron and pancreas homeobox 1 (Mnx1), formerly known as Hlbx9 or Hb9, is expressed early but, unlike the previously mentioned transcription factors, *Mnx1*-null mice fail to develop a dorsal pancreas, and the resulting pancreas has fewer and dysfunctional islets.[336,339,341] Similar phenotypic results were observed in humans with mutations in *MNX1*.



• **Fig. 23.12** Expression of main transcription factors during pancreatic embryogenesis. Knockout of *PDX1, MNX1* (previously called *HLXB9*), or *ISL1* is associated with early arrest of pancreatic development. *MNX1* knockout leads to failure of the pancreatic dorsal bud to develop with decreased β-cell number in the remnant pancreas. *HES1, PTF1A,* or neurogenin 3 *(NGN3)* disruption leads to aplasia or hypoplasia of the islets of Langerhans. Disruption of the downstream transcription factors impairs formation of the β cells or α cells. *SOX9* and *HNF3B* (not shown) are required for early foregut formation and pancreas specification. (Data from Habener JF, Kemp DM, Thomas MK. Minireview: transcriptional regulation in pancreatic development. *Endocrinology*. 2005;146:1025–1034.)

Hes family BHLH transcription factor 1 (Hes1) is a Notch signaling pathway component, and its loss in mice disrupts normal development of the pancreas but still allows formation of exocrine and endocrine cells.[340] During this period, the only hormone-positive endocrine cells produced are a small number of glucagon-positive cells. Whether these cells play a role in development and postnatal function and what that role might be is not understood.[336]

Starting at around e13.5 in the mouse, expression of the basic helix-loop-helix transcription factor neurogenin 3 (Ngn3) marks endocrine progenitor cells that will differentiate into the mature, hormone-producing cells of the islets of Langerhans.[336,339,340] Ngn3 is only highly expressed before hormone expression, and loss of *Ngn3* prevents the development of endocrine progenitor cells and consequently pancreatic islets. *Ngn3* knockout in the mouse leads to marked β-cell dysplasia and hypoplasia.[341] Ngn3 initiates a new set of developmental programs for endocrine cell lineage differentiation and maintenance. These include the LIM homeobox protein islet 1 (Isl1), paired box 4 and 6 (Pax4 and Pax6), aristaless related homeobox (Arx), and neuronal differentiation 1 (Neurod1), which are all lost in Ngn3-deficient mice. In mice, *Ngn3* knockout leads to complete absence of endocrine cells, whereas knockout of the lower pathway genes shown in Fig. 23.12 impairs specific islet cell differentiation.[341] *Nkx2.2, Nkx6.1, Pax4*, or *Pax6* loss of function results in endocrine cell agenesis or hypogenesis.[342–344] The competency of progenitors changes with time such that Ngn3$^+$ cells that arise early in development preferentially

differentiate into glucagon-positive cells while later $Ngn3^+$ cells give rise to insulin-positive or somatostatin-positive cells.[339]

During the later stages of β-cell differentiation, the MafB and MafA (V-maf musculoaponeurotic fibrosarcoma oncogene homologs B and A) transcription factors play a prominent role. Upon β-cell specification, the transcription of insulin is initiated and maintained by MafB, Pdx1, Neurod1, Pax6, and MafA. Additionally, Pax4, NKX6.1, and Pdx1 inhibit glucagon expression thereby preventing the expression of glucagon by β cells.[339]

Arx is one of the first known transcription factors to specifically mark the glucagon-producing α-cell lineage, and its loss prevents development of α cells with a concomitant increase in β and δ cells.[339] Indeed, a competitive reciprocal interaction between Arx and Pax4 is involved in the decision of cell fate between α and β cells. While Arx is necessary for the early specification of the α cell and directly maintains α-cell mass, it is not directly involved in glucagon expression. The Pax4-related factor Pax6 is expressed by both α and β cells. Pax6-deficient mice lack α cells. In addition to its role in insulin transcription in the β cell, Pax6 also coordinates glucagon transcription in the α cell directly by binding to the glucagon promoter and indirectly by inducing expression of other transcription factors such as c-Maf, MafB, and Neurod1, which also activate glucagon expression. A third factor that is crucial for later α-cell development is forkhead box A2 (Foxa2), which plays a role downstream of Arx and Pax6.[339]

A de novo heterozygous mutation in FOXA2 has been described in a child with congenital hyperinsulinism and congenital hypopituitarism with craniofacial dysmorphic features, choroidal coloboma, and endoderm-derived organ malformations in liver, lung, and gastrointestinal tract.[345,346] Expression profiling in human embryos by immunohistochemistry showed strong expression of hFOXA2 in endoderm-derived organs, including the pancreas, and transfection studies and Western blot assays demonstrated the causative role of FOXA2 in this syndrome. Upon successful specification of the α-cell lineage, pre-proglucagon expression is further promoted by forkhead box A1 (Foxa1), brain 4 (Brn4), and Isl1. Of all the transcription factors that can promote glucagon expression, expression of only two, Brn4 and MafB, is enriched in adult mouse α cells compared to β cells.[339]

More recent studies have compiled gene regulatory networks for pancreas development integrating data from multiple studies, including mutation analysis, gene expression, cell lineage tracing, and biochemical gene regulation. These networks include all the previously mentioned genes but also others such as *Tle2, Dll, Onecut1, BMP7,* and *SOX9.*[340] The transcription factor Glis-similar 3 (Glis3) has been implicated in the development of neonatal type 1 and type 2 diabetes.[347] Loss of functional *Glis3* leads to a severe reduction in both β-cell and pancreatic polypeptide cell numbers postnatally, as well as formation of cystic pancreatic ducts. Ngn3, glis-, and Glis3 represent the relatively "late actors" as their expression is restricted to the establishment of defined endocrine progenitor cells (or endocrine and ductal cells in the case of Glis3).

In humans, mutations in *GLIS3, NeuroD1, PDX1, PTF1A,* and *GATA6* are associated with neonatal diabetes mellitus due to pancreatic agenesis; mutations in *EIF2AK3* (encoding translation initiation factor 2-alpha kinase 3), *HNF1B* (hepatocyte nuclear factor-1β), *MNX1*, *NKX2.2,* and *RFX6* lead to neonatal diabetes due to pancreatic hypoplasia.[348–351] Recessive mutations in a distal enhancer of *PTF1A* also cause pancreatic agenesis.[352]

Functional Development of the Endocrine Pancreas

The human fetal pancreas is identifiable by 4 weeks of gestation and α and β cells can be recognized by 8 to 9 weeks. Insulin, glucagon, somatostatin, and pancreatic polypeptide are measurable by 8 to 10 weeks of gestation.[353] α Cells are more numerous than β cells in the early fetal pancreas and reach a relative peak at midgestation; β cells continue to increase throughout the second half of gestation so that, by term, the ratio of α to β cells is approximately 1:1. Both α and β cells have opposing roles in glucose homeostasis, although they derive from a common progenitor and share many proteins important for glucose sensing and hormone secretion. Mouse models have underlined the similarities between the two cell types by showing that β-to-α as well as α-to-β transdifferentiation can take place under certain experimental circumstances, revealing a remarkable degree of plasticity among the different pancreatic endocrine cells.[339]

The β cell is functional by 14 weeks of human gestation, and fetal pancreatic insulin content exceeds that of the adult throughout most of pregnancy. The insulin content of the pancreas increases from less than 3.6 pmol/g (0.5 U/g) at 7 to 10 weeks to 30 pmol/g (4 U/g) at 16 to 25 weeks of gestation and 93 pmol/g (13 U/g) near term; the concentration in the adult pancreas is approximately 14 pmol/g (2 U/g).[354] Endocrine cells are dispersed throughout the exocrine tissues by 20 weeks and the islets of Langerhans are clearly differentiated by 31 weeks.

Fetal glucose metabolism is largely independent of insulin and glucagon.[355–357] In physiologic doses, glucagon does not increase hepatic glucose production, probably because of a paucity of fetal hepatic glucagon receptors.[357] Insulin receptors are present in a variety of fetal tissues at levels generally exceeding those of the adult, but downregulation of insulin receptor binding does not occur during fetal hyperinsulinemia, in contrast with observations in adult animals. Acute hypoglycemia or hyperglycemia is not associated with significant alterations in either insulin or glucagon concentrations.

Although the fetal β cell is functional by 14 to 24 weeks of gestation, secretion of insulin into the bloodstream by the fetal pancreas is low. Insulin release from the fetal rat pancreas in vitro in response to glucose or pyruvate is minimal but can be stimulated by leucine, arginine, tolbutamide, or potassium chloride, indicating that parts of the secretory mechanism are functional in the fetus.[354,355,358] Insulin secretion in adult islets is mediated by two or more mechanisms, including stimulation of the adenylate cyclase system with production of cAMP and inhibition of potassium efflux, which leads to depolarization of the cell membrane and opening of voltage-dependent calcium channels. Calcium channel activation does not occur in fetal islets in response to initiators of insulin release that cause depolarization of adult islet cells.[358] Infusion of glucose or arginine to pregnant women fails to provoke fetal insulin secretion at midgestation or near term, and plasma insulin concentrations in the late human fetus are relatively unresponsive to high glucose concentrations before the onset of labor.[354] Similar observations have been made in the monkey: Neither glucose nor arginine stimulated fetal insulin release near term, but glucagon evoked prompt insulin production.[354] Late in gestation in the ovine fetus, epinephrine inhibits insulin release through a receptor pathway.[354]

In the anencephalic human fetus, the endocrine pancreas develops normally if maternal carbohydrate metabolism is not impaired; β-cell hypertrophy and hyperplasia do not occur in the anencephalic fetus or in decapitated fetal rabbits exposed to chronic hyperglycemia. This lack of β-cell response to hyperglycemia may be the result of deficiency of GH or IGF1, or both, because GH stimulates insulin gene expression and may play a permissive role in β-cell hyperplasia and hypertrophy.[338] Chronic

fetal hyperglycemia does evoke hyperinsulinemia and glucagon suppression, and chronic hypoglycemia may inhibit fetal insulin and promote fetal glucagon release.[343]

Pancreatic glucagon concentrations are relatively high in fetal plasma and increase progressively with fetal age.[354,355] The fetal pancreatic glucagon content at midgestation is approximately 6 μg/g, compared with an adult level of 2 μg/g. As is true for insulin, the capacity for glucagon secretion is blunted in the fetus. Hyperglycemia does not suppress fetal plasma glucagon concentrations in rats, monkeys, or sheep, and acute hypoglycemia does not evoke glucagon secretion in the rat fetus. Amino acids, which are important secretagogues for insulin and glucagon in the adult, probably have little role in modulating insulin and glucagon secretion in the preterm fetus, but infusion of alanine into women at term increases both maternal and cord blood glucagon concentrations, indicating a fetal glucagon response to amino acids in the term fetus. Catecholamines also evoke glucagon release in the near-term ovine fetus.[354]

Thus fetal pancreatic islet cells, although histologically mature and capable of hormone synthesis and hyperplasia, are relatively immature functionally at birth with regard to their capacity to secrete insulin and glucagon. The rapid maturation of responsiveness to glucose in the neonatal period in both premature and mature infants suggests that this blunted state may be a secondary result of the relatively stable fetal serum glucose concentrations maintained by placental transfer of maternal glucose rather than a primary, temporally fixed, maturation process. Alternatively, the lack of any enteric signal to the pancreas from feeding via release of incretins may also account for this stability. The blunted capacity for insulin and glucagon secretion has been related to a deficient capacity of the fetal pancreatic islet cells to generate cAMP or to rapid destruction of cAMP by phosphodiesterase, or both.[354]

In rodents, a period of a rapid increase in β-cell mass at the time of birth and shortly thereafter has been observed; this rapid change is attenuated by a period of apoptosis before adult mass is achieved. β-cell mass is more difficult to determine from a developmental standpoint in humans. At birth in the human, there are some 200 to 300 × 10^6 β cells, which is approximately one-third of the population present in adulthood. However, most of the actual mass change takes place in the newborn period and is associated with changes in β-cell size rather than number.[359] Thereafter, there is rapid further expansion in terms of cell numbers, but the waxing and waning of the β-cell mass, particularly with pregnancy, is poorly understood. How much β-cell mass is a determinant of progression to type 2 diabetes mellitus is unclear.

Insulin and glucagon are normally not necessary for substrate metabolism in the fetus.[355] Fetal energy needs are met by a continuous intravenous supply of glucose across the placenta, fetal glucose uptake being directly related to both the maternal blood glucose concentration and the transplacental gradient. There is little endogenous glucose production. The fetal respiratory quotient is approximately 1, which suggests that glucose is the primary energy substrate for the fetus. Other substrates, such as amino acids and lactate, may also be used in the human as in the sheep fetus and these, together with glucose, are stored as fat and glycogen in preparation for birth. Early in gestation, hepatic metabolism and substrate utilization appear to be independent of insulin and to be modulated in an autoregulatory fashion by glucose.[354] In addition, the constant supply of glucose normally precludes the necessity for endogenous gluconeogenesis, and gluconeogenic enzyme activities are low in the fetal liver.

Glycogen storage in the fetus is modulated by fetal glucocorticoids and probably by placental lactogen (hPL). Fetal insulin plays a role near term, when insulin also has the capacity to increase fetal glucose uptake and lipogenesis.[354,355] Insulin receptors are present on most fetal cells in higher numbers than on adult cells; moreover, hyperinsulinemia fails to downregulate fetal insulin receptors.[354] Fetal hepatic glucagon receptors, in contrast, are reduced in number, and fetal liver is relatively resistant to the glycemic effect of glucagon. These conditions tend to potentiate the fetal anabolic milieu during the period of rapid growth in the last trimester of gestation.

Pancreatic Regulation of Blood Glucose

During the neonatal period, the placental source of glucose is lost. A normal term infant has an immediate postnatal fall in blood glucose concentration during the first 2 to 4 hours from values close to maternal concentrations to around 2.5 mmol/L (45 g/dL) postpartum.[360,361] Low concentrations are usually transient, asymptomatic, and part of the normal adaptation to extrauterine life. Counterregulatory hormones rapidly become active, and these changes result in stabilization of blood glucose concentrations, although adult concentrations are not reached until approximately 72 hours of life.[360] Plasma glucagon concentrations rise in association with a rapid increase in functionally coupled glucagon receptors. The increase in plasma catecholamines coincident with parturition may be responsible for some of these changes; catecholamines stimulate glucagon and inhibit insulin release.[344,357,362] Phosphoenolpyruvate carboxykinase (PEPCK) activity also increases during this period. Thus gluconeogenesis is readily demonstrable in the newborn, where nearly 10% of glucose utilization is accounted for by gluconeogenesis from alanine.

Plasma free fatty acid concentrations increase postnatally because of the effects of catecholamines and chemical thermogenesis. Oxidation of fatty acids probably provides cofactors (acetyl coenzyme A and the reduced form of nicotinamide adenine dinucleotide) required for gluconeogenesis, as well as sparing glucose for utilization by crucially dependent tissues such as the brain.[344] Failure of this sequence of physiologic changes can lead to hypoglycemia, which is most common in the first few hours after birth. It has been reported that lactate may be neuroprotective in at-risk babies with hypoglycemia within the first 48 hours after birth, a time period when ketone bodies are unlikely to provide protection.[363] Neonatal hypoglycemia is common and is treated with supplemental feeds or intravenous glucose. Early feeding for prevention of hypoglycemia in at-risk babies (including infants of mothers with diabetes, small and large for gestational age, and preterm) is the mainstay of management. Dextrose gel is effective in treating neonatal hypoglycemia without affecting breastfeeding. The Pre-hPOD Study (*h*ypoglycemia *p*revention with *o*ral *d*extrose) reports the first randomized controlled trial (RCT) of prophylactic oral dextrose in at-risk babies, showing reduced incidence of neonatal hypoglycemia.[364]

Neonatal Diabetes

The neonatal diabetes phenotype encapsulates numerous subtypes, which involve severe disruption in β-cell function. Neonatal diabetes can be permanent and require lifelong treatment or may be transient, in which case the diabetes may spontaneously remit before 18 months of age (60% of cases) (or be so mild as not to require treatment) but will often relapse, usually during adolescence. Both have an incidence of approximately 1 in 200,000 to 250,000.[365]

Transient neonatal diabetes mellitus (TNDM) may be due to overexpression of an imprinted gene on chromosome 6q24 in the majority of cases, whereas activating mutations of *KCNJ11* and *ABCC8*, encoding for the two subunits (Kir6.2 and sulfonylurea receptor 1 [SUR1]) of the pancreatic β-cell ATP-sensitive inward rectifier potassium (K-ATP) channel account for 25% of cases. Mutations in the *INS* gene, encoding pre-proinsulin, are rarer, as are mutations in *ZPF57*, which regulates gene methylation. A genetic cause can be identified in approximately half of patients with NDM. Intrauterine growth retardation (IUGR) is another feature of TNDM, although not in TNDM due to K-ATP channelopathies. After remission, diabetes mellitus recurs in approximately 50% of cases.

Permanent neonatal diabetes mellitus (PNDM) is most often due to activating mutations in *KCNJ11* or *ABCC8*, resulting in overactivation of potassium channels and thus impaired insulin secretion. These patients are responsive to sulfonylurea therapy and do not require insulin treatment. Other causes of PNDM include *INS* and *GCK* mutations. Mutations in *EIF2AK3* (eukaryotic translation initiation factor 2-alpha kinase 3) lead to Wolcott-Rallison syndrome of which other features include skeletal abnormalities, liver dysfunction, cardiac and renal abnormalities, developmental delay, epilepsy, and neutropenia. *FOXP3* mutations result in IPEX (immune dysregulation, polyendocrinopathy, on X chromosome), which can lead to PNDM. Other monogenic causes of PNDM include mutations in genes encoding for zinc finger protein GLIS3, PAX6, the thiamine transporter SLC19A2, glucose transporter GLUT2 (encoded by *SLC2A2*),[366] NeuroG3, and NeuroD1, and genes important for pancreas development: *PTF1A* (hypoplasia of pancreas and cerebellum), *PDX1* (pancreas agenesis, endocrine and exocrine hormone deficiency),[367] *RFX6*, acting downstream of NeuroG3 (hypoplasia of pancreas, gallbladder, intestinal atresia, intractable diarrhea),[368,369] GATA4,[370] and GATA6.[367] Recently, mutations in *MNX1* (previously called HLXB9) and *NKX2-2*, coding for key transcription factors for pancreas development, were identified as monogenic causes for PNDM in humans.[350] Neonatal diabetes that does not respond to sulfonylurea therapy is best treated with continuous subcutaneous insulin infusion via a pump using rapid-acting insulin analogues. Dilution of insulin to fill the pump is often required for neonates and infants. Demand of insulin is so small, and feeding so frequent, that treatment with multiple daily injections is difficult.[371,372]

Although most cases of neonatal diabetes involve isolated diabetes, many of the known monogenic causes are characterized by a variety of syndromic features. Increased attention to the primarily genetic nature of early-onset diabetes has resulted in an expanding list of causal genes and an expansion of phenotypic characteristics in syndromic forms.

Hyperinsulinemic Hypoglycemia of Infancy

Hyperinsulinemic hypoglycemia (HH) is the most frequent cause of persistent and recurrent hypoglycemia in neonates and infants. It is due to unregulated secretion of insulin and can be transient or permanent.[373,374] Transient forms lasting for days are associated with maternal diabetes mellitus, maternal sulfonylurea treatment, and glucose infusions during labor. Poorly controlled maternal diabetes mellitus is associated with fetal macrosomia, an increased risk of spontaneous abortion, and fetal malformation. Maternal hyperglycemia also leads to hyperinsulinism and β-cell hyperplasia in the infant. Infants of diabetic mothers are prone to polycythemia, renal vein thrombosis, hypocalcemia, respiratory distress syndrome, jaundice, persistent fetal circulation, cardiomyopathy, congenital heart disease, and malformations of other organs.

Transient hyperinsulinism due to intrauterine growth retardation, perinatal asphyxia, and BWS can last for days to months and may require treatment.[375] Permanent hyperinsulinism can histologically be divided into focal and diffuse forms, which are inherited in, respectively, a sporadic and autosomal manner. To date, causative mutations in 12 different genes involved in β-cell insulin release have been described in congenital hyperinsulinism (CHI), for example, *ABCC8, KCNJ11, GLUD1, GCK, HADH, SLC16A1, HNF4A,* and *UCP2*.[374–376] *ABCC8* and *KCNJ11* encode for the subunits (Kir6.2 and SUR1) of the pancreatic β-cell ATP-sensitive K^+ channel.[377] However, the molecular basis of HH is unclear in up to 60% of patients.[375,376,378] Owing to their sensitivity to ATP, K^+ channels convert intracellular metabolic signals into membrane excitability in the following cascade. Glucose is transported into the cell by glucose transporters; intracellular glucokinase catalyzes an essential irreversible step for glycolysis. Intracellular glucose metabolism raises the cytosol ATP/adenosine diphosphate (ADP) ratio, which inhibits the SUR1 component of the K^+ channel, resulting in K^+ channel closure, which subsequently results in membrane depolarization, opening of voltage-gated calcium channels, and calcium influx, which then triggers the release of insulin from stored granules. Mutations in *ABCC8* and *KCNJ11* can thus result in oversecretion of insulin. The hyperinsulinism/hyperammonemia (HI/HA) syndrome is due to gain-of-function mutations in glutamate dehydrogenase (GDH) and is characterized by both fasting hypoglycemia and hyperinsulinemic hypoglycemia due to protein ingestion.[379] Certain syndromes have been associated with hyperinsulinism: overgrowth syndromes (BWS, Sotos, Simpson-Golabi-Behmel), chromosomal abnormalities (trisomy 13, mosaic Turner syndrome), growth failure syndromes (Kabuki syndrome, Costello syndrome), syndromes with abnormal calcium homeostasis (Timothy syndrome), congenital disorders of glycosylation, congenital hypoventilation syndrome, and a contiguous gene deletion, including *ABCC8* (Usher syndrome).[375]

The rarest forms of CHI have recently been associated with inactivating mutations in the uncoupling protein 2 gene *(UCP2),* a mitochondrial inner membrane protein,[380] and recently the spectrum of genetic causes for HH has been extended with the reported co-occurrence of congenital hyperinsulinism and congenital polycystic kidney disease, caused by a promoter mutation in the phosphomannomutase 2 gene *(PMM2)*.[381]

At the time of hypoglycemia, a hypoglycemia screen, including glucose, insulin, C-peptide, acetoacetate, and β-hydroxybutyrate, will easily identify congenital hyperinsulinism.

Diagnostic criteria are a glucose infusion rate more than 8 mg/kg/minute, a laboratory blood glucose less than 3 mmol/L with detectable serum insulin or C-peptide, low serum ketone bodies, and low serum fatty acids.

First-line treatment is diazoxide and chlorothiazide, and second-line treatment is octreotide and glucagon. Patients who are diazoxide insensitive need further investigation with a combined computed tomography (CT)/^{18}F-DOPA positron emission tomography (PET) scan to assess for focal or diffuse lesions. Focal lesions can be cured with surgery, whereas diffuse lesions require near-total pancreatectomy, which usually does not result in a cure and may lead to diabetes mellitus.[377]

Tremendous advances are being made in the field of hyperinsulinemic hypoglycemia, for example, use of rapid molecular genetic testing, application of novel imaging techniques (6-[fluoride-18]

fluoro-levodopa [^{18}F-DOPA] PET-CT, glucagon-like peptide 1 [GLP1] receptor imaging), and development of new medical therapies (e.g., long-acting octreotide formulations, mammalian target of rapamycin [mTOR] inhibitors, GLP1 receptor antagonists) and laparoscopic surgical techniques.[373,374]

Parathyroid/Calcitonin System

Embryology

During the fifth to sixth week of intrauterine development, the embryonic pharynx is marked externally by four branchial clefts of ectoderm origin and internally by five branchial pouches of endoderm origin. The branchial arches of mesoderm origin are in between them. Together these structures comprise the branchial apparatus, which normally undergoes involution leaving behind derivatives, including the thyroid gland, parathyroid glands, thymus, ultimobranchial body, eustachian tube, middle ear, and external auditory canal.[382]

The parathyroid glands develop as epithelial thickenings of the dorsal endoderm of the third and fourth branchial pouches. The superior parathyroid glands are derived from the fourth branchial pouch, which also gives rise to the thyroid gland. The third branchial pouch gives rise to the inferior parathyroid glands and the thymus. The parathyroid glands remain intimately connected with their respective branchial pouch derivatives, and parathyroid gland development from the third and fourth pharyngeal pouches proceeds in synchrony with thyroid embryogenesis.[21,383]

The third pouches encounter the migrating thyroid anlage, and the parathyroid anlagen are carried caudally with the thyroid gland, finally coming to rest at the lower poles of the thyroid lobes as the inferior parathyroid glands.[21,383] The fourth pouches encounter the thyroid anlage later and come to rest at the upper poles of the thyroid lobes as the superior parathyroid glands.[21,383] The individual parathyroid glands increase in diameter from less than 0.1 mm at 14 weeks of gestation to 1 to 2 mm at birth. Most of the parathyroid gland parenchyma is composed of chief cells (also known as the parathyroid gland principal cells). These play a critical role in calcium homeostasis by sensing changes in extracellular calcium concentration and releasing the appropriate amount of PTH to correct or maintain normal blood calcium concentrations. The chief cells spend most of their time inactive due to normal calcium homeostasis. Near term, fetal parathyroid cells are largely composed of inactive chief cells, with only a few intermediate chief cells containing occasional secretory granules. The fifth pouches contribute paired ultimobranchial bodies that are incorporated into the developing thyroid gland as the parafollicular or C cells that secrete calcitonin. The calcitonin content of C cells in the neonatal thyroid gland is as high as 540 to 2100 mU/g of tissue, values as much as 10 times those observed in the normal adult gland.[384] Both endocrine systems are functional during the second and third trimesters.

Transcription Factors Involved in Development of Parathyroid Glands

The molecular signaling pathways that are involved in determining the differentiation of the pharyngeal pouch endoderm into parathyroid cells are being elucidated by studies of patients with hypoparathyroidism and appropriate mouse models. These studies have revealed important roles for several transcription factors, which include members of the homeobox (Hox) and paired box (Pax) families. Disruption of the *Hox15* gene in mice results in parathyroid gland aplasia, indicating that this gene functions as part of the genetic cascade programming normal thyroid-parathyroid gland development. Deletions of genes encoding several transcription factors in mice result in developmental anomalies that include parathyroid defects, although such abnormalities have not yet been reported in man. The transcription factors include members of the Hox (Hoxa3, which results in parathyroid agenesis, and Pbx1, a protein that acts as a cofactor of HOX transcription factors) and Pax (Pax 1,3,9) families, the eyes absent homologue (Eya1) and sine oculis homeobox homologue (Six1).[385]

Human disorders with congenital hypoparathyroidism have been found in association with mutations and deletions in *GATA3* (hypoparathyroidism, deafness, renal dysplasia [HDR] syndrome), *AIRE1* (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy [APECED] syndrome or autoimmune polyglandular syndrome type 1 [APS1]), and tubulin folding cofactor E *(TBCE)* gene (Kenny-Caffey syndrome). *TBX1* (a DNA-binding transcriptional factor of the T-box family that is known to have an important role in vertebrate and invertebrate organogenesis and pattern formation) and *CRK1* mutations have been associated with 22q11 deletion syndrome.[386]

Isolated hypoparathyroidism can be found in association with mutations in *PTH*, *GCMB* (the human homologue of the mouse glial cells missing 2 *[Gcm2]* gene, which is expressed exclusively in the parathyroid glands) and the SRY-related HMG-box gene 3 *(SOX3)*.[385] X-linked recessive hypoparathyroidism has been reported in two multigenerational, related kindreds with a deletion-insertion downstream of *SOX3* likely to exert a position effect on *SOX3* expression. These findings point to a potential role for the *SOX3* gene in the embryologic development of the parathyroid glands from the pharyngeal pouches.

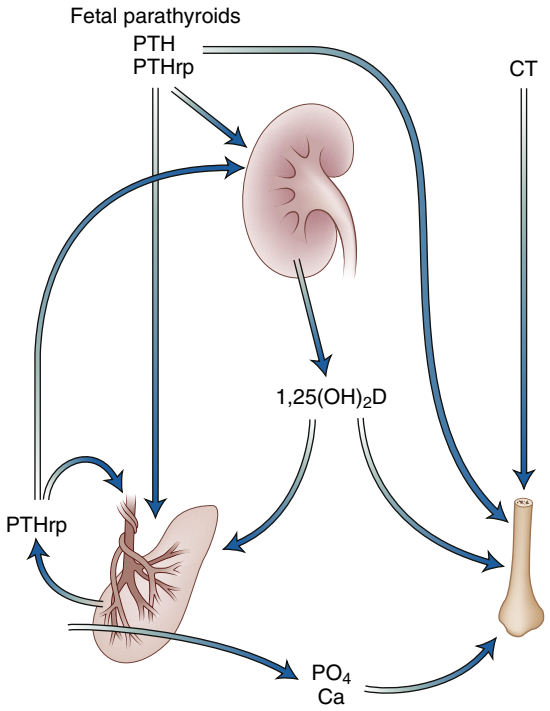
Fetal and Neonatal Calcium Metabolism

Parathyroid hormone produced by the parathyroid glands is an 84–amino acid sequence that stimulates bone resorption, renal tubular absorption, and synthesis of calcitriol in the kidneys. There is a negative feedback loop with ionized calcium and calcitriol. PTH-related protein (PTHrp) binds to and activates PTH receptors. This is expressed in the uterus, placenta, amnion, umbilic cord, lactating breast, and fetal parathyroid glands. In animal models, despite maternal hypocalcemia induced by a calcium-restricted diet, vitamin D deficiency, parathyroidectomy, or a vitamin D receptor deletion model, fetuses were reported to be normocalcemic. The calcium concentration in the fetus is therefore independent of the maternal calcium concentration (Fig. 23.13).

Studies in fetal sheep and monkeys and measurements in human preterm and term infants indicate that the majority of calcium is transported in the third trimester. Concentrations of total calcium (averaging 2.75–3 mmol/L in the third trimester) and ionized calcium, as well as phosphate and magnesium, are higher in the fetal circulation compared to maternal, demonstrating active transport of calcium across the placenta against a concentration gradient.[387,388] Nearly all the calcium transferred to the fetus is involved in mineralization of the fetal skeleton.[389]

Calcium enters the placental trophoblast at the brush-border membrane primarily via the TRPV6 channel. Transport is facilitated by calbindin D9K, and calcium is released to the fetal circulation through the calcium pump PMCA3. Inorganic phosphate enters the trophoblast probably via the sodium-phosphate cotransporter NaPiIIb.[387] A three-step model of transcellular placental calcium transport has been proposed[387,390]:

1. Influx of calcium ions from the maternal circulation involving voltage-dependent calcium channels (primarily transient



• **Fig. 23.13** Proposed actions of parathyroid hormone (PTH), PTH-related protein (PTHrp), and calcitonin (CT) in the fetus. PTHrp and perhaps PTH from the parathyroid glands and PTHrp from the placenta act on the placenta to promote calcium (Ca) and phosphate (PO_4) transport from the maternal to the fetal circulation to maintain the relative fetal hypercalcemia and the high rate of fetal bone formation during the last half of gestation. PTHrp also acts on the kidney to promote 1-hydroxylation of 25-hydroxycholecalciferol to $1,25(OH)_2D$ (1,25-dihydroxyvitamin D, calcitriol), which augments placental calcium transport and promotes fetal bone growth. High fetal CT levels tend to promote bone accretion. See text for details. (From Kovacs CS. Bone metabolism in the fetus and neonate. *Pediatr Nephrol*. 2014;29:793–803.)

receptor potential cation channel, subfamily V, member 6 [TRPV6], which is a calcium channel that opens in the maternal-facing basement membrane of the syncytiotrophoblast to enable calcium entry into cells) at the apical brush-border membrane of the trophoblast cells at the maternal-placental interface.

2. Movement of calcium through trophoblastic cytosol, involving intracellular calcium-binding proteins (mainly calbindin D9K), which transport calcium to the basal membrane.
3. Efflux of calcium ions from cytosol across the basolateral placental membrane involving a membrane ATP-dependent calcium pump (PMCA3, at the placental-fetal interface), which transports calcium into the fetal circulation.

Calcium and phosphate are maintained at a higher set-point in the fetus than in the pregnant mother, especially in the third trimester, to meet the demands of fetal bone mineralization. In late gestation, fetal serum concentrations of calcium and phosphate are maintained above the maternal concentrations by approximately 1.2 to 2 mg/dL and 1.5 mg/dL, respectively.[388] Although the fetal concentration of Mg is also increased compared with the maternal value, the gradient is less (~0.12 mg/dL)[388] than those of calcium and phosphate. Although the molecular mechanism of transplacental calcium transport has been well studied, little is known about the transport mechanism of phosphate and magnesium.

Fetal serum phosphate is also higher than maternal serum concentrations. The type IIb sodium-dependent inorganic phosphate transporter (NaPiIIb) is expressed in embryonic visceral and parietal endoderm as well as in labyrinthine cells of the placenta and likely has a role in fetal phosphate homeostasis.[387] Little is known of the mechanism of placental magnesium transport.

The placenta is impermeable to PTH, PTHrp, and calcitonin, but 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D (calcitriol) are transported across the placenta, and free vitamin D concentrations in fetal blood are similar to or higher than maternal values.[391,392]

The fetal sheep has low circulating concentrations of PTH but can increase serum PTH concentrations in response to a fall in serum calcium concentration induced by ethylenediaminetetraacetic acid (EDTA) and can respond promptly to infused calcium with increased serum calcitonin concentrations. In this model, fetal parathyroidectomy decreases placental calcium transport and lowers fetal serum calcium.

Although PTH has no effect on placental calcium transport, PTHrp is present in fetal tissues and placenta and stimulates calcium transport. The principal PTH receptor is PTHR1, which has equal affinity for PTH and PTHrp. A second receptor, PTHR2, is present in CNS and binds PTH but not PTHrp, whereas PTHrp action in the placenta occurs likely through a distinct receptor that binds PTHrp but not PTH.[387,393]

The PTH concentration in the fetus near the end of gestation is lower (<4.72 pg/mL) than maternal or nonpregnant adult concentrations. In cord blood, the concentration of PTHrp is 15-fold higher than that of PTH at term and plays a critical role in perinatal calcium homeostasis and maintenance of the higher fetal calcium set-point.[390,394] In the fetus, PTHrp is produced in many tissues, including the placenta. PTH plays an essential role in regulating calcium and bone homeostasis in the adult, but whether PTH is required at all for regulating fetal-placental mineral homeostasis and skeletal development is uncertain. It is unknown whether the human placenta produces PTH. Since maternal PTH concentrations are suppressed during pregnancy compared with those in the nonpregnant adult, fetal PTH concentrations appear to be strongly suppressed. Little PTH is detectable in fetal plasma using immunoassays, whereas bioassays showed significant bioactivity.[395] Parathyroid glands are active in the human fetus from about 12 weeks of gestation, and thyroparathyroidectomy in the fetal sheep causes a rapid decrease in fetal plasma calcium concentration and a loss of the placental calcium gradient.[391] Although the fetal PTH concentrations are very low, fetal PTH is thought to be important because fetal mice lacking parathyroid glands, PTH, or the PTH/PTHrp receptor are hypocalcemic and have undermineralized skeletons.[389,396,397] The principal factor responsible for this bioactivity would therefore appear to be PTHrp rather than PTH itself.

Other factors are involved in maintenance of fetal serum calcium concentrations because knockout of the mouse gene for PTH-PTHrp also results in hypocalcemia in the presence of normal or increased placental calcium transport.[390,398] PTH and PTHrp through the PTH/PTHrp receptor presumably modulate fetal skeletal calcium flux, calcium excretion through the fetal kidney, and perhaps reabsorption of calcium from amniotic fluid. PTHrp has a major role in fetal bone development and metabolism as well as fetal calcium homeostasis. PTHrp knockout mice display increased ossification of the basal portion of the skull, long bones, vertebral bodies and pelvic bones, and mineralization of the normally cartilaginous portions of the ribs and sternum; as

a result of the cartilaginous mineralization, the animals die of asphyxiation in the early neonatal period.[390,394,399,400]

The skeletal chondrodysplasia is more severe in combined PTH plus PTHrp or PTH/PTHrp receptor gene–ablated animals.[397,401] In the mouse, knockout of the calcitonin gene or the vitamin D receptor gene leads to postnatal osteosclerosis or osteomalacia, respectively; the pups appear normal at birth.[402] Studies have demonstrated that *Pthrp*-null fetuses had hypocalcemia (equal to maternal blood concentration), hypomagnesemia, hyperphosphatemia, reduced fetal-placental calcium transfer, normal amniotic fluid calcium content, and normal skeletal mineral content,[389] but the absence of parathyroids caused a more substantial reduction in serum calcium and skeletal mineral content than absence of PTHrp despite the fact that PTH normally circulates at low concentrations in the fetus.

PTH-null mice have enlarged parathyroids that are incapable of making PTH, whereas *Gcm2*-null mice lack parathyroids but have PTH that arises from the thymus. *PTH*-nulls served as a model of complete absence of PTH, whereas *Gcm2*-nulls are a model of severe hypoparathyroidism. Simmonds and colleagues demonstrated that PTH contributes to fetal mineral homeostasis because a fetal hypoparathyroid phenotype results with hypocalcemia, hypomagnesemia, hyperphosphatemia, low amniotic fluid mineral content, and reduced skeletal mineral content in its absence.[389] PTH regulates the placental expression of genes involved in calcium and other solute transfer and may contribute to the regulation of placental calcium transfer. PTH may contribute to placental gene expression and function through both endocrine/systemic (parathyroid-derived) and paracrine (placental-derived) pathways. Although parathyroid hormone–related protein (PTHrP) acts in concert with PTH to regulate fetal mineral homeostasis and placental calcium transfer, unlike PTH, it does not upregulate in response to fetal hypocalcemia.

The fetal parathyroid-placental axis promotes maternal-fetal transfer of bone mineral and accretion of fetal bone mineral. It seems likely that fetal PTH and presumably PTHrP act on the fetal kidney to stimulate 1α-hydroxylation of 25-hydroxyvitamin D and that 1,25-dihydroxyvitamin D participates in modulating placental calcium transport. $1,25(OH)_2D$ or $24,25(OH)_2D$ also play a role in fetal cartilage growth and bone mineral accretion.[403] Thus PTHrP and to a lesser extent PTH in the ovine fetus appear to augment maternal-to-fetal calcium transport across the placenta and thus provide for the high rate of bone mineral accretion in the latter half of pregnancy.

During pregnancy, maternal 25(OH)D crosses the placenta, so the fetal concentrations of 25(OH)D reach 75% to 100% of the maternal concentration at term.[404] Fetal $1,25(OH)_2D$ concentrations, however, are lower than that in the mother (<50%).[405] The maternal concentration of $1,25(OH)_2D$ during pregnancy is twofold to threefold higher than that in the nonpregnant adult. It is suggested that the maternal kidneys contribute to the abundance of $1,25(OH)_2D$ in the pregnant mother.[406] Nephrectomy in the fetal sheep reduces fetal serum calcium concentrations, and this can be prevented by prior administration of 1,25-dihydroxyvitamin D. Moreover, infusion into the sheep fetus of antibody to $1,25(OH)_2D$ reduced the placental calcium gradient.[391] The fetal kidney can produce $1,25(OH)_2D$ and the placenta contains $1,25(OH)_2D$ receptors, as well as a vitamin D–dependent calcium-binding protein.[400] It is thought that the synthesis of $1,25(OH)_2D$ in the fetus is suppressed by the high concentrations of calcium and phosphate and low concentrations of PTH.

The high blood concentrations of calcitonin in the fetus, probably resulting from the chronic stimulation by fetal hypercalcemia, are thought to contribute to the fetal bone mineral accretion.[391,397] The predominant effect of calcitonin is to inhibit bone resorption, and the high fetal serum calcium concentrations coupled with high circulating calcitonin promote bone mineral anabolism.[397] Placental calcitonin production may contribute to the calcitonin in fetal plasma, but the persistence of high plasma concentrations in neonatal plasma argues for predominant fetal production. Thyroid C cells begin to differentiate at around week 12 of gestation,[407] and calcitonin is detectable at around week 15 of gestation. Fetal circulating calcitonin concentrations are approximately twofold higher than those in the mother.[388,408,409] The trophoblasts of the placenta also produce calcitonin and supply it to the fetus.[410] Since calcitonin does not cross the placenta in the mouse or rat,[411] fetal circulating calcitonin is derived from fetal sources. The major source of fetal circulating calcitonin has not been determined.

Calcium-Sensing Receptor (CaSR) and FGF23

The calcium-sensing receptor plays a pivotal role in systemic calcium metabolism by modulating production and secretion of calcium-regulating hormones such as PTH, calcitonin, FGF23 and vitamin D, and urinary calcium excretion.[412,413] The CaSR is present in parathyroid glands, renal tubules, bone and cartilage, and many other tissues. Magnesium binds to the CaSR and influences PTH secretion. Mutations within the *CaSR* result in either inactivation or overactivation of the receptor resulting in hypercalcemia or hypocalcemia, respectively.[414] Inactivating mutations lead to switching off of PTH secretion at a lower calcium concentration than usual and hypercalcemia ensues. Renal calcium secretion is reduced.

Hypercalcemia disorders related to inactivating mutations of the *CASR* gene are either heterozygous (autosomal dominant familial benign hypercalcemia [FBH], still named hypocalciuric hypercalcemia syndrome type 1) or homozygous (severe neonatal hyperparathyroidism). The differential diagnosis consists of the hypocalciuric hypercalcemia syndrome, types 2 (involving *GNA11*) and 3 (involving *AP2S1*), hyperparathyroidism, abnormalities of vitamin D metabolism involving *CYP24A1* and *SLC34A1* genes, and reduced GFR.

Hypocalcemia disorders, which are rarer, are related to heterozygous activating mutations of the *CASR* gene (type 1), consisting of autosomal dominant hypocalcemia (ADH), sometimes with a presentation of pseudo-Bartter syndrome. The differential diagnosis consists of the hypercalciuric hypocalcemia syndrome type 2, involving *GNA11* and other hypoparathyroidism etiologies. An online database to keep track of described mutations is available at http://www.casrdb.mcgill.ca.

Some conditions are particularly important in the neonatal period. When a neonate presents with abnormal calcium concentrations, assessment of calcium, albumin, phosphate, creatinine, alkaline phosphatase, vitamin D and urine Ca-creatinine ratio, and phosphate reabsorption in both the child and parents will help formulate the underlying diagnosis. Hypocalcemia resulting from fetal and maternal vitamin D deficiency is relatively common. Occasionally, severe neonatal vitamin D deficiency can be associated with a dilated cardiomyopathy, which is reversible.[415,416] Asphyxia can result in subcutaneous fat necrosis, which is due to release of 1,25(OH)D from macrophages and leads to hypercalcemia. Hyperhydration, steroid therapy, low calcium milk, and occasionally bisphosphonate treatment may be needed to reduce calcium concentrations.

FBH and familial hypocalciuric hypercalcemia (FHH) can present in neonates with mild to moderately elevated calcium concentrations, high normal (nonsuppressed) PTH, and a low urine Ca-creatinine ratio.[417] FHH may also present as neonatal hyperparathyroidism leading to bone disease and variable symptoms of hypercalcemia. This occurs especially if an affected infant has inherited the inactivating *CaSR* mutation from the father while the mother is normal. The fetus will consider the mother as hypocalcemic and therefore overproduce PTH. Cinacalcet therapy may help bind calcium and settle the hyperparathyroidism but parathyroidectomy is occasionally necessary. Hypocalcemic and hypercalcemic disorders may also be due to mutations in G protein subunit alpha 11 (*GNA11*), *AP2S1* (involved in G protein internalization), and calcium channels *TRPV5* and *TRPV6*.[418,419]

FGF23 is the principal hormone that regulates phosphate transport. It is primarily produced and secreted into the circulation by osteocytes and, after its activation through glycosylation by GALNT3, acts through FGFR1c and FGFR4 on renal tubules to increase phosphate excretion through the sodium-phosphate exchanger (NaPiIIC, SLC34A3).[420] α-Klotho acts as a cofactor for FGF23 to bind to FGFR1c, increasing specificity of FGFR1c for FGF23. FGF23 is inactivated by cleavage by subtilisin/furin-like enzyme. PHEX (phosphate regulating gene with homologies to endopeptidases on the X chromosome) regulates cleavage of FGF23 and *PHEX* mutations, and therefore renders FGF23 constitutively active. Besides increasing renal tubular phosphate excretion, FGF23 inhibits 1α hydroxylase, thus reducing 1,25 vitamin D activity. Loss of FGF23 or components of the FGF23 network (*GALNT3*, *FGF23, α-Klotho*) cause hyperphosphatemia, extraskeletal calcifications, and early mortality; excess FGF23 or pathway components (*FGF23, PHEX, SLC34A3,* subtilisins *[PCSKs],* phosphate transporters *NPT2a* and *NPT2c*) cause hypophosphatemia with rickets or osteomalacia.[421] However, FGF23 may not be important during fetal development. In a study by Ma and colleagues, FGF23 deficiency (*Fgf23*-null) and FGF23 excess (*Phex*-null) did not alter fetal phosphorous or skeletal parameters.[422] Although FGF23 is present in the fetal circulation at concentrations that may equal adult values, and there is robust expression of FGF23 target genes in placenta and fetal kidneys, FGF23 itself may not be an important regulator of fetal phosphorous metabolism.

Fetal Growth

The hormones most important for postnatal growth, T_4, GH, and gonadal steroids have a limited role in fetal growth, even though TR and GHR are expressed in many embryonic tissues, including the growth plates.[1] Placental hormones, including the human GH variant and placental lactogens (hPL), play a limited role; hPL may promote early embryonic growth and may stimulate IGF and insulin production.[1] IGF1 and IGF2 are produced by the placenta and may exert autocrine-paracrine actions on placental growth. IGF1, IGF2, and the IGF1 and IGF2 receptors are widely expressed in fetal tissues of mesenchymal, ectodermal, and endodermal origin and play critical roles in modulating normal fetal growth, including the nervous system.[423,424]

Insulin-Like Growth Factors

IGFs are involved in regulation of uterine and placental growth during pregnancy. In early embryonic and fetal development, IGF1, EGF, and estrogens are mitogens for endometrial stromal cells, and the endometrial contents of *IGF1* and *IGF1 mRNA* are high at implantation and during early embryogenesis in the sow.[425] Uterine *IGF1* and *IGF1 mRNA* levels decrease progressively with advancing gestation.[425] Placental tissue also contains *IGF1* and *IGF2 mRNAs,* significant concentrations of the respective proteins, and IGF1 receptors.[25] Autocrine and paracrine roles for the IGFs in uterine and placental tissues are postulated. IGF1 and insulin are produced by embryonic tissues during the prepancreatic stage of mouse development; both factors stimulate growth of embryonic mouse cells.[426]

IGF2 is genomically imprinted and paternally expressed in the fetus and placenta. The mature IGF2 protein is generated from the biologically inactive pro-IGF2 peptide by the action of proprotein convertase 4. Previous studies have shown a role of IGF2 in determining placental nutrient supply and hence fetal growth.[427] In mutant mice lacking the imprinted placental-specific *IGF2* transcript, growth of the placenta is altered from early gestation, but fetal growth is normal until late gestation, suggesting functional adaptation of the placenta to meet the fetal demands. It is believed that this adaptation may be mediated by the altered expression of placental transporters GLUT3 and Slc38a4.[428]

Studies of transgenic mice with null mutations of the genes encoding *IGF1, IGF2,* or the *IGF1 receptor* have defined the role of these somatomedins; the birth weight of embryos lacking IGF1 or IGF2 is only 60% that of control mice. When both genes are inactive, birth weight is reduced by another 30%, and mice lacking the IGF1 receptor have birth weights averaging 45% of control values.[157] IGF2-deficient mice also manifest intrauterine growth retardation in association with a small placenta. They have near-normal postnatal growth but delayed bone development.[157] *IGF2-receptor* knockout fetal mice are 30% overweight, suggesting a negative growth-modulating effect of this receptor.

The normal growth in fetuses with both IGF1 receptor and *IGF2 receptor* knockout is caused by IGF1 signaling via the insulin receptor; combined IGF1, IGF2, and insulin receptor knockout results in severe intrauterine growth retardation and fetal death. Knockout of individual IGF-binding proteins (IGFBP) has little effect on fetal or placental growth.[157] In humans, mutations in *IGF1* or *IGF1R* are associated with intrauterine growth retardation,[429,430] suggesting that IGF1 signaling contributes significantly to fetal growth. Apart from growth retardation, these children also suffer from developmental delay, microcephaly, hypoglycemia, and sensorineural hearing loss.[428,429] Indeed, there is a relation between umbilic IGF1 concentration and birth weight in humans; maternal smoking reduces both umbilic IGF1 and birth weight.[431,432]

Recent work in mice suggests that IGF1 and IGF1R are required for late gestational lung maturation.[433] Hypomethylation of the 11p15 imprinted region has been associated with the phenotype of Silver-Russell syndrome (SRS).[434] This results in the relaxation of imprinting and biallelic expression of *H19* and downregulation of *IGF2*. Additionally, abnormal processing of IGF2 by proprotein convertase 4 in the placenta has been implicated in the etiology of fetal growth restriction.[435] Pregnant women carrying fetuses with intrauterine growth retardation (IUGR, low birth weight for gestational age) had higher concentrations of pro-IGF2 compared with controls. Recently, a genomic mutation of the human *IGF2* gene was described in four individuals from one family who exhibited severe intrauterine and postnatal growth retardation and signs of SRS.[436] The defect was a nonsense mutation with a predicted severe truncation of the prepro-IGF2; the disorder was paternally inherited and affected children had diminished IGF2 serum concentrations.

Murphy and colleagues reported severe intrauterine growth retardation and atypical diabetes mellitus secondary to insulin resistance in association with disruption of regulation of the *IGF2* gene.[437] On the other hand, overexpression of IGF2 as a result of loss of imprinting associated with uniparental disomy, *CDKN1C* gene loss of function, alteration in the KvLQT1 differentially methylated region (DMR), or microdeletions in the human H19 DMR are associated with overgrowth in the form of BWS.[438] As highlighted previously, activating mutations in the proliferating cell nuclear antigen (PCNA)–binding domain of *CDKN1C* have been shown to cause IMAGe syndrome.[181] Arboleda and colleagues demonstrated that missense mutations in the PCNA-binding domain have an inhibitory effect on growth and differentiation in vivo, via loss of binding of PCNA to CDKN1C.[181] The contrast between BWS and IMAGe mutations in *CDKN1C* highlights the dual and opposing effects of specific *CDKN1C* mutations.

IGF-binding proteins (BPs) are present as early as 5 weeks of gestation; prenatally as postnatally, the IGFs circulate in association with binding proteins.[157] High concentrations of circulating IGFBP1 are associated with fetal growth restriction in the mouse, as is overexpression of fetal IGFBP1 in the human.[439,440] IGFBP4, expressed in the maternal decidua and cleaved by its protease PAPP-A (pregnancy-associated plasma protein-A), inhibits IGF action. High maternal concentrations of IGFBP4 were recently shown to be related to fetal growth restriction.[435] During fetal and postnatal life, plasma concentrations of IGFs are relatively high compared with tissue concentrations.

In the fetus, IGF2 concentrations are five to six times higher than those of IGF1 in contrast to these concentrations in children and adults; concentrations of both increase progressively throughout gestation,[441] and fetal concentrations at term are 30% to 50% of the adult concentrations. The metalloproteinase pregnancy-associated plasma protein-A2 (PAPP-A2) regulates IGFBPs and is hypothesized to increase IGF1 bioactivity by specific proteolytic cleavage of IGFBP3 and 5. Mutations in *PAPPA-A2* have recently been shown to cause short stature due to low IGF1 availability as part of a novel syndrome of growth failure.[442] The two mutations described by Dauber and colleagues were shown to cause a complete absence of PAPP-A2 proteolytic activity, a significant increase in bound IGF1 and decreased IGF1 concentrations, highlighting the critical role of PAPP-A2 in releasing IGF1 from its BPs.[442]

In most studies, cord blood IGF1 concentrations correlate with birth size. Despite the fetal growth–enhancing effects of IGF2, blood concentrations are only weakly related to size at birth, largely because of the inhibiting effect of soluble IGF2 receptor (IGF2R)[424] but also because IGF2 appears to exert most of its growth effects in the earlier part of gestation. Soluble IGF2R is derived through proteolytic cleavage of the transmembrane region of the receptor in many tissues. IGF receptors have been identified as early as 5 weeks of gestation and are widespread in fetal tissues.[443] IGF1 stimulates glycogenesis in cultured fetal rat hepatocytes and induces formation of myotubes in cultured myoblasts. IGF2 is active in cultured muscle and neonatal rat astroglial cells. Insulin receptors are increased in fetal cells and are resistant to downregulation.

The control of IGF production differs in fetal and postnatal life. GH receptors are present, but receptors for hPL predominate in fetal tissue[61]; GH, which stimulates IGF1 production after birth, has a limited role in fetal IGF production.[423] GH does play a minor role in fetal growth, as reflected in the low IGF concentrations and slight reduction in birth weight and length in infants with GH resistance (Laron dwarfism) and growth hormone deficiency.[444] hPL stimulates IGF1 production and augments amino acid transport and DNA synthesis in human fetal fibroblasts and muscle cells.[61] IGF1 and IGF2 concentrations are reduced in fetuses of protein-starved pregnant rats, and the low IGF2 concentrations are reversed by hPL.[445]

Thyroidectomy of the third-trimester sheep fetus impairs skeletal muscle growth in association with a decrease in muscle *GH receptor mRNA* and *IGF1 mRNA* without an effect on IGF2 concentrations.[446] Glucocorticoids can inhibit fetal growth, presumably by inhibiting *IGF* gene transcription, but may also affect growth plate chondrocytes directly.[446] Indeed, nutrition is the major factor modulating IGF production in the fetus. IGF concentrations fall in suckling rats deprived of milk, and IGF1 and IGF2 concentrations are reduced in fetuses of protein-starved pregnant rats and placentally restricted sheep.[156,157] Recent work suggests that light stimuli alter circulating and brain IGF1 concentrations and control neuronal migration through increased IGF1 signaling.[447] Weekly intra-amniotic injections increase fetal growth of intrauterine growth–restricted sheep.[448] These data support the view that IGFs are important in embryonic and fetal growth and that in the fetus they are regulated, at least in part, by hPL and by nutritional substrate derived transplacentally. The high concentrations of IGF2 in fetal rat serum, the high concentrations of *IGF2* mRNA in fetal tissues, and the presence of a truncated form of IGF1 in human fetal brain tissue suggest unique developmental actions of these peptides.

Insulin

Insulin has been proposed to act as a fetal growth factor. Infants born to women with diabetes mellitus may have hyperinsulinemia associated with increased birth weight.[449] Most of this increased weight is accounted for by body fat; there is little increase in body length, but some organomegaly may occur. Infants with hyperinsulinemia caused by congenital hyperinsulinism or the BWS may also have increased somatic growth in utero; the human neonate is born large for gestational age, primarily due to increased lipogenesis mediated by insulin or IGF1 receptors. Conversely, the human fetus with pancreatic agenesis is small and has decreased muscle bulk and little or no adipose tissue.[449]

Mice with insulin or insulin receptor gene mutations have a 10% decrease in birth weight and early neonatal death with hyperglycemia and ketonemia.[157] Insulin receptor mutations in humans lead to severe intrauterine growth retardation and limited postnatal weight gain.[157] In contrast to mice, the human fetus during the latter half of gestation has a significant increase in adipose mass, and adipose tissue is highly sensitive to insulin. Treatment with IGF1 improves the clinical condition to some extent.[450]

Epidermal Growth Factor/Transforming Growth Factor

The EGF/TGFα system has been characterized in considerable detail.[451,452] EGF is a 6-kDa peptide product of a large, 1207–amino acid precursor molecule and acts through a 170-kDa membrane receptor glycoprotein. This has intrinsic tyrosine kinase activity like the IGF receptor, and tyrosine kinase–mediated autophosphorylation is a critical event in EGF signal transduction. TGFα, which has 35% amino acid homology with murine EGF and 44% homology with human EGF, also acts through the EGF receptor system.[453,454] Several additional family members have

been characterized, including amphiregulin, heparin-binding EGF, betacellulin, and neuregulins.[452] Three additional receptors are referred to as ErbB2, ErbB3, and ErbB4 in animals; the human receptors are called human EGF receptor 2 (HER2), HER3, and HER4.[452]

EGF, pre-pro-EGF mRNA, and EGF receptors are present in most tissues in the postnatal rodent, but mRNA levels are highest in salivary glands and kidneys. EGF and pre-pro-EGF mRNA levels are absent or low in the fetal mouse and remain low in mouse tissues during the early neonatal period.[453] Nonetheless, the EGF receptor knockout mouse exhibits epithelial immaturity and multiorgan failure with early death.[453] Tissue concentrations of EGF mRNA increase in the mouse during the first 2 months of postnatal life; indeed, concentrations of EGF in the salivary glands increase 1000s-fold between 3 weeks and 3 months of age. Mouse urinary levels increase 200-fold and kidney concentrations increase 10-fold between 1 week and 2 months of age. EGF concentrations in mouse ocular tissues increase 100-fold during the first week of life.[452] Liver EGF concentrations increase more slowly, as do serum concentrations, and there is a high degree of correlation between serum and liver EGF concentrations in the developing mouse.[452] Therefore the production of EGF in the rodent is accelerated during the early neonatal period, and it is during this time that most hormone-stimulated growth and development occur.

Fetal mouse and human tissues have high concentrations of TGFα.[451,454,455] Immunoreactive TGFα concentrations in mice are measurable at relatively high levels in lung, brain, liver, and kidney tissues in the fetal/neonatal rat, and the ontogenic pattern of TGFα is tissue specific; most late fetal tissues studied contained TGFα, and levels persisted or increased in most tissues through the period of growth and development.[454]

In rodents and sheep, EGF provokes precocious eyelid opening and tooth eruption in neonatal animals, stimulates lung maturation, promotes palatal development in organ culture, stimulates gastrointestinal maturation, evokes secretion of pituitary hormones (including GH, PRL, and corticotropin), and stimulates secretion of chorionic gonadotropin and placental lactogen by the placenta.[452,453]

Both EGF and TGFα compete for binding to the EGF receptor, and both factors accelerate eye opening and tooth eruption in the neonatal rodent, presumably through interaction with the same EGF receptor.[452] Considerable evidence suggests a role for the EGF family of growth factors in mammalian CNS development.[456] EGF, TGFα, neuregulins, and the EGF receptors are widely distributed in the nervous system.[452,457–460] EGF promotes proliferation of astroglial cells, acts as an astroglial differentiation factor, and enhances survival and outgrowth of selected neuronal cells.[457,458] Transgenic mice with a deficiency of neuregulin, ErbB2, ErbB3, or ErbB4 die in utero with cardiac anomalies and developmental anomalies of the hindbrain, midbrain, and ventral forebrain.[459,460]

EGF also plays an important role in rodent pregnancy. Maternal salivary gland and plasma EGF concentrations in the mouse increase fourfold to fivefold during pregnancy.[461] Removal of the salivary glands prevents the increase in plasma EGF and reduces the number of mice completing term pregnancy by 50%, decreases the percentage of live pups, and decreases the crown-rump length of fetuses delivered.[461] Administration of EGF antiserum to pregnant mice without salivary glands further increases the abortion rate, whereas administration of EGF improves pregnancy outcome.[461] Because maternal EGF is too large a molecule to traverse the placental barrier, an effect on maternal metabolism or on the placenta is likely.[461] The placenta is richly endowed with EGF receptors, and placental tissue binds and degrades EGF to constituent amino acids.[452] TGFα is also produced by the maternal decidua in rodents and stimulates proliferation of decidual tissue and decidual PRL production.

Other Factors

Additional growth factors involved in fetal growth and development include hematopoietic growth factors, platelet-derived growth factors (PDGFs), fibroblast growth factors, vascular endothelial growth factor (VEGF), and members of the TGFβ family.[157,462] The TGFβ superfamily of extracellular growth factors comprises more than 35 members, including TGFβ, the bone morphogenetic proteins (BMPs), growth and differentiation factors, activins, inhibins, müllerian-inhibiting substance, Nodal, and Lefty.[463] These ligands activate 12 transmembrane serine/threonine kinase receptors expressed in a variety of tissues. The family is critical for early embryonic development, left-right asymmetry, heart and vascular system development, craniofacial development, nervous system development, and skeletal morphogenesis and plays an important role in body composition and growth.

Hematopoietic growth factors are also active in the fetus during development; erythropoietin in fetal sheep is produced by the liver rather than the kidney, and erythropoietin gene expression in fetal sheep is regulated by glucocorticoids.[464] A switch to kidney production occurs after parturition.[465] Postnatally, thyroid hormones, testosterone, and hypoxia modulate erythropoietin production. PDGF represents a family of homodimers and heterodimers of PDGFA and PDGFB chains derived from two gene loci.[466] Two PDGF receptors have been characterized, PDGFα and PDGFβ. The genes for PDGF and its receptors are expressed in many tissues. *PDGFA* gene inactivation in mice leads to defects in lung, skin, intestine, testes, and brain resulting in early postnatal death.[466] *PDGFB* gene inactivation leads to microvessel disruption and leakage with hemorrhage, edema, and intrauterine death.

The FGF family of heparin-binding growth factors now includes 17 members with diverse effects on development, angiogenesis, wound healing, and other biologic systems.[467,468] These effects are mediated by ligand-activated tyrosine protein kinase receptors (FGFRs) transcribed from four related genes. Several receptor isoforms are products of alternative RNA splicing.[157,451] Targeted disruptions of *FGF* and *FGFR* genes in mice have defined critical roles in development.[157,467] FGF3-deficient mice show tail and inner ear defects. Knockout of the *FGF4* gene is lethal, leading to early death. Knockout of the *FGFR1* gene also leads to early fetal death. *FGF10* knockout mice die at birth because of pulmonary agenesis. Deficiency of FGF4, FGF8, FGF9, FGF10, or FGF17 is associated with limb deformities. FGF8 deficiency leads to abnormal left-right axis patterning. In mice, *FGFR3* knockout results in chondrocyte hypertrophy and increased bone length.[451] In humans, a variety of gain-of-function *FGFR* mutations are associated with chondrodysplasias and craniosynostosis syndromes.[451] On the other hand, loss-of-function mutations in both *FGFR1* and *FGF8* are associated with Kallmann syndrome or hypogonadotrophic hypogonadism; *FGF8* mutations are also associated with holoprosencephaly.[62,469,470] FGF, like EGF, stimulates the production of hCG from a choriocarcinoma cell line.[465] FGF21 is mainly produced by the liver but is also expressed in adipocytes and pancreas, and regulates glucose and lipid metabolism through pleotropic actions in these tissues and the brain.

During calorie restriction in mice, increased FGF21 causes growth attenuation and GH insensitivity. Additionally high concentrations of FGF21 may directly suppress growth plate chondrocyte proliferation and differentiation. A negative correlation between FGF21 concentration and linear growth has been reported, and high levels of FGF21 were associated with growth failure in preterm infants.[471] The mechanisms might involve GH resistance by GH-induced SOCS2 expression (which negatively regulates GH signaling) and inhibiting GH-induced signal STAT5 phosphorylation. These observations and the fact that the placenta contains FGF, NGF, TGFα, TGFβ, IGF1, and IGF2 suggest that the placenta plays an important role in modulating fetal growth.

Lastly, Wnt signaling, Notch signaling, BMP signaling, and hedgehog signaling play major roles in embryogenesis and fetal organ growth and development. These signaling pathways are also involved in bone development and growth and thus have a major effect on fetal size.[472]

Neutralization of Hormone Actions in the Fetus

After the period of embryogenesis, the fetal milieu is programmed to optimize body growth and organ development through an array of generalized and specialized growth factors with substrate supply maintained by the placenta. The endocrine and metabolic systems characterizing the extrauterine environment are programmed to maintain metabolic stability in a changing external environment. Hormonal systems in the fetus are programmed to maintain anabolism with minimal hormonal perturbation. Therefore production of catabolic and thermogenic hormones is limited, and the effects of the hormones altering metabolic substrate supply and distribution pathways are muted (Table 23.6).

Limitation of Hormone Secretion

The human fetal pancreas is functional during the second trimester, but secretion of insulin in response to glucose or pyruvate is minimal until the neonatal period.[354,355] Glucagon secretion is also blunted, although fetal blood glucagon concentrations are relatively high. Fetal islet hyperplasia and increased insulin secretion occur in response to chronic hyperglycemia (e.g., in the infant of a diabetic mother), and insulin release can be stimulated by acute fetal infusions of leucine, arginine, or tolbutamide.[355,358] Moreover, responsiveness of both insulin and glucagon secretion to glucose develops rapidly in the neonatal period. It is not clear whether the limited fetal islet cell responsiveness results from the relatively stable fetal serum glucose concentrations or from a temporally fixed maturation process.

Production of Inactive Hormone Metabolites

Throughout the second half of gestation, cortisol is metabolized in fetal tissues to inactive cortisone through the activity of 11βHSD2. The placenta is permeable to steroid hormones, including cortisol. During midgestation, placental 11βHSD2 activity is low, and some cortisol is transferred to the fetus. Placental 11βHSD2 activity increases during the second half of pregnancy under the control of placental estrogens, and enzyme activity near term is high.[25,194] Maternal-fetal cortisol transfer decreases progressively. In addition, although many adult tissues can convert cortisone to cortisol, conversion is limited during most of fetal life. Consequently, most of the cortisol that crosses the placenta or is produced by the fetus is inactivated to cortisone by the placenta or by fetal tissues.

Concentrations of cortisone in fetal plasma exceed those of cortisol by threefold to fourfold until after 30 weeks' gestation (see Fig. 23.5). Teleologically, this would help preserve the anabolic and growth-promoting milieu of the fetus and minimize premature maturational and parturitional effects of cortisol. After 30 weeks, the ratio of cortisol to cortisone in fetal tissues and plasma increases as a result of increased fetal secretion and decreased conversion of cortisol to cortisone within the placenta and fetal tissues.[194] Cortisol has an important maturational action on several fetal tissues near term.

Fetal thyroid hormone metabolism is characterized by conversion of active thyroid hormones to inactive rT_3 and inactive sulfated iodothyronines and by limited receptor and postreceptor responsiveness to thyroid hormone in selected tissues.[219,232] The placenta contains an iodothyronine inner-ring monodeiodinase that catalyzes conversion of maternal T_4 to rT_3. The fetal sheep liver and kidney, in contrast to the adult liver and kidney, manifest low concentrations of D1 outer-ring monodeiodinase activity, so conversion of T_4 to active T_3 is limited, and large amounts of inactive iodothyronine sulfoconjugates accumulate.[219,233] Consequently plasma T_3 concentrations in the fetus remain low until the last few weeks of gestation (see Fig. 23.8). Selected fetal tissues (brain, brown adipose tissue) have active D2 outer-ring monodeiodinase activities that contribute to local tissue T_3 concentrations; local T_3 is important in development, particularly in the hypothyroid fetus.[219,473] Near term and in the neonatal period in the human fetus, the dramatic increase in plasma T_3 concentrations, and presumably in T_3 production,

TABLE 23.6 Neutralization of Hormone Actions in the Fetus

PRODUCTION OF INACTIVE METABOLITES	
Active Hormone	**Inactive Metabolites**
Cortisol	Cortisone
Thyroxine (T_4)	rT_3, T_4S, rT_3S
Triiodothyronine (T_3)	T_3S, T_2

DELAYED EXPRESSION OR NEUTRALIZATION OF RECEPTORS	
Active Hormone	**Receptor**
Growth hormone (GH)	GHR
Thyroid hormone	TRα, TRβ
Catecholamines	βAR
Estrogens	ERα, ERβ
Glucagon	GR

LIMITED HORMONE SECRETION	
Active Hormone	**Secretory Cell**
Insulin	Islet cell β
Glucagon	Islet cell α

AR, Adrenergic receptor; *T_2*, diiodothyronine; *rT_3*, reverse T_3; *T_4S*, T_4 sulfate.

heralds the onset of thyroid hormone actions on growth and development and on metabolism (see Fig. 23.8).

Neutralization of Receptor Response

Selected ovine fetal tissues seem relatively unresponsive to thyroid hormones. Fetal ovine liver and kidney thermogenesis as evidenced by oxygen consumption, sodium-potassium pump (Na^+, K^+-ATPase) activity, and mitochondrial α-glycerophosphate activity is unresponsive to exogenous T_3 during the third trimester, and thyroid hormone responsiveness in a number of tissues (cardiac, hepatic, renal, and skin) develops only during the perinatal period.[474] β-Adrenergic receptor binding in heart and lung of the ovine fetus is unresponsive to T_3 late in the third trimester but increases in response to T_3 in the neonatal period.[21,474] In rodent species, in which development at birth is comparable to human fetal development at midgestation, pituitary GH concentrations become responsive to thyroid hormone only during the first weeks of extrauterine life.[475] Mouse submandibular gland EGF and NGF concentrations become responsive to thyroid hormone during the second week of life, as do urine and kidney EGF concentrations and hepatic EGF receptor levels.[476,477] Mouse skin EGF levels and EGF receptors are responsive during the first neonatal week.[478,479] Therefore, despite the presence of nuclear T_3 receptors in significant concentrations in developing rat and sheep fetuses, many thyroid hormone actions in these species are delayed.[480] The mechanism of this delayed thyroid hormone responsiveness is not clear; developmental programming of iodothyronine monodeiodinase expression and gene expression programming via unliganded TRs or TR-interacting corepressors probably all play a role.

The effect of high circulating concentrations of GH in the fetus is also limited. Fetal somatic growth is only partially GH dependent; indeed, the GH-deficient fetus has little or no growth retardation.[61,444] The paucity of fetal GH effects is due to delayed maturation of GH receptors or postreceptor mechanisms. In animals such as sheep, hepatic GH receptor binding appears only during the neonatal period.[61] Receptor deficiency may also be a factor in the limited PRL bioactivity in the fetus near term.[61]

There is less information on fetal hormone responsiveness in other systems. β-Adrenergic receptor binding in heart and lung of the sheep fetus is relatively low near term and increases in the neonatal period in response to thyroid hormones.[474] Premature lambs have an augmented plasma catecholamine surge at birth but have a relatively mild increase in plasma free fatty acid concentrations, which suggests reduced catecholamine responsiveness.[481] The high concentrations of progesterone and estrogens in fetal blood also seem to have limited effects in the fetus. Progesterone receptors are present in low concentration in fetal guinea pig kidney, lung, and uterus at midgestation and increase progressively until term.[482] ERs appear in neonatal rat uterus, oviduct, cervix, and vagina during the first 10 days of extrauterine life, and both *ERα* and *ERβ* mRNAs are present in human fetal tissues during the second trimester.[312,483] The human neonate often manifests mild breast enlargement at birth, and vaginal estrogenization may be evident in female infants at birth. Estrogen effects otherwise appear to be limited (see Table 23.6).

Programming of Fetal Endocrine Systems

It is becoming increasingly evident that the early environment can have a significant impact on the health of an individual throughout a lifetime. During the past several decades, the concept of the plasticity of fetal endocrine systems has evolved from experiments in several mammalian species indicating that hormonal programming occurs during a critical fetal or perinatal period of development. Our understanding of the mechanisms of programming is expanding with epigenetic processes clearly being implicated.

There is a growing list of examples. In the female rodent, transient neonatal androgen administration masculinizes the pattern of hypothalamic control of GnRH secretion and pituitary gonadotropin secretion. Furthermore, masculinization of adult behavior and sexual activity is seen, and the pattern of GH secretion is permanently altered with an increase in longitudinal bone growth and body weight.[484,485] Prenatal androgens program the timing of neuroendocrine puberty in sheep: The higher the dose of prenatal testosterone, the earlier the initiation of the pubertal LH rise.[486] Estrogen administration to pregnant rats during the last third of gestation produces cryptorchid male offspring and may permanently suppress spermatogenesis in adult males.[487] Transient levothyroxine administration to neonatal rodents leads to growth retardation, delayed puberty, decreased adult pituitary weight, decreased pituitary TRH concentrations, low serum thyrotropin levels, and decreased thyrotropin responsiveness to propylthiouracil challenge.[488,489] Administration of insulin or alloxan to neonatal rats produces permanent alteration of glucose tolerance.[490] A single dose of vasopressin to the neonatal rat permanently enhances the adult response to vasopressin.[5,32] Fetal exposure to high maternal glucocorticoid concentrations in the rat inhibits fetal growth and leads to subsequent hypertension in the offspring.[9] Moreover, it has been observed that the permanent programming can be transmitted to later generations, leading to the concept of epigenetic effects.[490,491]

The concept of fetal programming was extended with the observation of ecologic associations between fetal and early-life health indicators (e.g., birth size, infant mortality) and adult diseases. The concept advanced in the 1980s that adult diseases have fetal and perinatal genesis has been referred to as the Barker hypothesis.[492] There is now extensive documentation of the association of IUGR with an increased risk of later hypertension, insulin resistance, diabetes, and cardiovascular and coronary heart disease.[493–500] The programming involves epigenetic, neuroendocrine, hormonal receptor, and metabolic alterations involving the placenta and fetus.

Growth of the fetus involves complex interactions between epigenetic and genetic factors. Furthermore the impact of the environment is being increasingly recognized. It has been shown that airborne pollutants such as cadmium and cigarette smoke may alter thyroid hormone function, which is crucial for growth. A recent study examining the role of fine particle air pollution reported that fine particles with a diameter under 2.5 μm ($PM_{2.5}$) during the third trimester impacted on fetal thyroid function.[501] In cord blood, TSH and FT_4:T_3 ratio decreased with increasing exposure to $PM_{2.5}$ possibly via modulation of the hypothalamic-pituitary-thyroid axis indirectly via glucocorticoid activity as previously shown.[502]

An increasing range of epigenetic effects and corresponding clinical syndromes have been identified over recent years. Epigenetic effects include genetic imprinting. Imprinted genes are a class of genes in placental mammals and marsupials whose expression depends on the parental origin; they are expressed only from the paternal or the maternal gene copy but not biparentally. To date, more than 150 human genes have been shown to be imprinted,[503] but there are likely to be more.

Four classes of molecular changes are described in most imprinted disorders: uniparental disomy, chromosomal imbalances, aberrant methylation (epigenetic mutation), and genomic mutations in imprinted genes. These all alter expression of imprinted genes, but it is the parental allele affected by the mutation that determines phenotype. So far, genomic point mutations in imprinted genes have been reported only for Beckwith-Wiedemann, Silver-Russell, and Angelman syndromes, precocious puberty, and pseudohypoparathyroidism (PHP). Imprinting is controlled epigenetically (by factors such as nutrition) via DNA methylation, post-translational histone modification, chromatin structure, and noncoding RNAs. Imprinted loci often comprise several genes under epigenetic regulation leading to stage-specific and tissue-specific transcriptional activity in cells with identical DNA sequences. In the majority of imprinted disorders only the disease-specific loci are affected, but an increasing number have been reported to exhibit multilocus methylation imprinting disturbance (MLID),[504] the mechanism of which is at present unknown.

Many of the imprinted genes are involved in the control of fetal growth.[505] Paternally expressed imprinted genes tend to enhance fetal growth, and maternally expressed genes tend to suppress it. Knockout of paternally expressed genes for *IGF2, PEG1, PEG2,* and insulin results in IUGR, whereas knockout of the maternal genes *H19, IGF2R,* or overexpression of *IGF2* results in fetal overgrowth.[505] More recently, the identification of an *IGF2* mutation in patients with growth restriction indicates that IGF2 not only mediates prenatal growth but also contributes to postnatal growth and has pleiotropic effects.[436] A role of *IGF2* mutations in both overgrowth and growth restriction phenotypes is conceivable, as has been shown for functionally opposing mutations in *CDKN1C* in 11p15.5.[506] A loss of DNA methylation (LOM) at the *H19/IGF2* domain has been identified in over 50% of patients with SRS.[507] In contrast, gain of methylation at this domain is found in 10% of patients with BWS. Other genetic alterations, including modification of tandem repeats in the insulin gene, have been described.[508]

Hormones in the fetus are derived from the placenta, from the mother, from fetal endocrine glands, and from circulating precursors in fetal or placental tissues. These extensive networks linking maternal-placental-fetal endocrine interactions and the apparent plasticity of developing endocrine and metabolic systems facilitate endocrine system programming. As discussed earlier, the programming may be relatively system limited. Other examples include the observation many years ago that diethylstilbestrol administration to pregnant women increased the prevalence of vaginal adenocarcinoma in female offspring during the second and third decades of life.[498] More recently, it was shown that prenatal or neonatal diethylstilbestrol exposure in hamsters and mice perturbs normal uterine development by affecting the genetic pathways programming uterine differentiation and results in hyperplastic and neoplastic uterine lesions with increased levels of cJun, cFos, Myc, Bax, and Bclx.[509,510]

Excessive androgen exposure during fetal life has been associated with later polycystic ovary syndrome.[498] Hormonal programming is also demonstrable in cell lines and in unicellular organisms, in which a single exposure to a hormone can produce persistent alteration of the hormonal response characteristics or of prohormone processing.[511,512] Undernutrition during pregnancy in the rat results in the development of obesity, hyperinsulinemia, and hyperleptinemia during adult life; this phenotype is potentiated when the offspring are fed a high-fat diet. Neonatal leptin treatment normalized the programmed phenotype, indicating that metabolic programming may be reversible during the period of developmental plasticity.[513]

The effects of maternal undernutrition and fetal IUGR extend to several systems, and it is hypothesized that excessive maternal-fetal glucocorticoids play a significant programming role. Glucocorticoids have wide-ranging effects in the fetus, altering receptors, enzymes, ion channels, and transporters in a variety of cells and tissues in the late-gestation fetus, and they can induce programming of other endocrine systems. Throughout gestation, they modify *GLUT* gene expression in placenta and fetus, influence IGF and glucocorticoid receptor gene expression in various tissues, affect expression of several transcription factors, and affect a wide variety of enzymes in placenta, liver, kidney, intestine, and lung.[514] Intrauterine exposure to stress or high levels of endogenous or exogenous glucocorticoids has been shown to have molecular and structural impact on brain development, particularly limbic regions, impairing cognition and increasing anxiety and reactivity to stress.[515] Furthermore, recent studies have begun to show transgenerational effects in the rat model where in utero exposure to glucocorticoids resulted in two generations of offspring exhibiting decreased weight at birth compared to controls as well as abnormal glucose tolerance and behavior modifications.[491,516] These programming effects were transmitted by either maternal or paternal lines implying an epigenetic mechanism. The HPA axis seems to play an important role in the fetal programming of adult disease, but the pathologic mechanisms are largely unknown. In very preterm babies less than 32 weeks of gestation, a relative adrenal insufficiency is observed after birth, while adverse effects of chronic glucocorticoid excess are observed in adult life.[517,518] Maternal undernutrition, stress, and placental dysfunction are associated with increased maternal and fetal glucocorticoid concentrations, which contribute importantly to IUGR and programmed alterations in adult endocrine systems and metabolism.[496,514,519] Preweaning growth hormone treatment during a critical developmental window has been shown to prevent maternal undernutrition changes in postnatal growth pattern and related adiposity, normalizing body growth trajectory and reversing metabolic dysregulation in adult offspring.[520] These findings suggest that early intervention on the GH-IGF1 axis may attenuate or prevent long-term consequences of fetal programming.

Transition to Extrauterine Life

The transition to extrauterine life involves abrupt delivery from the protected intrauterine environment and succor by the placenta into the relatively hostile extrauterine environment. The neonate must initiate air breathing and defend against hypothermia, hypoglycemia, and hypocalcemia as the placental supply of energy and nutritional substrate is removed. Both the adrenal cortex and the autonomic nervous system, including the para-aortic chromaffin system, are essential for extrauterine adaptation. Longer term transition requires adaptation to an environment of intermittent nutrient supply and transient substrate deficiency and requires maturation of the secretory control mechanisms for the PTH-calcitonin system and the endocrine pancreas.

Cortisol Surge

In most mammals, a cortisol surge occurs near term and is mediated by increased cortisol production by the fetal adrenal and a decreased rate of conversion of cortisol to cortisone. Pepe and

Albrecht have proposed that the preterm fetal cortisol surge is due to the progressive stimulation by estrogens of placental 11βHSD2 activity and the subsequent increase in placental conversion of cortisol to cortisone.[194] The resulting decrease in maternal-to-fetal cortisol transfer results in stimulation of fetal CRH and corticotropin secretion through the negative-feedback control loop. The concomitant estrogen-stimulated increase in 11βHSD2 activity in fetal tissues potentiates the relative fetal cortisol deficiency and the CRH-corticotropin response.[194] Placental CRH may also potentiate fetal adrenal activation. Recent data suggest an increase in 11βHSD1 expression and activity in placenta and intrauterine fetal membranes during late gestation, with a consequent increase in local cortisol production in preparation for parturition.[521] The cortisol surge results in a number of actions (Fig. 23.14)[522–524]:

- Augments surfactant synthesis in lung tissue
- Increases lung liquid reabsorption
- Increases adrenomedullary PNMT activity, which in turn increases methylation of NE to epinephrine
- Increases hepatic iodothyronine outer-ring monodeiodinase activity and hence conversion of T_4 to T_3
- Decreases sensitivity of the ductus arteriosus to prostaglandins, facilitating ductus closure
- Induces maturation of several enzymes and transport processes of the small intestine
- Stimulates maturation of hepatic enzymes

In some cases, these events involve increased synthesis of specific proteins or enzymes. In other instances, such as the action on the ductus arteriosus, the mechanism remains obscure.

Secondary effects of cortisol also promote extrauterine adaptations. The increased T_3 concentrations stimulate β-adrenergic receptor binding, potentiate surfactant synthesis in lung tissue, and increase the sensitivity of brown adipose tissue to NE. The significance of prenatal cortisol is demonstrated by the effects of gene-targeted CRH or glucocorticoid receptor deficiency in mice; the progeny of homozygous CRH-deficient or glucocorticoid receptor–deficient animals die in the first 12 hours with lung dysplasia and surfactant deficiency.[525,526]

The adaptational effects of the prenatal cortisol surge have led to the current recommendation for prenatal corticosteroid therapy in pregnancies threatened by the risk of preterm delivery. Generally, preterm infants prenatally exposed to augmented glucocorticoid concentrations have lower overall morbidity and mortality than untreated infants.

Catecholamine Surge

Parturition also evokes a dramatic catecholamine surge in the newborn, resulting in extraordinarily high concentrations of NE, epinephrine, and dopamine in cord blood.[319] As discussed earlier, plasma NE concentrations exceed epinephrine concentrations because of peripheral and adrenomedullary and para-aortic catecholamine release. Cord blood NE concentrations of 15 nmol/L (2500 pg/mL) and epinephrine concentrations of 2 nmol/L (370 pg/mL) are common after spontaneous delivery of term infants.[319] Concentrations of 25 nmol/L (4200 pg/mL) of NE and 35 nmol/L (640 pg/mL) of epinephrine are common in cord blood of premature infants. These changes evoke critical cardiovascular adaptations, including increased blood pressure and cardiac inotropic effects and glucagon secretion, decreased insulin secretion, increased thermogenesis in brown adipose tissue, plasma free fatty acid concentrations, and pulmonary adaptation, including mobilization of pulmonary fluid and increased surfactant release.[319,521]

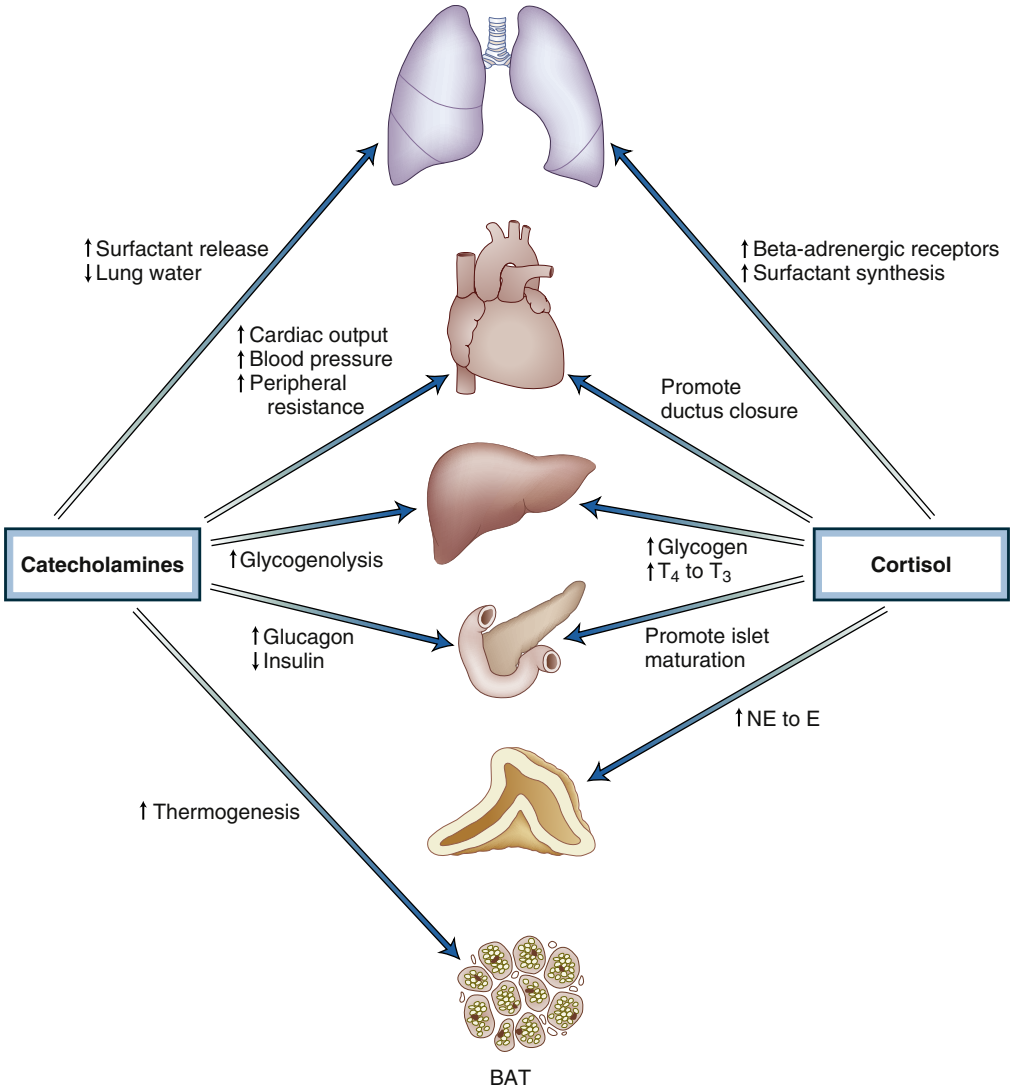
Thermogenesis in Neonatal Brown Adipose Tissue

Brown adipose tissue is the major site of thermogenesis in the newborn and is especially prominent in the mammalian fetus. The largest accumulations of brown adipose tissue envelop the kidneys and adrenal glands, and smaller amounts surround the blood vessels of the mediastinum and neck.[234] The mass of brown adipose tissue peaks at the time of birth and gradually decreases during the early weeks of life. Surgical removal of this tissue leads to neonatal hypothermia. NE, through β-adrenergic receptors, stimulates thermogenesis by brown adipose tissue, and optimal responsiveness of this tissue to NE is dependent on thyroid hormone.[527] Brown adipose tissue is rich in mitochondria containing a unique 32-kDa protein (thermogenin) that uncouples oxidation and phosphorylation of adenosine diphosphate, reduces ATP production, and consequently enhances thermogenesis.[234] Thermogenin is T_3 dependent, and brown adipose tissue contains a 5′-monoiodothyronine deiodinase that deiodinates T_4 locally to T_3.[234] Full maturation of catecholamine-stimulated cellular respiration in brown adipose tissue occurs before delivery in the ovine fetus and requires thyroid hormone.[234] Fetal thyroidectomy in this species leads to marked hypothermia, with low plasma free fatty acid concentrations and increased plasma epinephrine concentrations.[528] Basal brown adipose tissue thermogenesis and NE-stimulated and dibutyryl cAMP-stimulated thermogenesis are decreased by fetal thyroidectomy.

The rapid onset of thermogenesis in brown adipose tissue is essential for survival in newborn infants. Catecholamine release is the stimulus for brown adipose tissue thermogenesis in the early neonatal period, and responsiveness to catecholamines is markedly increased by cutting of the umbilical cord.[527] Fetal hypoxia and placental inhibitors, including prostaglandin E_2 and adenosine, appear to inhibit brown adipose tissue thermogenesis in utero.[527] Cord cutting, neonatal cooling, catecholamine stimulation, and augmented conversion of T_4 to T_3 in brown adipose tissue in the neonatal period are the essential features that mediate and condition newborn thermogenesis. It was previously thought that brown adipose tissue involuted soon after birth, but combined ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET) and CT scans have identified active brown adipose tissue in adults and shown a strong positive correlation between brown adipose tissue activity and the basal metabolic rate.[529–531]

Calcium Homeostasis

In utero, active calcium transport from the mother to the fetus takes place through the placenta, responsible for approximately 20% to 30% of calcium stores in the fetus. The neonate must adjust rapidly from a high-calcium environment regulated by PTHrp and calcitonin to a low-calcium environment that requires regulation by PTH and vitamin D. With removal of the placenta in term infants, plasma total calcium concentration falls, reaching a nadir of approximately 2.3 mmol/L (9 mg/dL),[390] and the ionized calcium concentration reaches a low level of about 1.2 mmol/L (4.8 mg/dL) by 24 hours of life.[532] Plasma PTH concentrations are relatively low in the neonatal period and are minimally responsive to hypocalcemia during the first 2 to 3 days of life. Calcitonin concentrations are high in cord blood (~2000 ng/L), increase further during the early neonatal period, and remain high for several days after birth.[390,533] The relatively obtunded PTH response and the high calcitonin concentrations lead to a period of 2 to 3 days of transient neonatal hypocalcemia.[533,534]



• **Fig. 23.14** Actions of cortisol and catecholamines during fetal adaptation to the extrauterine environment. The prenatal cortisol surge promotes functional maturation of several organ systems. The neonatal catecholamine surge triggers or potentiates many of the extrauterine cardiopulmonary and metabolic functional adaptations that are critical to extrauterine survival. *BAT,* brown adipose tissue; *E,* epinephrine; *NE*, norepinephrine; T_3, triiodothyronine; T_4, thyroxine.

Inhibition of calcitonin secretion and stimulation of PTH secretion gradually result in increased serum calcium concentrations in the neonate. The disappearance of PTHrp in the neonatal lamb is approximately coincident with the time of restoration of calcium concentrations to the adult range.[390] The mechanism of transition from PTHrp to PTH secretion by the neonatal parathyroid glands is not clear.

Calcium homeostasis is also affected in the human newborn by the low level of glomerular filtration that persists for several days.[533,534] In addition, renal responsiveness to PTH is reduced in the first few days of life. These factors limit phosphate excretion and predispose the neonate to hyperphosphatemia, particularly if the diet includes high-phosphate milk such as unmodified cow's milk. Premature infants compared with term infants tend to have lower PTH and higher calcitonin concentrations and more immature kidney function; in these infants, neonatal hypocalcemia may be more marked and prolonged, and the incidence of symptomatic hypocalcemia is higher. Birth asphyxia also predisposes the neonate to hypocalcemia.[534] Infants born to mothers with hypercalcemia related to hyperparathyroidism have a high incidence of symptomatic hypocalcemia. These infants have a more marked suppression of parathyroid function and a longer period of transient hypoparathyroidism in the neonatal period. PTH secretion and calcium homeostasis usually return to normal in 1 to 2 weeks in full-term infants and within 2 to 3 weeks in the small premature infant.

Glucose Homeostasis

Fetal energy needs are met by a continuous intravenous supply of glucose across the placenta, fetal glucose uptake being directly related to both the maternal blood glucose concentration and the transplacental gradient. There is little endogenous glucose production. Glucose and other substrates are stored as fat and glycogen in preparation for birth. During labor and delivery, the secretion of stress hormones such as glucocorticoids and catecholamines

causes a rise in fetal blood glucose concentrations, so that cord blood glucose concentrations are often high.

At delivery following clamping of the cord, the maternal supply of glucose ceases. A normal term infant has an immediate postnatal fall in blood glucose concentration during the first 2 to 4 hours from values close to maternal levels to around 2.5 mmol/L (45g/dL) in the initial hours postpartum.[354,355] Low concentrations are usually transient, asymptomatic, and part of the normal adaptation to extrauterine life. In term infants metabolic adaptation is compensated for by producing alternative fuels, and the transition to intermittent feeding and fasting with the introduction of milk feeds into the gut is accomplished with little external evidence of the magnitude of changes taking place. Counterregulatory hormones rapidly become active with high catecholamine, glucagon, GH, and glucocorticoid concentrations and a fall in insulin.[354,355] Insulin concentrations are low at birth and tend to fall further with hypoglycemia. The early glucagon response is short lived, but concentrations remain at about 100 ng/L for the first 12 to 24 hours; the glucagon/insulin ratio is high enough to stabilize glucose concentrations in the range of 2.8 to 4 mmol/L (50–70 mg/dL) during this period. The early glucagon and catecholamine surges deplete hepatic glycogen stores, so the return of plasma glucose concentrations to normal after 12 to 18 hours requires maturation of hepatic gluconeogenesis under the stimulus of a high plasma glucagon to insulin ratio.[355] Glucagon secretion gradually increases during the early hours after birth, especially with protein feeding, which stimulates gut glucagon release and pancreatic glucagon secretion.[354,355] Together these changes result in stabilization of blood glucose concentrations, although adult concentrations are not reached until approximately 72 hours of life.

Failure of this sequence of physiologic changes can lead to hypoglycemia, which is most common in the first few hours after birth. Severe hypoglycemia occurs if the normal relationship of these hormones is disturbed but the net effect of the changes is to stabilize the blood glucose concentration at a lower level during the first few hours while milk feeding is initiated. The availability of ketone bodies allows a sparing effect of glucose for brain utilization. The process of adaptation is, however, incomplete and is compromised when an infant is born prematurely or following intrauterine growth retardation.

Premature infants have more severe and more prolonged hypoglycemia because of reduced glycogen stores and impaired hepatic gluconeogenesis. Infants born to diabetic mothers have more severe neonatal hypoglycemia because of relative hyperinsulinism. In the healthy term infant, glucose homeostasis is achieved within 5 to 7 days of life; in premature infants, 1 to 2 weeks may be required.

Other Hormonal Adaptations

Delivery of the placenta results in decreases in fetal blood concentrations of estrogens, progesterone, hCG, and hPL. The fall in estrogen concentrations presumably removes the major stimulus to fetal pituitary PRL release, and PRL concentrations decrease within several weeks. The relatively delayed fall may be due to lactotrope hyperplasia in the fetal pituitary or to delayed maturation of hypothalamic dopamine secretion. The gradual fall of GH concentrations during the early weeks of life is due to delayed maturation of hypothalamic-pituitary feedback control of GH release.[57] In the neonatal primate, there are concomitant decreases in plasma GH concentrations and GH responsiveness to exogenous GHRH.[535] The mechanisms remain unclear; changes in secretion or in pituitary sensitivity to GHRH or somatostatin, or both, may be involved. IGF1 and IGF2 concentrations fall to infantile values within a few days, presumably because of the removal of placental hPL and placental IGF production (see Fig. 23.5).

In male infants (see Fig. 23.10), after a transient fall in testosterone concentrations as the hCG stimulus abates, pituitary LH secretion rebounds modestly, and there is a secondary surge of plasma testosterone that persists at significant levels for several weeks.[57,536] This surge is mediated by hypothalamic GnRH; blockade of neonatal activation of the pituitary-testicular axis with a GnRH agonist in neonatal monkeys ablates the neonatal increments in LH and testosterone.[537] Such a blockade also results in subnormal increments in plasma LH and testosterone concentrations and subnormal testicular enlargement at puberty in these animals, suggesting that neonatal GnRH release with pituitary-testicular activation may be critical for normal sexual maturation of male primates.[537] In females, a transient, secondary surge in FSH may transiently elevate estrogen concentrations.

Delivery results in a reversal of the high fetal cortisone/cortisol ratio, and plasma cortisol concentrations are higher in the neonate despite relatively lower plasma corticotropin concentrations (see Fig. 23.7). Presumably this increase is due to decreased inhibition of adrenal 3βHSD by estrogen and perhaps to removal of a placental CRH action on fetal pituitary corticotropin release. Plasma DHEAS and DHEA concentrations fall as the fetal adrenal atrophies.

The increase in serum thyrotropin concentrations during the early minutes after birth is due to cooling of the neonate in the extrauterine environment.[21,219] In term infants, the thyrotropin surge peaks at 30 minutes at a concentration of about 70 mU/L (see Fig. 23.7). This peak evokes increased secretion of T_4 and T_3 by the thyroid gland. In addition, increased conversion of T_4 to T_3 by liver and other tissues maintains the T_3 concentration in the extrauterine range of 1.6 to 3.4 nmol/L (105–220 ng/dL). The re-equilibration of thyrotropin concentrations to the normal extrauterine range is probably a result of the readjustment of prevailing serum T_3 concentrations and maturation of feedback control of thyrotropin by thyroid hormones during the early weeks of life.[21,538] Production of rT_3 by fetal and neonatal tissues abates by 3 to 4 weeks of age, at which time serum rT_3 reaches adult concentrations.

Maternal and Fetal Medicine

Advances in fetal imaging, genomics, and minimally invasive techniques, as well as a better understanding of the intrauterine endocrine milieu and natural history of many endocrine diseases, have revolutionized the management of fetal and neonatal endocrine conditions. This progress has set the stage for disease diagnosis, therapy for fetal endocrine and metabolic disorders, management of disorders of fetal growth, and diagnosis and management of perinatal or neonatal endocrine dysfunction. In addition, understanding of developmental endocrinology is increasingly relevant to management strategies for premature infants, and infants and children with fetal growth retardation, and for understanding of the pathogenesis of adult endocrine and metabolic diseases. Up to 18% of babies are born preterm, and over the last decades survival has increased. Understanding the endocrine physiology in the extreme preterm and establishment of normal reference ranges has significant clinical value. To that effect, a biobank for blood samples of infants 24 to 32 weeks has recently been established.[539]

We are now entering an era of direct access to and management of the intrauterine environment with provision of medical and surgical fetal therapy, entailing both potential advantages and adverse effects.[540] With expansion of the application and scope of amniotic fluid fetal cell sampling, maternal plasma DNA analysis, and the advent of fetal visualization and intrauterine fetal blood sampling, direct access for fetal diagnosis is now possible.[541] Noninvasive technologies for fetal evaluation are realizing the promise of lower risk yet robust diagnostics; examples include sampling and analysis of free fetal DNA from maternal blood and analysis of fetal products accessible at maternal sites. After birth, definitive assessment of prenatal environmental and/or drug exposures to the fetus can be retrospectively assessed by analysis of meconium, hair, and other alternative matrices.[542] Intrauterine diagnosis of fetal adrenal and thyroid disorders has become the standard of care,[543,544] followed by the prospect of in utero treatment, although this is often controversial. Management of hypothyroid fetal goiter, for example, poses significant risks (including miscarriage) associated with both diagnostic amniocentesis/cordocentesis and intra-amniotic thyroid hormone therapy.[545] These risks often preclude large-scale studies of optimal antenatal treatment.

Intravenous nutritional supplementation of fetal sheep can prevent some forms of growth retardation, and chronic fetal therapy through indwelling pumps is feasible in animal fetuses.[546] These approaches, coupled with increasing availability of synthetic hormones and growth factor agonists and antagonists, facilitate direct fetal endocrine therapy. In addition, intrauterine stem cell transplantation has been successful in the correction of congenital hematologic diseases. The fetus in early gestation is a favorable recipient of cellular therapy, and fetal cell transplantation may be applicable to therapy for selected endocrine and metabolic disease.[547] Studies involving the endocrine pancreas, for example, have demonstrated long-term circulating human insulin following intrauterine stem cell transplantation in sheep, with potential translation as diabetic cellular therapy.[548]

Finally, there is a growing experience with fetal and neonatal gene therapy in animals,[549] although this field remains a challenging frontier of medicine. Intraplacental gene therapy, for example, is a novel strategy that takes advantage of an organ that will be discarded at birth. The results of preliminary studies in the rabbit recently have demonstrated that placental gene therapy may be an effective therapy for intrauterine growth restriction, for which no treatment is currently available.[550]

The rapid evolution of maternal-fetal medicine raises complex ethical and medicolegal challenges related to what constitutes innovative treatment versus human experimentation, with or without the umbrella of "medical research." There inevitably exists a grey zone between these black and white classifications, highlighting the need for clear, responsible guidelines and thoughtful, safe practice delivering excellence in care.[551]

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24 Disorders of Sex Development

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CHAPTER OUTLINE

KEY POINTS

- Disorders of sex development (DSDs) can present to different health professionals at different ages, but pediatric and adult endocrinologists play a central role in diagnosis, support, and management.
- DSDs represent a broad range of conditions with many underlying causes; understanding the basic biology of sex development and steroidogenesis can help in elucidating the causes of these conditions.
- Achieving a diagnosis is important for predicting the natural history of specific conditions, identifying associated features, monitoring endocrine function and tumor risk, advising about fertility potential, and counseling families about inheritance patterns. In some situations, a diagnosis might influence sex designation.
- A range of special biochemical tests and genetic analyses can help reach a specific diagnosis in most steroidogenesis disorders. Currently, a genetic diagnosis is reached in less than half of children with gonadal dysgenesis.
- A multidisciplinary team approach is key in providing coordinated management from diagnosis through later life. An experienced psychologist or allied professional can help support families and young people in the early years, as well as following transition to adult services. Support groups also play an important role.
- Some DSDs present in teenage years or even in adulthood. Adult endocrinologists have a crucial role to play in managing young people who present in adulthood, as well as for long-term follow-up of individuals with DSDs diagnosed in childhood. A supportive and sensitive approach is essential.

Defined as "conditions in which chromosomal, gonadal, or anatomical sex is atypical,"[1] disorders of sex development (or differences in sex development [DSDs]) represent a broad range of conditions that can present to many different health professionals at different stages of life.

In the newborn period, approximately 1 in every 4500 babies has atypical (ambiguous) genitalia and cannot be immediately designated as male or female without further expert evaluation. However, DSDs can present in many other ways, such as discordance between prenatal karyotype and appearance of the external genitalia at birth; bilateral inguinal hernias; during evaluation for associated syndromic features (e.g., renal) in childhood; virilization, absence of pubertal development, or primary amenorrhea in teenage years; or even later in life with infertility. Therefore many different health professionals may be involved in DSDs, and all should be aware of the range of conditions, how they might present, and the principles of their management.[1,2]

The investigation and management of DSDs require a multidisciplinary team with experience in these conditions.[2,3] The pediatric endocrinologist plays a key role within this team in the childhood and teenage years, whereas adult endocrinologists need to be involved in transitioning care and in the management of long-term issues such as hormone replacement and bone health. In addition to input from urologists, gynecologists, biochemists, clinical pathologists, radiologists, and geneticists, it is becoming increasingly clear that experienced psychological support is essential for individuals with DSDs and their families at key points in their lives. The past decade has seen changing terminology and attitudes (Table 24.1), but DSD still has significant stigma, and engagement with support groups and the DSD community is increasingly important to define the best pathways of care at local and national levels.

In keeping with previous editions of this textbook, this chapter will first describe the development of the reproductive systems, then present an overview of the range of conditions that can be classified as DSDs, and finally consider approaches to investigation and management of DSDs at different ages.[4]

Development of the Reproductive Systems

Reproductive system development begins at 4 to 5 weeks after conception in humans and is complete with the achievement of secondary sex characteristics and fertility (i.e., production of viable gametes) after puberty. Sex development is a dynamic process that requires the interaction of many genes, proteins, signaling molecules, paracrine factors, and endocrine stimuli.[5–9] Marked differences in the basic mechanisms of sex determination, differentiation, and reproductive strategy have evolved in different species, with variability in sex chromosome complement, gonad development, and gametogenesis throughout the animal kingdom.[10,11] In this chapter, we focus on the basic mechanisms of reproductive development in humans. We also include some important insights obtained from studies of normal and transgenic mice, although these are reviewed extensively elsewhere. More detailed explanation of pituitary gonadotrope development is provided in Chapter 23 and of normal and disordered puberty in Chapter 26.

TABLE 24.1 Proposed Revised Nomenclature

Previous Terms	Proposed Terms
Intersex	Disorders of sex development (DSDs)
Male pseudohermaphrodite Undervirilization of an XY male Undermasculinization of an XY male	46,XY DSD
Female pseudohermaphrodite Overvirilization of an XX female Masculinization of an XX female	46,XX DSD
True hermaphrodite	Ovotesticular DSD
XX male or XX sex reversal	46,XX testicular DSD
XY sex reversal	46,XY complete gonadal dysgenesis

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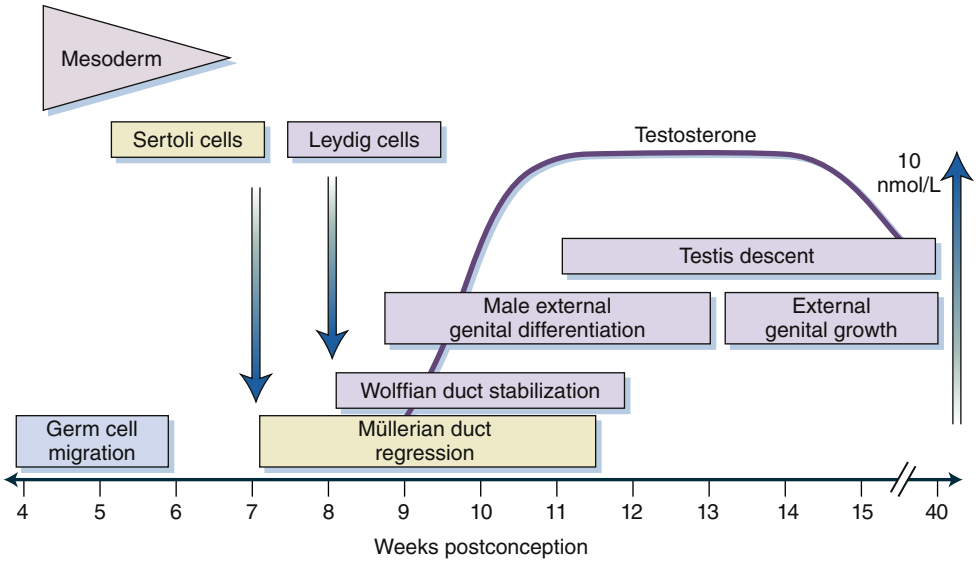
Sex Determination and Sex Differentiation

Sex determination is the process whereby the bipotential gonad develops into a testis or an ovary. Sex differentiation refers to the development of the internal and external genitalia, as directed by the production of peptide hormones and sex steroids by the developing gonad.

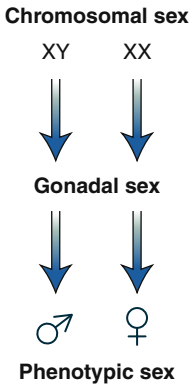
In the typical male, the process of sex differentiation involves androgenization of the external genitalia (leading to formation of the penis and scrotum), regression of müllerian structures (leading to absence of the uterus, fallopian tubes, and the upper two-thirds of the vagina), stabilization of wolffian structures (leading to development of seminal vesicles, vasa deferentia, and epididymides), and descent of the testes from their origin in the urogenital ridge to their final position in the scrotum (Fig. 24.1).

Ovarian hormonal secretion in fetal life has little if any effect on sex differentiation. At the time of puberty, estrogen synthesis stimulates breast and uterine development, and eventually coordinated activity of the reproductive endocrine axis results in regular menstrual cycles. Defects in ovarian development therefore usually manifest in adolescence with absent puberty. Ovarian development and differentiation have been viewed in the past as a "default" or passive process, but ovarian development in fact involves many active processes. Studies of gene expression show that a specific complement of genes is implicated in ovarian development and integrity, some of which (e.g., *RSPO1, NR2F2*) may actively antagonize testis differentiation.[6,12–14] Even the concepts of a fixed and quiescent population of ovarian germ cells at birth and of absent ovarian steroidogenesis have been challenged.[15,16]

In typical sex development, sex determination and sex differentiation can be divided into three major components: chromosomal sex (i.e., the complement of X and Y chromosomes), gonadal sex (i.e., presence of testes, ovaries, or both), and phenotypic or anatomic sex (i.e., presence of male and/or female external



• **Fig. 24.1** Events temporally related to sex differentiation in the male fetus. Mesoderm refers to the tissue source for Sertoli and Leydig cell formation. The continuous line depicts the rise in fetal serum testosterone, with a peak concentration of about 10 nmol/L (300 ng/dL).



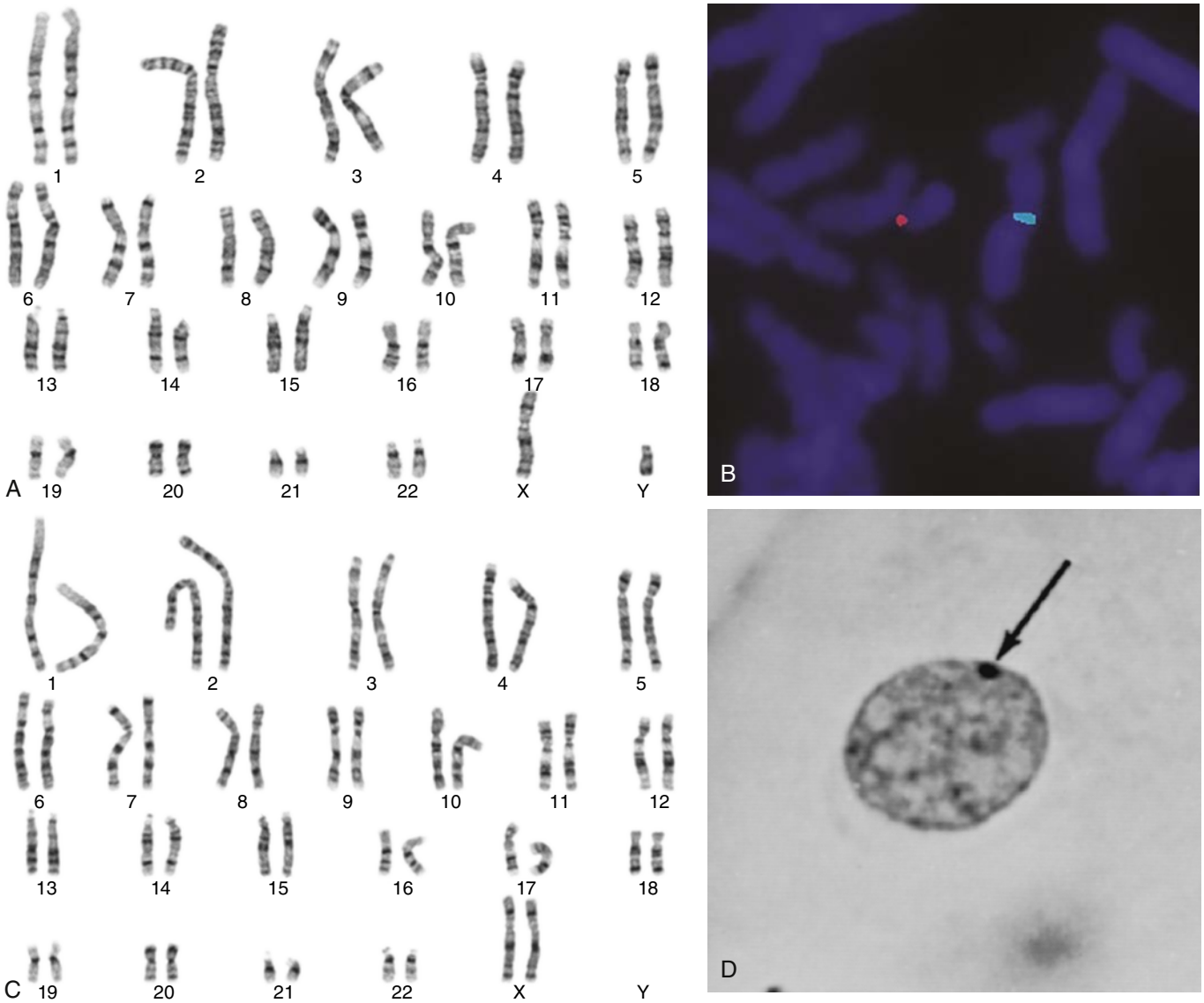
• **Fig. 24.2** Division of typical sex development into three major components provides a useful framework for diagnosis and classification. Chromosomal sex refers to the sex-chromosome complement. Gonadal sex refers to the presence of a testis or ovary after the process of sex determination. Phenotypic (anatomic) sex refers to the appearance of the external genitalia and internal structures after the process of sex differentiation.

and internal genitalia) (Fig. 24.2). Though consideration of sex development in terms of chromosomal sex, gonadal sex, and phenotypic (anatomic) sex can be a useful way of understanding the processes involved in reproductive development, none of these processes absolutely defines a person's sex, and gender and psychosexual development are influenced by several biologic factors as well as by environmental and social influences.

Chromosomal Sex

Chromosomal sex describes the complement of sex chromosomes present in an individual (e.g., 46,XY or 46,XX). In humans, the usual complement of 46 chromosomes consists of 22 pairs of autosomes (identified numerically from 1 to 22 based on decreasing size) and a pair of sex chromosomes (XX or XY) (Fig. 24.3). Other species have different numbers of chromosomes, and they may have different types of sex chromosomes or sexually dimorphic autosomes.[10,11]

In humans, chromosomal sex is usually determined at the time of fertilization, when two haploid gametes (an ovum and a sperm,



• **Fig. 24.3** Cytogenetic and fluorescence in situ hybridization (FISH) studies. (A) Typical male (46,XY) G-banded karyotype. (B) FISH analysis of a male (46,XY) using fluorescent probes directed against SRY (sex-determining region on the Y chromosome, spectrum red) and against the X centromere (spectrum green). (C) Typical female (46,XX) G-banded karyotype. (D) Photomicrograph shows the X chromatin body (Barr body, *arrow*) in the nucleus of buccal mucosa cells from a 46,XX female (thionine stain; original magnification, ×2000). (A–C, courtesy Lee Grimsley and Jonathan Waters, MD, North East London Regional Cytogenetics Laboratory, Great Ormond Street Hospital NHS Trust, London, UK.)

with 23 chromosomes each) fuse to generate a diploid zygote (with 46 chromosomes). Gametes are derived from germ cells, which initially replicate their chromosome complement and then undergo a series of two meiotic divisions (meiosis I and meiosis II) to produce haploid ova or sperm. Normal ova have a single X chromosome. Normal sperm contain a single Y chromosome or a single X chromosome, resulting in a 46,XY or 46,XX zygote, respectively, after fertilization.

Nondisjunction is the failure of a pair of sister chromatids to separate during anaphase.[17,18] Meiotic nondisjunction during gametogenesis can result in ova or sperm with gain or loss of sex chromosomal material. Fertilization by such gametes can give rise to a zygote with an imbalance in sex chromosome number, called *sex chromosome aneuploidy*. For example, a zygote with a single X chromosome (i.e., 45,X) has Turner syndrome, and the presence of an extra X causes Klinefelter syndrome (47,XXY) or triple X syndrome (47,XXX, trisomy X).[18] Zygotes with no X chromosomal material (e.g., 45,Y) are nonviable.

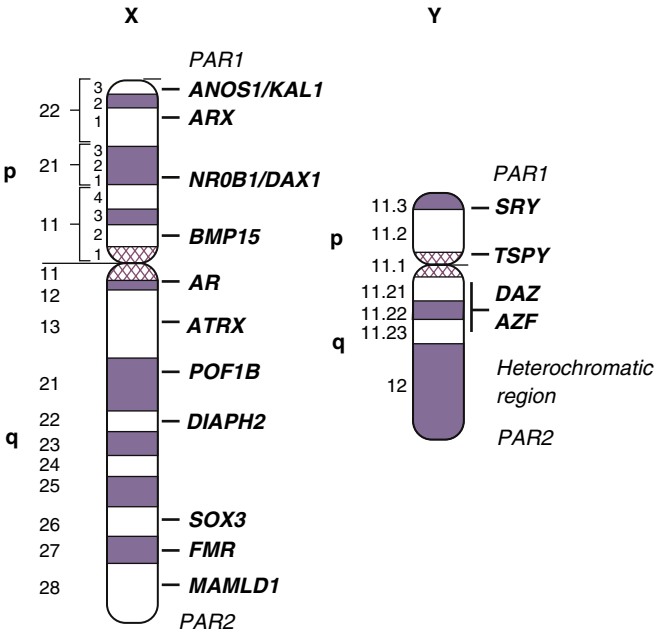
Mitotic nondisjunction can occur in the zygote (i.e., after fertilization), resulting in an imbalance in sex chromosome number in a subset of somatic cells, and this is called *sex chromosome mosaicism* (e.g., 45,X/46,XY). In such cases, the two (or more) cell lines originate from a single zygote. This situation differs from *chimerism*, which is the existence of two or more cell lines with different genetic origins in one individual. Chimerism can occur through several mechanisms, including double fertilization (dispermy) of a binucleate ovum, fusion of two complete zygotes or morulae before implantation, or fertilization by separate sperm of an ovum and its polar body. If the different cell lines have different sex chromosomes, a 46,XX/46,XY karyotype occurs. This form of true *sex chromosome chimerism* is very rare in humans. The consequences of some of these events in humans are discussed later (see "Sex Chromosome Disorders of Sex Development").

The Y Chromosome

Although the Y chromosome was initially thought to be inert, detection of a 46,XY karyotype in males and a 47,XXY karyotype in men with Klinefelter syndrome provided evidence that the Y chromosome is responsible for male sex determination.

The human Y chromosome is approximately 60 megabases (Mb) long and represents only 2% of the human genome (Fig. 24.4).[19,20] The Y chromosome consists of a heterochromatic region that is highly variable and largely genetically inactive, a conserved male-specific region, and autosomally derived regions that are estimated to have been added approximately 80 to 130 million years ago. The male-specific regions have undergone rapid evolution, with marked differences even between humans and chimpanzees.[21] It is thought that Y-chromosome genes encode around 57 proteins. Although some of these genes have putative roles in growth, cognition, and tooth development, several genes in the male-specific region are involved in reproductive development, function, and pathology. For example, a cluster of genes at Yq11.22 (e.g., the *AZFc* region) is essential for spermatogenesis, and genes within the gonadoblastoma locus (e.g., *TSPY*) increase the risk of malignancy when present in dysgenetic gonads (see Fig. 24.4).[22,23]

The euchromatic portion of the Y chromosome consists of a Y-specific segment and regions at the distal ends of the short and long arms, called the *pseudoautosomal regions* (PARs) (see Fig. 24.4).[19,24] These PARs are homologous to the distal ends of the short and long arms of the X chromosome and are the only regions involved in pairing and recombination during meiosis.

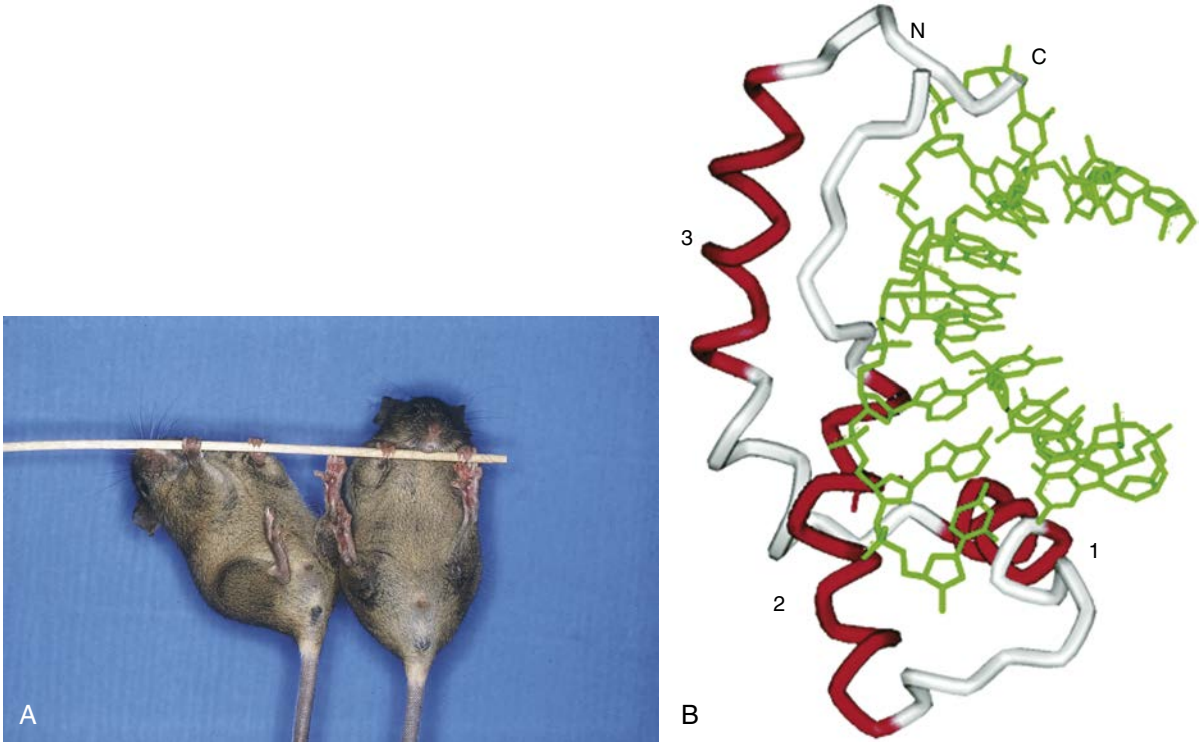


• **Fig. 24.4** Schematic diagrams of the X chromosome *(left)* and Y chromosome *(right)* show key regions and genes involved in sex development and reproduction. *ANOS1/KAL1,* Kallmann syndrome type 1; *AR,* Androgen receptor; *ARX,* aristaless-related homeobox, X-linked; *ATRX,* α-thalassemia, X-linked mental retardation; *AZF,* azoospermia factor; *BMP15,* bone morphogenetic protein 15; *DAZ,* deleted in azoospermia; *DIAPH2,* human homolog of the Drosophila diaphanous gene; *FMR,* fragile X, mental retardation; *MAMLD1,* mastermind-like domain containing 1 (CXorf6); p, short arm; *NR0B1/DAX1,* dosage-sensitive sex reversal congenital adrenal hypoplasia critical region on the X chromosome type 1; *PAR,* pseudoautosomal region; *POF1B,* actin-binding protein, 34 kDa; *q,* long arm; *SOX3,* SRY-related HMG box 3; *SRY,* sex-determining region Y; *TSPY,* testis-specific protein Y.

This process is essential for proper distribution of recombined sex chromosomal material to daughter cells as well as for maintaining dosage sensitivity of X-Y pairs, as these regions are not subject to dosage compensation (i.e., gene inactivation). PAR1 (distal short arm, Yp and Xp) contains at least 15 genes, including the homeobox gene *SHOX*. *SHOX* haploinsufficiency contributes to the short stature associated with Turner syndrome, Xp– or Yp– deletions, and Léri-Weill syndrome (i.e., dyschondrosteosis).

The search for a testis-determining factor on the Y chromosome began more than 50 years ago. In 1987, Mardon and Page proposed that the sex-determining function of the Y chromosome is located within a 140-kb segment of the short arm, within the Y-specific euchromatic portion.[25] The *ZFY* gene was the initial candidate in this region. However, in 1989, Palmer and colleagues described several 46,XX males who had Y-to-X translocations of Y-chromosomal material that was distal (telomeric) to the *ZFY* locus; this focused attention on a 35-kb region of the Y chromosome close to the pseudoautosomal boundary.[26] This region contained a gene encoding a putative transcription factor subsequently called *sex-determining region Y (SRY)* (see Fig. 24.4).

A series of elegant studies in mice and humans established *SRY* as the primary Y-chromosomal testis-determining gene.[27–29] The first definitive proof came with the generation of transgenic XX mice specifically expressing the *SRY* locus (14 kb); some of these mice had a male phenotype, developed testes (without spermatogenesis), and showed male sexual mating behavior (Fig. 24.5).[30] This work was supported by reports of deletions



• **Fig. 24.5** (A) The XXSRY+ mouse *(right)* has testis development and a male phenotype, providing convincing evidence that SRY (sex-determining region on the Y chromosome) is a testis-determining gene. A normal XY male littermate is shown for comparison *(left)*. (B) Model of the structure of the SRY high-mobility group (HMG) box bound to DNA. The HMG domain contains three α-helices *(red)*, which adopt an L-shaped conformation. Binding of this region of SRY to the minor groove of DNA *(green)* causes it to bend and unwind. (A, courtesy Professor Robin Lovell-Badge, National Institute of Medical Research, London, UK; B, from Harley VR, Clarkson MJ, Argentaro A. The molecular action and regulation of the testis-determining factors, SRY [sex-determining region on the Y chromosome] and SOX9 [SRY-related high-mobility group (HMG) box 9]. *Endocr Rev*. 2003;24:466–487, used with permission of The Endocrine Society, Copyright 2003.)

and loss-of-function mutations in *SRY* in humans with 46,XY complete gonadal dysgenesis (Swyer syndrome) (see later discussion).[28,31,32] The structure and function of the *SRY* gene and SRY gene product are discussed later.

The X Chromosome

The X chromosome is a relatively large and gene-rich chromosome compared with the Y chromosome, and it consists of about 160 Mb of genomic deoxyribonucleic acid (DNA) (see Fig. 24.4).[19,33,34] This DNA contains 5% of the haploid genome and approximately 850 protein-encoding genes. Several genes on the X chromosome play an important role in sex development in males and females, gametogenesis, and hypothalamic-pituitary (gonadotrope) function (e.g., androgen receptor *[AR]*, *ANOS1* [also called *KAL1*], DAX1 *[NR0B1]*, *MAMLD1*, *SOX3*). However, most X-linked genes are unrelated to reproductive function and have a diverse range of cellular functions.

The X chromosome contains PARs at the distal end of each arm, similar to the Y chromosome (see Fig. 24.4).[19] These regions and several genes in their boundaries function with their homologs on the Y-chromosome PARs in an autosomal fashion. However, as large numbers of genes on the X chromosome are located outside the PARs and do not have homologs on the Y chromosome, a process must exist to maintain the balance in copy number (i.e., gene dosage) of these genes between males with a single X chromosome and females with two X chromosomes. This process is called *X inactivation*.

The first insight into X inactivation came after the identification in 1949 of the X chromatin body (i.e., Barr body) in a proportion of cells in females (see Fig. 24.3). This X chromatin is derived from one of the two X chromosomes in interphase nuclei of these somatic cells. Grumbach and colleagues showed that the X chromosome giving rise to X chromatin completes DNA synthesis later than any other chromosome.[35] These findings led to the concept that only one X chromosome is genetically active during interphase, whereas the other X chromosome is heterochromatinized and relatively inactive. This change in activation state occurs in early gestation in humans (12–18 days, late blastocyte stage) and is a multistep process, regulated by the gene *XIST* and the *TSIX* antisense transcript, leading to stable and epigenetic silencing of genes on all but one X chromosome (Lyon hypothesis).[36] However, female germ cells beyond the stage of oogonia are exempt from X inactivation, demonstrating a need for a second X chromosome for oocyte development.

X inactivation occurs randomly in different cells.[37] After inactivation has occurred, the inactive state of that particular X chromosome is transmitted to all descendants of that cell so that XX individuals effectively function as genetic mosaics for X-linked traits. If the initial population of cells is small, skewed X inactivation can occur as a chance event despite random inactivation.

In these situations, heterozygous female carriers of an X-linked disorder may manifest symptoms of the condition. A subset of genes on the X chromosome may also be imprinted and only expressed from one allele. Furthermore, recent data suggest that several other X-chromosome genes (especially on the short arm) may escape X inactivation, potentially in a tissue-specific manner, and that sex chromosome gene dosage may regulate autosomal gene networks.[38,39] All these phenomena might influence phenotype variability of X-linked conditions or sex chromosome aneuploidy.

Gonadal Sex

Gonadal sex refers to whether the gonadal tissue developed as a testis or an ovary. The principal embryologic and morphologic changes involved in gonadal development are shown in Fig. 24.6 and have been described in detail elsewhere.[6–8,40]

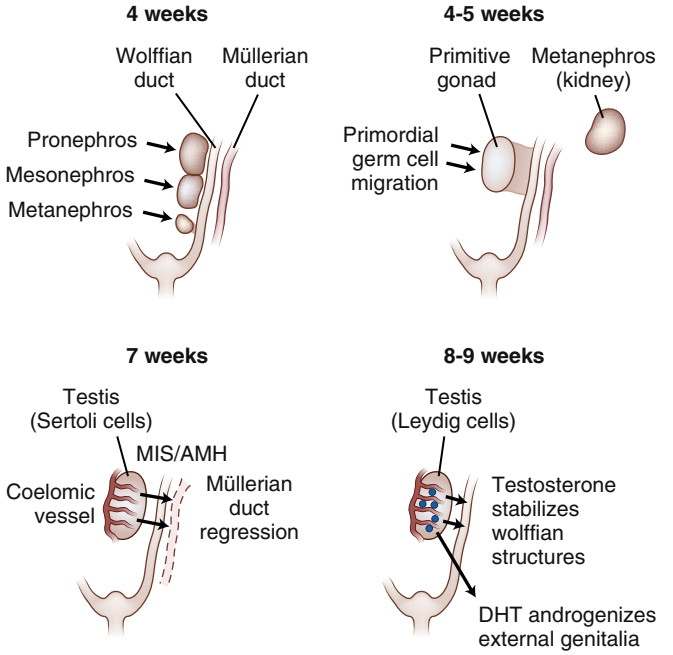
The Bipotential Gonad

The primitive gonad arises together with the adrenal gland from a condensation of the medioventral region of the urogenital ridge at approximately 4 to 5 weeks after conception in humans (see Fig. 24.6). The primitive gonad separates from the adrenal primordium at about 5 weeks but remains bipotential (indifferent) until about 42 days after conception.

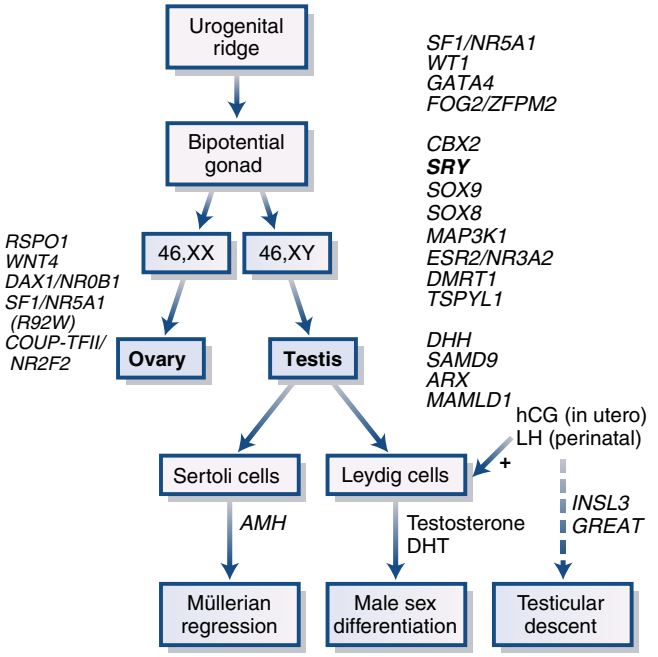
Several important genes are expressed in the developing urogenital ridge in mice that facilitate formation of the bipotential gonad; they include *Emx2*, *Lim1*, *Lhx9*, *M33/Cbx2*, *Pod1*, *Six1/4*, *Map3k4*, *Wt1*, and *Nr5a1/Sf1*.[41–47] Deletion of these genes causes gonadal dysgenesis in mice and can be associated with abnormalities in other organs (e.g., kidney, brain). Only some of them have been associated with human DSDs to date (e.g., *WT1*, *CBX2*, *NR5A1/SF1*) (see "46,XY Disorders of Sex Development") (Fig. 24.7).[48] These factors may affect gonad/testis development at multiple stages (Fig. 24.8).

WT1 (11p13) is a gene that encodes a four–zinc finger transcription factor expressed in the developing genital ridge, kidney, gonads, and mesothelium.[49] Homozygous deletion of *Wt1* in mice prevents gonad and kidney development.[50] It is thought that at least 24 WT1 isoforms exist due to mRNA splicing variants and complex post-translational modifications.[51] The two most common variants are an isoform with alternative splicing of exon 5, resulting in insertion of an additional 17 amino acids in the middle of the protein, and an isoform that uses an alternative splice donor site for exon 9, resulting in the addition of three amino acids (lysine, threonine, and serine; called +KTS) between zinc fingers 3 and 4. It is thought that +KTS and –KTS isoforms have different cellular functions and differential effects on gonad and renal development.[52] The ratio of +KTS to –KTS isoforms may be important in testis development, with the +KTS isoform having a cell-autonomous role in regulating SRY expression and influencing cellular proliferation and Sertoli cell differentiation.[53] Wt1 also regulates expression of *Sf1* and *Sox9* in mice and may oppose β-catenin (Ctnnb1) pathways.

In humans, *WT1* transcripts can be detected in the indifferent gonadal ridge when it first forms at 32 days after ovulation.[54] Deletions or mutations of *WT1* cause well-defined syndromes. Haploinsufficiency of *WT1* due to deletion of the chromosomal locus containing *WT1* and *PAX6* (11p13) causes WAGR syndrome (Wilms tumor, aniridia, genitourinary abnormalities, and mental retardation).[55] Dominant-negative point mutations in *WT1* cause Denys-Drash syndrome (gonadal dysgenesis, early-onset nephropathy, and predisposition to Wilms tumor),[56] whereas mutations in the exon 9 splice site of *WT1*, causing an altered ratio of +KTS to –KTS isoforms of WT1, result in Frasier syndrome (gonadal dysgenesis, late-onset nephropathy, and predisposition to gonadoblastoma) (see "46,XY Disorders of Sex Development" and Fig.



• **Fig. 24.6** Schematic representation of the principal morphologic and functional events during early gonad or testis development in humans. *DHT,* dihydrotestosterone; *MIS/AMH,* müllerian-inhibiting substance/anti-müllerian hormone. (Modified from Achermann JC, Jameson JL. Testis determination. *Top Endocrinol*. 2003;22:10–14. Used with permission of Chapterhouse Codex.)



• **Fig. 24.7** The flow chart provides an overview of the major events involved in sex determination and sex differentiation. Mutations or deletions in several genes reported to cause disorders of sex development in humans are shown. *hCG,* human chorionic gonadotropin; *LH,* luteinizing hormone.

24.21 later).[57,58] Some phenotypic overlap in the latter two conditions exists.

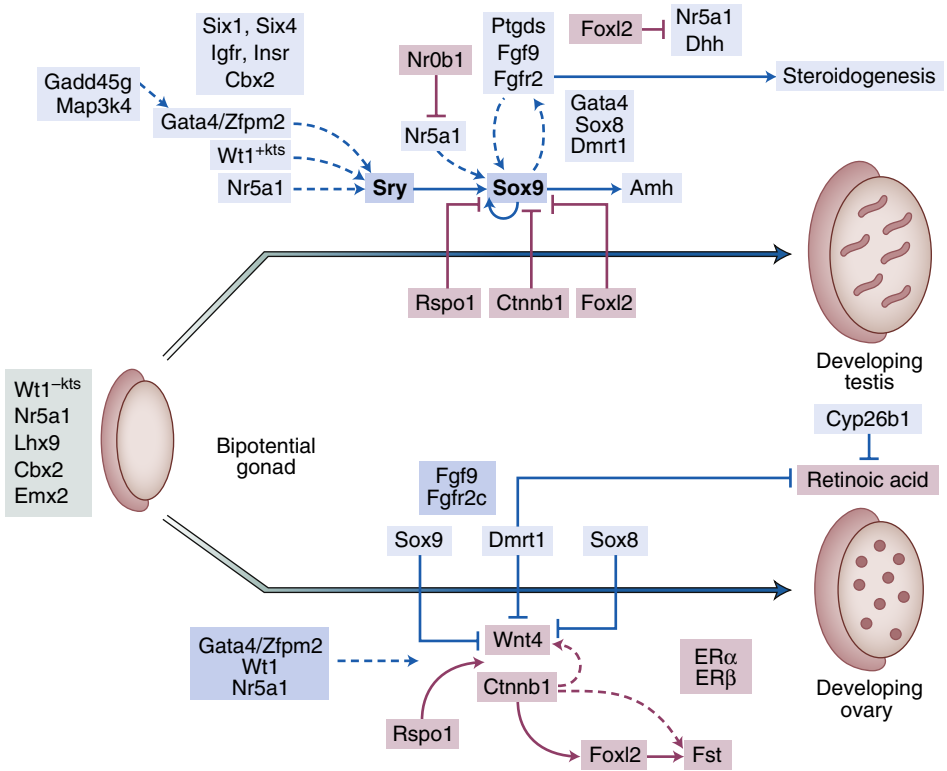
Another key transcription factor expressed in the urogenital ridge is steroidogenic factor 1 (SF1, encoded by *NR5A1*).[59] SF1 is a member of the nuclear receptor superfamily and regulates the transcription of at least 30 genes known to be involved in gonadal development, adrenal development, steroidogenesis, and reproduction. In mice, complete deletion of the gene encoding Sf1 results in apoptosis of the developing gonad and adrenal gland during early embryonic development, resulting in impaired androgenization and persistence of müllerian structures in XY animals.[60] Other features of these homozygous-deleted animals include hypogonadotropic hypogonadism, abnormalities of the ventromedial hypothalamus, and late-onset obesity in adult animals rescued by adrenal transplantation.[61] Heterozygous animals have reduced gonadal size and impaired adrenal stress responses.[62] Data for mice suggest that Sf1 plays a critical role in the generation of a population of Sf1+ progenitor cells in the gonad before sex determination occurs, as well as in promoting testis development by facilitating SRY regulation of *SOX9* expression (discussion to come).[63,64]

SF1 is expressed during the early stages of urogenital ridge formation in humans (32 days after ovulation).[54] Consistent with the mouse phenotype, heterozygous and homozygous loss-of-function mutations affecting key DNA-binding motives have rarely been described in patients with primary adrenal failure and severe 46,XY gonadal dysgenesis (see later discussion).[65,66] In contrast, haploinsufficiency of *NR5A1* due to heterozygous disruptive variants is now established as a relatively frequent cause of 46,XY DSD with normal adrenal function.[67]

Although SF1 was originally thought to play a less significant role in the ovary compared with the testis, studies in mice show that SF1 is also an important regulator of ovarian integrity and function as well as in the development of early progenitor cell populations in the ovary.[68–70] Loss of function or haploinsufficiency of *NR5A1* is associated with primary ovarian insufficiency (POI), whereas recurrent heterozygous variants affecting the same codon in SF1 (p.Arg92) are associated with 46,XX testicular and ovotesticular DSD,[71–76] suggesting that SF1 can also act as a switch between testis-development and ovary-development pathways.

Primordial Germ Cell Migration

Primordial germ cells (PGCs) are the embryonic precursors of gametes (spermatocytes or ova). Surprisingly, in all species, PGCs arise some distance from the developing gonad and undergo a process of migration during the early stages of embryogenesis.[77,78] In humans, PGCs arise from pluripotent epiblast cells and are initially located in the 24-day embryo in a region of the dorsal endoderm of the yolk sac, close to the allantoic evagination (see Fig. 24.6). After mitotic division, PGCs migrate into the primitive gonad (between 4 and 5 weeks postconception [wpc]) under the influence of signaling molecules, receptors, and extracellular matrix proteins such as KIT, the KIT ligand KITLG (formerly called Steel), β_1-integrin, E-cadherin, WNT5A/ROR2, KIF13B, interferon-induced transmembrane protein 1 (IFITM1), and IFITM3.[79,80] Hindgut expansion may also regulate or facilitate this process. Gonadal colonization is mediated by CXCL12 (previously called SDF1) and its receptor CXCR4 and influenced by CXCR7.



• **Fig. 24.8** Overview of some of the molecular events thought to be involved in the development of the bipotential gonad, as well as in testis determination and ovary development. These data are based mostly on studies in mice. Sry is the principal testis-determining factor, but many other factors interact downstream to support testis development and repress ovary development, or vice versa.

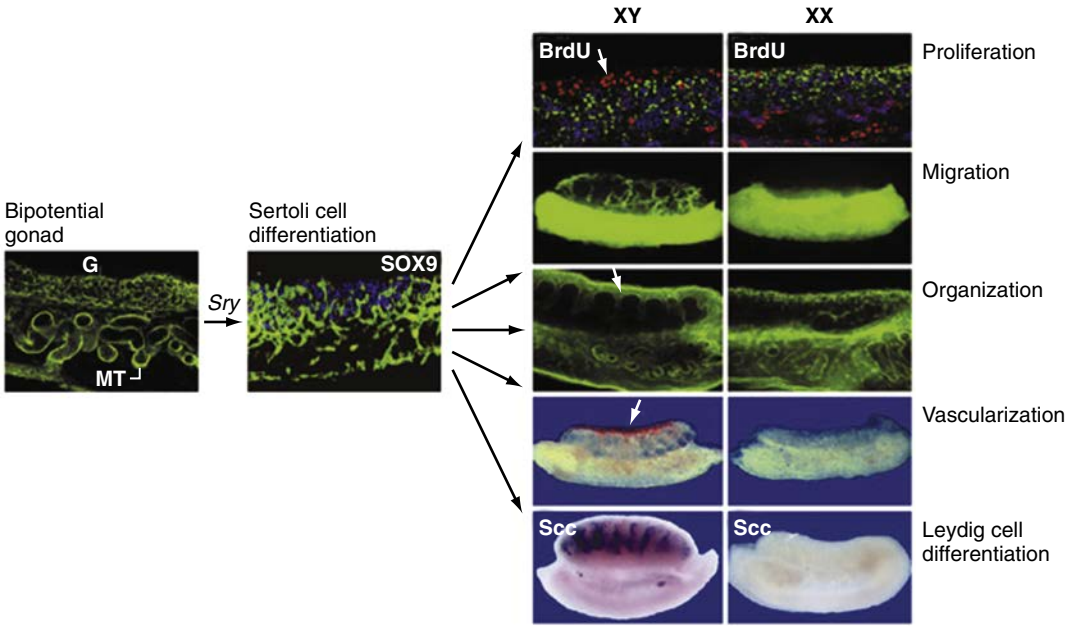
In the first few months of gestation, PGCs undergo multiple cycles of mitotic division. In the testis, a self-renewing population of germ cells exists. These undifferentiated PGCs are maintained by factors such as POU5F1 (also called OCT4), but they commit to differentiation in response to the expression of specific signaling molecules and transcription factors. After several cycles of mitotic division, these cells enter mitotic arrest.[81] Subsequent testicular development can occur in the absence of this germ cell population.[82] Meiosis does not occur until the onset of spermatogenesis in puberty (see Chapter 19).

In the developing ovary, primordial ova (oogonia) undergo mitotic expansion in the first few months of gestation (5–24 weeks), followed by meiotic division (8–36 weeks) and a process of meiotic arrest (oocytes). Although it was originally thought that entry into meiosis occurred autonomously, data suggest that retinoic acid signaling from the mesonephros stimulates this process.[83,84] Male germ cells may be protected from this signal by their location within the testis cord and by Sertoli cell expression of the cytochrome P450 isoenzyme 26B1 (CYP26B1), which breaks down retinoic acid. Meiotic arrest occurs in the first prophase, when the chromatids of homologous pairs have begun to separate but are fixed by chiasmata (diplotene stage). The presence of these PGCs and subsequent meiotic oocytes is critical for the differentiation of prefollicular cells into follicular cells and for the maintenance of ovarian development (see Chapter 17). More detailed insight into the stages of human PGC development is emerging from single-cell transcriptomic studies.[85]

More than 6 million oogonia and prophase oocytes exist in the developing ovary at about 16 weeks of gestation, and formation of oogonia from PGCs ceases by the seventh month. At that stage, some oocytes remain in undifferentiated nests, whereas others associate with somatic pregranulosa cells to form primitive or primordial follicles. However, approximately 80% of oogonia fail to form follicles and undergo apoptosis, so that only 1 million germ cells are present in the ovary at the time of birth. The resting primordial follicles can remain in that stage of development throughout the woman's reproductive life, and meiosis progresses only in response to ovulation of the graafian follicle, which occurs approximately 400 times in a woman's reproductive lifetime.

Testis Determination

Testis determination begins about 6 wpc in humans and consists of several distinct genetic and morphologic events.[5–7] One of the first and most significant events in testis determination is a transient wave of SRY expression through the hitherto undifferentiated gonad at approximately 42 days postconception (dpc) (Fig. 24.9; also see Fig. 24.8).[12,86] Initially, this occurs in the center of the gonad, followed by expression in cells located at the cranial and caudal poles. SRY expression must reach a certain threshold within a definite time window for testis development to occur.[87,88]



• **Fig. 24.9** Key morphologic changes in the developing testis in mice. No morphologic differences between the XY and XX gonad are seen during the bipotential gonad stage at 10.5 to 11.5 days postconception (dpc) *(far left)*. In XY gonads, *SRY* expression is followed by expression and nuclear localization of SOX9 *(blue)* in pre-Sertoli cells *(middle)*, resulting in Sertoli cell differentiation by 11.5 dpc (vasculature and germ cells are labeled with platelet endothelial cell adhesion molecule [PECAM] and appear *green*). Between 11.5 and 12.5 dpc, distinct changes occur in the XY gonad *(near right column)*, which are not seen in the XX gonad *(far right)*. These changes include proliferation of coelomic epithelial cells (measured by BrdU incorporation; *red, arrow*); migration of cells from the mesonephros (shown by recombinant culture of a wild-type gonad and a mesonephros in which the cells express green fluorescent protein); structural organization of testis cords (detected by laminin deposition, *green*); male-specific vascularization (by light microscopy with blood cells indicated by an *arrow*); and Leydig cell differentiation (detected by mRNA in situ hybridization for the steroidogenic enzyme P450scc). *BrdU,* bromodeoxyuridine; *MT,* basal lamina of mesonephric tubules; *G,* gonad. (From Brennan J, Capel B. One tissue, two fates: molecular genetic events that underlie testis versus ovary development. *Nat Rev Genet.* 2004;5:509–521. Used with permission of Macmillan Publishers, Ltd.)

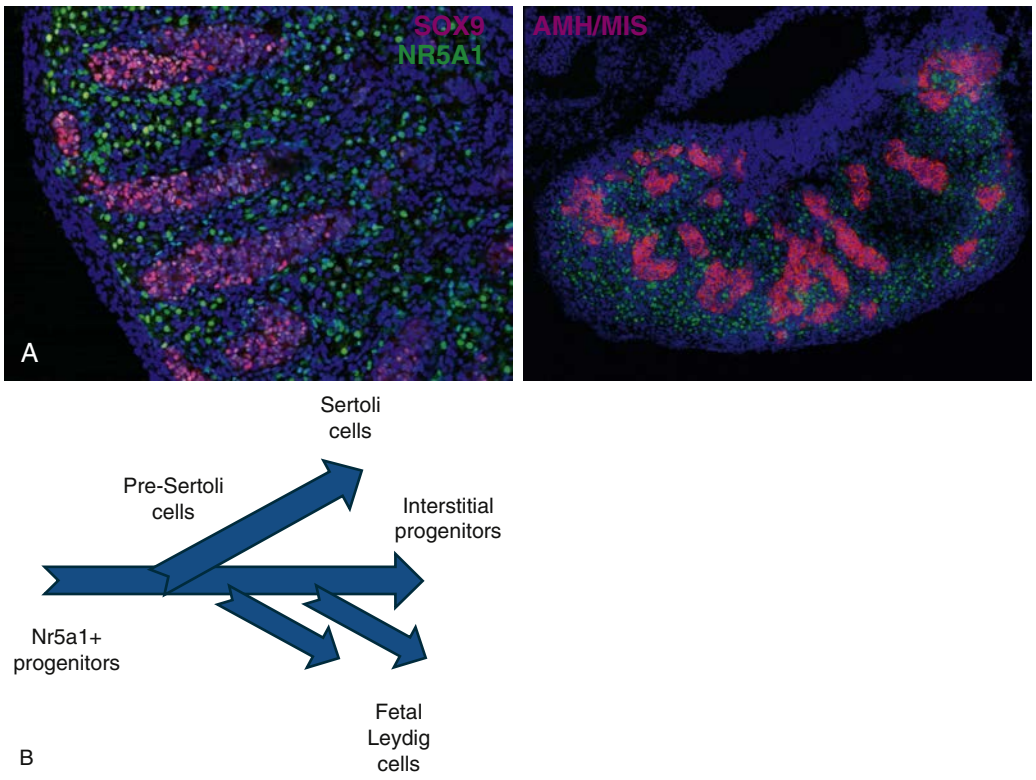
Expression levels peak from approximately day 44, when testicular cords are first visible. Thereafter, low-level SRY expression in humans is confined to Sertoli cells (day 52), where it persists into adulthood.

In humans, *SRY* is a single-exon gene (Yp11.3) that encodes a 204–amino acid, high-mobility group (HMG)–box transcription factor.[87] Mutations in *SRY* have been reported in approximately 10% to 15% of patients with sporadic or familial 46,XY gonadal dysgenesis (see "46,XY Disorders of Sex Development" and Fig. 24.22 in later discussion). As described earlier, the presence of *SRY* (due to translocation of *SRY* to the X chromosome or an autosome) or transgenic expression of *SRY* is sufficient to induce testis development in XX humans and mice (see "The Y Chromosome" and Fig. 24.5).

Mutations and deletions in *SRY* tend to cluster within the region encoding the HMG box. The HMG box is a 79–amino acid structure that has moderate homology with SRY in other species (~70%) and with the HMG box of related SOX (SRY-like HMG box) proteins (60%) (see "46,XY Disorders of Sex Development" and Fig. 24.22 later).[87] The HMG box consists of three α-helices, which are able to adopt an L-shaped or boomerang-shaped configuration (see Fig. 24.5). The HMG box binds to specific response elements (AACAAT/A and variants) in the minor groove of DNA and induces a 40-degree to 85-degree structural bend in its target, depending on the sequence. The precise function of protein-directed DNA bending is not known, although this interaction results in minor groove expansion, DNA unwinding, and altered base stacking. These effects likely alter DNA architecture in chromatin and may permit the interaction of other protein complexes with the DNA, resulting in activation or repression. Other important domains in SRY are two nuclear localization signals that can interact with calmodulin and importin-β to regulate cellular localization; several serine residues in the amino (N)-terminal of SRY that can undergo phosphorylation and influence DNA binding; and a carboxy (C)-terminal, 7–amino acid motif that interacts with PDZ domains of SRY-interacting protein 1 (SIP1).[89–93]

Although *SRY* was convincingly shown to be the primary testis-determining gene more than 20 years ago, relatively little is known about the regulation of *SRY* expression. Some studies have shown that SF1, WT1, GATA4, and ZFPM2 (FOG2) can all regulate *SRY* promoter activity in vitro, and MAP3K4 and insulin-related signaling pathways may be important, but the exact mechanisms that activate *SRY* in vivo remain relatively poorly understood (see Fig. 24.8).[47,94–98]

The downstream targets of *SRY* are also not entirely elucidated, but it seems likely that the most significant actions of SRY occur through upregulation of SOX9.[12] SOX9 is an SRY-related HMG box factor comprising three exons (509 amino acids).[99–102] In humans, SOX9 becomes strongly localized to the developing sex cords at 44 to 52 days after ovulation and is expressed in Sertoli cells thereafter (Fig. 24.10; also see Fig. 24.9).[86] SOX9 is also expressed in developing cartilage under the regulation of parathyroid hormone–related protein (PTHrP)/Indian hedgehog signaling pathways. Heterozygous mutations or deletions in *SOX9* cause campomelic dysplasia, a severe skeletal dysplasia that is



• **Fig. 24.10** (A) Expression of SOX9 *(left panel)* and AMH/MIS *(right panel)* in primitive seminiferous tubules of human fetal testis at 9 weeks postconception. NR5A1 (SF1) is shown in *green*. (B) Model for fetal testis lineage development based on single cell transcriptomic studies in the mouse. (A, modified from Del Valle I, Buonocore F, Duncan AJ, et al. A genomic atlas of human adrenal and gonad development. *Wellcome Open Res*. 2017;2:25; B, modified from Stévant I, Neirijnck Y, Borel C, et al. Deciphering cell lineage specification during male sex determination with single-cell RNA sequencing. *Cell Rep*. 2018;22:1589–1599.)

associated with variable gonadal dysgenesis in approximately 75% of patients.[99,100] Mutations have been found in the HMG box of SOX9, in the C-terminal transactivation domain, and in a region that interacts with heat shock proteins (e.g., HSP70) (see "46,XY Disorders of Sex Development" and Fig. 24.22 later).[101,102]

The regulatory region of the *SOX9* promoter is very large. Breakpoints have been reported up to 350 kb from the start of the *SOX9* gene in patients with campomelic dysplasia and gonadal dysgenesis. Studies have shown that Sry and Sf1 can synergistically regulate *Sox9* expression in mice and in vitro through a testis-specific enhancer region (TESCO).[64,103] Recently, several additional SOX9 enhancer regions have been characterized that are regulated by SRY/SF1 or through SOX9 autoregulation (e.g., Enh13)[104] and which may be involved in maintaining SOX9 expression at critical stages over time.

SOX9 can direct testicular development independently of SRY and function as a testis determination gene in its own right. Transgenic expression of *Sox9* in mice results in testis development in XX animals, and the XX *odsex (Ods)* mouse develops as a male owing to disruption of a regulatory element 1 Mb upstream of *Sox9* that causes testis-specific overexpression of Sox9 during development.[105,106] Similar disruption of an upstream *cis*-regulatory region resulting in SOX9 overexpression causes 46,XX testicular DSD,[107] and overexpression of SOX9 due to duplication of 17q24.3-q25.1 or duplications in the SOX9 promoter region have also been reported in 46,XX individuals with testicular or ovotesticular DSD.[108–110] In mice, *Sox9* may mediate its effects through direct interactions with other target genes (e.g., *Fgf9, Ptgds, Amh*) to promote testis development pathways and through the degradation of β-catenin to suppress inhibitors of testis development (see Fig. 24.8). Autoregulatory loops with the abovementioned target genes may also be important in maintaining SOX9 expression.

Around the time of SRY and SOX9 expression, the developing testis undergoes a series of distinct cellular and morphologic changes (see Fig. 24.9). Understanding of these processes has resulted largely from studies in mice, although data are also emerging from humans at a global level as well as from single-cell transcriptomic studies.[5,12,85,111] As outlined earlier, the first stage of testis development in mice involves a proliferation of Nr5a1/Sf1-positive somatic cells that results in an increase in pre-Sertoli cell precursors and, ultimately, Sertoli cell differentiation. This process is influenced by growth factors such as fibroblast growth factor 9 (Fgf9) and the receptor Fgfr2, as well as *DMRT1* (double sex, Mab3–related transcription factor 1; 9p24.3), a 373–amino acid protein that is homologous to the sex development *doublesex* gene of *Drosophila* and the *Mab3* gene of *Caenorhabditis elegans*, and SOX8.[112] These primitive Sertoli cells coalesce with peritubular myoid cells (i.e., flat, smooth muscle–like cells) to form primary sex cords, which then condense to form primitive seminiferous cords at about 7 wpc in humans.

Sex cord development is supported by a striking reorganization of the gonadal vasculature that occurs in the developing testis but not in the ovary (see Fig. 24.9).[5,113] These changes include the development of a discrete coelomic vessel, restriction of endothelial cells to the interstitial space between the sex cords, and increased branching of blood vessels. The development of these vascular systems is influenced by growth factor signaling systems, such as platelet-derived growth factor receptor-α (PDGFRα)/prostaglandin D_2 synthase (PTGDS), and can be repressed by the Wnt4/β-catenin/follistatin (Fst) systems in mice.[113] These changes in vascular architecture play an important role in determining cellular patterning and organization in the developing testis, in supporting paracrine interactions, and in the export of androgens from the developing fetal Leydig cells to the perineal and systemic circulation.

A fetal Leydig cell population also differentiates around this time from in the developing testis, supported by Nr5a1/Sf1 and following the loss of Tcf21 and Nr2f2/Coup-TFII from interstitial progenitors. Other key factors involved in this process that have been associated with human phenotypes include *DHH* (desert hedgehog), *ARX* (aristaless-related homeobox, X-linked gene), and *MAMLD1* (mastermind-like domain–containing 1, previously called *CXORF6*).[114–118]

Our understanding of the genetic events underlying cell fate is being advanced by newer techniques such as single-cell transcriptomics. These events have been reviewed recently and new data are emerging rapidly in this field.[40,63,111] These approaches are showing that the early Nr5a1+ common progenitor lineage first gives rise to a Sertoli cell lineage, followed by fetal Leydig cell lines, with a progenitor cell lineage maintained in the interstitium that has capacity for steroidogenic fate. A summary of these events is shown in Fig. 24.10.

Ovary Development

For many years, ovary development was thought to be a constitutive (default) process because the gonad differentiates as an ovary in the absence of a Y chromosome (and specifically lack of *SRY*). However, several studies in mice and humans have now shown that a similar number of genes are differentially expressed in the developing ovary at critical early stages of development compared to the testis.[12,13] Therefore ovarian development can be viewed as an active process that requires expression of a set of specific genes and factors to promote this process as well as to prevent testicular development. Considerable mutual antagonism between these pathways exists (see Fig. 24.8).

The initial stages of ovary development require establishment of the bipotential gonad. As in the testis, several key genes are required for supporting the early granulosa-cell lineage *(Gata4/Zfpm2*, *Wt1*, *Nr5a1)* (see Fig. 24.8). Subsequently, WNT signaling pathways play a key role in promoting ovarian development as well as in repressing testis development, mostly through antagonism of SOX9. RSPO1/R-spondin 1 and WNT4 stabilize β-catenin. Loss of either *RSPO1* or *WNT4* has been reported in association with XX ovotesticular DSD or hyperandrogenism in humans.[119,120]

Other key factors involved in ovary development in the mouse are Foxl2 and follistatin. Foxl2 is involved in maintaining or enforcing the ovary program and granulosa-cell fate, and loss of *Foxl2* in mice is associated with postnatal transdifferentiation of ovary into testis.[121] In humans, loss of *FOXL2* is associated with blepharophimosis-ptosis-epicanthus inversus syndrome (BPES). Women with BPES can have variable POI, but ovotesticular DSD has not been reported. The role of follistatin in human ovary development is not well established.

Recent reports of ovotesticular DSD associated with specific variants in *NR5A1*/*SF1* and *NR2F2* suggest that both these transcription factors play a role in maintaining the balance between ovarian and testicular differentiation, but more data regarding the specific mechanisms are needed,[71,122] as well as the role of related estrogen receptors (see Fig. 24.8). It has been proposed that *NR2F2* and *NR5A1* may be involved in supporting theca cell fate in the ovary by maintaining a stromal progenitor lineage (similar to that in the testis that gives rise to Leydig cells), but data from patients suggest that the ovotestis has more profound development of testicular structures beyond just Leydig cells.

As with testis development, single-cell transcriptomics data and lineage-tracing studies are starting to map out the genetic events underlying development of germ cells and pregranulosa cells, as well as theca cell origins in the mouse and human ovary. Current knowledge has been reviewed extensively recently, but it is likely major new insights will emerge in the future in this area.[40]

Phenotypic or Anatomic Sex

The developing gonad produces several steroid and peptide hormones that mediate sexual differentiation and result in the phenotypic sex seen at birth. Alfred Jost first showed the importance of fetal testicular androgens in this process in 1947.[123] In his classic experiments, Jost demonstrated that surgical removal of the gonads during embryonic development of the rabbit resulted in development of female reproductive characteristics, regardless of the chromosomal sex of the embryo.

Male Sex Differentiation

Sertoli Cells and Müllerian Regression. Sertoli cells play a key role in supporting germ cell survival, and they produce two important peptide hormones: antimüllerian hormone (AMH also known as müllerian-inhibiting substance [MIS]) and inhibin B. AMH, a glycoprotein homodimer, is a member of the transforming growth factor-β (TGFβ) superfamily and is first secreted in humans from about 7 to 8 wpc under the regulation of key transcription factors such as SOX9, SF1, WT1, and GATA4 (see Figs. 24.1, 24.6, and 24.10).[124,125] AMH causes regression of müllerian structures (i.e., fallopian tubes, uterus, upper two-thirds of the vagina) by its paracrine action on the AMH type 2 receptor (AMHR2).

Müllerian structures appear to be maximally sensitive to AMH between 9 and 12 weeks after conception, a time when the developing testis is producing peak concentrations of AMH but before the onset of significant AMH production by the developing ovary. Boys with mutations in the *AMH* or *AMHR2* genes can present with persistent müllerian duct syndrome (PMDS), which can present with undescended testes but otherwise typical male external genitalia. Severe forms of 46,XY gonadal dysgenesis can also result in persistent müllerian structures due to impaired Sertoli cell development and AMH release. In some cases, a hemiuterus is present if AMH release is affected on only one side (the same side as the hemiuterus); but in these cases, androgenization of the external genitalia is usually also impaired so that these children present with atypical genitalia. In contrast, defects confined to Leydig cell steroidogenesis in 46,XY DSD are not associated with persistent müllerian structures because Sertoli cell production of AMH is unaffected. In boys, a small müllerian remnant sometimes persists as a testicular appendage or as a prostatic utriculus or utricular remnant.

Inhibin B is also a member of the TGFβ superfamily and exerts negative feedback on pituitary follicle-stimulating hormone (FSH) secretion (but not secretion of luteinizing hormone [LH]), but it is unclear whether inhibin B has any local role during testis development.

Fetal Leydig Cells and Steroidogenesis. Fetal Leydig cells develop within the interstitium of the developing testis from common precursor cells. There is a marked upregulation of steroidogenic genes at around 53 to 57 dpc, followed by androgen synthesis and secretion (see Fig. 24.1).[12,126] An expansion in fetal Leydig cells occurs between 14 and 18 weeks of gestation, resulting in increased testosterone secretion at about 16 weeks.[126,127] Fetal Leydig cell steroidogenesis is stimulated by placental human chorionic gonadotropin (hCG) during the first trimester of pregnancy, but the developing hypothalamic-gonadotrope system produces significant amounts of LH from about 16 to 20 weeks of gestation.[128,129]

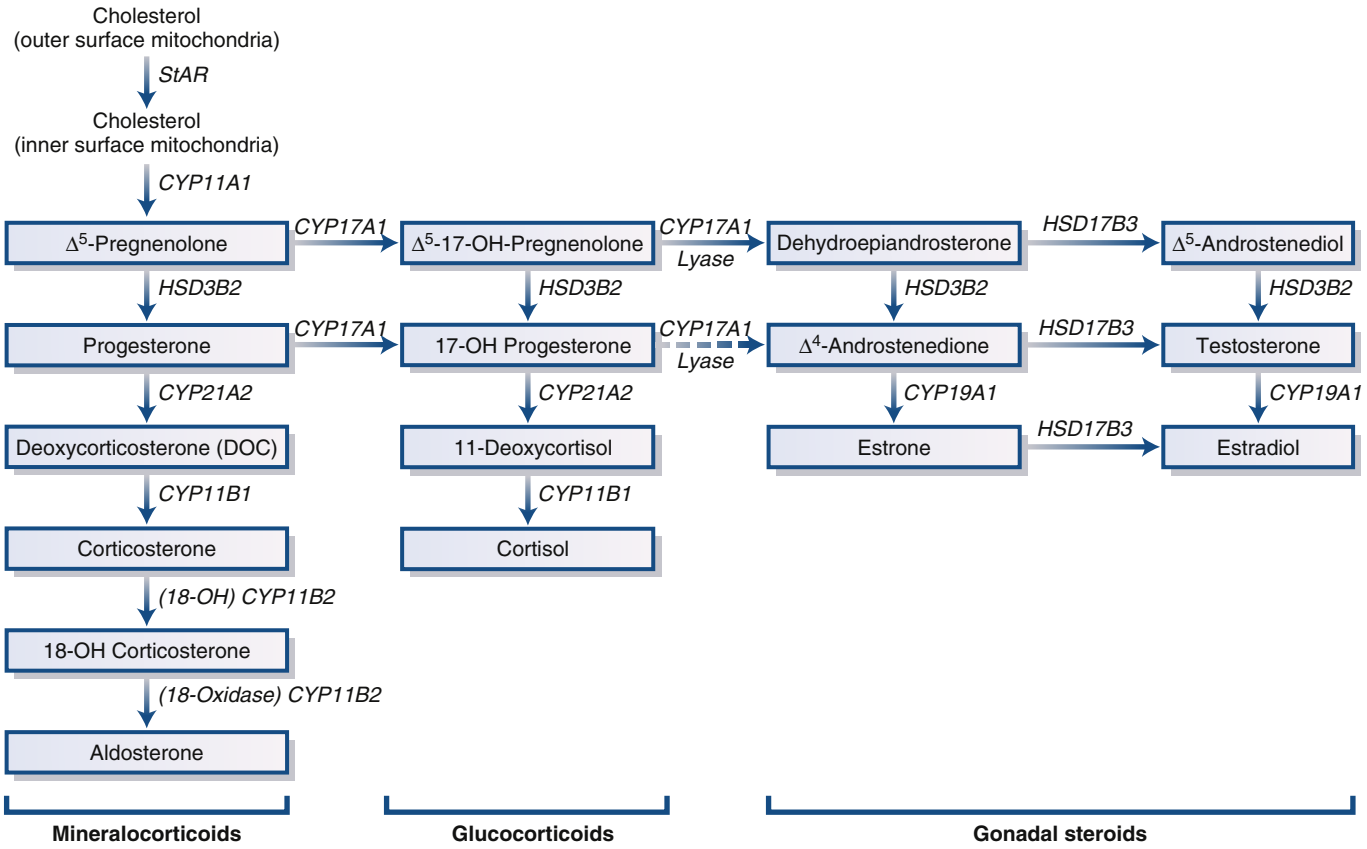
The pathways of testicular steroidogenesis are shown in Fig. 24.11 and overlap extensively with adrenal steroidogenesis pathways (see Chapter 15). The role of individual enzymes is discussed in relation to individual steroidogenic defects later in this chapter and in several excellent reviews.[130] In brief, cholesterol is taken up into Leydig cells by low-density lipoprotein or high-density lipoprotein receptors or is generated de novo by cholesterol synthesis pathways or from cholesterol ester. Stimulation of the LH/hCG receptor increases the ability of steroidogenic acute regulatory protein (StAR) to facilitate movement of cholesterol from the outer to the inner mitochondrial membrane.[131] The first and rate-limiting step in steroid hormone synthesis involves three distinct reactions: 20α-hydroxylation, 22-hydroxylation, and cleavage of the cholesterol side chain to generate pregnenolone and isocaproic acid. These steps are catalyzed by a single enzyme, P450scc (CYP11A1).

Pregnenolone is converted to progesterone by the microsomal enzyme 3β-hydroxysteroid dehydrogenase type 2 (3βHSD2), or it can undergo 17α-hydroxylation by P450c17 (CYP17) to yield 17-hydroxypregnenolone. CYP17 also has 17,20-lyase activity, which can cleave the C17,20 carbon bond of 17-hydroxypregnenolone to generate dehydroepiandrosterone (DHEA). This 17,20-lyase activity is favored by the presence of Δ^5-substrates (e.g., 17-hydroxypregnenolone), redox partners such as P450 oxidoreductase (POR) and cytochrome b_5, and serine phosphorylation of CYP17. These reactions are facilitated by the relative abundance of these factors in Leydig cells in humans, and the main pathway to androgen production is through conversion of 17-hydroxypregnenolone to DHEA rather than through conversion of 17-hydroxyprogesterone (17-OHP) to androstenedione.[132] Subsequent testosterone production can occur through conversion of DHEA to androstenedione by 3βHSD2, followed by the 17β-hydroxysteroid dehydrogenase action of 17βHSD3 to generate testosterone, or through the intermediate metabolite androstenediol (see Fig. 24.11).

Testosterone undergoes conversion to dihydrotestosterone (DHT) by 5α-reductase type 2 in peripheral tissues. DHT's high-affinity action on the androgen receptor results in androgenization of the external genitalia. Studies based on the phenotype of patients with POR deficiency and the fetal tammar wallaby have proposed that an alternative pathway to DHT production may exist in the human fetal testis (see "P450 Oxidoreductase Deficiency"): the so-called backdoor pathway (Fig. 24.12).[133,134]

Local production of testosterone stabilizes wolffian structures such as the epididymis, vas deferens, and seminal vesicle, whereas the potent metabolite DHT induces androgenization of the external genitalia and urogenital sinus (Fig. 24.13). In the male, the urogenital sinus gives rise to the prostate and prostatic urethra, the genital tubercle develops into the glans penis, the urogenital (urethral) folds fuse to form the shaft of the penis, and the urogenital (labioscrotal) swellings fuse to form the scrotum (Figs. 24.14 and 24.15).[135]

Testosterone and DHT mediate their effects through the androgen receptor (AR). The AR is a member of the superfamily of ligand-dependent nuclear receptor transcription factors encoded by a gene (*AR*, also known as *NR3C4*) on the X chromosome (Xq11-q12).[136] Like other nuclear receptors, it contains a highly conserved central DNA-binding domain (DBD) and a C-terminal ligand-binding domain (LBD) (Fig. 24.16). In addition, the AR uniquely contains an extended N-terminal domain (NTD) that



• **Fig. 24.11** Schematic diagram shows the steroid biosynthetic pathways leading to androgen production in the testis. In humans, the main pathway to androgen production is through conversion of 17-hydroxypregnenolone to dehydroepiandrosterone (DHEA) rather than through conversion of 17-hydroxyprogesterone to androstenedione. Subsequent testosterone biosynthesis can occur through conversion of DHEA to androstenedione (by 3β-hydroxysteroid dehydrogenase type 2 [3βHSD2]), followed by the actions of 17α-hydroxysteroid dehydrogenase type 3 (17βHSD3) to generate testosterone, or through the intermediate metabolite androstenediol. During male sex development, testosterone undergoes local conversion to dihydrotestosterone by 5α-reductase type 2 *(not shown)*. The high-affinity action of dihydrotestosterone on the androgen receptor results in androgenization of the external genitalia. The pathways responsible for mineralocorticoid and glucocorticoid synthesis are present in the adrenal gland. Additional or alternative pathways to dihydrotestosterone production may exist in the fetal testis.

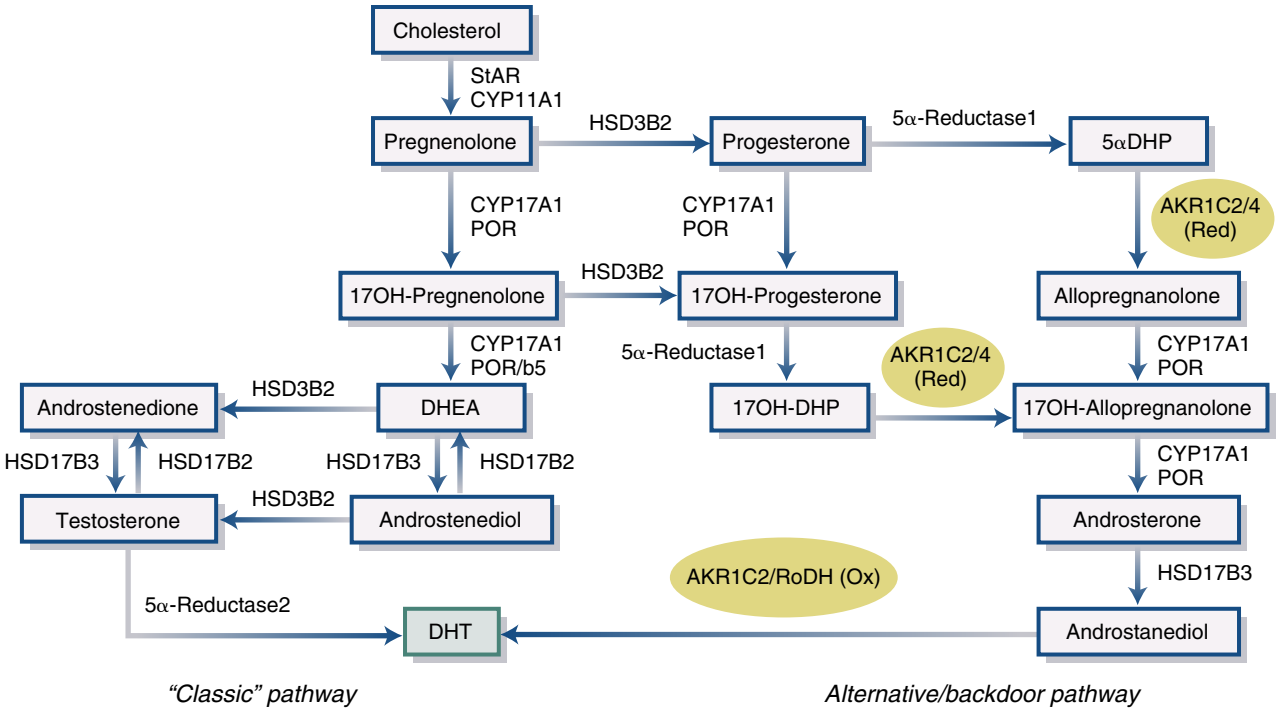
includes polyglutamine (CAG) and polyglycine (GGN) repeats that can vary in number; the length of these repeats can modulate AR activity, with a longer stretch of repeats associated with decreased AR activity.

The DBD contains cysteine residues that coordinate zinc atoms to form two zinc fingers, the first of which contains a P-box that enters the major groove of DNA to form specific base-pair contacts that are identical for all classic steroid receptors. The second zinc finger containing the D-box is involved in protein-protein interactions and stabilizes the unit for receptor dimerization. This binding requires preferential recognition of androgen response elements (AREs) composed of an inverted repeat of DNA sequences related to 5′-AGAACA-3′.[137]

The AR in the unliganded state is located in the cytoplasm and is complexed to heat shock proteins such as HSP70 and HSP90 and to co-chaperone proteins such as FKBP4 (also called FKBP52). Binding of ligand to its receptor results in dissociation from these complexes to allow translocation of the AR into the nucleus, where it binds to DNA target sequences as a homodimer.

The crystal structure of the AR LBD bound to natural and synthetic androgens is shown in Fig. 24.16C. Helix 12, in the presence of ligand, undergoes a conformational change to fold back on top of the ligand hydrophobic pocket to capture the ligand, thus slowing the rate of ligand-receptor dissociation. This trapping effect by helix 12 enables recruitment of transcriptional coactivators.

Activation of transcription involves motif activation function 1 (AF1) in the NTD and motif activation function 2 (AF2) in the LBD. AF1 is ligand independent, whereas AF2 is ligand dependent and interacts with p160 steroid receptor coactivators such as NCOA1, NCOA2, and NCOA3.[138] A relatively unique feature of the AR is interaction between the N-terminal and C-terminal domains (N-C interaction). In the presence of its primary physiologic ligands, testosterone and DHT, the AR recruits diverse coregulators that cooperate in the activation of transcription of androgen-regulated genes (see Fig. 24.16). N-terminal sequences comprising amino acid residues 23 to 27 (FQNLF) and residues 435 to 439 (WHTLF) participate in the AR N-C interaction to stabilize the AR and slow dissociation of the ligand. Further modulation of AR function occurs post-translationally by processes such as phosphorylation and sumoylation.



• **Fig. 24.12** The classic and alternative (backdoor) pathways of dihydrotestosterone (DHT) synthesis. The classic pathway leading to synthesis of DHT is *shown on the left*. The alternative pathway potentially involved in DHT synthesis is *shown on the right*. The alternative pathway involves the actions of additional enzymes: 5α-reductase, type 1 (5α-reductase 1, encoded by *SRD5A1*), AKR1C2 (3α-reductase, type 3) and possibly AKR1C4 (3α-reductase, type 1), and RoDH (3-hydroxyepimerase, encoded by *HSD17B6*). *DHEA,* dehydroepiandrosterone; *DHP,* dihydroprogesterone; *HSD,* hydroxysteroid dehydrogenase; *POR,* P450 oxidoreductase; *StAR,* steroidogenic acute regulatory protein. (From Flück CE, Meyer-Böni M, Pandey AV, et al. Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *Am J Hum Genet*. 2011;89:201–218.)

The action of the AR is further modulated by interaction with coregulatory proteins that function as coactivators or corepressors.[138] Agonist-induced changes in LBD organization enable coactivator recruitment through their LXXLL motifs.[139] The AR exhibits a selective preference for the ARA70, ARA55, and ARA54 coregulators, which contain FXXLF motifs related to the aforementioned amino acid residues in the N-terminus.

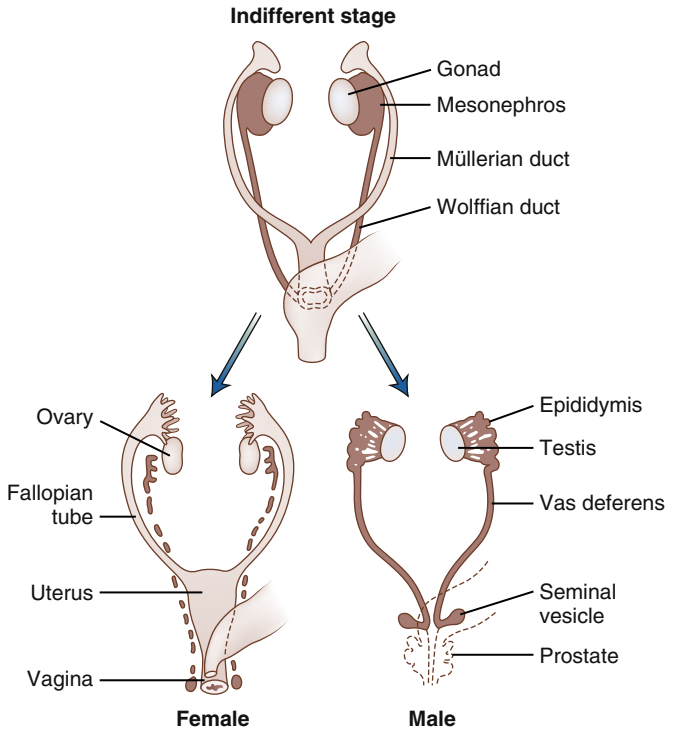
Relatively little is known about AR targets in the developing wolffian structures (which are testosterone responsive) and in the key target tissues such as the developing external genitalia (which are DHT responsive). Studies in mice have revealed a number of factors that are necessary for development of the wolffian ducts (e.g., Gdf7, Bmps4, Bmps7, Bmps8a, Bmps8b, Hoxa10, Hoxa11) and for growth of the genital tubercle (e.g., Fgfs, Shh, Wnts, Hoxa13, Hoxd13, Bmp/noggin, ephrin signaling). Impaired androgen action occurs in several syndromic conditions and may reflect defects in genes that mediate target-tissue responsiveness and genital tubercle growth (e.g., *HOXA10*, *HOXA13*).

Testicular Descent. Testicular descent is a two-stage process that starts at about 8 weeks of gestation and is usually complete by the middle of the third trimester.[140] The initial transabdominal stage of testicular descent (8–15 weeks) involves contraction and thickening of the gubernacular ligament and degeneration of the craniosuspensory ligament. This stage is mediated by the testis itself after secretion of factors such as insulin-like 3 (INSL3, a relaxin-like factor) and its G protein–coupled receptor, GREAT (also called LGR8 or RXFP2).[141] The subsequent transinguinal (or inguinoscrotal) phase of testicular descent (25–35 weeks) is primarily driven by androgens. The genitofemoral nerve and its neurotransmitter, calcitonin gene–related peptide (CGRP), have also been implicated in this process.

Subsequent Testicular Development. During the second and third trimesters, the testes show several distinct morphologic changes, including a reduction in fetal Leydig cell mass and elongation and coiling of seminiferous cords. There is no further significant development of germ cells during this time, and seminiferous cords do not canalize until later in childhood. Nevertheless, certain developmental insults can affect the testis at this stage. For example, testicular regression syndrome (also called vanishing or absent testis syndrome) is most likely a late fetal event, because boys with this condition have adequate androgenization and no müllerian structures, indicating normal testicular function in early gestation (see "Anorchia and Cryptorchidism").

Female Sex Differentiation

The processes of female sex differentiation do not involve significant morphologic changes in the external genitalia. Müllerian structures persist to form the fallopian tubes, uterus, and upper portion of the vagina (see Fig. 24.13). Normal uterine development in mice occurs even in the absence of the ovary and requires a host of factors for uterine development (e.g., Pax2, Lim1, Emx2, Wnt4/Lp, Hoxa13) and differentiation (e.g., Wnt7a, Hoxa10, Hoxa11, Hoxa13, progesterone and estrogen receptors).[142] The lack of local testosterone production leads to degeneration of wolffian structures. The urogenital sinus develops into the urethra and lower portion of the vagina, which in the absence of testosterone



• **Fig. 24.13** Embryonic differentiation of female and male genital ducts from wolffian and müllerian primordial tissue before descent of the testes into the scrotum. In females, müllerian structures persist to form the fallopian tubes, uterus, and upper portion of the vagina. The lower portion of the vagina and urethra are derived from the urogenital sinus. In males, wolffian structures develop into the epididymides, vasa deferentia, and seminal vesicles, whereas the prostate and prostatic urethra are derived from the urogenital sinus. In some cases, a small müllerian remnant can persist in males as a testicular appendage.

become separated by the vaginal plate, resulting in two separate orifices for these structures. The genital tubercle develops into the clitoris, the urogenital (urethral) folds form the labia minora, and the urogenital (labioscrotal) swellings form the labia majora (see Figs. 24.13 and 24.14).

In contrast to the testis, the developing ovary does not express FSH or LH/hCG receptors until after 16 weeks of gestation. At about 20 weeks of gestation, plasma concentrations of FSH reach a peak and the first primary follicles are formed.[129] By 25 weeks of gestation, the ovary has developed definitive morphologic characteristics. Folliculogenesis can proceed, and a few graafian follicles have developed by the third trimester.[8] Although some studies have suggested that the early fetal ovary can produce steroids and express aromatase, the amount of estrogen secreted by the developing ovary is likely to be insignificant compared with placental estrogen synthesis, and ovarian estrogen production does not seem to have significant effects on sex development until the time of puberty.[16]

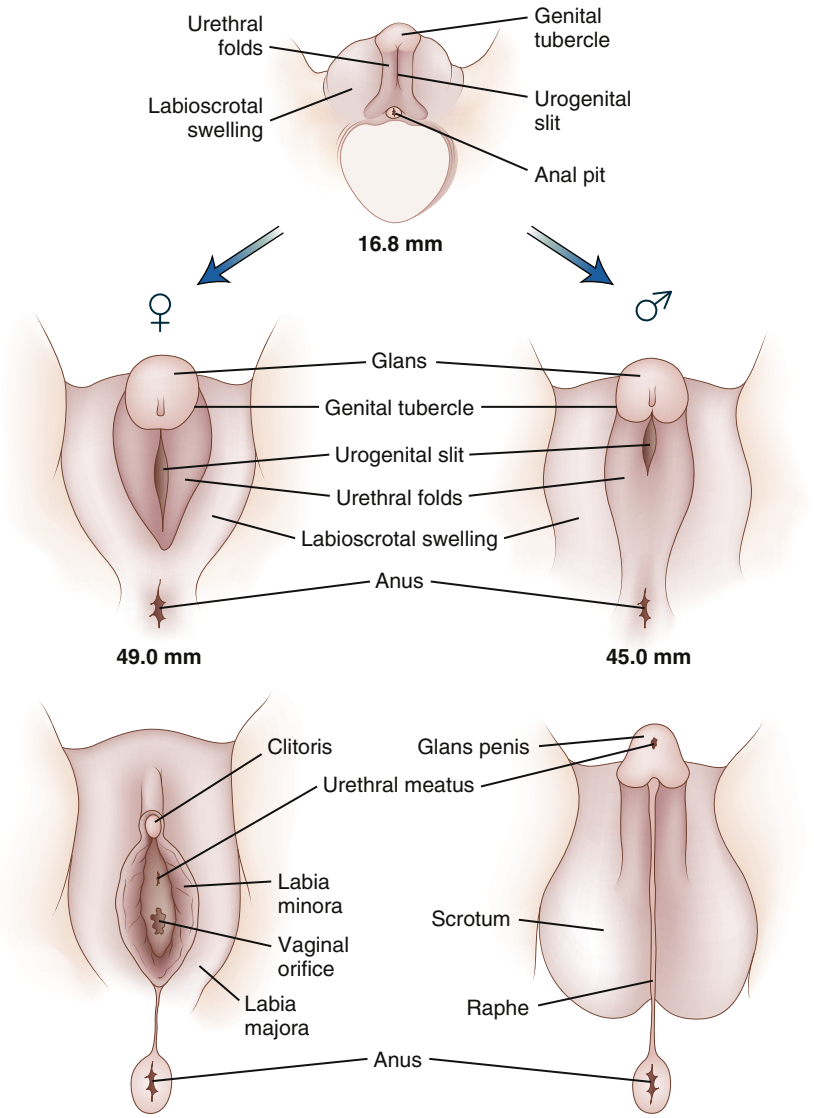
Several conditions can affect 46,XX sex development in utero. Exposure of the fetus to androgens results in androgenization of the external genitalia. The local testosterone concentration typically is not sufficient to stabilize wolffian structures because the androgens are usually adrenal in origin. Most frequently, androgenization of the 46,XX fetus results from disorders of adrenal steroidogenesis (e.g., deficiency of 21-hydroxylase [CYP21], 11β-hydroxylase, POR, or 3β-hydroxysteroid dehydrogenase). Rare causes of androgenization include (placental) aromatase deficiency, glucocorticoid resistance, testicular and ovotesticular DSD, and maternal virilizing tumors (e.g., luteoma of pregnancy). With the exception of testicular/ovotesticular DSD, these conditions result in exposure to androgens but not AMH, so a uterus is usually present. Exposure to certain chemical agents in pregnancy has been proposed as a cause of fetal androgenization, but data are limited. For other developmental abnormalities of the female genital tract (e.g., Mayer-Rokitansky-Küster-Hauser [MRKH] syndrome) see "46,XX Disorders of Sex Development."

Psychosexual Development

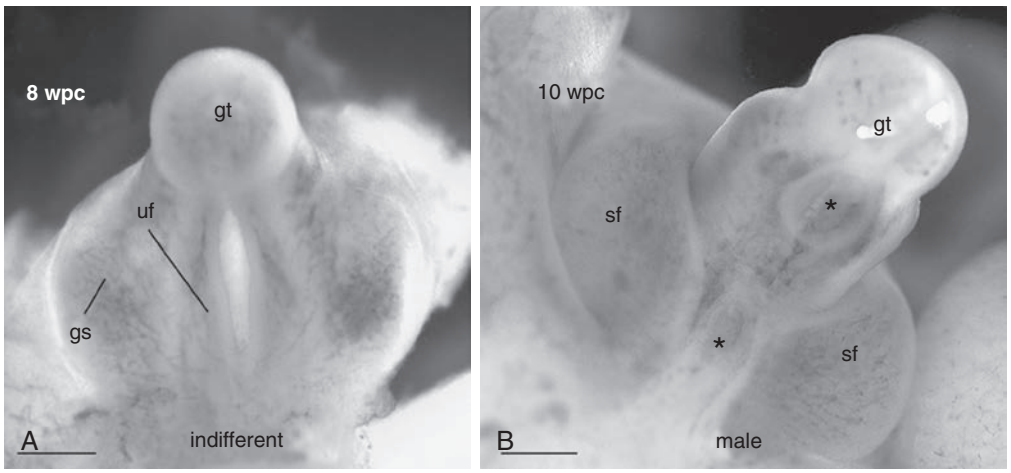
Psychosexual development is traditionally viewed as having several distinct components (Table 24.2). *Gender identity* refers to a person's self-identification as male, female, or a nonbinary gender (e.g., intermediate to male and female, combined male and female, agender). For some individuals, gender identity may be fluid. *Gender role* (sex-typical behaviors) describes the expression or portrayal of psychological characteristics and behaviors that are sexually dimorphic within the general population, such as toy preferences, physical aggression, and choices of activities and occupation. *Gender expression* refers to whether outward appearance conforms more closely to male or female societal norms. *Sexual orientation* refers to romantic and erotic interests (e.g., heterosexual, bisexual, homosexual) and includes behavior, fantasies, and attractions and may or may not correspond to actual sexual activity.

The past 50 years have seen a number of opposing theories about the origins of psychosexual development and debate about the relative contributions of chromosomes, hormones, brain structure, and societal and family influences on the various components. Much of this work has focused on the study of rodents and nonhuman primate species. For example, Young and colleagues first showed in 1959 that exposure of guinea pigs to testosterone during pregnancy resulted in altered mating behavior of female offspring.[143] These effects may be most pronounced during a critical window of exposure and, in rodents and some primates, may depend in part on aromatization of the androgens to estrogens, receptor availability, and social environment.[144] More recently, interest has focused on the role of genes and chromosomes in sex behavior. For example, studies of differential gene expression patterns in the developing mouse brain have shown upregulation of different X and Y chromosomal genes in early embryonic life, even before the onset of significant androgen secretion by the developing testis in males.[145] Studies of mice in which *Sry* was deleted ($XYSRY^{-}$) or transgenically expressed (XXSRY) showed certain neuroanatomic differences between XY and XX mice independent of gonad development and endocrine status.[146] These findings suggest that factors related to chromosome complement have at least the potential to affect psychosexual development independent of sex hormone action, and a model integrating both factors may be more appropriate.[147]

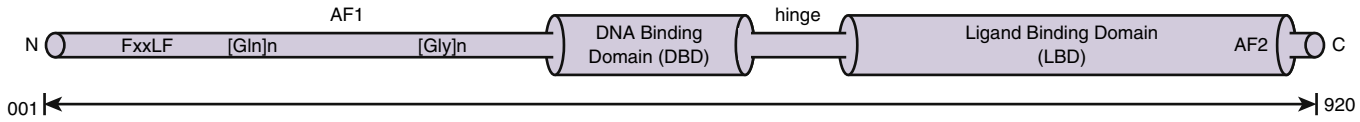
Understanding the complex issues related to psychosexual development in humans is even more challenging, especially because gender identity cannot readily be assessed in nonhuman species. For many years, it was thought that gender identity would be concordant with the sex designated at birth, provided that the child was raised unambiguously, and appropriate surgical procedures and hormone therapies were instituted concordant with the gender chosen. This theory assumed psychosexual neutrality at birth, but it has been challenged by a refocusing on the potential importance of prenatal (e.g., endocrine) and innate (e.g., chromosomal) influences on psychosexual development.[148,149] Direct data to assess such effects



• **Fig. 24.14** Differentiation of male and female external genitalia. (Modified from Spaulding MH. The development of the external genitalia in the human embryo. *Contrib Embryol Carnegie Inst*. 1921;13:69–88.)



• **Fig. 24.15** Differentiation of male external genitalia in humans between 8 and 10 wpc. (A) Undifferentiated human external genitalia at 8 wpc. (B) Differentiation of scrotal folds and fusion of the urethral folds *(asterisks indicate patent regions on either side)* at 10 wpc. *gs,* genital swelling; *gt,* genital tubercle; *sf,* scrotal folds; *uf,* urethral folds. Scale bars: 500 μm. (From Goto M, Piper Hanley K, Marcos J, et al. In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. *J Clin Invest*. 2006;116:872–874, used with permission of the American Society for Clinical Investigation, Copyright 2006.)



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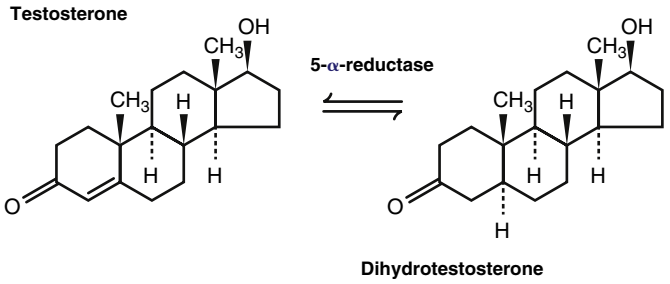

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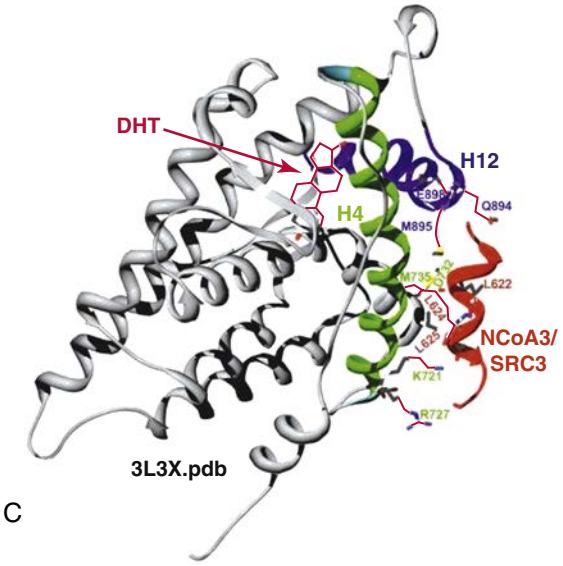

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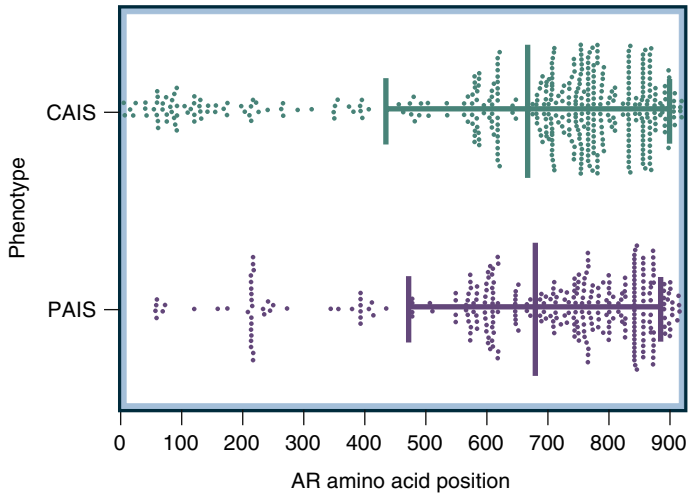


D

• **Fig. 24.16** (A, B) Structure of the androgen receptor (AR) in keeping with nuclear receptors in general. The AR uniquely has an extended amino-terminal domain, which contains the FxxLF, polyglutamine, and polyglycine motifs, which influence transactivation in response to testosterone and its 5α-reduced metabolite, dihydrotestosterone. (C) The crystal structure of the LBD (ligand-binding domain) is shown bound to the ligand, DHT, which initiates a conformational change involving helix 12 and enables recruitment of coactivators such as NCoA3/SRC3. (D) The gene distribution of mutations associated with CAIS and PAIS are shown (see also Fig. 24.31). *CAIS,* complete androgen insensitivity syndrome; *PAIS,* partial androgen insensitivity syndrome. (From Mongan NP, Tadokoro-Cuccaro R, Bunch T, et al. Androgen insensitivity syndrome. *Best Pract Res Clin Endocrinol Metab*. 2015;29:569–580.)

TABLE 24.2 Components of Psychosexual Development

Term	Explanation
Gender identity	Self-identification of gender as male, female, or other
Gender expression/role	Expression of gender-stereotypical behavior Aggression Parenting rehearsal Peer and group interactions Labeling (e.g., "tomboy") Grooming behavior
Sexual orientation	Pattern of sexual and romantic attraction

in humans are limited, but studies in women with complete androgen insensitivity syndrome (CAIS), in whom the karyotype is 46,XY but psychosexual development is almost always female, argue for a major role for androgens and a lesser role for Y chromosome genes alone in human psychosexual development.[150] However, the gender characteristics of individuals with a Y chromosome and a partial degree of androgen exposure or responsiveness (e.g., partial androgen insensitivity syndrome [PAIS], 17β-hydroxysteroid dehydrogenase deficiency, 5α-reductase deficiency) can vary, and long-term gender identity can be difficult to predict.[151,152] It has been suggested that the degree of androgenization of the external genitalia may serve as a marker of in utero testosterone exposure for not only the genitals but also the brain. Indeed, in a study of 46,XY individuals with PAIS, a greater degree of genital

androgenization generally correlated with increased scores on a composite gender index that incorporated assessments of both gender identity and gender-stereotypical behaviors, but there was wide variability for the most ambiguous phenotypes.[153]

Prenatal exposure to androgens can also influence psychosexual development in 46,XX individuals.[149,154] Girls with congenital adrenal hyperplasia (CAH) who have more marked genital androgenization are more likely to play more with boys' toys, with long-term effects evident into adulthood.[155] Prenatal androgen exposure can also be associated with other psychological characteristics, such as sexual orientation.[156] However, the association between high prenatal androgen exposure and gender identity is generally less marked, except perhaps in some cases of (ovo)testicular DSD and potentially severe deficiencies of aromatase or 11β-hydroxylase.[157] Although gender dissatisfaction (i.e., unhappiness with sex designated at birth) is more common in individuals with DSD, more than 90% of 46,XX individuals with CAH who are raised as girls later identify as female.[149,158] The small number of 46,XX men with CAH were usually raised male and diagnosed late. However, more 46,XX individuals with CAH and raised as girls may later identify as male than originally thought.[159,160]

Social environment and gender of rearing may also play a role in psychosexual development. Evidence for this comes from case series of 46,XY individuals with genital anatomy that was disrupted either developmentally (e.g., due to pelvic wall anomalies such as cloacal exstrophy) or iatrogenically (e.g., penile ablation) but who otherwise had normal testosterone production and responsiveness.[161,162] Historically, many of these individuals were raised as girls. Long-term follow-up of these patients has shown that many continued to identify as female, though a significant proportion identified as male gender or were uncomfortable with a female gender identity. Of note, of those raised as boys, all continued to identify with the male gender in late adolescence and adulthood, and current practice favors raising 46,XY patients as boys.

Collectively, these studies show that karyotype, prenatal androgen exposure, and designated gender may all influence gender identity. However, gender identity cannot be reliably predicted from any of these factors. Furthermore, because gender identity, sex-typical behavior, and sexual orientation are separate components of psychosexual development, it is important to appreciate that an interest in same-sex relationships (relative to sex of rearing) or strong cross-sex interest in an individual with DSD is not necessarily an indication of incorrect gender designation.[3]

Challenges in assessing gender identity in young children make it difficult to know when this is established, although it is thought to begin between 18 and 36 months and possibly younger.[163] Many of the sex differences in brain structures reported at late childhood, puberty, or adulthood are not seen in early childhood and are therefore not useful for guiding sex designation.[164,165] There may be some plasticity in psychosexual development, as is evident from studies of some patients with conditions such as 5α-reductase deficiency and 17β-hydroxysteroid dehydrogenase deficiency who may change their gender role in adolescence. Furthermore, some individuals with DSD do not fit into a binary model of gender. In several countries, important legal changes have taken place to acknowledge this, such as the option not to define gender as male or female on passports. Nevertheless, many societies and individuals still have a very binary view of gender, and freedom of expression remains difficult for many people who do not feel they fit a binary model.

Development of the Hypothalamic-Pituitary-Gonadal Axis in the Fetus

Development of the hypothalamic-pituitary-gonadal (HPG) axis is described in detail in Chapter 23; points that are particularly relevant to DSD are reviewed here.

Fetal hypothalamic-gonadotrope development occurs from 6 wpc in humans and occurs in parallel with the processes of sex determination and differentiation. This involves migration of gonadotropin-releasing hormone (GnRH)–producing neurons from the olfactory placode through the cribriform plate to the fetal hypothalamus, development of the hypothalamic nuclei, formation of the anterior pituitary from Rathke pouch, and specification and maturation of pituitary gonadotropes that are capable of releasing LH and FSH as part of a functional HPG axis. Defects in these systems can result in a range of clinical conditions, such as Kallmann syndrome, congenital isolated hypogonadotropic hypogonadism, and gonadotropin insufficiency as part of a multiple pituitary hormone deficiency, which are reviewed elsewhere (Chapters 8, 23, and 26).

It is generally thought that pulsatile release of LH and FSH does not occur and therefore does not influence the gonad until about 16 to 20 wpc, at which stage fusion of the penis is complete.[129] In male fetuses, the main effects of gonadotropins are to support the latter stages of testicular steroidogenesis involved in elongation of the penis and to support the transinguinal aspects of testicular descent. Consequently, boys with congenital gonadotropin insufficiency may have a small penis (micropenis) and/or bilaterally undescended testes. These children might need monitoring for important associated features such as renal agenesis in Kallmann syndrome or growth hormone or adrenocorticotropic hormone (ACTH) insufficiency (causing hypoglycemia) in panhypopituitarism. Classically, boys with hypothalamic or pituitary gonadotropin insufficiency do not have hypospadias, as fusion of urethral folds has occurred before 20 weeks. Rarely, hypospadias occurs with HPG disorders, potentially reflecting loss of factors that influence the HPG axis at multiple levels.[166,167]

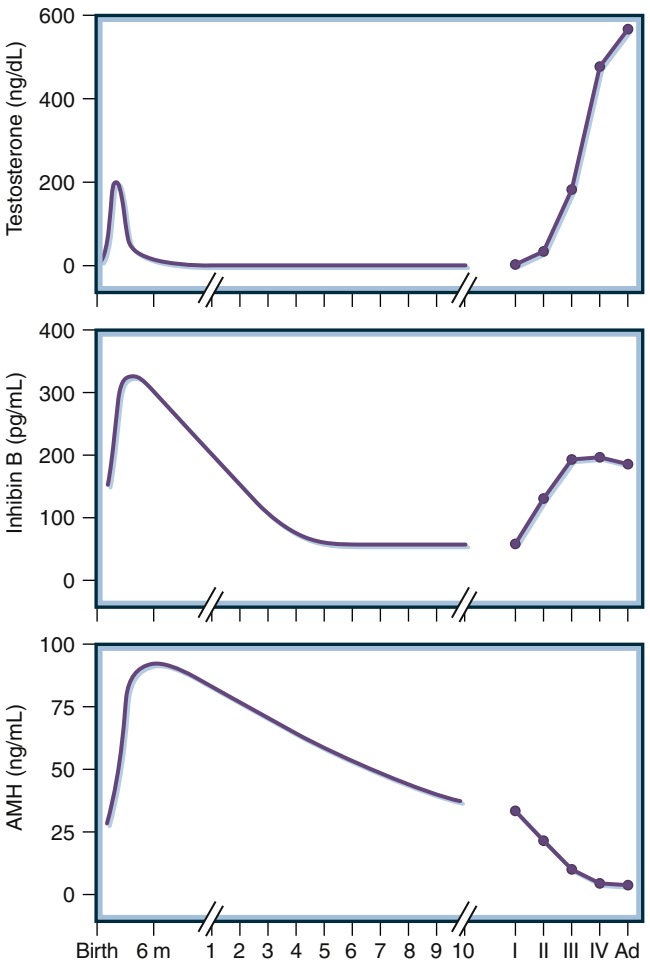
The Hypothalamic-Pituitary-Gonadal Axis in Infancy and Childhood

At birth, the infant is removed from the influence of maternal and placental hormones and undergoes a series of distinct endocrine changes.

Postnatal Endocrine Changes in Boys

Testosterone can be detected at birth, with levels falling during the first few days of life. Thereafter, a reactivation of the HPG axis occurs and results in serum testosterone that is typically measurable by 1 to 2 weeks of life and peaks at midpubertal levels between 2 and 3 months after birth (Fig. 24.17).[168,169] This peak of testosterone is associated with an acceleration in penile growth and may be linked in part to early postnatal maturation of gonocytes in the testis.[169] The HPG axis then becomes relatively quiescent from around 6 months of age until the onset of puberty, and serum concentrations of LH, FSH, and testosterone are low during this time.

Inhibin B concentrations are similarly high at birth and in infancy, fall during the first 2 years of life to low but still detectable levels, then rise again with the onset of puberty (see Fig. 24.17).[170,171] In contrast, AMH concentrations remain high from birth through childhood and only decline with the onset



• **Fig. 24.17** Typical postnatal changes are shown for testosterone, antimüllerian hormone (AMH), and inhibin B in normal males from birth to adulthood. Pubertal stages (I–IV) are indicated. Conversions: testosterone, ng/dL × 0.0347 for nmol/L; AMH, ng/mL × 7.14 for pmol/L; ranges may vary with the assay, so only indicative values are provided to show trends over time.

of puberty (see Fig. 24.17).[124,125] Inhibin B and especially AMH can therefore be useful markers of active testicular tissue (specifically, Sertoli cells) in boys with cryptorchidism, anorchia, and 46,XY DSD even when assessed between the periods of robust HPG activity in infancy and at puberty. INSL3 assays may provide a useful additional marker of Leydig cell function in the future.[172,173]

Postnatal Endocrine Changes in Girls

The early postnatal endocrine events in girls are less well characterized. Placental estrogen exposure can result in breast development before birth, and a small episode of menstrual bleeding can occur several days after birth from withdrawal of estrogen and progesterone. Girls also have a discrete activation of the HPG axis in infancy that declines by 1 to 3 years of age. Detectable concentrations of estradiol (5–20 pg/mL [20–80 pmol/L]) and inhibin B (50–200 pg/mL) can be measured in the first few months of life, and surprisingly high concentrations of FSH with marked interindividual variability can be found during infancy and early childhood (median, 3.8 IU/L; range, 1.2–18.8 IU/L [2.5–97.5%] at 3 months of age in healthy term girls).[174] Throughout childhood, FSH remains detectable, but LH is low. AMH is very low until puberty. Inhibin A, which is made exclusively by the ovary, has been proposed as a test of ovarian tissue in the newborn period in children with possible ovotesticular DSD, but this hormone is below the limits of detection in many normal term newborn girls, and FSH stimulation may be needed to detect it.[175]

Disorders (Differences) of Sex Development

DSDs can have a wide range of presenting phenotypes, depending on the underlying condition and its severity. Individuals with these conditions can present to many different health care professions, including neonatologists, geneticists, urologists, gynecologists, and internists. The classic presentation is an infant born with ambiguous genitalia, but not all conditions are apparent at birth. For example, 46,XY individuals with complete 17α-hydroxylase/17,20-lyase deficiency may first present in early adolescence with hypertension and delayed puberty, or a young woman with CAIS (46,XY) may first present to a gynecologist with amenorrhea.

Significant progress in understanding the molecular basis of gonadal development has occurred during the past 30 years. Several single-gene disorders causing gonadal dysgenesis in humans have been described, and many more candidate genes are emerging from studies in mice. The percentage of patients with disorders of gonadal development who can be diagnosed at the molecular level is increasing with the use of next-generation sequencing (NGS) of gene panels or whole exomes (currently around 25–40%),[166,176–179] and molecular diagnosis of classic disorders of steroidogenesis can be reached in most situations. Defining the exact basis of a patient's DSD can have important implications for sex designation, predicting response to treatment (e.g., androgen supplementation), screening for associated features (e.g., adrenal dysfunction), assessing the risk of tumorigenesis, determining likely fertility options, and providing long-term counseling for the individual and family. However, long-term outcome studies are often inadequate, and an evidence-based approach to management is not possible in many cases.

Nomenclature and Classification of Disorders of Sex Development

DSD has been defined as "congenital conditions in which development of chromosomal, gonadal, or anatomic sex is atypical."[1] This definition is wide ranging enough to cover nonendocrine conditions such as cloacal exstrophy but sufficiently specific not to embrace, for example, disorders of puberty. The consensus statement that introduced the term DSD further suggested classification of DSD by karyotype (Table 24.3): sex chromosome variations (sex chromosome DSD), disorders of testis development and of androgen synthesis and action (46,XY DSD), and androgen excess and disorders of ovary development (46,XX DSD). Although karyotype does not define a person's gender, rapid assessment of karyotype can be a very useful stepping stone for focusing investigations and to start to counsel the family about likely causes and outcomes.

Sex Chromosome Disorders of Sex Development

Differences in the number of sex chromosomes (i.e., sex chromosome aneuploidy) can be considered *sex chromosome DSDs.*

TABLE 24.3 Example of a DSD Classification System

Sex Chromosome DSD	46,XY DSD	46,XX DSD
A: 47,XXY (Klinefelter syndrome) and variants B: 45,X (Turner syndrome) and variants C: 45,X/46,XY (mosaicism) and variants D: 46,XX/46,XY (chimerism)	A: Disorders of gonadal (testis) development Complete or partial gonadal dysgenesis (e.g., *SF1/NR5A1, WT1, GATA4, FOG2/ZFPM2, CBX2, SRY, SOX9, SOX8, MAP3K1, ESR2/NR3A2, DMRT1, TSPYL1, DHH, SAMD9, ARX, MAMLD1/CXorf6*) Ovotesticular DSD Testis regression	A: Disorders of gonadal (ovary) development Gonadal dysgenesis Ovotesticular DSD (e.g. *NR5A1, NR2F2, RSPO1*) Testicular DSD (e.g., *SRY*+, dup *SOX9,* dup *SOX3, NR5A1, NR2F2, RSPO1, WNT4*)
	B: Disorders in androgen synthesis or action Disorders of androgen synthesis Luteinizing hormone (LH) receptor mutations Smith-Lemli-Opitz syndrome StAR protein mutations Cholesterol side-chain cleavage *(CYP11A1)* 3β-hydroxysteroid dehydrogenase 2 *(HSD3B2)* 17α-hydroxylase/17,20-lyase *(CYP17)* P450 oxidoreductase *(POR)* Cytochrome b_5 *(CYB5A)* Aldo-keto reductase 1C2 *(AKR1C2)* 17β-hydroxysteroid dehydrogenase *(HSD17B3)* 5α-reductase 2 *(SRD5A2)* Disorders of androgen action Androgen insensitivity syndrome Drugs and environmental modulators	B: Androgen excess Fetal 3β-hydroxysteroid dehydrogenase 2 *(HSD3B2)* 21-hydroxylase *(CYP21A2)* P450 oxidoreductase *(POR)* 11β-hydroxylase *(CYP11B1)* Glucocorticoid receptor mutations Fetoplacental Aromatase (CYP19) deficiency Oxidoreductase (POR) deficiency Maternal Maternal virilizing tumors (e.g., luteomas) Androgenic drugs
	C: Other Syndromic associations of male genital development (e.g., cloacal anomalies, Robinow, Aarskog, hand-foot-genital, popliteal pterygium) Persistent müllerian duct syndrome Vanishing testis syndrome Isolated hypospadias Cryptorchidism *(INSL3, GREAT)* Environmental influences	C: Other Syndromic associations (e.g., cloacal anomalies) Müllerian agenesis/hypoplasia (e.g., MKRH) Uterine abnormalities (e.g., MODY5) Vaginal atresias (e.g., McKusick-Kaufman) Labial adhesions

CYP, Cytochrome P450 isoenzyme; *DSD,* disorders of sex development; *MODY5,* maturity-onset diabetes of the young type 5; *MRKH,* Mayer-Rokitansky-Küster-Hauser; *StAR,* steroidogenic acute regulatory (protein).

These conditions include Klinefelter syndrome (47,XXY and variants), Turner syndrome (45,X and variants), trisomy X (47,XXX and variants), XYY syndrome (47,XYY and variants), 45,X/46,XY mosaicism and its variants, and true sex chromosome chimerism (46,XY/46,XX) (Table 24.4). A 45,Y cell line is nonviable.

There can be ambiguous genitalia at birth if a Y chromosome or Y chromosome fragment is present in some but not all cells due to sex chromosome chimerism or mosaicism, but Klinefelter syndrome (unless four or more X chromosomes are present) and classic Turner syndrome in which there is no Y chromosomal material do not typically cause ambiguous genitalia. In many cases of classic sex chromosome aneuploidy, the diagnosis is made in adolescence or adult life during evaluation of characteristic features, impaired pubertal development, or infertility. More detailed descriptions of the features and long-term management of some of these conditions are provided in the relevant chapters (e.g., Turner syndrome in Chapter 26, Klinefelter syndrome in Chapters 19 and 26).

Klinefelter Syndrome and Its Variants

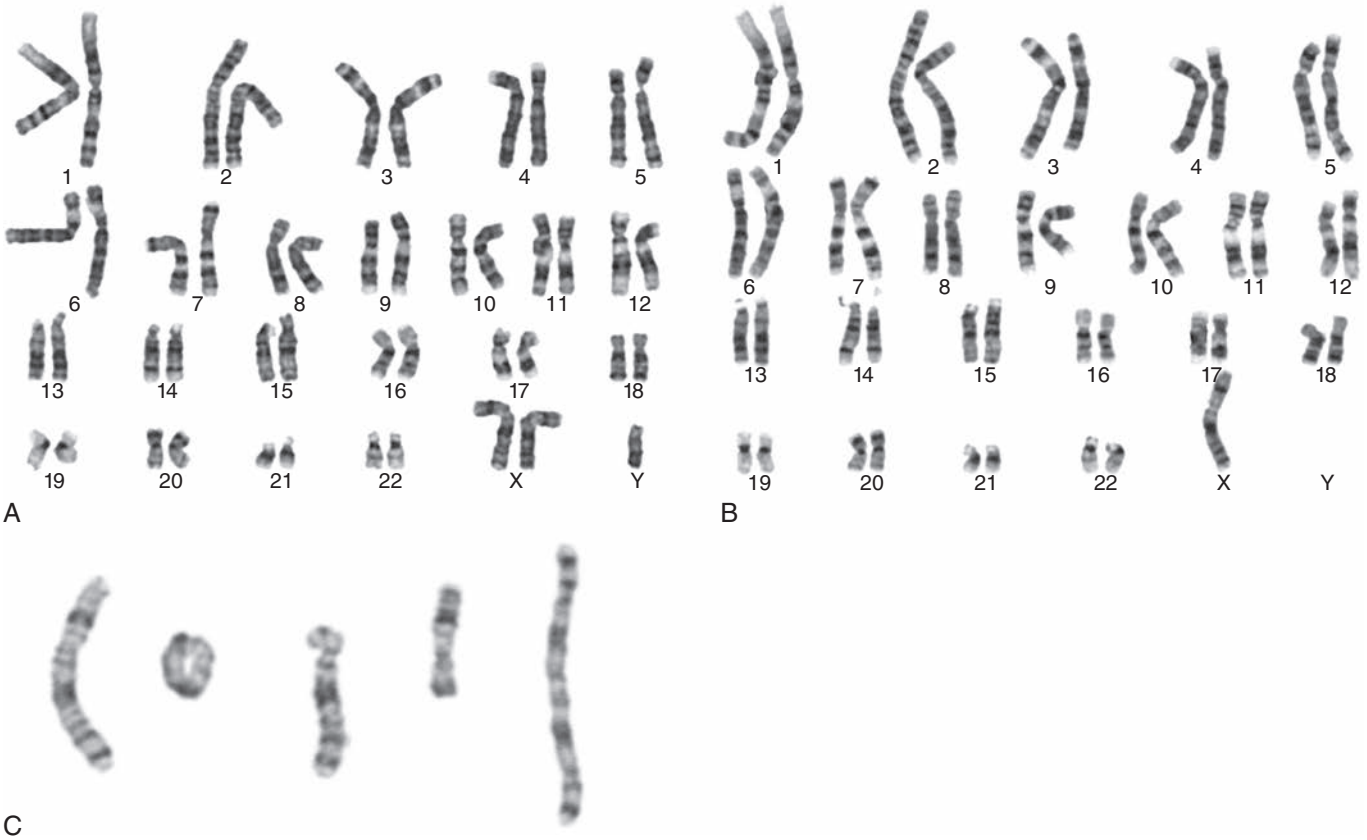
Klinefelter syndrome and its variants are the most common forms of sex chromosome aneuploidy, with a reported incidence of 1 in 500 to 1 in 1000 live male births.[180] This incidence may be increasing.[181] The classic form of Klinefelter syndrome is associated with a 47,XXY karyotype and is caused by meiotic nondisjunction of the sex chromosomes during gametogenesis (Fig. 24.18).[18] This abnormality occurs during spermatogenesis in approximately 50% of patients and during oogenesis or postzygotic division in approximately 50%.[180] Mosaic forms of Klinefelter syndrome (46,XY/47,XXY) likely represent mitotic nondisjunction within the developing zygote and are thought to occur in approximately 10% of individuals with Klinefelter syndrome. Other chromosomal variants of Klinefelter syndrome (e.g., 48,XXXY) can be seen.

The clinical features of Klinefelter syndrome and its variants are summarized in Table 24.4.[180] In the most obvious situations, a young man may be diagnosed because of small testes, gynecomastia, poor androgenization at puberty, eunuchoid proportions,

TABLE 24.4 Clinical Features of Sex Chromosome DSDs

Condition	Karyotype	Gonad	Internal Genitalia	Features
Klinefelter syndrome	47,XXY and variants	Hyalinized testes	No uterus	Small testis, azoospermia, hypoandrogenemia; tall stature and increased leg length; increased incidence of learning difficulties, language delay, obesity, breast tumors, varicose veins, impaired glucose tolerance
Turner syndrome	45,X and variants	Streak gonad or immature ovary	Uterus	*Childhood:* Lymphedema, shield chest, web neck, low hairline; cardiac defects and coarctation of the aorta; renal and urinary abnormalities; short stature, cubitus valgus, hypoplastic nails, scoliosis; otitis media and hearing loss; ptosis and amblyopia; nevi; autoimmune thyroid disease; visuospatial learning difficulties *Adulthood:* Pubertal failure, primary amenorrhea; hypertension; aortic root dilatation and dissection; sensorineural hearing loss; increased risk of CVD, IBD, colon cancer, thyroid disease, glucose intolerance and diabetes mellitus, osteoporosis (note that some of these may be related to estrogen deficiency)
Mixed gonadal dysgenesis	45,X/46,XY and variants	Testis or dysgenetic gonad	Variable	Increased risk of gonadal tumors; short stature; features of Turner syndrome may be present
Ovotesticular DSD	46,XX/46,XY chimerism	Testis, ovary, or ovotestis	Variable	Possible increased risk of gonadal tumors

CVD, Cardiovascular disease; *DSDs,* disorders of sex development; *IBD,* inflammatory bowel disease.



• **Fig. 24.18** G-banded karyotypes of Klinefelter syndrome (47,XXY) (A) and Turner syndrome (45,X) (B). (C) Structural changes of the X chromosome seen in variants of Turner syndrome *(from left to right):* normal X; ring chromosome (r[X][p22.3q22]); short-arm deletion (del[X][p21]); long-arm deletion (del[X][q21.31]); isochromosome (I[X][q10]). (Images courtesy Lee Grimsley and Jonathan Waters, MD, North East London Regional Cytogenetics Laboratory, Great Ormond Street Hospital NHS Trust, London, UK.)

or infertility. Other features, such as learning difficulties, speech and language delay, behavioral issues, and altered motor development, may occur, and early educational support focusing on any specific areas of difficulty is important.[182,183] It is likely that the clinical detection of Klinefelter syndrome based on postnatal karyotyping is biased toward detection of those individuals with a more severe phenotype, and it is estimated that as few as 25% of men with Klinefelter syndrome may be diagnosed throughout their life span. However, this pattern may change as diagnosis of Klinefelter syndrome on prenatal genetic testing becomes increasingly common.

The development of testes and a male phenotype in individuals with Klinefelter syndrome provides important evidence for the key role of the presence of the Y chromosome (rather than X chromosome number) in testis determination and subsequent prenatal androgen production. However, testicular function may not be completely intact; micropenis and hypospadias may be presenting features in some cases, and some studies report mildly elevated FSH concentrations during the postnatal "mini-puberty" together with testosterone levels in the lower half of the normal range.[184]

A more predictable elevation in gonadotropin concentrations (e.g., FSH, LH) occurs in the periadolescent period in patients with Klinefelter syndrome, after activation of the HPG axis.[185,186] By midadolescence, plasma concentrations of FSH are increased in 90% of patients with Klinefelter syndrome, and plasma concentrations of LH are increased in 80%. Other serum markers of testicular function (e.g., peripubertal inhibin B, midpubertal INSL3) are often below the normal ranges.[173,186] Although puberty usually starts at a normal age with an appropriate rise in testosterone in classic Klinefelter syndrome, serum testosterone levels off around midpuberty with an increase of gonadotropins. In the majority of young adults, serum testosterone is in the lower half of the reference range.[185,187] Testes usually remain small and firm; the median length and volume are 2.5 cm and 3 mL, but most are smaller than 3.5 cm (range 1–7 mL).[185] Testes typically appear inappropriately small for the degree of androgenization. The serum estradiol concentration is often elevated, which contributes to the gynecomastia observed during the adolescent period.

Testicular biopsy is not warranted clinically because the diagnosis can usually be made on karyotyping from peripheral blood cells. However, studies in which testicular histologic examination has been obtained report germ cell depletion, progressive hyalinization of seminiferous tubules, and Leydig cell hyperplasia after chronic LH stimulation.[185] Testosterone levels need careful surveillance during puberty.[186] A significant proportion of individuals with Klinefelter syndrome receive testosterone supplementation to fully induce puberty and to support sex characteristics, libido, and bone mineralization into adult life. Psychological support and educational support may be needed, as well as attention to potential long-term issues such as diabetes. Management of Klinefelter syndrome in adolescence and adulthood is discussed in Chapter 19.

Although some cases of spontaneous fertility have been reported for mosaic forms of Klinefelter syndrome (46,XY/47,XXY), the prospects for fertility in classic Klinefelter syndrome have historically been poor. Occasionally, sperm can be obtained from ejaculate, and this should always be assessed, but most men have azoospermia. However, in the past decade, testicular sperm extraction (TESE, or micro-TESE) has allowed successful sperm retrieval for approximately 40% to 50% of men with classic Klinefelter syndrome (47,XXY) in specialist centers, independent of age, testicular volume, or hormonal profile.[188] Sperm retrieval rates also seem to be similar in those with and without previous testosterone treatment.[189] Subsequent intracytoplasmic sperm injection (ICSI) has led to successful pregnancy and live birth in approximately 40% to 50%.[185,188] The risk of transmission of the sex chromosome aneuploidy seems to be low, although an increase in some other chromosomal changes is seen, and preimplantation genetic diagnosis is often offered.

Turner Syndrome and Its Variants

Turner syndrome is the second most frequent form of sex chromosome aneuploidy, with an incidence of approximately 1 in 2500 live female births.[190] The classic form of Turner syndrome is associated with a 45,X karyotype, which occurs in approximately one-half of individuals with this condition (see Fig. 24.18). Mosaic forms of Turner syndrome (45,X/46,XX) account for approximately one-fourth of the patients, and the remainder have structural abnormalities of the X chromosome such as long-arm or short-arm deletions, isochromosomes, or ring chromosomes (see Fig. 24.18).[190]

A 45,X chromosomal constitution may be the consequence of nondisjunction or chromosome loss during gametogenesis in either parent that results in a sperm or ovum that lacks a sex chromosome. Although errors in mitosis in normal zygotes often lead to mosaicism, a purely 45,X constitution may arise at the first cleavage division from anaphase lag with loss of a sex chromosome or, less often, from mitotic nondisjunction with failure of the complementary 47,XXX or 47,XYY cell line to survive.[17] An estimated 2% of all zygotes have a 45,X karyotype, and approximately 7% of spontaneous abortuses have a 45,X karyotype, making this the most frequent chromosomal anomaly in humans.

The clinical features of Turner syndrome are highly variable, and the age at diagnosis can be similarly variable. For example, a prenatal diagnosis of Turner syndrome may be made incidentally on prenatal genetic testing or after the detection of increased nuchal translucency on fetal ultrasound scanning.[190] In early infancy, the diagnosis should be considered in females with lymphedema, nuchal folds, low hair line, and/or left-sided cardiac defects. Unexplained growth failure or characteristic somatic features (e.g., abnormal nails, shield chest, abnormal carrying angle, recurrent ear infections) may point to the diagnosis during childhood, and Turner syndrome should be considered in all girls with pubertal delay or pubertal failure associated with hypergonadotropic hypogonadism. The clinical features of Turner syndrome are summarized in Table 24.4, and diagnosis and management are discussed in Chapters 25 and 26. Growth hormone treatment can reduce the severity of short stature. Timely and appropriate introduction of estrogens is necessary in adolescence to ensure optimal growth, bone health, and psychosocial development as well as adequate breast and uterine development, thereby optimizing the opportunity to potentially carry a pregnancy by ovum donation in the future.[190–192] However, careful assessment of cardiovascular risk before and during pregnancy is essential.[193] Women with Turner syndrome benefit from dedicated transition in adolescence and long-term follow-up with a focus on issues such as cardiovascular, bone, and reproductive health and hearing.[190,194–198]

Girls with classic Turner syndrome show ovarian dysgenesis. Studies of Turner syndrome embryos have shown normal germ

cell migration and normal ovarian development until the third month of gestation.[199] Then, accelerated germ cell apoptosis and subsequent oocyte atresia occur, resulting in progressive degeneration of the ovary in the prenatal or postnatal period. With these gonadal changes, LH and FSH tend to rise in late childhood, after activation of the hypothalamic pulse generator, and sometimes even earlier. Nevertheless, sufficient estrogen synthesis for puberty to commence in adolescence occurs in approximately 25% of girls with Turner syndrome (10% of those with 45,X; 30–40% of those with 45,X/46,XX mosaicism), and menstruation occurs in about 2% of cases.[194,200] AMH may be a useful marker of ovarian reserve and a predictor of ovarian insufficiency.[201] Gonadectomy is not usually required except when a Y fragment containing the *TSPY* locus is present; in such patients, the risk of gonadoblastoma is increased.[202]

Oocyte cryopreservation has been used successfully in several women with Turner mosaicism and is a fertility preservation option for young women with persistent ovarian function.[190,203] Ovarian cortical tissue preservation is feasible but requires surgery and is currently considered experimental. Routine fertility preservation in girls under 12 years of age is not recommended.[190]

Risk from pregnancy is high in women with Turner syndrome due to cardiovascular defects and other comorbidities, and detailed assessment and counseling prior to and during any potential pregnancy are mandatory.[190,203]

45,X/46,XY Mosaicism and Variants

A mosaic 45,X/46,XY karyotype probably arises through anaphase lag during mitosis in the zygote, although Y chromosomal abnormalities are sometimes seen, and interchromosomal rearrangements with loss of structural abnormal Y material may be a common mechanism for variants of this condition. Although the classic form of this condition is associated with 45,X/46,XY mosaicism, 45,X/47,XYY, 45,X/46,XY/47,XYY, and other mosaic karyotypes can occur.

The true prevalence of this condition is unknown (see Table 24.4). Because individuals with 45,X/46,XY mosaicism are more likely to be referred for further assessment if they have atypical genitalia, most series of patients reported in the literature have probably reflected this bias; studies based on nonselected prenatal karyotyping have shown that most children with 45,X/46,XY mosaicism appear male.[204–206]

The phenotypes associated with 45,X/46,XY mosaicism are highly variable. The gonads can develop as dysgenetic ovaries (ovarian-like stroma with sparse primordial follicles) or streak gonads (similar to those seen in Turner syndrome), normal or dysgenetic testes, or very rarely as ovotestes (with testicular and dysgenetic ovarian components within a single gonad).

Depending on the degree of Leydig cell activity (if any), the gonads may be positioned anywhere along the pathway of testicular descent, with streak gonads more likely to be intraabdominal and well-formed testes more likely to be in the inguinoscrotal region. Marked differences in gonadal development and histologic appearance can sometimes be seen between the right and the left sides (referred to as *mixed gonadal dysgenesis*) or even within a single gonad.[207]

All possible genital phenotypes have been reported, ranging from typical female external genitalia or mild clitoromegaly through all stages of ambiguous genitalia to hypospadias or typical male external genitalia.[204,207–210] Asymmetry of the external genitalia strongly suggests asymmetric gonadal development (i.e., mixed gonadal dysgenesis).

Müllerian structures may be present if there is absent or impaired AMH production by Sertoli cells, and these structures are also often asymmetric. The presence of a hemiuterus and fallopian tube on the side of the more dysgenetic gonad in some cases of mixed gonadal dysgenesis provides important evidence for the paracrine actions of AMH on developing müllerian structures.

Somatic features associated with a 45,X/46,XY karyotype are also highly variable and do not always correlate well with the gonadal phenotype.[204,207,209,210] Approximately 40% of children have clinical features reminiscent of Turner syndrome, such as short stature, nuchal folds, low-set hairline, and cardiac and renal abnormalities.[207] In other cases, short stature may be the only somatic manifestation. Ongoing monitoring for features associated with Turner syndrome (e.g., thyroid function, hearing, and cardiac anomalies; see Chapter 26) is therefore applicable for this group of individuals.[190] Furthermore, many families benefit from the psychological support and education that can be linked to specialist services.

Sex designation can be difficult in individuals with 45,X/46,XY, and several factors should be considered, including probable gender identity, genital appearance and urogenital anatomy, fertility and reproductive options, risk of gonadal malignancy, and potential need for hormone replacement. Recommendation for sex designation should be handled like any other intervention, with a careful discussion of risks, benefits, and potential outcomes. Most infants with female or minimally androgenized genitalia are raised as female, and the presence of a uterus or hemiuterus may allow the potential for pregnancy by ovum donation in the future, though predicting future function can be difficult. Furthermore, as with girls with classic Turner syndrome, there may be risks associated with pregnancy (by ovum donation) due to cardiovascular anomalies. Intraabdominal streak-like (i.e., flat, scar-like gonads) and dysgenetic gonads are thought to pose a significant risk of germ cell malignancy and should be removed.[208,210,211] The risk of germ cell tumors in individuals with 45,X/46,XY DSD is estimated to be 15% to 40%.[212] Estrogen replacement is required to induce breast and uterine development in adolescence, and the addition of progestins allows menstruation if a uterus is present. Growth hormone treatment has been used when short stature is present, but no large trials have been performed to assess this group of patients. Usually there is a significantly reduced growth spurt during puberty, and some studies have suggested use of GH from earlier in childhood may optimize growth.[209,213] Similarly, limited long-term outcome data on gender identity or psychosexual functioning are available.

Infants with hypospadias are usually raised as male. Testosterone can sometimes be given to promote phallic growth in infancy, and hypospadias repair is usually offered as a one-stage or two-stage procedure, depending on the severity of the hypospadias. Attempts should be made to perform orchidopexy as a one-stage or two-stage procedure because there may be a significant risk of malignancy in these gonads. Gonadectomy should be considered for gonads that cannot be placed in a palpable position; some have advocated for close monitoring, but imaging is not able to detect early neoplastic changes in abdominal gonads.[208,210,214] Gonads that can be secured within the scrotum need careful monitoring by palpation/self-examination and imaging to detect changes such as microlithiasis.[214] At the time of orchidopexy, and in adolescence, biopsy can be used to assess for premalignant changes and estimate the risk of germ cell cancer. Puberty should be carefully monitored to ensure adequate endogenous testosterone production. Most boys enter puberty spontaneously, but some then

develop androgen insufficiency requiring testosterone supplementation.[210] Reduced final height is invariable, but there is a beneficial response to growth hormone in some boys.[215]

Designation of sex and management of a 45,X/46,XY child with *highly ambiguous genitalia* is a difficult situation for families and physicians, and long-term outcome data for this group are scant. Individuals raised female are infertile, usually have no uterus, require gonadectomy because of tumor risk, and are likely to undergo urogenital surgery. Those raised as male often undergo multiple hypospadias operations, may have poor corporal tissue, are infertile if dysgenetic gonads are present that need to be removed and often infertile even if the gonads are intact, and may have a significantly reduced height potential. There is limited data on gender identity outcomes; in one series of 11 children with mixed gonadal dysgenesis raised as girls, 6 later declared a male gender identity.[216] Detailed assessment of each child is important, and an individualized approach by an experienced multidisciplinary team is important for long-term monitoring, management, and support. Long-term outcome data from larger studies may provide better guidance on the management of this group of individuals in the future.

A 45,X/46,XY mosaic karyotype can also be associated with a male phenotype and apparently normal testis development. Initial cases of typical-appearing males with a 45,X/46,XY karyotype were described after screening of family members as potential bone marrow transplant donors, but later studies of amniocentesis showed that 90% of infants with a 45,X/46,XY karyotype have normal male genitalia and apparently normal testes, and postnatally these individuals may be diagnosed because of short stature or infertility.[205,206,217,218] There seems to be variable correlation between the degree of mosaicism on peripheral blood sampling and gonadal or somatic phenotype. Monitoring of gonadal function and growth, assessment for premalignant changes of the gonads, and cardiac and renal screening are advised in individuals with a typical male phenotype or minor genital anomalies because they are at risk for abnormal growth, Sertoli cell dysfunction, gonadal germ cell cancer, and cardiac and renal malformations.[217]

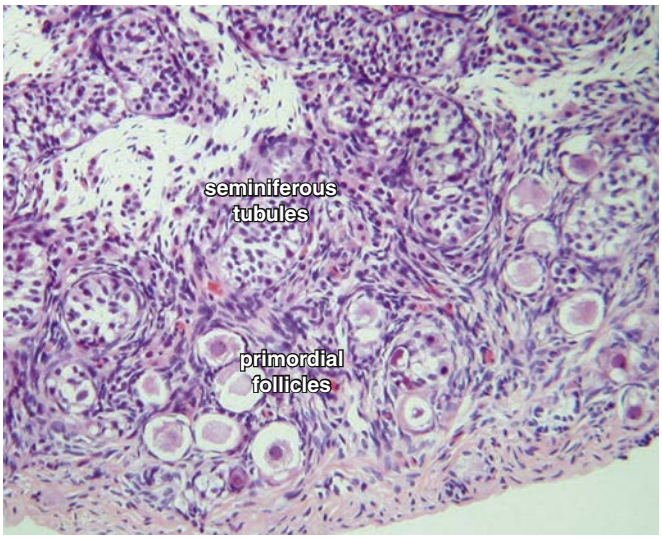
Ovotesticular Disorders of Sex Development: 46,XX/46,XY Chimerism and Variants

The diagnosis of ovotesticular DSD (previously referred to as true hermaphroditism) requires the presence of ovarian tissue (containing follicles) and testicular tissue in the same or the opposite gonad (Fig. 24.19; see Table 24.4). Gonadal stroma arranged in whorls, similar to what is found in a typical ovary but lacking oocytes, is a common finding in a dysgenetic or streak gonad and is not considered sufficient evidence to designate the rudimentary gonad as an ovotestis.

Ovotesticular DSD is an uncommon condition in the United States and Europe, occurring in less than 5% of babies referred because of atypical genitalia, but is a common cause of atypical genitalia in South Africa.[218a] Ovotesticular DSD can occur in several different scenarios. The classic diagnosis is 46,XX/46,XY chimerism (sometimes caused by double fertilization or ovum fusion; see "Chromosomal Sex"), which is found in approximately 20% to 30% of children with ovotestes in our center and occurs in all ancestral backgrounds (Table 24.5).[219,220] Somewhat more prevalent is ovotesticular DSD associated with a 46,XX karyotype. In a proportion of cases this can occur due to p.Arg92 variants in NR5A1/SF1, loss of function of RSPO1 or NR2F2/COUP-TFII, or upregulation of expression of SRY, SOX9, or SOX3.[71,72,122,221,222] Of note, 46,XX ovotesticular DSD is much more prevalent in children from sub-Saharan Africa; the molecular etiology for this is not currently known (see Table 24.5). The molecular basis of these conditions will be discussed later in the relevant section (see "46,XX Ovotesticular and 46,XX Testicular Disorders of Sex Development"). Finally, ovotestes have rarely been reported in several conditions associated with 46,XY gonadal dysgenesis (e.g., MAP3K1, SOX9), and fertile 46,XY women have been described.[223–225]

Here we will discuss basic principles of management of ovotesticular DSD based on 46,XX/46,XY chimerism, which are applicable to the other forms of the condition. However, it is important to remember that a Y chromosome (containing the spermatogenic locus) is necessary for potential sperm production, so 46,XX children with ovotestes will not be fertile males.

Patients with ovotesticular DSD often have asymmetric gonadal development and sometimes may be subclassified according to the type and location of the gonads.[4] Three forms are possible: *lateral* (20%) with a testis on one side and an ovary on the other, *bilateral* (30%) with testicular and ovarian tissue present bilaterally, and *unilateral* cases (50%) with an ovotestis present on one side and an ovary or testis on the other. It has been suggested that the left gonad is more likely to develop as an ovary, whereas the right gonad is more likely to develop as a testis. An ovary is



• **Fig. 24.19** Ovotestis, showing immature seminiferous tubules lined with Sertoli cells and germ cells *(upper left)* and ovarian tissue with primordial follicles *(lower right)* (hematoxylin-eosin stain; original magnification, ×400). (Courtesy Neil Sebire, MD, Great Ormond Street Hospital NHS Trust, London, UK.)

TABLE 24.5 Relative Frequency (%) of Different Karyotypes in Ovotesticular DSD (True Hermaphroditism)

Location	46,XX/46,XY	46,XX	46,XY
North America	21	72	7
Europe	41	52	7
Africa	—	97	—

DSD, Disorders of sex development.

Adapted from Krob G, Braun A, Kuhnle U. True hermaphroditism: geographical distribution, clinical findings, chromosomes and gonadal histology. *Eur J Pediatr*. 1994;153:2–10.

likely to be in its normal anatomic position, whereas a testis or ovotestis can be anywhere along the pathway of testicular descent and is often found in the inguinal region.

Differentiation of the genital tract and development of secondary sex characteristics vary in ovotesticular DSD.[226,227] Most patients who present early have ambiguous genitalia or significant hypospadias. Cryptorchidism is common, but often at least one gonad is palpable, usually in the labioscrotal fold or inguinal region, and is often associated with an inguinal hernia. The differentiation of the genital ducts usually follows that of the gonad, and a hemiuterus or rudimentary uterus is often present on the side of the ovary or ovotestis.

Breast development at the time of puberty is common in ovotesticular DSD.[227] Menses occur in a significant proportion of cases when a uterus is present, and ovulation and pregnancy have been reported in a number of patients with a 46,XX karyotype when an ovary is present. However, progressive androgenization can occur in girls with testicular tissue, which can result in voice changes and clitoral enlargement during adolescence if left untreated. Individuals reared as male often present with hypospadias and undescended testes, although bilateral scrotal ovotestes have been reported. These individuals can experience significant estrogenization at the time of puberty and may have cyclic hematuria if a uterus is present. Spermatogenesis is rare, and interstitial fibrosis of the testis is common. As noted, fertility requires Y chromosomal genes other than *SRY*, so boys with 46,XX ovotesticular DSD will be infertile.

Although ovotesticular DSD is rare, the diagnosis should be considered in all patients with ambiguous genitalia. A 46,XX/46,XY karyotype strongly suggests the diagnosis, but the detection of a 46,XX or 46,XY karyotype does not exclude the diagnosis, especially if a 46,XX baby has genital asymmetry. Pelvic imaging with ultrasound or magnetic resonance imaging (MRI) is useful for visualizing internal genitalia. The presence of testicular tissue may be detected by measurement of basal testosterone, AMH, and inhibin B in the first months of life and by measuring basal AMH thereafter. Ovarian tissue is more difficult to detect in early childhood, although estradiol, inhibin A, and follicular response to repeated injection of recombinant human FSH can provide useful information.[175] Examination under anesthesia and laparoscopy may provide the most detailed information about internal structures and allow a biopsy to confirm the diagnosis of ovotesticular DSD when other forms of DSD have been excluded.[228,229] However, the biopsy sometimes does not sample all the tissues present in a gonad.[229]

The management of ovotesticular DSD depends on the age at diagnosis, genital development, internal structures, and reproductive capacity. Either male or female designation may be appropriate for the young infant. Individuals with a 46,XX karyotype, little virilization, and a uterus are likely to have functional ovarian tissue, and female designation is likely to be appropriate. If androgenization is not desired by the individual, functional testicular tissue could potentially be removed before puberty and monitored postoperatively by measuring serum AMH and testosterone levels. Although still experimental, options for cryopreserving gonadal tissue are being explored. Potentially, GnRH agonists could be used to temporarily delay puberty if the young person needs more time to understand the condition and be involved in decisions about gonadectomy (whether complete or partial). The risk of malignant transformation in ovarian tissue of 46,XX patients is not known but is likely to be low given the absence of Y chromosomal material.

A male sex designation may be more appropriate if there is significant clitorophallic development and testicular tissue, and müllerian structures are absent or very poorly formed. Appropriate counseling should be provided and evidence of a likely male gender seen before considering prepubertal removal of ovarian tissue within an ovotestis. Again, cryopreservation of gonadal tissue may emerge as an option but is currently experimental. GnRH agonists or aromatase inhibitors might be considered on a trial basis if more time is needed to make decisions. Remnant müllerian structures can be removed by an experienced surgeon if there are problems with hematuria or urinary retention, although extreme care is needed not to disrupt the blood supply to any testis/testes being preserved, and often there is no harm in leaving müllerian structures intact. The testicular tissue is usually well differentiated in 46,XX ovotesticular DSD so that the risk of germ cell tumors is estimated to be low.[22]

Gender identity is also an important consideration in patients with ovotesticular DSD who first present in late childhood or adolescence due to androgenization in girls or estrogenization in boys. In most cases, gender identity is consistent with gender of rearing. After appropriate counseling, the discordant gonad and dysgenetic tissue can be removed to prevent unwanted androgenization in girls and estrogenization in boys. Sex hormone supplementation may be required for complete pubertal development and in adult life.

46,XY Disorders of Sex Development

The 46,XY DSDs can be categorized as disorders of testis development, disorders of androgen synthesis, disorders of androgen action, and other conditions affecting sex development (Table 24.6; see also Fig. 24.7).

Disorders of Testis Development

Disorders of testis development can have a spectrum of phenotypes and presentations. *Complete testicular dysgenesis*, a condition sometimes called *Swyer syndrome*, is associated with a complete lack of androgenization of the external genitalia and persistent müllerian structures due to insufficient AMH production. In contrast, *partial gonadal dysgenesis* may be associated with a range of phenotypes, ranging from clitoromegaly to ambiguous or atypical genitalia to isolated hypospadias. A uterus or uterine remnant and vagina may be present. Subtle forms of testicular dysgenesis can present with testicular regression, a small penis, or male infertility in the absence of an external genital phenotype.

Several single-gene disorders have been described in patients with various degrees of testicular dysgenesis. Table 24.6 summarizes these factors, and the role of many of these factors in development has already been discussed (see "Testis Determination"). Although associated features can sometimes help to direct genetic analysis, often there are no other findings, and a genetic diagnosis is currently reached in only about 20% to 40% of individuals with 46,XY testicular dysgenesis.

Single-Gene Disorders

Steroidogenic Factor 1: NR5A1. SF1 (encoded by *NR5A1*) is a member of the nuclear receptor superfamily that regulates the transcription of at least 30 genes involved in gonadal development, adrenal development, spleen development, steroidogenesis, and reproduction.[59,230] *NR5A1* mutations were first reported in two 46,XY individuals with female external genitalia, persistent müllerian structures, and primary adrenal failure.[65,66] The first variant was a de novo heterozygous p.Gly35Glu change in the P-box primary DNA-binding region of SF1; the second was a recessively inherited homozygous p.Arg92Gln mutation in the A-box secondary DNA-binding region (Fig. 24.20). These changes resulted

TABLE 24.6 Overview of Important Genes Involved in DSDs

Gene	Protein	OMIM	Locus	Inheritance	Gonad	Müllerian Structures	External Genitalia	Associated Features/Variants
A. Causes of 46,XY DSD								
Disorders of Gonadal (Testicular) Development: Single-Gene Disorders								
WT1	TF	607102	11p13	AD	Dysgenetic testis	±	Female, ambiguous or hypospadias	Wilms tumor, renal abnormalities, gonadal tumors (WAGR, Denys-Drash, and Frasier syndromes)
NR5A1 (SF1)	Nuclear receptor TF	184757	9q33.3	AD/AR (SLD)	Dysgenetic testis (variable)	±	Female, ambiguous, or hypospadias	More severe phenotypes include primary adrenal failure; milder phenotypes have isolated partial gonadal dysgenesis or impaired androgenization or both
GATA4	TF	600576	8p23.1	AD (SLD)	Dysgenetic testis (variable)	–	Female, ambiguous, or hypospadias/micropenis	Cardiac defects (e.g., septal defects, tetralogy of Fallot)
ZFPM2 (FOG2)	Coregulator	603693	8q23.1	AD	Dysgenetic testis (variable)	±	Female or ambiguous	Congenital heart defects. Learning and language difficulties, autism spectrum disorder in one individual.
CBX2	Polycomb protein	602770	17q25.3	AR	Ovary (one case)	+	Female	
SRY	TF	480000	Yp11.3	Y	Dysgenetic testis or ovotestis	±	Female or ambiguous	
SOX9	TF	608160	17q24-q25	AD	Dysgenetic testis or ovotestis	±	Female or ambiguous	Campomelic dysplasia (17q24 rearrangements have a milder phenotype than point mutations)
SOX8	TF	605923	16p13.3	AD, de novo	Dysgenetic testis (variable)	±	Female or ambiguous	16p deletion can have additional features
MAP3K1	Signaling molecule	600982	5q11.2	AD	Dysgenetic testis (variable)	±	Female, ambiguous, or hypospadias/micropenis	
NR3A2 (ESR2)	Nuclear receptor TF	601663	14q23.2	AD/AR	Dysgenetic testis	±	Female or ambiguous	Variable (anal atresia, blepharophimosis, dysmorphic features)
DMRT1	TF	602424	9p24.3	AD	Dysgenetic testis	+	Female	
TSPYL1	? Chromatin remodeling	604714	6q22.1	AR	Dysgenetic testis	–	Female or ambiguous	Sudden infant death
DHH	Signaling molecule	605423	12q13.1	AR	Dysgenetic testis, testis (Leydig)	–	Female or ambiguous	Minifascicular neuropathy in several patients
SAMD9	Growth repressor	617053	7q21.2	AD, de novo	Dysgenetic testis, testis (Leydig)	-	Female, ambiguous or hypospadias	MIRAGE syndrome (myelodysplasia, infections, restriction of growth, adrenal hypoplasia, genital phenotypes, enteropathy)
ARX	TF	300382	Xp22.13	X	Dysgenetic testis (Leydig)	–	Ambiguous	X-linked lissencephaly, epilepsy, temperature instability
MAMLD1 (CXORF6)	Unknown	300120	Xq28	X	Normal (Leydig cell dysfunction)	–	Hypospadias	

Continued

TABLE 24.6 Overview of Important Genes Involved in DSDs—cont'd

Gene	Protein	OMIM	Locus	Inheritance	Gonad	Müllerian Structures	External Genitalia	Associated Features/Variants
Disorders of Gonadal (Testicular) Development: Chromosomal Changes Involving Key Candidate Genes[a]								
DMRT1	TF	602424	9p24.3	Monosomic deletion	Dysgenetic testis	±	Female or ambiguous	Mental retardation
ATRX	Helicase (? chromatin remodeling)	300032	Xq13.3	X	Dysgenetic testis	–	Female, ambiguous, or male	α-thalassemia, mental retardation
NR0B1 (DAX1)	Nuclear receptor TF	300018	Xp21.3	dupXp21	Dysgenetic testis or ovary	±	Female or ambiguous	
WNT4	Signaling molecule	603490	1p36.12	dup1p35	Dysgenetic testis	+	Ambiguous	Mental retardation
Disorders in Hormone Synthesis or Action								
DHCR7	Enzyme	602858	11q13.4	AR	Testis	–	Variable	Smith-Lemli-Opitz syndrome: coarse facies, second-third toe syndactyly, failure to thrive, developmental delay, cardiac and visceral abnormalities
LHCGR	G protein receptor	152790	2p16.3	AR	Testis	–	Female, ambiguous, or micropenis	Leydig cell hypoplasia
STAR	Mitochondrial associated protein	600617	8p11.2	AR	Testis	–	Female, ambiguous, or micropenis	Lipoid CAH (primary adrenal failure), pubertal failure
CYP11A1	Enzyme	118485	15q24.1	AR	Testis	–	Female or ambiguous	CAH (primary adrenal failure), pubertal failure
HSD3B2	Enzyme	201810	1p13.1	AR	Testis	–	Ambiguous	CAH, primary adrenal failure, ↑ Δ^5:Δ^4 ratio
CYP17A1	Enzyme	202110	10q24.3	AR	Testis	–	Female, ambiguous, or micropenis	CAH, hypertension due to DOC (except in isolated 17,20-lyase deficiency)
POR (P450 oxidoreductase)	CYP enzyme electron donor	124015	7q11.2	AR	Testis	–	Male or ambiguous	Mixed features of 21-hydroxylase deficiency, 17α-hydroxylase/17,20-lyase deficiency, and aromatase deficiency; sometimes associated with Antley-Bixler craniosynostosis
CYB5A	Cofactor	613218	18q22.3	AR	Testis	–	Ambiguous or hypospadiac	Methemoglobinemia
AKR1C2 (AKR1C4)	Enzyme	600450	10p15.1	AR (? digenic)	Testis	–	Variable	
HSD17B3	Enzyme	605573	9q22.23	AR	Testis	–	Female or ambiguous	Partial androgenization at puberty, ↑ ratio of androstenedione to testosterone
SRD5A2	Enzyme	607306	2p23.1	AR	Testis	–	Ambiguous or micropenis	Partial androgenization at puberty, ↑ ratio of testosterone to DHT

Androgen receptor (NR3C4)	Nuclear receptor TF	313700	Xq12	X	Testis	–	Female, ambiguous, micropenis, or normal male	Phenotypic spectrum from complete AIS (female external genitalia) to partial AIS (ambiguous) to normal male genitalia/infertility
AMH	Signaling molecule	600957	19p13.3	AR	Testis	+	Normal male	Persistent müllerian duct syndrome (PMDS)
AMH receptor	Serine/threonine kinase transmembrane receptor	600956	12q13.13	AR	Testis	+	Normal male	Male external genitalia, bilateral cryptorchidism
B. Causes of 46,XX DSD								
Disorders of Gonadal (Ovarian) Development								
SRY	TF	480000	Yp11.3	Translocation	Testis or ovotestis	–	Male or ambiguous	
SOX9	TF	608160	17q24	dup17q24 or deletion of regulatory region	Not determined	–	Male or ambiguous	
SOX3	TF	313430	Xq27.1	dup Xq27 or del of regulatory region	Testis (variable)	–	Male	Additional features if large duplication
NR5A1 (SF1)	Nuclear receptor TF	184757	9q33.3	AD or de novo (affecting codon 92)	Testis or ovotestis	-	Male or ambiguous	
NR2F2 (COUP-TFII)	Nuclear receptor TF	107773	15q26.2	AD or de novo	Testis or ovotestis	-	Male or ambiguous	Cardiac defects, diaphragmatic hernia, BPES
RSPO1	Thrombospondin (Wnt signaling)	609595	1p34.3	AR	Testis or ovotestis	–	Male	Palmar-plantar hyperkeratosis, squamous cell carcinoma
WNT4	Wnt signaling	611812	1p36.12	AR	Testis or ovotestis	–	Male or ambiguous	SERKAL syndrome
Androgen Excess								
HSD3B2	Enzyme	201810	1p13.1	AR	Ovary	+	Clitoromegaly (mild)	CAH, primary adrenal failure, partial androgenization due to ↑ conversion of DHEA

Continued

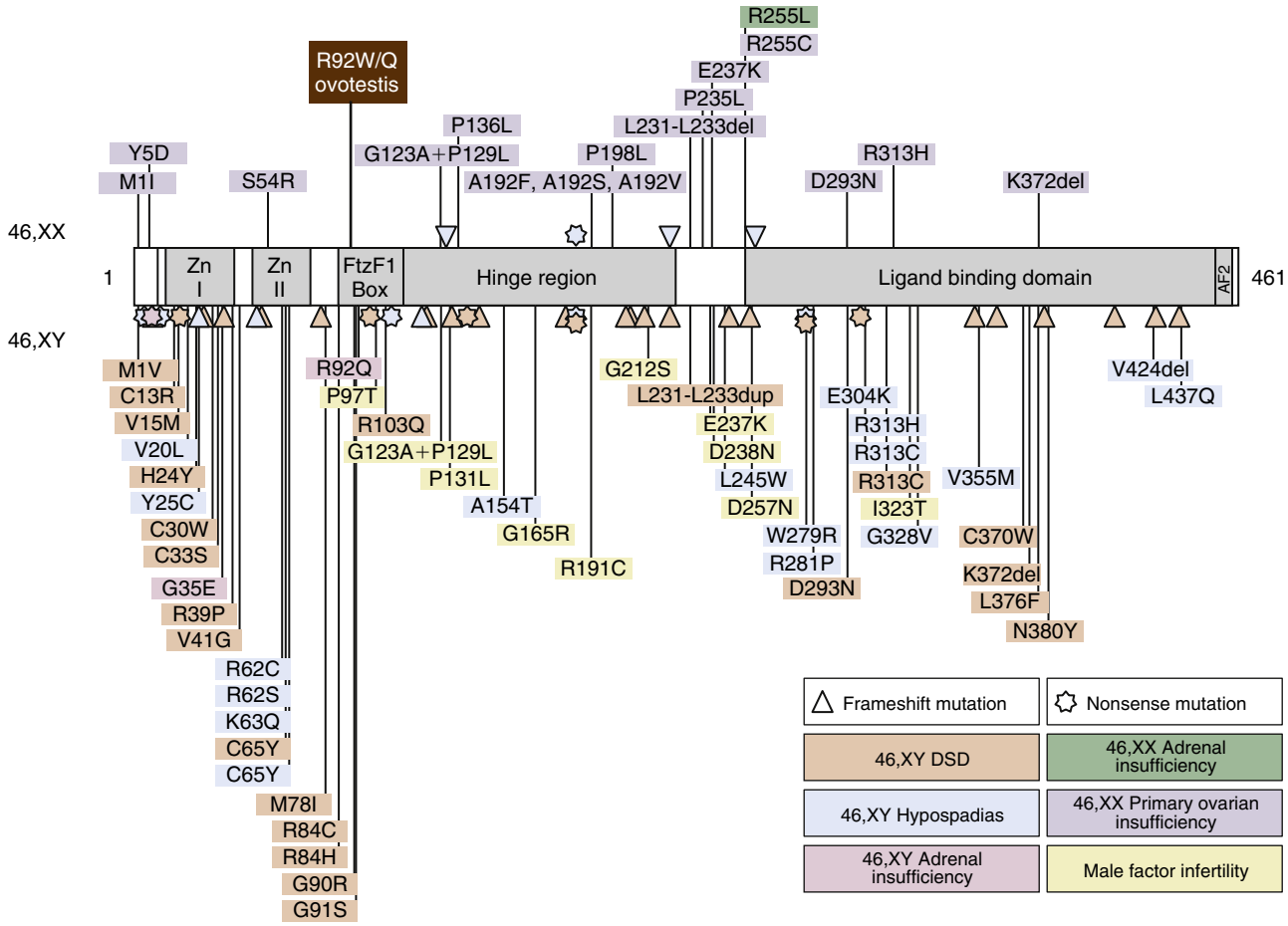
TABLE 24.6 Overview of Important Genes Involved in DSDs—cont'd

Gene	Protein	OMIM	Locus	Inheritance	Gonad	Müllerian Structures	External Genitalia	Associated Features/Variants
CYP21A2	Enzyme	201910	6p21.33	AR	Ovary	+	Ambiguous; rarely Prader V	CAH, phenotypic spectrum from severe salt-losing forms associated with adrenal failure to simple virilizing forms with compensated adrenal function, ↑ 17-OHP
CYP11B1	Enzyme	202010	8q24.3	AR	Ovary	+	Ambiguous; rarely Prader V	CAH, hypertension due to ↑ 11-deoxycorticosterone
POR (P450 oxidoreductase)	CYP enzyme electron donor	124015	7q11.23	AR	Ovary	+	Normal or ambiguous	Mixed features of 21-hydroxylase deficiency, 17α-hydroxylase/17,20-lyase deficiency and aromatase deficiency; associated with Antley-Bixler craniosynostosis
CYP19A1	Enzyme	107910	15q21.2	AR	Ovary	+	Ambiguous	Maternal androgenization during pregnancy, absent breast development at puberty except in partial cases
Glucocorticoid receptor[b] (NR3C1)	Nuclear receptor TF	138040	5q31.3	AR	Ovary	+	Normal or ambiguous	↑ ACTH, 17-OHP, cortisol, mineralocorticoids, and androgens; failure of dexamethasone suppression

[a]Chromosomal rearrangements likely to include key genes are included.

[b]Note: Patient heterozygous for a mutation in *CYP21*.

–, Absent; +, present; *ACTH,* adrenocorticotropic hormone; *AD,* autosomal dominant (often de novo mutation); *AIS,* androgen insensitivity syndrome; *AR,* autosomal recessive; *BPES,* blepharophimosis-ptosis-epicanthus inversus syndrome; *CAH,* congenital adrenal hyperplasia; *CYP,* cytochrome P450 enzyme; *DHEA,* dehydroepiandrosterone; *DHT,* dihydrotestosterone; *DOC,* 11-deoxycorticosterone; *DSDs,* disorders of sex development; *17-OHP,* 17-hydroxyprogesterone; *LHCGR,* luteinizing hormone (LH) or human chorionic gonadotropin (hCG) receptor; *OMIM, Online Mendelian Inheritance in Man*; *SERKAL,* sex-reversal with kidney, adrenal, and lung dysgenesis; *SLD,* sex-limited dominant; *TF,* transcription factor; *WAGR,* Wilms tumor, aniridia, genitourinary anomalies, and mental retardation.



• **Fig. 24.20** Schematic diagram of steroidogenic factor 1 (SF1) shows key domains and mutations associated with a gonadal phenotype. The Gly35Glu (heterozygous) and Arg92Gln (homozygous) changes affect DNA-binding regions of the protein and are associated with marked underandrogenization, dysgenetic gonads, müllerian structures, and primary adrenal failure. Heterozygous frameshift *(triangles)* and nonsense mutations *(stars)*, as well as missense mutations, have been described in 46,XY individuals with a spectrum of disorders of sex development (DSD) phenotypes *(below the structure drawing)* and in women with primary ovarian insufficiency *(above the structure drawing)*. (From Suntharalingham JP, Buonocore F, Duncan A, et al. DAX-1 (NR0B1) and steroidogenic factor-1 (SF-1, NR5A1) in human disease. *Best Pract Res Clin Endocrinol Metab*. 2015;29:607–619. Based on Lin L, Philibert P, Ferraz-de-Souza B, et al. Heterozygous missense mutations in steroidogenic factor-1 [SF1/Ad4BP, NR5A1] are associated with 46,XY disorders of sex development with normal adrenal function. *J Clin Endocrinol Metab*. 2007;92:991–999, used with permission of The Endocrine Society, Copyright 2007.)

in impaired DNA binding. The combination of gonadal and adrenal phenotype is uncommon, and it initially seemed that disruption of SF1 would be a rare cause of DSD in humans.

However, the past decade has seen a different picture emerging as an ever-increasing number of monoallelic (heterozygous) nonsense, frameshift, and missense mutations in *NR5A1* have been associated with a spectrum of 46,XY DSD conditions in individuals with normal adrenal function (see Fig. 24.20).[59,67,231,232] These changes usually result in haploinsufficiency of SF1. A range of phenotypes is seen, most commonly mild gonadal dysgenesis and significantly impaired androgenization, where alterations in SF1 are found in approximately 15% of cases.[59] These variations usually arise de novo but may be inherited from the mother in a sex-limited dominant fashion (i.e., the mother carries the mutation but is unaffected) or even from the father and occasionally may be autosomal recessive.[66] In some situations, there is a family history of ovarian insufficiency, and females in the family may be at risk of developing ovarian insufficiency in the future.[68] Loss of SF1 function is also reported in some boys with severe hypospadias and undescended testes (~5%), bilateral anorchia, and a small penis and in men with infertility (~2%).[233–235] Oligogenic effects of other genes have also been proposed to influence phenotype.[236,237] Therefore variable loss of SF1 activity is associated primarily with different degrees of testicular or ovarian dysfunction in humans. Adrenal function may need to be monitored over time, but at present, adrenal insufficiency does not seem prevalent. In addition, splenic abnormalities have been described, ranging from asplenia to polysplenia.[230,238] Although their prevalence among patients with *NR5A1* mutations is currently unclear, assessment of spleen anatomy and function by ultrasound and search for Howell-Jolly bodies in blood has been recommended given the serious consequences that (functional) asplenia may have.[238] Defining the molecular basis in different families can be important for counseling, especially about the potential risk of ovarian insufficiency,

and for identifying those males who might need surveillance for potential androgen insufficiency or a decline in fertility with age. *NR5A1* mutations can also result in ovotesticular DSD (see "Ovotesticular Disorders of Sex Development").

Wilms Tumor 1 Gene: *WT1*. *WT1* (11p13) is a four–zinc finger transcription factor expressed in the developing genital ridge, kidney, gonads, and mesothelium.[49] The WT1 protein has several different isoforms that have complex roles in sex development, as outlined previously and shown in Fig. 24.21. The important role of WT1 in human testis development has been confirmed through the description of various WT1 mutations in patients with WAGR syndrome, Denys-Drash syndrome, and Frasier syndrome.

WAGR syndrome is caused by deletion of a region of chromosome 11p13.[239] The resultant phenotype is likely to be the consequence of haploinsufficiency of *WT1* together with loss of developmental genes such as *PAX6*, which is involved in eye development.[55] Renal abnormalities include childhood Wilms tumor in about half of children; renal agenesis has been reported, and renal dysfunction often occurs after adolescence. Genitourinary abnormalities are usually relatively mild and include bilateral cryptorchidism, micropenis, and occasionally hypospadias. Careful ophthalmic support is needed for the aniridia or iridic hypoplasia, and cataracts or corneal clouding can occur. Developmental delay can occur with larger chromosomal deletions, and obesity may be a feature if the deletion is large and includes the *BDNF* gene (WAGRO syndrome).

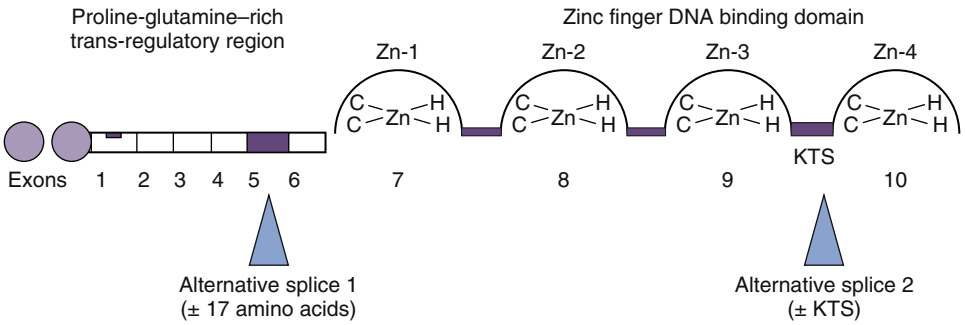
Denys-Drash syndrome is characterized by gonadal dysgenesis, severe congenital or early-onset nephropathy (i.e., diffuse mesangial sclerosis), and predisposition to Wilms tumor.[56] Most 46,XY patients with Denys-Drash syndrome present with genital ambiguity or severe penoscrotal hypospadias in the newborn period, although typical male or female genital appearance has been described. The presence or absence of müllerian structures depends on the degree of Sertoli cell dysfunction. Denys-Drash syndrome usually results from heterozygous de novo point mutations in *WT1* that have a dominant negative effect on the function of the wild-type protein. These point mutations usually affect the DNA-binding region (zinc fingers) of WT1. The risk of early-onset renal failure is high, and Wilms tumor usually develops in the first few years of life. There is an increased risk of germ cell tumors.[212] The prevalence of *WT1* mutations in boys with penoscrotal hypospadias and undescended testes has been reported to be as high as 7%.[240]

Frasier syndrome usually results from heterozygous mutations in the donor splice site of exon 9 of *WT1*.[57,58] These changes are predicted to result in an imbalance in the ratio of +KTS to –KTS isoforms of WT1. Frasier syndrome is typically characterized by streak gonads, a 46,XY female phenotype with müllerian structures, and later-onset nephropathy (i.e., focal segmental glomerulosclerosis) that usually progresses to renal failure in the second decade of life. There is a high risk of gonadal tumors (gonadoblastoma and dysgerminoma) in patients with Frasier syndrome.[241] In practice, Denys-Drash syndrome and Frasier syndrome may represent a continuum of phenotypes rather than distinct conditions.[242] Severe forms of these conditions can present with congenital nephrotic syndrome, whereas milder variants have been reported, for example, in a man with hypospadias and late-onset nephropathy.[243] Taken together, these cases highlight the importance of considering this diagnosis in 46,XY DSD and of performing urinalysis for proteinuria in children with 46,XY DSD. Meacham syndrome (i.e., DSD, cardiac defects, and diaphragmatic hernia) may also result from changes in *WT1*.[244]

Management of patients with *WT1* mutations includes monitoring and treatment of renal function and assessment for Wilms tumor, as well as gonadectomy in individuals with Frasier syndrome and in patients with Denys-Drash syndrome who have a Y chromosome if the gonads cannot be monitored.[22]

Gata-Binding Protein 4: GATA4. GATA4 is a four–zinc finger transcription factor involved in early testis development and heart development. Haploinsufficiency of *GATA4* or heterozygous loss-of-function changes are well established in a range of cardiac defects such as atrial and ventricular septal defects. More recently, heterozygous *GATA4* mutations have been shown to cause testicular dysgenesis with or without cardiac anomalies.[245] These mutations can be transmitted by a mother in a sex-limited dominant manner. Reported changes may disrupt interactions between GATA4 and its cofactor, friend of GATA 2 (FOG2, also known as ZFPM2).

Friend of GATA2 (FOG2): ZFPM2. FOG2 is a key coregulator of GATA4, and missense mutations in the gene encoding FOG2 *(ZFPM2)* have been described in individuals with 46,XY gonadal dysgenesis.[166,246,247] The point mutations affect interactions with GATA4, and some have been described previously in patients with cardiac defects, highlighting the variable penetrance of gonadal and cardiac phenotypes following disruption of the GATA4/FOG2 complex.



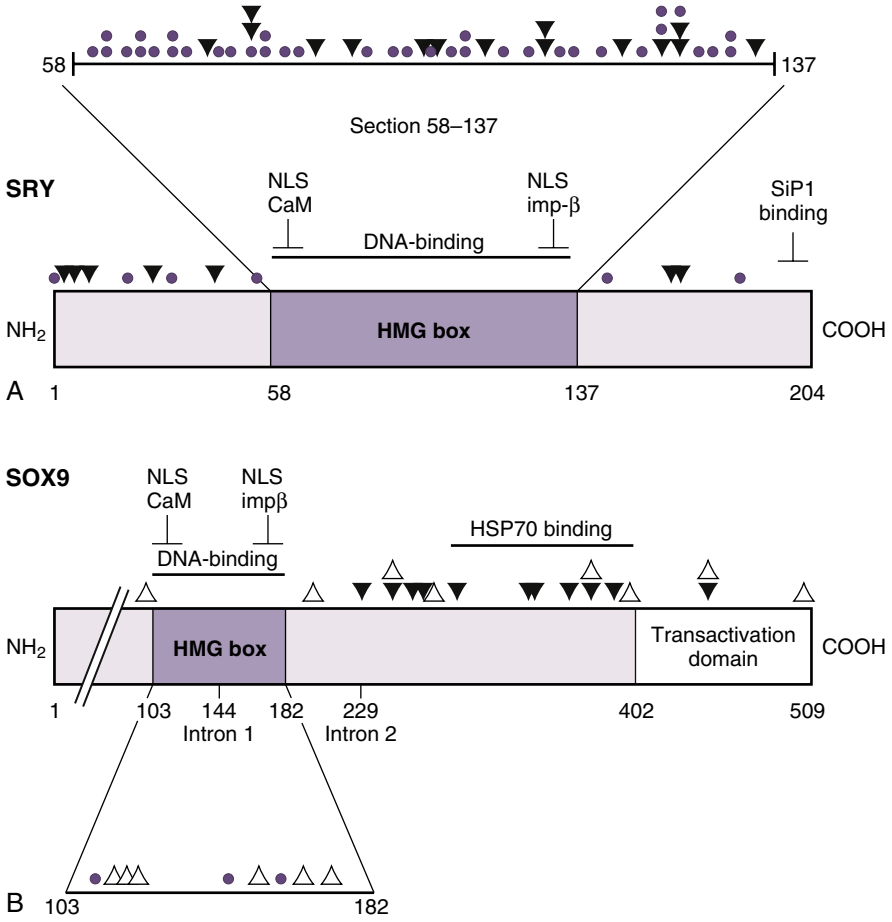
• **Fig. 24.21** Schematic diagram shows the structure of WT1 and the changes associated with exon 5 and exon 9 (addition of lysine, threonine, and serine) in +KTS isoforms. Many point mutations associated with Denys-Drash syndrome are located within zinc fingers 2 and 3 (especially Arg394). Mutations affecting the exon 9 splice site are associated with Frasier syndrome. (Modified from Koziell A, Grundy R. Frasier and Denys-Drash syndromes: different disorders or part of a spectrum? *Arch Dis Child*. 1999;81:365–369, used with permission of the BMJ Publishing Group, Copyright 1999.)

Chromobox Homolog 2: CBX2. Chromobox homolog 2 *(CBX2)* is a human homolog of the polycomb protein M33. Deletion of its gene causes XY sex reversal in mice. Loss-of-function mutations in *CBX2* were described in a 46,XY girl with a uterus and ovaries who was diagnosed by prenatal karyotyping, but at present, *CBX2* variants in humans are rare.[48]

Sex-Determining Region of the Y Chromosome: SRY. The sequence of events leading to the identification of *SRY* as the primary testis-determining gene and the actions of SRY in testis development were described earlier. SRY is a 204–amino acid HMG box transcription factor that is encoded by a single exon on the Y chromosome (Yp11.3) (see Fig. 24.5).[87] The discovery of inactivating mutations in SRY in patients with 46,XY gonadal dysgenesis confirmed the key role played by this factor in testis determination in humans.[31]

Approximately 10% of individuals with the complete form of 46,XY gonadal dysgenesis have inactivating mutations in *SRY*.[87] Most of the mutations occur in the HMG box DNA-binding domain of the SRY protein (Fig. 24.22), a region that is involved in binding and bending of DNA.[87] Rare mutations in the 5′ and 3′ flanking regions occur.[248] The HMG box contains at least two nuclear localization signals that bind calmodulin/exportin 4 and importin B. Mutations in these nuclear localization signal domains in the HMG box of SRY result in failure to transport the SRY protein into the nucleus, and subtle alterations in nuclear import and export can lead to XY gonadal dysgenesis.[90] Most individuals with *SRY* mutations are girls who have no pubertal development (Swyer syndrome). Testicular tumor risk in this group is high.

SRY Box 9: SOX9. Heterozygous mutations of the autosomal *SOX9* gene (17q24-q25) cause campomelic dysplasia.[99,100] Features of this condition include bowed long bones, hypoplastic scapulae, a deformed pelvis, 11 pairs of ribs, a small thoracic cage, cleft palate, macrocephaly, micrognathia, hypertelorism, and a variety of cardiac and renal defects. Death from respiratory distress often occurs in the neonatal period, but long-term survival has been reported.



• **Fig. 24.22** Structure of human SRY and SOX9 proteins and a selection of reported mutations. (A) Diagram of SRY. The high-mobility group (HMG) box is an 80–amino acid, DNA-binding domain with nuclear localization signals *(NLS)* at either end, one of which binds calmodulin (CaM) or exportin-4 and the other importin-β (imp-β). The last seven amino acids of SRY can bind to either of the PDZ domains found in SRY-interacting protein 1 (SiP1). The *solid circles* indicate missense mutations reported in the SRY protein that affect testicular development and cluster within the HMG box. Nonsense and frameshift mutations in SRY are indicated by *solid triangles*. (B) Diagram of SOX9. SOX9 has an HMG box with two NLSs, similar to SRY. However, SOX9 is encoded by three exons, binds to heat shock protein 70 (HSP70), and has a transactivation domain at the carboxy-terminal end, unlike SRY. Selected mutations causing 46,XY DSD and campomelic dysplasia are indicated by the *solid circles* (missense) and *solid triangles* (nonsense and frameshift). Mutations that cause only campomelia or a bony phenotype in 46,XY males or affect 46,XX females are indicated by *open triangles*.

SOX9 is an important testis-determining gene, and it is the key regulator of testis determination downstream of *SRY* (see "Testis Determination"). Consistent with this hypothesis, three-fourths of 46,XY patients with heterozygous *SOX9* mutations have dysgenetic gonads, but a wide range of genital phenotypes can be seen, from typically male to typically female appearance.[87] Histologic examination of the gonads from 46,XY patients with ambiguous or female external genitalia shows a similarly wide range of gonadal phenotypes, including streak gonads, dysgenetic testes, ovotestes, or even ovaries.[223] Müllerian structures may or may not be present, depending on the degree of gonadal dysgenesis. 46,XX females with *SOX9* mutations have normal external genitalia and apparently normal ovaries.

The locus for campomelic dysplasia with 46,XY DSD was mapped to 17q24.3-q25.1 after studies of three patients with balanced de novo reciprocal translocations, and *SOX9* was proposed as a candidate gene based on expression studies in the mouse. Subsequently, missense, nonsense, frameshift, and splice junction mutations have been detected in *SOX9* in patients with campomelic dysplasia with or without gonadal dysgenesis.[87] These mutations are usually heterozygous de novo changes. In one kindred, multiple siblings were affected due to germline mosaicism for a *SOX9* mutation in a parent.[223] The gonadal phenotype in this kindred varied in the two affected 46,XY siblings: One of them had dysgenetic gonads; the other was reported to have normal ovaries.

The *SOX9* gene has three exons and two introns; it encodes a 509-residue protein that contains an HMG box with 71% homology to that of the SRY protein and a C-terminal, *trans*-activation domain (see Fig. 24.22). Unlike SRY, in which most mutations are located within the HMG box, SOX9 mutations are located throughout the protein, with little relation between functional domains and phenotype. Chromosomal translocations that disrupt regulatory elements upstream of the *SOX9* promoter can be associated with a less severe phenotype or campomelic dysplasia without gonadal abnormalities, or 46,XY DSD without a skeletal phenotype. Heterozygous changes that allow residual DNA binding and transactivation may also be associated with the acampomelic variant and variable or absent DSD.

SRY Box 8: SOX8. *SOX8* (16p13.3) is closely related to *SRY* and *SOX9* and is coexpressed with *NR5A1* and *SOX9* in Sertoli and Leydig cells during the early stages of human testis determination.[249] Deletions and point mutations in *SOX8* have recently been described in individuals with testicular dysgenesis, sometimes as part of a chromosome 16p-deletion syndrome. An enrichment of rare variants in *SOX8* has also been found in individuals with a spectrum of reproductive phenotypes, including oligozoospermia and primary ovarian insufficiency.[249]

Mitogen-Activated Protein Kinase Kinase Kinase-1: MAP3K1. MAP3K1 is one of several kinase signaling pathways involved in organogenesis, and heterozygous splice site or point mutations in *MAP3K1* have been described in several families and individuals with impaired testis development.[97] MAP3K1 is expressed in early testis cords and Sertoli cells in mice, and disruption of *MAP3K1* has been described in diverse phenotypes ranging from 46,XY complete testicular dysgenesis to hypospadias and micropenis with cryptorchidism. Familial cases have a clear autosomal dominant inheritance. *MAP3K1* mutations may be one of the more common causes of 46,XY DSD, and routine screening has been recommended in all patients with 46,XY complete or partial gonadal dysgenesis.[225]

Estrogen Receptor β: ESR2/NR3A2. A homozygous *ESR2* mutation has been reported in an individual with 46,XY DSD characterized by absence of gonads, uterus, and vagina, as well as dysmorphic features, eye abnormalities, and anal atresia; heterozygous mutations were identified in two individuals with 46,XY complete and partial gonadal dysgenesis without syndromic features.[250] It was hypothesized that the *ESR2* variants may lead to increased activity of the MAPK pathway through nonclassic signaling, thereby altering gonadal fate.

Doublesex-Related and Mab3-Related Transcription Factor 1: DMRT1. *DMRT1*, a gene with sex-specific homologs in *Drosophila* (*doublesex* gene) and *Caenorhabditis elegans (Mab3)* that is expressed in early gonad development, plays an important role in supporting testis development.[112,251] Deletions of the region of chromosome 9p24-pter containing *DMRT1* are associated with 46,XY gonadal dysgenesis.[252] More recently, a partial deletion of *DMRT1* and a dominant-negative point mutation in *DMRT1* have been seen in patients with 46,XY DSD.[253,254]

Testis-Specific Protein, Y-Linked–Like 1 Gene: TSPYL1. An association between 46,XY gonadal dysgenesis and sudden infant death syndrome was characterized in a large Amish kindred and named *sudden infant death, dysgenetic testes* (SIDDT).[255] The gene responsible for this autosomal recessive condition, *TSPYL1*, encodes a protein of unknown function that may be involved in chromatin remodeling. Additional variants of *TSPYL1* have been described in patients with 46,XY DSD.[256]

Desert Hedgehog: DHH. The hedgehog signaling pathways play an important role in many aspects of neuronal, skeletal, and endocrine development. A homozygous mutation in the desert hedgehog gene *(DHH)* was originally reported in a patient with partial gonadal dysgenesis and minifascicular neuropathy.[116] Subsequently, a number of *DHH* changes have been found in patients with complete 46,XY gonadal dysgenesis or more often Leydig cell defects, with or without apparent neurologic features.[117,166]

Sterile α-Motif Domain-Containing Protein 9: SAMD9. SAMD9 is an endosomal protein that negatively regulates growth and development. Gain-of-function variants in SAMD9 have been reported to cause impaired testicular function and 46,XY DSD as part of a complex multisystem growth restriction disorder, MIRAGE syndrome (myelodysplasia, infections, restriction of growth, adrenal hypoplasia, genital phenotypes, enteropathy).[257,258] Children are often born preterm and have high mortality. Somatic rescue due to monosomy 7 or secondary somatic loss-of-function changes in SAMD9 itself can modify the phenotype, but monosomy can be associated with the development of myelodysplastic syndrome. The testicular phenotype most likely represents a Leydig cell defect.[258]

Aristaless-Related Homeobox, X-Linked Gene: ARX. ARX is a transcription factor that plays a central role in neuronal migration, and the *Arx* knockout mouse has a profound myelination defect. Mutations in *ARX* have been found in X-linked lissencephaly ambiguous genitalia (XLAG) syndrome.[114] This unusual form of lissencephaly is associated with severe epilepsy and thermal instability. The genital abnormality most likely represents a defect in Leydig cell function. Additional *ARX* mutations have been described in patients with neurologic defects (e.g., infantile spasms) without significant DSDs.

Mastermind-Like Domain-Containing 1: MAMLD1. *MAMLD1* (formerly called *CXORF6*) is a gene on the X chromosome that encodes a protein expressed in the developing testes. Hemizygous mutations in *MAMLD1* were originally described in boys with isolated severe hypospadias, but a range of phenotypes has since been described, although it is unclear whether the reported *MAMLD1* mutations suffice to explain the phenotype in

all individuals.[118,259,260] *MAMLD1* disruption results in a defect in fetal Leydig cell development and function.

Chromosomal Rearrangements Associated With Gonadal Dysgenesis

Abnormalities of genital development are associated with a number of chromosomal deletions, duplications, and rearrangements. The most frequently seen changes are deletions of 9p24-pter (affecting *DMRT1*, described earlier), 10q25-qter, and Xq13 and duplications of Xp21.

Terminal deletions of chromosome 10 (10q25-qter) are frequently associated with urogenital abnormalities and sometimes with complete gonadal dysgenesis.[261] The gene in this locus has not been identified; *FGFR2* is a candidate. Deletions of Xq13.3 and of the tip of chromosome 16p cause α-thalassemia mental retardation (ATR) syndromes that may have gonadal dysgenesis as part of the phenotype.[262] The Xq13.3 locus contains the transcription factor gene *ATRX* (also known as *XH2* or *XNP*). The *SOX8* gene is located on 16p, and rearrangements of this locus have been implicated in a variety of conditions with impaired gonadal development and/or function (see previous discussion).[249,263]

Duplications of the Xp21.3 region that contains the *DAX1 (NR0B1)* gene can cause 46,XY partial or complete gonadal dysgenesis.[264] The role of *DAX1* and the *WNT4* pathway (duplication 1p35) in opposing testis development was discussed previously (see "Development of Reproductive Systems").[264,265]

Several other studies using array comparative genomic hybridization or single-nucleotide polymorphism (SNP) analysis have been reported and have proposed other candidate regions for testicular dysgenesis.[266,267]

Syndromic Causes of 46,XY Disorders of Sex Development

In addition to the specific syndromes outlined earlier, various degrees of testicular dysgenesis and impaired genital development (e.g., hypospadias, cryptorchidism, scrotal transposition) are seen in many discrete syndromes.[268,269] In some situations, a genetic basis has been identified, but in many cases the cause is unknown. Syndromic associations of 46,XY DSD may be more prevalent than originally thought and have been reviewed in detail elsewhere.[268,269]

46,XY DSD is often associated with intrauterine growth restriction (IUGR).[270] Monozygotic twins can show disparate genital development, with the growth-restricted twin having ambiguous genitalia and the larger twin appearing as a normal male. The mechanism of this association is unclear and may represent a shared genetic cause or a common epigenetic or somatic developmental event affecting fetal growth, placental function, and reproductive development. More common genetic causes of 46,XY DSD (e.g., mutations of SRY, SF1, androgen receptor, and steroidogenic enzymes) are rarely found in this group of IUGR patients (personal observation).

Genes Involved in Central Hypogonadism

Central causes of hypogonadism resulting in LH and FSH insufficiency do not typically cause hypospadias, as the early stages of fetal testis steroidogenesis are autonomous or driven by hCG. However, boys can have combined central defects together with hypospadias or DSD, and potential disease-causing variants in several genes have been reported, which may affect the HPG axis at multiple levels (e.g., *CHD7*, *ANOS1*/*KAL1*, *WDR11*, *PROK2*, *PROKR2*, *FGF8*, and *FGFR1).*[166,167]

Potential Novel Genes and Oligogenic Effects

Whole-exome array and next-generation sequencing approaches have identified potential pathogenic variants in genes related to testis development (e.g., *WWOX*), but the significance of some of these findings is not yet clear. Furthermore, data emerging from these approaches suggest that oligogenic effects involving changes in more than one gene may also be associated with gonadal dysgenesis or may influence the severity of the phenotype in some situations.[166,236,237]

Disorders of Androgen Synthesis

Defects anywhere along the pathway of androgen synthesis and target organ action can result in impaired androgenization and 46,XY DSD (formerly referred to as male pseudohermaphroditism) (see Table 24.6 and Fig. 24.11). Because many of the steps of androgen synthesis overlap with those of glucocorticoid synthesis, some of the enzymatic deficiencies that cause androgen deficiency also cause congenital adrenal hyperplasia. In all of these conditions, because Sertoli cell secretion of AMH is intact, müllerian structures are absent.

Cholesterol Synthesis Defects: Smith-Lemli-Opitz Syndrome

Smith-Lemli-Opitz syndrome is caused by a deficiency of 7-dehydrocholesterol reductase (3β-hydroxysterol Δ7-reductase [DHCR7]), the phylogenetically conserved sterol-sensing domain–containing enzyme required for the last step in the biosynthetic pathway from acetate to cholesterol. Cholesterol is necessary as a substrate for steroid-hormone synthesis, and intermediates of cholesterol synthesis may also have important interactions with hedgehog signaling pathways.

Smith-Lemli-Opitz syndrome has a broad phenotypic spectrum that typically includes microcephaly, developmental delay, cardiac defects, ptosis, upturned nose, micrognathia, cleft palate, polydactyly, syndactyly of toes (especially the second and third toes), severe hypospadias, micropenis, and growth failure.[271] Abnormalities of the external genitalia occur in approximately 65% of 46,XY patients and vary from micropenis and hypospadias to complete absence of androgenization, resulting in a female phenotype.

The syndrome is diagnosed by finding elevated plasma levels of 7-dehydrocholesterol (7-DHC) and low levels of cholesterol. The *DHCR7* gene maps to 11q12-q13, and more than 150 mutations have been described.[272] Measurement of serum 7-DHC should be considered in all underandrogenized males with relevant phenotypic features, however mild. Testis development is apparently normal, and normal, elevated, or low concentrations of plasma testosterone have been described in affected male infants with intact HPG function. Compromised adrenal function can sometimes occur.

Luteinizing Hormone Receptor Mutations

Mutations in the LH/hCG receptor cause impaired responsiveness to hCG and LH, resulting in Leydig cell agenesis or hypoplasia.[273] Phenotypically, the external genitalia vary from a female appearance to a male with micropenis (Table 24.7). Rudimentary wolffian derivatives may be present, even in some patients with severely underandrogenized external genitalia. This finding may reflect some early hCG-independent mechanisms of testosterone synthesis between 8 and 10 weeks of gestation. Small, undescended testes are usually found in the inguinal region in the most severe forms of this Leydig cell hypoplasia. Patients with milder phenotypes may have appropriately descended testes of relatively normal

TABLE 24.7 Clinical Features of Leydig Cell Hypoplasia in 46,XY Individuals

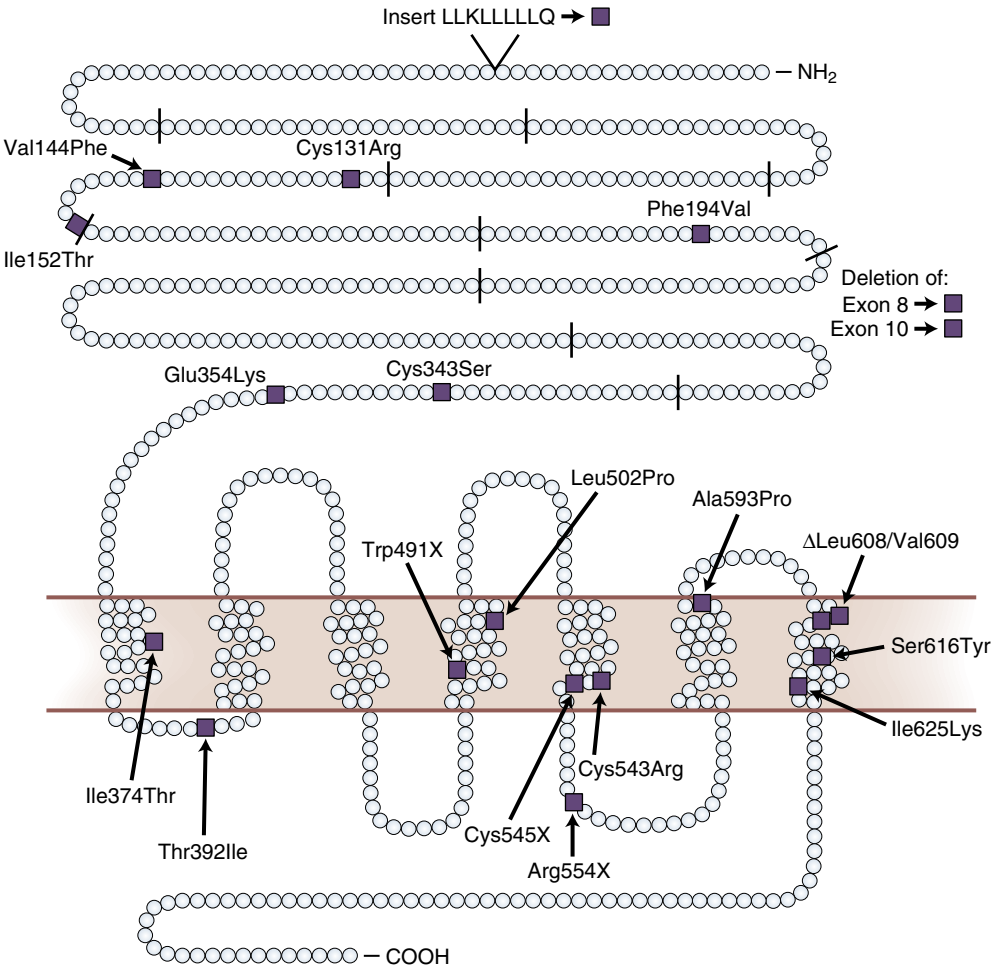
Karyotype	46,XY
Inheritance	Autosomal recessive; mutations in *LHCGR* gene
Genitalia	Female, hypospadias, or micropenis
Wolffian duct derivatives	Hypoplastic
Müllerian duct derivatives	Absent
Gonads	Testes
Biochemical and physiologic features	Underandrogenization with variable insufficiency of sex hormone production at puberty
Hormone profile	Low T and DHT; elevated LH (and FSH); exaggerated LH response to LHRH stimulation; poor T and DHT response to hCG stimulation

DHT, Dihydrotestosterone; *FSH,* follicle-stimulating hormone; *hCG,* human chorionic gonadotropin; *LH,* luteinizing hormone; *LHCGR,* LH or hCG receptor; *LHRH,* luteinizing hormone–releasing hormone; *T,* testosterone.

size because the Leydig cell population contributes to only about 10% of testicular volume. On histologic examination, the testes lack distinct Leydig cells in prepubertal patients, though Leydig cells may be difficult to visualize even in normal prepubertal testes. Postpubertal patients have absent or decreased numbers of Leydig cells without Reinke crystalloids, normal-appearing Sertoli cells, and discrete seminiferous tubules with spermatogenic arrest. This observation highlights the important role of intratesticular testosterone in the final stages of sperm maturation.

The typical biochemical profile of patients with Leydig cell hypoplasia includes elevated basal and GnRH-stimulated LH (and FSH) levels in early infancy or at puberty due to loss of sex steroid negative feedback. In childhood, when the GnRH pulse generator is relatively quiescent, basal LH levels may still sometimes be detected above the normal range. Plasma levels of androstenedione and testosterone are low, with little or no response to prolonged hCG stimulation. Plasma LH falls after testosterone administration. Less marked biochemical changes can occur with milder forms of this condition.

More than 30 different homozygous or compound heterozygous mutations have been reported in the LH/hCG receptor gene *(LHCGR)* in individuals with various forms of this condition (Fig. 24.23).[274,275] The original reports of Kremer and colleagues[274] and Latronico and associates[275] described homozygous



• **Fig. 24.23** Diagram of the luteinizing hormone/human chorionic gonadotropin (LH/hCG) receptor with selected inactivating missense mutations shown by *squares*. Most of these changes are associated with marked underandrogenization. However, the changes at residues 616 and 625 in the seventh transmembrane domain are associated with a milder phenotype of micropenis.

Ala593Pro and Arg554Ter mutations, respectively, in 46,XY phenotypic females with Leydig cell hypoplasia, hypergonadotropic hypogonadism, and no testosterone response to hCG stimulation. An affected 46,XX sister showed normal sexual maturation at puberty but an elevated LH level and amenorrhea, demonstrating that the LH receptor is not necessary for estrogen synthesis but is necessary for normal ovulation in females. These mutations impair hCG stimulation of intracellular cyclic adenosine monophosphate (cAMP) in vitro through disturbances in hCG binding, intracellular signaling, and/or receptor stability and trafficking, depending on the nature of the change. Defects in a cryptic exon of the LH receptor (exon 6A) have also been described as a cause of 46,XY DSD.[276] Partial loss-of-function mutations in the LH/hCG receptor causing milder phenotypes such as micropenis have tended to localize within the seventh transmembrane domain (Ser616Tyr, Ile625Lys) (see Fig. 24.23).[275,277] Individuals with complete Leydig cell hypoplasia are usually reared as females and require estrogens at puberty. Gonadectomy is usually performed, though the risk of gonadal malignancy is unknown. If a male sex designation is chosen, testosterone supplements may be given in early infancy and to support puberty.

Steroidogenic Acute Regulatory Protein Defects

Steroidogenic acute regulatory protein is a 30-kDa mitochondrial protein that is present in the adrenal gland and gonads. It plays a key role in facilitating the rapid movement of cholesterol from the outer to the inner mitochondrial membrane.[131,278] This process is necessary to allow de novo steroid biosynthesis in response to ACTH or angiotensin II in the adrenal gland or an LH pulse in the gonad. Although a limited amount of cholesterol transfer is StAR independent (14%), this protein plays a central role in the acute regulation of adrenal and gonadal steroidogenesis. StAR is not necessary for placental progesterone production, unlike P450scc (CYP11A1, discussed next).

Consistent with these actions, patients with recessively inherited defects in StAR develop a severe form of primary adrenal failure called *lipoid congenital adrenal hyperplasia* (lipoid CAH).[279,280] Patients with this condition tend to present with severe glucocorticoid deficiency (e.g., hypoglycemia, hyperpigmentation) early in life and progressive mineralocorticoid insufficiency resulting in hyponatremia, hyperkalemia, dehydration, acidosis, and collapse (Table 24.8). Presentation is often between 3 weeks and 3 months of age. Little or no C18, C19, and C21 steroids (which include estrogens, androgens, and progesterone/glucocorticoids/mineralocorticoids, respectively) are detectable in plasma or urine, even after corticotropin or hCG stimulation. Females (46,XX) with lipoid CAH have typical female genitalia. In 46,XY individuals, mutations in *STAR* typically cause a marked deficiency in testosterone synthesis by fetal Leydig cells so that typical female genitalia are seen. Testes may be abdominal, inguinal, or labial. Müllerian structures have regressed, and there is a blind vaginal pouch. A karyotype should be performed in all apparent girls presenting with early-onset adrenal failure.

The typical histologic finding in lipoid CAH is lipid accumulation within steroidogenic cells. In the steroid-deficient state, the tropic drive by ACTH, angiotensin II, and LH causes increased cholesterol uptake and synthesis by steroidogenic cells. Coupled with the inability of StAR to facilitate cholesterol movement into mitochondria, this leads to marked accumulation of cholesterol in cells and results in the appearance of enlarged, lipid-laden adrenal glands sometimes seen on MRI or computed tomography (CT). Eventually, cholesterol accumulation causes engorgement and results in structural disruption superimposed on the functional disruption—the "two-hit" hypothesis (Fig. 24.24).[280]

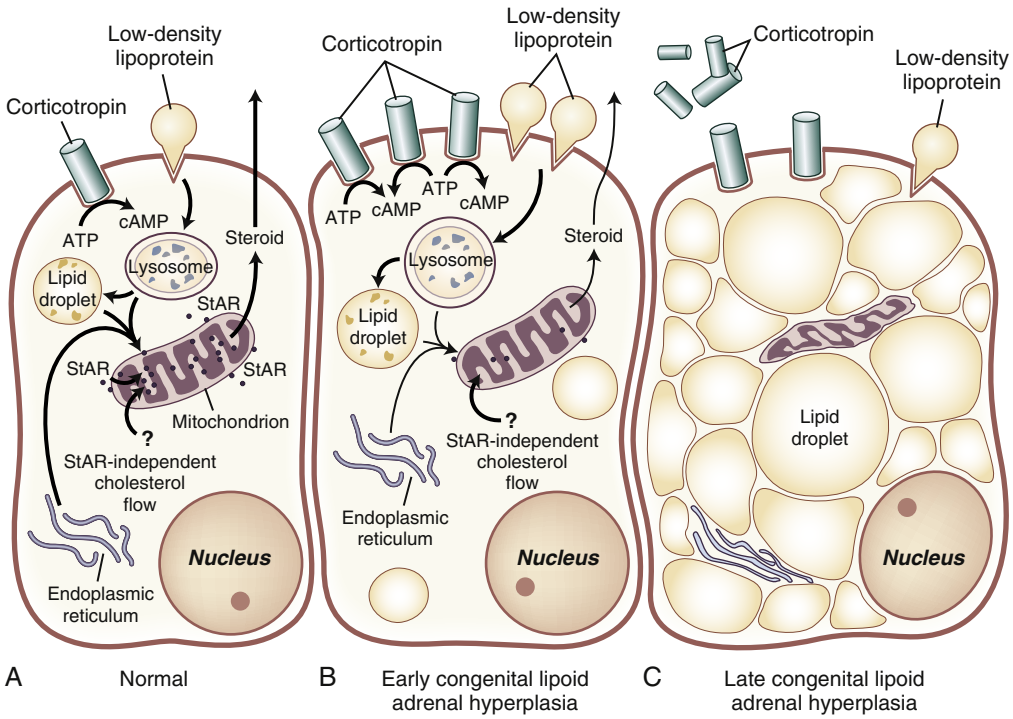
TABLE 24.8 Clinical Features of Lipoid CAH in 46,XY Individuals

Karyotype	46,XY
Inheritance	Autosomal recessive; mutations in *STAR* gene
Genitalia	Female; sometimes ambiguous, hypospadias, or male
Wolffian duct derivatives	Hypoplastic or normal
Müllerian duct derivatives	Absent
Gonads	Testes
Biochemical and physiologic features	Severe adrenal insufficiency in infancy with salt loss; lack of pubertal development; rare nonclassic cases associated with isolated glucocorticoid deficiency
Hormone profile	Usually deficiency of glucocorticoids, mineralocorticoids, and sex steroids except in nonclassic cases in which a predominant defect in glucocorticoid production is seen (similar to familial glucocorticoid deficiency)

CAH, Congenital adrenal hyperplasia; *StAR*, steroidogenic acute regulatory (protein).

The two-hit hypothesis explains why 46,XX girls with lipoid CAH show evidence of estrogenization and breast development at puberty but have progressive hypergonadotropic hypogonadism.[280,281] Follicular cells are relatively quiescent in utero and before puberty and are therefore undamaged. They are recruited at the beginning of each cycle, and a small amount of estradiol can be produced as a result of StAR-independent mechanisms. This can occur until the follicular cells are engorged and rendered nonfunctional. Puberty can occur, but any cycles are anovulatory. Without treatment, polycystic ovaries and progressive ovarian insufficiency failure usually ensue.

Although more than 40 different StAR mutations have been described in patients from around the world, lipoid CAH is especially prevalent in Japan and Korea, where it is the second-most common steroidogenic disorder after CYP21 deficiency (Fig. 24.25). Most Japanese patients and virtually all Korean patients harbor the Gln258Stop mutation, which is estimated to be carried by 1 in 300 Japanese people.[282] Other geographic clusters include the Leu260Pro mutation in patients of Swiss ancestry, Arg182His in Eastern Saudi Arabia, and Arg182Leu in Palestinians. Most of these mutations result in complete loss of function. However, a nonclassic form of lipoid CAH has been described that is caused by point mutations in StAR; in this form, StAR retains approximately 20% function.[283] These patients present with progressive glucocorticoid deficiency between 2 and 4 years of age; affected males have normal androgenization of the external genitalia but may be at risk of hypoandrogenism or reduced fertility later in life. StAR mutations with intermediate function may also be associated with hypospadias and milder adrenal failure.[284] These partial loss-of-function changes tend to affect amino acids (codons 187, 188, 192, 221) that lie around the cholesterol-binding pocket.[285]



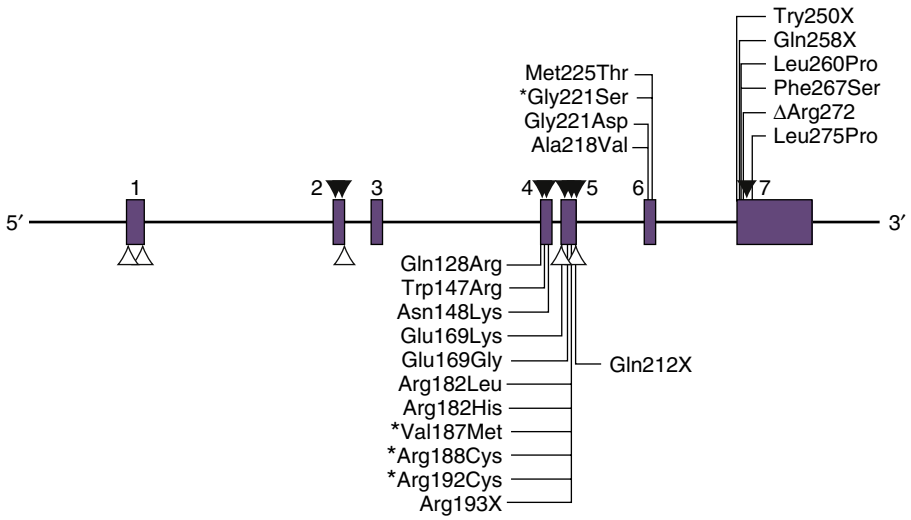
• **Fig. 24.24** Model of the steroid-synthesizing cell (adrenal/gonadal) showing conversion of cholesterol to steroids. (A) Cholesterol from low-density lipoprotein, from cholesterol esters stored in lipid droplets, and from endogenous synthesis in the endoplasmic reticulum is transported from the outer mitochondrial membrane to the inner membrane. This transport is facilitated by the steroidogenic acute regulatory protein (StAR) and by other StAR-independent mechanisms. In the mitochondria, steroid synthesis begins with the conversion of cholesterol to Δ^5 pregnenolone by the enzyme CYP11A1 (P450scc). (B) In patients with lipoid congenital adrenal hyperplasia, a mutation in the gene encoding StAR results in little or no activity of the mutant StAR, greatly reducing cholesterol transport into the mitochondria. Low levels of steroidogenesis by mechanisms independent of StAR can occur; however, increased secretion of corticotropin (or luteinizing hormone or follicle-stimulating hormone) results in cholesterol accumulation in the cells as lipid droplets. (C) Continued stimulation and resultant accumulation of cholesterol cause engorgement of these cells, with mechanical and chemical perturbation of cell function. This results in primary adrenal insufficiency and impaired androgen biosynthesis by fetal Leydig cells. Females with lipoid congenital adrenal hyperplasia feminize at puberty and menstruate but have progressive hypergonadotropic hypogonadism. This may occur because the follicular cells are relatively quiescent in utero and before puberty and therefore are undamaged. At the beginning of each cycle, follicles are recruited, and a small amount of estradiol can be produced as a result of StAR-independent mechanisms. This can occur until the follicular cells are engorged and rendered nonfunctional. *ATP,* Adenosine triphosphate; *cAMP,* cyclic adenosine monophosphate. (From Bose HS, Sujiwara T, Strauss JF III, et al. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. *N Engl J Med*. 1996;335:1870–1878, used with permission of the Massachusetts Medical Society, Copyright 1996.)

Management of classic lipoid CAH includes glucocorticoid and mineralocorticoid replacement and salt supplementation in early life. Gonadectomy is usually performed in individuals with a 46,XY karyotype; although limited data are available, neoplastic germ cell changes have been reported.[286,287] Males with nonclassic lipoid CAH need careful follow-up to ensure adequate testosterone production in puberty and adulthood. Estrogen treatment is given to induce puberty and is administered to 46,XX females when gonadal insufficiency occurs.

P450 Side-Chain Cleavage Enzyme Deficiency

P450scc (CYP11A1) is the mitochondrial enzyme that converts cholesterol to pregnenolone by three distinct enzymatic reactions: 20α-hydroxylation, 22-hydroxylation, and cleavage of the cholesterol side chain. P450scc is therefore responsible for the first and rate-limiting step in steroid synthesis, which is necessary for pregnenolone and progesterone production by the placenta, for mineralocorticoid, glucocorticoid, and androgen production by the adrenal glands, and for sex steroid production by the gonads.

Although a natural model of lipoid CAH due to P450scc deficiency exists in the rabbit, it was thought that severe loss of P450scc activity in humans would be incompatible with survival. Placental progesterone production is necessary to support pregnancy after the second trimester in higher primates (due to the so-called luteoplacental shift) but not in rodents. However, complete disruption of P450 activity due to a homozygous frameshift mutation in *CYP11A1* has been reported in a 46,XY infant with female external genitalia and severe early-onset, salt-losing adrenal failure.[288] An increasing number of other mutations in P450scc have also been described, initially in 46,XY phenotypic females with severe salt-losing adrenal failure (Table 24.9).[289,290] More recently,



• **Fig. 24.25** Diagram of selected mutations identified in the *STAR* gene associated with lipoid congenital adrenal hyperplasia. Numbered *solid boxes* depict the exons. The three-letter abbreviations for amino acids are used to indicate the position of missense mutations; *X* indicates a nonsense (stop) mutation; insertions and deletions resulting in frameshift mutations *(solid triangles)* and splice site mutations *(open triangles)* are shown. Although *STAR* mutations are common in Japan, Korea, and regions of the Middle East, an increasing number of sporadic changes in the steroidogenic acute regulatory (StAR) protein are being detected in other countries. Missense mutations at residues 187, 188, 192, and 221 *(asterisks)* have been associated with a nonclassic late-onset phenotype of glucocorticoid insufficiency.

TABLE 24.9 Clinical Features of CYP11A1 Deficiency in 46,XY Individuals

Karyotype	46,XY
Inheritance	Autosomal recessive; mutations in *CYP11A1* gene
Genitalia	Female; rarely ambiguous or hypospadias
Wolffian duct derivatives	Hypoplastic or normal
Müllerian duct derivatives	Absent
Gonads	Testes (or absent)
Biochemical and physiologic features	Severe adrenal insufficiency in infancy with salt loss ranging to milder adrenal insufficiency with onset in childhood; prematurity associated in one case
Hormone profile	Usually deficiency of glucocorticoids, mineralocorticoids, and sex steroids

CYP11A1, Cytochrome P450 side-chain cleavage enzyme.

partial loss-of-function defects in P450scc have been described in boys with hypospadias who developed adrenal failure in late childhood, and milder changes in *CYP11A1* have been reported in children with primary adrenal insufficiency without DSD.[291–293] This condition is now well established as a cause of combined adrenal and gonadal failure as well as isolated adrenal insufficiency. Long-term follow-up of patients with hypospadias or isolated adrenal insufficiency may be needed to monitor testicular function and fertility. Molecular analysis is needed to differentiate this P450scc deficiency from StAR deficiency, though it seems that patients with P450scc deficiency rarely have significant lipoid enlargement of the adrenal glands.[290]

3β-Hydroxysteroid Dehydrogenase/$\Delta^{4,5}$-Isomerase Type 2 Deficiency

3βHSD type 2 deficiency is a rare, autosomal recessive cause of CAH that affects both adrenal and gonadal steroid production. It is one of the few conditions that can cause atypical genitalia in both 46,XX and 46,XY individuals. This autosomal recessive disorder is a consequence of mutations in *HSD3B2*, the gene encoding the 3βHSD/$\Delta^{4,5}$-isomerase type 2 isozyme, which is expressed mainly in the adrenals and gonads. This enzyme catalyzes a crucial step in the biosynthesis of all steroid hormones, the conversion of Δ^5 steroids to Δ^4 steroids (see Fig. 24.11).[294] The other 3βHSD isoenzyme in humans, 3βHSD1, is expressed in the placenta and in peripheral tissues such as the skin (mainly sebaceous glands), breast, and prostate; it is not associated with CAH.

Classic 3βHSD2 deficiency is subdivided into salt-losing and non–salt-losing forms (Table 24.10). Severely disruptive changes in 3βHSD2 cause salt-losing adrenal insufficiency soon after birth. In 46,XY patients, the external genitalia are usually atypical, with small penis, severe hypospadias, partial labioscrotal fusion, and urogenital sinus. Mutations that severely disrupt enzymatic activity usually present with salt loss (Fig. 24.26).[295–297] In contrast, mutations such as Ala245Pro retain considerable enzyme activity (2–10%) and have been found in males with penoscrotal hypospadias and no salt loss. A late-onset form of 3βHSD2 deficiency usually manifests as premature pubarche and idiopathic hirsutism in females. The biochemical profile of classic 3βHSD2 deficiency is an elevated 17-hydroxypregnenolone, which is present at levels greater than 100 nmol/L at baseline or after ACTH stimulation.[298] An elevated ratio of Δ^5 to Δ^4 steroids is sometimes but not always seen; adrenal production of Δ^4 steroids such as

17-OHP and androstenedione is decreased, but serum concentrations may be elevated in 3βHSD2 deficiency. This apparent paradox arises because of peripheral conversion of Δ^5 to Δ^4 steroids by 3βHSD1 activity. In a neonatal screening program, an elevated 17-OHP level in a salt-losing person with 3βHSD2 deficiency may be potentially misdiagnosed as 21-hydroxylase CYP21A2 deficiency.[299,300] As the ratio of Δ^5 to Δ^4 steroids is not always elevated in 3βHSD2 deficiency, the ratio of 17-hydroxypregnenolone to cortisol has been proposed as a more sensitive screen for this condition.[301] However, a 17-hydroxypregnenolone assay is not always readily available, and early genetic testing may be an alternative.

TABLE 24.10 Clinical Features of HSD3B2 Deficiency in 46,XY Individuals

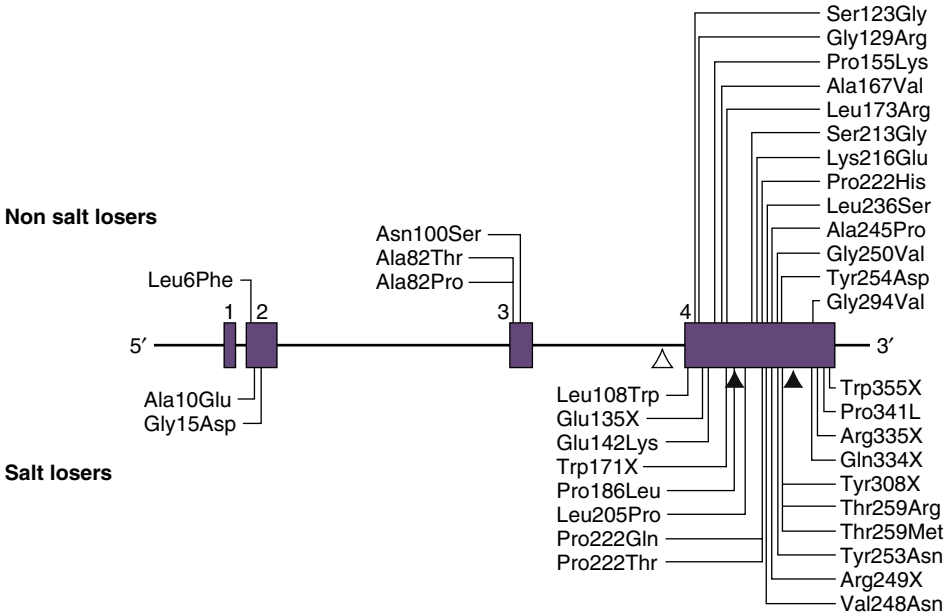
Karyotype	46,XY
Inheritance	Autosomal recessive; mutations in *HSD3B2* gene
Genitalia	Ambiguous; hypospadias
Wolffian duct derivatives	Normal
Müllerian duct derivatives	Absent
Gonads	Testes
Biochemical and physiologic features	Severe adrenal insufficiency in infancy; poor virilization at puberty with gynecomastia *Mild form:* no mineralocorticoid deficiency, premature adrenarche → mild virilization
Hormone profile	Increased concentrations of Δ^5C21 and C19 steroids (e.g., 17-hydroxypregnenolone/cortisol ratio response to corticotropin, 17-hydroxypregnenolone, DHEA suppressible by dexamethasone)

DHEA, Dehydroepiandrosterone; *HSD3B2,* 3β-hydroxysteroid dehydrogenase type 2.

Children with 3βHSD deficiency need glucocorticoid and sometimes mineralocorticoid replacement. A recent study of the Amish population, in which the c.35G>A founder effect is seen, has suggested that some children need only physiologic doses of glucocorticoids to suppress ACTH and, in turn, adrenal androgen production, whereas others need higher, supraphysiologic doses.[300] Undertreatment can be associated with advanced skeletal maturation and early puberty due to sex steroid excess in childhood, whereas overtreatment can cause iatrogenic complications.[300] Gynecomastia can occur at puberty in affected males. This is presumably the result of 3βHSD1-mediated peripheral conversion of Δ^5C19 steroids to Δ^4C19 steroids and aromatization to estrogens.[302] Normal puberty has been reported in males with a null mutation in the *HSD3B2* gene, but puberty needs close monitoring to ensure adequate androgens are produced, and data on fertility are still limited.[302]

17α-Hydroxylase/17,20-Lyase Deficiency

P450c17 (CYP17) is a microsomal enzyme with 17α-hydroxylase and 17,20-lyase activity that is expressed in the adrenals and gonads but not in the placenta or in ovarian granulosa cells.[303,304] The 17α-hydroxylase action of P450c17 catalyzes the conversion of pregnenolone (Δ^5) to 17-hydroxypregnenolone and the conversion of progesterone (Δ^4) to 17-OHP (see Fig. 24.11). The 17,20-lyase action of P450c17 can convert 17-hydroxypregnenolone



• **Fig. 24.26** Diagram of the 3β-hydroxysteroid dehydrogenase type 2 gene *(HSD3B2)* shows the mutations that result in deficiency of the enzyme. Changes associated with salt loss are shown below and non–salt loss above. The numbered *solid boxes* depict the exons. Mutations are subdivided according to their association with salt-losing and non–salt-losing states. The three-letter abbreviations for amino acids are used to indicate the position of missense mutations; *X* indicates a nonsense (stop) mutation; insertions and deletions resulting in frameshift mutations *(solid triangles)* and splice site mutations *(open triangles)* are shown.

(Δ^5) to DHEA and 17-OHP (Δ^4) to androstenedione (see Fig. 24.11). P450c17 is bound to the smooth endoplasmic reticulum, where it accepts electrons from a specific flavoprotein, reduced nicotinamide adenine dinucleotide phosphate (NADPH)–P450 oxidoreductase (POR). The 17,20-lyase activity of P450c17 is favored by the presence of Δ^5 substrates, redox partners such as POR and cytochrome b_5, and serine phosphorylation. Unlike in rodents, the 17,20-lyase activity of human P450c17 on Δ^5 substrates is 50 times more efficient than its activity on Δ^4 substrates; therefore very little androstenedione is formed directly from 17-OHP, and the principal pathway to androgen production is through DHEA.[132] P450c17 also has 16α-hydroxylase activity.

Defects in P450c17 action can result in two different forms of CAH. Most often, combined 17α-hydroxylase/17,20-lyase deficiency is seen, but rare cases of isolated 17,20-lyase deficiency have been reported (Tables 24.11 and 24.12).[305]

Combined 17α-hydroxylase/17,20-lyase deficiency is a rare, autosomal recessive form of CAH, although an increasing number of cases are being reported from many different countries.[306] A prevalence of approximately 1 case per 50,000 individuals is reported in some areas, but in general, the prevalence is lower than this. The classic phenotype of complete combined 17α-hydroxylase/17,20-lyase deficiency is that of a phenotypic female (46,XX, or underandrogenized 46,XY) who presents with an absence of secondary sex characteristics and hypergonadotropic hypogonadism at puberty and is found to have low-renin hypertension and hypokalemic alkalosis (see Table 24.11).

The classic phenotype and underlying biochemistry can be explained by the enzyme deficiency (see Fig. 24.11).[305] A defect in 17α-hydroxylation in the adrenal cortex and gonads results in impaired synthesis of 17-OHP and 17-hydroxypregnenolone and therefore of cortisol, androgens, and estrogens. Decreased cortisol synthesis causes increased ACTH secretion, which results in excessive secretion of 17-deoxysteroids by the adrenal cortex, including the mineralocorticoids 11-deoxycorticosterone (DOC), corticosterone, and 18-hydroxycorticosterone. Excess DOC secretion eventually leads to hypertension, hypokalemic alkalosis, and suppression of the renin-angiotensin system. Diminished aldosterone synthesis and secretion are sometimes reported. Corticosterone is a weak glucocorticoid; the high plasma concentrations in this disorder prevent the signs and symptoms of cortisol deficiency (e.g., hypoglycemia) and moderate the secretion of ACTH.

Affected 46,XX females have normal typical female internal and external genital tracts, but the ovaries cannot secrete estrogens at puberty, resulting in absent breast development and hypogonadism with elevated plasma FSH and LH levels. The lack of adrenal and ovarian androgens can result in little or no growth of pubic and axillary hair. In affected 46,XX individuals, the ovaries have a high proportion of atretic follicles, and some ovaries contain enlarged follicular cysts.

Affected 46,XY individuals with complete combined 17α-hydroxylase/17,20-lyase deficiency who are diagnosed in adolescence usually have female external genitalia and a blind vaginal pouch (see Table 24.11). Testes may be intraabdominal, in the inguinal canal, or in the labioscrotal folds. Inguinal hernias are common, müllerian structures are absent, and wolffian derivatives are hypoplastic. Bone age is frequently delayed because of decreased sex steroid production, and prolonged linear growth can lead to tall stature. Pubic and axillary hair is absent or sparse, and hypergonadotropic hypogonadism results in a failure to develop secondary sex characteristics at puberty. Excessive secretion of DOC and corticosterone usually leads to low-renin hypertension and hypokalemic alkalosis, as in 46,XX girls with this condition.

Complete 17α-hydroxylase/17,20-lyase deficiency is associated with a variety of pathogenic variants in the *CYP17* gene that cause complete loss of function in assays of enzyme activity. These changes include a range of missense, frameshift, and nonsense mutations (Fig. 24.27). A common mutation is the 4-bp

TABLE 24.11 Clinical Features of Combined CYP17 Deficiency in 46,XY Individuals

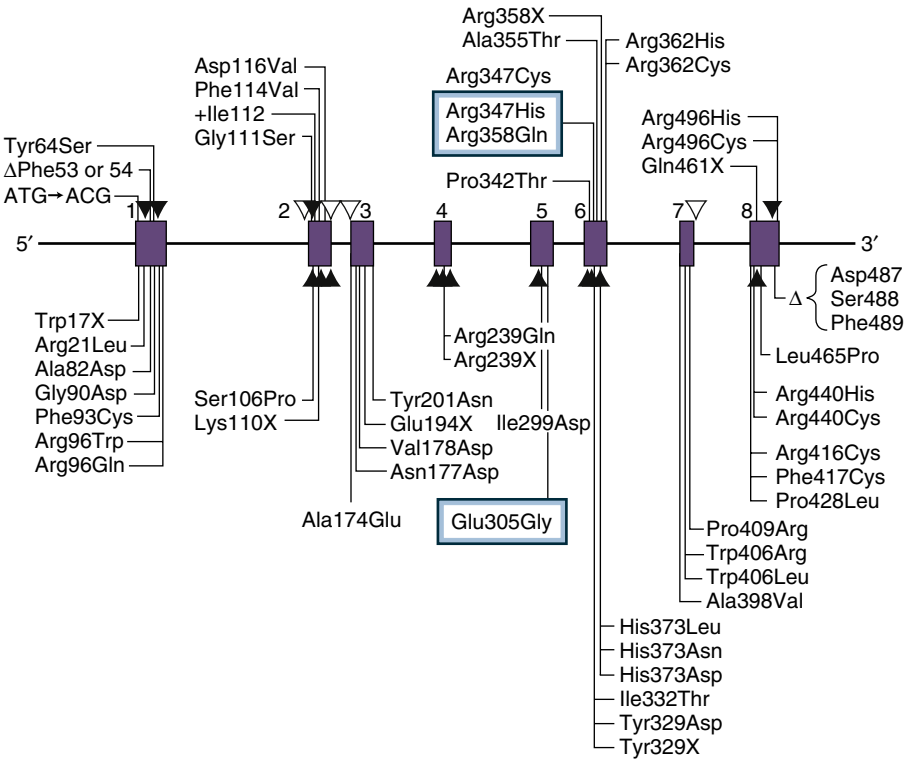
Karyotype	46,XY
Inheritance	Autosomal recessive; mutations in *CYP17* gene
Genitalia	Female, ambiguous, or hypospadias
Wolffian duct derivatives	Absent or hypoplastic
Müllerian duct derivatives	Absent
Gonads	Testes
Physiologic features	Absent or poor virilization at puberty, gynecomastia, hypertension
Hormone profile	Decreased T; increased LH and FSH; increased plasma deoxycorticosterone, corticosterone, and progesterone; decreased plasma renin activity Low renin hypertension with hypokalemic alkalosis

CYP17, 17α-Hydroxylase/17,20-lyase; *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone; *T,* testosterone.

TABLE 24.12 Clinical Features of Isolated 17,20-Lyase Deficiency in 46,XY Individuals

Karyotype	46,XY
Inheritance	Autosomal recessive; mutations in *CYP17* gene, usually affecting key redox domains
Genitalia	Female, ambiguous, or hypospadias
Wolffian duct derivatives	Absent or hypoplastic
Müllerian duct derivatives	Absent
Gonads	Testes
Physiologic features	Absent or poor virilization at puberty; gynecomastia
Hormone profile	Decreased plasma T, DHEA, androstenedione, and estradiol; abnormal increase in plasma 17-hydroxyprogesterone and 17-hydroxypregnenolone; increased LH and FSH; increased ratio of C21 deoxysteroids to C19 steroids (DHEA, androstenedione) after hCG stimulation

CYP17, 17α-Hydroxylase/17,20-lyase; *DHEA,* dehydroepiandrosterone; *FSH,* follicle-stimulating hormone; *hCG,* human chorionic gonadotropin; *LH,* luteinizing hormone; *T,* testosterone.



• **Fig. 24.27** Diagram of selected mutations in the *CYP17* gene causing 17α-hydroxylase/17,20-lyase deficiency. The numbered *solid boxes* depict the exons. The three-letter abbreviations for amino acids are used to indicate the position of missense mutations; *X* indicates a nonsense (stop) mutation; insertions and deletions resulting in frameshift and splice site mutations are shown by *solid triangles* and *open triangles*, respectively. All of these mutations cause 17α-hydroxylase deficiency. Several missense mutations, such as those at codons 305, 347, and 358 *(boxes)*, have been associated with isolated 17,20-lyase deficiency.

duplication in exon 8, which is shared by Mennonites and individuals in the Friesland region of the Netherlands and is attributed to a founder effect originating in Friesland. Other geographic clusters include an in-frame deletion of residues 487 to 489 in Southeast Asia and the Arg362Cys and Trp406Arg missense mutations found among Brazilians of Portuguese and Spanish ancestry, respectively.[306] However, many different changes occur in other populations and are located throughout the enzyme.[307]

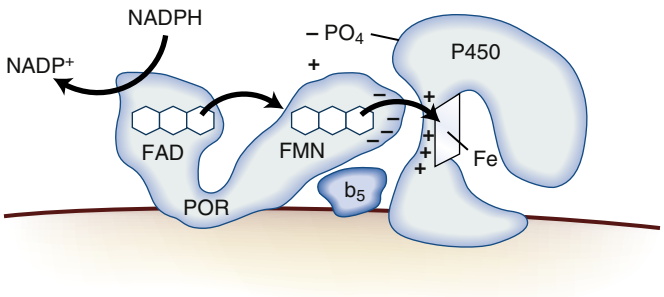
Partial forms of combined 17α-hydroxylase/17,20-lyase deficiency have been described. This condition most frequently manifests in a 46,XY infant with ambiguous genitalia or severe hypospadias for whom the steroid profile is consistent with the diagnosis of P450c17 deficiency. Hypertension may or may not be present in partial forms of combined 17α-hydroxylase/17,20-lyase deficiency, and aldosterone secretion may be normal or even elevated. Corticosterone levels, which are usually 50-fold to 100-fold higher than normal, provide adequate glucocorticoid effects and prevent symptoms of cortisol deficiency. The development of male secondary sex characteristics at puberty may be incomplete, and gynecomastia is often seen. This rare condition has been associated with a phenylalanine deletion at codon 53 or 54 and several missense changes.[305,308]

Isolated 17,20-lyase deficiency has been reported in a small number of patients.[305,309,310] These 46,XY individuals usually have genital ambiguity, normal secretion of glucocorticoids and mineralocorticoids, and marked reduction in sex steroid synthesis (see Table 24.12). The first two patients shown to have a molecular defect in P450c17 harbored homozygous point mutations (Arg347His, Arg358Gln) that specifically interfered with 17,20-lyase activity by changing the distribution of surface charges in the redox-partner binding site.[311] Other patients have been reported with similar mutations or with a point mutation (Glu305Gly) that alters the conformation of the substrate-binding site.[312]

The diagnosis of 17α-hydroxylase deficiency is strongly supported by the discovery of low-renin hypertension and hypokalemic alkalosis and by a lack of secondary sex characteristics at puberty. Plasma concentrations of corticotropin, DOC, corticosterone, and progesterone are high, and those of 17α-OHP, cortisol, and gonadal steroids are low. Replacement therapy with physiologic doses of glucocorticoids suppresses DOC and corticosterone secretion and normalizes serum potassium levels, blood pressure, and plasma renin and aldosterone levels. The hypokalemia can be associated with life-threatening cardiac arrhythmia, so careful monitoring and treatment in the acute phase are needed. Gonadectomy is usually performed in 46,XY patients who have a female gender identity and can consent. A gonadoblastoma and an invasive mixed germ cell tumor have been reported in the gonads of two 46,XX females with this condition.[306,313] Appropriate gonadal steroid replacement therapy is indicated at puberty.

Cytochrome b_5 Deficiency

A splice site mutation in the 17,20-lyase redox partner cytochrome b_5 was first reported in a 46,XY child with ambiguous genitalia and methemoglobinemia, although extensive hormone data were not reported.[314] Homozygous nonsense and missense mutations in this gene *(CYB5A)* have now been described in 46,XY children with severe hypospadias and a biochemical profile consistent



• **Fig. 24.28** Role of P450 oxidoreductase (POR) in electron transfer to microsomal (type II) P450 enzymes. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) interacts with POR, which is bound to the endoplasmic reticulum, and transfers a pair of electrons to the FAD moiety. This change in charge results in altered conformation, which allows the electrons to pass from the FAD to the FMN moiety. After further realignment, the FMN domain can interact with the redox partner binding site of the P450 enzyme (e.g., P450c17, P450c21, P450c19), permitting electron transfer to the active heme group of the enzyme, which results in substrate catalysis. The interaction of POR and the P450 enzymes is coordinated by negatively charged acidic residues on the surface of the FMN domain of POR and positively charged basic residues in the redox partner binding site of the P450 enzyme. In the case of human P450c17, this interaction is facilitated by the allosteric action of cytochrome b_5 and by serine phosphorylation of P450c17. *FAD,* flavin adenine dinucleotide; *FMN,* flavin mononucleotide. (From Miller WL. Minireview: regulation of steroidogenesis by electron transfer. *Endocrinology*. 2005;146:2544–2550, used with permission of The Endocrine Society, Copyright 2005.)

TABLE 24.13 Clinical Features of P450 Oxidoreductase Deficiency in 46,XY Individuals

Karyotype	46,XY
Inheritance	Autosomal recessive; mutations in *POR* gene
Genitalia	Ambiguous; hypospadias or male
Wolffian duct derivatives	Absent or hypoplastic
Müllerian duct derivatives	Absent
Gonads	Testes
Biochemical and physiologic features	Variable androgenization at birth; variable virilization at puberty, especially in girls; glucocorticoid deficiency; no severe mineralocorticoid deficiency; features of Antley-Bixler syndrome (craniosynostosis, skeletal dysplasia) in some cases
Hormone profile	Evidence of combined CYP17 and CYP21 insufficiency; normal or low cortisol with poor response to ACTH stimulation; elevated 17-OHP; T low

ACTH, Adrenocorticotropic hormone; *CYP,* cytochrome P450 enzyme; *17-OHP,* 17-hydroxyprogesterone; *POR,* P450 oxidoreductase; *T,* testosterone.

with isolated 17,20-lyase deficiency and in a family with multiple affected members and a range of genital phenotypes.[315,316] Methemoglobin was elevated above the normal range in all these cases, but it did not result in clinical signs.

P450 Oxidoreductase Deficiency

POR is a membrane-bound flavoprotein that plays a central role in electron transfer from NADPH to P450 enzymes (Fig. 24.28).[317] POR is crucial in the 17,20-lyase reaction of P450c17, and it interacts with all 57 microsomal P450 enzymes, including P450c21 (21-hydroxylase) and P450c19 (aromatase), and with many others involved in hepatic drug metabolism.

A potential role for POR in human steroidogenesis emerged after the description of several patients with apparent combined deficiencies of CYP17 and CYP21,[318] who presented not only with ambiguous genitalia and unusual patterns of combined steroidogenic defects but also with Antley-Bixler syndrome, a form of skeletal dysplasia that is characterized by craniosynostosis, brachycephaly, midface hypoplasia, proptosis, choanal stenosis, radioulnar or radiohumeral synostosis, femoral bowing, and arachnodactyly.

The first recessively inherited human mutations in *POR* were described in 2004, and a wide range of phenotypes has been described in patients (Table 24.13).[133,319,320] At the most severe end of the spectrum is ambiguous genitalia due to apparent combined CYP17 and CYP21 deficiency, with or without Antley-Bixler syndrome. Milder defects in POR have also been seen in women with a phenotype resembling polycystic ovary syndrome and in men with mild gonadal insufficiency, sometimes with subtle skeletal features. Two common mutations are emerging: Arg287Pro is the most prevalent mutation in patients of European ancestry, whereas the Arg457His mutation is common in Japan.[320,321] Activating mutations in fibroblast growth factor receptor 2 (FGFR2) have also been reported in association with Antley-Bixler syndrome; these patients do not have ambiguous genitalia or steroidogenic defects.

Most patients with POR deficiency have normal electrolytes and mineralocorticoid function (see Table 24.13). Cortisol insufficiency may be present, or if basal levels are adequate, the response to ACTH stimulation is reduced.[321] The serum 17-OHP concentration is usually elevated, with a variable response to ACTH stimulation, and levels of sex steroids tend to be low. POR deficiency can be associated with ambiguous genitalia in both 46,XY and 46,XX individuals. Underandrogenization of 46,XY males can occur due to disturbed 17,20-lyase activity during fetal Leydig cell steroidogenesis. The partial androgenization of 46,XX infants is more prevalent and may be the result of a disturbance in aromatase activity, because POR is an electron donor for this enzyme and aromatase deficiency causes prenatal androgenization of the developing 46,XX fetus (see "Aromatase Deficiency"). Alternatively or additionally, a "backdoor" pathway of androgen biosynthesis has been described in certain species, such as the tammar wallaby.[133,134] In this model system, 17-OHP can be converted to DHT without the use of androstenedione or testosterone as an intermediate. Emerging data indicate that this pathway may also be functional during human development. Puberty can be variable in children with POR deficiency, with delayed or disordered puberty that is especially common in girls. This diagnosis should be considered in any child with skeletal features and hypospadias (46,XY) or clitoromegaly (46,XX) or who does not progress through puberty.[322]

3α-Reductase Type 3 and 3α-Reductase Type 1: AKR1C2 and AKR1C4

The "backdoor" pathway from 17-OHP to DHT involves the activity of AKR1C2 (aldo-keto reductase family 1 member C2) (see Fig. 24.12). Variants in *AKR1C2*, sometimes in combination

with changes in *AKR1C4*, have been described in several members of a family with various degrees of 46,XY underandrogenization who were originally thought to have 17,20-lyase deficiency.[323] Compound heterozygous variants or rearrangements in *AKR1C2* were found in another unrelated child with 46,XY DSD. These variants were inherited in a sex-limited recessive manner.[134,323] These findings were taken as evidence that the "backdoor" pathway plays a role in fetal androgen synthesis in humans as well as in wallabies. These studies also highlight that biochemical 17,20-lyase deficiency can have several molecular causes: CYP17A1, CYB5A, POR, and possibly AKR1C2/4.[310]

17β-Hydroxysteroid Dehydrogenase Type 3 Deficiency

The 17β-hydroxysteroid dehydrogenase reaction (also called the 17-ketosteroid reductase reaction) is mediated by isozymes that catalyze the reduction of androstenedione, DHEA, and estrone to testosterone, Δ^5 androstenediol, and estradiol, respectively, as well as the reverse reaction (see Fig. 24.11). The family of 17βHSD comprises at least 14 isoenzymes that have physiologic relevance in a range of human tissues and disorders such as breast cancer, prostate cancer, and endometriosis. The *HSD17B3* gene on chromosome 9q22 contains 11 exons and encodes the type 3 isoenzyme, which is expressed primarily in the testes, where it favors the conversion of the weak androgen androstenedione to the more biologically active testosterone (see Fig. 24.11). 17βHSD isoenzymes other than 17βHSD3 play a role in estrogen synthesis in the ovary.

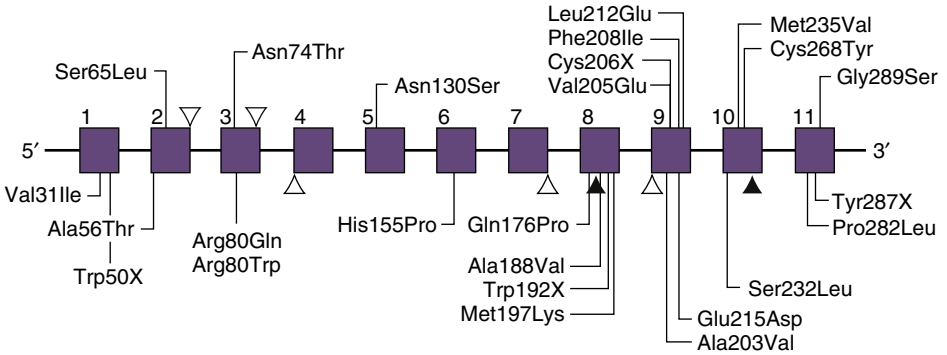
Deficiency of 17βHSD3, which was originally called 17-ketosteroid reductase, was first reported as an autosomal recessive cause of 46,XY DSD by Saez and colleagues (Fig. 24.29).[324] Many cases have been reported, and the phenotype is well characterized (Table 24.14).[325,326] Most affected 46,XY children have female-typical external genitalia at birth, although a few infants present with clitoromegaly or ambiguous genitalia. The testes are usually located in the inguinal canal. Müllerian structures are absent, and there is a blind vaginal pouch. The wolffian ducts are often stabilized to form epididymides, vasa deferentia, seminal vesicles, and ejaculatory ducts, likely due to paracrine androgenic effects of high concentrations of androstenedione.[327,328] Affected infants are usually designated female and may mistakenly be assumed to have CAIS. The phenotype of 17βHSD3 deficiency is limited to 46,XY individuals; 46,XX individuals with biallelic *HSD17B3* mutations are asymptomatic and fertile. The chances of having an affected child are twice as high if the mother has biallelic changes, which is why affected 46,XY siblings with 17βHSD3 deficiency are more common than might be expected.

Significant virilization usually occurs at puberty in 46,XY 17βHSD3 deficiency in the form of clitoral enlargement, hirsutism, deepening of the voice, and muscle development. These symptoms and signs may be the presenting features of this condition if it was not diagnosed earlier in life.[329] These changes are largely due to a pubertal increase in androstenedione production by the gonad and subsequent extraglandular conversion of androstenedione to testosterone. It has been speculated that this is mediated by genetic or environmental induction of enzyme activities of other 17βHSD isoenzymes, such as the type 5 isoenzyme (also known as 17βHSD5 and AKR1C3).[330] The testes may also have partial 17βHSD3 activity in some cases. In a large cohort of patients from a consanguineous population in the Gaza

TABLE 24.14 Clinical Features of HSD17B3 Deficiency in 46,XY Individuals

Karyotype	46,XY
Inheritance	Autosomal recessive; mutations in *HSD17B3* gene
Genitalia	Female → ambiguous; blind vaginal pouch
Wolffian duct derivatives	Present
Müllerian duct derivatives	Absent
Gonads	Testes (usually undescended)
Physiologic features	Virilization at puberty (phallus enlargement, deepening of voice, and development of facial and body hair); gynecomastia variable
Hormone profile	Increased plasma estrone and androstenedione; decreased ratio of plasma testosterone/androstenedione after hCG stimulation test; increased plasma FSH and LH levels

FSH, Follicle-stimulating hormone; *hCG,* human chorionic gonadotropin; *HSD17B3,* 17β-hydroxysteroid dehydrogenase 3; *LH,* luteinizing hormone.



• **Fig. 24.29** Diagram of the 17β-hydroxysteroid dehydrogenase type 3 gene *(HSD17B3)* shows the mutations that result in deficiency of the enzyme. The numbered *solid boxes* depict the exons. The three-letter abbreviations for amino acids are used to indicate the position of missense mutations; *X* indicates a nonsense (stop) mutation; insertions and deletions resulting in frameshift mutations *(solid triangles)* and splice site mutations *(open triangles)* are shown.

Strip, the phallic lengths were described as reaching 4 to 8 cm.[331] The 17βHSD3 mutation reported in this population (Arg80Gln) is associated with 15% to 20% retention of normal 17βHSD3 activity (see Fig. 24.29). Gynecomastia occurs at puberty because of estrogens derived from the conversion of androstenedione to estrone by aromatase in extraglandular tissue and the subsequent conversion of estrone to estradiol by the action of the 17βHSD1 or 17βHSD2 isoenzymes.

The typical biochemical profile in 17βHSD3 deficiency is an elevated androstenedione level relative to testosterone (see Table 24.14). Expressed as a ratio of testosterone to androstenedione (after an hCG stimulation test before puberty), values less than 0.8 are typically found in these patients; however, this is not a universal finding presumably because of the extratesticular action of other 17βHSD isoenzymes, and diagnosis based on hormone measurements alone can be challenging.[326,332,333] Testicular vein sampling at the time of gonadectomy shows a markedly increased androstenedione gradient relative to testosterone, though this test is not routinely performed. Early genetic analysis can be useful and can avoid the need for hCG stimulation in some children.[334,335]

The range of mutations reported in patients with 17βHSD3 deficiency is shown in Fig. 24.29. Most are missense mutations. Expression studies of the mutant enzymes in heterologous cells usually show complete absence of activity in the conversion of androstenedione to testosterone compared with the normal enzyme. One recent study from a single center specializing in adult DSD found that approximately one-fourth of a cohort of women with partially virilized 46,XY DSD had mutations in *HSD17B3*.[336] The degree of androgenization was less than that typically seen in 5α-reductase deficiency, and many had been labeled as having PAIS. The diagnosis could only be made by genetic analysis.

Sex designation can be particularly difficult in children with 17βHSD3 deficiency because the external genital appearance correlates even less well with future gender identity than in other DSDs. Some babies have had early gonadectomy and been brought up as girls. In other settings (e.g., in Gaza), where affected patients typically adopted a male identity at puberty, some patients were designated male at diagnosis and were treated early with testosterone.[337] An alternative approach would be to raise the child as a girl initially and delay any interventions until late childhood or early adolescence so that the family and child have more time to consider the options and the child's gender is clearer. Careful monitoring is needed for the onset of puberty and androgen production if the child identifies as female, and gonadectomy may be considered to prevent undesired androgenization.

Those children who first present with virilization at puberty may declare a male gender identity in some cases. Gender changes at puberty have been reported in 39% to 64% of patients reared as girls in one study, but in our experience the rates are lower.[151,152,336] Other young people may identify as having a nonbinary gender. If androgenization is undesired, gonadectomy offers a definitive treatment; another potential option is short-term, reversible blockade of androgenization with an antiandrogen or puberty suppression with a GnRH agonist to provide time for appropriate counseling and to ensure the young person is involved in the decision making. Careful assessment and support from a multidisciplinary team and experienced psychologist are important, and long-term follow-up is important for hormone replacement and support.

Steroid 5α-Reductase Type 2 Deficiency

Steroid 5α-reductase type 2 deficiency in 46,XY individuals is also characterized by normally differentiated testes and male internal ducts, but external genitalia may be more ambiguous at birth than in 17βHSD deficiency. Classic features of this enzyme deficiency are summarized in Table 24.15.

The description of a genetic variant in *SRD5A2,* the gene that encodes steroid 5α-reductase type 2, in the Dominican Republic and Mexico and analysis of the biochemical and molecular features underline the importance of DHT in the development of the male phenotype.[338–340] At birth, there is typically a bifid scrotum, a urogenital sinus, a blind vaginal pouch, and a clitoris-like hypospadiac phallus. Testes differentiate normally and are located in the inguinal canal or in the labioscrotal folds. No müllerian structures are present. The wolffian ducts are stabilized so that the epididymides, vasa deferentia, and seminal vesicles are well differentiated; the ejaculatory ducts usually terminate in the blind vaginal pouch. The prostate is hypoplastic. Up to a third of cases may present with isolated hypospadias.[341]

At puberty, there is a striking degree of virilization: The voice deepens, muscle mass increases, the phallus lengthens to 4 to 8 cm, the bifid scrotum becomes rugated and pigmented, and the testes enlarge and descend into the labioscrotal folds. Postpubertal affected individuals do not have acne, temporal hair recession, or enlargement of the prostate. They rarely develop gynecomastia, in contrast to individuals with 17βHSD3 deficiency or AIS. There is normal libido with penile erections. Histologic examination of the testes shows Leydig cell hyperplasia and decreased spermatogenesis. Infertility is caused by a composite of failure to transform spermatogonia into spermatocytes, the adverse effect of a cryptorchid testis, and the specific role of DHT in regulating semen volume and viscosity.[342] Nevertheless, some individuals with 5α-reductase deficiency have a normal sperm count. One man in the Dominican cohort fathered a child after intrauterine insemination, and two affected brothers in a Swedish family were spontaneously fertile after hypospadias repair performed in childhood.[343,344] Gender changes occur frequently in 5α-reductase deficiency, particularly in individuals who do not undergo gonadectomy. Rates of social gender change in three cohorts ranged from 12% to 50% and seemed related to the age at diagnosis, with social gender

TABLE 24.15 Clinical Features of 5α-Reductase Type 2 Deficiency in 46,XY Individuals

Karyotype	46,XY
Inheritance	Autosomal recessive; mutations in *SRD5A2* gene
Genitalia	Usually ambiguous with small, hypospadiac phallus; blind vaginal pouch
Wolffian duct derivatives	Normal
Müllerian duct derivatives	Absent
Gonads	Normal testes
Physiologic features	Decreased facial and body hair, no temporal hair recession, prostate not palpable
Hormone profile	Decreased ratio of 5α/5β C21 and C19 steroids in urine; increased T/DHT ratio before and after hCG stimulation; modest increase in plasma LH; decreased conversion of T to DHT in vitro

DHT, Dihydrotestosterone; *hCG,* human chorionic gonadotropin; *LH,* luteinizing hormone; *SRD5A2,* steroid-5α-reductase type 2; *T,* testosterone.

change being less common in the cohort where individuals were diagnosed at a younger (prepubertal) age.[345] Male sex designation is now increasingly chosen when the diagnosis is made in infancy.[345,346] An alternative approach similar to that sometimes used for 17β-hydroxysteroid dehydrogenase deficiency would be to rear the child as female with the gonads kept intact pending further informed discussions before puberty. Females (46,XX) homozygous for 5α-reductase deficiency undergo normal puberty and have normal fertility.[347]

The biochemical profile in 5α-reductase deficiency typically shows an elevated testosterone-to-DHT ratio basally or after hCG stimulation (see Table 24.15). Various ratios have been suggested as diagnostic, but none is unequivocal. A ratio exceeding 10:1 (when testosterone and DHT are expressed in the same units) had a sensitivity and specificity of 78% and 72%, respectively, in a cohort of 90 patients investigated for this enzyme deficiency.[348] Serum LH and FSH levels may be normal or elevated after puberty. The most reliable biochemical test (undertaken after 3 months of age) is analysis of a urinary steroid profile by gas chromatography and mass spectrometry to demonstrate a diminished ratio of urinary 5α/5β-reduced C19 and C21 steroids.[349,350] The diagnosis can still be confirmed biochemically after gonadectomy because of persistent effects on 5α/5β-reduced C21 steroids.[351] Early genetic testing is also useful to make the diagnosis or to confirm it when suspected from the biochemical profile.

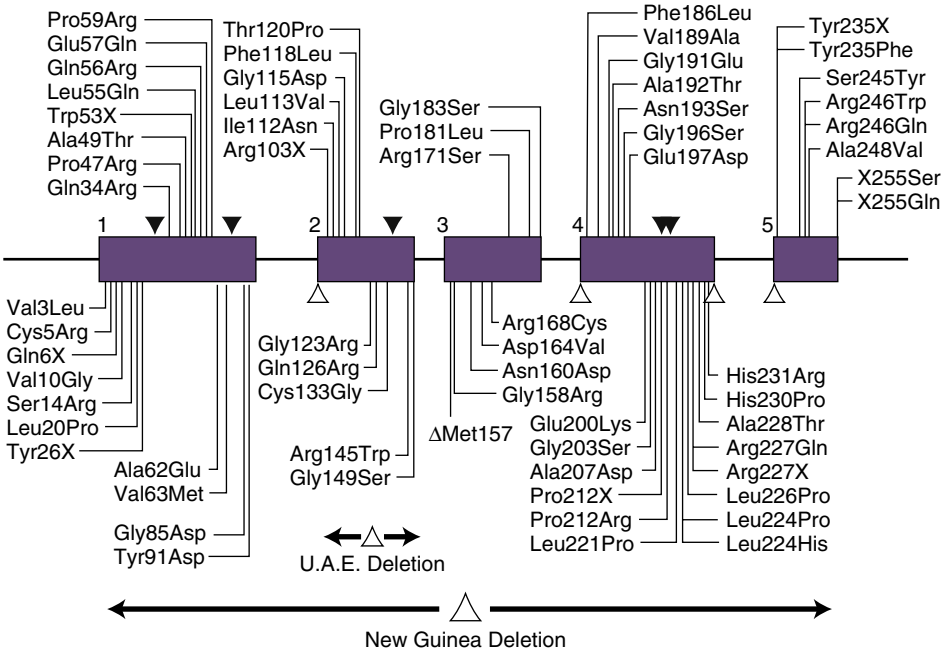
Early diagnosis of 5α-reductase type 2 deficiency is important because of its bearing on sex designation. The natural history of this condition, with a tendency for change to a male gender role with virilization at puberty, indicates that male sex designation is now a frequent option even when the external genitalia are relatively severely underandrogenized at birth.[352] The enzyme deficiency can masquerade as PAIS in newborns.[353] DHT, which may be applied topically increases penile length and facilitates repair of the hypospadias, but commercial availability may be limited.[354]

Two microsomal 5α-reductase enzymes catalyze the NADPH-dependent conversion of testosterone to DHT. 5α-reductase type 2 is a 254–amino acid protein encoded by the *SRD5A2* gene on chromosome 2p23. The type 2 isozyme is expressed predominantly in the primordia of the prostate and external genitalia but not in the wolffian ducts until after their differentiation into the male internal genital ducts.[355] The type 1 isoenzyme is expressed in skin, including human genital skin fibroblasts. The action of this isoenzyme may contribute to the virilization that occurs in 5α-reductase–deficient patients at puberty.[356]

5α-reductase type 2 deficiency is transmitted as an autosomal recessive trait. 5α-reductase type 2 deficiency is genetically heterogeneous, and the more than 60 mutations detected in the *SRD5A2* gene are distributed among all five exons (Fig. 24.30). Most are missense mutations; a complete gene deletion is found in the New Guinea population. There is a predominance of mutations in exon 4, mostly localized between codons 197 and 230, where the effect is complete inactivity of the mutant enzyme. Consanguinity is common, but a significant number of cases are compound heterozygotes. Male heterozygotes are not affected.

Disorders of Androgen Action

Androgen insensitivity syndrome (AIS) is the paradigm of a clinical disorder resulting from hormone resistance.[136] A detailed description



• **Fig. 24.30** Diagram of the 5α-reductase type 2 gene *(SRD5A2)* shows the mutations that result in 5α-reductase deficiency. The numbered *solid boxes* depict the exons. The three-letter abbreviations for amino acids are used to indicate the position of missense mutations; X indicates a nonsense (stop) mutation; insertions and deletions resulting in frameshift and splice site mutations are shown by *solid triangles* and *open triangles*, respectively. Large deletions found in affected United Arab Emirates (U.A.E.) and New Guinea populations are shown. (Adapted from Grumbach MM, Hughes IA, Conte FA. Disorders of sex differentiation. In: Larsen PR, Kronenberg HM, Melmed S, et al, eds. *Williams Textbook of Endocrinology,* 10th ed. Philadelphia, PA: Saunders; 2003, with additional data provided courtesy of Dr. Julianne Imperato-McGinley, Department of Medicine, Weill Medical College of Cornell University, Ithaca, NY.)

of the role of androgens in sex development and the molecular actions of the androgen receptor are provided earlier in this chapter.

Complete Androgen Insensitivity Syndrome

CAIS is an X-linked condition that typically presents in an adolescent female who has breast development and a pubertal growth spurt but who has not had menarche (Table 24.16). Pubic and axillary hair is absent or scant, and the uterus is absent as a result of normal AMH action, although there may be müllerian remnants. The wolffian ducts are stabilized in many patients, with a well-developed vas deferens and epididymis observed when gonadectomy is performed.[328] Estimates of the prevalence of CAIS range from 1 case in 20,400 to 1 in 99,000 individuals with a 46,XY karyotype.[357] The main differential diagnosis in an adolescent with a 46,XY karyotype is complete gonadal dysgenesis (Swyer syndrome), which is distinguished from CAIS by (1) absence of gonadal sex steroid production, resulting in poor breast development, and (2) absence of AMH production, resulting in retention of müllerian structures in Swyer syndrome.[358] CAIS may present in early infancy with bilateral inguinal or labial swellings that on evaluation are found to be partially descended testes. 17βHSD3 deficiency (or rarely 5α-reductase deficiency or SF1 deficiency) may also present this way. Bilateral inguinal hernias are rare in girls, of whom 1% to 2% are estimated to have CAIS.[359] Girls with this type of hernia should undergo evaluation for the presence of a Y chromosome by fluorescence in situ hybridization (FISH) or a full karyotype. If the hernial sac contains gonads, a biopsy can be performed together with cytogenetic studies.[360] Presently, with fetal chromosomal analysis being commonplace, a mismatch between chromosomal sex and the external genital phenotype at birth can be the presenting feature of CAIS.

Partial Androgen Insensitivity Syndrome

PAIS is characterized by incomplete androgenization resulting from a partial biologic response to androgens (Table 24.17). The phenotype for PAIS is highly variable and may include penoscrotal hypospadias, micropenis, and bifid scrotum. The testes may be undescended. The most severe form of PAIS manifests as isolated clitoromegaly. The milder end of the spectrum includes isolated hypospadias; PAIS does not present as isolated micropenis. As with other forms of AIS, müllerian structures are absent. Gynecomastia is common at puberty. The differential diagnosis for a PAIS-like phenotype is lengthy and includes partial gonadal dysgenesis (e.g., due to an *SF1*/*NR5A1* mutation or 45,X/46,XY mosaicism) and partial defects in androgen biosynthesis (e.g., due to LH receptor, 17βHSD, or 5α-reductase deficiencies).

Minimal or Mild Androgen Insensitivity Syndrome

This category of AIS is generally associated with typical male genitalia but can present with gynecomastia at puberty and infertility in adulthood.[361] Cancer of the breast is rare in males, but the risk is increased in MAIS as well as in those with PAIS raised male. In contrast, breast cancer has not been reported in women with CAIS. A mild form of androgen insensitivity is also found in spinal and bulbar muscular atrophy (Kennedy syndrome), a condition caused by hyperexpansion of the N-terminal polyglutamine repeat region of the androgen receptor, which can be associated with gynecomastia and reduced fertility.[362]

Hormone Profiles in Androgen Insensitivity Syndromes

In pubertal and adult individuals with CAIS, serum testosterone is within or above the adult male range with a moderately but inappropriately increased concentration of LH due to loss of negative feedback from androgens (though negative feedback from estrogens remains intact).[363] Serum estradiol is usually within and sometimes above the adult male reference range due to aromatization of androgens.[363] The concentration of sex hormone–binding globulin (SHBG), which is suppressed by

TABLE 24.16 Clinical Features of Complete Androgen Insensitivity Syndrome

Karyotype	46,XY
Inheritance	X-linked recessive; mutations in *AR* gene
Genitalia	Female with blind vaginal pouch
Wolffian duct derivatives	Often present, depending on mutation type
Müllerian duct derivatives	Absent or vestigial
Gonads	Testes
Physiologic findings	Scant or absent pubic and axillary hair; breast development at puberty; primary amenorrhea
Hormone and metabolic profile	Increased LH and testosterone levels; normal or increased estradiol (for male reference range); FSH levels often normal or slightly increased; resistance to androgenic and metabolic effects of testosterone

AR, Androgen receptor; *FSH*, follicle-stimulating hormone; *LH*, luteinizing hormone.

TABLE 24.17 Clinical Features of Partial Androgen Insensitivity Syndrome

Karyotype	46,XY
Inheritance	X-linked recessive; mutations in *AR* gene
External genitalia	Ambiguous with blind vaginal pouch → isolated hypospadias → male with infertility (mild AIS)
Wolffian duct derivatives	Often normal
Müllerian duct derivatives	Absent
Gonads	Testes (usually undescended)
Physiologic features	Decreased to normal axillary and pubic hair, beard growth, and body hair; gynecomastia common at puberty
Hormone and metabolic profile	Increased LH and testosterone concentrations; normal or increased estradiol (for men); FSH levels may be normal or slightly increased Partial resistance to androgenic and metabolic effects of testosterone

AIS, Androgen insensitivity syndrome; *AR*, androgen receptor; *FSH*, follicle-stimulating hormone; *LH*, luteinizing hormone.

androgens and enhanced by estrogens, is similar to that found in 46,XX females. Serum AMH concentration is typically in or above the normal male range in CAIS, a finding that distinguishes CAIS from gonadal dysgenesis, in which AMH levels are low.[364]

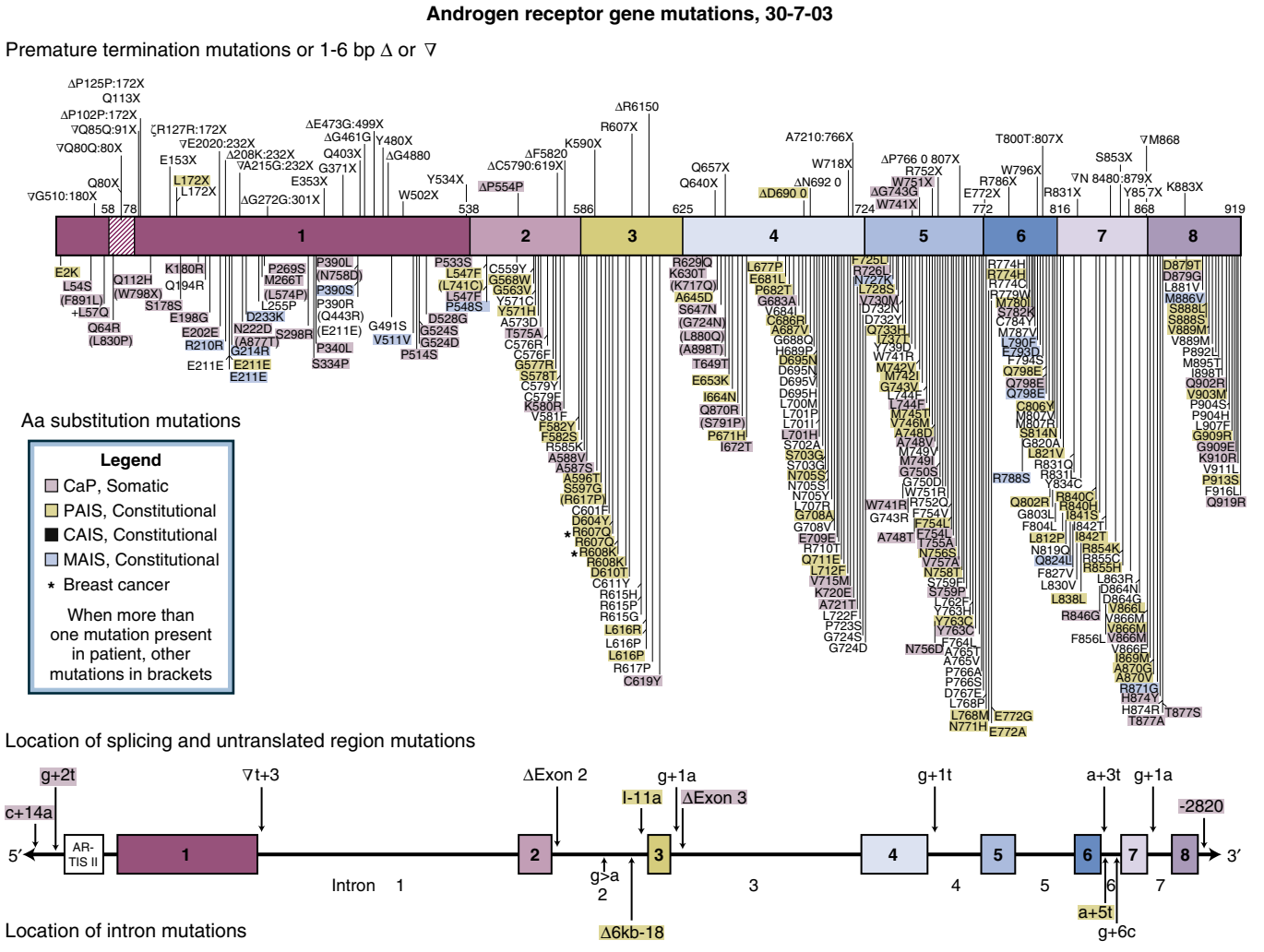
During the first few months of life, male infants have an active reproductive endocrine system with robust production of LH and testosterone; this period is known as *minipuberty*. For reasons that are unclear, this early reproductive endocrine activity is absent in most infants with CAIS (but not in PAIS),[365] and this may be a distinguishing feature of CAIS.

Molecular Pathogenesis of Androgen Insensitivity Syndromes

Information about mutations that affect the AR is recorded on an International Mutation Database at McGill University[366] (Fig. 24.31). More than 400 germline mutations are recorded for AIS; the database also records somatic mutations associated with prostate cancer. There is no specific hot spot for mutations, but certain locations, such as exons 5 and 7 within the LBD, are affected more frequently. Approximately 20% of mutations are located in the DBD. The most common functional AR defect results from disruption of the hydrophobic ligand-binding pocket, which is necessary for repositioning of helix 12 to form the AF2 coregulator interaction surface.

The phenotype of individuals with AIS can vary according to different substitutions at the same codon. For example, phenylalanine at codon 754 in helix 5 has a side chain that points away from the ligand-binding pocket. When substituted for a valine, the mutant AR is transcriptionally inactive and causes CAIS.[367] In contrast, serine and leucine substitutions result in a PAIS phenotype, explained by some residual transcriptional activity as measured in vitro. Linking the DBD to the LBD is a flexible hinge region defined by residues 628 to 669. This region stabilizes receptor interaction with selective androgen response elements and signals nuclear localization. Deletion of the hinge region by site-directed mutagenesis results in enhanced gene transcription, suggesting that it has a role in repression.[368]

There can be considerable heterogeneity in the phenotypic expression of a particular mutation, sometimes even within families. A mutation at codon 703 in exon 4 of the LBD, which changes a serine to a glycine, is reported in four individuals listed in the McGill database. One patient had typical female genitalia consistent with CAIS. The other three all had ambiguous genitalia consistent with PAIS, but the degree of androgenization of



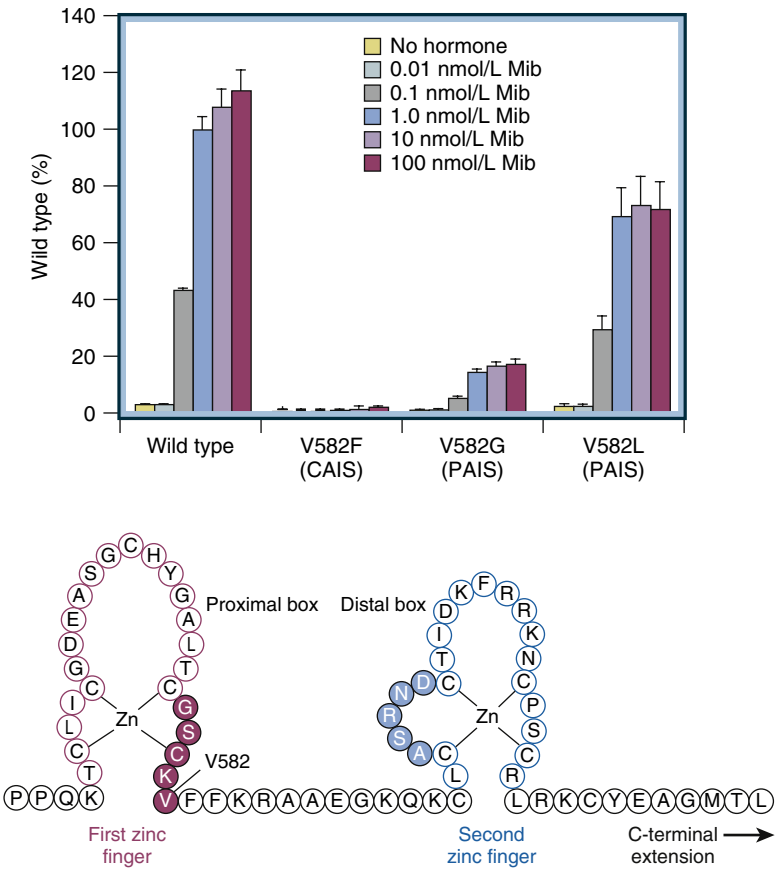
• **Fig. 24.31** Overview of androgen receptor (AR) mutations that cause different forms of androgen insensitivity syndrome (AIS). *CAIS,* complete androgen insensitivity syndrome; *MAIS,* minimal or mild androgen insensitivity syndrome; *PAIS,* partial androgen insensitivity syndrome. (From the McGill Androgen Receptor Gene Mutation Database. Available at http://www.androgendb.mcgill.ca.)

the external genitalia was sufficiently variable that two were raised male and the third was raised female.[369] About 30% of mutations in AIS are de novo. Such mutations arise from a single mutational event in a parental germ cell (the mother in the case of AIS) or as germ cell mosaicism in the maternal gonad. When the mutation arises at the postzygotic stage, the index case is a somatic mosaic. Perhaps one-third of de novo mutations arise at the postzygotic stage, giving rise to expression of mutant and wild-type AR in different target tissues. This may explain some of the phenotypic variability in PAIS.[370]

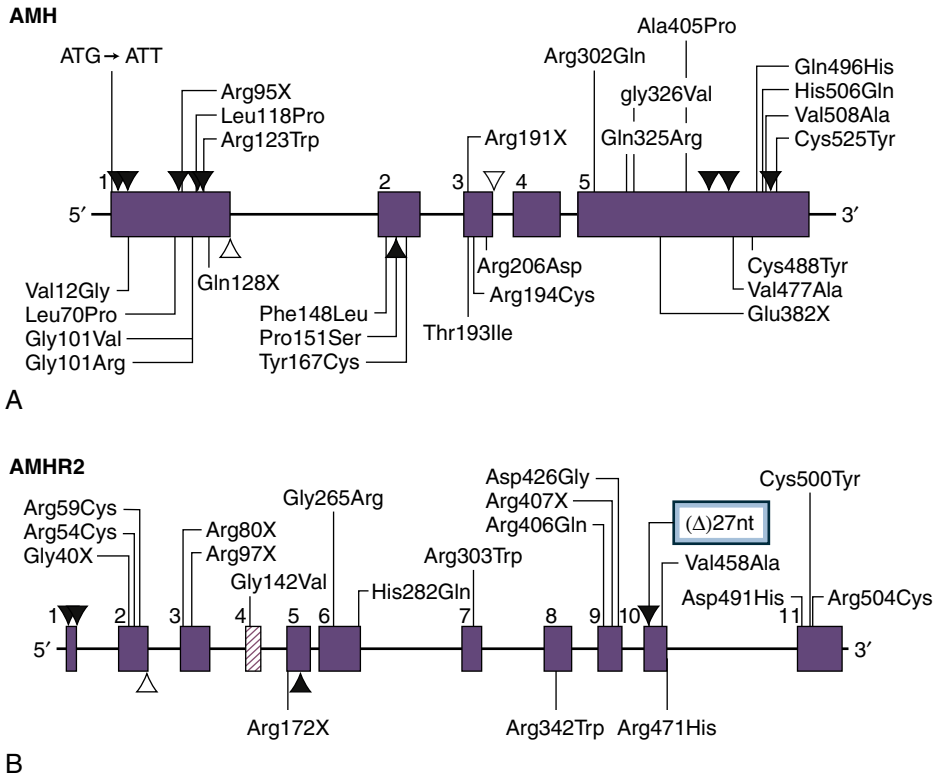
Implicating a variant in *AR* as the cause of AIS generally requires proof of pathogenicity when it is novel and particularly with a PAIS phenotype. A commonly used assay assesses transcriptional activity using a promoter-luciferase system after transient transfection of the variant *AR* in an androgen receptor–negative cell line (such as Cos-1, Hela, CV1, or the PC3 prostate cancer cell line). Fig. 24.32 illustrates the application of such an assay for analysis of three different amino acid substitutions at codon 582 in the DBD.[136] A range of phenotypes from CAIS to severe and mild PAIS is found, depending on the nature of the substitution. Furthermore, the data demonstrate that high doses of androgens can overcome the resistance of the mutant AR. This has particular relevance for patients with PAIS who are raised male and may require large doses of androgens to induce puberty or to stimulate spermatogenesis. In addition to functional studies in vitro, structure-guided modeling of the androgen receptor can be used to predict the likely consequences of an *AR* mutation (see Fig. 24.16C). However, the N-terminal domain has a disordered flexible structure that has yet to be analyzed by crystallography. Nonsense mutations have long been identified in the NTD, but improved sequencing techniques have now identified a number of missense mutations generally associated with milder forms of AIS. It is possible that the structural flexibility of the NTD enables the AR to tolerate amino acid substitutions with little functional consequence. Analysis of such mutations provides a means to better define the structure-function relationship of the N-terminal domain.[371] The discovery of novel mutations is more common now that full sequencing of the *AR* gene is more straightforward.[372]

Androgen Insensitivity Syndromes Without an Androgen Receptor Mutation

An *AR* gene mutation is identified in about 95% of individuals when the clinical and biochemical phenotypes are consistent with CAIS.[373] There may also be mutations outside the *AR* coding region, such as the mutation in the 5′ untranslated region that was recently reported in two unrelated individuals with CAIS, which generates an upstream open reading frame and results in reduced AR protein levels.[374] In addition, in some individuals with a phenotype consistent with AIS, no *AR* mutation is identified, but assessment of DHT-dependent transcriptional induction of an



• **Fig. 24.32** Phenotypes differing according to mutation at codon 582 in first zinc finger of the DNA-binding domain (DBD). The effects of substitution of a valine (V) with phenylalanine (F), glycine (G), and leucine (L) are shown in a transactivation assay *(upper panel)* with the synthetic androgen, mibolerone. The linear stretch of amino acids composing the DBD is *shown in the lower panel*. *CAIS,* complete androgen insensitivity syndrome; *PAIS,* partial androgen insensitivity syndrome. (From Hughes IA, Davies JD, Bunch TI, et al. Androgen insensitivity syndrome. *Lancet*. 2012;380:1419–1428.)



• **Fig. 24.33** (A) Diagram of selected mutations in the antimüllerian hormone (AMH) gene that cause persistent müllerian duct syndrome. The *numbered solid boxes* depict the exons. The three-letter abbreviations for amino acids are used to indicate the positions of missense mutations; X indicates a nonsense (stop) mutation; insertions and deletions resulting in frameshift and splice site mutations are shown by *solid triangles* and *open triangles*, respectively. (B) Diagram of selected mutations in the gene for the AMH receptor type 2 *(AMHR2)*. The *numbered solid boxes* depict the exons. Exons 1 to 3 encode the extracellular domain of the receptor. Exon 4 *(diagonal lines)* encodes the transmembrane domain, and exons 5 through 11 encode the intracytoplasmic domain. Different forms of mutation are depicted, as in (A). The Δ27nt *(open box)* is a 27-nucleotide deletion, the most common *AMHR2* mutation causing the persistent müllerian duct syndrome. (A, redrawn from Imbeaud S, Carré Eusebe D, Rey R, et al. Molecular genetics of the persistent müllerian duct syndrome: a study of 19 families. *Hum Mol Genet.* 1994;3:125–131, used with permission of Oxford University Press; B, redrawn from Imbeaud S, Belville C, Messike-Zeitoun L, et al. A 27 base-pair deletion of the antimüllerian type II receptor gene is the most common cause of the persistent müllerian duct syndrome. *Hum Mol Genet.* 1996;5:1269–1277, used with permission of Oxford University Press.)

AR target gene (i.e., apolipoprotein D) in cultured genital skin fibroblasts shows a pattern consistent with functional androgen insensitivity.[375]

In contrast, the likelihood of finding a pathogenic *AR* variant in those with a PAIS phenotype is much lower, even when other causes of a similar phenotype have been excluded.[369] The genital phenotype in patients without an AR mutation, as based on a validated external masculinization score (EMS), is no different than that in infants with PAIS with a proven AR mutation, but gynecomastia is much less common.[376–378] Boys with an AR mutation are more likely to have poorer outcome than those without a mutation.[378] Birth weight for gestational age is significantly lower in mutation-negative patients compared with infants with PAIS who have an *AR* gene mutation.[270,379] It has been suggested that this form of 46,XY DSD with fetal growth restriction is a discrete category that as yet remains unexplained. The term PAIS is best reserved for individuals with *AR* mutations. Individuals with 17βHSD3, 5α-reductase deficiency, and especially SF1 mutations might also have been mislabeled as PAIS in the past. Although there is evidence that the AR can interact with more than 200 unique coregulator proteins, no mutations have been identified when screening these proteins in AIS, in contrast to findings in prostate cancer.[380]

Management of Androgen Insensitivity Syndromes

Sex designation and gender of rearing are uniformly female in CAIS. Inguinal hernias need repair when presenting in infancy. There is an option then to perform gonadectomy at this early stage, but most patients, families, and providers now favor late gonadectomy, as this approach enables spontaneous puberty to occur, allows the patient to be involved in the decision-making process, and does not substantially increase the risk for malignancy (discussed later in chapter). If gonadectomy is performed before puberty, hormone induction of puberty is similar to that used for girls with other causes of hypogonadism, typically starting around 10 to 11 years of age; the precise timing should consider the patient's wishes, family history of pubertal timing, growth and the degree of skeletal maturation as assessed by bone age X-ray, and whether gonadotropins are starting to rise. In the

absence of a uterus, hormone replacement can be continuous, unopposed estrogen, with no need for a progestin. Some adults with CAIS report improved well-being when androgen replacement is also given; the mechanism of this androgenic effect is not understood. One double-blind crossover trial found increased sexual desire with testosterone compared with estradiol treatment but otherwise found no difference in mental-health outcomes.[381] Bone mineral density of the lumbar spine in women with CAIS with intact gonads is lower than in typical 46,XX women, indicative of the direct effect of androgens on bone mineralization,[382] and women who have undergone gonadectomy have lower bone mineral density than those with intact gonads. Optimal estrogen therapy is essential as well as adequate calcium, vitamin D, and physical exercise. A small increase in fractures has been reported in AIS, although this study did not show any difference in underlying osteoporosis.[383]

There is an increased risk of gonadal germ cell tumors in 46,XY DSD, including AIS. Germ cell maturation is delayed in the abnormal microenvironment. Some of the immature germ cells may subsequently mature, and while many may undergo apoptosis, some may retain fetal characteristics and become premalignant/malignant. Within the testicular environment, the premalignant lesion is termed *germ-cell neoplasia in situ* (GCNIS) (previously called *carcinoma in situ* [CIS] or *intratubular germ cell neoplasia unclassified* [ITGNU]). GCNIS may progress to invasive germ cell tumors, most commonly a seminoma. Recently, the stage between maturational delay and GCNIS has been termed *pre-GCNIS*.[384] GCNIS cells express immunohistochemical markers such as placenta-like alkaline phosphatase (PLAP), c-KIT (the receptor for the stem cell factor SCF), POU5F1 (a transcription factor also called OCT3/4), and NANOG. However, in individuals with DSD, germ cells frequently show delayed maturation and prolonged expression of these markers. Therefore additional histologic criteria have been developed to distinguish maturational delay from GCNIS (see "Tumor Risk and DSDs" later in the chapter). In addition, SCF (or KIT ligand [KITLG]) has been proposed as a marker specific for germ cell neoplasia that is not expressed in germ cells with maturational delay.[384] Nearly all GCNIS eventually progress to invasive germ cell cancer in individuals without DSD, but it is hypothesized that this progression requires androgenic activity and may therefore be much more uncommon in AIS.[384,385] In CAIS the risk of developing GCNIS or a germ cell tumor before puberty is low (in the order of 0.8–2%).[386,387] Hence there is a general preference to delay gonadectomy until young adulthood to enable spontaneous puberty to occur. The risk of pre-GCNIS, GNIS, or malignancy is estimated to be 10% to 14% in adolescent and adult individuals with CAIS.[385,387] Some adults with CAIS elect to retain the gonads, though monitoring can be difficult, especially in case of abdominal gonads (see "Tumor Risk and DSDs").

Surgery to bring abdominal gonads to a more superficial location is a potential option; a biopsy can be taken at the time of surgery to assess the risk of germ cell cancer.[214] GCNIS and seminoma usually do not secrete tumor markers such as βhCG and α-fetoprotein (AFP), and GCNIS cannot be detected using ultrasound or MRI.[388] Noninvasive monitoring may potentially become available with the development of techniques to measure embryonic microRNAs in serum, but this method is also currently unable to detect GCNIS.[389] Common findings in CAIS gonads on MRI include Sertoli cell adenomas and epididymal cysts.[388]

Surgery in CAIS is generally confined to gonadectomy. For vaginal hypoplasia, self-dilatation is the first-line approach.[390,391] This technique is effective in lengthening the vagina for most CAIS women but requires time; thus it should be started when a girl is thinking about sexual relations and when there is clear focus and motivation. A specialist nurse or gynecologist with experience of vaginal dilatation is important to provide support and guidance, and several useful written materials are becoming available (e.g., www.dsdteens.org). In situations in which self-dilatation is not appropriate or possible, alternative forms of vaginoplasty, such as the laparoscopic Vecchietti procedure, may be offered.[390]

The early management of PAIS centers on sex designation and the subsequent plan and timing for surgery. There has been an increasing trend in recent decades to choose a male gender of rearing.[392] A recent prospective study of boys with PAIS showed the value of the EMS at birth as a predictor of pubertal development.[393] Those with an EMS of 5 or more started puberty spontaneously. Further studies on this cohort of 27 boys with PAIS involved functional analysis of the mutant ARs using reporter gene assays. In contrast to the EMS as a predictor, the results were not a reliable indicator of whether androgens would be required to induce puberty. However, such assays may have a place in guiding the magnitude of high-dose androgens sometimes required in adulthood in men with PAIS. Several surgical procedures may be required to correct hypospadias and relocate the testes in a scrotal sac. Gynecomastia is an almost universal finding as boys with PAIS reach puberty. This raises the possibility of prevention by the use of antiestrogens and aromatase inhibitors and thereby avoids the need for surgical reduction mammoplasty, but long-term safety has not been established. The risk of germ cell tumor of the testis had previously been reported to be high in PAIS, but more recent reports suggest a lower risk, comparable to that in CAIS.[378,394] When scrotal in position, testes should be monitored by regular self-examination and periodic imaging by ultrasound. It has been suggested that bilateral testicular biopsies should be undertaken at the time of orchidopexy and after puberty to determine the presence of GCNIS.[395] In view of the risk of malignant progression, gonadectomy is advised if GCNIS is discovered, although an alternative may be irradiation of bilateral in situ lesions.

When an infant with PAIS is designated a female sex, gonadectomy will be required before puberty if the adolescent wishes to prevent virilization. If gonadectomy is performed, estrogen replacement is needed to induce puberty and for hormone replacement thereafter. Whether and when to perform genitoplasty is a subject of controversy and is discussed in "Surgery and DSDs." In MAIS, clinical presentation occurs either in adolescence because of gynecomastia or in adulthood because of male factor infertility. Reduction mammoplasty is required for the gynecomastia. There is one report of successful fertility after testicular sperm extraction in a man with MAIS.[396,538]

Other Conditions Affecting 46,XY Sex Development

Persistent Müllerian Duct Syndrome

AMH is a glycoprotein homodimer that is secreted by the Sertoli cells of the developing testes from approximately 7 wpc. It acts through the AMH type 2 receptor between 8 and 12 wpc to cause regression of the müllerian duct (see "Development of Reproductive Systems").[124]

AMH is encoded by a 2.75-kb gene containing 5 exons in the region of chromosome 19p13.3. The AMHR2 is a serine/threonine kinase with a single transmembrane domain that is encoded by an 11-exon gene on 12q13. Exons 1 to 3 code for the signal sequence and the extracellular domain of the AMHR2, exon 4 codes for the transmembrane domain, and exons 5 through 11 code for the intracellular serine/threonine domain.

Persistent müllerian duct syndrome is a condition in which 46,XY males have well-developed testes and normal male external genitalia but also have müllerian duct derivatives. The diagnosis is often not made until a fallopian tube and uterus are incidentally encountered in patients undergoing inguinal hernia repair, orchiopexy, or abdominal surgery. In the more prevalent form, a boy presents with bilaterally undescended testes, and the uterus, tubes, and testes are in the pelvis. In other situations, a hernia contains a partially descended or scrotal testis, and the ipsilateral fallopian tube and uterus are in the hernia, which is known as *hernia uteri inguinale*. In some instances, the contralateral testis and tube also are present in the hernial sac, known as *transverse testicular ectopia*.

Approximately one-half of all genetically proven cases of PMDS result from defects in AMH, and half are caused by mutations in its receptor (Fig. 24.33).[397–399] *AMH* gene mutations are most commonly found in Mediterranean and North African countries,[400] and most familial mutations are homozygous. In contrast, mutations in the gene encoding AMHR2 are more commonly found in Northern Europe and the Middle East. Europeans are often compound heterozygous for mutations involving a 27-bp deletion in exon 10.[401,402] Measurement of serum AMH levels can provide a useful means for guiding genetic analysis; patients with PMDS caused by mutations of the *AMH* gene typically have low or undetectable levels of serum AMH (except for the p.Gln496His mutation thought to affect receptor binding), whereas AMH concentrations are normal for age or elevated in patients with mutations of *AMHR2*. Approximately 12% of patients with PMDS have an idiopathic form, with no mutation in *AMH* or *AMHR2*; they often have associated malformations.[399]

The main aim of management of PMDS is to prevent testicular cancer and preserve fertility in males, which can be difficult because of the anatomic findings. An increased prevalence of testicular degeneration has been described, which probably results from torsion of the testes and/or their anatomic location. Anatomic abnormalities of the epididymis and the vas deferens are common. Infertility may result from late orchiopexy or from mechanical problems associated with entrapment of the vas deferens in the müllerian derivatives. Treatment consists of early orchidopexy, which often requires removal of mullerian structures, although it should not be attempted to remove these in toto as this will likely damage the genital ducts or blood supply. Men with high pelvic testes rarely have successful orchidopexy, and many of these individuals are androgen deficient. Increasing rates of testicular malignancy have been reported in adult men with this condition, which further complicates long-term management of gonads that cannot be orchidopexed.[399] Fertility is rare, but successful TESE-ICSI has been reported in isolated cases.[399]

Hypospadias

Hypospadias is incomplete fusion of the penile urethra due to an arrest in development of the urethral spongiosum and ventral prepuce.[403] The normal embryologic resolution of penile curvature may also be interrupted, resulting in chordee. It is a common congenital anomaly with an estimated birth prevalence of 3 to 4 cases per 1000 live births. Although it has been suggested that the rate of hypospadias has been increasing, this is not borne out in contemporaneous studies.[404] The cause is unknown in most cases, but it is assumed that there is an interplay of genetic and environmental factors.[405–407] Mutations in candidate genes such as *MAMLD1, NR5A1,* and the *AR* are found in a minority of cases.[408] There is a familial clustering of cases in hypospadias, with a 7% incidence of one or more additional affected family members.[409] Four chromosomal regions have been identified in familial hypospadias by fine-mapping analysis of genes involved in steroid metabolism.[410] A genome-wide association study involving a total of 2978 cases and 7298 controls of European ancestry identified 18 genetic loci associated with hypospadias at genome-wide significance.[407] None of these loci lay near known 46,XY DSD genes, so distinct pathways may underlie the susceptibility to hypospadias.

Hypospadias is associated with increased maternal age, multiple births, maternal exposure to diethylstilbestrol in utero, paternal subfertility, maternal vegetarian diet, maternal smoking, assisted reproductive techniques, paternal exposure to pesticides, and fetal growth restriction.[411] The association with low birth weight is remarkably consistent across all studies of idiopathic hypospadias.[412]

Hypospadias may be the only phenotype in mild forms of 46,XY DSD, but it is not entirely clear when an evaluation for DSD is needed in a child with isolated hypospadias. Recommendations have been made to conduct such an evaluation if the hypospadias is severe (midshaft or more proximal) or if there are additional genital findings (e.g., unilateral or bilateral cryptorchidism, micropenis, anomalies of the scrotum), but the prevalence of DSD conditions with each of these presentations has yet to be reported.

Management is generally surgical. The urethral meatus is relocated to the glans, and the penis is straightened by correcting any chordee to give a forward-directed urinary stream and the ability to have sexual intercourse. Numerous techniques are described; repairs may require more than one stage, usually starting at 6 to 18 months of age or deferred until 4 years or later. Delaying surgery allows the child to be involved in decisions regarding surgery, but older age at surgery has been reported to be a risk factor for complications.[413] A course of testosterone is often given to induce penile growth prior to surgery, particularly if micropenis is present. The severity of the hypospadias may not be apparent until release of the chordee. Complications include fistula formation, meatal stenosis, urethral stricture, and residual chordee. Follow-up studies in adulthood indicate a less satisfactory cosmetic appearance compared to control subjects, shorter penile length, more dysfunction with voiding, and a lower urinary flow rate.[414] These problems were more evident in severe forms of hypospadias.

Anorchia and Cryptorchidism

The term *testicular regression syndrome* (also called *vanishing testis syndrome*) is used to describe the phenotype of bilateral anorchia in a male infant with otherwise typical-appearing genitalia. The phenotype suggests the presence of testes in early gestation functioning appropriately to differentiate male external genitalia, induce müllerian duct regression, and stabilize wolffian duct development. Bilateral anorchia with a normally differentiated but small phallus (micropenis) is a variant of the syndrome and may represent a form of testicular dysgenesis. The cause of testicular regression syndrome is unknown, but an interruption of the testis blood supply by a torsion or vascular occlusion event in utero has been proposed. Familial cases suggest that there may be a genetic cause in some cases. Surgical exploration and histologic findings typically show nubbins of fibrous tissue devoid of any testicular tissue attached to a blind-ending vas deferens.

Diagnosis is based on a combination of biochemical tests, imaging studies, and/or surgical exploration. An undetectable serum AMH level coupled with elevated serum levels of gonadotropins (particularly FSH) is highly predictive of the absence of

testes.[415] If such findings are present, there may be no need for surgical exploration. Androgen replacement is required to induce puberty at around 11 to 12 years of age. Testicular prostheses can be inserted if and when the young person desires. It is unclear whether the testicular nubbins pose a risk for gonadal tumors, but most studies suggest that the risk is low; whether nubbins should be removed surgically remains a subject of debate.

Testes that have not descended at birth (i.e., cryptorchidism) present the most common congenital abnormality in boys, affecting 2% to 9% of term infants.[140,416] Because testicular descent occurs late in gestation, undescended testes are a common finding in premature baby boys. There is epidemiologic evidence of an increasing prevalence to 6% in populations studied in the United Kingdom.[417] Longitudinal studies demonstrate the expected spontaneous descent of the testis by early infancy but an unexpected reascent of the testis to a cryptorchid position in later childhood. It is now recognized that there are just as many boys with acquired cryptorchidism as there are with congenital cryptorchidism.[418] There is a strong association with low birth weight, disorders of the pituitary-gonadal axis, and a number of syndromes, but in the majority of situations the cause of maldescent is unknown. Unilateral cryptorchidism does not require an evaluation to identify an underlying cause, but bilateral cryptorchidism could be a sign of hypogonadotropic hypogonadotropism, which could in turn be a sign of combined pituitary hormone deficiency that would require prompt diagnosis and treatment to avoid neurocognitive deficits due to untreated central hypothyroidism and hypoglycemia due to ACTH and growth hormone deficiency. In addition, bilateral nonpalpable gonads can rarely be the presenting feature of 46,XX congenital adrenal hyperplasia. Unilateral and bilateral cryptorchidism in the context of other signs of underandrogenization, such as hypospadias, also warrants a DSD evaluation. Mutations in *INSL3* and the gene for its receptor, *RXFP2*—factors involved in the transabdominal phase of testis descent—were reported in a few boys with cryptorchidism.[419] Cord blood levels of INSL3 but not testosterone are decreased in idiopathic cryptorchidism, particularly in infants whose testes descended by 3 months of age.[420]

Congenital and acquired forms of cryptorchidism differ in their management. Orchidopexy is the recommended treatment in the former after 6 months but preferably no later than 12 to 18 months of age according to recent guidelines.[421,422] There is no consensus on the role of hormonal treatment with GnRH or hCG due to poor-quality evidence and lack of long-term data.[422] It is not routinely recommended because complete testicular descent is often not achieved. Both positive and negative potential effects of hormonal treatment on germ cells and testicular volume have been reported. The European Urology Association/European Society for Paediatric Urology guidelines recommend offering hormonal treatment to boys with bilateral undescended testes to possibly improve further fertility potential, whereas the Canadian Urological Association–Pediatric Urologist of Canada guideline considers such treatment experimental.[422,423] hCG has been shown to induce descent to a lower level in the majority of testes, so as neoadjuvant therapy it may improve testis position before surgery to increase the chances of successful orchidopexy.[424] Some also recommend orchidopexy for acquired cryptorchidism, but an alternative approach is to wait until puberty because in more than 50% of cases the testes descend spontaneously at this time.[425] There is substantial evidence to indicate that the relative risk of germ cell tumor development in adulthood associated with cryptorchidism is more than halved if orchidopexy is undertaken before puberty.[426] Early orchidopexy is also beneficial for testis development and spermatogenesis, although the evidence that this is translated into improved fertility is lacking.[427]

Anatomic Defects of the Pelvis and Penis

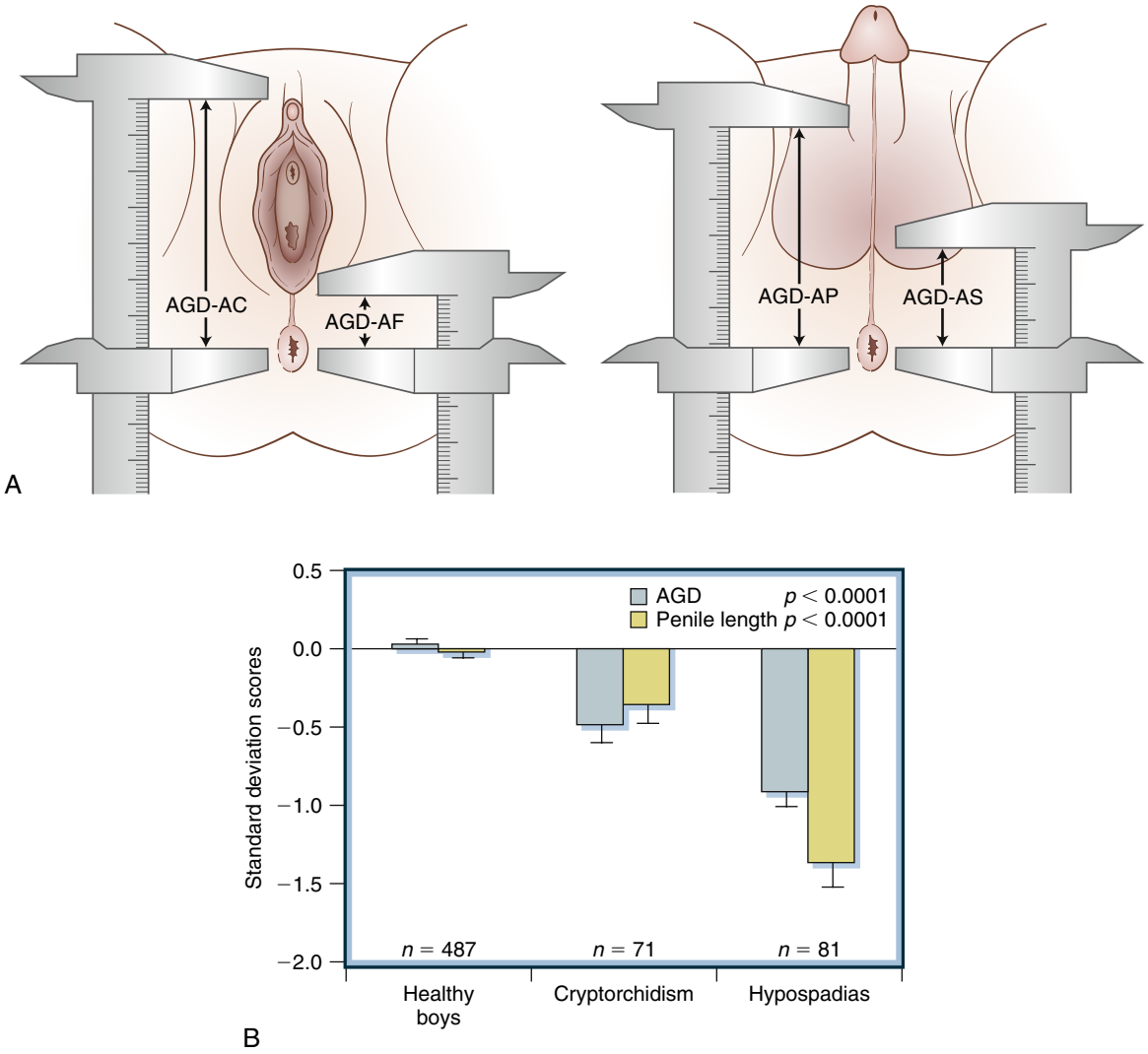
Cloacal exstrophy is an important developmental condition that can affect genital development as part of a complex anatomic anomaly involving the bladder, usually with associated gastrointestinal, anorectal, and skeletal features. *Penile agenesis* is a very rare isolated defect in penile development. In both these conditions, there is usually typical androgen synthesis by the testes, and androgen responsiveness in the brain, so a male gender identity is most likely.

Endocrine Disruptors

The proposed increase in male reproductive tract disorders (testis cancer, abnormal spermatogenesis, cryptorchidism, and hypospadias) led to the concept that testicular dysgenesis syndrome (TDS) of fetal origin might be triggered by lifestyle factors and exposure to endocrine-disrupting chemicals (EDCs) operating during the fetal and perinatal periods.[428,429] The US Environmental Protection Agency has defined an EDC as an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental processes.[430] The types of compounds generically include detergents, pesticides, and cosmetics with postulated modes of action comprising estrogenic and antiandrogenic effects. Such a broad definition and the ubiquity of thousands of compounds in the environment, which may have endocrine-disrupting effects, pose an enormous challenge for scientific studies to prove that adverse health effects occur in humans, and, if so, what measures can be taken for prevention. Analgesics commonly used during pregnancy, such as paracetamol/acetaminophen and ibuprofen, have also been suggested as potential endocrine disruptors interfering with testicular and ovarian development.[431–434]

Classic toxicological methods used in animals are not apposite for human studies, but application of a phenotypic marker long used by toxicologists testing EDCs in animals has been validated in humans for epidemiologic studies.[435] Anogenital distance (AGD) is significantly reduced in isolated hypospadias and cryptorchidism in comparison with normative data (Fig. 24.34).[436] As an exemplar of the application of this marker in epidemiologic studies in reproductive development, prenatal exposure to phthalates widely used as plasticizers in many products was associated with reduced AGD in males at birth.[437,438] Xenografts of human fetal testis tissue in animals is a recent developed methodology which usefully informs on chemical effects relevant to human reproductive development.[439] Another approach is the study of occupational exposure where there is evidence of increased risk of hypospadias and cryptorchidism in offspring of parents working with pesticides, industrial chemicals, cosmetics, and several other potentially toxic substances.[440,441]

The relevance of these epidemiologic findings to the assessment of an individual child with DSD remains uncertain. There is no consensus yet about low-dose effects on health from the combination of a myriad of chemicals to which humans are exposed over the lifespan or the optimal toxicological methodology to measure exposure.[442,443] An expert panel has identified compelling evidence for male infertility attributable to phthalate exposure and estimated the financial burden that accrues to the European Union from the consequences of needing additional assisted reproductive technology procedures.[444] A similar analysis can be undertaken to



• **Fig. 24.34** Measurement of anogenital distance (AGD). (A) Different landmarks used for measurement in female and male infants. The distance from the posterior fourchette (AF) and from the clitoris (AC) to the midpoint of the anus in females and from the cephalad insertion of the penis (AP) and from the junction of the scrotal and perineal (AS) skin to the midpoint of the anus in males are commonly used methods to determine AGD in epidemiologic studies. (B) Measurements of penile length and AGD in boys with cryptorchidism and hypospadias compared with normal male infants. (Redrawn from Thankamony A, Lek N, Carroll D, et al. Anogenital distance and penile length in infants with hypospadias or cryptorchidism; comparison with normative data. *Environ Health Perspect*. 2014;122:207–211, used with permission of Environmental Health Perspectives.)

estimate the additional cases of cryptorchidism requiring orchidopexy. Adopting the precautionary principle to reduce exposure to chemicals to as low as is reasonably practicable is a sensible approach in the face of so much uncertainty about the adverse effects of EDCs on human health. This applies particularly to pregnant women who may need to take an analgesic periodically.

46,XX Disorders of Sex Development

46,XX DSDs can be divided into disorders of ovarian development, disorders of steroid hormone synthesis leading to androgen excess, and other conditions affecting sex development (see Table 24.3).

Disorders of Ovarian Development

Disorders of ovarian development (i.e., ovarian dysgenesis or resistance) usually manifest as absent puberty due to the absence of estrogen production. Other disorders of gonadal development can result in a 46,XX individual with an ovary containing testicular tissue (i.e., 46,XX ovotesticular DSD) or a testis capable of producing enough testosterone for a male phenotype and/or sufficient AMH to regress the uterus (i.e., 46,XX testicular DSD).

Ovarian Dysgenesis

Ovarian dysgenesis most often occurs as a result of sex chromosome aneuploidy (e.g., Turner syndrome; 45,X and variants) with progressive follicular apoptosis. Mutations in many genes can result in POI, but each accounts for only a small proportion, and often no cause of POI is identified. Two recent reviews provide an excellent overview of the various groups of genes that are involved.[445,446] Some of these genes are important for formation of the indifferent gonad; these include *NR5A1* and *WT1*, which have been discussed earlier. *FOXL2* plays a role in early ovarian

determination, and mutations are associated with POI, usually but not always, as part of the blepharophimosis, ptosis, epicanthus inversus syndrome. Other genes are involved in meiosis and DNA repair, such as cohesins (e.g., *STAG3*), and various helicases (e.g., *MCM8* and *HFM1*). Mutations in these genes disrupt meiotic progression of oocytes and result in oocyte degeneration. They form an important group of genes to consider in women with POI, as mutations in these genes are associated with an increased risk of various cancers. A group of genes involved in mitochondrial functions cause sensorineural deafness in combination with POI, also known as Perrault syndrome and other neurosensory syndromes (e.g., *POLG*). Other syndromic causes include premature aging syndromes (e.g., *LMNA*) and skeletal syndromes (e.g., *BMPR1B*). Mutations in genes involved in the formation and maturation of the primordial follicles, such as *SOHLH1*, *SOHLH2*, *NUP107*, *FIGLA,* and *NOBOX*, as well as those involved in the next hormone-dependent step, maturation and growth from primary to ovulatory follicles, such as *ESR1*, *FSHR*, *LHCGR,* and *BMP15*, cause nonsyndromic POI. One of the most common monogenic causes is a premutation of *FMR1*, found in approximately 2% to 6% of women with sporadic POI. *SOX8* mutations have recently been identified in 5% of a cohort of females with POI.[249]

46,XX Ovotesticular and 46,XX Testicular Disorders of Sex Development

In rare situations, the developing ovary may contain testicular tissue (i.e., 46,XX ovotesticular DSD) or may even develop as a functioning testis (i.e., 46,XX testicular DSD). In 46,XX ovotesticular DSD, the patient usually presents with ambiguous genitalia at birth and progressive virilization at puberty if the testicular component is not removed. In contrast, 46,XX testicular DSD is usually associated with a male phenotype at birth and absence of müllerian structures. However, infertility occurs in both situations as crucial spermatogenesis genes on the Y chromosome are absent, and testicular androgen production may not be sufficient to support full pubertal development or may decrease with age, so careful monitoring is needed. In some circumstances ovotesticular DSD and testicular DSD may represent a spectrum of the same molecular condition.

The presentation, endocrinology, and management of 46,XX ovotesticular DSD is similar to those of ovotesticular DSD due to 46,XX/46,XY chimerism, as described earlier (see "Sex Chromosome Disorders of Sex Development"). However, 46,XX males cannot achieve male fertility. As discussed, a 46,XX karyotype is the most frequent finding in ovotesticular DSD, especially in patients from sub-Saharan Africa.[219,220] Familial cases have been described, and it is likely that a genetic basis exists in some situations. Indeed, mutations affecting a specific residue (p.Arg92Trp or p.Arg92Gln) of NR5A1 (SF1) have recently been identified in a number of individuals with ovotesticular/testicular DSD from diverse ancestral backgrounds (see "Gonadal Sex") (see Fig. 24.20).[71,72] These specific changes initiate a testis development pathway, possibly through loss of repressor activity, and account for up to 10% of ovotesticular DSD. Disruption of another nuclear receptor, NR2F2 (COUP-TFII), has also been reported in ovotesticular/testicular DSD and cardiac defects, sometimes with additional features (e.g., diaphragmatic hernia, BPES).[122] Homozygous mutations of the ovarian-testis repressor gene *RSPO1* (encoding R-spondin 1) can cause ovotesticular/testicular DSD with eye and skin abnormalities and hearing impairment.[222,447]

In contrast, patients with 46,XX testicular DSD often present first with male factor infertility, although sometimes hypospadias is present. In some cases, a family history is found. Because different family members can have different phenotypes, it is possible that 46,XX testicular DSD represents the most severe end of the spectrum of ovarian transdifferentiation phenotypes. Up to 80% of 46,XX males harbor translocations of Y-chromosomal material containing *SRY*, the testis-determining factor. This finding helped considerably in mapping the *SRY* gene in the first instance (see "The Y Chromosome"), and in some situations, residual ovarian tissue (ovotestis) develops. Individuals with *SRY* translocations can be diagnosed by FISH analysis using a probe directed to that gene (see Fig. 24.3). A number of *SRY*-negative cases have been reported. Sometimes, these arise from the same mutations in NR5A1 or NR2F2 that can cause 46,XX ovotesticular DSD. Duplication of the genetic locus containing *SOX9* or deletions or rearrangements in distant regulatory regions that result in *SOX9* overexpression,[107–109] and similar alterations in the X chromosome resulting in overexpression of *SOX3,* have also been described.[448] In addition, it has been hypothesized that overexpression of *SOX10* may be the cause of 46,XX ovotesticular/testicular DSD in two individuals with an increased copy number of the 22q13 area.[449] Loss-of-function mutations in *RSPO1* have been reported in individuals with 46,XX testicular DSD as well as in ovotesticular DSD. Severe disruption of *WNT4* can result in 46,XX ovotesticular/testicular DSD as part of the SERKAL syndrome, which also encompasses renal, adrenal, and lung dysgenesis.[450]

Disorders of Androgen Excess

The steroid biosynthetic disorders causing androgen excess and 46,XX DSD are summarized in Table 24.6. Although CYP21 deficiency is by far the most common cause of this condition, alternative diagnoses should be considered because approaches to counseling and management may vary.

3β-Hydroxysteroid Dehydrogenase Type 2 Deficiency

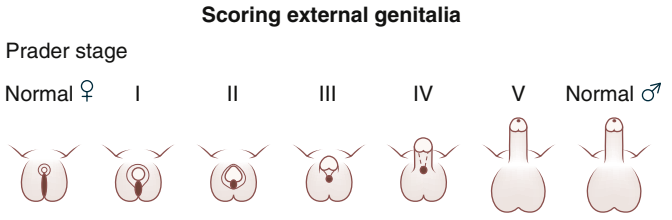
3βHSD/$\Delta^{4,5}$-isomerase catalyzes the conversion of Δ^5 steroids to Δ^4 steroids and is required for the generation of mineralocorticoids, glucocorticoids, and more potent androgens (e.g., testosterone, DHT) by the adrenal glands and gonads (see Fig. 24.11). The actions of 3βHSD and the consequences of deficiency of the type 2 enzyme (3βHSD2) were described earlier because, in 46,XY individuals, this deficiency results in adrenal insufficiency with variable defects in androgenization.

Defects in 3βHSD2 can also cause a genital phenotype in 46,XX females. Severe, recessively inherited defects in enzyme function can cause mild clitoral enlargement at birth in girls with glucocorticoid deficiency with or without salt loss. This mild androgenization occurs not as a direct androgenic effect of the excess DHEA but as a result of conversion of DHEA and other Δ^5-3β-hydroxy-C19-steroids to testosterone by 3βHSD1 in the placenta and in the peripheral tissues of the fetus. This conversion, coupled with the limited capacity of the placenta to aromatize androgens to estrogens early in gestation, can increase circulating levels of androgens in the female fetus and cause modest clitoromegaly in a minority of patients. Other girls have normal genitalia but show adrenal features. Milder, nonclassic forms of 3βHSD2 deficiency can cause premature pubarche in girls. Breast development can occur at puberty in affected females, presumably by peripheral conversion of Δ^5C19 steroids to Δ^4C19 steroids by 3βHSD1 expressed principally in the liver and peripheral tissues and by the subsequent aromatization of androgens to estrogens.

The diagnosis of 3βHSD2 deficiency can be challenging in nonclassic forms of this condition.[299] The levels of Δ^5 steroids (e.g., 17-hydroxypregnenolone, DHEA, and its sulfate, DHEAS) usually are increased, and the ratio of Δ^5 steroids to Δ^4 steroids (e.g., the ratio of 17-hydroxypregnenolone to cortisol) is markedly increased, especially after stimulation with intravenous ACTH. The urinary steroid profile can also be informative because 17-ketosteroids and especially DHEAS and 16-hydroxy-DHEAS are elevated. Although adrenal production of 17-OHP is reduced due to the enzymatic block, plasma concentrations of 17-OHP can actually be increased as a result of peripheral conversion of $\Delta^5$17-hydroxypregnenolone to 17-OHP by the 3βHSD type 1 enzyme. This finding may lead to confusion with other forms of CAH such as CYP21A2 deficiency or POR deficiency. Mild forms of 3βHSD2 deficiency may manifest in a fashion similar to that of virilizing adrenal tumors. Suppression of the increased plasma and urinary levels of C19 and C21 3β-hydroxysteroids by glucocorticoids distinguishes 3βHSD deficiency in such cases. Treatment for 3βHSD deficiency is glucocorticoid, mineralocorticoid, and salt supplementation, as appropriate, and estrogen replacement to induce puberty, if needed.[300]

21-Hydroxylase Deficiency

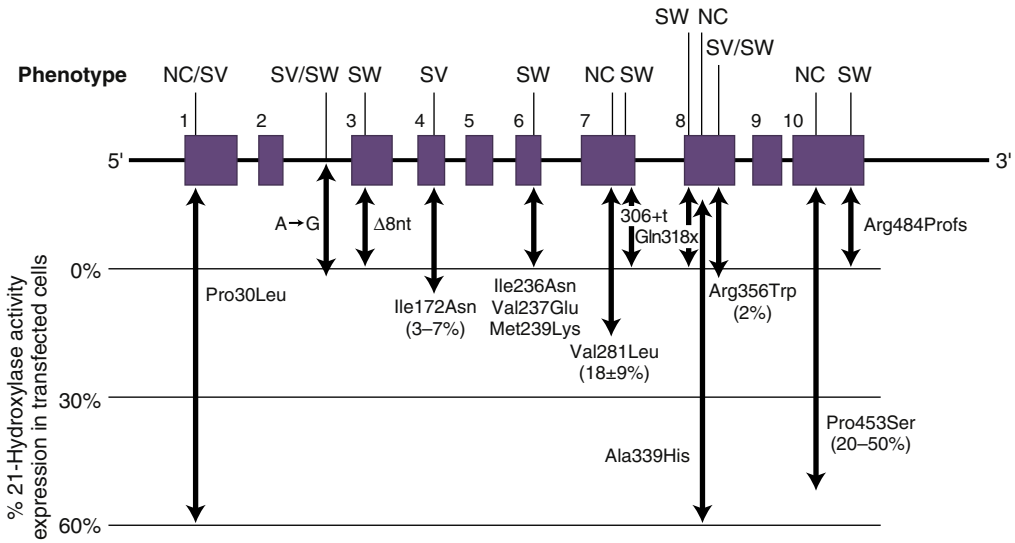
CYP21A2 deficiency (see Fig. 24.11) is one of the most common causes of ambiguous genitalia of the newborn. It is a disorder of adrenal steroidogenesis that affects around 1 in 15,000 children, with most 46,XX patients presenting at birth due to virilization of the genitalia (see Chapter 15).[451,452]



• **Fig. 24.35** Prader classification of the degree of androgenization in a 46,XX individual with congenital adrenal hyperplasia.

A 46,XX fetus with CYP21A2 deficiency can become androgenized to various degrees, as illustrated by the Prader classification (Fig. 24.35). High fetal concentrations of 17-OHP, androstenedione, and testosterone are the hallmark of CAH due to CYP21A2 deficiency. More than 75% of patients with classic CAH, characterized by cortisol deficiency, are also salt losers because of their inability to synthesize sufficient mineralocorticoids. This is sometimes referred to as *salt-wasting* 21-hydroxylase deficiency. Other children do not develop such significant salt loss and are termed *simple virilizing*. A third form is *nonclassic* 21-hydroxylase deficiency, which is characterized by mild subclinical mineralocorticoid and glucocortiocoid deficiency but varying degrees of excess androgen production, and usually presents with premature pubarche, irregular menses, or hirsutism but may also be asymptomatic. Salt loss is usually caused by mutations/deletions of the *CYP21A2* gene on chromosome 6p21.3 that result in a completely inactive enzyme. The concordance between genotype and phenotype for the single-gene disorder is reasonably strong but not invariable (Fig. 24.36). In patients who are compound heterozygotes, the phenotype is usually determined by the milder allele. More than 90% of cases result from *CYP21A2* deletion or one of nine mutations derived from recombination with the nonfunctional pseudogene *CYP21P* (see Fig. 24.36). About 5% of *CYP21A2* mutations arise spontaneously.[453] Worldwide, around 100 non–pseudogene-derived mutations are reported for the *CYP21A2* allele.

The diagnosis of CYP21A2 deficiency in a newborn with ambiguous genitalia is readily confirmed by markedly elevated serum 17-OHP concentrations (>300 nmol/L) after the first 48 hours. Sick and/or preterm infants can have moderately increased steroid levels, so ACTH stimulation may be needed to resolve any



• **Fig. 24.36** Diagram of the *CYP21* gene and locations of the mutations that cause more than 90% of cases of 21-hydroxylase deficiency. The *numbered boxes* depict the exons. The three-letter abbreviations for amino acids are used; *X* indicates a nonsense (stop) mutation. An adenine (A) to guanine (G) transition in intron 2 causes a common splice site mutation. Other mutations include an 8-nucleotide deletion (Δ8nt) in exon 3, a thymidine insertion at codon 306 (306+t), and a guanine (G) to cytosine (C) transition at codon 484. The activities of the mutant enzymes, expressed as a percentage of the wild type, are indicated on the vertical axis and in parentheses for some missense mutations. Historic codon numbering is shown. Current classification of codon numbering is usually n + 1 (e.g., Pro30Leu is Pro31Leu, Ile 172Asn is Ile173Asn). *NC,* nonclassic form; *SV,* simple virilizing; *SW,* salt wasting.

diagnostic confusion. The affected male usually has no alerting clinical signs at birth and, if a salt loser, does not decompensate with hyponatremia, hyperkalemia, and weight loss until 1 to 2 weeks after birth.[452] Newborn screening programs to measure blood spot 17-OHP concentrations soon after birth have been introduced in some countries.[454] Elevated 17-OHP can also be seen in conditions such as 3βHSD deficiency, 11β-hydroxylase deficiency, and P450 oxidoreductase deficiency, although the levels are usually not as high as in 21-hydroxylase deficiency.

Treatment of 21-Hydroxylase Deficiency

Prompt diagnosis and treatment of 21-hydroxylase deficiency are required to replace adrenal steroids and to suppress androgen production. Details of adrenal treatment are discussed in Chapter 15, and the approach to diagnosis and initial management are provided later (see "Investigation and Management of Disorders of Sex Development"). 21-hydroxylase deficiency is a potentially life-threatening condition, and the atypical genitalia give an important sign that this diagnosis should be considered.

Of relevance to DSD is the potential to prevent ambiguous genitalia of the newborn through prenatal treatment with dexamethasone, although this treatment is considered experimental.[455,456] Androgenization can be prevented if dexamethasone is administered to the mother early enough in gestation, indicating an intact fetal pituitary-adrenal axis that is responsive to negative-feedback regulation. Treatment is effective if dexamethasone is started by 6 to 7 weeks of gestation but has side effects in treated mothers and fetuses. Traditionally, karyotype analysis and *CYP21* genotype analysis have not been available until weeks 9 to 11, when chorionic villus biopsy can be performed. This means that seven unaffected or male fetuses would need to be treated with dexamethasone during this initial period for every one affected female fetus who might benefit. However, recent advances in the detection of circulating free fetal DNA in the maternal serum have increased the sensitivity and specificity of determining fetal chromosomal sex much earlier in pregnancy.[457] Furthermore, using parental haplotype analysis by targeted massively parallel sequencing of the *CYP21* locus, it has been possible to determine the genotype of the fetus earlier in pregnancy so that fewer unaffected females would be treated.[458] With this approach CAH can be diagnosed using a maternal blood sample drawn at 6 weeks of gestation, although this awaits validation in larger studies.[459] These are all potentially promising advances to prevent exposure of unaffected babies to dexamethasone, if parents do decide to have dexamethasone treatment in pregnancy in a center that offers it.

Several concerns have been raised about the potential effects of dexamethasone on the developing fetus. Although cognitive and motor development based on questionnaires appeared not to be adversely affected by exposure to dexamethasone, direct examination of children who had been exposed to dexamethasone from gestational age 6 to 7 weeks did show adverse effects on verbal working memory compared with control subjects.[460–463] All of these studies were observational, and a meta-analysis emphasized the paucity of good-quality, long-term follow-up data to inform a risk-benefit analysis.[464] Cognitive function may be more affected in girls than in boys.[465] Limited long-term data in older children, adolescents, and adults have suggested that any changes may not persist.[466,467] However, this aspect should be considered when counseling families about prenatal treatment. Clinical practice guidelines have suggested that prenatal therapy should only be undertaken using protocols approved by institutional review boards and at centers capable of collecting long-term outcome data as part of multicenter studies.[468,469]

P450 Oxidoreductase Deficiency

POR is a membrane-bound flavoprotein that plays a central role in electron transfer from NADPH to all microsomal P450 enzymes, including CYP17, CYP21, and CYP19 (aromatase) (see Fig. 24.28). Defects in POR can cause apparent combined CYP17 and CYP21 deficiency, with or without Antley-Bixler syndrome, a form of craniosynostosis. These conditions were described earlier (see "Disorders of Androgen Synthesis").[320,322] POR deficiency can be associated with ambiguous genitalia in both 46,XX and 46,XY infants. The androgenization of 46,XX fetuses may result from a defect in aromatase activity or occur through a proposed backdoor pathway of DHT production that does not involve androstenedione or testosterone as intermediates.[134] Children with this condition usually have cortisol deficiency, but mineralocorticoid function is relatively preserved. Careful monitoring of puberty is needed as this can be delayed or disrupted.[320]

11β-Hydroxylase Deficiency

11β-hydroxylase (CYP11B1) deficiency can cause profound androgenization in an affected 46,XX fetus and accounts for approximately 5% of patients with CAH.[470,471] 11β-hydroxylase deficiency typically presents with ambiguous genitalia in the 46,XX newborn, although in some situations the androgenization may be so marked that the baby is thought to be a boy with a small penis and undescended testes.[472] Subsequently in childhood there can be hyperandrogenism and precocious puberty, with accelerated growth and skeletal maturation. Hypertension occurs in two-thirds of cases as prolonged accumulation of 11-deoxycorticosterone, which has some mineralocorticoid activity, leads to salt and fluid retention, and plasma renin is suppressed. Milder changes in 11β-hydroxylase cause a nonclassic form of the condition, similar to nonclassic 21-hydroxylase deficiency, which presents mostly with hyperandrogenism.[473] The biochemical diagnosis can be made by urine steroid profile and through a pattern of reduced cortisol and elevated intermediates such as 11-deoxycorticosterone.

11β-hydroxylase is encoded by the *CYP11B1* gene, which is located on chromosome 8q21-22 in tandem with *CYP11B2*, which encodes aldosterone synthase, the enzyme catalyzing the conversion of deoxycorticosterone to corticosterone and then to aldosterone. Some *CYP11B1* mutations that cause 11β-hydroxylase deficiency are shown in Fig. 24.37. Most changes are missense mutations. Arg448 appears to be a relative hot spot for mutations, with Arg448His possibly a founder mutation in the Moroccan Jewish population. Treatment of 11β-hydroxylase deficiency includes glucocorticoid replacement to reduce ACTH drive and in some situations antihypertensive treatment.

Familial Glucocorticoid Resistance

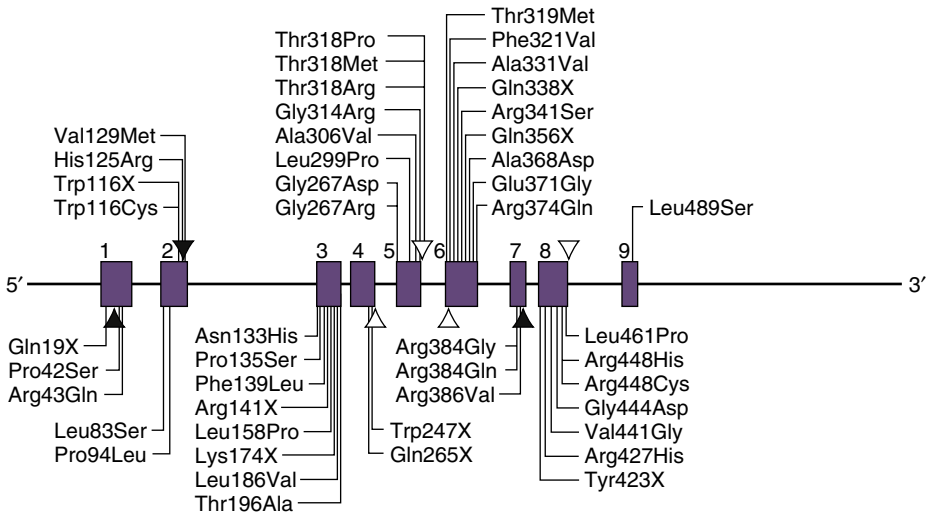
Glucocorticoid resistance is a rare disorder that is usually caused by sporadic heterozygous mutations in the glucocorticoid receptor α-isoform (GRα), encoded by the *NR3C1* gene.[474] Partial end-organ insensitivity to glucocorticoid action coupled with impaired feedback mechanisms results in excess ACTH secretion and elevated circulating cortisol levels without the clinical features of Cushing syndrome. In many cases, elevated levels of mineralocorticoids cause hypertension and hypokalemia, and elevated levels of adrenal androgens cause hirsutism and acne. Although classic glucocorticoid resistance is not associated with atypical genitalia,

one homozygous *NR3C1* mutation (Val571Ala) was reported in a Brazilian girl who had a large clitoris, posterior labioscrotal fusion, and a urogenital sinus at birth.[475] This mutation caused marked reduction in GRα function without complete loss of receptor activity. She also harbored a heterozygous mutation in *CYP21*, which may have influenced the phenotype.

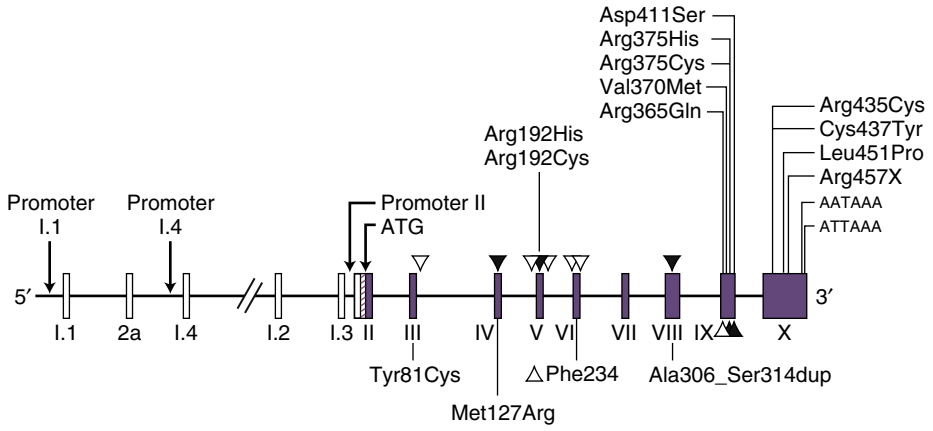
Aromatase Deficiency

Aromatase (CYP19A1), a cytochrome P450 enzyme, is the only enzyme known to catalyze the conversion of androgens (C19 steroids) to estrogens (C18 steroids) in vertebrate species.[476] Aromatase is expressed in many tissues, including the placenta, ovary, brain, bone, vascular endothelium, breast, and adipose tissue, where it is regulated by a number of tissue-specific promoters to convert testosterone to estradiol and androstenedione to estrone (Fig. 24.38). Aromatase plays a crucial role in the local production of estrogens and in the synthesis of circulating estrogens from the ovary at the time of puberty.

Aromatase deficiency due to recessively inherited mutations in *CYP19* has been described in approximately 40 girls with 46,XX DSD (see Fig. 24.38). The clinical and biochemical features of this condition underscore the key role aromatase plays in the



• **Fig. 24.37** Diagram of the *CYP11B1* gene and locations of selected mutations causing 11β-hydroxylase deficiency. The *numbered solid boxes* depict the exons. The three-letter abbreviations for amino acids are used; X indicates a nonsense (stop) mutation. A deletion of cytosine (C) at codon 32 and the addition of two nucleotides at codon 394 cause frameshift mutations; insertions and deletions resulting in a frameshift and splice site mutations are shown by *solid triangles* and *open triangles*, respectively.



• **Fig. 24.38** Diagram of the *CYP19* gene and selected mutations causing aromatase deficiency. The *numbered solid boxes* represent translated exons. The septum in the open box in exon II represents the 3′ acceptor splice junction for the untranslated exons. Multiple alternative promoters and the untranslated exons *(open boxes)* are indicated. The three-letter abbreviations for amino acids are used to indicate the positions of missense mutations; X indicates a nonsense (stop) mutation; insertions and deletions resulting in frameshift and splice site mutations are shown by *solid triangles* and *open triangles*, respectively. In addition to the mutations causing classic aromatase deficiency, a homozygous Arg435Cys mutation and deletion of a phenylalanine residue at position 234 are both associated with a partial aromatase insufficiency phenotype. (Modified from Morishima A, Grumbach MM, Simpson ER, et al. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metab*. 1995;80:3689–3698, used with permission of The Endocrine Society, Copyright 1995.)

fetoplacental unit, and this condition is sometimes referred to as *placental aromatase deficiency*. Aromatase plays a critical role in protecting the fetus and mother from excessive androgen exposure during gestation. In the absence of aromatase, estrogen cannot be synthesized by the placenta, and large quantities of placental testosterone and androstenedione are transferred to the fetal and maternal circulation, resulting in androgenization of the female fetus and virilization of the mother during pregnancy.[477]

Females (46,XX) with aromatase insufficiency are born with clitoromegaly, various degrees of posterior fusion, scrotalization of the labioscrotal folds, and in some infants a urogenital sinus with a single perineal orifice.[478–480] There is often a striking history of maternal virilization after the second trimester of pregnancy (e.g., acne, hair growth, voice changes) coupled with elevated maternal androgen levels, which usually resolve after the infant is born, but maternal virilization does not always occur.[481,482] As expected for a steroidogenic defect, affected girls (46,XX) have normal müllerian structures because AMH is not produced. The histologic picture of the ovaries in infancy is normal, but under increased FSH stimulation in the absence of ovarian aromatase, multiple enlarged follicular cysts develop. At puberty, affected females have hypergonadotropic hypogonadism, typically fail to develop female secondary sex characteristics, and exhibit progressive virilization. Plasma androstenedione and testosterone levels are elevated, and estrone and estradiol levels are low or not measurable. The hypergonadotropism and the multiple ovarian cysts respond to estrogen replacement therapy, but in some cases, temporary treatment with an antiandrogen is necessary.

In addition to a role in the reproductive axis, aromatase deficiency has implications for bone development, metabolism, and immune function, as determined by the long-term follow-up of the small numbers of women (46,XX) and men (46,XY) with aromatase deficiency and by studies of aromatase-knockout mice. Males present only after puberty and have tall stature, delayed bone maturation and delayed epiphyseal fusion, and osteopenia, demonstrating that estrogens are essential for normal skeletal maturation and proportions and for the prevention of osteoporosis in males and females.[483] Hyperinsulinemia and abnormal plasma lipids have also been reported in aromatase deficiency, which may in part reflect estrogen insufficiency and/or testosterone overproduction but may also reflect specific actions of aromatase itself. The finding of apparently normal psychosexual development in the three aromatase-deficient adolescent or adult patients and in a man with an estrogen receptor defect suggests that estrogen does not play a critical role in sex differentiation of the human brain, as has been reported in nonprimate mammals.

CYP19A1 mutations cause aromatase deficiency in males and females and are inherited in a recessive fashion (see Fig. 24.38). Functional assays of aromatase activity have shown severe loss of enzyme function (~0.3%) in nearly all cases associated with classic aromatase deficiency; in one case, approximately 1% activity was found for an Arg435Cys change in a compound heterozygous state together with a null Cys437Tyr mutation.[480] Partial aromatase deficiency has also been described; a homozygous Arg435Cys change has been reported in a girl who presented with androgenized genitalia at birth and showed some limited breast development during puberty, and deletion of a single phenylalanine residue (Phe234del) causing partial loss of aromatase activity was described in an androgenized 46,XX individual who achieved significant breast development (to Tanner stage 4) during puberty.[484]

Thus a spectrum of phenotypes may be seen with aromatase insufficiency in humans. This important diagnosis should be considered in all androgenized 46,XX infants when more common forms of CAH (e.g., CYP21 deficiency) and ovotesticular DSD have been excluded. A history of maternal virilization in pregnancy should always be sought but may not always be present, and increased levels of Δ^4 androstenedione, testosterone, and DHT and low levels of plasma estriol, urinary estriol, and amniotic fluid estrone, estradiol, and estriol may be detected. The diagnosis can be difficult to make during childhood years, when the HPG axis is relatively quiescent, and genetic analysis may be needed.

Maternal Androgen Excess

Maternal sources of androgens that may virilize a female fetus may be endogenous from adrenal and ovarian tumors or exogenous from maternal exposure to androgenic compounds. Danazol, a synthetic derivative of ethisterone with androgenic, antiestrogenic, and antiprogestogenic activities, is used in diverse conditions such as endometriosis, benign fibrocystic breast disease, and hereditary angioedema and in women with unexplained subfertility. It crosses the placenta and is contraindicated in pregnancy in view of reports that a female fetus may become androgenized.[485] Ovarian causes of virilization include primary malignancy and benign lesions such as luteoma and hyperreactio luteinalis. Recurrence in subsequent pregnancies and maternal virilization can occur with luteomas.[486] A case of a similar pattern of recurrent hyperandrogenization without evidence of placental aromatase deficiency has been reported.[487]

Other Conditions Affecting 46,XX Sex Development

Several syndromic associations can cause developmental genital abnormalities in 46,XX females. Complex pelvic anomalies such as cloacal exstrophy can affect both sexes and require major reconstructive surgery for bladder and bowel function and for the lower genital system.

Abnormalities in uterine development can result in bicornate uterus (i.e., Fryns syndrome), uterine hemiagenesis or hypoplasia, or uterine agenesis. These conditions can be part of Mayer-Rokitansky-Küster-Hauser syndrome, which may be associated with vaginal and/or ovarian agenesis as well as with renal, cardiac, and cervical spinal abnormalities, as in MURCS (müllerian, renal, and cervical spine syndrome).[488,489] The cause of these conditions is unknown for most women, although some familial cases have been described, and a mutation in *WNT4* was reported in a patient with absent müllerian structures (uterus and upper vagina), unilateral renal agenesis, and mild hyperandrogenemia.[120] Uterine abnormalities have also been associated with maturity-onset diabetes of the young type 5 (MODY5, *HNF1B*) and with vaginal abnormalities in patients with hand-foot-genital syndrome *(HOXA13)* and McKusick-Kaufman syndrome (*MKKS*, formerly called *BBS6*). Because a prominent clitoris can be associated with conditions such as Fraser syndrome (not to be confused with Frasier syndrome) or neurofibromatosis, careful evaluation is necessary before a hyperandrogenic cause is diagnosed.

Other common conditions that may be mistaken for a more serious underlying disorder include apparent clitoromegaly seen in premature or formerly premature babies or when little labial adipose tissue is present. Assessment by a surgeon or physician with experience in recognizing normal variability in clitoral size is important. Sometimes this form of isolated clitoromegaly can persist into childhood.[490] Labial adhesions are a common finding in female infants. They often resolve spontaneously, although estrogen cream can be used to accelerate the process. Transient

menstrual bleeding during the first week of life can be seen frequently in female infants with the withdrawal of large amounts of estrogens and progesterones after birth. This can be alarming for parents but rapidly resolves, and no treatment is needed.

Investigation and Management of Disorders of Sex Development

DSDs can present at many ages and to a wide range of different health professionals. Some of the most common presentations are shown in Table 24.18, and the range of conditions discussed in our center over a 2-year period is shown in Table 24.19. The potential diagnosis of DSD has major implications for the child and the family. Most people will never have heard of DSD or have thought that they may be faced with, for example, a situation in which they cannot be told immediately whether their newborn is a boy or a girl. Talking about such issues can be difficult, and sensitive and positive support and education from all health professionals is essential to provide parents with knowledge and confidence.[491]

Specialist multidisciplinary (or interdisciplinary) team (MDT) involvement with families, young people, and adults with DSD is important.[1–3,348,492] The pediatric (or adult) endocrinologist plays a central role in the team with support from psychologists, urologists, and other health professionals (Fig. 24.39).[3,492,493] The structure and available expertise vary depending on the center. Specialist MDTs are usually based only at large centers, so effective communication between the team and those health professionals managing the initial presentation is crucial. Sometimes regional or national networks (e.g., the Scottish Genital Anomalies Network) are needed to provide support where the population is scattered geographically or to link experts in different centers together, with meetings held by teleconference rather than in person.[494] It is important to keep the primary care physician informed. It is also useful for families to have a clearly defined point of contact if they need to ask further questions.

Although there is often uncertainty at the start, a general diagnosis can usually be reached following a series of careful assessments and basic investigations. Reaching a more specific diagnosis can be important in some situations and may take longer and involve more detailed investigations or genetic tests (discussed later). Every person is different, and many DSD-related conditions can have a spectrum of presentations, so it is also important to take an individualized approach to the child and the family.

The following is a discussion of the investigation and management of DSDs at different ages. Several overarching themes are subsequently highlighted. More extensive details for specific conditions are discussed earlier in the chapter.

TABLE 24.18 Common Presentations of DSDs at Different Ages

Presentation	Feature	Examples
Prenatal	Karyotype-phenotype discordance	Complete androgen insensitivity, 46,XY complete gonadal dysgenesis, 46,XX testicular DSD with SRY translocation
Neonatal	Atypical genitalia	21-hydroxylase deficiency (46,XX), 45,X/46,XY mosaicism, partial AIS, 5α-reductase deficiency, SF1/*NR5A1* (46,XY)
	Salt-losing crisis	21-hydroxylase deficiency, 3βHSD deficiency (46,XX) StAR, CYP11A1, 3βHSD deficiency, SF1/*NR5A1* (46,XY)
Childhood	Inguinal hernia	Complete AIS
	Androgenization	21-hydroxylase deficiency, 11β-hydroxylase deficiency
	Associated features	Wilms tumor
Puberty	Androgenization	17βHSD deficiency, 5α-reductase deficiency (rarely SF1/*NR5A1*, partial AIS, ovotestes)
	Absent puberty	Complete gonadal dysgenesis (Swyer syndrome), 17α-hydroxylase deficiency
Postpuberty	Amenorrhea	Complete AIS
Adult	Tumors	Minimal AIS, SF1/*NR5A1*, etc.

AIS, Androgen insensitivity syndrome; *CYP*, cytochrome P450 enzyme; *DSDs*, disorders/differences of sex development; *HSD*, hydroxysteroid dehydrogenase; *SF1*, steroidogenic factor 1; *StAR*, steroidogenic acute regulatory (protein).

Prenatal Diagnosis

Prenatal diagnosis of DSD is increasingly common as fetal ultrasonography improves and as more people have karyotype analysis or cell free fetal DNA for various reasons in pregnancy.[495] Although some prospective parents choose not to know the sex of their baby, many couples ask whether they are having a boy or a girl, and issues can arise when the genital appearance is unclear on the scan or when the appearance of the genitals is discordant with the known karyotype. These situations can generate great anxiety, and early contact with the DSD MDT is helpful to support the parent(s). Sometimes prenatal genetic testing such as a microarray is considered, especially when multiple congenital anomalies are suspected on ultrasound. However, prenatal genetic testing can occasionally produce inaccurate results that are not confirmed on karyotyping of the baby (e.g., in cases of twin fetal demise). Also, genital appearance at birth can differ from that seen on prenatal scans. Support to deal with the uncertainty and explanations of what will happen if their baby does have atypical genitalia are important, and discussion of specific diagnoses is best deferred until after birth. Nonetheless, prenatal suspicion of DSD can have benefits as it gives parents time to learn about possible conditions and to think how they will deal with the situation as long as support is provided in a sensitive manner.

The Newborn With Atypical Genitalia

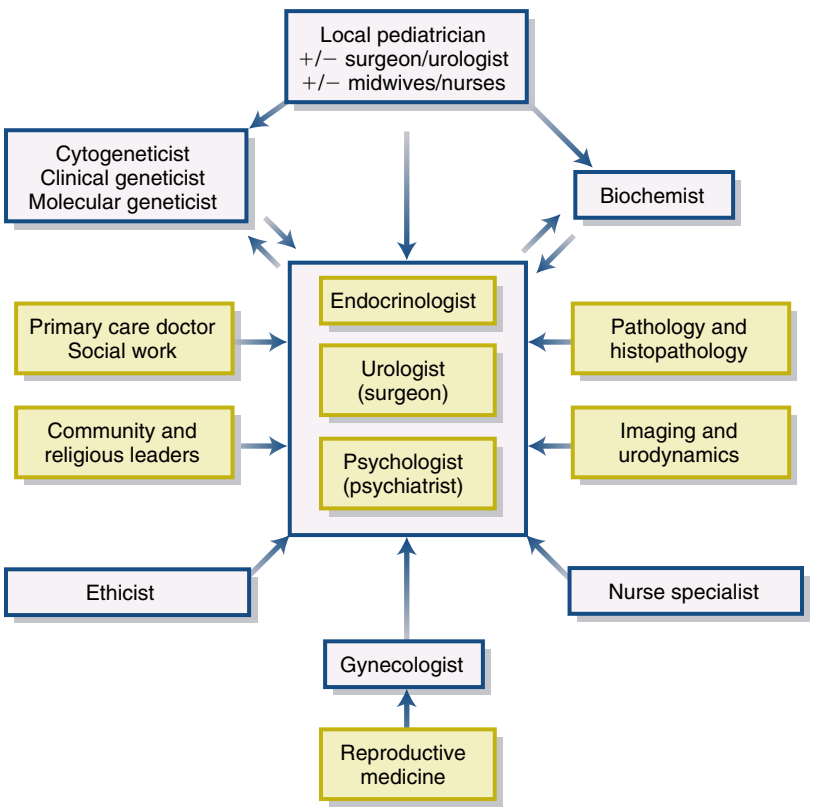
The most established presentation of DSD occurs when a newborn baby has atypical (or ambiguous) genitalia, and it is not possible to say immediately whether the baby is a boy or a girl. Sometimes atypical genital features are first discovered on the first full newborn examination rather than immediately at birth. Further investigations such as a karyotype are usually needed, and specialist review may be required. It is estimated that around 1 in 4500 babies cannot have a sex designated immediately at birth, although the exact incidence is not known. Some presentations

TABLE 24.19 The Range of Diagnoses of DSD Seen in a Single Center Over 2 Years

Sex Chromosome DSD (n = 13)	46,XY DSD (n = 65)	46,XX DSD (n = 23)
A: 47,XXY (Klinefelter syndrome and variants) (2)	A: Disorders of gonadal (testis) development	A: Disorders of gonadal (ovary) development
B: 45,X (Turner syndrome and variants) (2) (both Y fragment)	Complete gonadal dysgenesis (7)	Ovotesticular DSD (1)
C: 45,X/46,XY mosaicism (mixed gonadal dysgenesis and variants) (8)	Partial gonadal dysgenesis (8)	Testicular DSD (1)
D: 46,XX/46,XY (chimerism/mosaicism) (1)	Steroidogenic factor 1 (2)	
	B: Disorders in androgen synthesis or action	B: Androgen excess
	Disorders of androgen biosynthesis	Fetal
	StAR (1)	21-hydroxylase (10)
	17βHSD3 (2)	11β-hydroxylase (2)
	5α-reductase 2 (6)	Fetoplacental
	Disorders of androgen action	Maternal
	CAIS (6)	
	PAIS (5)	
	C: Other	C: Other
	Syndromic associations of male genital development (11) (e.g., chromosomal variants, skeletal, lung, skin, gastrointestinal, Cornelia de Lange, CHARGE)	Syndromic associations (e.g., cloacal anomalies) (1)
		Müllerian agenesis (1)
	Cloacal anomalies (1)	Clitoromegaly, possible clitoromegaly or clitoral variants (6)
	IUGR/preterm/hypospadias (4)	Ovarian cysts (1)
	Persistent müllerian duct syndrome (3)	
	Vanishing testis syndrome (2)	
	Isolated severe hypospadias (4)	
	Micropenis (1)	
	Bilateral undescended testes (2)	

CAIS, Complete androgen insensitivity syndrome; *CHARGE,* coloboma, heart defect, atresia choanae, retarded growth and development, genital hypoplasia, ear anomalies (deafness); *DSDs,* disorders of sex development; *IUGR,* intrauterine growth restriction; *PAIS,* partial androgen insensitivity syndrome; *StAR,* steroidogenic acute regulatory (protein).

From Brain CE, Creighton SM, Mushtaq I, et al. Holistic management of DSD. *Best Pract Res Clin Endocrinol Metab.* 2010;24:335–354.



• **Fig. 24.39** Overview of the multidisciplinary team (MDT). (From Brain CE, Creighton SM, Mushtaq I, et al. Holistic management of DSD. *Best Pract Res Clin Endocrinol Metab*. 2010;24:335–354.)

may be mistaken for a DSD condition, such as labial swelling after a breech delivery, undescended testes, or mild hypospadias, which can affect up to 1 in 300 male infants. Examples of genital features that should trigger consideration of a DSD condition are outlined in Table 24.20. In particular, any child with nonpalpable testes needs careful review because this child could—rarely, but importantly—be a 46,XX baby with severe, life-threatening CAH.

History and Examination

A careful history is important but must be done sensitively and rarely points to an immediate diagnosis. Key points to consider include aspects of the pregnancy such as growth restriction and preeclampsia, maternal acne or hair growth in pregnancy (which would suggest aromatase or POR deficiency), and any prenatal tests or scans performed. Theoretically, medications and drugs may have an effect on the fetus (e.g., 5α-reductase inhibitors), and assisted reproduction or the need for fertility treatment might indicate an underlying and variably penetrant genetic cause. A careful family history should include any history of adrenal conditions, known CAH or infant death, other known DSD, hypospadias, or infertility (with attention to X-linked patterns that could suggest androgen insensitivity syndrome), but such issues may not be known or widely discussed. Consanguinity is associated with an increased risk of autosomal recessive conditions such as many of the steroidogenic enzyme deficiencies. It is important to understand the parents' thoughts and beliefs and not to generate additional anxiety by insensitive questions.

A careful physical examination is needed but should be done sensitively and only after the parents have had some time to be with their baby. Key features to note are the length of the phallus, consistency of the corpora and glans, extent of chordee; scrotal transposition (extension of the scrotal folds above the phallus), features of the foreskin (hooded, excess skin), position of the urethral opening; presence or absence of a separate vaginal opening, rugosity (wrinkling of the scrotal skin, also called scrotalization) and degree of midline fusion of the labioscrotal folds, and size and position of any palpable gonads (likely to be testes). Genital appearances can sometimes be misleading because of chordee (bending and tethering of the phallus) and/or a large suprapubic fat pad, so palpation as well as inspection is important. Various scales such as the external masculinization score, the Prader scale, and the Quigley scale can be used to summarize findings and are particularly useful for research purposes, but it is also important to note and document individual features (Fig. 24.40).[376]

TABLE 24.20 Problems in Newborns That Merit Investigation for DSDs

Ambiguous (atypical) genitalia
Apparent female genitalia
Enlarged clitoris
Posterior labial fusion
Inguinal/labial mass
Apparent male genitalia
Nonpalpable testes
Isolated penoscrotal hypospadias
Severe hypospadias, undescended testes, micropenis
Family history of DSD, such as complete AIS
Discordance between genital appearance and prenatal karyotype

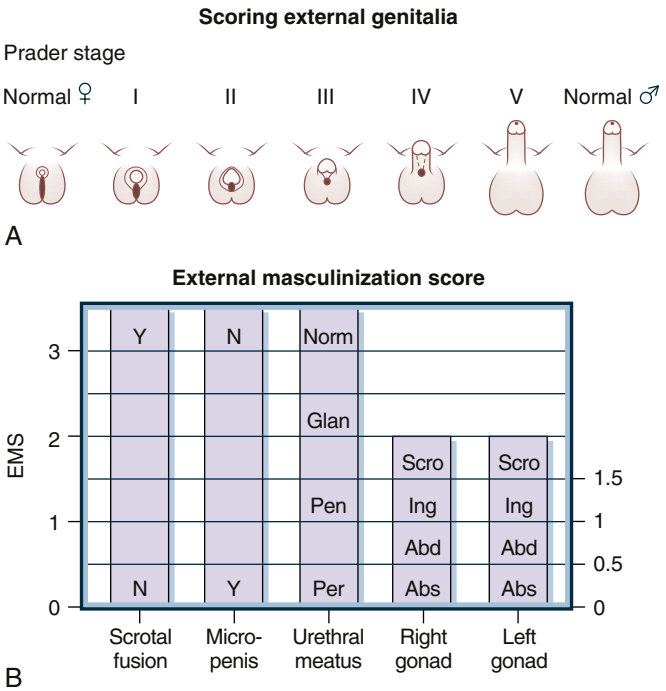
AIS, Androgen insensitivity syndrome; *DSDs*, disorders of sex development.

Asymmetry of the genitalia can suggest mixed gonadal dysgenesis associated with 45,X/46,XY mosaicism and can also be seen in partial gonadal dysgenesis or ovotesticular DSD. Other findings such as low birthweight; position of the anus; skeletal, cardiac, or other congenital anomalies; and dysmorphic features are important to note. Hyperpigmentation is a rare but important sign of primary adrenal insufficiency due to elevated ACTH, but increased labioscrotal pigmentation can often be seen in babies of Asian or African ancestry.

The Initial Approach to the Baby With Atypical Genitalia

A useful mnemonic for the initial approach is BASIC: *b*onding, *a*drenal insufficiency, *s*ex designation, *i*maging, and *c*ytogenetics. Each of these is introduced briefly here and discussed more extensively elsewhere.

Bonding between the parents and baby can be disrupted by the shock of the situation or if the newborn is taken away to an intensive care nursery without good reason. Allowing parents time to focus on their new baby is important, and putting the genital issue aside at times is key. At the same time, the impact of the situation should not be trivialized; striking the right balance requires an individualized approach involving frequent feedback from parents. Nursing staff can play a crucial role in helping families initially by engaging with them and acknowledging the stress that uncertainty can bring while also addressing routine newborn issues such as establishing feeding, changing diapers, and sleeping.



• **Fig. 24.40** (A) Prader staging for scoring the degree of androgenization of the external genitalia in a female infant with congenital adrenal hyperplasia. (B) The external masculinization score (EMS) is used to assess the degree of underandrogenization in an individual with 46,XY DSD. The score is based on the presence or absence of a micropenis and bifid scrotum, the location of the urethral meatus (normal, glandular, penile, perineal), and the position of the testes (scrotal, inguinal, abdominal/absent). (B, from Ahmed SF, Khwaja O, Hughes JA. The role of a clinical score in the assessment of ambiguous genitalia. *BJU Int*. 2000;85:120–124.)

Staff members themselves may feel uncomfortable and may also need support and guidance.

Adrenal insufficiency is associated with some causes of DSD and is potentially life threatening if not promptly recognized and treated. The common scenario is a 46,XX baby with 21-hydroxylase deficiency, which occurs in about 15% to 20% of babies with atypical genitalia, more in some studies. Adrenal insufficiency can also occur in 46,XY babies (e.g., StAR, CYP11A, HSD3B2, CYP17A1, NR5A1) but is rare. Monitoring for hypoglycemia, hyponatremia, and hyperkalemia is necessary, but the electrolyte changes usually take several days to manifest. During this time the infant can be kept with its mother if otherwise well. Micropenis and/or undescended testes may be caused by gonadotropin deficiency, which can be associated with deficiency of other pituitary hormones, including ACTH.

Sex designation should be undertaken as soon as possible but should not be rushed. Parents sometimes have strong preconceptions about their baby, especially if they have been told prenatally to expect a boy or a girl, and/or may want a child of a specific sex to fit their desired family structure. If wrong decisions are made, it can be very traumatic to reverse them, so keeping an open mind from the start and avoiding premature recommendations is critical.[496] In the interim, it is advisable to use neutral terms such as "phallus" rather than "penis" or "clitoris," and to refer to the child as "your baby," "your child," or "little one" rather than "he" or "she."

Imaging is useful but can be operator dependent, and results can sometimes be deceptive. Pelvic ultrasound or MRI can show internal structures, but structures are not always clearly visualized, a utricular remnant (commonly found in boys with hypospadias) can be confused with a uterus, and lymph nodes can be confused with testes.[228,497] Parents and providers need to know the limitations of these tests.

Cytogenetics, or a rapid assessment of sex chromosome complement, is essential. This testing has been done traditionally by fluorescence in situ hybridization for X-chromosome and Y-chromosome markers (see Fig. 24.3), but in some centers is now being performed by quantitative fluorescent polymerase chain reaction (qfPCR) on DNA. The relevant laboratories should be informed of the urgency of the test, and samples should be transported quickly. An attempt to look for mosaicism should be made by determining the genotype in a sufficient number of cells, but FISH on a second tissue (such as a buccal smear) may be necessary to detect mosaicism. Rapid karyotyping can help with the next stages of management and investigation, and a result is usually available within 2 working days.

Support for the Parents

Providing parents with adequate support in the first days while awaiting results is essential.[491] It can be useful to stress that many other parents are in a similar position each year, that specialists who have knowledge and experience are available, and that the situation will become clearer in the next days. Expressions of anxiety by the staff may exacerbate anxiety in the parents. On the other hand, excessive nonchalance may be viewed as trivializing a serious situation. Parents need to be provided with information about the tests performed and a realistic expectation of when results will be available. They should be encouraged to ask questions and to rediscuss information that is not clear or difficult to understand. Sometimes recording conversations can be helpful so that families have an opportunity to listen to information again.

Being repeatedly asked by family and friends if they have a boy or a girl can be highly stressful for parents. There is no single strategy that suits all parents, but many seek the safe haven of the hospital and will try to delay announcing the birth until the situation is clearer. It is reasonable to ask friends for some private time and to say that they will be in touch soon with more information. It often helps parents to confide in one or two close friends or family members; sometimes parents can be under pressure with work, and siblings may have questions or need care. Several resources have been developed to help support parents during this time.[498] It is important to emphasize to parents that they need to look after themselves, too.

Sex Designation

Most people wish to raise their child as either a boy or a girl. Sufficient information should be gathered so that parents can be informed and educated to make the best decisions for their child. This should be done as quickly as practical but without rushing decisions. The karyotype per se does not determine gender identity, but it is a useful starting point for focusing investigations and reaching a diagnosis (see Table 24.3 for classification of DSDs). A broad diagnosis is often sufficient for designating sex, and a more specific diagnosis can be reached later. Within the context of the karyotype and diagnosis, useful points to consider are the child's future gender identity, functional anatomic aspects such as likely urologic and sexual function, potential endocrine function (whether consistent or inconsistent with designated sex), fertility options, and the risk of gonadal tumors. Many of these questions are difficult to answer, and in some situations more investigations may be undertaken to inform the parents, but often the choice is relatively straightforward based on findings from physical examination, imaging studies, and karyotype. Current recommendations are for 46,XX individuals with congenital adrenal hyperplasia to be raised as girls, though some have suggested that those with highly androgenized genitalia may be raised as boys.[499] A female sex designation is appropriate for 46,XY individuals with complete androgen insensitivity syndrome or complete gonadal dysgenesis (Swyer syndrome), who have female-appearing external genitalia and are highly likely to identify as women in adulthood; a female sex designation is probably also appropriate for those with minimal androgenization (e.g., isolated clitoromegaly). Particularly challenging situations arise in babies with limited phallic development and a 46,XY karyotype who have intact testosterone responsiveness (such as 17β-hydroxysteroid dehydrogenase deficiency, 5α-reductase deficiency, and partial testicular dysgenesis) or with mixed internal and external phenotypes (e.g., 45,X/46,XY or various forms of ovotesticular DSD). In the past there was a greater focus on the genitalia and a tendency to raise children with a very small phallus as female. More recently, there has been a trend to raise more 46,XY children as male and to look at other aspects of the child such as likely gender identity, possible fertility, and endocrine function.[392] Sometimes, cultural influences can be important for the parents.[500] In most countries there is a time limit to register the birth of a baby (e.g., 42 days in England and Wales), but this can usually be deferred or done without defining sex (e.g., in Germany).

Investigations for DSDs

Once the initial analysis of sex chromosomes is available, several investigations can be undertaken to reach a more specific diagnosis or to obtain a more detailed assessment of urogenital anatomy and future function.[2,492] It is difficult to develop rigid guidelines for investigations because local availability and affordability of tests around the world vary greatly.[501] Details of some of the commonly used tests for DSD are shown in Table 24.21, and potential investigations based on karyotype are shown in Fig. 24.41. It is important to consider what the likelihood of a specific diagnosis might be. An overview of diagnoses discussed in our setting is shown in Table 24.19, but in areas of the world with greater consanguinity, the relative prevalence of recessive conditions such as CAH is greater. There may also be hotspots of specific diagnoses due to founder effects (e.g., 5α-reductase in the Dominican Republic), so knowledge of the local prevalence of certain conditions is important. Genetic testing is more widely available and may sometimes avoid the need for invasive tests or prolonged endocrine stimulation, but it has certain drawbacks, which are discussed later.[335]

Chromosomal DSD

In our experience, approximately 10% to 15% of newborn referrals have a chromosomal DSD (usually 45,X/46,XY), and an individualized approach is needed.[3,207] This includes assessment of anatomy and gonadal function and, if indicated, screening for Turner syndrome–associated features (e.g., renal and cardiac conditions) (see Fig. 24.41). Sometimes an early examination under

TABLE 24.21 Potential Investigations for DSDs

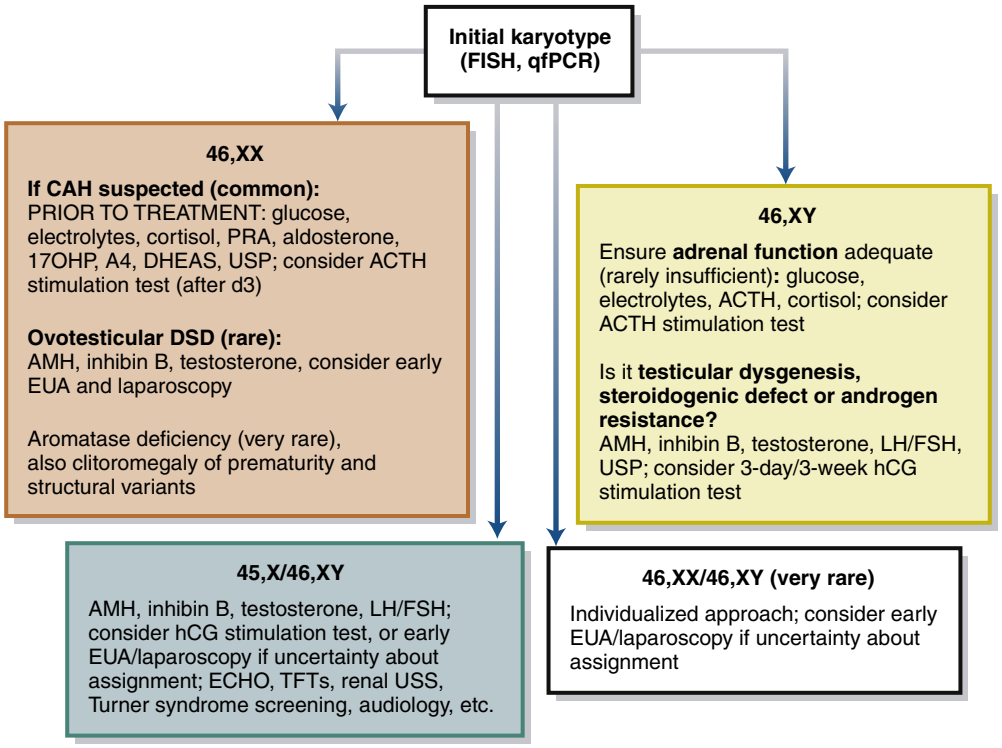
Approach	Test	Uses
Genetics	FISH[a] (X-specific and Y-specific probes)	Rapid analysis of sex chromosome complement on cells
	qfPCR[a]	Rapid analysis of sex chromosome signal in DNA
	Karyotype[a]	Analysis of sex chromosomes and autosomes in cells with ability to look for mosaicism by screening multiple cells, as well as detection of major deletions, duplications, and balanced translocations
	Array CGH or SNP microarray[a]	Analysis of chromosome signal across the genome, with ability to detect smaller copy number variants but not balanced translocations, using DNA
	Multiple ligation probe-dependent amplification	Analysis of the loss or gain of specific exons or whole genes on a predefined panel of probes, such as for DSD genes, using DNA
	Single-gene analysis	Sanger sequencing and analysis of individual genes that are highly likely to be the cause of DSD based on incidence and clinical and biochemical features (e.g., *CYP21A2*)
	Targeted panel sequencing	Analysis of large numbers of known DSD-causing genes using high throughput sequencing of DNA
	Whole-exome sequencing	Analysis of all the coding exons in the DNA, which may show changes in known, putative, or novel DSD-associated genes, using high-throughput sequencing
Endocrine	Routine serum biochemistry[a]; urinalysis[a]	May reveal a salt-losing crisis or associated renal disorder (e.g., WT1)
	17-hydroxyprogesterone,[a] 11-deoxycortisol, 17-hydroxypregnenolone	May help to diagnose CAH or reveal a specific block in an adrenal pathway relevant to DSD
	Renin, ACTH	May show a salt-losing state or primary adrenal insufficiency
	Testosterone,[a] androstenedione, DHT; in the basal state or following hCG stimulation	Indicates the degree of androgen production and ratios of androgens and may help to diagnose a block in androgen production consistent with a specific diagnosis (e.g., 17βHSD or 5α-reductase deficiencies); can also reveal androgen production in ovotesticular DSD
	Gonadotropins	May indicate an underlying block in steroidogenesis or androgen insensitivity (LH), or impaired Sertoli cell function (FSH), or gonadal dysgenesis (FSH and LH)
	AMH, inhibin B	Can be useful markers of testicular integrity: AMH is detectable throughout childhood and is reduced in testicular dysgenesis or absent in case of streak gonads or anorchia; AMH may be high in AIS or reduced androgen production due to steroidogenic defects; AMH may help to reveal the presence of testicular tissue in 46,XX (ovo)testicular DSD
	Urinary steroids by GC/MS	Can be used to diagnose specific steroidogenic defects in the newborn period (e.g., 21-hydroxylase deficiency, 11β-hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase deficiency, P450 oxidoreductase deficiency, 17α-hydroxylase deficiency); can reveal 5α-reductase deficiency only after 3–6 months of life
	Dynamic tests: ACTH stimulation	Used to assess the adrenal gland stress response (quantitative) and can be coupled with measurement of steroid metabolites or poststimulation urine steroid analysis to study ratios of metabolites (diagnostic)
	hCG stimulation	Used in short (e.g., 3 day) or prolonged (e.g., 3 week) formats to assess androgen production (quantitative) and androgen biosynthesis pathways (diagnostic); can also be used to assess the presence of testicular tissue (e.g., anorchia, ovotestis), although AMH is now more often used initially
	FSH stimulation test	Rarely used to investigate the presence of ovarian tissue by measuring inhibin A and estradiol response

TABLE 24.21 Potential Investigations for DSDs—cont'd

Approach	Test	Uses
Imaging	Abdominopelvic and renal ultrasound[a]	Can reveal the size, position, and structure of gonads (especially testes), the presence of müllerian structures, and associated changes (such as renal size or anomalies)
	MRI	Sometimes used to assess internal structures, especially in adolescence
	Cystourethroscopy, sinogram	Can reveal the structure of the bladder, vagina, and common channel
Surgical	Laparoscopy	Can reveal internal structures by direct visualization, such as gonads and müllerian structures
	Gonadal biopsies	Can be used to determine the nature of gonads, especially if dysgenetic testes or ovotestes are suspected

[a]Indicates first-line investigations for which results are available within days. For images of G-banded karyotypes and FISH analysis, see Fig. 23.3.

ACTH, Adrenocorticotropic hormone; *AIS,* androgen insensitivity syndrome; *AMH,* antimüllerian hormone; *CAH,* congenital adrenal hyperplasia; *CGH,* comparative genomic hybridization; *DHT,* dihydrotestosterone; *DSDs,* disorders of sex development; *FISH,* fluorescent in situ hybridization; *FSH,* follicle-stimulating hormone; *GC-MS,* gas chromatography–mass spectrometry; *hCG,* human chorionic gonadotropin; *LH,* luteinizing hormone; *MRI,* magnetic resonance imaging; *qfPCR,* quantitative fluorescent polymerase chain reaction; *SNP,* single-nucleotide polymorphism.



• **Fig. 24.41** Overview of potential investigations for a newborn with atypical genitalia or disorder of sex development (DSD) once the initial karyotype or assessment of sex chromosome complement has been made. *A4,* androstenedione; *ACTH,* adrenocorticotropic hormone; *AMH,* antimüllerian hormone; *CAH,* congenital adrenal hyperplasia; *DHEAS,* dehydroepiandrosterone sulfate; *ECHO,* echocardiogram; *EUA,* examination under anesthetic; *FISH,* fluorescent in situ hybridization; *FSH,* follicle-stimulating hormone; *hCG,* human chorionic gonadotropin; *LH,* luteinizing hormone; *17-OHP,* 17-hydroxyprogesterone; *PRA,* plasma renin activity; *qfPCR,* quantitative fluorescent polymerase chain reaction; *TFT,* thyroid function test; *USP,* urine steroid profiling; *USS,* ultrasound screening.

anesthesia and laparoscopy is performed to assess anatomy and to determine gonadal characteristics and histologic features.

46,XX DSD

The majority of babies with a 46,XX DSD have 21-hydroxylase deficiency, although other rarer forms of CAH, ovotesticular DSD, and clitoromegaly associated with prematurity are seen. Children have variable genital appearances; sometimes the baby may have a clitoris that is naturally large (Table 24.22) or appears slightly more prominent or with excess clitoral skin. However, any 46,XX baby with atypical genitalia must be assumed to be at risk of adrenal insufficiency until proved otherwise. Serial electrolytes should be monitored and tests to confirm or refute the diagnosis

TABLE 24.22 Anthropometric Measurements of the External Genitalia (Mean ± SD)

Population	Age	Stretched Penile Length (cm) or Clitoral Length (mm)	Penile Width (cm) or Clitoral Width (mm)	Testicular Volume (mL) or Perineum Length (mm)
Male				
United States	30 weeks GA	2.5 ± 0.4 cm		
United States	Full term	3.5 ± 0.4 cm	1.1 ± 0.1 cm	0.52 mL (median)
Japan	Term 14 years	2.9 ± 0.4 cm 8.3 ± 0.8 cm		
Australia	24–36 weeks GA	2.27 + (0.16 × GA) cm		
China	Term	3.1 ± 0.3 cm	1.07 ± 0.09 cm	
India	Term	3.6 ± 0.4 cm	1.14 ± 0.07 cm	
North America	Term	3.4 ± 0.3 cm	1.13 ± 0.08 cm	
Europe	Adult	13.3 ± 1.6 cm		16.5 to 18.2 mL
Female				
United States	Full term	4.0 ± 1.24 mm	3.32 ± 0.78 mm	
United States	Adult, nulliparous	15.4 ± 4.3 mm		
United States	Adult	19.1 ± 8.7 mm	5.5 ± 1.7 mm	31.3 ± 8.5 mm

GA, Gestational age; *SD*, standard deviation.

Data from Cheng PK, Chanoine JP. Should the definition of micropenis vary according to ethnicity? *Horm Res*. 2001;55:278–281; Feldman KW, Smith DW. Fetal phallic growth and penile standards for newborn male infants. *J Pediatr*. 1975;86:395–398; Fujieda K, Matsuura N. Growth and maturation in the male genitalia from birth to adolescence. II. Change of penile length. *Acta Paediatr Jpn*. 1987;29:220–223; Lloyd J, Crouch NS, Minto CL, et al. Female genital appearance: "normality" unfolds. *BJOG*. 2005;112:643–646; Oberfield SE, Mondok A, Shahrivar F, et al. Clitoral size in full-term infants. *Am J Perinatol*. 1989;64:53–54; Schonfield WA, Beebe GW. Normal growth and variation in the male genitalia from birth to maturity. *J Urol*. 1942;48:759–777; Tuladhar R, Davis PG, Batch J, et al. Establishment of a normal range of penile length in preterm infants. *J Paediatr Child Health*. 1998;34:471–473; Verkauf BS, Von Thron J, O'Brien WF. Clitoral size in normal women. *Obstet Gynecol*. 1992;80:41–44; Zachmann M, Prader A, Kind HP, et al. Testicular volume during adolescence: cross-sectional and longitudinal studies. *Helv Paediatr Acta*. 1974;29:61–72; from Hughes IA, Houk C, Ahmed SF, et al. Consensus statement on management of intersex disorders. *Arch Dis Child*. 2006;91:554–562, used with permission.

should be undertaken according to local protocol and assay availability (such as 17-OHP, basal and stimulated cortisol, ACTH, plasma renin activity, and urine steroid profiling). Results can be inaccurate before 3 days of age using conventional assays due to interfering substances, although liquid chromatography/tandem mass spectrometry (LC/MS-MS) assays may be more useful if available.[502,503] Steroid replacement treatment can be started once the diagnosis is clear and any additional samples obtained. Of note, 17-OHP can be mildly elevated in other forms of CAH (e.g., 11β-hydroxylase deficiency, 3βHSD, POR), and investigation of steroid intermediates (e.g., 11-deoxycortisol, 11-deoxycorticosterone, 17-hydroxypregnenolone) and urine steroid profiling can be useful in these situations.[350] If all adrenal investigations are negative, rare diagnoses such as 46,XX ovotesticular DSD and aromatase deficiency should be considered and AMH measured to investigate for testicular tissue.[492] The presence of ovarian tissue can be difficult to assess in early infancy without laparoscopy or even biopsy. Occasionally, follicles can be seen on imaging, or there can be a rise in estradiol and inhibin A following recombinant FSH stimulation, but such tests are rarely performed.[175]

46,XY DSD

The most common newborn karyotype in our experience is 46,XY, which occurs in approximately 60% of babies with atypical genitalia. These children can have a range of conditions, including variable forms of partial testicular dysgenesis, disorders of androgen synthesis and action, or other diagnoses such as proximal hypospadias of unknown cause or a DSD-associated syndrome. In some situations, the diagnosis might influence decision making. For example, some clinicians would advocate raising a baby with 5α-reductase deficiency as a boy and giving dihydrotestosterone cream, whereas a child with androgen resistance causing a more severe form of PAIS might be raised as a girl. Basic investigations in the 46,XY baby can include karyotype or array analysis looking for established chromosomal alterations (e.g., 9q deletion, 10p deletion, Xp21 duplication) and basal assessment of testis integrity (e.g., AMH/MIS, androgens).[2,125] Additional diagnostic tests to assess androgen synthesis include basal androgens (DHEA, androstenedione, testosterone, DHT) and short-term and long-term hCG-stimulated androgens.[504] Basal androgens may be low initially and can be reassessed during minipuberty, around age 6 to 8 weeks. The ratios of intermediates after hCG stimulation can be useful in diagnosing specific blocks, such as 17β-hydroxysteroid dehydrogenase deficiency (testosterone to androstenedione ratio often <0.8) and 5α-reductase deficiency (testosterone to dihydrotestosterone ratio often >10–20).[349] However, these assays may not be accurate in the first few days of life unless performed by LC/MS-MS, and diagnosing these conditions by androgen ratios or urine steroid profiling can be difficult in the first few

months of life, possibly because of other pathways or isoenzymes influencing the results.[350] Adrenal disorders do occur but are rare. Most children with complete blocks in steroid production (e.g., StAR, CYP11A1) do not present with atypical genitalia but rather present as a phenotypic girl with a salt-losing crisis, and the 46,XY karyotype is only found afterward. Atypical genitalia at birth can be a sign of *partial* defects in high steroidogenic enzymes (e.g., partial StAR, CYP11A1) or *partial* 17α-hydroxylase insufficiency (CYP17A1), as well as classic presentations of 3β-hydroxysteroid dehydrogenase deficiency (3βHSD2) or P450 oxidoreductase deficiency (POR). It is currently unclear whether detailed investigations of adrenal function are justified in all boys with 46,XY DSD, but an adrenal diagnosis should at least be considered.

Genetic Testing and DSDs

Genetic testing plays an increasingly important role in the diagnostic process.[505] It is important for reaching a specific diagnosis (for both the individual and other affected family members), as well as counseling about the risk of recurrence in future children, defining the need to look for associated features, and in some situations providing guidance on issues such as potential gender identity, endocrine function and fertility, and tumor risk.[334] Reaching a specific genetic diagnosis can provide a sense of resolution and reduce uncertainty but may generate anxiety if no cause is found or if incidental genetic information is uncovered. Parents may experience guilt or blame for transmitting a mutation to their child. Individuals and families should have a clear understanding about what genetic tests are being performed and what the potential benefits and disadvantages might be. Genetic testing can be expensive and may not be available or affordable locally, especially as a clinical service.[334]

Approaches to genetic testing are changing rapidly with new technologies for the analysis of chromosomes as well as individual genes. In some centers, sex chromosome FISH is being replaced by qfPCR, and microarray or array-based comparative genomic hybridization (CGH) approaches are being used to replace or supplement traditional karyotype analysis. These technologies can detect much smaller copy number variations than traditional G-banded karyotypes but do not detect balanced translocations and, as with traditional karyotyping, may miss low levels of mosaicism. Multiplex ligation-dependent probe amplification (MLPA) is a method of looking for specific copy number changes (deletions or duplications) in known DSD genes (e.g., duplication of *NR0B1*, or loss of exons of other genes) that are too small to be detected on arrays.

Analysis of single genes is also changing with the development of next-generation sequencing approaches. Traditionally, candidate genes for a condition were amplified one by one by PCR and analyzed by Sanger sequencing. This direct sequencing approach is still the method of choice when there is a clear candidate gene from biochemical analysis (e.g., *HSD17B3, SRD5A2*), especially when the presence of a pseudogene can make sequencing difficult (e.g., *CYP21A2*).[334] However, this approach is relatively time consuming and expensive, particularly if there are several candidate genes. Newer approaches involve using NGS to sequence panels of genes relevant to DSD or sequencing all of the exome or even whole genomes.[176,506] These methods can provide high throughput analysis but are still expensive, generate large amounts of data (including so-called bystander or incidental data for other genes, which may or may not be desired), and are still mostly only available as research tools, although increasingly these approaches are used in clinical testing. Positive findings can be validated by focused clinic testing, which can then provide useful information for the family and clinician. Interpretation of identified variants in DSD genes can be challenging and may require detailed information on anatomy and endocrine function in the affected individual, genetic analysis in family members (to look at segregation of the variant with the phenotype), or (in vitro) functional studies of the variant.

Given the advances in genetics and increasing recognition that DSD can be a feature of many well-defined syndromes, a clinical geneticist can be a very valuable member of the MDT, especially when a child needs to be examined for additional syndromic features or when the family needs to be counseled about genetic testing and the significance of any results.

Presentation During Childhood

DSD can occasionally present during the childhood years. Sometimes a parent may notice genital differences for the first time in childhood or feel inguinal masses that turn out to be testes. A small proportion of inguinal hernias in girls are found to contain testes, most often in patients with CAIS. Sometimes associated features present for the first time in childhood; for example, a small proportion of boys with penoscrotal hypospadias develop an abdominal mass due to a Wilms tumor and are found to have Denys-Drash syndrome (due to mutations in *WT1*).

Presentation During Adolescence

Another common time for DSD to present is during adolescence in one of three well-recognized ways: (1) a girl who experiences spontaneous virilization at puberty, (2) a girl with absence of pubertal development, and (3) a girl who has primary amenorrhea after having normal breast development.[2] Although the situation is not as urgent as in the newborn who may be at risk of salt-wasting CAH, it is equally if not more important to handle the situation with sensitivity, as the young person is aware of the issues as well as the parents and must be involved in decision making and consent.

The conditions that are typically associated with virilization at puberty are 5α-reductase deficiency type 2 and 17β-hydroxysteroid dehydroxygenase deficiency type 3. Alternative diagnoses include cases in which previous clitoromegaly may have been overlooked in partial testicular dysgenesis (e.g., mutations in *SF1*/*NR5A1*),[507] partial androgen insensitivity syndrome, or testicular/ovotesticular DSD. Usually the diagnosis is relatively straightforward based on serum concentrations and ratios of androgens, urine steroid profiling, and genetic testing.[2] It is best to avoid further stimulation with hCG tests if virilization is undesired, and some have proposed that puberty can be blocked for a period with a GnRH analog, or if not available, antiandrogens to provide time to obtain a genetic diagnosis, educate and counsel the young person and the family, and give them time make a decision. An MDT approach is invaluable, and the input of an experienced psychologist or expert in gender medicine is key. A proportion of young people with 5α-reductase deficiency will choose to transition from female to male, and this has also been reported for some individuals with 17-HSD deficiency.[151,152] Many girls with these conditions continue to identify as females and often want to have their gonads removed; appropriate counseling about risks,

benefits, and fertility options is essential. If gonads are dysfunctional or removed, puberty will need to be induced with estrogens. Gathering and sharing the information and providing psychological support over a period of time can help ensure that the young person makes the best choices for the future.

Another presentation in adolescence is a girl who does not have any pubertal development. This situation may represent many diagnoses in a 46,XX girl, including constitutional pubertal delay, hypogonadotropic hypogonadism, and congenital or acquired (e.g., autoimmune) primary ovarian insufficiency. Absence of pubertal development may also be the presenting feature of sex chromosomal DSD (Turner syndrome) or a 46,XY DSD (complete gonadal dysgenesis or 17α-hydroxylase/17,20-lyase deficiency). In complete testicular dysgenesis (Swyer syndrome), both Leydig cell production of testosterone and Sertoli cell production of AMH are impaired. As a result, müllerian structures are typically present. The immature uterus may be difficult to see, even on MRI scan, but may become more easily visualized following estrogen treatment (the "clandestine" uterus).[508] Dysgenetic gonads, which may appear streak-like (i.e., flat and scar-like), have a high tumor risk even in prepubertal children and should therefore be removed. True streak gonads (i.e., gonads that consist of fibrous tissue and lack germ cells and support cells) are not at risk of germ cell cancers (GCC) by definition, but this can only be confirmed at histologic examination. The underlying cause of complete gonadal dysgenesis may be a range of genetic conditions (see Table 24.6), but a cause is often not found. Puberty can be induced with estrogens. In contrast, 46,XY girls with 17α-hydroxylase/17,20-lyase deficiency have intact Sertoli cell function and AMH production, and as a result they do not have a uterus. The block in adrenal steroidogenesis can be associated with hypertension and hypokalemia, which can cause arrhythmias, so this is a rare but important diagnosis to make. Estrogen treatment is needed. The gonads are typically undescended but not dysgenetic, and the risk for GCC in pubertal children is likely to be low, but there may be an increased risk of germ cell tumors in late adolescence and adulthood. Two large studies of gonadal tumors in XY DSD individuals, which included 42 females with 17α-hydroxylase/17,20-lyase deficiency, reported one dysgerminoma, two Sertoli cell tumors, and one Leydig cell tumor.[509,510] A decision about gonadectomy should be made after discussing these risks.

The third common presentation is with primary amenorrhea in a girl who has experienced breast development. Acquired hypogonadotropic hypogonadism should be considered, and primary ovarian insufficiency may also be associated with some breast development but absent menarche. Hormone measurements, a karyotype, and pelvic ultrasound are often useful initial investigations.[2] If a uterus is visualized, obstruction of the outflow tract is likely, such as imperforate hymen; this is usually accompanied by cyclic abdominal pain. In the absence of a uterus, the diagnosis is most likely a form of uterine agenesis, such as MRKH syndrome if the karyotype is 46,XX and complete androgen insensitivity syndrome (CAIS) if the karyotype is 46,XY. Girls with CAIS often have reduced pubic and axillary hair. Detailed discussion about the management of these conditions is provided earlier in this chapter (see "Complete Androgen Insensitivity Syndrome").

Presentation During Adulthood

Occasionally, DSD may first present in adulthood. Examples include mild variants in DSD genes associated with male factor infertility or ovarian insufficiency (e.g., *NR5A1*), conditions such as persistent müllerian duct syndrome when a uterine-like structure is found incidentally in a man, or when a person with a more typical form of DSD presents to health professionals later in life, sometimes after migration from a medically underserved area or from one country to another.

Information Sharing, Transitioning, and Adult Services

Information sharing (disclosure) is an important part of educating people about their condition and giving them insight into the future. In the past, there was more secrecy around DSD, but most research supports sharing information in an age-appropriate manner over time.[511] Sometimes parents need considerable help in how to deal with questions and concerns, and psychological support is important to allow them to feel confident in discussing these issues with their child.[491,512,513] Education is an ongoing process and must account for ongoing changes with the child's cognitive development.[511,514] Different families may prefer different strategies, but being prepared for an open dialogue when opportunities arise usually works well. Sometimes a psychologist or pediatric endocrinologist can provide information at key stages and support the family in reinforcing information or answering questions as they come up. It is important to discuss what terminology the child and parents prefer; most people prefer the use of the specific diagnosis rather than the general term DSD.[515]

During childhood, growth and development are monitored. In adolescence, hormone replacement therapy may be indicated in cases of gonadal dysfunction, or as discussed, in some situations puberty may be blocked to delay the progression of virilization.

Transitioning the care of young people from pediatric to adult services is another important aspect of the care of DSD management.[516,517] Often the pediatric endocrinologist will induce puberty before transfer to an adult endocrinologist, but it may be useful to involve the adult provider at an earlier stage so that the young person can build trust with the doctor. In childhood and later, any genital examinations should be performed only when necessary and ideally by someone with experience who will be involved in long-term care. Photography should be avoided unless absolutely necessary and then only with consent.

As more young people transition into adult services, the need for specialist adult centers for DSDs is becoming apparent. Standardized follow-up has been proposed to detect and treat health problems associated with DSD in adulthood.[514] The team still largely includes an endocrinologist, psychologist, urologist, and gynecologist, and radiologists can be important team members, especially when discussing young people with retained gonads or complex anatomy. Multidisciplinary clinics in which all team members are available are useful for coordinating care and reducing the number of hospital visits and time away from work. The key issues in long-term management of DSDs include hormone replacement, bone health, sexual activity, relationship issues, genital/urinary issues, psychological well-being, fertility/family building, and other factors that contribute to overall quality of life.[518–520] Some data are now emerging on outcomes in adulthood for general medical issues and quality of life (see later).

Support Groups and Information

Support groups can also have a very important role in providing additional information for children, adolescents, and adults with

DSD and for bringing members of the community together.[521] There are well-established support groups in several countries for general aspects of DSD as well as for specific conditions such as CAH and AIS (for a recent overview, see Lee and colleagues[522]). Increasingly accessible information about DSD is becoming available through the internet, such as the biology of DSDs,[523] support for families (e.g., dsdfamilies.org),[498,524] and information for young people about DSDs (e.g., dsdteens.org).[525] It is useful to guide young people and families in how to search for information, as general search terms such as *sex* can result in many inappropriate hits. Patients and families should also be counseled that there is great variability within and between DSD conditions, and thus some information may not be applicable.

Tumor Risk and DSDs

The gonads of individuals with DSD with Y chromosomal material containing the GBY region are at risk of malignant change, but the incidence varies widely, depending on the underlying diagnosis (Table 24.23). Precise risk assessment per diagnosis is hampered by the lack of a molecularly confirmed diagnosis in various studies, report bias, inconsistent criteria to define precursor lesions, and the practice of early gonadectomy that has limited information on the natural history. In general, forms of DSD with impaired or arrested gonadal development have a high risk of germ cell cancers, whereas forms with normal testis development but reduced androgen synthesis or action have a lower risk, especially in childhood.[384] Scrotal testes are thought to be at lower risk of GCC development than undescended testes.[384] True streak gonads (i.e., gonads that consist of fibrous tissue and lack germ cells) are by definition not at risk of GCC, but this can only be concluded at histologic examination. Streak-like gonads (i.e., gonads that appear scar-like and lack endocrine function) may in fact contain germ cells, and these dysgenetic gonads are at risk of GCC.

Various subtypes of GCC exist: seminomatous cancers, on the one hand, classified as seminoma in the testis, dysgerminoma in the ovary and dysgenetic gonad, and germinoma when located extragonadally; nonseminomatous cancers, on the other hand, including teratoma, yolk sac tumor, choriocarcinoma, and embryonal carcinoma.[384] The development of GCC is preceded by abnormal germ cell development.

In poorly differentiated gonads, germ cells may be present without the formation of follicles or seminiferous tubules; this is known as *undifferentiated gonadal tissue*. These germ cells may develop into a gonadoblastoma, which is the precursor lesion of a dysgerminoma.[384] If the gonad is more testicularized, the germ cells are present in seminiferous tubules, although these may remain immature due to reduced synthesis or action of androgens. Delayed germ cell maturation is often seen in DSD and is characterized by luminal gonocytes that show prolonged expression of markers such as POU5F1 (also known as OCT3/4) that are normally only expressed during early life. Once these germ cells have migrated to the basal membrane but continue to express POU5F1, coexpress TSPY in a heterogeneous pattern, and show focal KITLG expression, they are classified as pre-GCNIS.[384] If germ cells show cytonuclear atypia with homogeneous TSPY expression and diffuse KITLG expression, then GCNIS is diagnosed.[384] GCNIS is the precursor lesion of GCC in an undervirilized testis. GCNIS was previously known as *carcinoma in situ, testicular intraepithelial neoplasia,* or *intratubular germ cell neoplasia, unclassified*.

In the general population, GCNIS is thought to progress to invasive GCC in the majority of cases, but this progression seems rare in CAIS, suggesting that androgens influence this progression.[384,385]

The risk of GCC can be estimated using information from clinical assessment, endocrine and genetic evaluation, and imaging. Gonadal biopsy is the gold standard, although it is important

TABLE 24.23 Risk of Germ Cell Malignancy According to Diagnosis

Risk Group	Disorder	Malignancy Risk (%)	Recommended Action	No. Studies	No. Patients
High	GD[a] (+Y)[b] intraabdominal	15–35	Gonadectomy[d]	12	350
	Partial AIS[c] nonscrotal	50	Gonadectomy[d]	2	24
	Frasier syndrome	60	Gonadectomy[d]	1	15
	Denys-Drash syndrome (+Y)	40	Gonadectomy[d]	1	5
Intermediate	Turner syndrome (+Y)	12	Gonadectomy[d]	11	43
	17βHSD	28	Watchful waiting	2	7
	GD (+Y)[b] scrotal	Unknown	Biopsy[e] and irradiation?	0	0
	Partial AIS scrotal gonad	Unknown	Biopsy[e] and irradiation?	0	0
Low	Complete AIS[f]	2	Biopsy[e] and ?	2	55
	Ovotesticular DSD	3	Testis tissue removal?	3	426
	Turner syndrome (–Y)	1	None	11	557
No (?)	5α-reductase	0	Unresolved	1	3
	Leydig cell hypoplasia	0	Unresolved	1	2

[a]GD including disorders not further specified, 46XY, 45X/46XY, mixed, partial, and complete.

[b]GBY region positive, including the *TSPY* gene.

[c]More recent reports suggest a lower risk, comparable to that in CAIS.[378,394]

[d]At time of diagnosis.

[e]At puberty, allowing investigation of at least 30 seminiferous tubules, preferentially diagnosis based on OCT3/4 immunohistochemistry.

[f]This risk estimate concerns postpubertal individuals; the risk is lower before puberty.[387]

AIS, Androgen insensitivity syndrome; *DSDs,* disorders of sex development; *GD,* gonadal dysgenesis; *17βHSD,* 17β-hydroxysteroid dehydrogenase.

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that appropriate immunohistochemical markers are used and that the pathologist has a high level of expertise, and one should be aware that a biopsy may not be representative of the entire gonad. Genetic predisposition to GCC development is thought to play a role; SNPs in candidate genes for disease development have been investigated as predictors of GCC risk but were found to have insufficient discriminatory value to use in clinical practice.[394]

The highest risk for early gonadoblastoma occurs with Frasier syndrome (caused by mutations in *WT1*), for which the risk may be as high as 60%.[212]

In individuals with gonadal dysgenesis due to a 45,X/46,XY karyotype, the prevalence of gonadoblastoma has been estimated to be 15% to 40%.[212]

The risk of GCC is lower in AIS[385] (see "Disorders of Androgen Action"). Fewer data are available on the risk in conditions affecting steroidogenesis, but the risk seems relatively low but not negligible.[212] Other tumors such as Sertoli cell adenomas are common in CAIS.

When counseling patients and their family about surveillance and/or treatment, the MDT should take into account the risk of gonadal GCC based on diagnosis, age of the individual, location of the gonad and results of gonadal biopsy if available, options for relocating the gonad, options for reliable surveillance, endocrine potential of the gonad and whether this endocrine function is desired by the individual, fertility potential of the gonad, risk of surgery, and side effects of hormone replacement therapy.[214,384,526]

Because of the high risk for early tumor development, prophylactic gonadectomy in infancy or early childhood is typically recommended for dysgenetic gonads and streak-like gonads that are undescended and cannot be placed in a lower position where they can be monitored. Serial monitoring with physical examination, and for older individuals self-examination, and imaging (e.g., ultrasound) may be more appropriate for gonads that are descended or can be placed in an extraabdominal, preferably scrotal position, and that exhibit more robust endocrine function. In conditions such as CAIS with a lower risk of germ cell tumors and where GCC generally do not occur before puberty, gonadectomy is deferred until after puberty; after appropriate counseling, some individuals may prefer to leave the gonads in situ in adulthood, although monitoring of abdominal gonads is difficult. GCNIS cannot be detected directly on ultrasound, although microlithiasis, especially when associated with inhomogeneous testis parenchyma, may be suggestive.[385] Abdominal gonads may not be visualized on ultrasound, and while MRI has been suggested as an alternative, this cannot reliably detect GCNIS either.[385] An alternative may be to move abdominal gonads to a more superficial location[214] (see "Complete Androgen Insensitivity Syndrome"). Standard circulating tumor markers (e.g., α-fetoprotein, βhCG, lactate dehydrogenase) are of limited use in detecting GCNIS or seminomas. Novel serum biomarkers such as microRNAs (e.g., miR371-3 and miR302/367) may be useful to detect malignant lesions but currently cannot detect premalignant lesions.[385]

Surgery and DSDs

Surgical approaches to DSD were once regarded as standard management, but there is much greater consideration now about the need for surgery and its timing.[527,528]

Surgery can potentially be performed on the gonads, the urogenital tract, and the genitals. The indication, timing, and choice of any potential procedure should be made on an individual basis and can be influenced by the underlying diagnosis. The MDT should inform the patient and family of all treatment options and their potential benefits and risks and should take into account medical, psychological, social, and cultural aspects so that a well-informed shared decision can be made.[529]

Some procedures are functionally important (e.g., to prevent hematocolpos or to mitigate high tumor risk), whereas others such as clitoral reduction surgery might be viewed as cosmetic. Some people believe that early surgery can be beneficial because the tissues are easier to operate on and heal better and that parents can make decisions for their child, acting in the child's best interest, whereas others feel that surgery should be deferred until a time when a young person can be part of the decision-making and consent process.[528–531] The psychological implications of these decisions can be great. Some parents experience great distress because of the appearance of their child's genitals.[532] Performing early surgery to make the appearance of the genitalia more typical may make some parents feel more comfortable, but the child may later regret the parents' decisions, especially if there is impaired sexual function or sensation or if further surgeries are required. On the other hand, bringing up a child with very atypical genitalia protects the child's rights to bodily integrity but needs strong positive parenting and support, and it is not well understood how well young people cope in these situations as they mature.

For a child born with atypical genitalia and raised female, the parents should be guided through a balanced discussion about all options. The most common scenario where clitoral reduction surgery is considered is in 46,XX girls with CAH. In a baby with CAH there is often considerable reduction in the size of the enlarged clitoris once androgen exposure is reduced by adrenal suppression, and the clitoris may also become less prominent as the child grows, so a period of observation is appropriate before decisions about surgery are made.[530] Psychological support in dealing with worries such as diaper changing should be provided, and in many situations surgery may not be considered. Outcome studies involving assessment of genital sensitivity and sexual function in women with CAH show impairment related to previous feminizing genital surgery.[531,533] Although surgical procedures have changed, with greater emphasis on nerve sparing, these data have had a bearing on decisions taken during infancy and childhood for the surgical management of conditions such as CAH, and long-term outcome data are awaited for newer approaches. In centers that offer surgical options, it is generally accepted that clitoroplasty should be reserved only for the most severe degree of clitoromegaly, and in many situations vaginal surgery is deferred until after puberty. In adulthood, vaginal lengthening in conditions such as CAIS and the MRKH syndrome can often be achieved by dilator treatment alone.[390] If a surgical vaginoplasty is performed, the Vecchietti procedure is often preferred.

Subjects with DSD raised male usually undergo surgery to correct hypospadias and orchidopexy for testis maldescent. Hypospadias repair can be scheduled between 6 and 18 months of age or deferred until approximately 4 years of age or later. More than one procedure is often required, and complications such as stenosis, fistula, or need for redo surgery are relatively frequent. The surgeon should provide complete information on these procedures to the parents and ensure that their expectations are realistic.[534] Many boys with severe hypospadias undergo surgery in early life, as performing these approaches in adolescence may be more difficult and associated with a higher rate of complications, but there is a lack of data for optimal timing and outcomes.[535] Open discussions with the parents and later with the child are needed, and boys may need more support in childhood and adolescence than is usually currently offered.

The topic of gonadectomy before puberty in 46,XY individuals raised female to prevent virilization and in others with DSD to avoid gonadal tumors has previously been mentioned. The irreversible nature of such a procedure has created uncertainty among professionals, particularly when the procedure is performed before the affected child can be engaged in discussions and in situations where there could be a potential for future fertility or endocrine function. Temporary suppression of puberty using GnRH analogues may be used to delay a decision on gonadectomy.[528] Gonadectomy is another issue that requires the collective discussion of the multidisciplinary team and may include input from an ethicist. The practice of cryopreserving excised gonads with unrealistic expectations for preservation of reproductive potential should be viewed cautiously, especially because current knowledge is based primarily on the gonadal effects of cancer therapies.[536] If tissue is preserved, there needs to be a clear dialogue about likely outcomes and options. However, germ cell number and viability may decline with time, so there could be a limited window of opportunity.[536] Furthermore, reproductive technologies are changing rapidly, there are highly publicized stem cell approaches such as germ cell reprogramming reported in animals, and successful birth following cryopreservation of (normal) prepubertal testis has been achieved in monkeys.[537] Whether these technologies will eventually provide useful clinical approaches remains to be seen.

Fertility/Family Building

Many forms of DSD are associated with reduced fertility or infertility. Successful use of assisted reproductive techniques has been reported in some cases, such as TESE and/or ICSI in men with PAIS or 5α-reductase deficiency,[396,538,539] and a few pregnancies using donor oocytes have been reported in women with 46,XY DSD who have a uterus.[540,541] Uterus transplantation has recently been shown to make pregnancy possible for women who do not have a uterus, resulting in live birth in a woman with MRKH syndrome.[542] Alternative options to build families include the use of donor sperm, adoption, or foster care. It is important to provide individuals with information on the possibilities in their specific situation; a recent study found that provision of such information is suboptimal.[543]

DSD in Resource-Limited Countries

Many of our approaches to DSD are being developed within the context of large medical centers and experienced MDTs with ready access to biochemical assays such as AMH, imaging, and state-of-the-art genetic tests. In reality, many children and adults with DSD live in countries with less access to resources or with different social and cultural pressures.[501] Each clinician needs to work with the tests and treatments that are available and affordable to optimize their ability to assess, diagnose, and treat individuals with DSD. Knowledge of the local prevalence of certain forms of DSD and genetic hotspots can be very important, as well as relevant social, cultural, and religious factors that might influence the way a child and the family view DSD. Limited education about DSD is available to clinicians in some countries, but tools such as the European Society for Paediatric Endocrinology (ESPE) e-learning may be valuable.[544] The Paediatric Training Centres in Africa (PETCA) established in 2008 to provide specialist training in endocrinology and diabetes for qualified pediatricians is beginning to pay dividends with more than 100 additional qualified specialists distributed among 13 sub-Saharan African countries.[545]

Outcome Studies

There is such a wide range of conditions included under the umbrella of DSD that it is difficult to obtain an overarching assessment of the health and quality of life in adulthood for individuals who have lived with DSD from childhood. Standardizing longitudinal assessment in individuals with DSD across centers should facilitate future outcome studies.[514]

Most data regarding sexual function and quality of life come from women with CAH who had various genitoplasty procedures during their childhood.[546,547] The debate continues regarding the timing of any surgery for girls with CAH and whether one-stage or two-stage surgery is the preferred option.[548] Adult endocrinologists are now undertaking detailed studies of the comorbid conditions associated with CAH and its medical treatment (see also Chapter 15). The UK Congenital Adrenal Hyperplasia Adult Study Executive (CaHASE) studies on cohorts of more than 200 adults show poorer health status relating to increased obesity, hypertension, insulin resistance, osteoporosis, impaired fertility, and reduced quality of life.[549–551] Despite improvements in growth achievement in recent years, shorter adult height was one of the adverse outcomes, particularly in relation to hypertension. Similar findings were found in outcome studies of a Swedish population of adults with CAH.[552] The Swedish CAH population also had lower educational achievement and reduced income, had increased disability allowance and sick leave, were less likely to be married, and had fewer children compared to control subjects.[553] The medical comorbid conditions may result from long-standing inadequate control of CAH by replacement glucocorticoids, estimated to be the case in more than a third of adults. Studies of modified-release hydrocortisone preparations that mimic more closely the cortisol circadian rhythm are showing promising results, with a reduction in the total hydrocortisone daily dose required for adequate adrenal androgen suppression.[554]

There are fewer outcome studies available for adults with conditions within the category of 46,XY DSD, but data are beginning to emerge. A nationwide study of females with 46,XY DSD in Denmark showed that morbidity is not increased when diagnoses directly related to the DSD itself were excluded.[383] A Swedish study reported a higher rate of psychiatric morbidity in women with CAIS and CGD compared to controls but similar to that in women with primary ovarian insufficiency.[555]

Quality-of-life studies have recently been reported in populations of German, Italian, and Brazilian individuals with 46,XX DSD and 46,XY DSD and of either male or female social gender, so study cohorts are quite heterogeneous.[556–558] In general, quality of life was satisfactory, there was no evidence of gender dysphoria, and psychosocial adjustment was better in younger people when compared with an older generation of DSD individuals. Among the 46,XY DSD group in the Brazilian population, those with a male social gender had a better quality of life than those with a female social gender (e.g., the group with CAIS). There were more difficulties overall in partnerships and sexual relationships. Another study from Germany, which included individuals with both 46,XX and 46,XY DSD, showed evidence of dissatisfaction with health care services available, especially in the group with 46,XY DSD.[559] In a study that focused on comparing women with either CAIS or MRKH syndrome, there was greater lack of sexual confidence and sexual satisfaction in women with CAIS, whereas those with MRKH syndrome, while apprehensive in sexual situations, reported being satisfied with their sex life.[560] Danish women with 46,XY DSD had lower rates of cohabitation

and motherhood compared to female controls, but education and income were similar to or higher than in controls.[383] In contrast, Danish men with 46,XX DSD had poorer education and reduced income in some age groups.[561]

As discussed, a recent study of more than 400 46,XY subjects subdivided according to clinical diagnoses of PAIS, disorders of gonadal development (partial gonadal dysgenesis), and disorders of androgen synthesis (such as 5α-reductase deficiency) has shown an increasing trend for babies with atypical genitalia to be raised as boys.[392] Therefore outcome studies are needed for these groups of individuals. A recent review of 46,XY DSD provided mainly anecdotal data on issues such as gender identity and sex redesignation, whether males were satisfied with the appearance of their genitalia, and the prevalence of sexual dysfunction.[562] Overall, males were satisfied with their gender, and rarely did male-to-female gender redesignation occur in adulthood. Most males had some concern about the appearance of the genitalia, and many were dissatisfied with sexual function. Another small Dutch study reported poor outcome in terms of penile size and sexual function, although their overall body image and psychosexual functioning was no different from control subjects.[563]

There is a paucity of information about outcome in specific causes of 46,XY DSD, such as PAIS.[565,566] Often this label is applied without confirmation that the phenotype is due to a mutation in the *AR* gene; as a result, other diagnoses may have been included inadvertently. A French multicenter study reported on 15 adults with PAIS who had been monitored since birth.[567] The EMS (see Fig. 24.40) was very low and penile length was markedly reduced in adulthood irrespective of additional androgen treatment. All boys developed gynecomastia in adolescence, and sexual function was severely impaired. An international study of 29 males with PAIS who were at least 16 years old and a more recent study of 27 males with PAIS studied prospectively also confirmed universal development of gynecomastia.[378,393] Micropenis was common at initial presentation but less so when assessed after puberty. Onset of puberty was often spontaneous, although androgen treatment may be required subsequently.

There has been considerable progress in many aspects of DSD since the previous edition of this chapter. What is clearly needed now are outcome studies in larger cohorts of individuals with DSD, particularly within the 46,XY DSD category. A major step toward that goal is the establishment of international registries for DSD studies (I-DSD) and allied activities such as evaluation of long-term care and outcomes in DSD (DSD-Life), interactive networks for activities in DSD (DSDnet), European Reference Networks for rare conditions such as DSD, and a DSD Translational Research Network (DSD-TRN).[568] Such networking should provide the means to gather more data on the individual rare causes of DSDs across the lifespan that can then be translated into improved management and outcome.

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25

Normal and Aberrant Growth in Children

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CHAPTER OUTLINE

KEY POINTS

- Height is an important vital sign to obtain during childhood because deviations from the normal linear growth pattern may indicate an underlying disorder.
- An intact hypothalamic (growth hormone–releasing hormone [GHRH])/pituitary (growth hormone [GH])/insulin-like growth factor 1 (IGF1) axis, adequate nutrition, and absence of significant systemic disease are requirements for normal linear growth.
- A comprehensive past medical, family, and social history with assessment of an accurate growth velocity is required for the initial investigation of abnormal growth. Laboratory and radiologic investigations include an evaluation for occult systemic disease and exclusion of hormonal abnormalities.
- Successful treatment of the underlying disorder or correction of hormone deficiency(ies) improves linear growth.
- Treatment of short stature with growth-promoting agents may improve linear growth in select patients with intact GHRH/GH/IGF1 axis function.

Normal Growth

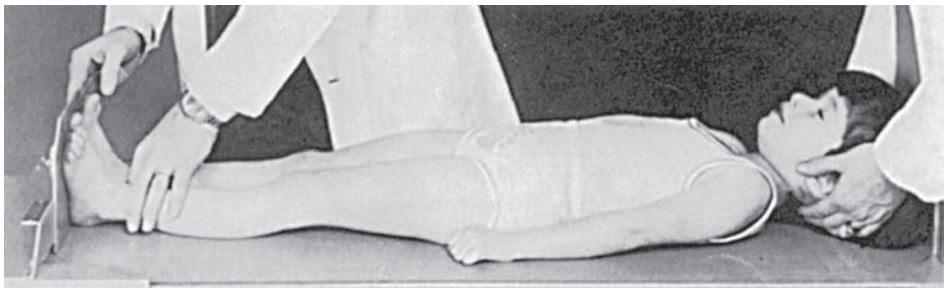
Overview

Growth is a fundamental, intrinsic aspect of childhood health. It is also a complex yet tightly regulated process. An individual's final height and the path taken to reach that end point are significantly determined by that person's genetic composition. But growth and final height can also be affected by external factors, including the quality and quantity of nutrition, and by psychosocial factors. This process is regulated by multiple hormones and growth factors interacting with an array of membrane receptors that activate seemingly redundant intracellular signaling cascades. Yet, as complex as this process is, 1 standard deviation (SD) of adult height represents about only 4% of the mean adult height.

Whether linear growth occurs as a continuous process or with periodic bursts of growth and arrest[1–4] has been hard to characterize definitively. There do appear to be seasonal variations of growth, with slower growth in autumn and winter and greater growth in spring and early summer.[5,6] Some normal children have a broad growth channel, with many showing diverse but characteristic growth tracks.[7] Nonetheless, even though the process of growth is multifactorial and complex, children usually grow in a remarkably predictable manner. Deviation from such a normal pattern of growth can be the first manifestation of a wide variety of disease processes, including endocrine and nonendocrine disorders and involving virtually any organ system of the body. Therefore frequent and accurate assessment of growth is of primary importance in the care of children.

Measurement

Assessment of growth requires accurate and reproducible determinations of height. Supine length is routinely measured in children younger than 2 years of age, and erect height is assessed in older children. It can be useful to measure both length and height in children between 2 and 3 years of age to allow comparisons with prior length measurements and to begin to record height measurement for ongoing comparisons. The inherent inaccuracies involved in measuring length in infants are often obscured by the rapid skeletal growth during this period. For measurement of supine length (Fig. 25.1), it is best to use a firm box with an inflexible board, against which the head lies, and a movable footboard, on which the feet are placed perpendicular to the plane of the supine length of the infant. Optimally, the child should be



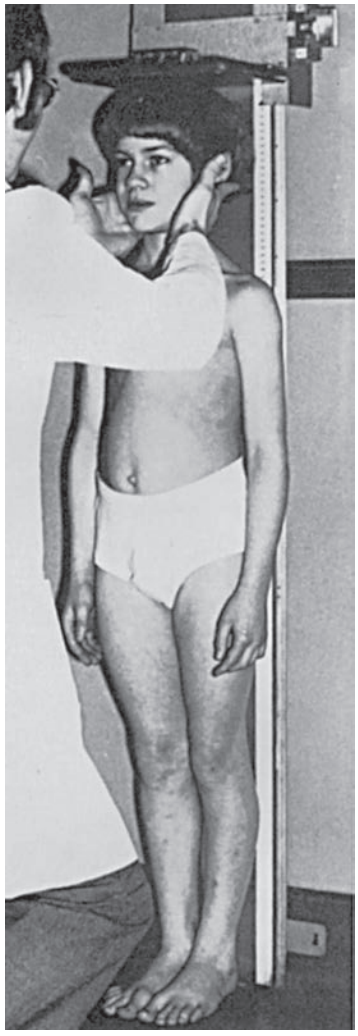
• **Fig. 25.1** Technique for measuring recumbent length. (A device suitable for measurement of length of infants can be purchased from Raven Equipment Limited, Essex, UK.) (Photograph courtesy Noel Cameron.)

relaxed, the legs should be fully extended, and the head should be positioned in the Frankfurt plane, with the line connecting the outer canthus of the eyes and the external auditory meatus perpendicular to the long axis of the trunk.

When children are old enough (and physically capable) to stand erect, it is best to use a wall-mounted Harpenden stadiometer similar to that designed by Tanner and Whitehouse for the British Harpenden growth study. The traditional measuring device of a flexible arm mounted to a weight balance is notoriously unreliable and does not provide accurate serial measurements.

As with length measurements in infants, positioning of the child in the stadiometer is critical (Fig. 25.2). The child should be fully erect, with the head in the Frankfurt plane; the back of the head, thoracic spine, buttocks, and heels should touch the vertical axis of the stadiometer, and the heels should be together. Every effort should be made to correct discrepancies related to lordosis or scoliosis. Ideally, serial measurements should be made at the same time of day, because standing height may undergo diurnal variation.

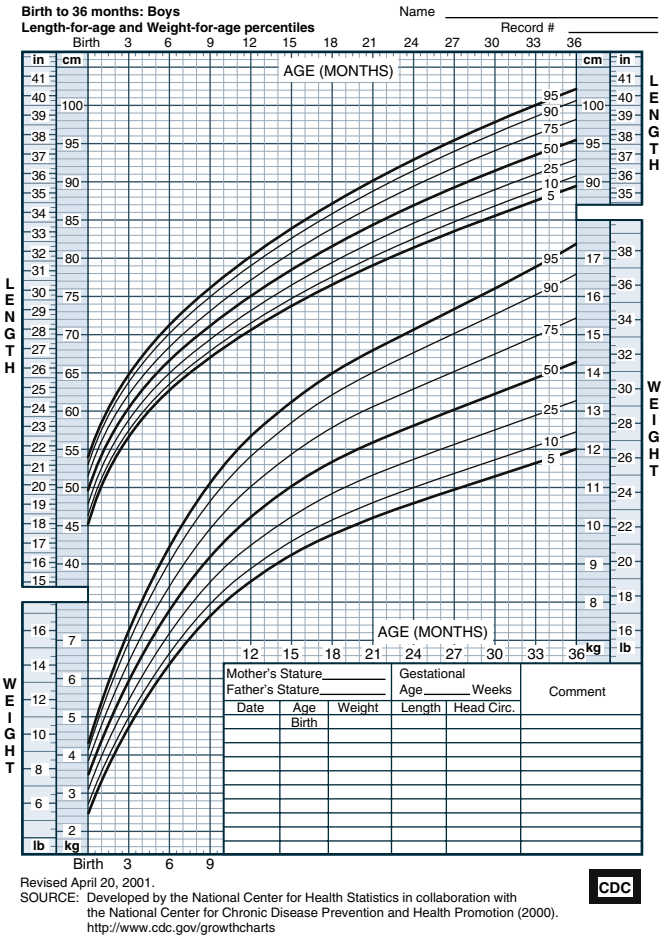
Height determinations should be performed by a trained individual rather than an inexperienced member of the staff. We recommend that lengths and heights be measured in triplicate, that variation should be no more than 0.3 cm, and that the mean height should be recorded. For determination of height velocity when several measurements are being made within a short period, the same individual should perform the determinations to eliminate interobserver variability. Even when every effort is made to obtain accurate height measurements, a minimum interval of 6 months is necessary for meaningful height velocity computation. Nine to 12 months of data are preferable so that errors of measurement are minimized and the seasonal variation in height velocity is assimilated into the data.



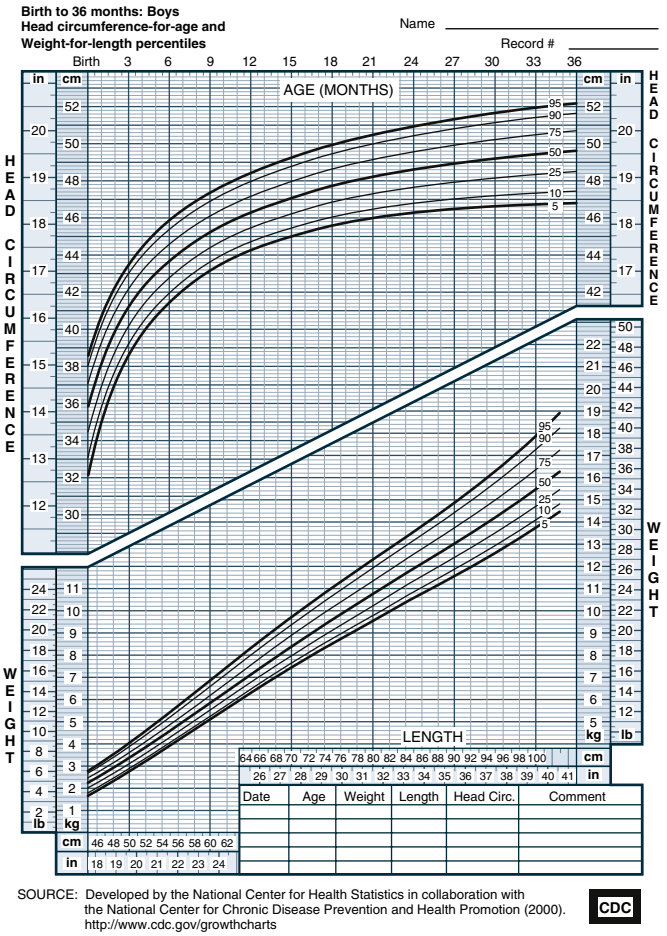
• **Fig. 25.2** Technique for measuring erect height using the Harpenden stadiometer with direct digital display of height. (Devices of this type are available from Holtain Ltd., Wales, UK, and Seritex Inc., Carlstadt, NJ.)

Growth Charts

Evaluation of a child's height must be done in the context of normal standards. Most American pediatric endocrine clinics use the cross-sectional data provided by the National Center for Health Statistics (NCHS), which were originally introduced in 1977. Revised and updated growth charts have been available on the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/growthcharts) since 2000 (Figs. 25.3 through 25.8).[8] The data for these charts encompass measurements obtained in the United States between 1963 and 1995, and they include a broader representation of the US population for all measures than was available in earlier charts. Growth charts based on the World Health Organization (WHO) data gathered from 1997 throughout 2003 should be used to monitor the growth of children under 2 years of age. While the data on length are very similar between the CDC and WHO curves, the CDC curves describe higher weight gain that, among other things, reflects the lower prevalence of breastfed infants in the CDC data and is not felt to represent optimal growth.[9]



• **Fig. 25.3** Length-for-age and weight-for-age percentiles for boys (birth to 36 months). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion, 2000. http://www.cdc.gov/growthcharts.)



• **Fig. 25.4** Head circumference-for-age and weight-for-length percentiles for boys (birth to 36 months). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion, 2000. http://www.cdc.gov/growthcharts.)

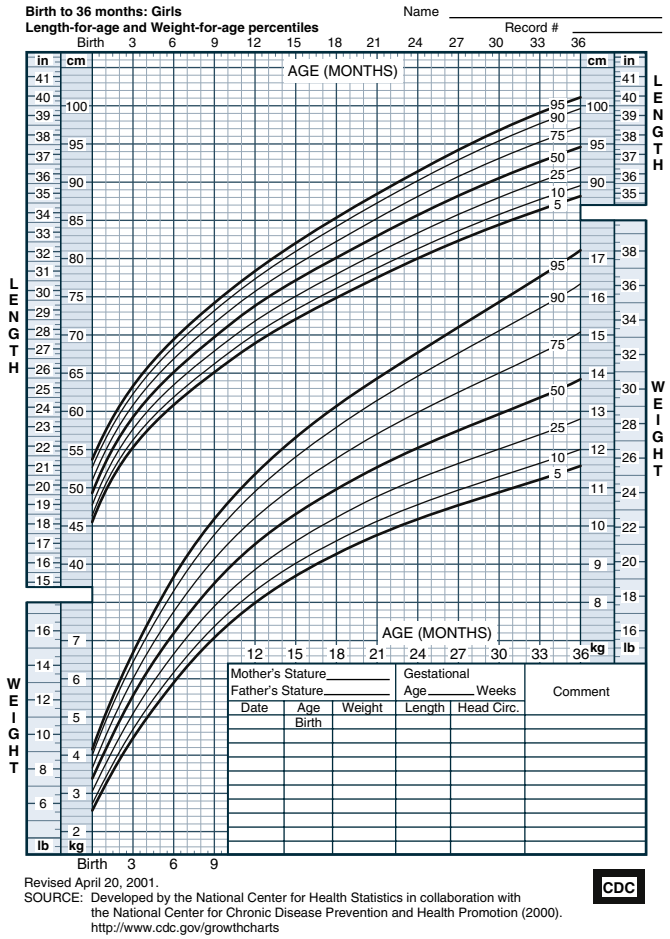
These charts allow comparison of individual children with the 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles of normal American children. There are, however, two limitations of these charts when applied to the individual child. First, they do not satisfactorily define children below the 3rd or above the 97th percentiles—the very children in whom it is most critical to define the degree to which they deviate from the normal growth centiles. However, the NCHS data tables (also available on the CDC website) can be used to compute standard deviation scores (SDS). For example, a short child below the 3rd percentile can be described more precisely as being, for example, approximately 4.2 SDS below the mean for age. A height SDS for age is calculated as follows: The SDS equals the child's height, minus the mean height for normal children of the child's age and gender, divided by the SD of the height for normal children of this age and gender. Second, cross-sectional data are of greater value during infancy and childhood than in adolescence, because differences in the timing of pubertal onset can considerably influence normal growth rates. To address this issue, Tanner and Davies[10] developed longitudinal growth charts in an effort to construct the curve shapes with centile widths obtained from a large cross-sectional survey, thus accounting for variability in the timing of puberty. Such charts are of particular value in assessing growth during adolescence and puberty and for plotting sequential growth data for any individual child.

The data from cross-sectional and longitudinal growth studies have been used to develop *height velocity* standards (Figs. 25.9 and 25.10). It is important to emphasize that carefully documented height velocity data are invaluable in assessing a child with abnormalities of growth. There is considerable variability in normal height velocity of children at different ages; however, between the age of 2 years and the onset of puberty, children grow with remarkable fidelity relative to the normal growth curves. Any crossing of percentile curves on the height chart during this age period should be considered abnormal and warrants further evaluation.

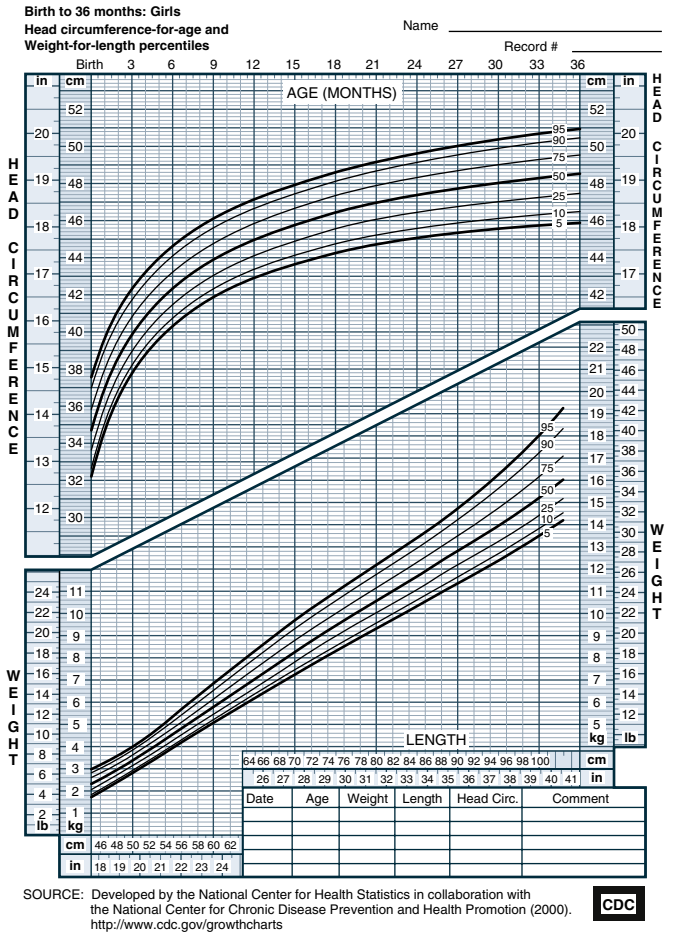
Syndrome-specific growth curves have been developed for a number of clinical conditions associated with growth failure, such as Turner syndrome (TS),[11] achondroplasia,[12] and Down syndrome.[13,14] Such growth profiles are invaluable for tracking the growth of children with these clinical conditions. Deviation of growth from the appropriate disease-related growth curve suggests the possibility of a second underlying cause, such as acquired autoimmune hypothyroidism in children with Down syndrome or TS.

Body Proportions

Many abnormal growth states, including both short stature and excessive stature, are characterized by *disproportionate* growth. The



• **Fig. 25.5** Length-for-age and weight-for-age percentiles for girls (birth to 36 months). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion, 2000. http://www.cdc.gov/growthcharts.)



• **Fig. 25.6** Head circumference-for-age and weight-for-length percentiles for girls (birth to 36 months). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion, 2000. http://www.cdc.gov/growthcharts.)

following determinations should be made as part of the evaluation of short stature:

1. Occipitofrontal head circumference
2. Lower body segment: distance from top of pubic symphysis to the floor
3. Upper body segment: the difference between total height and lower body segment (it can also be measured as the sitting height, subtracting the height of the chair or stool)
4. Arm span

Published standards exist for these body proportion measurements, which must be evaluated relative to the patient's age.[15] The ratio of upper segment to lower segment ranges from 1.7 in the neonate to slightly less than 1.0 in the adult (Fig. 25.11).

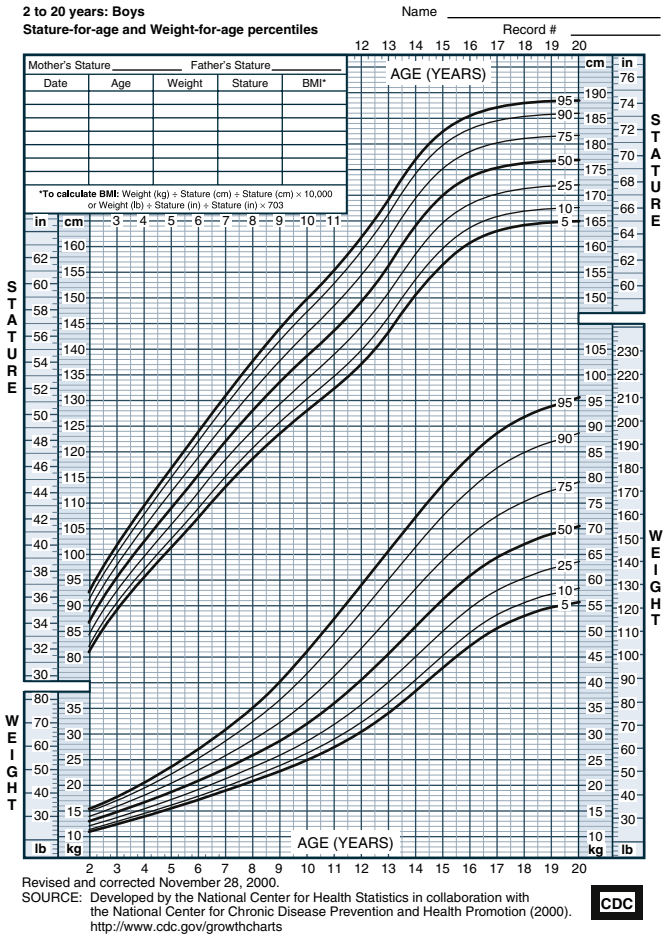
Parental Target Height

Genetic factors are important determinants of growth and height potential. Therefore it is useful to assess a patient's stature relative to that of siblings and parents. Tanner and associates developed a growth chart that factored parents' heights into the evaluation of the heights of children ages 2 to 9 years.[16] One can also calculate a child's expected final height based on the parents' heights by calculating the midparental height. This is the average of the parents' heights, after accounting for the average difference in height between adult men and women (13 cm). In other words, a boy's midparental height equals the average of his parents' heights plus 6.5 cm, and a girl's midparental height equals the average of her parents' heights minus 6.5 cm.

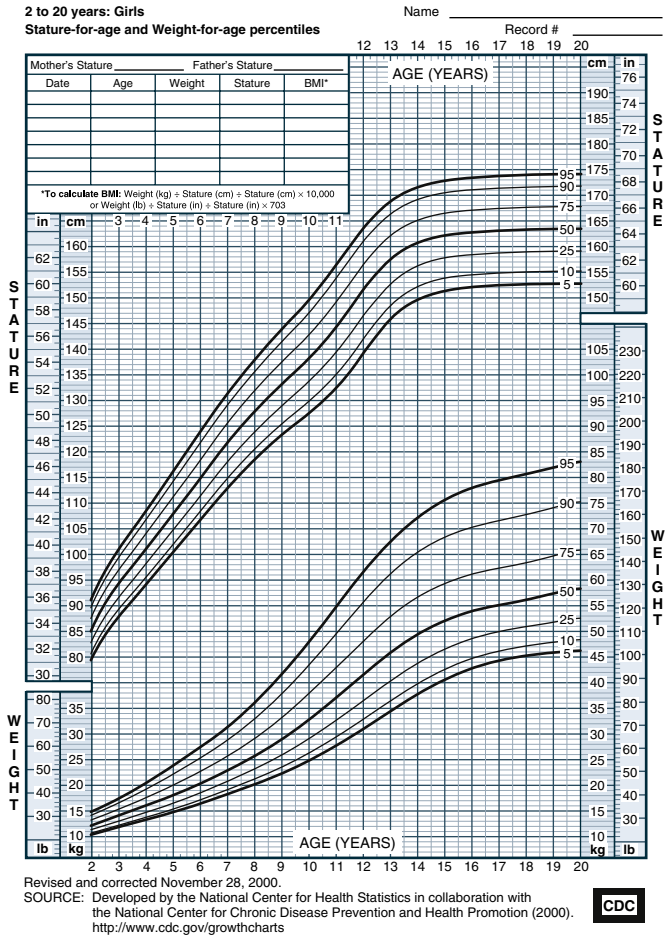
Because of regression to the mean,[17,18] children of short parents are likely to be less short than their parents, and children of tall parents are likely to be less tall than their parents. Therefore a child's genetic *target height range* centers on the point that represents 80% of the difference between the child's midparental height and the mean adult height for the child's gender.[17] For example, if a boy's father is 168 cm tall and his mother is 153 cm tall, his midparental height is 167 cm, which is 10 cm below the mean height of adult men (177 cm). Therefore the boy's target height range centers on 169 cm, which is 8 cm below the mean adult height for men. In more than 95% of children, the adult height falls within 10 cm of the point thus calculated.[16,17] In children with extremely short stature (≥3 SD), the father's height may more strongly correlate with the patient's height, and the mother's height may more greatly influence birth length.[19]

Skeletal Maturation

The growth potential in the tubular bones can be assessed by evaluation of the progression of ossification within the epiphyses. The



• **Fig. 25.7** Stature-for-age and weight-for-age percentiles for boys (2 to 20 years). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion, 2000. http://www.cdc.gov/growthcharts.)



• **Fig. 25.8** Stature-for-age and weight-for-age percentiles for girls (2 to 20 years). (Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion, 2000. http://www.cdc.gov/growthcharts.)

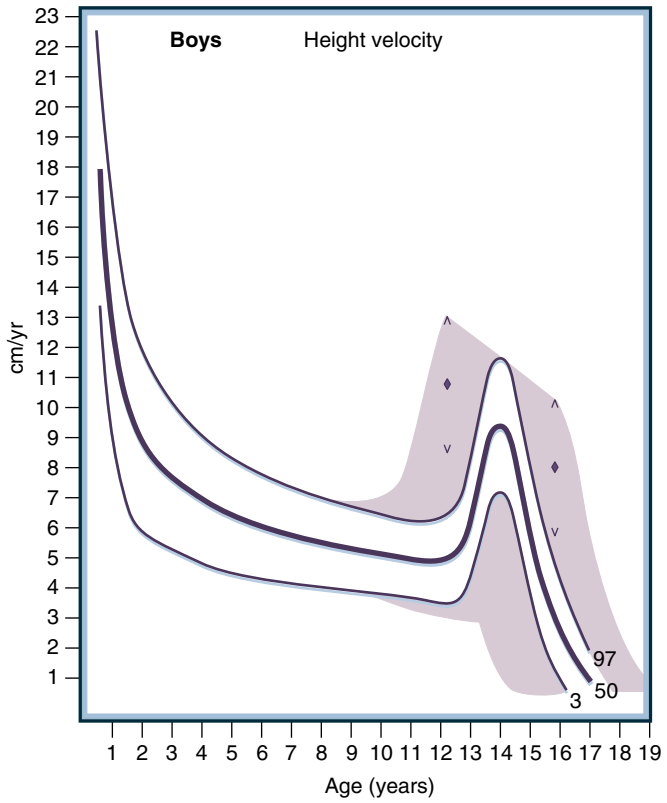
ossification centers of the skeleton appear and progress in a predictable sequence in normal children, and this skeletal maturation can be compared with normal age-related standards. This forms the basis of the *bone age* or *skeletal age*, a quantitative determination of net somatic maturation that serves as a mirror of the tempo of growth and maturation. The bone age also reflects the degree of growth plate senescence and is therefore a useful adjunct in estimating growth opportunity (i.e., the ultimate adult height), as discussed later in this chapter.

Not all of the factors that determine the normal pattern of skeletal maturation have been identified, but genetic factors and multiple hormones, including thyroxine, growth hormone (GH), and gonadal steroids, are involved.[20] Ultimately, growth cessation occurs after exhaustion of the proliferative capacity of the growth plate chondrocyte.[21] Estrogen plays an important role in this process: Animal studies have indicated that estrogen accelerates growth plate senescence,[22] and studies in patients with mutations of the gene for the estrogen receptor[23] or for the aromatase enzyme[24,25] demonstrated that estrogen is primarily responsible for epiphyseal fusion.[26]

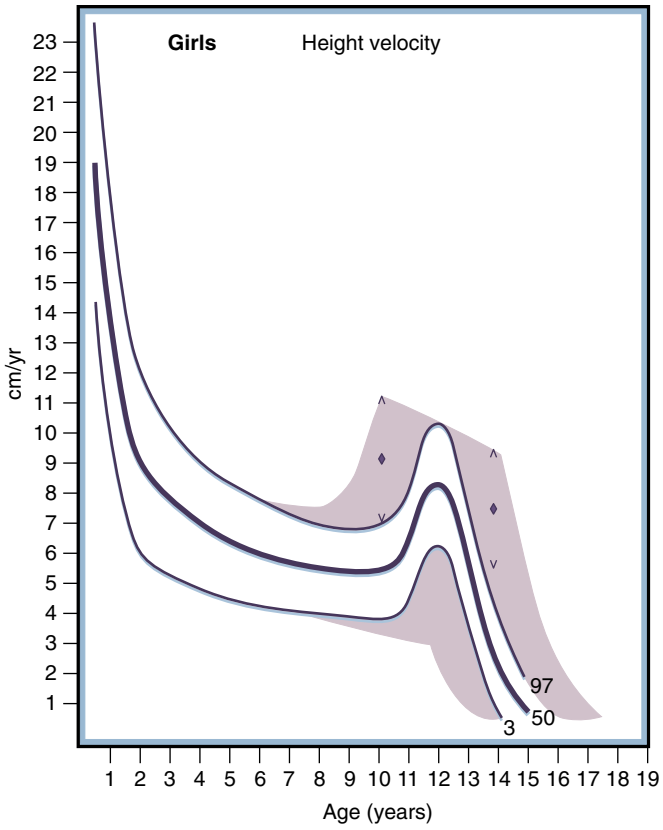
Phases of Normal Growth

Growth occurs at differing rates during intrauterine life, early and middle childhood, and adolescence and then ceases after fusion of long bone and vertebral epiphyseal growth plates. Karlberg and associates resolved the normal linear growth curve into three additive, partially superimposable phases[27,28]: an "infancy" phase, starting in midgestation and then rapidly decelerating until about 3 to 4 years of age; a "childhood" phase, of slowly decelerating growth through early adolescence; and a sigmoid-shaped "puberty" phase that comprises the adolescent growth spurt. Prenatal growth averages 1.2 to 1.5 cm per week but varies dramatically (Fig. 25.12); the midgestational length growth velocity of 2.5 cm per week falls to almost 0.5 cm per week immediately before birth. Growth velocity (see Figs. 25.9 and 25.10) averages about 15 cm per year during the first 2 years of life; it then slows to approximately 6 cm per year during middle childhood. During this time, a normal child's height, plotted on a growth curve, typically remains within a given growth channel; that is, it does not cross percentile lines on the growth curve.

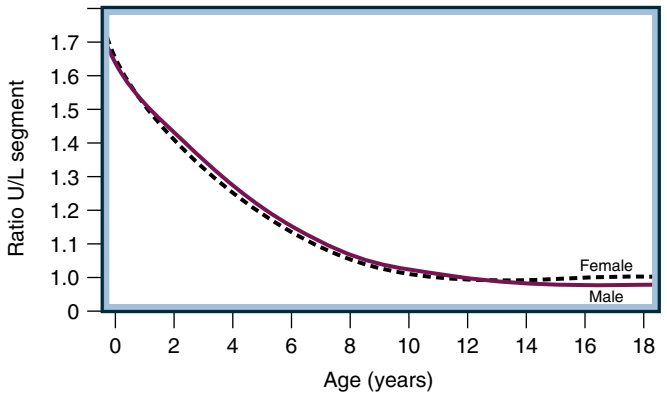
Prepubertal growth is similar between boys and girls. The height difference between men and women, an average of 13 cm, is accounted for by two factors. First, boys grow for an average of 2 years longer than girls, because girls have an earlier onset of puberty and, consequently, earlier cessation of growth. Therefore prepubertal growth is greater for boys; they are 8 to 10 cm taller when their puberty starts, compared with girls' heights when their puberty starts.[17] Second, boys achieve a greater maximal pubertal growth velocity than girls, giving them 3 to 5 cm greater pubertal growth. The time of onset of puberty varies in normal children,



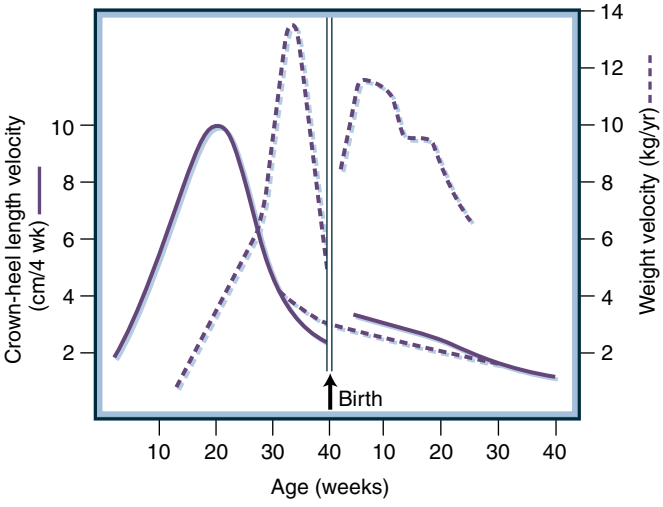
• **Fig. 25.9** Height velocity chart for boys constructed from longitudinal observations of British children. The 97th, 50th, and 3rd percentile curves define the general pattern of growth during puberty. Shaded areas indicate velocities in those children with peak velocities occurring up to 2 standard deviations before or after the average age depicted by the percentile lines. (*Up arrows*, *diamonds*, and *down arrows* mark, respectively, the 97th, 50th, and 3rd percentiles of peak velocity when the peak occurs at these early or late limits.) (Modified from charts prepared by J.M. Tanner and R.H. Whitehouse from data published in references 10 and 30. Reproduced with permission of J.M. Tanner and Castlemead Publications, Ward's Publishing Services, Herts, UK.)



• **Fig. 25.10** Height velocity chart for girls constructed from longitudinal observations of British children. The 97th, 50th, and 3rd percentile curves define the general pattern of growth during puberty. Shaded areas indicate velocities in those children with peak velocities occurring up to 2 standard deviations before or after the average age depicted by the percentile lines. (*Up arrows*, *diamonds*, and *down arrows* mark, respectively, the 97th, 50th, and 3rd percentiles of peak velocity when the peak occurs at these early or late limits.) (Modified and reproduced with permission of J.M. Tanner and Castlemead Publications, Ward's Publishing Services, Herts, UK.)



• **Fig. 25.11** Upper/lower segment ratio from birth to 18 years of age. (Data from Wilkins L. The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence. Springfield, IL: Charles C. Thomas; 1957.)



• **Fig. 25.12** Rate of linear growth and weight gain in utero and during first 40 weeks after birth. Length velocity is expressed in centimeters per week. The *solid line* depicts the actual linear growth rate; the *dashed line* connecting the prenatal and postnatal length velocity lines depicts the theoretic curve for no uterine restriction late in gestation. The *lighter dashed line* depicts weight velocity. (From data in Tanner JM. *Fetus Into Man.* Cambridge, MA: Harvard University Press; 1978.)

resulting in a normal variation in the timing of the pubertal growth spurt. However, in most normal children, the final height is not influenced by the chronologic age at onset of the pubertal growth spurt because the additional time for prepubertal growth that occurs when puberty is late is balanced by the fact that pubertal growth is smaller the later it occurs (see Figs. 25.9 and 25.10). After puberty, chondrocyte proliferation in the growth plate slows and senescence occurs due to depletion of stem-like cells in the resting zone of the growth plate.[29,30]

There are two variants of normal growth whose characteristic patterns are such that children exhibiting these growth variants are often evaluated for a growth disorder. These two variants are crossing linear percentiles of infancy and constitutional delay of growth and development (CDGD). In many cases, it is difficult to differentiate children with these normal growth variants from children with a growth disorder.

Crossing Linear Percentiles of Infancy

As in postnatal growth, genetic and environmental factors are important determinants of fetal growth and of the size of an infant (weight and length) at birth. However, there are significant differences between the factors affecting birth size and those affecting childhood growth and adult stature. There is much less correlation between length at birth and ultimate adult height than between length at birth and height in the later years of childhood.[31]

Parental stature affects birth length, as it does adult stature; this is an indicator of the genetic influence on growth. Although maternal and paternal stature contribute equally to childhood growth and adult stature, the effect of maternal stature may predominate over that of paternal stature on length at birth.[32,33] However, some studies have found equal effects of maternal and paternal stature on birth size.[34] Maternal nutrition and health have significant effects on fetal growth. Maternal weight has a positive effect, and smoking during pregnancy has a negative effect. Maternal diabetes has a strong positive effect on fetal growth. It is possible for the prenatal determinants of growth to result, for example, in a child who will ultimately grow to below-average stature but is above average size at birth. For this reason, it is common for infants' lengths to cross percentiles on the growth curve. Indeed, it is more common for the growth of an infant to cross percentiles than to follow a single percentile from birth into childhood: Approximately one-third of infants have lengths that cross percentiles moving upward on the growth curves, and approximately one-third have lengths that cross percentiles moving downward.[33,35] Most normal infants crossing percentiles do so in the first 6 to 12 months, although some normal infants cross percentiles after 1 year of age.

Infants who are small at birth can be divided into (1) those who are small only because of prematurity and are therefore of a size appropriate for gestational age (AGA) and (2) those who are small for gestational age (SGA). SGA is usually defined as a birth weight or length (or both) below the 3rd percentile (or, sometimes, below the 10th percentile) for gestational age.[36] Some infants born SGA represent the small percentage of individuals whose genetic potential leads to small size at birth, and they can be expected to remain small throughout childhood and adulthood. However, many infants are SGA due to intrauterine growth retardation (IUGR) and have a genetic potential that would not be expected to result in small adult stature. Most infants who are born small, either AGA or SGA, have catch-up growth and achieve lengths greater than the 3rd percentile within the first 2 years of life. However, up to 10% of SGA infants do not show such catch-up growth.[37,38] The pathologic aspects of IUGR and of SGA in infants who do not have catch-up growth are discussed later.

Constitutional Delay of Growth and Development

CDGD is a normal variant of growth.[39] It describes the growth pattern of children who will experience a later than average timing of puberty. Their birth size is normal, and their final height is within their genetic potential. However, during most of their childhood, they grow at a height percentile below that expected based on their genetic potential. Typically, these children have a low growth velocity during the first years of life, crossing downward on the length percentile growth curves, so that by 2 years of age their heights are at or slightly below the 5th percentile. After age 3 years, their growth rate is typically normal, so that their height growth usually remains parallel to the 5th percentile until adolescence, although the height SDS may gradually drift slightly lower during the middle childhood years in some cases.[39,40] Their height diverges even further from that of average children during the early teen years due to the declining prepubertal growth velocity of these children compared with the accelerating growth rate of children with an average timing of the onset of puberty.

Ultimately, children with CDGD have a late growth spurt, consistent with their late puberty, and this brings their height into the normal adult range. Final height is often in the lower part of the parental target height range, and few patients exceed the parental target height,[41–43] although this finding is probably at least in part the result of a selection bias of the children examined for such studies. However, there is evidence that a delayed growth spurt may adversely affect growth of the spine, resulting in a decrease in the final ratio of the upper to the lower segment and perhaps contributing to a limited final height.[44] Studies have also reported that prepubertal boys with CDGD have decreased bone mineral density (BMD),[45] although by young adulthood the majority of the BMD deficit is lost.[46,47]

Secular Changes in Height

There are surprisingly few data concerning the stature of modern humans before the measurement of military recruits became customary in the 18th century. Skeletal remains from the last ice age appear to indicate that adult stature 10,000 to 20,000 years ago was not substantially different from that of contemporary adults, although this record is obviously fragmentary.[48] It has been suggested that a reduction in stature was observed with the introduction of agriculture approximately 5000 years ago, with growth attenuation resulting from the combined effects of nutrient deficiency, population growth, and spread of infectious diseases.

Military recruits in the 18th and 19th centuries were clearly shorter than those of today, although it must be recognized that soldiers were commonly recruited from the lower socioeconomic classes, and poor health and nutrition would have contributed to both poor growth and late maturity.[49] Whereas men in the 20th century averaged 5 to 10 cm greater in height than those in the 18th century for whom we have records, much of this height gain has occurred over the past 100 years and probably reflects the dramatic improvement in overall nutrition and health seen in the Western world. This upward trend in height appears to have stopped in many developed countries in the early 21st century.[50]

Therefore secular changes in height appear to reflect fundamental alterations in the standard of living rather than major genomic differences among populations; future economic advances in developing countries can be predicted to lead to improvement in adult stature and a reduction in international differences in growth.

Endocrine Regulation of Growth

The Hypothalamic-Pituitary Axis: Embryogenesis and Anatomy

The pituitary gland is central to the regulation of mammalian growth. The pituitary gland develops from oral ectoderm in response to inductive signals from the neuroepithelium of the ventral diencephalon and intrinsic signaling gradients determining expression patterns of pituitary-specific transcription factors in the developing anterior pituitary gland.[51] The primordium of the anterior pituitary, Rathke pouch, forms as an upward invagination of a single-cell-thick layer of ectoderm that contacts the neuroectoderm of the primordium of the ventral hypothalamus at embryonic day 8.5 (E8.5) in the mouse embryo[52] and can be identified by the third week of pregnancy in humans. The neurohypophysis (posterior pituitary) originates in the neural ectoderm of the floor of the forebrain, which also develops into the third ventricle. During anterior pituitary development, overlapping but regionally specific and temporally distinct patterns of homeobox transcription factor expression lead to the sequential appearance of the terminally differentiated cell types from E12.5 to birth.[52]

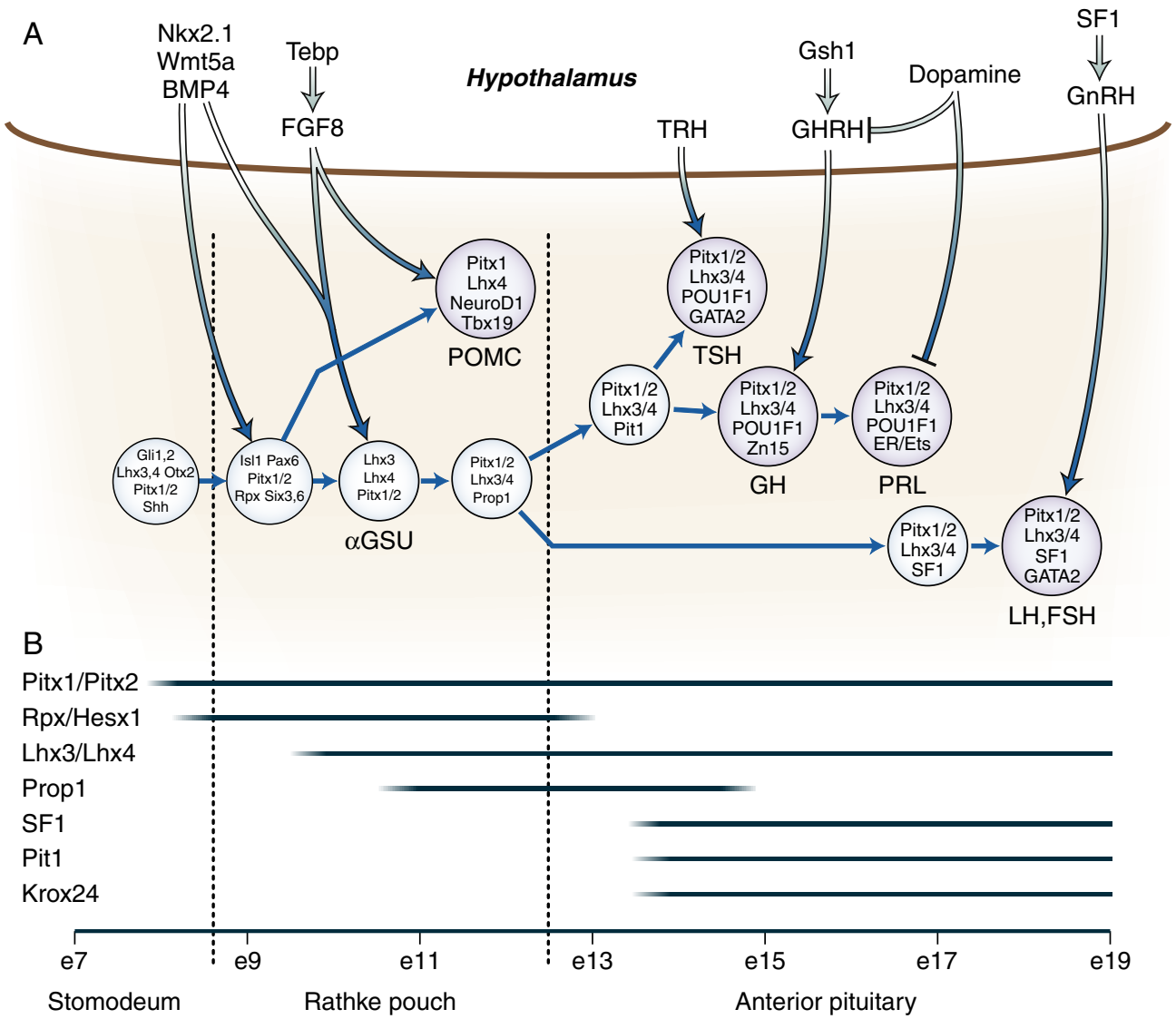
The initiation of anterior pituitary gland development depends on the competency of the oral ectoderm to respond to inducing factors from the neural epithelium of the ventral diencephalon.[53] The bone morphogenetic protein 4 (BMP4) signal from the ventral diencephalon is the critical dorsal neuroepithelial signal required for organ commitment of the anterior pituitary gland. Wnt5a and fibroblast growth factor 8 (FGF8) are also expressed in the diencephalon in distinct overlapping patterns with BMP4. Subsequently, a BMP2 signal arises from the boundary of a region of oral ectoderm in which Sonic hedgehog (SHH) expression, initially expressed uniformly in the oral ectoderm, is selectively excluded from the developing Rathke pouch. Also expressed are Gli1 and 2, Lhx3, and Pitx1 and 2, which are important in progenitor pituitary cell type development. The ventral-dorsal BMP2 signal and the dorsal-ventral FGF8 signal appear to create opposing activity gradients that are suggested to dictate overlapping patterns of specific transcription factors underlying cell lineage specification. The various extensions of these transcription factors in their fields are theorized to combinatorially determine the specific cell types. The FGF8 gradient determines the dorsal cell phenotypes,[53,54] and dorsally expressed transcription factors include Hesx1, Nkx-3.1, Six3, Pax6,[55] and prophet of Pit1 (PROP1).[56] The attenuation of Hesx1 is required for the expression of PROP1. Temporally specific attenuation of the BMP2 signal is required for terminal differentiation of the ventral cell types, and ventrally expressed transcription factors include islet-1 (Isl1), Brn4, P-Frk, and GATA2.[53,56,57] Pit1 (encoded by the gene *POU1F1*) is expressed upon the attenuation of PROP1 expression and required for somatotroph, lactotroph, and thyrotroph development,[58,59] whereas the orphan nuclear receptor steroidogenic factor 1 (SF1) is selectively expressed in the gonadotrophs.[60,61]

The ventral-dorsal gradient induces GATA2 in a corresponding gradient in presumptive gonadotrophs and thyrotrophs, and high levels of GATA2 in the most ventral aspect of the developing anterior pituitary directly or indirectly restrict expression of *POU1F1* out of the presumptive gonadotrophs. In the absence of Pit1, GATA2 expression appears sufficient to induce the entire set of transcription factors that are typical of the gonadotroph cell type, including SF1, P-Frk, and Isl1. Conversely, the absence of GATA2 dorsally is critical for differentiation of Pit1-positive cells to somatotroph/lactotroph fates. It is hypothesized that the level of GATA2 expression in the thyrotrophs is below the threshold required to inhibit activation of the *POU1F1* gene early enhancer, permitting the emergence of a Pit1+, GATA2+ cell that results in the thyrotroph fate.[57] Pax6 has a role in the sharp boundary of attenuation of the ventral signals that dictate thyrotroph and gonadotroph cell lineages. In the absence of Pax6, the ventral lineages, particularly thyrotrophs, become dorsally extended at the expense of somatotroph and lactotroph cell types,[55] and Pax6 mutant mice are GH and PRL deficient.[62]

The earliest marker of the anterior pituitary is expression of the glycoprotein α-subunit (αGSU), which appears at E11.5 in the mouse. These αGSU-positive cells also express the transcription factor Isl1 and mark a population of differentiating thyrotrophs that disappears after birth.[54,58,63,64] αGSU is expressed in mature thyrotrophs and gonadotrophs. At E12.5, corticotrophs start to differentiate and produce pro-opiomelanocortin (POMC).[54,64] Intensified cell proliferation within Rathke pouch results in formation of a visible nascent anterior pituitary lobe on E12.5.[52] Definitive thyrotrophs are observed at 14.5 days postconception (dpc), characterized by the expression of *Tshb* at E14.5 and followed by the expression of GH and prolactin (PRL) in somatotrophs and lactotrophs, respectively, at E15.5. The gonadotrophs are the last cell type to develop, at E16.5, marked by expression of luteinizing hormone (LH) and later by follicle-stimulating hormone (FSH). Eventually the mature gland is populated by at least five highly differentiated cell types: Ventrally to dorsally, they are the gonadotrophs, thyrotrophs, somatotrophs, lactotrophs, and corticotrophs.[53] Ultimately, some of these same transcription factors are also involved in the cell-specific expression and regulation of the gene products of these pituitary cell types, with corticotrophs producing adrenocorticotropin hormone (ACTH), thyrotrophs producing thyrotropin (thyroid-stimulating hormone [TSH]), gonadotrophs producing gonadotropins (LH and FSH), somatotrophs producing GH, and lactotrophs producing PRL. The developmental factors that play an in vivo role in pituitary gland development and differentiation are shown in Fig. 25.13.

In humans, GH-producing cells can be found in the anterior pituitary gland by 9 weeks of gestation,[65] and vascular connections between the anterior lobe of the pituitary and the hypothalamus develop at about the same time,[66] although hormone production can occur in the pituitary in the absence of connections with the hypothalamus. Somatotrophs can be demonstrated in the pituitary in anencephalic newborns.[67]

In the newborn, the pituitary weighs about 100 mg. In the adult, the mean weight is about 600 mg, with a range of 400 to 900 mg; the pituitary is slightly heavier in women than in men and increases during pregnancy.[68] The mean adult pituitary size is 13 × 9 × 6 mm.[69] The anterior pituitary normally constitutes 80% of the weight of the pituitary. The pituitary resides in the sella turcica, immediately above and partially surrounded by the sphenoid bone. The volume of the sella turcica is a good index of pituitary size and may be reduced in the child with pituitary hypoplasia. The optic chiasm is located superior to the pituitary gland, so suprasellar growth of a pituitary tumor may initially manifest with visual complaints or evidence of decreases in peripheral vision. Furthermore, development of the neurohypophysis and the pituitary are intimately related, leading to potential anatomic associations of central nervous system (CNS) abnormalities with pituitary hypoplasia. For example, septo-optic dysplasia is associated with several



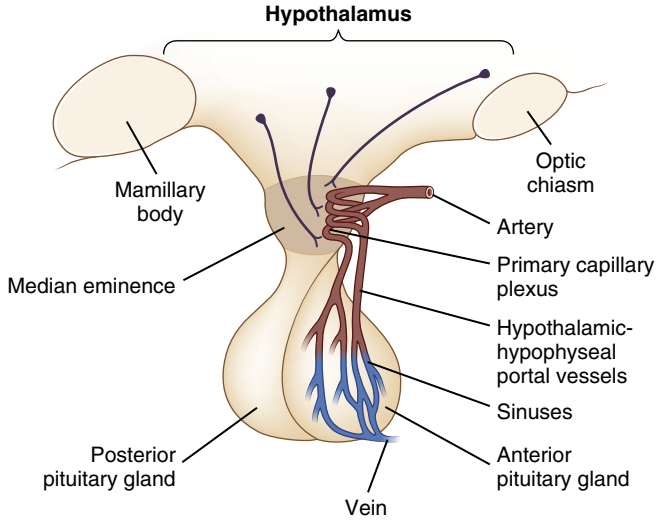
• **Fig. 25.13** Development of pituitary cell lineages. (A) Schematic representation of pituitary cell precursors shows the expression of prevalent transcription factors at each stage of development. Terminally differentiated cells are shown as larger and shaded circles together with the hormones produced (lineage-specific transcription factors are highlighted in bold in these cells). The interaction with transcription factors and signaling molecules in the hypothalamus is also depicted. Transcription factors are represented in lowercase (except for SF1 and GATA2), whereas signaling molecules appear in uppercase. (B) The timing of appearance and disappearance of pituitary transcription factors during mouse embryogenesis. *αGSU,* glycoprotein α-subunit; *BMP4,* bone morphogenic protein 4; *e,* embryonic day; *ER,* estrogen receptor; *FGF8,* fibroblast growth factor 8; *FSH,* follicle-stimulating hormone; *GATA2,* GATA binding factor 2; *GHRH,* growth hormone–releasing hormone; *GnRH,* gonadotropin-releasing hormone; *Hesx1,* homeobox expressed in ES1 cells (Rathke pouch homeobox, Rpx); *LH,* luteinizing hormone; *Nkx2.1,* NK2 homeobox 1; *POMC,* pro-opiomelanocortin; *PRL,* prolactin; *SF1,* steroidogenic factor 1; *Tebp,* telomere binding protein; *TRH,* thyrotropin-releasing hormone; *TSH,* thyroid-stimulating hormone (thyrotropin); *Wmt5a,* wingless type MMTV integration site family, member 5A. (Reprinted with permission from Lopez-Bermejo A, Buckway CK, Rosenfeld RG. Genetic defects of the growth hormone-insulin-like growth factor axis. *Trends Endocrinol.* 2000;11:43.)

CNS anatomic abnormalities and pituitary hormone deficiencies. For this reason, children with congenital blindness or nystagmus should be monitored for hypopituitarism.

The anterior pituitary receives controlling signals from the hypothalamus through the portal circulatory system (Fig. 25.14).[66] The hypothalamus integrates signals from other brain regions and the environment, resulting in the release of factors that control pituitary hormone synthesis and secretion. Hypothalamic neurons that synthesize peptides terminate in the infundibulum, enter the primary plexus of the hypophyseal portal circulation, and are transported via the hypophyseal portal veins to the capillaries of the anterior pituitary. This portal system in the pituitary stalk provides a means of communication between the neurons of the hypothalamus and the anterior pituitary.

Growth Hormone–Releasing Hormone

Growth hormone–releasing hormone (GHRH) is a 44–amino acid peptide hormone[70,71] secreted by neurons located in the arcuate nucleus (ARC) of the hypothalamus.[72] GHRH is the main hypophysiotropic neuropeptide responsible for the generation and maintenance of pulsatile GH secretion.[73,74] Regulation of GH production by GHRH is mediated largely at the level of transcription and is enhanced by increases in intracellular cyclic adenosine monophosphate (cAMP) levels. Evidence includes administration of GHRH antagonists resulting in an impairment of GH pulsatility, ablation of the ARC nucleus resulting in loss of GH secretion,[74,75] and administration of synthetic GHRH increasing GH secretion.[75] GHRH binds to GHRH receptors (GHRH-R) in somatotropes to activate multiple intracellular signaling pathways to stimulate somatotroph cell proliferation, differentiation, and growth[76,77] as well as to stimulate the secretion and synthesis of GH (Fig. 25.15).[78,79] The GHRH receptor is a member of the G protein–coupled receptor (GPCR) family BIII.[80] GH signaling pathways engaged by GHRH-R activation include adenylate cyclase (AC), increasing cAMP production, which in turn leads to an increase in protein kinase A (PKA) activity,[81–83] intracellular and extracellular Ca^{2+}, NOS/NO/GC/cGMP, and PKC/PLC pathways.[81,84] In a dwarf transgenic mouse model with diminished GHRH production, pituitary somatotroph proliferation is markedly decreased.[85,86] Transgenic mice that overexpress GHRH grow at a faster rate than control mice.[87]



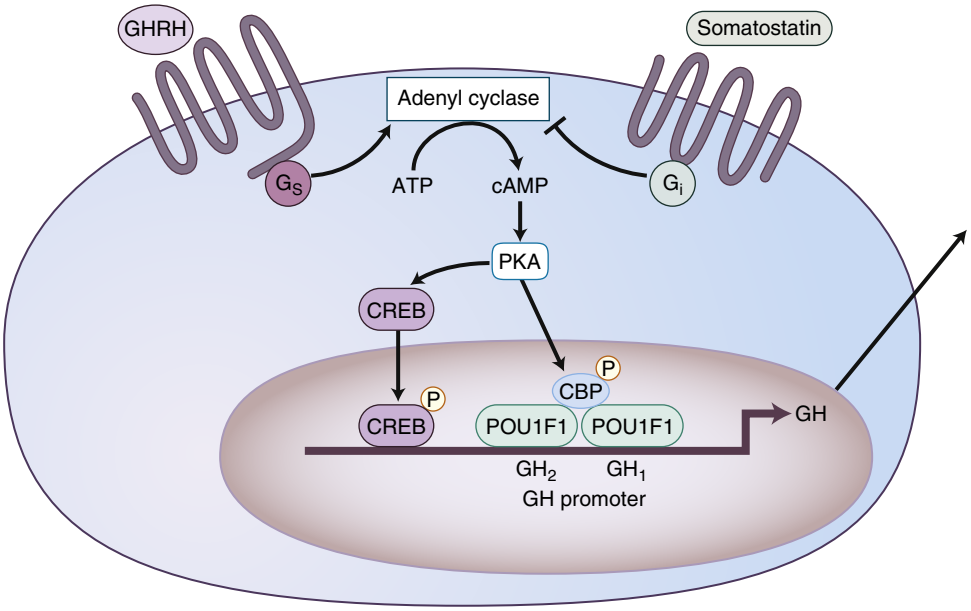
• **Fig. 25.14** The main components of the hypothalamic-pituitary portal system. (From Guyton AC, Hall JC. *Human Physiology and Mechanisms of Disease,* 6th ed. Philadelphia: WB Saunders; 1997:600, used with permission.)

Somatostatin (SST)

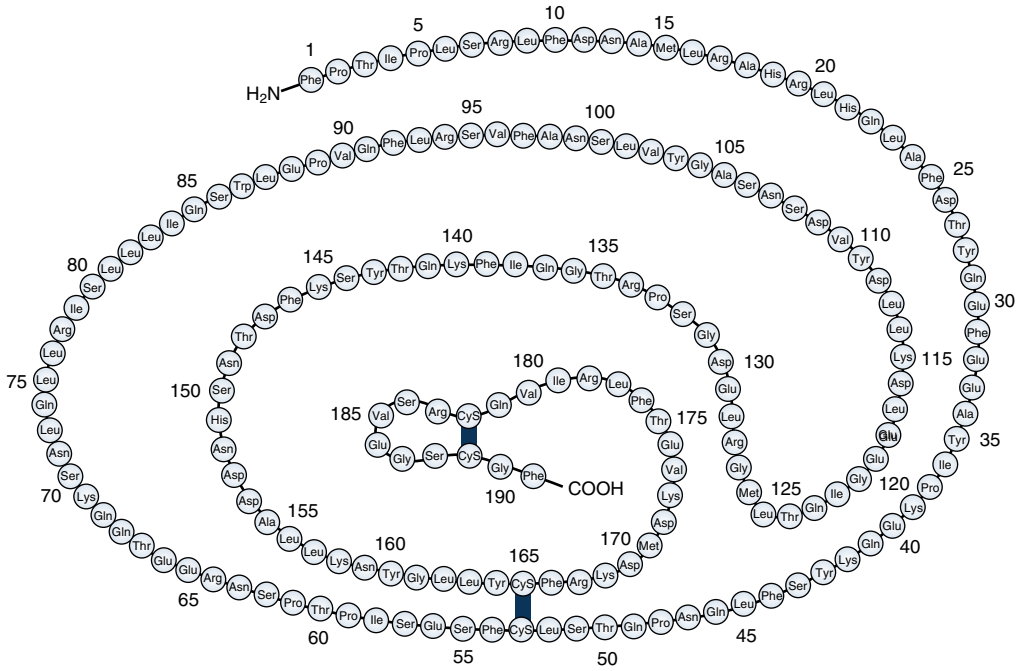
SST or somatotropin-release inhibitory factor (SRIF) is derived from a 116 precursor that produces two different cyclic forms, somatostatin-14 and somatostatin-28, by alternative post-translational processing. Somatostatin binds to at least five receptor subtypes (SST1–5 receptors), which are GPCRs with seven transmembrane domains. Subtypes 2 and 5 are the predominant types found in the pituitary.[88–90] SST binding results in the recruitment of several downstream signaling pathways, including AC, protein phosphatases, cyclic guanosine monophosphate (cGMP)–dependent protein kinases, and calcium in addition to other ion channels.[91–93] SST inhibits GH release and antagonizes the stimulatory effects from GHRH or ghrelin.[73]

Growth Hormone

Human GH is produced as a single-chain, 191–amino acid, 22-kDa protein containing two intramolecular disulfide bonds (Fig. 25.16). GH shares sequence homology with PRL, chorionic somatomammotropin (CS, placental lactogen), and a 22-kDa GH variant (GH-V) that is secreted only by the placenta and



• **Fig. 25.15** Schematic diagram summarizing the main signaling pathways in the somatotroph involved in the response to GHRH and somatostatin. GHRH binding to its receptor increases intracellular cAMP. Activation of the PKA pathway results in binding of POU1F1 to specific promoter sequences on the GH gene to increase transcription. Somatostatin binding to its receptor decreases intracellular cAMP.



• **Fig. 25.16** Covalent structure of human growth hormone. (From Chawla RK, Parks JS, Rudman D. Structural variants of human growth hormone: biochemical, genetic and clinical aspects. *Annu Rev Med*. 1983;34:519–547.)

differs from pituitary GH by 13 amino acids. The genes encoding these proteins most likely evolved from a common ancestral gene, despite being located on different chromosomes (chromosome 6 for PRL and chromosome 17 for GH).[94] The genes for GH, PRL, and placental lactogen share a common structural organization, with four introns separating five exons. The GH subfamily contains five members, with genes located on a 78-kilobase (kb) section of chromosome 17; the 5′ to 3′ order of the genes is GH, a CS pseudogene, CS-A, GH-V, and CS-B. Normally, about 75% of GH produced by the pituitary is of the mature, 22-kDa form. Alternative splicing of the second codon results in deletion of amino acids 32 through 46, yielding a 20-kDa form that normally accounts for 5% to 10% of pituitary GH.[94] The remainder of pituitary GH includes desamidated and *N*-acetylated forms and various GH oligomers.

Growth Hormone Pulsatility

The pulsatile pattern characteristic of GH secretion reflects the interplay of the two hypothalamic regulatory peptides, GHRH and somatostatin, with modulation by other putative GH-releasing factors.[95,96] Somatostatin appears to have its major effect on the timing and amplitude of pulsatile GH secretion and lesser effects on the regulation of GH synthesis. The pulsatile secretion of GH in vivo is believed to result from a simultaneous reduction in hypothalamic somatostatin release and increased GHRH release.[97] Conversely, a trough of GH secretion occurs when somatostatin is released in the face of diminished GHRH activity. The coordinated secretion of GH pulses in response to GH secretagogues results from a continuum of GH cells geographically connected by adherens junctions as revealed by three-dimensional reconstructive microscopy.[98]

Regulation of the reciprocal secretion of GHRH and somatostatin is imperfectly understood. The hypothalamus integrates signals for stress, sleep, hemorrhage, fasting, hypoglycemia, and exercise through the secretion of multiple neurotransmitters and neuropeptides to regulate the release of these hypothalamic factors and ultimately to influence GH secretion. This physiologic phenomenon forms the basis for a number of GH-stimulatory tests used in the evaluation of GH secretory capacity or reserve. GH secretion is also influenced by a variety of nonpeptide hormones, including androgens,[99] estrogens,[100] thyroxine,[101] and glucocorticoids.[102,103] The mechanisms by which these hormones regulate GH secretion may involve actions at the hypothalamus and the pituitary. For example, hypothyroidism and glucocorticoid excess may each blunt spontaneous and provocative GH secretion. Gonadal steroids appear to be responsible for the rise in GH secretion that characterizes puberty.

Ghrelin

Synthetic hexapeptides capable of stimulating GH secretion are termed *GH secretagogues*.[80] These peptides stimulate GH release and enhance the GH response to GHRH, although they work at receptors distinct from those for GHRH, at hypothalamic and pituitary sites. Kojima and colleagues[104] identified a natural ligand called *ghrelin*, a 28–amino acid protein with the serine 3 residue *n*-octanoylated. It is produced mainly by the oxyntic cells of the stomach (and throughout the gastrointestinal tract[105]) and in the hypothalamus, heart, lung, and adipose tissue.[106,107] Ghrelin has a potent, dose-related, GH-releasing effect[108] and potentiates the GHRH-dependent secretion of GH. At the hypothalamic level, it increases GHRH secretion and inhibits somatostatin neurons.[81,109–111] GH release results from the binding of ghrelin to the growth hormone secretagogue 1a receptor (GHSR1a) on somatotrophs in the pituitary[108] and on GHRH-containing neurons in the hypothalamus.[112] The coupling of ghrelin to the GHSR1a receptor results in activation of multiple signaling cascades, including phospholipase C (PLC), protein kinase C (PKC), PKA,[113] intracellular and extracellular Ca^{2+}, or mitogen-activated

protein kinases.[110,114,115] Many studies have demonstrated that ghrelin has a wide range of effects, including on immune function, cognition, other anterior pituitary hormones (including gonadal axis regulation), bone metabolism, gastrointestinal motility, cell proliferation, and the cardiovascular system.[111,116–123]

However, it is difficult to separate the direct effects of ghrelin from those related to GH secretion. Although ghrelin has documented physiologic effects in vivo, GHSR1a knockout mice have a phenotype similar to that of wild-type animals, suggesting that ghrelin does not have a role in growth. However, compensatory mechanisms may provide an explanation for these findings.[124,125]

More recently, studies have documented a positive correlation between ghrelin and anthropometric parameters in the first months of life, a finding that strengthens the hypothesis that ghrelin exerts an influence on growth.[126] Two reports of mutations in GHSR1a in familial short stature provide evidence to the contrary.[127,128] Further studies indicate that the aging process may be associated with decreased expression of GHSRs in the hypothalamus[129] and with systemic concentration of ghrelin.[130] In addition to direct effects on linear growth, ghrelin has been shown to increase energy stores by stimulating appetite and affecting peripheral glucose and lipid metabolism.[131–133] These data suggest that ghrelin is an important stimulus for nutrient allocation for growth and metabolism and a central component of the GH regulatory system. Orally active ghrelin analogues have been considered as therapeutic agents in the treatment of GH deficiency (GHD), because they may provide a more physiologic approach to increasing the pulsatile release of endogenous GH compared with a single daily dose of recombinant human GH. However, there has been no definite evidence of the therapeutic efficacy of ghrelin analogues in the treatment of GHD states.

Pituitary Adenylate Cyclase–Activating Polypeptide

Pituitary adenylate cyclase–activating polypeptide (PACAP) is a hypothalamic peptide that has been shown to be effective in releasing GH from cultured pituitary cells. It belongs to a superfamily of hormones that includes glucagon, secretin, glucagon-like peptide 1 (GLP1), GLP2, GHRH, vasoactive intestinal polypeptide (VIP), peptide histidine methionine (PHM), and glucose-dependent insulinotropic polypeptide (GIP). Studies on the role of PACAP on GH release have been contradictory, some reporting a stimulatory action while others showing no effect on GH release.[73] PACAP increased both cAMP production and GH release in human somatotrope tumor cell lines[134] involving activation of voltage-operated/gated Ca^{2+} channels and signaling through the AC/PKA pathway.[73,135] However, studies in humans did not reveal an induction of GH release after intravenous PACAP administration.[136]

PACAP binds to several G protein–coupled receptors with differential affinity for PACAP or VIP. PACAP type 1 receptors (PAC1R) are more specific for PACAP, while VPAC1 and VPAC2 receptors bind VIP and PACAP isoforms with similar affinity. Further complexity is added by at least five alternative splice products of PAC1R alternative splicing, which have differential affinity for PACAP isoforms and induce different signaling pathways.[137,138] PACAP receptors (PAC1R) are found throughout the brain and in peripheral tissues.[137,138] Gene knockout of the specific PACAP receptor (PAC1R) resulted in a 60% mortality rate in the PAC1R null mice in the first 4 weeks after birth, providing insight into the importance of PACAP, even though other superfamily members may compensate some of its functions.[139] The surviving knockout mice showed reduced glucose-stimulated insulin release and glucose intolerance. This observation suggests that PACAP is important in carbohydrate metabolism, potentially through GH.

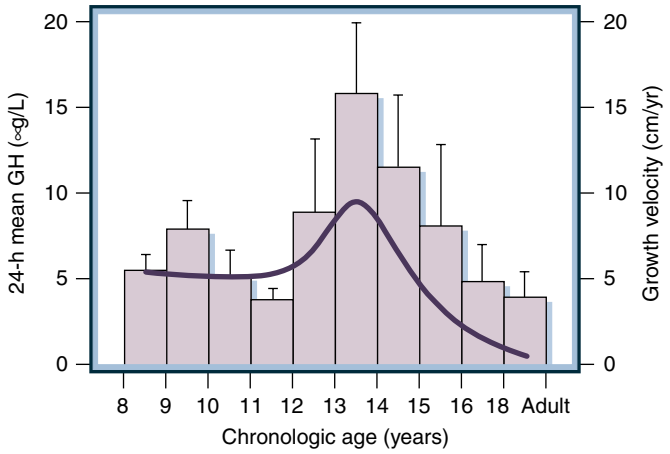
The synthesis and secretion of GH are also regulated by the insulin-like growth factor (IGF) peptides. Receptors specific for IGF1 and IGF2 have been identified in the hypothalamus and pituitary.[140] Inhibition of GH secretion by IGF1 or IGF2, or both, has been demonstrated,[141,142] and spontaneous GH secretion is diminished in humans treated with synthetic IGF1.[143]

Growth Hormone Secretion in Humans

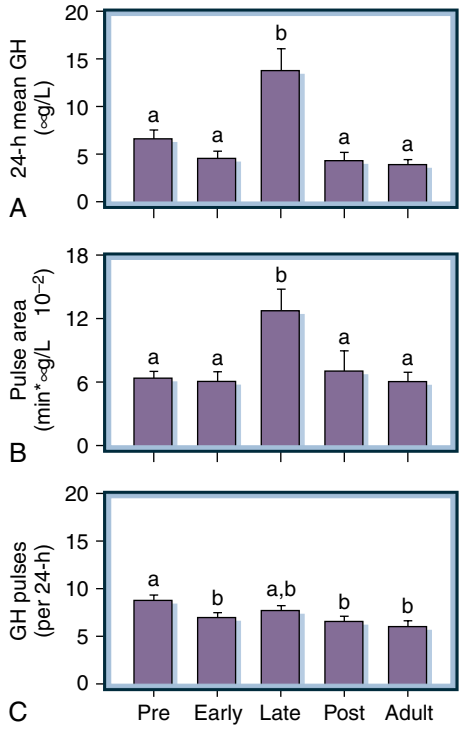
The episodic release of GH from the pituitary somatotrophs results in intermittent increases in serum levels of GH separated by periods of low or undetectable levels, during which time GH secretion is minimal.[97,144] The pulsatile nature of GH secretion has been demonstrated by frequent serum sampling coupled with the use of sensitive immunofluorometric or chemiluminescent assays of GH.[144] GHRH is primarily responsible for the generation and maintenance of pulsatile/episodic GH secretion.[73,74] Under normal circumstances, serum GH levels are less than 0.04 mg/L between secretory bursts. It is impractical to assess GH secretion by random serum sampling. Extensive sampling studies at different ages in normal persons and in many abnormal conditions have defined GH pulses, basal secretion, and diurnal variability. Computer programs have been developed to indicate whether changes in GH levels in various life periods and under diverse clinical circumstances occur because of a change of secretory mass or pulse frequency, altered clearance, or a combination of these processes.[144,145] Deconvolution techniques allow accurate estimates of the quantity of GH secreted per burst, GH clearance kinetics, and pulse amplitudes and frequencies, as well as an overall calculation of endogenous GH production. Approximate entropy, a model-free measure, is applied to quantify the degree of orderliness of GH release patterns.[146] The impact of the specific nature of pulsatile GH secretion on its biologic actions is under study.[144,145] For example, it appears that better statural growth is associated with large swings of GH output of relatively uniform magnitude in an irregular sequence (high approximate entropy).[147,148]

GH-secreting cells have been identified by 9 to 12 weeks of gestation, and immunoreactive pituitary GH is present by 7 to 9 weeks of gestation.[149] Fetal pituicytes secrete GH in vitro by 5 weeks, before the hypothalamic-portal vascular system is differentiated. PitT1 mRNA and Pit1 protein are expressed by at least 6 weeks of gestation; their abundant presence early in gestation suggests an important role in cytodifferentiation and cell proliferation.[150] GH can be identified in fetal serum by the end of the first trimester, with peak levels of approximately 150 mg/L in midgestation.[149] Serum levels fall throughout the latter part of pregnancy and are lower in full-term than in premature infants, perhaps reflecting feedback by the higher serum levels of IGF peptides that characterize the later stages of gestation.[151]

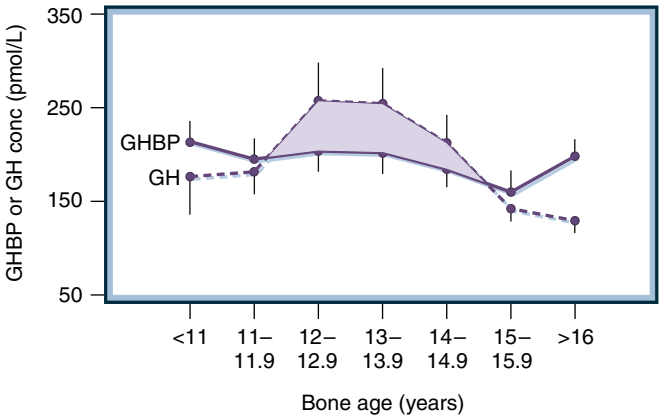
Mean levels of GH decrease from values of 25 to 35 mg/L in the neonatal period to approximately 5 to 7 mg/L through childhood and early puberty.[152] Twenty-four-hour GH secretion peaks during adolescence, undoubtedly contributing to the high serum levels of IGF1 that are characteristic of puberty. The increase in GH production during middle to late puberty is caused by enhanced pulse amplitude and increased mass of GH per secretory burst, rather than by a change in pulse frequency (Figs. 25.17 and 25.18).[145,146,152] Greater irregularity in GH secretion corresponds to greater linear growth.[153] In the face of stable levels of the growth hormone–binding protein (GHBP),[154] the enhanced pubertal GH production appears to be associated with higher



• **Fig. 25.17** Relation between 24-hour mean growth hormone (GH) levels and age in boys and men. The bars represent the 24-hour mean and standard error (+SE) values of GH *(left axis)* obtained from 60 24-hour GH profiles of healthy boys and men subdivided according to chronologic age. An idealized growth velocity curve reproduced from the 50th percentile values for whole-year height velocity of North American boys[16] is superimposed. (From Martha PM Jr, Rogol AD, Veldhuis JD, et al. Alterations in the pulsatile properties of circulating growth hormone concentrations during puberty in boys. *J Clin Endocrinol Metab.* 1989;69:563–570.)



• **Fig. 25.18** (A) The 24-hour and standard error (+SE) levels of growth hormone (GH) for groups of normal boys at varied stages of pubertal maturation. (B) The mean (+SE) area under the GH concentration-vs.-time curve for individual GH pulses, as identified by the cluster pulse detection algorithm. (C) The number of GH pulses (+SE), as detected by the cluster algorithm, in the 24-hour GH concentration profiles for boys in each of the pubertal study groups. Notice that the mean 24-hour GH concentration changes are largely mediated by changes in the amount of GH secreted per pulse, rather than the frequency of pulses. In each panel, bars bearing the same letter are statistically indistinguishable. (From Martha PM Jr, Rogol AD, Veldhuis JD, et al. Alterations in the pulsatile properties of circulating growth hormone concentrations during puberty in boys. *J Clin Endocrinol Metab.* 1989;69:563–570.)



• **Fig. 25.19** Levels of growth hormone (GH) and growth hormone–binding protein (GHBP) measured in normal pubertal boys throughout adolescence. The GHBP levels do not significantly change during puberty, but there is a significant increment of GH production and therefore of GH levels during this same time. These data suggest that there may be greater amounts of "free GH" during this period, leading to greater production of insulin-like growth factor 1. (Based on data from Martha PM Jr, Rogol AD, Blizzard RM, et al. Growth hormone-binding protein activity is inversely related to 24-hour growth hormone release in normal boys. *J Clin Endocrinol Metab*. 1991;73:175–181; Martha PM Jr, Rogol AD, Veldhuis JD, et al. Alterations in the pulsatile properties of circulating growth hormone concentrations during puberty in boys. *J Clin Endocrinol Metab.* 1989;69:563–570.)

levels of "free" GH (Fig. 25.19), potentially facilitating the delivery of IGF1 to target tissues. This enhanced activity of the GH-IGF axis contributes to the insulin resistance that occurs during puberty.[155] Production of GH and IGF begins to decline by late adolescence and continues to fall throughout adult life. Normal young adult men experience 6 to 10 GH secretory bursts per 24 hours, a value similar to that observed in younger children and adolescents.[144,152] On the other hand, 24-hour GH production rates for normal men range from 0.25 to 0.52 mg/m^2 surface area,[103] about 20% to 30% of pubertal levels; this is largely due to decreased GH pulse amplitude with age.[152] Indeed, puberty may be considered, with some justification, a period of "physiologic acromegaly," whereas aging, with its decrease in GH secretion, has been termed the *somatopause*.[100,156]

Physiologic states that affect GH secretion, in addition to maturation and aging, include sleep,[157] nutritional status,[95,158] fasting, exercise,[159] stress,[159] and gonadal steroids. Maximal GH secretion occurs during the night, especially at the onset of the first slow-wave sleep period (stages III and IV). Rapid eye movement (REM) sleep, on the other hand, is associated with low GH secretion.[157] A circadian rhythm of somatostatin secretion, upon which is superimposed episodic bursts of GHRH release, may help explain the nocturnal augmentation of GH production.[160]

When testosterone was administered to boys with delayed puberty, spontaneous GH release was enhanced, but such a change was not duplicated by administration of nonaromatizable androgens, emphasizing the possible unique importance of estrogen in GH secretion.[161] The effects of testosterone on serum IGF1 levels may in part be independent of GH because individuals with mutations of the GH receptor (GHR) still experience a modest rise in serum IGF1 during puberty.[162] With a combination of deconvolution analysis, approximate entropy, and cosine regression analysis, Veldhuis and associates[146,161] carefully evaluated intensive GH sampling data derived from measurements in sensitive GH assays

in prepubertal and pubertal children of both genders. In addition to the amplified secretory burst mass caused by jointly increased GH pulse amplitude and duration, they found that sex steroids selectively affect facets of GH neurosecretory control: Estrogen increases the basal GH secretion rate and the irregularity of GH release patterns, whereas testosterone stimulates greater GH secretory burst mass and greater IGF1 concentrations.

Obesity is characterized by lowered GH production, reflected by a diminished number of GH secretory bursts and shorter half-life duration.[163,164] Obesity in childhood and adolescence is characterized by decreased GH production with normal IGF and increased GHBP levels and often by increased linear growth.[164] The hyperinsulinism associated with obesity causes lowered levels of IGF-binding protein 1 (IGFBP1) and, perhaps, higher levels of "free" IGF1.[165] Endogenous GH secretion and levels achieved during provocative tests in obese subjects[166] approximated the diagnostic range of GHD. Fasting increased both the number and the amplitude of GH secretory bursts, presumably reflecting decreased somatostatin secretion and enhanced GHRH release, while lowering GHBP concentrations. Rapid changes in levels of IGFBPs in response to altered nutrition and changes in insulin levels may modify the effect of IGF1 on its negative feedback and effector sites.[158,164] Body mass also influences GH production in normal prepubertal and pubertal children and adults.[167–169]

Peripheral Modulators of Growth Hormone

Glucocorticoids

Conflicting studies have shown that glucocorticoids (GCs) are able to either stimulate or inhibit GH secretion, depending on specific conditions.[170–178] During a short-term (1-hour) incubation, GCs inhibited GHRH-stimulated GH secretions due to an increase in SST secretion.[172,179–181] After a 3-hour treatment with dexamethasone in normal human subjects, GH release was stimulated, followed by an inhibition after 12 hours.[173,174] After 4 days of treatment with prednisone, GHRH- stimulated GH secretion was blunted.[175] Signaling is thought to involve the activation of cAMP/PKA or PKC signaling pathways and intracellular free calcium mobilization.[182]

Thyroid Hormones

Several studies have shown that an increase in thyroid hormone (TH) levels in humans inhibits GH release due to either an increase in SST tone or a direct effect to decrease GHRH release.[183,184] THs have also been shown to inhibit human growth hormone (hGH) gene expression and secretion of GH in somatotroph cell cultures.[185] T_3 treatment also decreased hGH RNA levels without an effect on GH protein in transgenic (171hGH/CS-TG) mice overexpressing the human GH gene.[186]

Insulin and IGF1

IGF1 and IGFBP3 have been shown to positively affect spontaneous 24-hour GH secretion in human volunteers.[187] IGF1 has a negative feedback role to the hypothalamus and pituitary to regulate GH secretion. Low doses of recombinant IGF1 infusion resulted in a blunted response to the fasting-stimulated GH secretion,[188] and its administration at physiologic doses decreased the GH response to GHRH without altering spontaneous GH levels.[189] Further, free and not total IGF1 levels may be the primary mediator of GH secretion[190]; however, a single dose of recombinant IGF1 does not affect basal or pulsatile GH release.[191]

Similarly, insulin reduces GH response to GHRH,[192] and the elevated insulin associated with obesity is thought to decrease GH levels.[193] IGF1 and insulin have a direct effect on the somatotropes to decrease GH mRNA levels and GH secretion.[194] Somatotrophs are suppressed by IGF1-mediated signalings through the PI3K, mTORC1, and MEK pathways and insulin by the PI3K signaling pathway.[176,195,196] Deletion of the IGF1 receptor from somatotrophs in a genetically engineered mouse line resulted in mice of normal weight and length with only slightly elevated GH messenger RNA (mRNA) and serum GH levels.[197]

Free Fatty Acids

Free fatty acids (FFAs) inhibit GHRH-stimulated GH secretion by suppressing GHRH and/or stimulating SST secretion, or a direct effect on somatotropes.[198–205]

Adipokines

The adipokines (leptin, adiponectin, and resistin) are members of the cytokine family of proteins, which are released from adipose tissues. Although studies have reported an effect of leptin or adiponectin on somatotrophs, the precise relevance is unknown. Adiponectin has been associated with GH pulse secretion; however, whether this is a direct or indirect effect is unknown.[206] Adiponectin has also been shown to decrease GHRH-stimulated, but not ghrelin-stimulated, GH release. Leptin and resistin both increase GH secretions. The adipokines all utilize the AC/PKA signaling pathway; leptin also signals through intracellular/extracellular calcium and PLC/PKC, adiponectin through intracellular/extracellular calcium, and resistin through the mTOR pathway.[207]

Estrogens

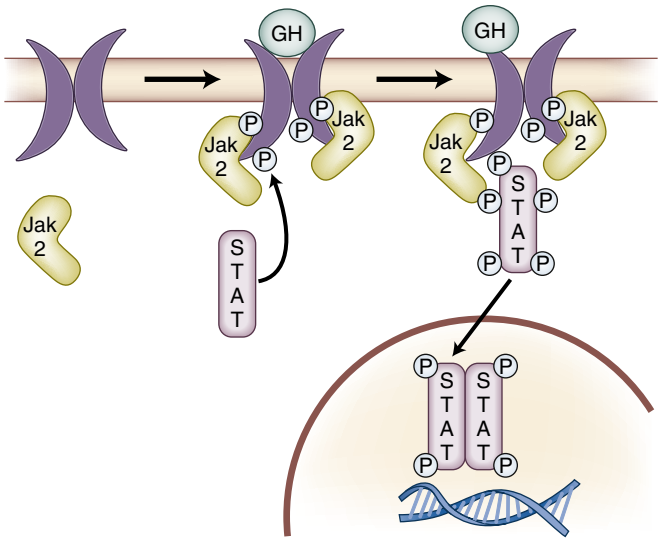
Estrogen receptors are expressed in somatotropes, and treatment with estradiol decreased IGF1 and elevated basal GH concentrations in men.[208] In postmenopausal women, estrogen treatment increased GH secretions and decreased IGF1 levels. These effects may be mediated directly or indirectly by negative feedback effects of IGF1.[209] In rodents, deletion of the estrogen receptor alpha resulted in a decrease in GH mRNA expression and secretion.[210] Both estrogen receptor α and β stimulate pituitary GH gene expression.

The Growth Hormone Receptor and Growth Hormone–Binding Protein

The gene for the human GHR is located on chromosome 5p13.1-p12, where it spans more than 87 kb.[211] The GHR gene *(GHR)* contains 10 exons: Exon 1 contains the 5′ untranslated region; exons 2 through 10 encode the three domains of the GHR—the extracellular ligand-binding domain, a single transmembrane domain, and a cytoplasmic domain for signal transduction.[212] The highest level of *GHR* expression is in the liver, followed by muscle, fat, kidney, and heart. The receptor is 638 amino acids long, with a predicted molecular weight of 70 kDa before glycosylation.

Two isoforms of the *GHR* have been found in humans—a full-length form and a form that has a deletion of exon 3 *(GHRd3)* with a loss of a 22–amino acid segment of the extracellular domain of the receptor.[213] The *GHRd3* allele is present in approximately 33% of the general population.[213] Several studies have investigated the clinical significance of the *GHRd3* allele on growth and GH responsiveness in specific disease states.

GH must be bound to a homodimer complex of GHR to activate its intracellular signaling pathways (Fig. 25.20). Whether dimerization of the GHR subunits occurs before or after GH binding is a matter of debate. It was initially thought that dimerization would occur only after GH binding; GH would bind to the first subunit, after which the GH-GHR complex would diffuse within



• **Fig. 25.20** A model depicting intracellular signaling intermediates induced by binding of growth hormone (GH) with the GH receptor (GHR). *Jak,* Janus kinase; *P,* phosphorylation; *STAT,* signal transducer and activator of transcription. (From Le Roith DC, Bondy S, Yakar J-L, et al. The somatomedin hypothesis. *Endocrine Rev.* 2001;22:53–74.)

the membrane until contacted by a second subunit, leading to receptor activation.[214] However, it was shown in live cells that the subunits of the GHR are constitutively dimerized in an inactive (i.e., unbound) state.[215] The GH binding sites on the extracellular domains of the two subunits are placed asymmetrically. Therefore once GH binds to both subunits, it induces rotation of the two subunits of the dimer that is transmitted via the transmembrane domain to the intracellular domain, allowing downstream kinase activation by transphosphorylation.

After binding to its receptor, GH stimulates phosphorylation of Janus kinase 2 (JAK2), a tyrosine kinase associated with the GHR (see Fig. 25.20). On recruitment or activation, the JAK2 molecule causes phosphorylation of critical tyrosines on the intracellular portion of the GHR, a sort of transphosphorylation. The phosphorylated tyrosines on the GHR provide docking sites for critical intermediary STAT (signal transducers and activators of transcription) proteins.[214] STAT proteins dock, via their Src homology 2 (SH2) domain to phosphotyrosines on ligand-activated receptors, such as the GHR. After docking, phosphorylation occurs on single tyrosines at the carboxy-terminus (C-terminal) of the protein. Then STATs dissociate from the GHR, dimerize, translocate to the nucleus, and bind to DNA through their DNA-binding domain to regulate gene transcription. There are seven known mammalian STATs; of these, STAT5B appears to be most critically involved in mediating the growth-promoting actions of the GHR.[216]

GHR signaling also leads to activation of extracellular-regulated kinase 1 (ERK1) and ERK2 to increase transcription.[217] How GHR activation promotes ERK1/2 activation is a matter of debate. A JAK2-independent but Src-dependent phosphorylation has been modeled,[167] as has a JAK2-dependent but Src-independent mechanism.[218] Experiments were conducted in different cell lines, so it is possible that the mechanisms of ERK1/2 activation by GHR are different in different cells. It is unclear what role these pathways play in the GH stimulation of growth.

GHBP prolongs the half-life of GH, presumably by impairing glomerular filtration, and modulates its binding to the GHR. It binds GH with high specificity and affinity but with low capacity; only about 45% of circulating GH is bound.[154] GHBP is derived from proteolytic cleavage of the extracellular domain of the receptor.[219] GHBP levels reflect GHR levels and activity; that is, low levels are associated with states of GH insensitivity.[154] Levels of GHBP are low early in life, rise through childhood, and plateau during the pubertal years and adulthood.[220] Impaired nutrition, diabetes mellitus, hypothyroidism, chronic liver disease, and inherited abnormalities of the GHR are associated with low levels of GHBP, whereas obesity, refeeding, early pregnancy, and estrogen treatment are associated with elevated levels of GHBP.[154] In general, GHBP levels reflect GHR levels and activity. Patients with GH insensitivity due to defects of the extracellular domain of the GHR have low GHBP levels, and GHBP levels can therefore be useful in identifying these individuals. Patients with GH insensitivity due to nonreceptor abnormalities, defects of the intracellular domain of the GHR, or inability of the receptor to dimerize may have normal levels of GHBP.[162]

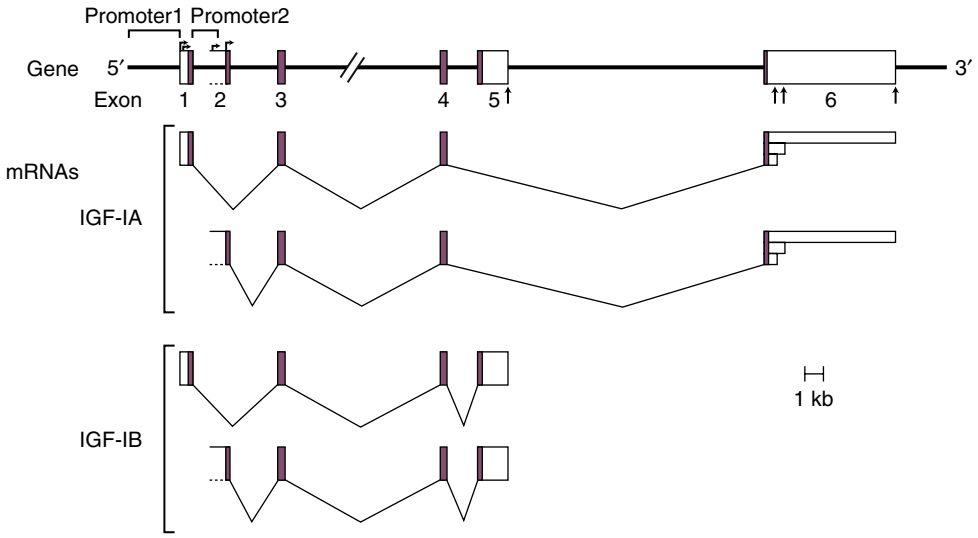
GHR signaling affects the transcription of many genes immediately (<3 hours after stimulation) and other genes over a longer period of stimulation. After acute GH stimulation in GH-deficient rats, hepatic genes immediately induced by GH included signal transducers (STAT3, gp130, p38), DNA repair proteins, receptor proteases, and metabolic regulators such as Igf1, Igfbp3, and Mct1.[221] GHR signaling is involved in regulation of expression of genes involved in carbohydrate, fat, and steroid metabolism.[222]

The study of mice that lack *Ghr* or downstream components of GHR signaling has shed light on the role of GH in normal physiology. *Ghr*$^{-/-}$ mice exhibit normal size at birth but have attenuated postnatal growth, with body weight about half of normal and length about two-thirds of normal.[223] *Ghr*$^{-/-}$ mice also exhibit delayed pubertal maturation, longer life span, and increased insulin sensitivity compared with a control group. Mice with *Ghr* deleted only in the liver have low IGF1 levels and high GH levels. These mice exhibit normal growth but have significantly lower bone density than controls. They also exhibit liver steatosis with insulin resistance and elevated serum free fatty acid levels,[224] showing the metabolic function of excessive GHR signaling independent of serum IGF1 signaling.

Deletion of *Jak2* in mice is embryonically lethal,[225] as is deletion of *Stat3*. Deletion of *Stat1* or *Stat5a* does not affect body size. However, deletion of *Stat5b* leads to decreased size in male but not female mice. The *Stat5a/b*$^{-/-}$ mouse is smaller than the *Stat5b*$^{-/-}$ mouse.[226] The combined *Igf1* and *Ghr*$^{-/-}$ mouse had a more severe attenuation of postnatal growth than mice with knockout of either gene alone, indicating that GH and IGF1 promote growth by both common and independent functions.[227] The liver-specific IGF1 knockout mouse with extremely low serum IGF1 levels has normal linear growth, suggesting IGF-independent actions of GH or paracrine production and effects of IGF1, or both.[228]

Historically, the anabolic actions of GH were thought to be mediated entirely by the IGF peptides (the so-called somatomedin hypothesis).[164] Although a majority of GH actions are mediated by IGFs, the opposite effects of GH and IGFs on metabolism and in knockout mouse models suggest that there are IGF-independent actions of GH. Indeed, the "diabetogenic" actions of GH are contradictory to the glucose-lowering effects of IGFs. In vitro studies suggest potential IGF-independent action of GH in the following tissues:

1. Epiphysis—stimulation of epiphyseal growth
2. Bone—stimulation of osteoclast differentiation and activity, stimulation of osteoblast activity, and increase in bone mass through endochondral bone formation



• **Fig. 25.21** Structure and expression of the human insulin-like growth factor 1 (IGF1) gene. The structure of the different human IGF1 messenger RNAs (mRNAs) is displayed below the map of the gene. Sites of pre-mRNA processing are indicated by the *thin lines*. Sites of differential polyadenylation are marked at the 3′ end of the gene by *vertical arrows* and in the mRNAs by *horizontal boxes* of varying length. (From Rotwein P. Structure, evolution, expression and regulation of insulin-like growth factors I and II. *Growth Factors.* 1991;5:3–18.)

3. Adipose tissue—acute insulin-like effects, followed by increased lipolysis, inhibition of lipoprotein lipase, stimulation of hormone-sensitive lipase, decreased glucose transport, and decreased lipogenesis
4. Muscle—increased amino acid transport, increased nitrogen retention, increased lean tissue, and increased energy expenditure

Insulin-Like Growth Factors

Historic Background. The IGFs (somatomedins) are a family of peptides that are, in part, GH dependent and mediate many of the anabolic and mitogenic actions of GH. They were originally identified in 1957 by their ability to stimulate [^{35}S]-sulfate incorporation into rat cartilage and were called *sulfation factors*.[164] In 1972, that term was replaced with *somatomedin*,[169] and purification of somatomedin from human serum yielded a basic peptide (somatomedin C) and a neutral peptide (somatomedin A).[229] In 1978, Rinderknecht and Humbel[230,231] isolated two active somatomedins from human plasma and, after demonstrating a striking structural resemblance to proinsulin, renamed them *insulin-like growth factors* (IGFs).

IGF Genes and Protein Structure. There are two IGFs circulating in humans, IGF1 and IGF2. IGF1 is a basic peptide of 70 amino acids, and IGF2 is a peptide of 67 amino acids. The two peptides share 45 of 73 possible amino acid positions and have 50% amino acid homology to insulin.[164,230,231] Like insulin, both IGFs have A and B chains connected by disulfide bonds. The connecting C-peptide region is 12 amino acids for IGF1 and 8 amino acids for IGF2, bearing no homology for the C-peptide region of proinsulin. The structural similarity to insulin explains the ability of both IGFs to bind to the insulin receptor and the ability of insulin to bind to the type I IGF receptor (encoded by *IGF1R*). On the other hand, structural differences probably explain the failure of insulin to bind with high affinity to the IGFBPs.

Insulin-Like Growth Factor 1

Gene Regulation. The human IGF1 gene *(IGF1)* is located on the long arm of chromosome 12 and contains at least six exons (Fig. 25.21). Exons 1 and 2 encode alternative signal peptides, each containing several transcription start sites; that is, the multiple existing *IGF1* transcripts consist of either exon 1 or exon 2. Two different promoters regulated in a tissue-specific manner[232] control the use of exon 1 or 2. Exons 3 and 4 encode the remaining signal peptide, the remainder of the mature IGF1 molecule, and part of the trailer peptide (E peptide). Exons 5 and 6 encode alternatively used segments of the trailer peptide and 3′ untranslated sequences with multiple different polyadenylation sites. As a result, multiple mRNA species exist, allowing for tissue-specific, developmental, and hormonal regulation of *IGF1* gene expression.

GH is the primary regulator of *Igf1* transcription, resulting in a 20-fold rise in *Igf1* mRNA. There may be tissue-to-tissue variability in the extent of GH-induced expression of *Igf1* mRNA.[233,234] Estrogen also regulates *IGF1* transcription, as its administration to a *Ghr*$^{-/-}$ mouse can stimulate hepatic *Igf1* synthesis and growth.[223] The sex steroid effects on *IGF1* transcription play a role in the pubertal rise of IGF1 levels in humans[235] (see later discussion).

The mechanisms involved in regulation of IGF gene expression include the existence of multiple promoters, heterogeneous transcription initiation within each of the promoters, alternative splicing of various exons, differential RNA polyadenylation, and variable mRNA stability. The transcription factor STAT5B is the most critical mediator of GH-induced activation of *IGF1* transcription, as detailed earlier. Two adjacent STAT5B binding sites are present in the second intron of the rat *Igf1* gene, within a region that undergoes acute changes in chromatin structure upon GH treatment.[216]

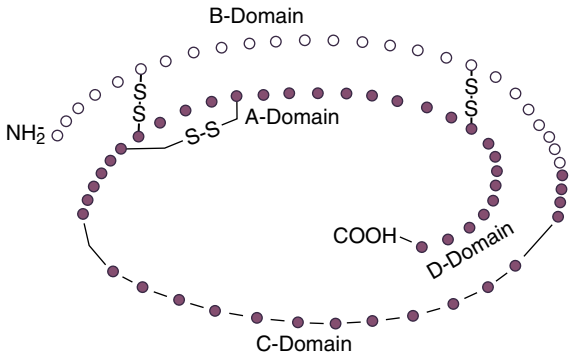
Once translated, IGF1 pre-propeptides require processing to form the mature IGF1 peptide (Fig. 25.22). Proteases from the subtilisin-related proprotein convertase family (SPC) cleave pro-IGF1.[236]

Serum Levels. In human fetal serum, IGF1 levels are relatively low and positively correlated with gestational age.[237] There is a reported correlation between fetal cord serum IGF1 levels and birth weight,[238] but this relationship is controversial.[239] IGF1 levels in newborn serum are typically 30% to 50% of adult levels and rise

during childhood. During puberty, IGF1 levels rise to two to three times the adult range. Levels during adolescence correlate better with Tanner stage or bone age than with chronologic age.[240] The pubertal rise in gonadal steroids may stimulate IGF1 production indirectly by contributing to a rise in GH secretion and directly by augmenting liver synthesis and secretion of IGF1. Girls with gonadal dysgenesis show no adolescent increase in serum IGF1, providing evidence of the association of the pubertal rise in IGF1 with the production of gonadal steroids.[241] As further evidence, patients with GH insensitivity due to GHR mutations exhibit a modest rise in IGF1 during puberty despite a decline in GH levels. After 20 to 30 years of age, serum IGF1 levels gradually and progressively fall, and this decline is implicated in the negative nitrogen balance, decrease in muscle mass, and osteoporosis of aging.[242]

Insulin-Like Growth Factor 2

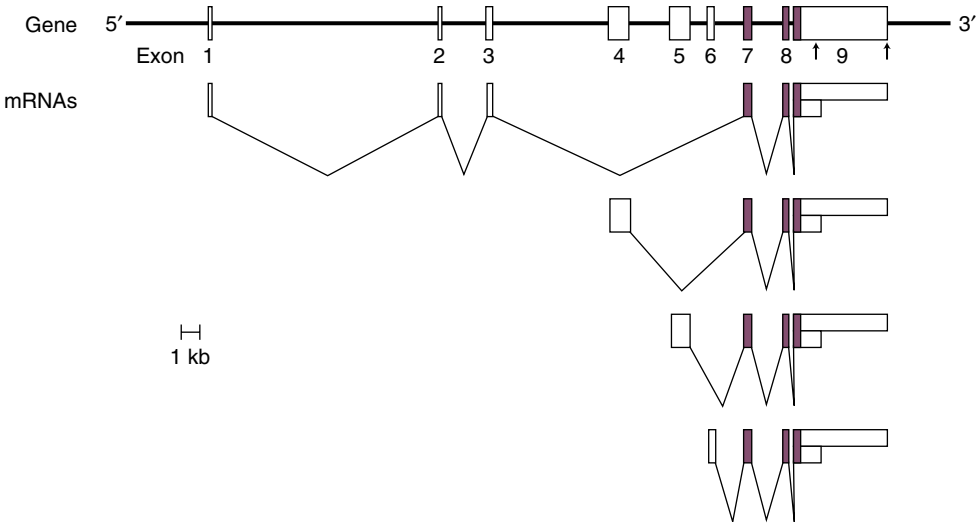
Gene Regulation. The gene for IGF2 *(IGF2)* is located on the short arm of chromosome 11, adjacent to the insulin gene, and contains 9 exons (Fig. 25.23). Exons 1 through 6 encode 5′ untranslated RNA, exon 7 encodes the signal peptide and most of the mature protein, and exon 8 encodes the C-terminal portion of the protein. As with *IGF1*, multiple mRNA species exist to allow for developmental and hormonal regulation of expression. *IGF2* mRNA expression is high in fetal life and has been detected as early as the blastocyst stage in mice.[243] Fetal tissues generally have high *IGF2* mRNA levels that decline postnatally.



• **Fig. 25.22** Structure of the insulin-like growth factor 1 (IGF1) peptide. See discussion in text. (From Yakar S, Wu Y, Setser J, et al. The role of circulating IGF-1. *Endocrine.* 2002;19:239–248.)

IGF2 is imprinted—that is, only one allele is active, depending on parental origin. In the case of *IGF2*, only the paternally expressed allele is active. Most imprinted genes occur in clusters with reciprocally imprinted genes, and *IGF2* is no exception. The noncoding gene *H19* is downstream from *IGF2* and is oppositely imprinted, meaning that only the maternal allele is expressed and the paternal allele is inactive. The promoters of *IGF2* and *H19* share a set of enhancers that act on either gene. On the paternal allele, the *H19* promoter region is methylated and thus inactivated (so-called epigenetic regulation of expression).[244] The *IGF2* promoter does not contain regions that can be methylated. Instead, upstream from the *H19* and *IGF2* promoter region is a so-called differentially methylated region (DMR). When it is methylated, binding of CCCTC binding factor (CTCF) is prevented, allowing enhancers to act on the *IGF2* promoter to activate transcription.[245] On the maternal chromosome, the DMR is not methylated, allowing CTCF to bind and preventing transcription (Fig. 25.24).[246]

The fact that *IGF2* is monoallelically expressed emphasizes the importance of gene dosage to normal physiology and development. Loss of imprinting of *IGF2* can lead to constitutively expressed *IGF2* mRNA and excessive IGF2. *IGF2* mRNA is expressed constitutively in a number of mesenchymal and embryonic tumors, including Wilms tumor,[247] rhabdomyosarcoma, neuroblastoma, pheochromocytoma, hepatoblastoma, leiomyoma, leiomyosarcoma, liposarcoma, and colon carcinoma.[248] Loss of imprinting of *IGF2* resulting in biallelic gene expression occurs in Beckwith-Wiedemann syndrome (BWS), which is characterized by fetal and neonatal overgrowth and an increased risk of childhood



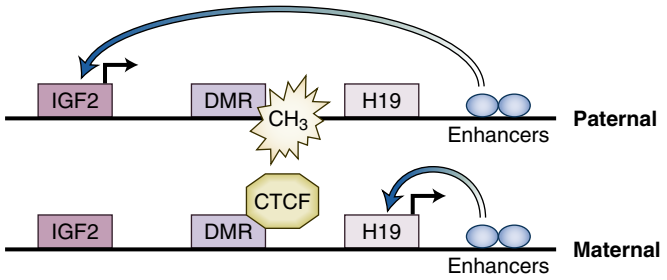
• **Fig. 25.23** Structure and expression of the human insulin-like growth factor 2 (IGF2) gene. The structure of different human IGF2 messenger RNAs (mRNAs) is displayed below the map of the gene. The patterns of mRNA processing are indicated by the *thin lines*. Sites of differential polyadenylation are marked at the 3′ end of the gene by *vertical arrows* and in the mRNAs by *horizontal boxes* of varying length. (From Rotwein P. Structure, evolution, expression and regulation of insulin-like growth factors 1 and 2. *Growth Factors.* 1991;5:3–18.)

cancers. Loss of imprinting in BWS results from mutations that affect imprinting in the region of chromosome 11 that contains *IGF2*,[249] methylation defects that cause hypermethylation of the region or duplication of the expressed paternal allele, resulting in increased *IGF2* expression, or paternal uniparental disomy (i.e., inheritance of only the expressing paternal allele).

Serum Levels. Human newborn levels of IGF2 are typically 50% of adult levels. By 1 year of age, adult levels are attained, and there is little if any subsequent decline, even up to the seventh or eighth decade.[250]

Insulin-Like Growth Factor Receptors

There are two types of IGF receptors, type I and type II (Fig. 25.25). Structural characterization of these receptors has provided documentation of the differences between these two forms.[251] The type I IGF receptor is closely related to the insulin receptor; both are heterotetramers comprising two membrane-spanning



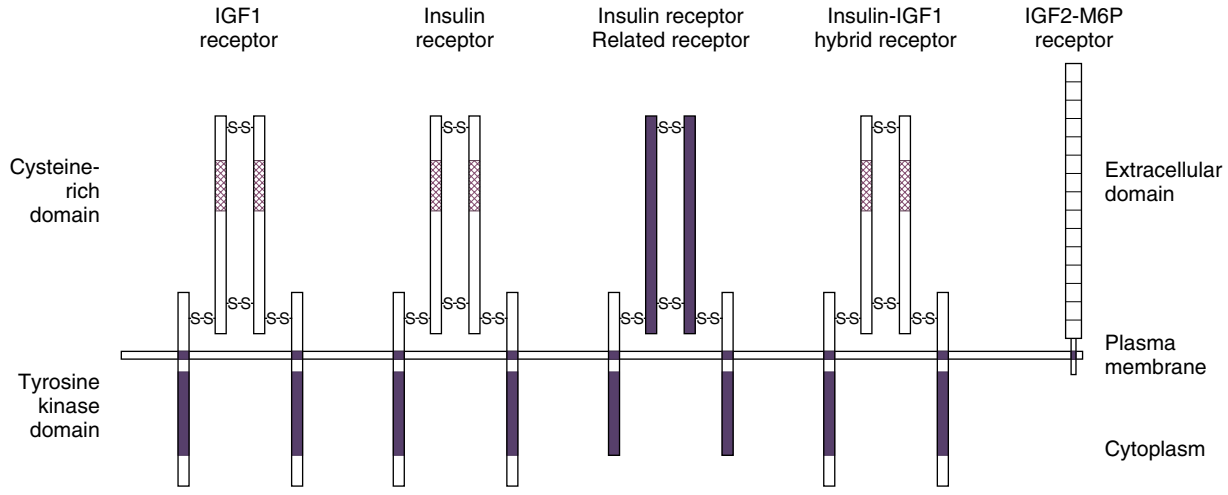
• **Fig. 25.24** Schematic of the imprinted region of the insulin-like growth factor 2 *(IGF2)-H19* locus. *CTCF,* CCCTC binding factor; *CH3,* methylation; *DMR,* differentially methylated region. (Adapted from Chao W, D'Amore P. IGF2: epigenetic regulation and role in development and disease. *Cytokine Growth Factor Rev.* 2008;19:111–120.)

α-subunits and two intracellular β-subunits. The α-subunits contain the binding sites for IGF1 and are linked by disulfide bonds. The β-subunits contain a transmembrane domain, an adenosine triphosphate (ATP)–binding site, and a tyrosine kinase domain, which constitute the presumed signal transduction mechanism for the receptor.

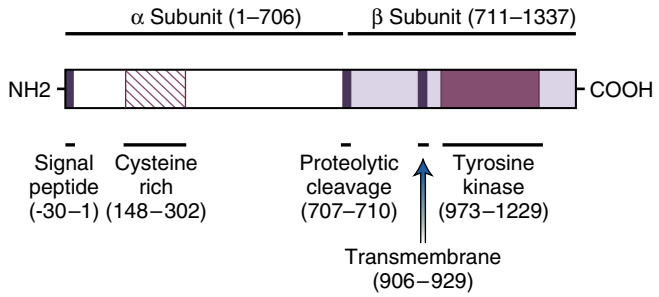
Although the type I IGF receptor has been commonly termed the *IGF1 receptor*, this receptor binds both IGF1 and IGF2 with high affinity, and both IGF peptides can activate tyrosine kinase by binding to the type I receptor. The affinity of the type I IGF receptor for insulin is usually 100-fold less, explaining the relatively weak mitogenic effect of insulin.

The mature IGF receptor peptide has 1337 amino acids with a predicted molecular mass of 151,869 kDa (Fig. 25.26). The translated αβ heterodimer is cleaved at an Arg-Lys-Arg-Arg sequence at positions 707 through 710. The released α-subunit and β-subunit, linked by disulfide bonds, then form the mature $(\alpha\beta)_2$ receptor, in which two α chains are joined by secondary disulfide bonds. The α-subunits are extracellular and contain a cysteine-rich domain that is critical for IGF binding. The β-subunit has a short extracellular domain, a hydrophobic transmembrane domain, and the intracellular tyrosine kinase domain with the ATP-binding site.

The gene for the type I IGF receptor *(IGF1R)* spans more than 100 kb of genomic DNA, with 21 exons; the genomic organization resembles that of the insulin receptor gene.[252] Exons 1 through 3 encode for the 5′ untranslated region and the cysteine-rich domain of the α-subunit that is involved in ligand binding. The remainder of the α-subunit is encoded by exons 4 through 10. The peptide cleavage site involved in generation of the α-subunit and β-subunit is encoded by exon 11, and the tyrosine kinase domain of the β-subunit is encoded by exons 16 through 20. It is in the latter region that *IGF1R* and the insulin receptor gene share the greatest sequence homology, ranging from 80% to 95%.



• **Fig. 25.25** Structure of the insulin-like growth factor (IGF) receptors. The insulin receptor and the IGF type 2 receptor are both heterotetrameric complexes composed of extracellular α-subunits that bind the ligands and β-subunits that anchor the receptor in the membrane and contain tyrosine kinase activity in their cytoplasmic domains. The tyrosine kinase domain of the insulin receptor–related receptor (IRR) is homologous to the tyrosine kinase domains of the insulin and IGF1 receptors. The carboxyl-terminal domain is deleted in the IRR. Hybrids consist of a hemireceptor from both insulin and IGF1 receptors. The IGF type 2/mannose-6-phosphate (M6P) receptor is not structurally related to the IGF1 and insulin receptors or to the IRR, having a short cytoplasmic tail and no tyrosine kinase activity. (From LeRoith D, Werner H, Geitner-Johnson D, et al. Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocr Rev.* 1995;16:143–163.)



• **Fig. 25.26** Structure of the human insulin-like growth factor type I receptor precursor. Molecular cloning of human IGF1 receptor complementary DNAs isolated from a placental library revealed the presence of an open reading frame of 4101 nucleotides. The 1367–amino acid polypeptide contains, at its NH_2-terminus, a 30–amino acid hydrophobic signal peptide that is responsible for the transfer of the nascent protein chain into the endoplasmic reticulum. After digestion by endopeptidases at a proteolytic cleavage site (Arg-Lys-Arg-Arg) located at residues 707 through 710, α-subunit and β-subunit are released and linked by disulfide bonds to yield the configuration of the mature heterotetrameric receptor. Also shown are the cysteine-rich domain of the α-subunit and the transmembrane and tyrosine kinase domains of the β-subunit. (From LeRoith D, Werner H, Beitner-Johnson D, et al. Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocr Rev.* 1995;16:143–163.)

IGF1R mRNA has been identified in virtually every tissue except liver.[253] The mRNA is most abundant in embryonic tissues and appears to decrease with age. *IGF1R* becomes widely expressed after implantation, consistent with the observation that this receptor is essential for normal fetal growth.

As with other growth factor receptor tyrosine kinases, binding of ligand (IGF1 or IGF2) induces receptor autophosphorylation of critical tyrosine residues in the type I IGF receptor.[221] Specifically, ligand binding to the α-subunits leads to activation of the tyrosine kinase domain of the β-subunits. Autophosphorylation appears to occur by transphosphorylation of sites on the opposite β-subunit.[254] A tyrosine proximal to the tyrosine kinase domain, Tyr 950, is part of a motif that, when deleted, reduces receptor autophosphorylation, affects receptor internalization, and inhibits postreceptor signaling; the adapter proteins Shc and insulin receptor substrate 1 (IRS1) bind to this domain.

Autophosphorylation and activation of the cytoplasmic region of the IGF type I receptor promotes recruitment or activation of several docking proteins, each of which activate distinct signaling pathways, with some overlap (Fig. 25.27). Proteins that dock onto the activated IGF type I receptor include members of the IRS family, Shc, p85 subunit of PI3 kinase, tyrosine phosphatase PTP1D, and mGRB10. Of these docking proteins, the pathways involving IRS and Shc are best characterized.

IRS1 is a 185-kDa protein that, when phosphorylated, contains specific phosphotyrosine motifs that can associate with proteins containing Src homology 2 domains such as the p85 subunit of PI3 kinase, growth factor receptor–bound protein 2 (Grb2), Syp (a phosphotyrosine phosphatase), and Nck (an oncogenic protein). Activation of Shc and Grb2 results in activation of the Ras, Raf, MAP kinase kinase, and S6 kinase pathways.[255] Binding of the p85 subunit of PI3 kinase leads to activation of the p110 subunit of PI3 kinase. This process then activates downstream phospholipid signal transduction pathways that include Akt. Activation of Akt leads to regulation of diverse cellular processes, including apoptosis, glucose transport and metabolism, protein synthesis, mitosis, and differentiation.[255] Phosphorylated Shc is a docking site for the SH2 domain of Grb2. Grb2 then binds SOS, a guanine nucleotide exchange factor that converts inactive Ras guanosine diphosphate (GDP) into guanosine triphosphate (GTP). GTP-bound Ras then recruits Raf, and Raf subsequently activates MAP kinase and mitogen and extracellular signal-related kinases (MEK) 1 and 2. Activation of these proteins ultimately regulates gene transcription. Given that insulin and IGF peptides activate similar signaling pathways through their own specific receptors, it is unclear how the cell distinguishes among these overlapping ligands. Whether the consequences merely reflect the relative levels of receptors and whether divergent downstream pathways exist for insulin and IGF action remain questions for future investigation.

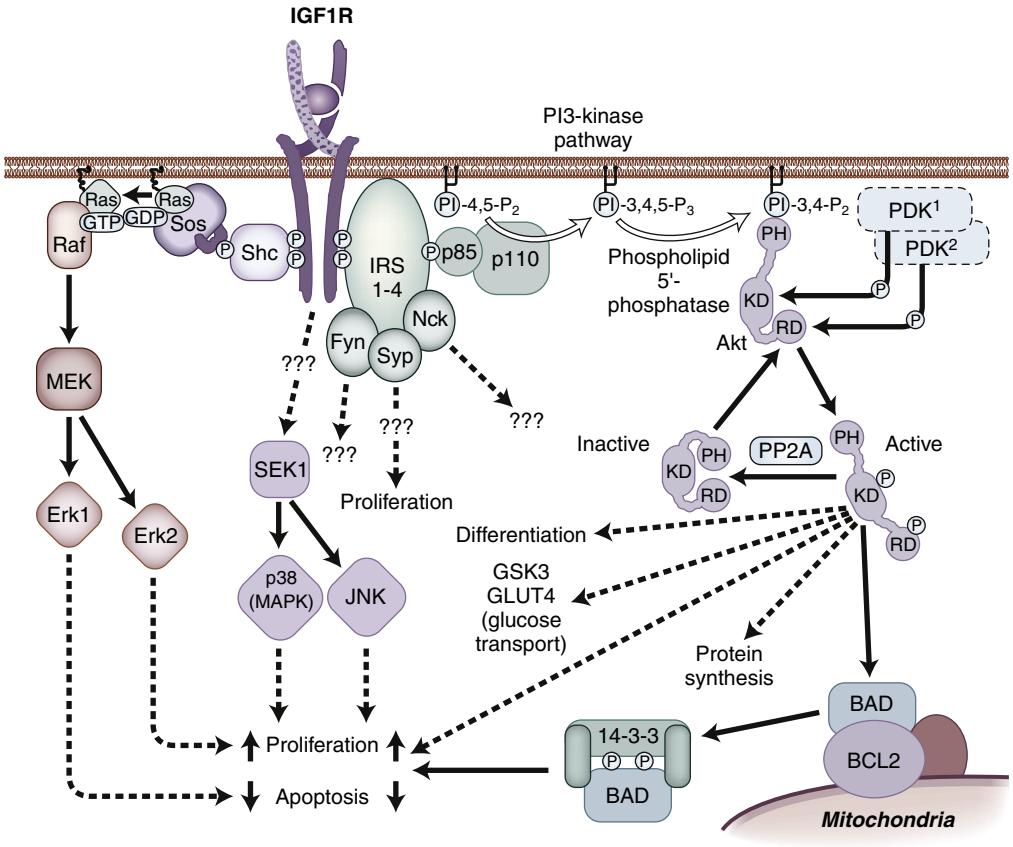
The α-subunit and β-subunit of the IGF receptor can form heterodimer with the α-subunit and β-subunit of the insulin receptor, forming hybrid receptors (see Fig. 25.25). Hybrid receptors have been found in significant amounts in most tissues. The hybrid receptor can bind either insulin of IGF1, but IGF1 may bind with higher affinity. No matter the ligand bound, autophosphorylation of the β-subunit of the insulin or IGF1 receptor occurs.[256] The physiologic significance of these hybrid receptors is unknown as studies thus far have been performed in vitro.

The type II IGF receptor bears no structural homology with either the insulin receptor or type I IGF receptors (see Fig. 25.25). The receptor does not contain an intrinsic tyrosine kinase domain or any other recognized signal transduction mechanism. The type II IGF receptor is identical to the cation-independent mannose-6-phosphate (M6P) receptor, a protein involved in the intracellular lysosomal targeting of acid hydrolases and other mannosylated proteins.[257] This common receptor is often referred to as the IGF2/M6P receptor. The gene for the IGF2/M6P receptor *(IGF2R)* is located on the long arm of chromosome 6. Exons 1 through 46 encode the extracellular region of the receptor, which contains 15 repeat sequences of 147 residues each. Exons 47 and 48 encode the 23-residue transmembrane domain and a small cytoplasmic domain consisting of only 164 residues.[258] Unlike *IGF2*, gene expression is biallelic in humans. Like *IGF2*, *IGF2R* expression is highest early in fetal development and declines postnatally.[258]

The IGF2/M6P receptor has an apparent molecular weight of 220,000 under nonreducing conditions and 250,000 after reduction, indicating that it is a monomeric protein.[259] The 15 repeat sequences contain cysteines to form intramolecular disulfide bonds necessary for receptor folding.[258] Repeat 11 binds IGF2, whereas repeats 3, 5, and 9 bind M6P. Because of receptor folding, it appears that the IGF2 binding site is on the opposite face to the M6P binding site.[260]

The IGF2/M6P receptor binds a variety of M6P-containing proteins, including lysosomal enzymes, transforming growth factor-β (TGFβ),[261] and leukemia inhibitory factor (LIF).[262] The IGF2/M6P receptor binds only IGF2 with high affinity, IGF1 binds to this receptor with lower affinity, and insulin does not bind at all.[263] The receptor participates in lysosomal enzyme trafficking between the trans-Golgi network and the extracellular space, regulates extracellular IGF2 and LIF levels, and plays a role in TGFβ activation (reviewed by El-Shewy and colleagues[264]). Mice null for the type II IGF receptor have macrosomia and fetal death, implicating a role in IGF2 degradation.[265]

The mitogenic and metabolic actions of IGF2 is mediated through the type I IGF receptor, because monoclonal antibodies directed against the IGF1 binding site on the type I IGF receptor inhibit the ability of both IGF1 and IGF2 to stimulate thymidine incorporation and cell replication.[266] Polyclonal antibodies that block IGF2 binding to the IGF2/M6P receptor do not block IGF2 actions.[267,268]



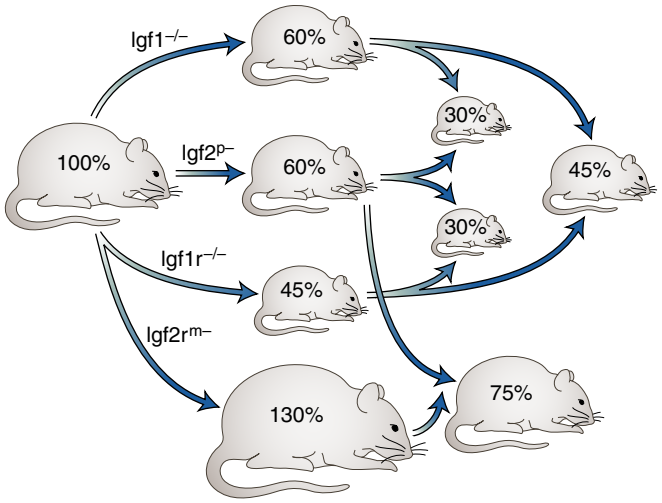
• **Fig. 25.27** Schematic representation of intracellular signaling pathways of the insulin-like growth factor type 1 (IGF1) receptor. On binding of IGF1, the IGF receptor undergoes autophosphorylation at multiple tyrosine residues. The intrinsic kinase activity of the receptor also phosphorylates insulin receptor substrate 1 (IRS1) at multiple tyrosine residues. Various SH domain–containing proteins, including phosphatidylinositol 3 (PI3) kinase, Syp, Fyn, and Nck associate with specific phosphotyrosine-containing motifs within IRS1. These docking proteins recruit diverse other intracellular substrates, which then activate a cascade of protein kinases, including Raf1, and one or more related kinases, including mitogen-activated protein kinase (MAPK), mitogen and extracellular signal-related kinase (MEK), and others. These protein kinases, in turn, activate various other elements, including nuclear transcription factors. Alterations in expression of various IGF1-responsive genes result in longer-term effects of IGF1, including growth and differentiation. This model of signal transduction cascades also shows a potential mechanism for inhibition of apoptosis. *BAD,* BCL2-associated agonist of cell death; *Erk,* extracellular signal-regulated kinase; *GDP,* guanosine diphosphate; *GLUT4,* glucose transporter 4; *GTP,* guanosine triphosphate; *JNK,* c-Jun N-terminal kinase; *KD,* catalytic kinase domain of Akt; *MEK,* mitogen-activated protein kinase; *P,* phosphorylation; *PH,* pleckstrin homology domain of Akt; *PP2A,* protein phosphatase 2A; *RD,* regulatory C-terminal tail of Akt; *SEK1,* serum-inducible and glucocorticoid-inducible protein kinase 1. (From Le Roith D, Bondy C, Yakar S, et al. The somatomedin hypothesis. *Endocr Rev.* 2001;22:53–74.)

Function-Targeted Disruption of IGF and IGF Receptor Genes

The in vivo role of the IGF axis in prenatal and postnatal growth was firmly established by a series of studies involving IGF and IGF receptor gene null mutations.[269] Unlike *Gh* or *Ghr* null mice that are near-normal size at birth, mice with deletions of *Igf1* or *Igf2* have birth weights approximately 60% of normal.[269] Fetal size is proportionately reduced, but mice with deletion of *Igf1* have a higher neonatal death rate than mice with *Igf2* deletion. Postnatally, mice with deletion of *Igf1* that survive the neonatal period continue to have growth failure, with weights 30% of normal by 2 months of age. When both *Igf1* and *Igf2* were disrupted, weight at birth was only 30% of normal and all animals died shortly after birth, apparently from respiratory insufficiency secondary to muscular hypoplasia (Fig. 25.28).

Mice lacking *Igf1* and the *Ghr* are only 17% of normal size.[227] Therefore both IGF1 and IGF2 are important in fetal growth, but GHR signaling may have IGF-independent actions on growth as well. The postnatal growth of *Igf1*$^{-/-}$ mice was poorer than that observed in mice with *Ghr* or *Ghrh* receptor mutations, indicating that both GH-dependent and GH-independent effects of IGF1 are necessary for normal growth.

Specific ablation of hepatic IGF1 production through the Cre/LoxP recombination system confirmed that the liver is the principal source of circulating IGF1, but the resulting 80% reduction in serum IGF1 levels had no effect on postnatal growth,[228,270] suggesting that postnatal growth is relatively independent of hepatic IGF1 production. Presumably, production of IGF1 from local chondrocytes or other tissues maintains adequate endocrine sources of IGF1 to account for growth preservation. Only modest decrement of postnatal growth is seen in mice null for *Igfals* (the gene encoding ALS).[271]



• **Fig. 25.28** Effects of the disruption of one or more genes of the insulin-like growth factor (Igf) system on fetal growth in mice, expressed as percentage of normal body weight. *Igf1*$^{-/-}$, Igf1 gene null mice; *Igf2*$^{p-}$, Igf2 paternal allele null mice; *Igf1r*$^{-/-}$, Igf1r gene null mice; *Igf2r*$^{m-}$, Igf2r maternal allele null mice. Mice with two source arrows are the combined genotype of the source arrow mice. (From Gicquel C, LeBouc Y. Hormonal regulation of fetal growth. *Horm Res.* 2006;65:28–33.)

By crossing the liver-derived IGF1 gene–deleted mice with ALS gene–deleted mice, 85% to 90% reduction in serum IGF1 occurred, with early postnatal growth retardation.[272] These findings suggest that postnatal growth is dependent on both hepatic and tissue IGF1, although definite conclusions are problematic in the face of elevated GH production and perturbations of the IGFBP system observed in these studies.

Deletion of *Igf1r* resulted in birth weights 45% of normal with 100% neonatal lethality.[273] Concurrent deletion of *Igf1* and *Igf1r* resulted in no further reduction in birth size compared with deletion of *Igf1r* alone; this is consistent with the hypothesis that all IGF1 actions are mediated via the IGF type I receptor.

Deletion of *Igf2r* in mice causes an increase in birth weight but death in late gestation or at birth.[265] Because this receptor normally degrades IGF2, increased growth reflects excess IGF2 acting through the IGF type I receptor, with variable accumulation of IGF2 in tissues. Deletion of *Igf2r* plus *Igf2* results in a birth weight 60% of normal, similar to the size of mice with knockout of *Igf2* alone, with no effect on neonatal survival.[274] Simultaneous knockout of the genes for *Igf2* and *Igf1r* causes further reduction of birth size to 30% of normal, suggesting that some of the fetal anabolic actions of IGF2 are mediated by another mechanism such as via placental growth or interactions with the insulin receptor. Indeed, specific deletion of *Igf2* in the placenta causes small placenta and growth retardation.[275]

These studies allow the following conclusions: (1) IGF1 is important for both fetal and postnatal growth; (2) IGF2 is a major fetal growth factor but has little role in postnatal growth; (3) the IGF type I receptor mediates anabolic actions of both IGF1 and IGF2; (4) the IGF type II receptor is bifunctional, serving to target lysosomal enzymes and to enhance IGF2 turnover; (5) IGF2 deletion results in impaired placental growth; and (6) IGF1 is the major mediator of GH effects on postnatal growth, although GH and GHR may have small IGF1-independent effects.

Insulin-Like Growth Factor–Binding Proteins

In contrast to insulin, the IGFs circulate in plasma complexed to a family of binding proteins that extend the serum half-life of the IGF peptides, transport the IGFs to target cells, and modulate the interactions of the IGFs with surface membrane receptors.[276] In addition, the IGFBPs have effects on cells independent of IGFs. The presence and various actions of the IGFBPs provide layers of regulation to the GH-IGF axis, greatly increasing its complexity (Fig. 25.29). In the following paragraphs, the structure of the IGFBP family is described first, followed by the role of IGFBPs in IGF physiology and the characteristics of individual IGFBPs.

Structure of IGFBPs

The six IGFBPs share structural homology in the N-terminal and C-terminal domains but have highly variable midsections, which probably accounts for the more specialized properties of the individual IGFBPs, such as cell dissociation, IGF-binding enhancement, and IGF-independent actions. In each of the conserved N-terminal and C-terminal domains, there are a high number of cysteine-rich residues with a conserved spatial order, implying that the disulfide bond–dependent secondary structure of the IGFBPs is also conserved.[277] Reduction of the disulfide proteins results in loss of IGF binding, further demonstrating the importance of the cysteine-rich region.

The N-terminal domain contains residues that have been shown to be essential for IGF binding to IGFBPs 1, 3, and 4.[278] The exact sequences in the C-terminal domain that are responsible for binding may differ among the IGFBPs. The highly variable midregion segment of the IGFBPs is the site of post-translational modifications such as glycosylation and phosphorylation and of proteolysis. Both the primary structure of IGFBPs and their post-translational modifications are responsible for the differential targeting to tissues: Glycosylation can affect cell interactions, phosphorylation can affect IGF1 binding affinity and susceptibility to proteases, and proteolysis can affect IGF/IGF receptor–dependent and IGF/IGF receptor–independent actions.[279]

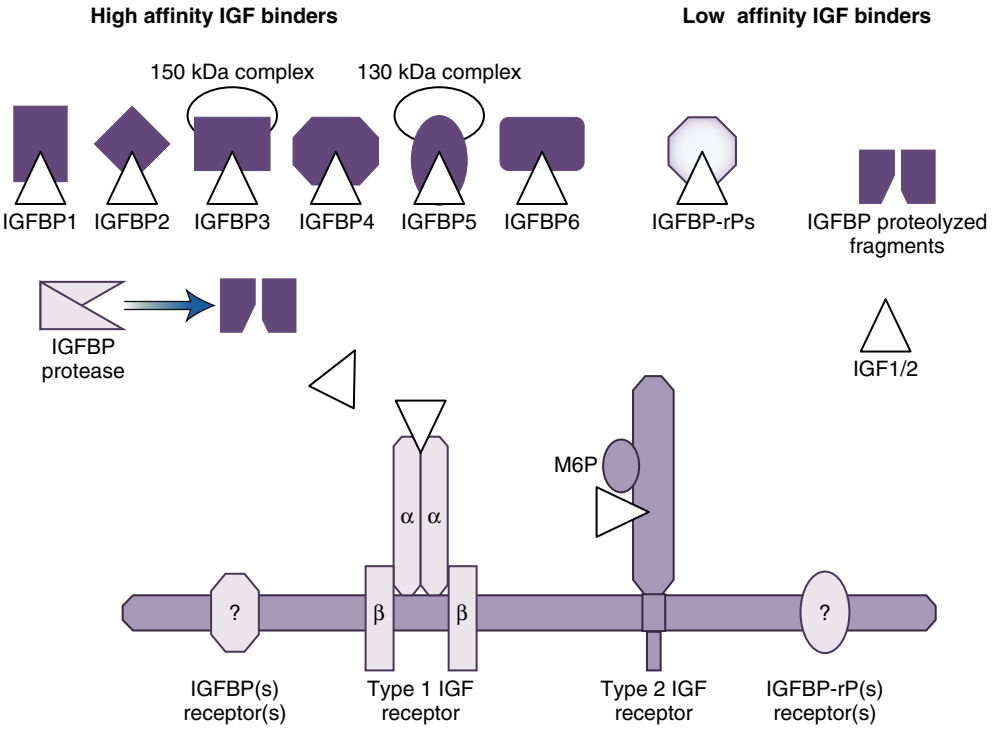
The three-dimensional structure of IGFBPs 1, 2, 4, and 5 details IGF-binding sites in the N-terminal and C-terminal domains. Crystallography indicates that the structure of the N-terminal domain is critical for IGF binding, whereas the C-terminal domain is important for inhibiting interactions between IGF1 and its receptor.[280,281]

Analysis of IGFBPs is complicated by the presence of IGFBP proteases that degrade IGFBP (Fig. 25.30).[282] Proteolysis of IGFBPs complicates their assay and must be taken into consideration when measuring the various IGFBPs in biologic fluids. Multiple IGFBP proteases exist, including calcium-dependent serine proteases, kallikreins, cathepsin, and matrix metalloproteases.[283,284] Proteolysis of IGFBPs releases IGFs to interact with cell surface receptors, thereby enhancing the mitogenic and anabolic effects of IGF peptides.

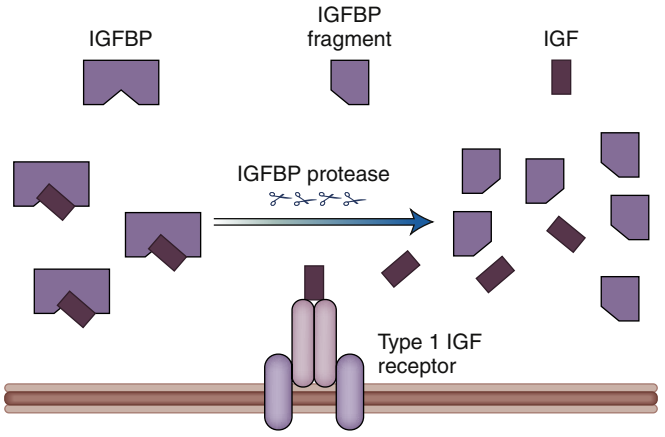
Role of IGFBPs in IGF Physiology

IGFBPs as Carrier Proteins

The IGFBPs complex almost all of the circulating IGF1 and IGF2 secondary to their high affinity for the IGFs (10^{-10} to 10^{-11} mol/L).[285] In adults, 75% to 80% of IGFs are carried in a ternary complex consisting of one molecule of IGF plus one molecule of IGFBP3 plus one molecule of the protein ALS.[286] This ternary complex is too large to leave the vascular compartment and thus extends the half-life of IGF peptides from approximately



• **Fig. 25.29** Schematic representation of the insulin-like growth factor (IGF) system, including IGF ligands (IGF receptors 1 and 2), binding proteins (both high-affinity and low-affinity binders), IGF-binding protein (IGFBP) proteases, type 1 and type 2 IGF receptors, and potential receptors for other IGFBPs and IGFBP-related proteins (IGFBP-rPs). *M6P,* mannose-6-phosphate. (From Hwa V, Oh Y, Rosenfeld RG. The insulin-like growth factor–binding protein [IGFBP] superfamily. *Endocr Rev.* 1999;20:761–787, copyright © Endocrine Society.)



• **Fig. 25.30** Schematic representation of the effect of insulin-like growth factor (IGF)–binding protein (IGFBP) proteases on IGF action. In this model, proteolysis of IGFBPs results in a reduction in their affinity for IGF ligands, leading to enhanced binding of IGF peptides by IGF receptors. (From Cohen P, Rosenfeld RG. The IGF axis. In Rosenbloom AL, ed. *Human Growth Hormone: Basic and Scientific Aspects.* Boca Raton, FL: CRC Press; 1995:43–58.)

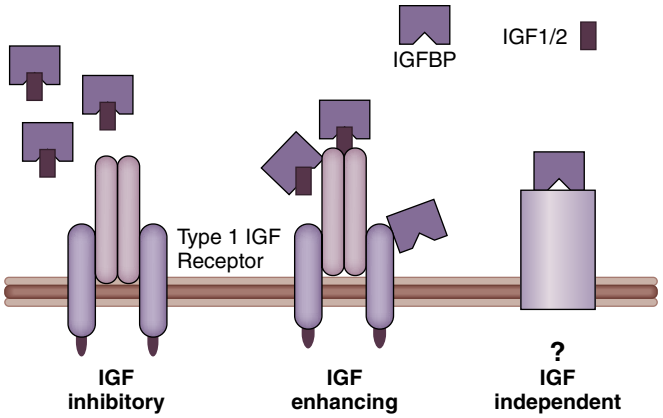
10 minutes for IGF alone to 12 to 15 hours for IGF in the ternary complex.[287] Binding of IGF to IGFBP3 in a binary complex extends the half-life of IGF to 1 to 2 hours as diffusion out of the vascular compartment may occur.[288]

Both IGFBP3 and ALS are GH dependent, providing an additional mechanism for GH regulation of the IGF axis. GH administration to GH-deficient patients shifts IGF to the ternary complex.[289] However, after IGF1 treatment alone, there is no increase in IGFBP3 levels, and ALS levels may decrease; thus IGF does not shift to the ternary complex.[290] In serum of patients with GHD or GH insensitivity, little IGF is present in the 150-kDa ternary complex; the IGF present is in the IGF-IGFBP3 binary complex or is bound by other IGFBPs such as IGFBPs 1, 2, 4, or 5.[291]

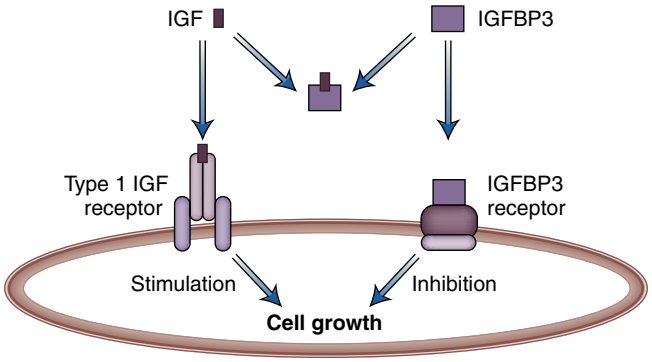
IGFBPs as Modulators of IGF Action

IGFBPs also regulate biologic actions by modulating the availability of IGFs (Fig. 25.31). The binding affinity of IGFs for IGFBPs is higher than for IGF receptors, and dissociation of IGFs from IGFBPs is required for IGFs to interact with IGF receptors.[285] Additionally, the concentration of IGFBPs is in excess, compared with IGFs, in many bodily fluids.[292] Dissociation of IGFs from IGFBPs is achieved by mass action, proteolysis, or other unknown mechanisms. Inhibition of IGF1 action by the presence of IGFBP4 occurs in vitro and in vivo.[293] IGFBPs 1, 3, and 5 have potentiating effects on IGF action, perhaps by facilitating the delivery of IGF to target receptors, as is for IGFBP3.[294]

The enhancing effects of IGFBP5 involve binding of IGFBP5 to extracellular matrix proteins, which reduces the affinity of IGFBP5 for IGF. Possibly, IGFBP5 binding to the extracellular matrix brings IGF close to the cell surface in a low-affinity complex, from which it can be released slowly to bind neighboring IGF receptors.[295] To complicate matters, it appears that the same IGFBP can potentiate or inhibit IGF action in vitro, depending on cell culture conditions, cell type, IGFBP dose, and post-translational modifications such as phosphorylation state.[296]



• **Fig. 25.31** Theoretic mechanisms of cellular actions of insulin-like growth factor (IGF)–binding proteins (IGFBPs). See text for details.



• **Fig. 25.32** Schematic diagram of insulin-like growth factor (IGF)–dependent and IGF-independent actions of IGF-binding protein 3 *(IGFBP3),* the latter being mediated through a putative membrane-associated IGFBP3 receptor.

It has also been proposed that IGFBP-IGF complexes can be stored in tissues to act in a paracrine manner. This has been demonstrated for IGFBP5 and bone matrix. IGFBP5 is produced by osteoblasts, and the IGFBP5-IGF complex can bind hydroxyapatite, possibly participating in bone turnover.[295]

IGFBP proteases, which degrade IGFBPs, are postulated to play a role in altering IGF availability by lowering affinities of IGFBPs for their ligand, thereby increasing the availability of IGFs to cell membrane receptors. The best characterized protease is pregnancy-associated plasma protein-A2 (PAPP-A2). *Papp-a2* knockout mice of postnatal growth restriction[297] and mutation of *PAPP-A2* causes growth retardation with high IGF1 levels in humans,[298] thus indicating the importance of these proteases for tissue IGF1 availability.

IGF-Independent Actions of IGFBPs

IGFBPs also have IGF-independent actions, such as growth inhibition of certain cell types, growth stimulation of tissues, direct induction of apoptosis, and modulation of the effects of other non-IGF growth factors. The mechanisms of IGF-independent actions are slowly being unraveled and include binding to IGFBP-specific cell surface receptors, binding to other cell surface receptors, and interaction with nuclear receptors (Fig. 25.32).

IGF-independent actions have been characterized for IGFBPs 1, 2, 3, and 5. IGFBP1 is implicated in cell motility and adhesion; IGFBP2 has mitogenic actions independent of IGF1 binding. In vitro, IGFBP3 administration or overexpression inhibits DNA synthesis and cell proliferation in conditions in which IGF1 or the IGF type I receptor is neutralized, suggesting IGF-independent inhibition by IGFBP3.[285] IGFBP5 has IGF-independent effects on osteoblasts.[279]

The IGFBPs also have IGF-independent effects via binding to IGFBP-specific membrane receptors for IGFBP3, IGFBP5, or other receptors such as the TGFβ receptor type V.[279] The downstream signaling pathways activated by the IGFBP-specific receptors are unknown, as are the possible interactions with IGF receptor signaling.

Characteristics of IGFBPs 1 Through 6

IGFBP1 was the first of the IGFBPs to be purified and to have its cDNA cloned. The gene is located on the short arm of chromosome 7, comprises four exons,[299] and is strongly expressed in decidua, liver, and kidney. It is the major IGFBP in fetal serum in early gestation, reaching levels as high as 3000 μg/L by the second trimester. Levels of IGFBP1 in newborn serum are inversely correlated with birth weight, suggesting an inhibitory role on fetal IGF action.

IGFBP1 may be involved in reproductive function, including endometrial cycling, oocyte maturation, and fetal growth.[300] It also appears to have an important metabolic role in that its gene expression is enhanced in catabolic states[250] while insulin suppresses IGFBP1 expression. Serum IGFBP1 levels correlate with insulin sensitivity. Low levels are associated with lower insulin sensitivity. Fasting levels predict risk of diabetes development in longitudinal cohorts.[301] IGFBP1 null mice have normal glucose homeostasis, but IGFBP1 transgenic mice are protected against insulin resistance when challenged with a high-fat diet. The cellular target(s) of IGFBP1 that mediate these effects are not known, as IGFBP1 can have IGF1-independent effects.

The *IGFBP2* gene is located on the long arm of chromosome 2 and is highly expressed in fetal tissues, particularly in the CNS.[302] It is the second most abundant IGFBP in the serum, behind IGFBP3. Unlike IGFBP1, its expression does not change with feeding. IGFBP2 transgenic mice have diminished postnatal weight gain and slightly reduced fasting insulin levels with protection against age-related insulin resistance.[301]

The *IGFBP3* gene is located on chromosome 7. IGFBP3 is composed of the conserved N-terminal and C-terminal domains and the variable midsection. The midsection is the site of N-linked glycosylation, which is not present in IGFBP1 or IGFBP2 and is the site of phosphorylation. This midsection is the site responsible for interaction with cell surfaces.[303]

IGFBP3 is the predominant IGFBP in adult serum, where it carries approximately 75% of the total IGF, primarily as part of the 150-kDa ternary complex consisting of IGF1, IGFBP3, and ALS. IGFBP3 and IGFBP5 are the only IGFBPs to form this complex. It is believed that formation of this ternary complex limits IGF access to target cells, while at the same time prolonging serum half-lives of both the IGF peptide and its binding protein.[304] Serum levels of IGFBP3 and ALS are reduced in patients with GHD or GH insensitivity, conditions in which assays for serum IGFBP3 have important diagnostic value. IGFBP3 is increased in states of GH excess and acromegaly.

IGFBP3 action is GH dependent, either directly or through regulation by IGF. IGF1 treatment of patients with GH insensitivity does not greatly alter serum IGFBP3 levels,[143] and GH treatment of GH-deficient patients does not increase serum levels. Whether these observations mean that GH has a direct effect on

IGFBP3 or reflects GH regulation of ALS and ternary complex formation is unclear, although it appears likely that both factors are contributory.

IGFBP3 associates with cell membrane proteins in a specific, cation-dependent manner and with high affinity.[279] Whether the cell membrane proteins constitute genuine IGFBP3 receptors remains to be determined, although they may mediate IGF-independent actions of IGFBP3.

IGFBP3 expression can be induced by cell cycle regulators and growth-inhibitory factors such as TNFα, TNFβ, retinoic acid, vitamin D, antiestrogens, and antiandrogens.[305] Like many genes, *IGFBP3* expression is affected by methylation and histone modification. Abnormal methylation or histone modification of the *IGFBP3* gene is present in many different types of human cancers (reviewed by Jogie-Brahim and colleagues[305]).

The *IGFBP4* gene is located on chromosome 17 and contains four exons. It is widely expressed in embryonic tissues, fibroblasts, osteoblastic cells, prostatic cells, ovarian cells, and liver. The circulating form is derived mostly from the liver. IGFBP4 inhibits IGF action in most tissues.[306] The inhibitory effect of IGFBP4 is reduced upon proteolysis by PAPP-A, as proteolysis of IGFBP4 results in an increase of free IGF1. Circulating IGF1 or IGF2 activates IGFBP4 proteolysis by inducing a conformational change allowing PAPP-A to access the cleavage site.[306]

The *IGFBP5* gene is located on chromosome 5 and contains four exons. IGFBP5 binds extracellular matrix proteins such as types III and IV collagen, laminin, and fibronectin, and it does so in response to binding of IGF1.[307] The affinity of IGFBP5 is reduced about sevenfold when the binding protein is associated with extracellular matrix, providing a potential mechanism for release of IGFs to cell surface receptors. Association of IGFBP5 with extracellular matrix protects it from proteolysis.[308] Unlike proteolysis of IGFBP4, which is enhanced by the addition of IGFs, degradation of IGFBP5 is inhibited by the binding of IGF peptides.[309]

The *IGFBP6* gene is located on chromosome 12 and contains four exons. Although IGFBP6 binds both IGF1 and IGF2, it has a significantly greater affinity for IGF2.[310] IGFBP6 is found in high levels in cerebrospinal fluid, as is IGFBP2, which also binds IGF2 with high affinity. IGFBP6 may have a role in regulating ovarian activity, perhaps by functioning as an antigonadotropin.[311]

Mice null for the individual IGFBPs exhibit modest, if any, alterations in growth. Modest decreases in organ size have been noted in IGFBP2 null mice,[312] whereas growth in IGFBP4 null mice is 85% to 90% of normal. Increases in other IGFBP levels were noted in these mice. Triple knockout mice null for IGFBPs 3, 4, and 5 exhibit lengths 80% of normal with IGF1 levels 45% of wild type.[313] Transgenic mice overexpressing the IGFBPs 1, 2, 3, and 4[314] exhibit growth retardation to varying degrees, demonstrating the role of the IGFBPs in sequestering IGF1 or inhibiting its actions. Mice overexpressing IGFBP1 and IGFBP3 exhibit impaired glucose tolerance and decreased fertility, further implicating a role for IGF1 or a separate role for these IGFBPs in glucose metabolism and reproduction.

Gonadal Steroids

Androgens and estrogens affect growth predominantly through two mechanisms: regulation of the GH-IGF1 axis and maturation of the epiphyseal growth plates. The adolescent rise in serum gonadal steroid levels is an important part of the pubertal growth spurt. In addition, it is the stimulation of epiphyseal fusion by the pubertal rise of gonadal steroid production that results in the ultimate cessation of linear growth. The details of the roles of androgens and estrogens in enhancing GH secretion and directly stimulating IGF1 production were discussed earlier.

Both androgens and estrogens increase skeletal maturation and accelerate growth plate senescence. However, most of these effects are due to the action of estrogen, with androgens acting indirectly after their conversion to estrogens by aromatase in extraglandular tissues, including locally within the growth plate. The primacy of the role of estrogen comes from animal studies[22] as well as reports of human subjects with mutations. A mutation of the estrogen receptor in a man was associated with tall stature and open epiphyses,[23] and similar findings were reported in patients with mutations of the gene encoding the aromatase enzyme.[315] In addition, variants of the estrogen receptor have been associated with height in males[316] and in females,[317] and increased aromatase expression results in short adult stature in males.[318,319]

Whereas most of the effects of androgen on growth are mediated through actions that occur after their aromatization to estrogen, there is evidence of androgen-specific effects. Notably, dihydrotestosterone, a nonaromatizable androgen, can accelerate linear growth in boys. This effect of androgen does not appear to be mediated by either GH or circulating IGF1, but it may be mediated by an increase in local IGF1 production.[320]

Gonadal steroids, along with GH and IGF1, contribute to the attainment of peak bone mass in adults. Again, this sex hormone effect is largely mediated by estrogen action.[321–323]

Thyroid Hormone

Thyroid hormone is of relatively little importance in the growth of the fetus, but it has significant effects on postnatal growth and bone maturation. Patients with hypothyroidism have decreased spontaneous GH secretion and blunted responses to GH provocative tests. Thyroid hormone also has a direct effect on chondrocytes and osteoblasts, which both express thyroid hormone receptors. Thyroid hormone regulates chondrocyte proliferation and stimulates terminal differentiation, mineralization, and angiogenesis.[324,325] In particular, thyroid hormone is essential for hypertrophic chondrocyte differentiation.[326] Postnatally, hypothyroidism can cause growth failure and delayed skeletal maturation, whereas hyperthyroidism can accelerate linear growth and skeletal maturation.

Glucocorticoids

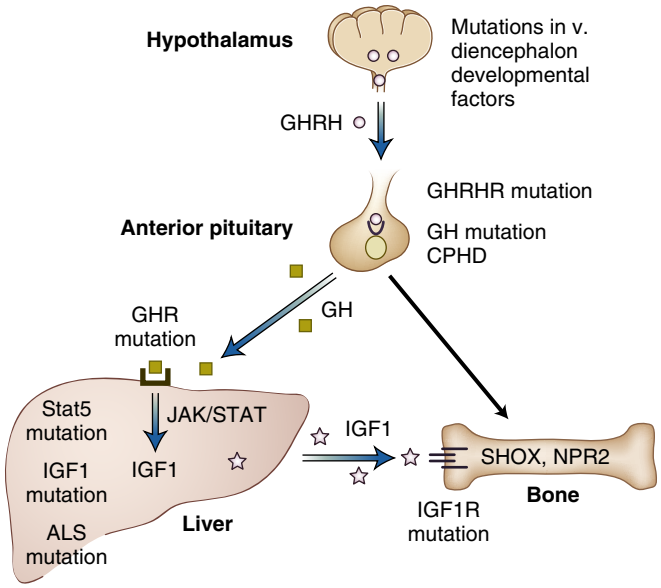
Glucocorticoids have both stimulatory and inhibitory effects on GH secretion, with the absolute effect depending on the timing and the glucocorticoid concentration. Glucocorticoid deficiency, as in Addison disease, leads to a decrease in GH secretion due to decreased expression of GHRH and GH secretagogue receptors.[170] Acute exposure to supraphysiologic levels of glucocorticoids decreases GH secretion within 1 hour followed by a subsequent transient increase in GH secretion.[170,327] Ongoing glucocorticoid excess then causes ongoing suppression of GH secretion. This decrease in GH secretion is due to an increase in somatostatin tone.[170] Glucocorticoids can also impair growth through direct actions at the growth plate by inhibiting local IGF1 production through suppression of chondrocyte GHR expression, impairment of chondrocyte IGF1 receptor expression,[170,328] alterations in IGFBP levels, and impairment of intracellular signaling.[329] Finally, glucocorticoids may stimulate apoptosis of growth plate chondrocytes.[320]

Additional indirect effects of excess glucocorticoids on growth can result from glucocorticoid inhibition of calcium absorption

TABLE 25.1 Causes of Growth Retardation

I. Disorders of the GH-IGF1 axis
  A. GH deficiency
    1. Hypothalamus
      a. Congenital disorders
      b. Acquired disorders
    2. Pituitary
      a. Congenital disorders
        (1) Combined pituitary hormone deficiencies
        (2) Isolated GH deficiency (IGHD)
      b. Acquired disorders
        (1) Craniopharyngiomas and other tumors
        (2) Histiocytosis X
  B. GH insensitivity
    1. Mutations in GHR signaling proteins and acid-labile subunit (ALS)
  C. Abnormalities of IGF1 and IGF1 receptor signaling
II. Growth disorders outside the GH-IGF1 axis
  A. Malnutrition
  B. Chronic disease
  C. Endocrine disorders
  D. Osteochondrodysplasias
  E. Chromosomal abnormalities
  F. Small for gestational age (SGA)
  G. Maternal and placental factors
III. Idiopathic short stature (ISS)

GH, Growth hormone; *IGF1,* insulin-like growth factor 1.



• **Fig. 25.33** The hypothalamic-pituitary-IGF axis: sites of established mutations. *ALS,* acid-labile subunit; *CPHD,* combined pituitary hormone deficiency; *GH,* growth hormone; *GHRH,* GH-releasing hormone; *GHRHR,* GHRH receptor; *IGF1,* insulin-like growth factor 1; *IGF1R,* IGF1 receptor; *JAK,* Janus kinase; *NPR2,* natriuretic peptide receptor 2; *SHOX,* short homeobox; *STAT,* signal transducer and activator of transcription; *v,* ventral. (Reprinted with permission from Lopez-Bermejo A, Buckway CK, Rosenfeld RG. Genetic defects of the growth hormone-insulin-like growth factor axis. *Trends Endocrinol Metab.* 2000;11:43.)

and reabsorption, with the development of secondary hyperparathyroidism.[325] In pubertal children, glucocorticoid excess can induce sex hormone deficiency, causing a loss of the normal growth stimulatory effect of these hormones.[325]

Pathologic Basis of Growth Retardation

A classification of growth retardation is presented in Table 25.1. Growth disorders can also be subdivided into (1) disorders of the hypothalamic-pituitary axis resulting in deficiency of GH, (2) disorders resulting in deficiency or resistance to the action of IGF1, (3) growth disorders that primarily affect the growth plate or are caused by chronic disease, and (4) idiopathic short stature (ISS), which is considered separately but may have a pathogenic basis in the GH-IGF1 axis or at the growth plate. A schematic diagram of known defects in the GH-IGF1 axis is shown in Fig. 25.33, and the known involved genes are listed in Table 25.2.

Disorders of the GH-IGF1 Axis

Growth Hormone Deficiency

Although it is not always possible to establish definitively whether hypothalamic or pituitary dysfunction is responsible for the hormone deficiency, these compartments are differentiated to facilitate discussion of the pathology. Table 25.3 indicates possible causes for deficiency in the GH-IGF axis. The term *idiopathic* is often used to designate lack of understanding of the basis for the GHD. Developmental or functional abnormalities of the hypothalamus account for most idiopathic cases of hypopituitarism, and recent molecular studies have begun to elucidate the molecular bases of these disorders. It is anticipated that most cases of idiopathic hypopituitarism will be defined at the genetic level in the future.

The Hypothalamus

Congenital Disorders

The primary hypothalamic neuropeptide responsible for synthesis and release of GH is GHRH. Somatostatin plays an antagonistic role in GH release. Synthesis of these two hypothalamic proteins is controlled by a series of neurochemicals, and the balance between them is responsible for the tight neuroendocrine control of GH biosynthesis. Mutations of some of the genes encoding hypothalamic peptides explain some cases of GHD due to hypothalamic dysfunction.

As noted earlier, patients with early-diagnosed congenital GHD frequently have an abnormal pituitary stalk, ectopia of the posterior pituitary, and hypoplasia of the anterior pituitary (Fig. 25.34). Anencephaly results in a pituitary gland that is small or abnormally formed and is frequently ectopic. Despite the loss of hypothalamic regulation, somatotrophs differentiate and proliferate, although in diminished overall mass.[67] During intrauterine life, serum GH and IGF1 levels are 30% to 50% of the normal range,[151] and pituitary GH content at birth is about 15% to 20% of normal,[149,151] with similarly low neonatal plasma GH levels.[330]

In most patients, so-called idiopathic hypopituitarism or GHD is presumed to be due to abnormalities of synthesis or secretion of hypothalamic hypophysiotropic factors.[331,332] In a number of reports, idiopathic GHD is associated with magnetic resonance imaging (MRI) findings of an ectopic neurohypophysis, pituitary stalk dysgenesis, and hypoplasia or aplasia of the anterior pituitary. Overall, those patients with the most striking abnormalities of the hypothalamic-pituitary region, largely those with combined pituitary hormone deficiency (CPHD), had the smallest anterior pituitary glands.[333] Patients with more severe deficiencies of GH have greater frequency of significant morphologic abnormalities.[334]

TABLE 25.2 Genetic Defects of the GH-IGF Axis Resulting in Somatotroph Dysfunction and GH Deficiency

Factor	Gene Function	Affected Cell Types	Clinical Phenotype	Mode of Inheritance
Mutations in Factors Resulting in Growth Hormone and Associated Hormone Deficiencies				
Hesx1	• Paired-like homeobox gene • Early marker for pituitary primordium and Rathke pouch • Requires Lhx3 for maintenance and PROP1 for repression	Somatotrophs, thyrotrophs, gonadotrophs (posterior pituitary may also be affected)	• Isolated GH deficiency or multiple hormone deficiency (including diabetes insipidus) • Puberty may be delayed • Associated with septo-optic dysplasia • Abnormal MRI findings: pituitary hypoplasia, ectopic posterior pituitary, midline forebrain abnormalities	AD, AR
Lhx3 (Lim3, P-LIM)	• Member of LIM-homeodomain family of gene regulatory proteins • Required for survival and proliferation of Rathke pouch • Activates αGSU promoter • Acts with Pit1 to activate TSHβ gene promoter	Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, possibly corticotrophs	• Patients may present with rigid cervical spine causing limited neck rotation • Hypoplastic anterior/intermediate pituitary lobe	AR
Lhx4	• A LIM protein with close resemblance to Lhx3 • Important for proliferation and differentiation of cell lineages • May have overlapping function with PROP1 and POU1F1	Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs	• Combined pituitary hormone deficiencies with predominant GH deficiency • Severe anterior pituitary hypoplasia, ectopic neurohypophysis	AD
SIX6	• Member of the *SIX/sine oculis* family of homeobox genes • Expressed early in hypothalamus, later in Rathke pouch, neural retina, and optic chiasma	Somatotrophs, gonadotrophs	• Bilateral anophthalmia • Pituitary hypoplasia • Associated with deletion at chromosome 14q22-23	Unknown
FGF8, FGFR1, PROKR2	• Ventral diencephalon	Somatotrophs, gonadotrophs	• Septo-optic dysplasia	AD

PITX2 (RIEG1)	• Bicoid-related homeobox gene expressed early in Rathke pouch
	• Important in maintaining expression of *Hesx1* and *PROP1*
		Somatotrophs, lactotrophs, thyrotrophs, reduced expression of gonadotrophs	• Associated with Rieger syndrome:
			  • Anterior-chamber eye anomalies
			  • Dental hypoplasia
			  • Protuberant umbilicus
			  • Mental retardation
			  • Pituitary dysfunction
				AD

PROP1 (Prophet of Pit1)	• Paired-like homeodomain transcription factor required for *PIT1* expression • Coexpressed with *Hesx1*	Somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, corticotrophs (delayed)	• Combined pituitary deficiencies (GH, TSH, PRL, and late-onset ACTH reported) • Gonadotropin insufficiency or normal puberty with later onset of deficiency • Several mutations noted in nonconsanguineous families	AR
POU1F1 (PIT1)	• Member of the POU transcription factor family • Important for activation of *GH1*, *PRL*, and *TSHβ* genes	Somatotrophs, lactotrophs, thyrotrophs	• Combined pituitary deficiencies (GH, TSH, PRL, and late-onset ACTH reported); TSH secretion may initially be normal • Pituitary hypoplasia	AD, AR
Otx2	• Bicoid-type homeodomain gene required for forebrain and eye development • Antagonizes *FGF8* and *SHH* expression • May have importance in activation of *Hesx1*	Somatotrophs, thyrotrophs, corticotrophs, and probably gonadotrophs	• Severe ocular malformation including anophthalmia • Combined pituitary hormone deficiencies with LH/FSH deficiency • Anterior pituitary hypoplasia with ectopic posterior pituitary	Unknown

TABLE 25.2 Genetic Defects of the GH-IGF Axis Resulting in Somatotroph Dysfunction and GH Deficiency—cont'd

Factor	Gene Function	Affected Cell Types	Clinical Phenotype	Mode of Inheritance
SOX2	• Member of SOXB1 subfamily as *SOX1* and *SOX3* expressed early in development	Somatotrophs, gonadotrophs (and, in animal models, thyrotrophs)	• Hypogonadotropic hypogonadism • Anterior pituitary hypoplasia • Bilateral anophthalmia/microphthalmia • Midbrain defects, including corpus callosum and hippocampus • Sensorineural defects • Esophageal atresia and learning difficulty	De novo
SOX3	• Member of SOX (SRY-related HMG box) • Developmental factor expressed in developing infundibulum and hypothalamus	Somatotrophs, additional anterior pituitary cell types	• Duplications of Xq26-27 in affected males (female carriers unaffected) • Variable mental retardation • Hypopituitarism with abnormal MRI • Anterior pituitary hypoplasia • Infundibular hypoplasia • Ectopic/undescended posterior pituitary • Abnormal corpus callosum • Murine studies suggest SOX3 dosage critical for normal pituitary development	X linked
Isolated Growth Hormone Deficiency				
GLI2	• Member of the *GLI* gene family; transcription factors that mediate SHH signaling	Somatotrophs	• Heterozygous loss-of-function mutations in patients with holoprosencephaly • Penetrance variable • Pituitary dysfunction accompanied by variable craniofacial abnormalities	Unknown
GHRHr	• Encodes GHRH receptor	Somatotrophs	• Short stature • Anterior pituitary hypoplasia	AR
GH1	• Encodes GH peptide • Several mutations shown to affect GH secretion or function	Somatotrophs	• Short stature • Abnormal facies • Presentation includes bioinactive GH	AR, AD, or X linked

ACTH, Adrenocorticotropic hormone; *AD,* autosomal dominant; *AR,* autosomal recessive; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GHRH,* growth hormone–releasing hormone; *αGSU,* glycoprotein α-subunit; *HMG,* high mobility group; *IGF,* insulin-like growth factor; *LH,* luteinizing hormone; *MRI,* magnetic resonance imaging; *PRL,* prolactin; *SHH,* Sonic hedgehog; *TSH,* thyroid-stimulating hormone (thyrotropin).

Although the increased incidence of breech presentation and birth trauma with neonatal asphyxia in congenital idiopathic hypopituitarism has led some to suggest an etiologic role for these occurrences,[335] the syndrome of pituitary stalk dysgenesis with congenital hypopituitarism is probably the result of abnormal development, and the perinatal difficulties are likely the consequence rather than the cause of the abnormalities. Findings of a similar MRI appearance in patients with septo-optic dysplasia,[149,336] in association with type I Arnold-Chiari syndrome and syringomyelia,[149,333] and possibly in holoprosencephaly,[149] and the occurrence of micropenis with congenital hypopituitarism[149,337,338] all support the concept that congenital hypopituitarism is a genetic or developmental malformation, not a birth injury. Further indirect evidence in studies[339] of isolated, complete anterior pituitary aplasia indicates that hypothalamic hypopituitarism and breech delivery are consequences of congenital midline brain defects, although perinatal residua of breech delivery may exacerbate ischemic damage to the hypothalamic-pituitary unit.

The MRI findings described for early-diagnosed patients with hypopituitarism are also found in children diagnosed at a later age. Most of these children have hypothalamic dysfunction as the cause of diminished pituitary hormone secretion. In the older group, as in the infants, structural, acquired hypothalamic, stalk, or pituitary abnormalities must be considered.

Holoprosencephaly. Holoprosencephaly, which is caused by abnormal midline development of the embryonic forebrain, usually results in hypothalamic insufficiency and has been associated with mutations in developmental proteins.[340–344] These mutations are associated with diminished signaling by SHH, a critical factor in forebrain development.[343] Hedgehog ligands bind to and activate the transmembrane receptor Patched (PTCH), which results in release of the coreceptor Smoothened (SMO), activating GLI transcription factors. SHH and FGF8 play a role in the induction of BMP2 and LHX, which are important for proliferation in the developing pituitary gland and are influenced by loss-of-function mutations of the *GL12* gene.[342,343]

Facial dysmorphism of holoprosencephaly ranges from cyclopia to hypertelorism and is accompanied by absence of the nasal septum, midline clefts of the palate or lip, and sometimes a single central incisor. GHD may be accompanied by other pituitary hormone insufficiencies.[345] The incidence of GHD is increased in cases of simple clefts of the lip or palate (or both),[346] and children with cleft palates who grow abnormally require further evaluation.

TABLE 25.3 GH-IGF1 Deficiency Syndromes

Congenital Growth Hormone Deficiency

GH deficiency resulting from hypothalamic dysfunction
Holoprosencephaly and septo-optic dysplasia
GH deficiency resulting from pituitary dysfunction
GH deficiency resulting from mutations of the GHRH receptor and GH
GH deficiency resulting from CPHD
Bioinactive GH

Acquired Growth Hormone Deficiency

GH deficiency resulting from CNS tumors, trauma, or inflammation
Circulating antibodies to GH that inhibit GH action

Congenital IGF1 Deficiency

Abnormalities of the GHR
STAT5 mutations
ALS mutations
IGF1 mutations
IGF1 receptor mutations

Acquired IGF1 Deficiency

Circulating antibodies to the GHR
Chronic disease states

ALS, Acid-labile subunit; *CHPD,* combined pituitary hormone deficiencies; *CNS,* central nervous system; *GH,* growth hormone; *GHR,* growth hormone receptor; *GHRH,* growth hormone–releasing hormone; *IGF1,* insulin-like growth factor 1.

Septo-Optic Dysplasia. In its complete form, the rare syndrome of *septo-optic dysplasia* (SOD) combines hypoplasia or absence of the optic chiasm or optic nerves (or both), agenesis or hypoplasia of the septum pellucidum or corpus callosum (or both), and hypothalamic insufficiency.[336,347,348] The extent of the anatomic and functional abnormalities can vary but usually are in parallel to each other.[347,348] GHD can occur by itself or in combination with deficiencies of TSH, ACTH, or gonadotropins. About 50% to 70% of children with severe anatomic defects have hypopituitarism or, at least, identifiable abnormalities of the GH-IGF axis,[349] and the diagnosis should be considered in any child who has growth failure associated with pendular or rotatory nystagmus or impaired vision and a small optic nerve disc. In some patients, a hypoplastic or interrupted pituitary stalk and ectopic posterior pituitary placement have been identified by MRI.[149,336,347] An increasing number of mutations in developmental transcription factors, including HESX1, SOX2, SOX3, and OTX2, are implicated in the pathogenesis of septo-optic dysplasia. Recently, mutations in genes known to be associated with hypogonadotropic hypogonadism, prokineticin receptor 2 (PROKR2), FGF8, and FGF receptor 1 (FGFR1) have been found to be associated with other pituitary hormone deficiencies along with septo-optic dysplasia.[350]

The varying conditions of environment and genetics likely contribute to the variable phenotype.[351,352] There is an increased incidence in offspring of young mothers, in first-born children, in areas of high unemployment, and in babies exposed to intrauterine medications, smoking, alcohol, or diabetes.[353]

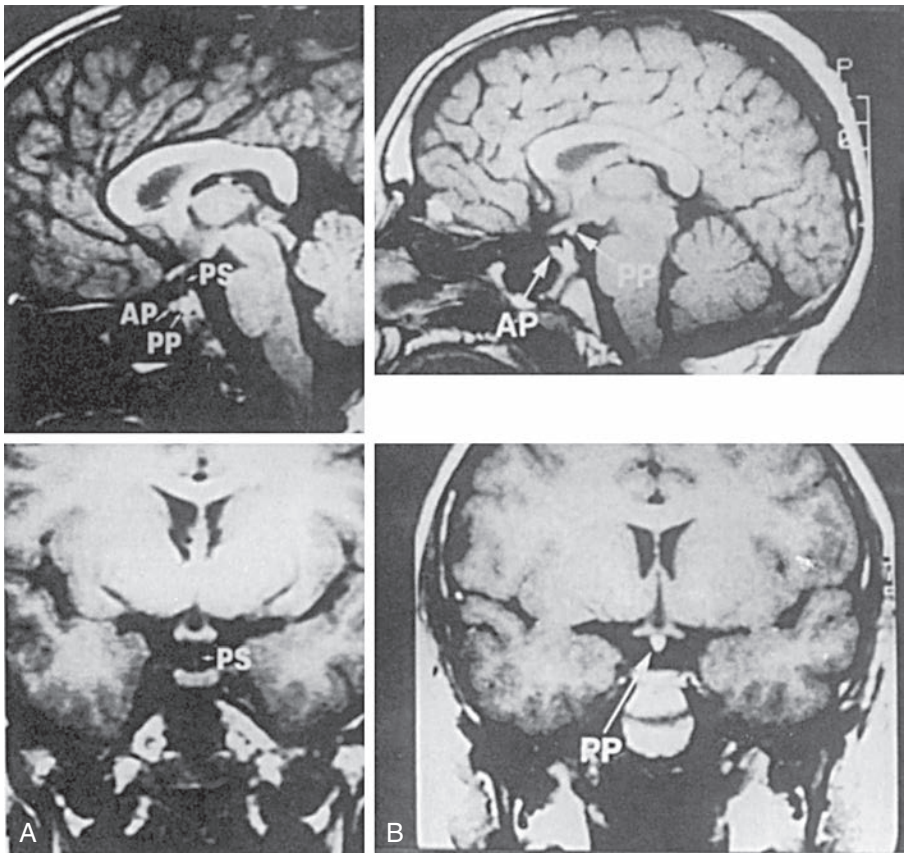
HESX1. The first homozygous missense mutation (N53C) has been detected within the homeobox domain of HESX1 in two siblings born to consanguineous parents with SOD.[354–361] Mutations of *HESX1*, a paired-like homeodomain gene that is expressed early in pituitary and forebrain development, are associated with familial forms of septo-optic dysplasia.[351,354,362,363] *HESX1*, also referred to as *RPX* (Rathke pouch homeobox), is a member of the paired-like class of homeobox genes and is essential for normal forebrain and pituitary formation.[364] It is one of the earliest known specific markers for the pituitary primordium and encodes for a developmental repressor with localization to the Rathke pouch.[357] TLE1 (the mammalian ortholog of the *Drosophila* protein Groucho) and the nuclear corepressor NCOR1 bind to HESX1 to exert repression.[365,366] *HESX1* downregulation is required for cell determination mediated by PROP1 to occur.[367] The LIM homeodomain proteins LHX1 and LHX3 are required for activation of the HESX1 promoter.[368]

Hesx1 null mutant mice display abnormalities in the forebrain, eyes, and other anterior structures such as the pituitary.[354] Mouse embryos heterozygous for both *Hesx1* and *Six3* null mutations have a mild phenotype, suggesting that these developmental factors control a switch in progenitor proliferation between repression by HESX1 and activation of PROP1.[369] These defects have similarity with human phenotypes such as septo-optic dysplasia and CPHD. Patients with septo-optic dysplasia may present with a wide spectrum of phenotypes associated with congenital hypopituitarism. Several homozygous and heterozygous mutations have been identified in *HESX1* (Online Mendelian Inheritance in Man [OMIM] no. 601802) in patients with hypopituitarism with variable phenotypes.[354,356,357,359,363,370–373]

Two siblings (born to consanguineous parents) with a severe septo-optic dysplasia phenotype, including anterior pituitary hypoplasia, an ectopic posterior lobe, agenesis of the corpus callosum, absent septum pellucidum, and optic nerve hypoplasia, were found to have a homozygous mutation at a highly conserved arginine residue of the homeodomain (p.R160C) that resulted in loss of DNA binding of the mutant protein. Another homozygous mutation, a threonine/isoleucine substitution at residue 26 (p.I26T), was identified in a child with GH and gonadotropin deficiency that later evolved to deficiencies of ACTH and TSH. She had no forebrain abnormalities and normal optic nerves, but on MRI had hypoplasia of the anterior pituitary and an undescended posterior pituitary. This mutation lies in a highly conserved engrailed homology domain (eh-1) that is required for transcriptional repression. Loss of repressor function was found to be caused by impaired interaction with the TLE1 corepressor. To determine the mechanisms by which these mutations cause the disorder, mice homozygous for these mutations were generated.[374] Mice homozygous for the p.R160C mutation displayed pituitary and forebrain defects similar to those in the *Hesx1* null embryos, indicating the critical role of HESX1 interactions with DNA in transcriptional repression during development. Mice homozygous for the p.I26T allele displayed pituitary defects and ocular abnormalities suggestive of a hypomorph allele, indicating the important role of TLE interaction in pituitary and ocular development.

Heterozygous mutations of *HESX1* have been identified in patients with hypopituitarism and septo-optic dysplasia and are usually associated with less affected phenotypes. Approximately 850 patients were studied for mutations in *HESX1,* including more than 300 patients with septo-optic dysplasia; 410 with isolated pituitary dysfunction, optic nerve hypoplasia, or midline neurologic abnormalities; and 126 with familial inheritance. The incidence of coding region mutations in *HESX1* within this population was about 1%, suggesting that mutations in *HESX1* are a rare cause of hypopituitarism and septo-optic dysplasia.[370]

Most of the HESX1 screenings performed in large CPHD cohorts failed to detect mutations. The only mutations



• **Fig. 25.34** Magnetic resonance imaging of infundibular dysgenesis. (A) T1-weighted sagittal and coronal images of the hypothalamic-pituitary area in a normal 8-year-old girl. The anterior (AP) and posterior pituitary (PP) lobes and the pituitary stalk (PS) are marked. (B) T1-weighted sagittal and coronal images of the hypothalamic-pituitary area of a 17-year-old boy with isolated growth hormone deficiency. The anterior pituitary lobe (AP) is hypoplastic, the posterior pituitary (PP) is ectopic, and the pituitary stalk is absent. (From Root AW, Martinez CR. Magnetic resonance imaging in patients with hypopituitarism. *Trends Endocrinol Metab.* 1992;3:283–287.)

identified were detected in sporadic subjects with an estimated global frequency of 0.45%.[375]

OTX2. Mutations in other genes have been associated with CNS anatomic abnormalities and hypopituitarism. *OTX2* is a homeobox gene that is expressed earliest in the neuroectoderm[376] cells of the forebrain and midbrain and encodes a transcription factor belonging to the orthodenticle family. This factor also plays a role in ocular development. Murine models harboring mutant *Otx2* genes demonstrate abnormal primitive streak organization and a headless phenotype.[376] OTX2 has a role in regulating early expression of HESX1 and is expressed in the pituitary to regulate POU1F1 (Pit1). Dateki and colleagues[377] identified a frameshift OTX2 mutation in a patient with bilateral anophthalmia and partial isolated GHD (IGHD) with minimal transactivation activity. A heterozygous OTX2 mutation was described in two unrelated patients with hypopituitarism; although initial studies demonstrated normal binding to HESX1 binding sites, the mutant *OTX2* gene was demonstrated to have decreased activation of the HESX1 promoter, suggesting a dominant negative effect leading to CPHD.[378] This relationship between the transcription factors OTX2 and HESX1 emphasizes the complexities of pituitary development and suggests that genetic causes may be multifactorial. Further studies have revealed two frameshift mutations, a nonsense mutation in two unrelated patients and a heterozygous microdeletion in a fifth patient.[377–385]

SOX3. A syndrome of X-linked hypopituitarism and mental retardation involving duplications of Xq26-27 encompassing *SOX3* (OMIM 313430) has been described in several pedigrees. SOX3 is a member of the SOX (SRY-related high mobility group box) family of transcription factors expressed in neuroepithelial progenitor and stem cells beginning in the earliest stages of embryogenesis involved in different developmental processes, such as gastrulation, neural induction, specification, and the differentiation of many cell types. Both the overexpression and underexpression of SOX3 can cause different phenotypes, ranging from IGHD to CPHD with ectopic neurohypophysis, anomalies of the corpus callosum with or without intellectual disability.[386–388] Microduplications and deletions of genomic regions containing SOX3 as well as expansion of a polyadenine tract (in one case, a deletion) have been reported.[389,393,394] The affected males have anterior pituitary and infundibular hypoplasia with an undescended posterior pituitary and abnormalities of the corpus callosum. Other abnormalities may include anophthalmia or microopthalmia, esoplageal atresia, and sensorineural hearing loss.[395,398,399] The GHD may also be associated with deficiencies of ACTH, TSH, or gonadotropins. Because *Sox3* is not expressed in Rathke pouch in the mouse, the anterior pituitary developmental defects are probably secondary to disruption of infundibular development.[351,386–389] Female carriers appear to be clinically unaffected, and no mutations have been found in patients with sex reversal, gonadal dysgenesis, or

infertility.[388–394] Synonymous changes have been found in children who inherited the variant from a clinically unaffected parent.[395,396] A patient was reported with isolated hypogonadotropic hypogonadism and a normal anterior pituitary suggesting that SOX2 may be involved in development and control of the hypothalamic neuronal reproductive axis. Indeed, SOX2 expression has been colocalized with LHX3 and HESX1.[374,395]

SOX2. Heterozygous mutations within *SOX2* in males have been associated with anophthalmia or microphthalmia and anterior pituitary hypoplasia. The resulting hormonal abnormalities include GH and gonadotropin deficiency. Some patients also present with genital abnormalities.[395] A wide variety of additional abnormalities may be present, including hypoplasia of the corpus callosum, hypothalamic hamartoma, hippocampal malformation, esophageal atresia, sensorineural hearing loss, and learning difficulties.[395–399] In addition to de novo heterozygous mutations, nonsynonymous changes have been identified in individuals who inherited the variant from a clinically unaffected parent.[395,396] A perplexing case of a patient with isolated hypogonadotropic hypogonadism but without anterior pituitary hypoplasia suggests that *SOX2* may be independently involved in hypothalamic neuronal development. The expression of *SOX2* has been found to overlap with that of *LHX3* and *HESX1* within Rathke pouch.[374,395,400]

GLI2. Heterozygous frameshift or nonsense GLI2 mutations have been reported in patients with holoprosencephaly. In addition, nonsynonymous GLI2 variants in patients with congenital hypopituitarism with CPHD and an ectopic posterior pituitary lobe without holoprosencephaly were found. Several patients with pituitary abnormalities, polydactyly, and abnormal facial features, also without holoprosencephaly, were reported to have truncating mutations. The pattern of inheritance of these mutations has been dominant with incomplete penetrance and variable expression.[401–403]

PROKR2. Patients with septo-optic dysplasia with CPHD have been found to have mutations in PROKR2, a known cause of isolated idiopathic hypogonadotropic hypogonadism and anosmia. Recently, a novel heterozygous substitution (c.742C>T;p.R248W) was identified in PROKR2 in a patient with panhypopituitarism and pituitary dysplasia.[350,404–406]

Acquired Disorders

Inflammation of the Brain or Hypothalamus. Bacterial, viral, or fungal infections may result in hypothalamic/pituitary insufficiency, and the hypothalamus or pituitary (or both) may also be involved in sarcoidosis.[407]

Tumors of the Brain or Hypothalamus. Brain tumors are a major cause of hypothalamic insufficiency,[408] especially midline brain tumors such as germinomas, meningiomas, gliomas, ependymomas, and gliomas of the optic nerve.[409] Although short stature and GHD are most often associated with suprasellar lesions in neurofibromatosis, they may also exist without such lesions. Whether growth impairment antedates the pathologic findings is not yet clear.[408] Metastases from extracranial carcinomas are rare in children, but hypothalamic insufficiency can result from local extension of craniopharyngeal carcinoma or Hodgkin disease of the nasopharynx. The laboratory diagnosis of GHD in children with brain tumors can be difficult because levels of IGF1 type I and IGFBP3 are poor predictors, especially in pubertal patients.[410] Craniopharyngiomas and histiocytosis can cause hypothalamic dysfunction (see later discussion).

Trauma of the Brain or Hypothalamus. Head trauma, resulting from boxing and various injuries, can cause IGHD or multiple anterior pituitary deficiencies. Some series of patients with GHD have indicated an increased incidence of birth trauma, such as breech deliveries, extensive use of forceps, prolonged labor, or abrupt delivery. Although GHD can be a consequence of a difficult delivery or hypoxemic perinatal period, it is more commonly associated with developmental deficiencies (discussed previously) or head trauma later in life. In a series of 22 head-injured adolescents and adults, almost 40% had some degree of hypopituitarism.[411–413]

Psychosocial Dwarfism. An extreme form of failure to thrive is termed *psychosocial dwarfism* or *emotional deprivation dwarfism.*[414–417] Most cases of failure to thrive can be traced back to a poor home environment and inadequate parenting, with improved weight gain and growth upon removal of the infant from the dysfunctional home. Some children have been reported, however, to show dramatic behavioral manifestations beyond those in the typical failure-to-thrive infant, namely, bizarre eating and drinking habits such as drinking from toilets, social withdrawal, and primitive speech.[414] Hyperphagia and abnormalities of GH production may be associated.[415] GH secretion is low in response to pharmacologic stimuli but returns to normal upon removal from the home. Concomitantly, eating and behavioral habits returned to normal, and a period of catch-up growth ensued. Careful assessment of endogenous GH secretion showed reversal of the GH insufficiency within 3 weeks, including enhancement of GH pulse amplitude and a variable increase of pulse frequency.[415] The reversibility of GH secretory defects and the later growth increment in the context of the clinical findings described previously confirm the diagnosis of psychosocial dwarfism.[415]

The neuroendocrinologic mechanisms involved in psychosocial dwarfism remain to be elucidated. GH secretion is abnormal, and ACTH and TSH levels may also be low, although some patients have high plasma cortisol levels. Even when GH secretion is reduced, treatment with GH is not usually of benefit until the psychosocial situation is improved. Management of the environmental causes of the growth failure is imperative and often associated with substantial growth. In our experience, although psychosocial dysfunction is a common cause of failure to thrive in infancy, the constellation of bizarre behaviors described in psychosocial dwarfism is rare.

The fact that GH production is impaired in adults with varied psychiatric disorders and the growth aberrations of functional GHD with psychosocial dwarfism together suggest that children with emotional problems may have impaired GH secretion and growth.[416] Indeed, depression in children, as adults, can lower GH production,[417] and in girls, anxiety disorders predict a modest height loss in adults.[416]

The Anterior Pituitary

As discussed earlier, many of the disease processes that impair hypothalamic regulation of GH secretion also impair pituitary function. Another group of abnormalities specifically affect pituitary somatotroph development and function.

Congenital Disorders. As many as 3% to 30% of patients with GHD have an affected parent, sibling, or child.[418] Inborn errors of the genes for nuclear transcription factors affecting hypothalamic-pituitary development, the GHRH receptor, or the GH gene can cause GHD and IGF deficiency.

Combined Pituitary Hormone Deficiency. During pituitary development, a series of transcription factors are expressed in a specific timeframe and in a spatial context. The result of cell differentiation and proliferation is a mature anterior pituitary gland with five distinct cell types.[352]

PITX2. *PITX2* (also known as *RIEG*) is a member of the bicoid-like homeobox transcription factor family that is closely related to the mammalian OTX genes expressed in the rostral brain during development; it is required at many stages of pituitary development.[352] Studies have shown that activation of the WNT signaling pathway or constitutive activation of β-catenin can induce *PITX2* expression. Further, protein inhibitors of activated STAT (PIAS) modulate PITX2 expression.[419] *PITX2* is expressed in thyrotrophs, gonadotrophs, somatotrophs, and lactotrophs but not in corticotrophs.[420]

PITX2 acts to activate the promoters of pituitary hormone target genes.[421,422] Homozygous loss of *PITX2* results in early embryonic lethality with pituitary development severely affected.[423–426] This is thought to be related to the control of cell cycle regulatory genes by PITX2.[427,428] Furthermore, the lack of *PITX2* also results in excessive cell death during early pituitary development, suggesting a role in cell survival.[429] A mouse line expressing a hypomorphic allele of *Pitx2* provided evidence that the extent of pituitary hypoplasia and cellular differentiation is proportional to the reduced dosage of *Pitx.*[426,430] In this model, the gonadotroph lineage was primarily affected and the numbers of differentiated somatotrophs and thyrotrophs were reduced, but the corticotroph population was unaffected.[430]

In a *Pitx2* overexpression model, the gonadotroph population was expanded, probably because of the role of *Pitx2* in the expression of gonadotroph-specific transcription factors GATA2, EGR1, and NR5A1 (SF1).[429] Mutations of *PITX2* have been described in patients diagnosed with Rieger syndrome, an autosomal dominant condition with variable manifestations, including anomalies of the anterior chamber of the eye, dental hypoplasia, a protuberant umbilicus, mental retardation, and pituitary abnormalities. PHX2 has been further associated with a role in left-right signaling, odontogenesis, cardiac development, and atrial fibrillation.[431–433] Mutations of *PITX2*, located within the homeodomain responsible for DNA binding, have been described, and several of these mutations show loss of DNA binding capacity.[434] A heterozygous mutation that changes the lysine at position 50 to glutamic acid in the homeodomain has been found to impart a dominant negative effect leading to a pronounced phenotype.[435]

SOX2. Heterozygous mutations in SOX2 (sex-determining region Y box 2) have been associated with eye abnormalities (i.e., anophthalmia, microphthalmia, and coloboma) and hypopituitarism characterized by anterior pituitary hypoplasia associated with GH and gonadotropin deficiency. Numerous nonsense, frameshift, and missense mutations, as well as chromosome deletions of SOX2, resulting in expression of SOX2 proteins with impaired function, have been identified.[352]

LHX3. LHX3 is a member of the LIM-type homeodomain protein family of transcription factors that feature two LIM domains in their amino terminus (N-terminal) and a centrally located homeodomain required for interaction with specific DNA elements on target genes. During development, expression of *LHX3*, which persists in the adult pituitary, is seen in the anterior and intermediate lobes of the pituitary, spinal cord, and medulla.[436] Murine models with targeted disruption of *Lhx3*, reporter transgenic mice, and mutants of LHX3[437] show depletion of thyrotrophs, gonadotrophs, and somatotrophs, suggesting that *Lhx3* is important for cell specification and proliferation.[438] Three LHX3 isoforms have been identified in humans: LHX3a, LHX3b, and M2LHX3.[439] Of these, LHX3a displays the greatest ability to activate the promoters of pituitary genes. LHX3 interacts with components of the inhibitor of histone acetyltransferase (INHAT) to modulate chromatin structure.[440] Patients with reported mutations in *LHX3* have deficits of GH, PRL, TSH, and gonadotropins, as well as abnormal pituitary morphology along with a rigid cervical spine that limits head rotation.[441–443] Other associated symptoms can include hearing loss and ACTH deficiency.

LHX3 mutations are a rare cause of reported hypopituitarism, and one study reported that the incidence of a homozygous *LHX3* mutation in patients studied with CPHD was 2.2%.[444] LHX3 mutations showed very low mutation frequency in genetic screening (0.3% in sporadic and 11.1% in familial cases). The two most northern counties of Sweden have an unexpectedly high prevalence of mutations, with carrier frequency estimated at 1 in 50, in apparently unrelated individuals, likely explained by a common ancestor dating back to the 17th century. Several other novel *LHX3* mutations in patients with CPHD demonstrating autosomal recessive inheritance have been reported and characterized.[441,442,444–448]

LHX4. LHX4 is another LIM homeodomain protein with homology to LHX3, and it is also expressed in the developing brain, including the cortex, pituitary, and spinal cord.[449] It contains two LIM domains in its N-terminus and a DNA-binding homeodomain.

Despite similarities in protein structure, the role of LHX4 in development is distinct from that of LHX3, as demonstrated by single and combined gene-deletion targeting in mice. Murine models with targeted deletion of *Lhx4* form a definitive Rathke pouch that arrests and results in a hypoplastic pituitary. In contrast to *Lhx3* gene knockout mice, *Lhx4*$^{-/-}$ mice contain all five differentiated cell types.[450,451] *Lhx3* expression is impaired in the *Lhx4* mutants, suggesting that *Lhx4* is required for cell survival, expansion of the pouch, and differentiation of pituitary-specific cell lineages. Since proper expression of Lhx4 is also crucial for the normal development of other organs such as lungs, *Lhx4*$^{-/-}$ mice die shortly after birth due to pulmonary failure, whereas heterozygous appear to be normal.

Several reports have described CPHD patients with evidence of a hypoplastic pituitary who harbored *LHX4* mutations.[436,452,453] These heterozygous mutations have been shown to result in proteins that are unable to bind DNA and activate pituitary gene expression.[454] Further studies have demonstrated a functional relationship between POU1F1 and LHX4 in the regulation of POU1F1 expression in specific pituitary cell types.[455] Also, several studies have suggested that LHX4 and PROP1 have overlapping functions in pituitary development.[450] Finally, in addition to pituitary hormone deficiencies, *LHX4* mutations have been implicated in structural abnormalities; patients with an *LHX4* mutation have been reported with abnormal MRI findings, including a hypoplastic anterior pituitary, ectopic posterior lobe, poorly developed sella turcica, and Chiari malformation.[456] In the nine cases described in the literature to date, large interfamilial and intrafamilial variabilities are present.[454,457–459] Recently a novel recessive mutation has been reported (pT126M), which is associated with a lethal form of congenital hypopituitarism. However, functional assay failed to show any difference between the mutant and the wild-type LHX4, making it difficult to implicate the mutation with pathogenicity.[460]

SIX6. *SIX6* is a member of the SIX/sine oculis family of homeobox genes that is expressed in retina, optic nerve, hypothalamus, and pituitary.[461] Murine expression studies of the TCF/LEF family of transcription factors during pituitary development demonstrated that *Six6* plays a role in proliferation of cells during early formation of Rathke pouch.[462] *SIX6* has also been shown

to interact with the Groucho family of transcriptional repressors.[463,464] Mice lacking *SIX6* demonstrate infertility.[465] *SIX6* has been mapped to chromosome 14q22-23, and patients with deletions of this chromosomal region display bilateral anophthalmia and pituitary anomalies.[466,467] Patients with anophthalmia/microphthalmia were shown to have several frequent polymorphisms of *Six6* and one potential causative missense mutation.[461] One case report implicated *SIX6* haploinsufficiency as being responsible for ocular and pituitary maldevelopment. Despite its importance in early development, further studies are required to determine whether *SIX6* mutations are present in patients with pituitary hormone deficiency.

ISL1. ISL1 is a member of the LIM homeodomain family of transcription factors, which are characterized by two tandemly repeated cysteine/histidine-rich LIM domains that are involved in protein-protein interactions. ISL1 has been shown to be a transcriptional regulator of LHX3.[468] Its expression is restricted to cells that will express the pituitary of glycoprotein hormone α-subunit (αGSU) gene.[469,470] Homozygous loss of *Isl1* in mice results in developmental arrest with no pouch formation.[471] To date, no human mutations in *ISL1* have been identified.

PROP1. Mutations in *PROP1*, a paired-like homeodomain transcription factor with expression restricted to the anterior pituitary during development, have also been found to result in CPHD.[472] Mutation of this gene is responsible for a form of murine pituitary-dependent dwarfism known as the Ames mouse.[473] The pituitary gland appears to be enlarged in mice bearing mutations in *Prop1*, although the mechanism is unclear.[473–476] In the end, a decrease in proliferation and apoptosis results in pituitary hypoplasia, similar to that seen in humans.[402,450,474–478] The switch from repression of target gene expression by HESX1 to activation by PROP1 is important for development of the POU1F1 (GH, PRL, and TSH) and gonadotroph lineages.[366,476,479] PROP1 and β-catenin have been shown to form a complex that represses *Hesx1* while activating *Pou1f1* expression.[480] PROP1 bends to a palindrome TAAT sequence as a dimer to actuate target gene expression.[481,482] The gonadotropin deficiency in the Ames dwarf remains unexplained, although treatment with thyroxine (T_4) or GH (or both) restored fertility in some male mice and restored sexual maturation, but not fertility, in female mice.[483,484]

Mutations of *PROP1* in humans result in GH, PRL, and TSH deficiencies, although failure in all cell lineages, including gonadotrophs and corticotrophs, has been reported.[485–487] The characterization of *PROP1* mutations is complex, because the phenotypes are variable and dynamic and hormone deficiencies may develop over time even in patients with similar genetic backgrounds.[485,488,489] Gonadotropin abnormalities are particularly diverse in that approximately 30% of patients have spontaneous pubertal development, including menarche, before ultimately developing hypogonadotropic hypogonadism.[485,490] Apparently normal growth without GH has also been found in a child with PROP1 deficiency.[491,492] The ACTH deficiency may develop in the fourth or fifth decade of life.[493] Striking variability has been described in pituitary size, with very large glands, possibly arising from the intermediate lobe,[494] producing a hyperintense T1-weighted signal occasionally demonstrated by MRI.[486,495,496] These glands may undergo involution, leaving a large empty sella in patients with complete anterior hypopituitarism, including ACTH deficiency.[497–499]

Many *PROP1* (chromosome 5q35, OMIM 601538) abnormalities have been identified, including missense, frameshift, and splicing mutations. A GA repeat in exon 2 (295-CGA-GAG-AGT-303) has been reported to be a "hot spot" in *PROP1;* any combination of a GA or AG deletion in this repeat region results in a frameshift in the coding sequence and premature termination at codon 109.[490,500] Similar abnormalities result from homozygous lesions at other sites on exon 2 and affect codons 73, 88, and 149.[490,500] Compound heterozygosity for two mutations was detected in 36% of children from four families; two different common deletions both led to a stop codon at position 109.[501] These mutations are predicted to result in loss of the DNA-binding and C-terminal transactivating domains of *PROP1*. Some missense mutations have been shown to retain partial activity.[486,487,502] Two mutations in the transactivating domain, not the DNA binding domain (W194 X Prop1, S156 insTProp1), were shown to differentially affect DNA binding and transactivation.[503]

To date, PROP1 mutations represent the most commonly known genetic cause of CPHD both in sporadic (6.7%) and familial cases (48.5%) with a global mutation frequency of 11% considering all the patients. A screen of 73 subjects (36 families) diagnosed with CPHD by Deladoey and associates identified 35 patients with *PROP1* gene defects, including three different missense mutations, two frameshift mutations, and one splice site mutation. In 12 of 36 unrelated families, defects were located in the region nt296 through nt302, suggesting a possible hot spot for *PROP1* mutations in CPHD.[490] Although *PROP1* mutations appear to be rare in sporadic cases, their prevalence is 29.5% in familial cases of CHPD, as reported by Turton and colleagues.[391,504] The mutation rate varies considerably among the different geographic areas: While Western European, US, Australian, and Japanese cohorts presented a mutation prevalence lower than 1%, the Eastern European and Russian cohorts showed a much higher frequency, reaching 64.8% in the Lithuanian population. Two variants, namely c.301_302delAG and c.150delA, are the most common mutations found in PROP1 and represent more than 90% of the mutated alleles in the Eastern European cohorts. Recently Dusatkova et al.[505] genotyped 21 SNPs flanking a 9.6-Mb region around PROP1 and demonstrated an ancestral origin for both variants that are carried on haplotypes spanning 0.2e0.3 Mb, showing that the most frequent PROP1 variants are not mutation hot spots as previously assumed but founder variants.[375,488,501,506–511]

There does not appear to be a strong correlation between phenotype and genotype.[490] Nyström and associates reported compound heterozygous mutations in the *PROP1* gene in twins with hypopituitarism and late ACTH deficiency.[512]

POU1F1. The *POU1F1* gene (chromosome 3p11, OMIM 173110) encodes Pit1, a member of a large family of transcription factors referred to as POU-domain proteins that is responsible for pituitary-specific transcription of genes for GH, PRL, TSH, and the GHRH receptor.[59,513–515] Pit1, a 290–amino acid protein, contains two domains, the POU-specific domain and POU-homeo domain; both are necessary for DNA binding and activation of GH and PRL genes and for regulation of the PRL, TSHβ, and POU1F1 genes.[516] Its expression is restricted to the anterior pituitary to control differentiation, proliferation, and survival of somatotrophs, lactotrophs, and thyrotrophs.[418,515–517] Pit1 regulates target genes by binding to response elements and recruiting coactivator proteins, such as cAMP response element–binding protein (CREB)–binding protein (CBP).[518] Gene expression microarray assays combined with chromatin immunoprecipitation (CHIP) were used to detect targets of POUIFI.[519]

Two mouse models were first reported to have GH, PRL, and TSH deficiencies associated with mutations or rearrangements of

the *Pit1* gene; these were the Snell (dw/dwS) and the Jackson (dw/dwJ) dwarf mice.[520,521] Many different mutations of the *POU1F1* gene have been found internationally in families with GHD and PRL deficiency and variable defects in TSH expression.[522–525] These mutations are transmitted as autosomal recessive or dominant traits and cause variable peptide hormone deficiencies with or without anterior pituitary hypoplasia.[522–528]

The most common mutation, present in about the 30% of the patients that carry a POU1F1 mutation, is an R271W substitution that affects the POU homeodomain, encoding a mutant protein that binds normally to DNA and acts as a dominant inhibitor of transcription.[528–531] Vertical transmission of the R271W mutation was shown, emphasizing the importance of diagnostic and therapeutic management during pregnancy.[532] Evidence from a patient with the R271W mutation suggests that Pit1 may have a role in cell survival.[531] Indeed, the mutation was used to target cell proliferation tumoral model systems. A patient diagnosed with GHD, along with dysregulation of PRL and TSH, was reported to have a lysine-to–glutamic acid mutation at codon 216 (K216E).[516] This mutant Pit1 binds to DNA and appears not to inhibit basal activation of GH and PRL genes; however, the mutant is unable to support retinoic acid induction of *POU1F1* gene expression. Another report suggested that CBP (p300) recruitment and Pit1 dimerization are necessary for target gene activation and that disruption of this process may account for the pathogenesis of CPHD.[533] All of the reported mutations involve sites affecting *POU1F1* DNA-binding, dimerization, or target gene transactivation.[524,525,528,534–542]

Phenotypic variability occurs among patients with apparently similar genotypes. It does not appear that ACTH or gonadotropin deficiencies occur, as is frequently the case with *PROP1* defects,[504] but adrenarche has been reported to be absent or delayed in patients with a POU1F1 mutation.[543] Circulating antibodies against Pit1 have been identified to be responsible for hypopituitarism similar to that caused by mutations.[544] Of the sporadic cases of CPHD, 1.6% are found to have POU1F1 mutations with no significant differences among populations. A higher frequency of POU1F1 mutations (21.6%) has been detected in familial cases.[375]

ARNT2

Six members of a consanguineous family with CPHD and microcephalia were found to have a homozygous frameshift mutation (c1373_1374dupTC) in aryl-hydrocarbon receptor nuclear translocator 2 (ARNT2).[545]

GRP161

Recently a homozygous mutation in GRP161, encoding the orphan G protein–coupled receptor 161, was identified in a family with CPHD and pituitary stalk interruption.[546]

Isolated Growth Hormone Deficiency. The incidence of IGHD is estimated to be 1 in every 3480 to 10,000 live births.[86,547–549] In most children with IGHD, no cause can be identified, and this group is often referred to as having idiopathic GHD. However, there is increasing recognition that genetic defects underlie some cases of GHD. It has been estimated that mutations in the GH and growth hormone receptor genes can be detected in up to 34% of familial cases of IGHD. Patients with GHD may develop deficiencies of additional anterior pituitary hormones.[550] Four forms of IGHD have been reported (see Table 25.2).[551] The classification system is based on clinical characteristics, inheritance patterns, and GH secretion but not necessarily on disease causation. IGHD was most recently reviewed by Alatzoglou and associates.[552]

The gene encoding GH *(GH1)* is located on chromosome 17q23 in a cluster of five highly homologous genes that includes two chorionic somatomammotropin genes CHS1 and CHS2, the placentally expressed growth hormone gene GH2, and a pseudogene, CSHP1. *GH1* and *GH2* differ in mRNA splicing pattern: *GH1* generates 20-kDa and 22-kDa proteins (of approximately equal bioactivity), whereas *GH2* yields a protein that differs from *GH1* by 13 amino acid residues.

The 22-kDa isoform includes the five exons with the complete biologic activity of GH. A cryptic in-frame splice site within exon 3 gives rise to alternative spliced transcripts lacking the first 45 bp of exon 3 and produces a 20-kDa peptide missing aa 32-46.[94] The 17.5-kDa form results from the complete skipping of exon 3 lacking aa 32-71 representing 1% to 5% of the total GH1 transcripts. Two isoforms lacking exons 3 and 4 encoding a 11.3-kDa and exons 2 through 4 encoding a 7.4-kDa peptide have been detected.[553–559]

IGHD Type I. IGHD type IA results primarily from large deletions, with rare frameshift and nonsense mutations of the *GH1* gene that prevents synthesis or secretion of the hormone.[552,560] IGHD IA is inherited as an autosomal recessive trait, and affected individuals have profound congenital GHD, including hypoglycemia in infancy and severe dwarfism by 6 months of age.[561] It has been reported that about 10% to 15% of subjects with a height below 4.5 SDS and severe IGHD carry GH1 gene deletions; however, geographic and patient selection criteria are present between studies. Frameshift and nonsense GH1 mutations have also been found in subjects with the IGHD IA phenotype.[561]

Because GH is not produced even in fetal life, patients are immunologically intolerant of GH and typically develop anti-GH antibodies when treated with either pituitary-derived or recombinant DNA–derived GH. When antibodies prevent patients from responding to GH, IGHD IA can be viewed as a form of GH insensitivity, and such patients are candidates for IGF1 therapy.[554,561–563]

The less severe form of autosomal recessive GHD, termed IGHD type IB, may also result from mutations or rearrangements of the *GH1* gene that causes production of an aberrant GH molecule that retains some function or at least generates immune tolerance. The phenotypic variability is greater than in IGHD IA.[551,552] These patients usually respond to exogenous GH therapy without antibody production. The very low frequency (1.7%) of *GH1* gene mutations in familial type IB IGHD suggests the importance of studying the *GH1* gene promoter region in patients with unexplained GHD.[564]

Mutations in *GHRHR* are also classified as IGHD type IB. Mutation of the gene for *Ghrhr* in its ligand-binding domain has been identified in the *little* mouse *(lit/lit)*[565] and results in dwarfism and decreased numbers of somatotrophs.[515,556,566] In this model, the fetal somatotroph mass is normal, and hypoplasia of the somatotrophs is evident only postnatally.[515,517,556,566] Such data suggest that GHRH is not an essential factor for fetal differentiation of the somatotrophs and that GHRH-independent cells persist or that mutation does not cause total loss of GHRH function.[567–569]

Wajnrajch and colleagues reported the first human cases of a mutation in the *GHRHR* gene in two cousins in a consanguineous Indian Muslim family with IGF deficiency and profound growth failure.[570] The gene defect, a nonsense mutation that introduced a stop codon at position 72 (E72X), resulted in a markedly truncated GHRHR protein that lacked the membrane-spanning regions and the G protein–binding site. The affected children had

undetectable GH release during standard provocative tests and after exogenous GHRH administration but responded to GH treatment. The same mutation was also identified in a reportedly unrelated Tamoulean kindred in Sri Lanka,[571] in a consanguineous kindred in Pakistan ("dwarfism of Sindh"),[572,573] and in 17 patients from one Muslim and four Hindu families in Western India.[574] The largest kindred in which a mutation of *GHRHR* was identified was a Brazilian family with a homozygous donor splice mutation (G to A at position +1) of exon 1.[86] This mutation disrupts the highly conserved consensus GT of the 50-donor splice site, generating a truncated GHRHR.[575,576]

A *GHRHR* missense mutation in exon 11 (R357C) was described in two consanguineous Israeli Arab families.[577] Patients in all of the groups had early growth failure with short stature (–4.5 to –8.6 SD), a high-pitched voice, and increased abdominal fat accumulation.[572,576] As expected, all of the patients demonstrated severely reduced or undetectable serum concentrations of GH in response to provocative GH stimulation, as well as very low serum concentrations of IGF1, IGFBP3, and ALS.[576] The adults manifest an unfavorable cardiovascular risk profile, which includes increased levels of low-density lipoprotein cholesterol and total cholesterol, elevated C-reactive protein, elevated blood pressure, and abdominal obesity. However, a perplexing study found no evidence of premature atherosclerosis or premature myocardial ischemia in these patients with *GHRHR* mutations.[578] The patients respond well to exogenous GH without antibody formation. Heterozygotes may have minimal height deficits and may show moderate biochemical deficiencies of the GH-IGF axis.[572] Despite extensive study, the geographic separation and ethnic differences among these patient groups do not suggest recent (>200 years) contact among the families from the Indian subcontinent. At present, the likely explanation for all four families is that of a "founder effect" or one-time mutation in each group followed by propagation within a geographically isolated gene pool.[86] In an analysis of 30 families with IGHD type IB, Salvatori and colleagues[579] found new missense mutations in transmembrane and intracellular domains of the *GHRHR* in three families (10%), with two affected members in each. Transfection experiments indicated normal cellular expression of these mutant receptors. Mutations in GHRH were recently reviewed by Corazzini and Salvatori.[580]

A recent dominant mutation with incomplete penetrance was reported due to a missense substitution (Val10Gly) in the signal peptide in three unrelated patients, resulting in a defect in receptor processing and translocation to the cell surface.

Most of the reported GHRHR mutations occur within consanguineous families, and of those a minority of the affected are compound heterozygotes. In a group of 65 children with IGHD IB, the GHRH receptor gene *(GHRHR)* was normal in domains coding for the extracellular region,[581] but mutations in transmembrane and intracellular gene domains were found in 10% of families with IGHD IB.[86,556,577,579,582–587]

IGHD Type II. IGHD type II is inherited as an autosomal dominant trait. The most common cause appears to be mutations that inactivate the 5′ splice donor site of intron 3 of the *GH1* gene, which contains multiple *cis*-acting splicing enhancers in the exon and intron required to activate canonic splice sites for intron 2 and 3 and silencing of the cryptic sites. The skipping of exon 3 results in a 17.5-kDa GH isoform mutant that has been shown to function in a dominant-negative manner to suppress intracellular accumulation and secretion of wild-type GH.[565,588,589] In patients with missense mutations in exon 4 or 5, clinical presentation is quite variable, with some evidence for reversibility of the impairment of intracellular GH storage and secretion by GH treatment.[590] Mullis and coworkers[591] studied 57 subjects from 19 families and found that patients with IGHD type II not only have a variable phenotype in terms of onset, severity, and progression of GHD but also may demonstrate later onset of ACTH or TSH deficiencies and pituitary hypoplasia. An extensive assessment of *GH1* gene mutations in short children with and without GHD revealed a substantial number of heterozygous mutations.[591] Recent studies characterizing the mechanism of the GH secretory defects and the increased expression of the 17.5-kDa isoform may lead to novel molecular therapies.[592]

IGHD Type III. IGHD type III, transmitted as an X-linked trait with associated hypogammaglobulinemia (XLA),[593] has not yet been related to a mutation of the *GH1* gene. A large Australian kindred demonstrated GHD with a variable spectrum of pituitary hormonal deficiencies that may have been caused by duplication of the Xq25.Xq28 region.[594]

A splicing mutation within the Bruton tyrosine kinase *(BTK)* gene in patients with XLA and IGHD has been reported. Mutations in *BTK,* a cytoplasmic tyrosine kinase expressed in B lymphocyte and myeloid cells, have also been identified in several patients with XLA but without IGHD.

SOX3. Mutations in SOX3, a member of the SOX (SRY-related high mobility group box) family of transcription factors on Xq27.1, have been found in families with IGHD with ectopic neurohypophysis, anomalies of the corpus callosum, and intellectual disability.[388] Several patients with GH deficiency and complex phenotypes with duplications in the proximal Xq chromosome have been reported.[595,596] A complete description of SOX3 can be found in the section on septo-optic dysplasia earlier in the chapter.

Bioinactive GH. Serum GH exists in multiple molecular forms, reflecting the consequences of alternative post-transcriptional or post-translational processing of the mRNA or protein, respectively. Some of these forms are presumed to have defects in the amino acid sequences required for the binding of GH to its receptor, and different molecular forms of GH may have varying potencies for stimulating skeletal growth, although this remains to be rigorously proved. Short stature with normal GH immunoreactivity but reduced biopotency has been suggested,[597,598] but the molecular abnormalities have been characterized in relatively few patients, and many cases of suspected bioinactive GH have not been rigorously proved.[599,600]

In one child with extreme short stature (–6.1 SDS), a mutant GH caused by a single missense heterozygous mutation (cysteine to arginine, codon 77 of *GH1*) bound with greater affinity than normal to GHBP and the GHR and inhibited the action of normal GH. The child grew more (6 vs. 3.9 cm/year) during a period of exogenous GH in moderate dosage. The father was found to have the same genetic abnormality but did not express the mutant hormone. In a second patient[600] with marked short stature (–3.6 SDS), a heterozygous alanine-to-glycine substitution in exon 4 of GH led to a substitution of glycine for arginine. This mutation was located in site 2 of GH molecular binding with its receptor and apparently led to failure of appropriate molecular rotation of the dimerized receptor and subsequent diminished tyrosine phosphorylation and the GH-mediated intracellular cascade of events. Bioactivity determined in a mouse B-cell lymphoma line was about 33% of immunoreactivity.[601] Exogenous GH substantially increased growth velocity (from 4.5 to 11 cm/year).

An Ile179Met substitution found in a short child was characterized by normal STAT5 activation but a 50% decrement in ERK activation.[217] This novel finding demonstrated the complexity of

the functional interaction of GH with its receptor, but because STAT5B is clearly the major (if not the sole) GH-dependent mediator of *IGF1* gene transcription, the role of this mutation is not clear. Six GH heterozygous variants with evidence of impairment of JAK/STAT activation were found by screening short children, suggesting that further studies are needed to determine the mechanism of interaction of GH with its receptor.[602] Because these variants occur in heterozygotes, the genotype-phenotype correlations are unclear. In one of the more convincing cases of bioinactive GH reported to date, Besson and associates[603] found a homozygous missense mutation (G705C) in a short child (–3.6 SDS) that resulted in the absence of two disulfide bridges. Both GHR binding and JAK2/STAT5 signaling activity were markedly reduced.

Some patients demonstrate a decrease in bioactivity (when measured by sensitive in vitro assays) but not in immunoreactivity. The absence of mutations suggests that abnormal post-translational modifications of GH or other peripheral mechanisms may be responsible.[604,605]

Acquired Disorders

Craniopharyngiomas and Other Tumors. Many tumors that impair hypothalamic function also affect pituitary secretion of GH. In addition, *craniopharyngiomas* are a major cause of pituitary insufficiency. These tumors arise from remnants of Rathke pouch, the diverticulum of the roof of the embryonic oral cavity that normally gives rise to the anterior pituitary. The diagnosis and treatment of craniopharyngioma have been recently reviewed. This tumor is a congenital malformation present at birth and gradually grows over the ensuing years. The tumor arises from rests of squamous cells at the junction of the adenohypophysis and neurohypophysis and forms a cyst as it enlarges; the cyst contains degenerated cells and may calcify but does not undergo malignant degeneration. The cyst fluid ranges from the consistency of machine oil to a shimmering, cholesterol-laden liquid, and the calcifications may be microscopic or gross. About 75% of craniopharyngiomas arise in the suprasellar region; the remainder resemble pituitary adenomas. Mutations in β-catenin have been found in patients with adamantinomatous craniopharyngiomas.[606]

Craniopharyngiomas can cause manifestations at any age from infancy to adulthood but usually manifest in middle childhood. The most common presentation results from increased intracranial pressure and includes headaches, vomiting, and oculomotor abnormalities. Visual field defects result from compression of the optic chiasm, and papilledema or optic atrophy may be present. Visual and olfactory hallucinations have been reported, as have seizures and dementia. Most children with craniopharyngiomas have evidence of growth failure at the time of presentation, and they are often found retrospectively to have had reduced growth since infancy.[607] GH and the gonadotropins are the most commonly affected pituitary hormones in children and adults, but deficiency of TSH and ACTH may also occur, and diabetes insipidus is present in 25% to 50% of patients.[607] Between 50% and 80% of patients have abnormalities of at least one anterior pituitary hormone at diagnosis.

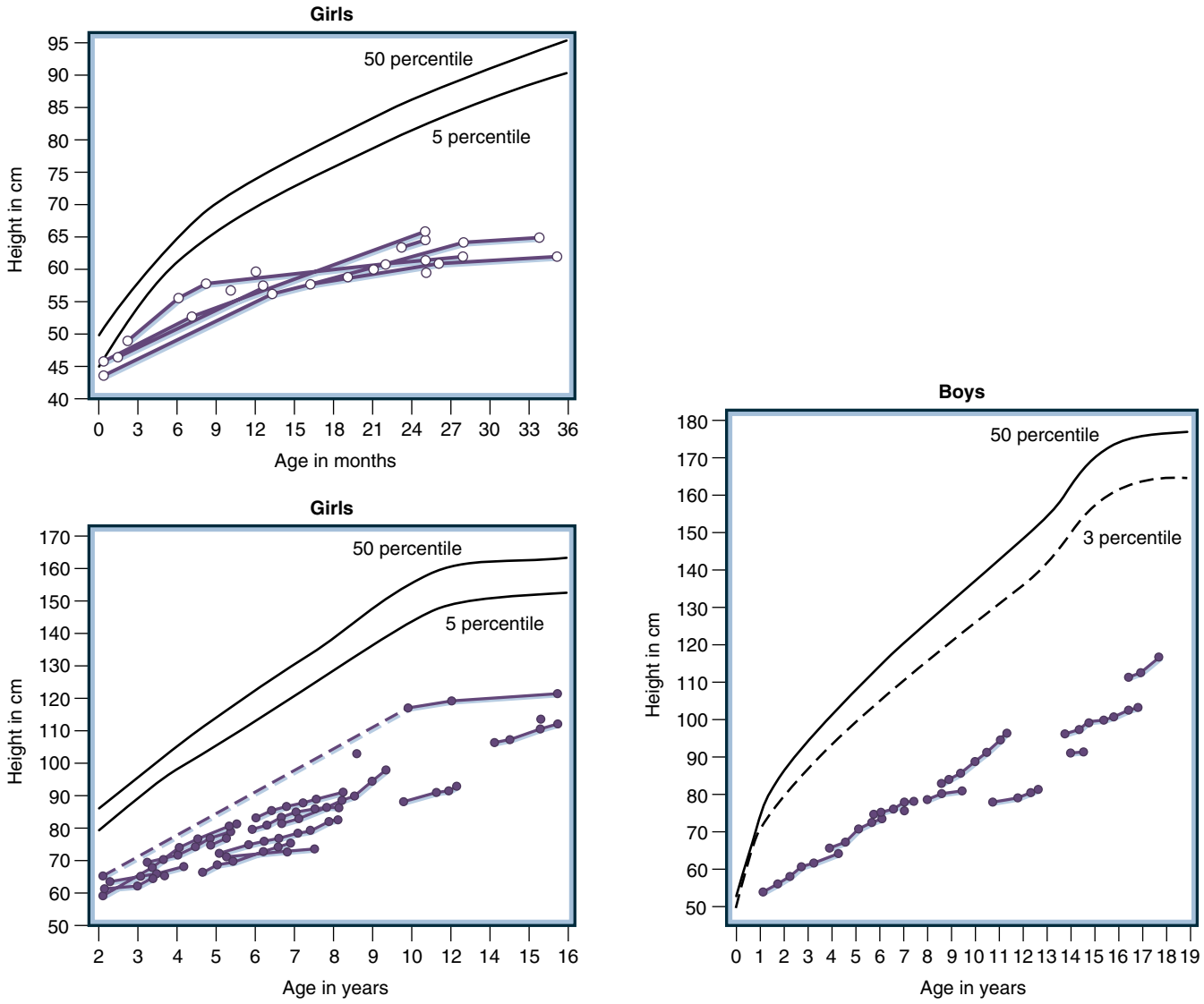
Cystic and solid components can be identified by MRI, and anatomic relationships can be delineated to help plan a rational operative approach. Operative intervention via craniotomy or transsphenoidal resection may result in partial or almost complete removal of the lesion. Postoperative irradiation is commonly used, especially if tumor resection was incomplete. In some patients, particularly those who become obese, a syndrome of normal linear growth without GH may occur. The metabolic syndrome, with evidence of insulin insensitivity and increased body mass index (BMI), is common and is a predictor of potential major long-term morbidity.[607–609] The long-term childhood and adolescent consequences of craniopharyngioma are substantial, with many quality-of-life issues exacerbating the hypopituitarism. Patients with a history of hypothalamic obesity associated with craniopharyngiomas undergoing brain surgery were favored to have sustained weight loss.

Pituitary adenomas (see Chapter 9) are infrequent during childhood and adolescence, accounting for fewer than 5% of patients undergoing surgery at large centers. Almost two-thirds of tumors immunochemically stain for PRL, and a small number stain for GH. GH-secreting pituitary adenomas are exceedingly unusual in youth. There is a variable experience as to the invasive nature of pituitary adenomas, but the prevailing opinion is that they are less aggressive in children than in adults. In 56 patients at the Mayo Clinic with non–ACTH-secreting adenomas removed transsphenoidally, macroadenomas were about one-third more frequent than microadenomas, with cases in girls outnumbering those in boys 3.3 to 1. The patients with macroadenoma had an approximately 50% incidence of hypopituitarism, compared with zero incidence in those patients with microadenomas; long-term cure rates were between 55% and 65% for both tumor sizes. Familial isolated pituitary adenoma (FIPA) accounts for about 2% of pituitary adenomas and is associated with aryl hydrocarbon receptor interacting protein (AIP) gene mutations. Gigantism is a feature of some patients with somatotropinomas.[610]

Histiocytosis X. The localized or generalized proliferation of mononuclear macrophages (histiocytes) characterizes Langerhans cell histiocytosis, a diverse disorder that occurs in patients of all ages, with a peak incidence at ages 1 to 4 years. Endocrinologists are more familiar with the term *histiocytosis X,* which includes three related disorders: solitary bony disease (eosinophilic granuloma), Hand-Schüller-Christian disease (chronic disease with diabetes insipidus, exophthalmos, and multiple calvarial lesions), and disseminated histiocytosis X (Letterer-Siwe disease, with widespread visceral involvement). These syndromes are characterized by an infiltration and accumulation of Langerhans cells in the involved areas, such as skull, hypothalamic-pituitary stalk, CNS, and viscera. Although these disorders, especially Hand-Schüller-Christian disease, are classically associated with diabetes insipidus, approximately 50% to 75% of patients in selected series have growth failure and GHD at the time of presentation. The degree of pituitary stalk thickness has been shown to correlate with long-term risk outcomes.[611] In contrast, a French national registry (n = 589) found GHD in 61 subjects, with overall endocrine dysfunction in 148. In the latter group, an evolving neurodegenerative syndrome (identified in 10% of patients with 15-year follow-up) seemed to be associated with pituitary involvement.[612] Only 1% of unselected children with Langerhans cell histiocytosis living in Canada during a 15-year period had GHD.[613]

Growth Hormone Insensitivity

Mutations in GHR Signaling Proteins and ALS. GH insensitivity, also known as *primary IGF1 deficiency,* encompasses a variety of genetic conditions characterized by growth failure, high serum GH levels, and very low serum IGF1 levels.[614] These findings, first described in siblings in 1966 by Laron and colleagues,[615] are also known as *Laron syndrome*. Most individuals with a diagnosis of classic GH insensitivity are from pedigrees of Mediterranean or Middle Eastern descent. Members of a well-described pedigree



• **Fig. 25.35** Height measurements for Ecuadorian children with insulin-like growth factor (IGF) deficiency resulting from growth hormone (GH) insensitivity. (From Rosenfeld RG, Rosenbloom AL, Guevara-Aguirre J. Growth hormone [GH] resistance due to primary GH receptor deficiency. *Endocr Rev.* 1994;15:369–390.)

from Ecuador were from a consanguineous population with Mediterranean origins.[616]

The phenotypic characteristics of classic GH insensitivity include growth failure evident at birth[615] with postnatal subnormal growth velocities and stature –4 to –10 SD below the mean (Fig. 25.35).[614] Patients also have subnormal head circumference, protruding forehead, abnormal upper-body to lower-body ratio, short extremities, and sparse hair (Table 25.4). There is delayed motor development, indicating the importance of IGF1 in cerebral development. The genitalia are small and puberty is delayed, but fertility is normal. Metabolically, the most striking feature of IGF1 deficiency is hypoglycemia with later development of obesity, relative hyperinsulinemia, and insulin resistance.[617] Individuals do not respond to exogenous GH, as determined by growth velocity, hypoglycemia incidence, or serum IGF1 or IGFBP1 levels.[618] GHBP activity is usually undetectable in sera of these patients,[619,620] but measurable levels correspond to higher final heights.

Liver biopsy performed on two affected patients demonstrated that microsomal cells did not bind recombinant GH, suggesting a defect in the GHR.[621] Both deletions and homozygous point mutations (missense, nonsense, and abnormal splicing) in the GHR have been described in persons with GH insensitivity.[622–624] Described gene deletions involve exons 3, 5, and 6.[625] Deletion of exons 5 and 6 results in a frameshift and a premature translational stop signal with consequent encoding of a receptor lacking most of the extracellular GH-binding domain.

More than 70 mutations of the *GHR* gene resulting in GH insensitivity have been reported to date.[626] Most of the mutations are in the extracellular (GH-binding) domain of the receptor and result in impaired ability of GH to bind to the receptor; this also leads to a deficiency of circulating GHBP, which is derived from the extracellular domain of the receptor. One reported mutation of the extracellular domain does not affect GH binding to the receptor but prevents dimerization of the receptor.[627] Homozygous mutations have also been reported in the transmembrane domain.[628,629] These mutations result in GH insensitivity with normal GH binding but lack of receptor transduction. Because the extracellular domain is intact and the mutant receptor protein

TABLE 25.4 Clinical Features of Growth Hormone Insensitivity

Parameter	Clinical Finding
Growth and Development	
Birth weight	Near-normal
Birth length	May be slightly decreased
Postnatal growth	Severe growth failure
Bone age	Delayed, but may be advanced relative to height age
Genitalia	Micropenis in childhood; normal for body size in adults
Puberty	Delayed 3 to 7 years
Sexual function and fertility	Normal
Craniofacies	
Hair	Sparse before age 7 years
Forehead	Prominent; frontal bossing
Skull	Normal head circumference; craniofacial disproportion due to small facies
Facies	Small
Nasal bridge	Hypoplastic
Orbits	Shallow
Dentition	Delayed eruption
Sclerae	Blue
Voice	High pitched
Musculoskeletal/Metabolic/Miscellaneous	
Blood glucose	Hypoglycemia in infants and children; fasting symptoms in some adults
Walking and motor milestones	Delayed
Hips	Dysplasia; avascular necrosis of femoral head
Elbow	Limited extensibility
Skin	Thin, prematurely aged
Bone mineral density	Osteopenia

apparently becomes detached from the cell receptor surface, GHBP levels are normal to elevated.

Mutations affecting the intracellular domain of the GHR also occur; these directly involve the intracellular domain leading to dominantly inherited GH insensitivity.[630–636] In the heterozygous mutations reported, truncation of the GHR results in absence of the intracellular domain. In vitro, this truncated GHR molecule behaves in a dominant negative manner, presumably by retaining an ability to dimerize with the normal GHR and thereby inhibiting GH-induced tyrosine phosphorylation of STAT5. Mutations resulting in C-terminal deletions of the intracellular domain of the GHR exhibit normal GH binding and JAK2 phosphorylation but impaired phosphorylation of STAT5B.[637,638]

That a dominant negative effect has been described for some mutations raises the question of whether heterozygosity for defects of the extracellular domain can also result in short stature. Heterozygosity for defects of *GHR* has been reported to cause some degree of relative GH insensitivity, with modest growth improvement occurring only with high doses of GH.[639–642] In addition, a truncated *GHR* splice variant has been described that functions as a dominant negative inhibitor of the full-length receptor and results in large amounts of GHBP, further downregulating GHR function.[643]

The most extensively studied polymorphism in the *GHR* gene is the deletion of exon 3 *(GHRd3)*, which is present in up to 50% of Caucasians. It has been proposed that GHRs without exon 3 bind GH with comparable affinity[644,645] but may transduce the signal with a different intensity in vitro.[644,645] Upon GH treatment, children with GHD, TS, or SGA with one or two copies of the *GHRd3* variant grow faster than those without the variant after correction for GH dosing. However, different studies reported different findings on whether GH-deficient children with the *GHRd3* variant experience faster growth.[646–648] Different GH dosing among studies and differences among the populations studied could account for the varying results.

Some patients with the phenotype of GH insensitivity but without mutations of the *GHR* gene may have mutations in downstream GHR signaling molecules. Homozygous mutations in the *Stat5B* gene cause GH insensitivity either by decreasing the phosphorylation of tyrosine[649] with resultant inability to dock with phosphotyrosines on GH-activated receptors, or to stably bind DNA,[650] or by causing an insertion in exon 10 leading to early protein termination.[651] A mutation in the SH2 domain of *Stat5b* has also been described, leading to an inability to induce gene transcription.[652] Patients with mutations in *Stat5b* have immune dysfunction and recurrent pulmonary infections, as Stat5B is involved in downstream signaling for multiple cytokines. Heterozygous STAT5b variants in the DNA-binding domain or the coiled-coil domain can lead to disruption of GHR signaling.[653] The mutated protein acts in a dominant negative manner because it can dimerize with wild-type STAT5b but cannot bind DNA to induce transcription. Affected patients had short stature, but immune dysfunction is not as pronounced as in persons with homozygous mutations in STAT5b.

Markedly reduced serum concentrations of IGF1 and IGFBP3 are present in persons with mutation in the ALS gene.[654] IGF1 and IGFBP3 are low as in patients with classic GH insensitivity, but the height deficit is less severe, with reported patients with heights between –3.6 and –0.3 SDS.[655] Affected male patients have delayed puberty and varying degrees of bone mineral density. Insulin insensitivity is present. Found mutations in the IGFALS gene are homozygous or compound heterozygous, all in exon 2. Since ALS levels are undetectable in affected patients, it is believed that the mutation prevents the protein from being expressed, secreted, or stable in serum.[655] Whether the relatively normal growth of these patients reflects the greater importance of locally produced IGF1 or altered kinetics of serum IGF1 in the face of reduced concentrations of binding proteins remains uncertain. The mechanism underlying the observed insulin resistance is also unknown but is postulated to be due to GH excess.[655]

Abnormalities of IGF1 and IGF1 Receptor Signaling. Woods and colleagues[656] described a 15-year-old boy with deletion of exons 4 and 5 of the *IGF1* gene that resulted in a truncated IGF1 molecule. The boy exhibited severe prenatal and postnatal (approximately –7 SDS) growth retardation unresponsive to GH in addition to sensorineural deafness, mental retardation, and microcephaly. He had normal IGFBP3 and GHBP levels,

undetectable IGF1 levels, and hyperinsulinism. On treatment with IGF1, the child experienced growth and improved metabolic parameters.[657] In a kindred of members with height SDS –4.0 and low but detectable IGF1, heterozygous mutation in the *IGF1* gene caused splicing of exon 4 and resultant protein truncation.[658] A child with height of –2.7 SDS and an IGF1 level in the low normal range was found to have a 260-kb heterozygous deletion of chromosome 12, which includes the *IGF1* gene.[659]

Inactivating Mutation of the IGF1 Gene. An adult with the same phenotype as the boy with the *IGF1* deletion but with markedly elevated serum IGF1 levels[660] had a homozygous point mutation in the *IGF1* gene. This mutation resulted in an IGF1 molecule with markedly reduced affinity for the IGF1 receptor that poorly stimulated autophosphorylation of the IGF1 receptor and activation of AKT or ERK.[661] Family members heterozygous for this mutation have significantly lower birth weight, final height, and head circumference, suggesting an effect of heterozygosity for this mutation on IGF1 function.

Primary Defects of IGF Transport and Clearance. Siblings from two families with high IGF1 and IGF2 levels, normal to high IGFBP3 levels, normal ALS levels, and height SDS scores between –2.2 and 1.06 were found to have mutation in the pregnancy-associated plasma protein A2 *(PPAP-A2)* gene.[298] The described mutations generate truncated proteins that cannot cleave IGFBP3 or IGFBP5. Because PAPP-A2 cleavage of IGFBP3 releases IGF1 from the ternary complex, free IGF1 levels in the affected persons were low. Serum from affected patients could not stimulate IGF1 receptor in vitro, further providing evidence of the loss of function induced by the mutation. That the IGF1 levels were elevated suggests that GH signaling was amplified. This amplification of GH signaling may account for the mild degree of height deficit seen in these patients compared to those with GH receptor defects. Treatment of the children with this mutation with rIGF1 resulted in increased growth rate after 1 year.[662] rIGF1 treatment's effect on adult height is unknown.

Primary Defects of IGF1 Receptor Production or Responsiveness. Patients with IUGR and postnatal growth failure, microcephaly, and mental retardation with normal to elevated serum IGF1 levels have been reported to have mutations in the IGF1 receptor gene leading to reduced binding of IGF1 to its receptor.[663,664] Fang and associates published a report of a patient with a missense mutation in *IGF1R* and height –5.9 SDS, elevated IGF1 levels, and resultant decreased activation of downstream signaling pathways.[665]

In leprechaunism, a syndrome of growth failure and insulin receptor dysfunction, there is variable IGF1 insensitivity.[666,667] The profound abnormality of the insulin receptor suggests that heterodimeric insulin and IGF1 receptor combinations might lead to failed activation of the IGF1 signaling cascade. The IGF1 receptor gene resides on 15q, so persons with deletions of the distal long arm of chromosome 15 or ring chromosome 15 have heterozygosity of the IGF1 receptor.[667,668] These patients may have IUGR and postnatal growth failure, but lack of a biologic response to IGF1 has not been conclusively demonstrated.[668] Therefore whether the growth failure is caused by altered levels of IGF1 receptor or is a result of the loss of other genes located on 15q remains to be determined.

Disorders Outside the Growth Hormone–IGF Axis

Many systemic disorders, if severe enough, can cause growth failure in children. Those that primarily alter hormones that directly regulate growth (e.g., thyroid hormone, glucocorticoids) can be understood based on the known actions of those hormones. Even in those disorders in which the pathology is not primarily within the endocrine system, there is often an underlying hormonal abnormality contributing to the growth failure. In some cases, the underlying disorder produces a secondary hormone deficiency. Those disorders in which a hormone deficiency cannot be identified may be thought of as demonstrating hormone resistance, because these children have growth failure in the presence of normal GH production.

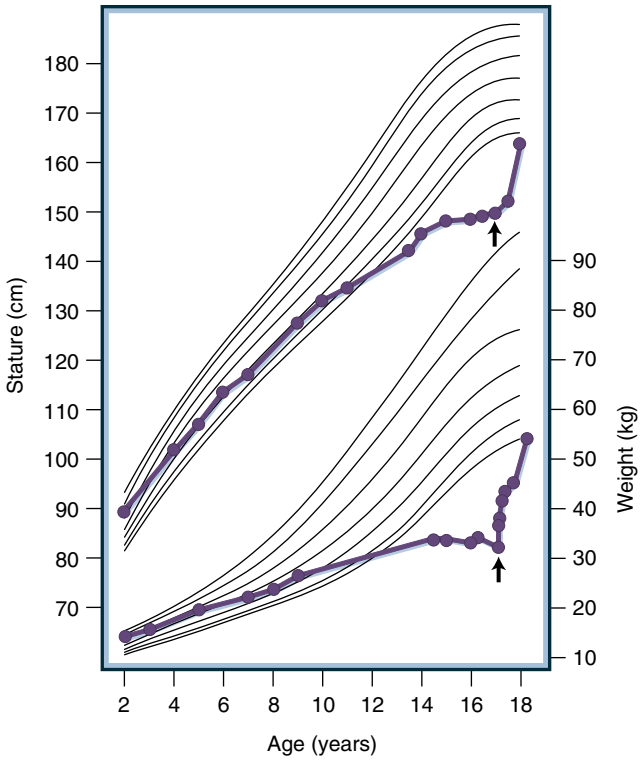
Malnutrition

Given the worldwide presence of undernutrition, inadequate intake of energy (calories), protein, or both is the most common cause of growth failure. *Marasmus* refers to an overall deficiency of calories, including protein malnutrition. Subcutaneous fat is minimal, and protein wasting is marked. *Kwashiorkor* refers specifically to inadequate protein intake, although it may also be characterized by some caloric undernutrition. Frequently, the two conditions overlap. Decreased weight growth usually precedes the failure of linear growth by a very short time in the neonatal period and by several years at older ages. Stunting of growth due to caloric or protein malnutrition in early life often has lifelong consequences, including diminished skeletal growth.[669]

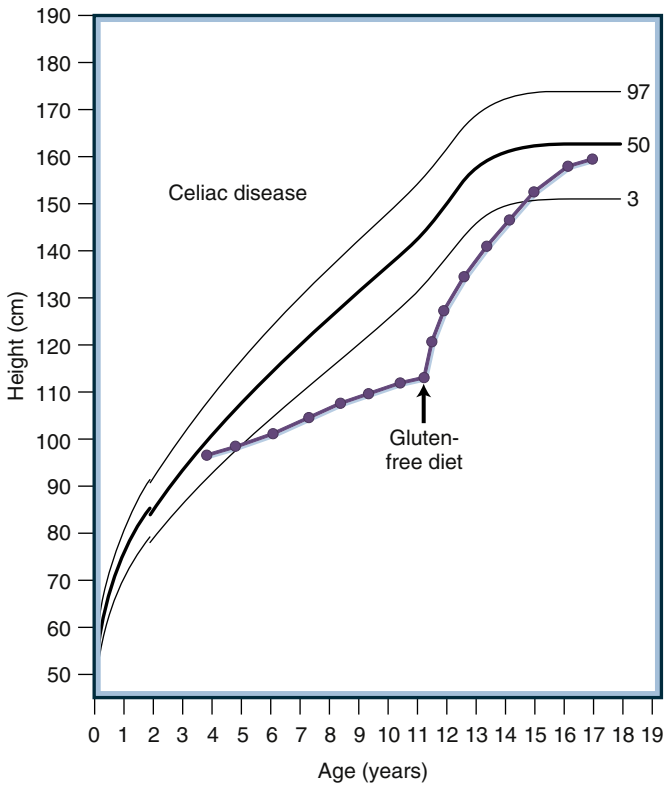
Both acute and chronic malnutrition affect the GH-IGF1 system. The impaired growth seen in malnutrition is usually associated with elevated basal or stimulated serum GH levels,[670,671] although in some cases of generalized malnutrition (marasmus), the GH levels are normal or low.[672] In both conditions, serum IGF1 levels are reduced.[671,673] The increase in GH levels is caused by a decrease in negative feedback by IGF1 and a decrease in somatostatin tone.[674] Malnutrition also results in increased ghrelin levels,[675,676] which could also contribute to an increase in GH secretion, although the role of ghrelin in regulating GH secretion remains unclear.[677] With serum IGF1 levels reduced despite normal or elevated GH levels, malnutrition is a form of GH insensitivity.[671] One cause of this insensitivity is a decrease in GHR expression, which is reflected in decreased serum GHBP levels.[670,678] In addition, fasting increases expression of FGF21, which may cause GH insensitivity by inhibiting STAT5.[679] This GH insensitivity may be an adaptive response, diverting scarce energy resources from growth toward use for acute metabolic needs. The low IGF1 minimizes stimulation of anabolism, whereas the direct actions of the elevated GH levels (e.g., lipolysis, insulin antagonism) may increase the availability of energy substrates.[671,680,681] These adaptive mechanisms are accompanied by changes in serum IGFBPs that further limit IGF action during periods of malnutrition.[670,682]

Inadequate calorie or protein intake complicates many chronic diseases that are characterized by growth failure. Anorexia is a common feature of renal failure and inflammatory bowel disease, and it also occurs with cyanotic heart disease, congestive heart failure, CNS disease, and other illnesses. Some of these conditions may be further characterized by deficiencies of specific dietary components, such as zinc, iron, and vitamins necessary for normal growth and development.

Undernutrition may also be voluntary, as occurs with dieting and food fads (Fig. 25.36).[682] Caloric restriction is especially common in girls during adolescence, in whom it may be associated with anxiety concerning obesity, and in gymnasts and ballet dancers. Anorexia nervosa and bulimia are extremes of "voluntary" caloric deprivation that are commonly associated with impaired growth before epiphyseal fusion, which may result in diminished



• **Fig. 25.36** Curves of weight and height for a child who had growth failure resulting from prolonged self-imposed caloric restriction due to a fear of becoming obese. Notice that crossing of percentiles on the weight curve preceded that on the height curve, and that, after caloric intake was normalized *(arrow)*, gain in weight occurred before improvement in linear growth. At the end of the prolonged period of caloric restriction, weight age (10.2 years) was less than height age (12 years). (From Pugliese MT, Lifshitz F, Grad G, et al. Fear of obesity: a cause of short stature and delayed puberty. *N Engl J Med.* 1983;309:513–518. Reprinted by permission of the *New England Journal of Medicine*.)



• **Fig. 25.37** Catch-up growth in a girl with gluten-induced enteropathy (celiac disease). After 8 years of growth impairment, the patient was placed on a gluten-free diet and demonstrated substantial catch-up growth, returning to the previous growth percentiles. (Courtesy J.M. Tanner.)

final adult height.[683,684] Adolescent bone mineral accretion is impaired, and significant osteopenia may persist into adulthood.[685] Later in adolescence, malnutrition may cause delayed puberty or menarche (or both), as well as a variety of metabolic alterations. In anorexia nervosa, hormonal profiles are similar to those in protein-energy malnutrition,[683,684,686] with high basal levels of GH. However, in contrast to chronic critical illness, in which there is an increase in nonpulsatile secretion but a decrease in pulsatile GH secretion, in anorexia both nonpulsatile and pulsatile secretion of GH are increased.[674] The GH secretion stimulated by insulin-induced hypoglycemia, dopaminergic agents, or acute administration of dexamethasone is impaired in patients with anorexia nervosa, whereas clonidine and arginine elicit normal GH responses. Finally, a paradoxical increase in GH release to intravenous glucose infusion has been described.[674] As in malnutrition in general, low levels of IGF1 and IGFBP3 are found in anorexia nervosa, indicating GH resistance. Similarly, GHBP levels are decreased,[674] indicating a decrease in GHR expression as a contributing factor to the GH resistance. The hormones of the GH-IGF1 axis return to normal levels with refeeding.[670,683,687]

A rare cause of failure to thrive in infants and young children is the diencephalic syndrome.[688] This syndrome is characterized by a marked impairment of weight gain, or even weight loss, but with normal linear growth (at least initially). It is caused by hypothalamic tumors. Similar to the findings in other causes of low weight for length or height, increased GH levels have been found in patients with diencephalic syndrome. As in patients with anorexia nervosa, GH levels paradoxically increase in response to a glucose load. However, in contrast to the increased GH secretion seen in malnutrition or anorexia nervosa, IGF1 levels are normal rather than decreased.[688] Therefore diencephalic syndrome does not demonstrate GH resistance in the same way as these other disorders.

Chronic or Systemic Diseases

Malabsorption and Gastrointestinal Diseases

Intestinal disorders that impair absorption of calories or protein cause growth failure, for many of the same reasons as malnutrition per se.[680,689,690] Growth retardation may predate other manifestations of malabsorption or chronic inflammatory bowel disease. Celiac disease (gluten-induced enteropathy) and regional enteritis (Crohn disease) should be considered in the differential diagnosis of unexplained growth failure. Serum levels of IGF1 may be reduced,[680,691] reflecting malnutrition, and it is critical to discriminate between these conditions and disorders related to GHD. Documentation of malabsorption requires demonstration of fecal wasting of calories, especially fecal fat, along with other measures of gut dysfunction such as the D-xylose or breath hydrogen studies.

In celiac disease, an immune-mediated disorder in which the intestinal mucosa is damaged by dietary gluten (Fig. 25.37), impaired linear growth may be the first manifestation of disease.[680] The degree of growth impairment may be similar in patients with or without gastrointestinal symptoms.[680] The incidence of childhood celiac disease in the United States is about 0.9%,[692] but its

prevalence is approximately 5% in children being evaluated for short stature.[693] Celiac disease also has an increased incidence in individuals with TS, insulin-dependent (type 1) diabetes mellitus (IDDM), Down syndrome, or Williams syndrome.[693] The onset and progression of puberty may be delayed, and menarche may be late.[694] Measurement of an immunoglobulin A (IgA) tissue transglutaminase antibody (tTG-IgA)[695] is the currently recommended screening test. This test has a sensitivity of up to 99%, with much higher specificity than IgA gliadin antibodies (AGA-IgA). However, tTG-IgA has significantly lower sensitivity in children younger than 18 months of age,[696] in whom it may be preferable to also measure AGA-IgA. When measuring IgA antibodies, it is necessary to measure total IgA levels to exclude IgA deficiency, particularly because IgA deficiency has an increased incidence in patients with celiac disease.[697] (In subjects with IgA deficiency, tTG-IgG may be used.[697]) Nonetheless, the diagnosis of celiac disease ultimately requires demonstration of the characteristic mucosal flattening in small bowel biopsy.[692] Gluten withdrawal is a highly effective treatment for celiac disease and results in rapid catch-up growth and decreased clinical symptoms during the first 6 to 12 months of treatment.[680,694] Low IGF1 and IGFBP3 levels return to normal during this period.[691,698] Most children who receive appropriate dietary management ultimately achieve a normal final height.[699,700]

Growth failure in Crohn disease, which correlates with disease severity,[701] is probably due to a combination of malnutrition from malabsorption, anorexia, nutrient loss, chronic inflammation,[690,701] inadequacy of trace minerals in the diet, and use of glucocorticoids. IGF1 levels are low, especially with impaired growth.[680,690] In an animal model, approximately one-half of the decrease in IGF1 levels was accounted for by undernutrition, with the other half attributable to the effects of inflammation.[702] One-third to two-thirds or more of children with Crohn disease have impaired growth at diagnosis. In some patients, the growth failure precedes clinical symptoms of bowel disease by a few years, with a significant number showing linear growth failure before any weight loss.[680,690,701,703,704] Adequate nutritional supplementation and surgical resection of the diseased intestine can lead to improved growth, but surgery is not always an option.[705–707] Osteopenia is common.[708,709] An elevated erythrocyte sedimentation rate, anemia, and low serum albumin are useful clues, but diagnosis of Crohn disease ultimately requires colonoscopy and biopsy, along with gastrointestinal imaging studies. Permanent impairment of linear growth and deficits of final height may occur in 30% of patients.[710] Approximately 20% of patients at adult height are more than 8 cm below the midparental target height.[711] Small, mostly uncontrolled trials of GH treatment in children with Crohn disease have had conflicting results: Some have shown improved growth velocity,[712,713] but others have not.[714] Some have shown improved body composition and BMD.[713] None of the reports, however, has extended beyond 2 years of treatment to determine whether there is a long-term benefit.

Chronic Liver Disease

Chronic liver disease in childhood can cause impaired linear growth. Decreased food intake, fat and fat-soluble vitamin malabsorption, and trace element deficiencies contribute to growth failure.[715–718] In addition, these children show evidence of GH resistance, having decreased levels of IGF1 and IGFBP3 and increased GH secretion.[719–722] IGF2 levels are also decreased, and IGFBP1 levels are increased.[719–721] Although the low IGF1 levels might be due to the impaired synthetic capacity of the liver, there is decreased expression of the GHR in cirrhotic liver, just as in malnutrition.[723] However, despite provision of adequate calories, insensitivity to the action of GH persists,[718,720] suggesting that the GH resistance of liver failure is not due solely to malnutrition. Liver transplantation prolongs life expectancy, and linear growth is variably improved in the early post-transplantation years.[717,721,724,725] Exogenous glucocorticoid administration presumably plays a major role in the continued growth retardation[717,725]; GH and IGF1 production are normal, but the amount of "free IGF" may be decreased because IGFBP3 levels are relatively high.[721,724] Post-transplantation growth is inversely correlated with age and directly correlated with the degree of growth impairment at transplantation.[716,717,724] Exogenous GH treatment enhances growth rates and increases median height SDS by 0.3 to 0.6 unit after 1 year of treatment,[472,726,727] with continued increases in height SDS for up to 5 years during treatment.[472]

Cardiovascular Disease

Congenital heart disease with cyanosis or chronic congestive heart failure can cause growth failure.[728–730] Although some children with congenital heart disease will have growth failure related to an underlying genetic disorder, the disease itself can cause growth failure. The growth failure in children with CHD is often due to malnutrition, which can have a number of causes in children with CHD. Frequently this is due to inadequate calorie intake due to feeding difficulties associated with the heart disease.[728,729,731] In addition, chronic congestive heart failure is associated with malabsorption that includes protein-losing enteropathy, intestinal lymphangiectasia, and steatorrhea. Greater cardiac and respiratory work requirement and the relatively higher ratio of metabolically active, energy-utilizing brain and heart tissue to the growth-retarded body mass (cardiac cachexia) cause an increased basal metabolic rate in these children.[732,733] Thus food intake that appears adequate for the child's weight is often inadequate for normal growth. Finally, in children with cyanotic CHD, the hypoxemia impairs cellular metabolism and growth. Decreased levels of IGF1 and IGFBP3[733–735] and normal levels of GH and hepatic GHRs in chronically hypoxemic newborn sheep[735] suggest GH insensitivity distal to the GHR.

In the past, up to 30% of children with congenital heart disease had heights and weights that fell below the 3rd percentile for age.[730] In the developing world, up to 90% of children with CHD continue to suffer growth failure.[729] In contrast, in the developed world the impact of CHD on the growth of children has been nearly eliminated owing to the ability to make an early diagnosis, as well as on improvements in supportive care and the early surgical correction of these lesions. The nutritional management of these infants before surgical correction includes the use of calorie-dense feedings because of the need to restrict fluids, calcium supplementation because of the use of diuretics that can cause calcium loss in the urine, and iron to maintain an enhanced rate of erythropoiesis. Early surgical correction restores normal growth, frequently after a phase of catch-up growth with normalization of energy expenditure.[728,730,732,736]

Renal Disease

All conditions that impair renal function can impair growth.[737–740] Uremia and renal tubular acidosis can cause growth failure before other clinical manifestations become evident. The growth impairment results from multiple mechanisms, including inadequate formation of 1,25-dihydroxycholecalciferol (1,25-dihydroxyvitamin D_3, calcitriol) with resultant osteopenia, decreased caloric

intake, loss of electrolytes necessary for normal growth, metabolic acidosis, protein wasting, insulin resistance, chronic anemia, compromised cardiac function, and impairment of GH and IGF production and action. In nephropathic cystinosis, acquired hypothyroidism contributes to the inadequate growth.[741] Between 60% and 75% of patients with chronic renal failure treated before the GH therapeutic era had final adult heights more than 2 SD below the mean.[742]

The effects of renal failure on the GH-IGF1 axis are complex, and there is evidence for both GH and IGF1 resistance. Children and adolescents have normal or elevated circulating levels of GH, depending on the degree of renal failure.[737,738,743–745] The increased GH levels result from both an increase in GH secretory bursts and a decrease in renal GH clearance.[746] Serum IGF1 and IGF2 levels are usually normal in patients with renal failure.[737,745,747,748] Early reports of decreased serum IGF levels in uremia were an artifact caused by inadequate separation of IGF from IGFBPs before assay.[749] However, the normal IGF1 levels in the face of elevated GH levels denotes GH resistance, which is also indicated by the finding of decreased hepatic IGF1 production.[750] The mechanism for GH resistance includes decreased GHR gene expression in the liver and in the growth plate.[747,751] There is also evidence that the uremic state causes a postreceptor defect in GH signal transduction by diminishing phosphorylation and nuclear translocation of GH-activated STAT proteins.[746,752] Although a defect in IGF1 receptor signaling has been demonstrated in renal failure,[751] the more important mechanism for decreased IGF1 action in renal failure is alterations in the serum level of IGFBPs that decrease the bioavailability of IGF1. IGFBPs 1, 2, 4, and 6 are increased.[737,738,751,753–756] In addition, low-molecular-weight IGFBP3 fragments, which have decreased affinity for IGF1, accumulate as a result of reduced renal clearance.[751] In nephrotic syndrome, an additional contribution to growth failure may come from reduced serum levels of IGF1 and IGFBP3 resulting from urinary loss of IGF-IGFBP complexes.[739] Finally, glucocorticoid therapy that may be used for treatment of the renal disease can exacerbate growth retardation by diminishing GH release and blunting IGF1 action at growth plates.[755,757,758]

After successful renal transplantation, growth may completely return to normal.[759,760] Based on data from the large cohort of patients in the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS), children who underwent transplantation before 6 years of age showed catch-up growth for the subsequent 1 to 2 years, followed by a plateauing of their growth rate.[759] In contrast, children older than 6 at the time of transplant have not shown catch-up growth; their final height is determined by their height at the time of transplantation.[759] In spite of this, final height in children requiring renal transplant has improved dramatically in the past 25 years so that the final heights of children in the NAPRTCS registry that were transplanted in the years 2002 to 2010 had a final height of –0.94 SDS, compared with final heights averaging –1.93 SDS in those transplanted between 1987 and 1991. This improvement appears to be driven almost completely by the improved height at the time of transplantation: –1.5 SDS in 2009 versus –2.5 SDS in 1987.[759] The importance of height at the time of transplantation in determining final adult height, despite the complex post-transplantation health issues, confirms the value of improving growth velocity and absolute height before transplantation. Additional factors that impact growth after transplantation include the function of the allograft and the use of glucocorticoids in the immunosuppressive regimen. Immunosuppressive regimens with alternate-day glucocorticoid treatment, rather than daily treatment,[760,759] utilizing a glucocorticoid withdrawal approach[759,761] and regimens that avoid glucocorticoids have all been associated with improved post-transplant growth rates.[759] There are conflicting data on whether sirolimus impairs growth in comparison to tacrolimus.[759] Growth-retarded post-transplantation children receiving daily or alternate-day glucocorticoid treatment have decreased GH secretion, normal levels of IGF1 and IGFBP1, and elevated levels of IGFBP3. They differ from patients with end-stage renal disease in that IGFBP1 levels are not strikingly elevated, perhaps because of altered glucose tolerance and hyperinsulinism due to chronic glucocorticoid therapy.[755]

Hematologic Disorders

Chronic anemias, such as sickle cell disease, are characterized by growth failure.[762] In general, the decrease in height and weight is greater in adolescent years than earlier because the onset of the adolescent growth spurt is delayed and menarche is late.[762–764] However, the adolescent growth and final adult height in patients with sickle cell disease may be normal.[764] The causes of growth retardation probably include suboptimal nutrition and hypogonadism.[765] Impaired oxygen delivery to tissues, increased work of the cardiovascular system, and energy demands of increased hematopoiesis are likely contributors to impaired nutrition. Long-term chronic transfusion therapy as part of stroke prevention treatment is associated with enhanced growth.[766] The GH-IGF1 system probably does not have a primary role in the growth impairment of sickle cell anemia, although abnormalities in the GH-IGF1 system have been described.[763]

In thalassemia, in addition to the consequences of chronic anemia, endocrine deficiencies can result from chronic transfusions and accompanying hemosiderosis.[767] Despite vigorous efforts to maintain hemoglobin levels near normal and to avoid iron overload, growth failure is still a common feature of thalassemia, especially in male adolescents.[768] The patients tend to show body disproportion, with truncal shortening but normal leg length. It is likely that anemia, impaired IGF1 synthesis, hypothyroidism, gonadal failure, and hypogonadotropic hypogonadism all contribute to growth failure in this disorder. GH insensitivity in some cases is suggested by generally adequate GH production with low IGF1 levels.[769,770] Several groups have reported data on treatment of thalassemia patients in whom GH production seemed diminished. In most patients, GH treatment increased growth, at least initially.[769,771] In a longer term study (average duration, 59 months) starting with young patients (7.2 years of age), an increased growth velocity was maintained throughout the treatment period[772]; when treatment was initiated at an older age (13.6 years), final height was not improved.[773] A small number of adults with thalassemia are found to have continued GHD, so GH treatment for cardiac and bone health reasons may be important in this disease.[774]

About half of the patients in the International Fanconi Anemia Registry have short stature. GHD was demonstrated by provocative testing (22 of 48 patients) or with assessment of endogenous secretion (13 of 13) in a group with a mean height of –2.23 SDS.[775] In pure red blood cell aplasia,[776] approximately 30% of patients demonstrated growth retardation. The frequency increased with age (42% in individuals >16 years) and with treatment programs such as chronic transfusions or glucocorticoids.

Inborn Errors of Metabolism

Inborn errors of metabolism are often accompanied by growth failure that may be pronounced. Glycogen storage disease, the mucopolysaccharidoses, glycoproteinoses, and mucolipidoses

are characterized by poor growth. Many inborn metabolic disorders are also associated with significant skeletal dysplasia. In a small number of patients with organic acidoses, such as methylmalonic and propionic acidurias, IGF1 levels are low and GH levels are normal, suggesting a possible state of GH insensitivity related to nutritional status.[777] Preliminary data suggest that exogenous GH treatment may improve the metabolic status of such children.[777,778]

Pulmonary Disease

Growth can be retarded in children with asthma, including those who have not received glucocorticoid therapy. The lowered growth in asthmatic children does not appear to be associated with abnormalities of the GH-IGF1 axis.[779] Impaired nutrition and increased energy requirements, along with chronic stress, especially with nocturnal asthma and enhanced endogenous glucocorticoid production, cause poor linear growth. However, despite this growth impairment, there is at most only a small difference in final height achieved by those with asthma, with the majority of the growth failure due to a delay in puberty.[780] Systemic glucocorticoid therapy, which is rarely required, can be expected to impair growth. Intermittent glucocorticoid therapy is usually not associated with impaired final height. Alternate-day or aerosolized glucocorticoid therapy often ameliorates growth retardation and can be associated with an accelerated catch-up phase.[781,782] The large Childhood Asthma Management Program investigated the effect of inhaled corticosteroids (ICS) on the growth of children with asthma. Although growth rate declined with the initiation of ICS, it subsequently normalized, and the final height in children treated with ICS for 4 to 6 years was only 1.2 cm below that in control subjects.[783] ICS can, however, suppress the hypothalamic-pituitary axis, and investigation for this is appropriate in children who have a more marked suppression of growth with ICS treatment.[780]

Bronchopulmonary dysplasia (BPD), a sequela of neonatal respiratory distress syndrome and prematurity, has an incidence as high as 35% in infants with very low birth weight (<1500 g).[784] The use of dexamethasone in the treatment of BPD in neonates causes a transient cessation of growth[785] and has engendered long-term concern for neurodevelopment and somatic growth.[786] Growth in surviving infants is poor through early childhood,[787–789] but the defect usually disappears by 8 years of age.[790–792] Long-term hypoxemia, poor nutrition, chronic pulmonary infections, and reactive airway disease are responsible for the poor early growth.

In patients with cystic fibrosis (CF), chronic pulmonary infection with bronchiectasis, pancreatic insufficiency with exocrine and endocrine inadequacy, malabsorption, and malnutrition all contribute to decreased growth and late sexual maturation. Over 20% of individuals with CF under 25 years of age will have heights or weights below the 10th percentile.[793] With newborn screening for CF, the failure to thrive that was a common presenting feature is now less likely to be seen, although these infants still demonstrate some growth failure.[794] Infants who are not identified by newborn screening often show marked growth failure before diagnosis followed by catch-up growth after diagnosis. The linear growth rate during childhood is generally normal before puberty,[795] followed by delayed puberty, including a delayed and attenuated pubertal growth spurt. Adult height in individuals with CF is slightly decreased compared with population norms by approximately –0.2 to –0.7 SD.[795–797] The GH-IGF1 axis in CF patients shows evidence for some degree of acquired GH insensitivity with lowered mean IGF1 and elevated GH levels.[798] The degree of growth retardation is related most closely to the severity and variability of the pulmonary disease rather than to pancreatic dysfunction.[799,800] While the degree of steatorrhea does not correlate well with growth impairment, improved nutrition programs do enhance the overall clinical picture.[801,802] Endocrine abnormalities, such as failure of both alpha and beta islet cells with decreased glucagon and insulin production, do not seem to influence prepubertal growth patterns in children with CF. Alterations of vitamin D metabolism, while potentially affecting skeletal mineralization, do not diminish growth.[803]

There is the potential for the anabolic effects of GH treatment to improve the health in patients with CF. A number of short-term trials of up to 12 months with GH treatment have been reported.[793,804,805] These studies have demonstrated that GH treatment of CF patients increases height, weight, and lean body mass. In addition, some measures of lung function are slightly improved, notably forced vital capacity, although this improvement did not always exceed that expected based on the improved growth.[805] Although some studies have demonstrated a decreased rate of hospitalization in GH-treated patients, there has not been clear evidence of a decrease in pulmonary exacerbations.[805,804] These studies, as well as reports of uncontrolled treatments of up to 4 years, have not raised serious safety concerns of GH treatment in CF patients. Specifically, there has not been an increase in glucose intolerance or diabetes mellitus, although in some studies fasting glucose levels were increased.[804–806] Although the current data demonstrate the possibility that GH treatment will benefit patients with CF, there are not yet sufficient data to indicate that GH treatment improves long-term health outcomes in these patients.

Chronic Inflammation and Infection

Poor growth is a characteristic feature of chronic inflammatory disease and recurrent serious infection. The impaired growth associated with such disorders as Crohn disease, CF, and asthma, in which inflammatory processes may be significant, has already been discussed. Inflammatory states are associated with increased levels of numerous cytokines. Interleukin 6 (IL6), specifically, has been implicated in this growth impairment. De Benedetti and colleagues,[807] studying juvenile rheumatoid arthritis in humans and in a transgenic murine model expressing excessive IL6, demonstrated an IL6-mediated decrease in IGF1 production. IL6 has been demonstrated to activate SOCS3; this provides a pathway for inflammation to inhibit IGF1 production because SOCS3 is a negative regulator of the JAK2/STAT5 GH signal transduction pathway.[702,808,809] IL6 may also cause a decrease in serum IGF1 levels by increasing its clearance through a decrease in IGFBP3 levels.[702] In addition, cytokines can affect the endocrine system at many other levels,[810,811] impairing mineral and nutrient metabolism and the growth and remodeling of bone.[812]

Exposure to human immunodeficiency virus (HIV) in children and adolescents occurs through perinatal transmission, blood transfusions, drug usage, and sexual contact. Growth failure is a cardinal feature of childhood acquired immunodeficiency syndrome (AIDS).[813–817] However, HIV-infected infants and children show growth failure even before demonstrating severe immune dysfunction.[818,819] Weight, length, and head circumference are all affected, although weight-for-height may be normal.[815,817,820] Before the era of highly active antiretroviral therapy (HAART), height growth velocity was associated with survival, independent of either $CD4^+$ T-cell lymphocyte count or viral load, with HAART therapy normalizing growth in most studies.[820] Studies of

the GH-IGF1 axis in HIV-infected children have shown evidence of decreased GH secretion, GH resistance, and IGF1 resistance; both normal and low levels of GH and IGF1 are seen.[818] Lipodystrophy associated with HAART therapy occurs in children, although less commonly than in adults, and decreased GH secretion has been demonstrated in HIV-associated lipodystrophy,[818] potentially contributing to impaired growth. HIV-infected children frequently have delayed puberty, which could contribute to their linear growth failure. In a short-term treatment trial with standard doses of GH, height and weight growth increased and protein catabolism diminished, without any adverse effect on viral burden.[821]

Endocrine Disorders

Hypothyroidism

Untreated severe congenital hypothyroidism results in profound growth failure. With proper treatment, however, children with congenital hypothyroidism reach a height appropriate for their genetic potential.[822]

Acquired hypothyroidism during childhood may also result in growth failure that can range from subtle to profound, depending on the severity and duration of the hypothyroidism. Growth failure may be the most prominent manifestation of hypothyroidism in children.[823] The poor growth is more apparent in height than in weight gain, so these children tend to be overweight for height. Rivkees and coworkers[823] reported a mean 4.2-year delay between the slowing of growth and the diagnosis of hypothyroidism; at diagnosis, girls were 4.04 SD below and boys 3.15 SD below the mean height for their age. Skeletal maturation is delayed in those children in whom the hypothyroidism was sufficient to retard growth, with the bone age at diagnosis corresponding to the age at onset of the hypothyroidism.[823] Body proportion is immature, with an increased upper-body to lower-body segment ratio. Although chronic hypothyroidism is usually associated with delayed puberty, precocious puberty and premature menarche can occur in hypothyroid children (see Chapter 26).

In those children with severe growth failure, treatment with thyroid hormone results in rapid catch-up growth. This growth is typically accompanied by marked skeletal maturation. In cases of prolonged severe hypothyroidism, the advancement of skeletal maturation with treatment can exceed the growth acceleration, resulting in a compromised adult height.[823] The deficit in adult stature correlates with the duration of hypothyroidism before initiation of treatment. Catch-up growth may be particularly compromised if therapy is initiated near puberty.[824]

As expected, hyperthyroidism has effects on growth opposite to those of hypothyroidism: It results in accelerated growth and epiphyseal maturation. Children with hyperthyroidism present with an increased height and advanced bone age. In neonates, hyperthyroidism can result in craniosynostosis. However, despite the advanced bone age at diagnosis, the final height of children treated for hyperthyroidism remains normal in relation to genetic potential.[825]

Diabetes Mellitus

Although weight loss may occur immediately before the onset of clinically apparent IDDM, children with new-onset diabetes are frequently taller than their peer group, possibly because GH and insulin levels are increased during the preclinical evolution of the disease.[826–828] Most children with IDDM, even those with marginal control,[829] grow quite normally, especially in prepubertal years, although growth velocity may decrease during puberty.[830] However, growth failure can occur in diabetic children with long-standing poor glycemic control.[831,832] The Mauriac syndrome[833] describes children with poorly regulated IDDM, severe growth failure, and hepatosplenomegaly due to excess hepatic glycogen deposition. This type of growth retardation has become increasingly rare with modern diabetes care.

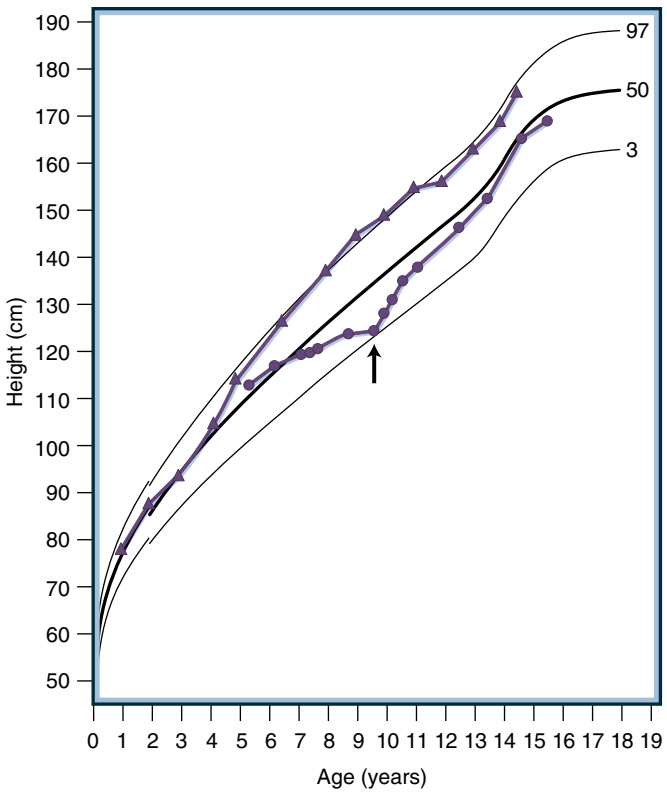
Many pathophysiologic processes, including malnutrition, chronic intermittent acidosis, increased glucocorticoid production, hypothyroidism, impaired calcium balance, and end-organ unresponsiveness to either GH or IGF, may contribute to growth failure in IDDM.[826,834–836] IGF1 and IGFBP3 levels are diminished in the face of enhanced GH production,[837–841] reflecting acquired GH insensitivity. GHBP levels are decreased,[834,840] supporting the concept of impaired GHR number or function. Furthermore, IGFBP1 is normally suppressed by insulin, and hypoinsulinemia results in elevated serum IGFBP1 levels that may inhibit IGF action.[612,840,842–844] In contrast to the situation in adolescents and adults, IGFBP1 levels are not elevated in well-growing prepubertal children.[844] On the contrary, increased IGFBP3 proteolysis may enhance the bioactivity of the available IGF1.[613,841] Most children with IDDM attain normal cellular nutrition and growth factor action despite intermittent hypoinsulinemia and derangements of peripheral indices of the GH-IGF1 system.

Even though glycemic control is inversely correlated with IGF1 level,[835,837,840,845] the correlation between glycemic control and growth is weak. After conflicting reports as to the influence of glycemic regulation on growth,[826,827,846,847] a longitudinal study[847] of 46 children whose diabetes began before age 10 indicated that initial heights at diagnosis were normal and that the final height SDS was minimally reduced from that at onset. In boys, despite a delay of about 2.5 years in onset of puberty, total pubertal height gain was normal. In girls with diabetes, however, total pubertal height gain was diminished and the age at menarche was delayed; the effects of altered insulin and IGF1 levels on ovarian function have not been assessed in such patients. Chronic metabolic control did not correlate with the pubertal height gain or with the normal final height. Nevertheless, good glycemic control may improve growth at certain maturational periods such as puberty.[826,839,848]

Cushing Syndrome: Glucocorticoid Excess

Glucocorticoid excess impairs skeletal growth, interferes with normal bone metabolism by inhibiting osteoblastic activity, and enhances bone resorption.[849–851] These effects are related to the duration of steroid excess,[852] regardless of whether the Cushing syndrome is due to ACTH hypersecretion, adrenal tumor, or glucocorticoid administration.

Even modest doses of oral glucocorticoids can inhibit growth; these doses may be as low as 3 to 5 mg/m^2 per day of prednisone or 12 to 15 mg/m^2 per day of hydrocortisone (i.e., only slightly above what is considered physiologic replacement).[853] The "toxic" effects of glucocorticoids on the epiphysis may persist to some degree after correction of chronic glucocorticoid excess, and patients frequently do not attain their target height.[854,855] The longer the duration and the greater the intensity of glucocorticoid excess, the less likely it is that catch up growth will be completed. Alternate-day glucocorticoid treatment decreases but does not eliminate the risk of growth suppression.[782,856,857] Inhaled glucocorticoids given for the treatment of asthma have an even lower risk of growth suppression, but even at modest doses (e.g., 400 μg/day of beclomethasone,[858] 200 μg/day of fluticasone,[858] or 400



• **Fig. 25.38** Growth curves of two boys with obesity. The boy depicted by the *circles* had cortisol excess related to Cushing disease. He had onset of rapid weight gain associated with a decrease in linear growth velocity at age 7. The diagnosis was made, and an adrenalectomy *(arrow)* was performed at age 9.5 years, with an almost immediate increase in growth rate and striking catch-up growth. The boy whose growth is depicted by *triangles* had exogenous obesity. At age 9.5 years, his weight was approximately the same as that of the patient with Cushing disease, but his height was at the 97th percentile, reflecting the enhancement of linear growth in individuals with exogenous obesity.

μg/day of budesonide[783]), they can cause at least temporary slowing of growth. However, inhaled corticosteroids do not appear to significantly impair final height.[783]

GH therapy can overcome some of the growth-inhibiting effects of excess glucocorticoids. The GH-induced increase in growth rate is inversely related to the glucocorticoid dose,[859] with one small study finding no benefit of GH treatment for prednisone doses greater than 0.35 mg/kg per day.[860] GH or IGF1 administration can diminish many of the catabolic effects of excess glucocorticoids.[850,861,862]

Adrenal tumors secreting large amounts of glucocorticoids can produce excess androgens, which may mask the growth-inhibitory effects of glucocorticoids. In addition, Cushing syndrome in children may not cause all the clinical signs and symptoms associated with the disorder in adults and may manifest with growth arrest. However, Cushing syndrome is an unlikely diagnosis in children with obesity because exogenous obesity is associated with normal or even accelerated skeletal growth, and growth deceleration is usually evident by the time other signs of Cushing syndrome appear (Fig. 25.38). In a series of 10 children and adolescents treated for Cushing disease with surgery and cranial irradiation, post-therapy GHD was common and mean final height was –1.36 SDS.[863]

Pseudohypoparathyroidism: Albright Hereditary Osteodystrophy

Albright hereditary osteodystrophy (AHO) is caused by mutations in the stimulatory GTP-binding protein, $G\alpha_s$. It exists both with and without multihormone resistance—termed *pseudohypoparathyroidism type 1A* (PHP1A) and *pseudo-pseudohypoparathyroidism* (pseudo-PHP). This condition is discussed in detail in Chapter 29 but is included here because short adult height is a common feature.[864] AHO is characterized by obesity (more marked in PHP1A than in pseudo-PHP[865]), short metacarpals, subcutaneous ossifications, round facies, and cognitive impairments. Patients with AHO, whether they have PHP1A or pseudo-PHP, typically have short adult stature. Many patients with PHP1A demonstrate GHD due to resistance to GHRH.[866,867] Interestingly, patients with PHP1B, which is caused by a methylation defect in the *GNAS* gene that encodes $G\alpha_s$, have PTH and TSH resistance like PHP1A patients, but have neither the AHO phenotype nor GHRH resistance.[868] Although AHO patients have evidence of GHD, their growth pattern suggests that there is another contributor to growth failure as they have modest growth failure in early and middle childhood, with early epiphyseal fusion contributing to their short final height.[866,869] Consistent with this likely multifactorial cause for short stature, preliminary data in patients with PHP1A found an increase in growth rate with GH treatment, but without an increase in final height, as the few children reaching final height appeared to have an absence of a pubertal growth spurt.[870]

Rickets

In the past, hypovitaminosis D was a major cause of short stature and was often associated with other causes of growth failure, such as malnutrition, prematurity, malabsorption, hepatic disease, or chronic renal failure (see Chapter 29). In isolated nutritional vitamin D deficiency, breastfed infants typically have poor exposure to sunlight and are not nutritionally supplemented with vitamin D. Characteristic skeletal manifestations of rickets include frontal bossing, craniotabes, rachitic rosary, and bowing of the legs. With vitamin D supplementation, whether due to prescribed supplementation or a broadened diet, amelioration of the early decrease of linear growth velocity typically occurs. The association of vitamin D receptor gene polymorphism with birth length, growth rate, adult stature, and BMD[871–874] emphasizes the importance of vitamin D in normal growth.

Hypophosphatemic Rickets

Hypophosphatemic rickets is typically an X-linked dominant disorder caused by decreased renal tubular reabsorption of phosphate related to mutations in the phosphate-regulating endopeptidase gene, *PHEX*, located on chromosome Xp22.1. Other hypophosphatemic syndromes include autosomal-dominant hypophosphatemic rickets, hereditary hypophosphatemic rickets with hypercalciuria, and tumor-induced osteomalacia (see Chapter 29).[875] In all of these conditions, there are increased levels of FGF23, a major phosphaturic agent.[876] The features are usually more severe in boys and include short stature,[776,877] prominent bowing of the legs, and sometimes rachitic signs.[878]

The metabolic and skeletal abnormalities cannot be overcome by vitamin D therapy alone. Treatment requires oral phosphate replacement, but such therapy may result in poor calcium absorption from the intestine. The addition of calcitriol to oral phosphate increases intestinal phosphate absorption and prevents hypocalcemia and secondary hyperparathyroidism. Such combined therapy

TABLE 25.5 Classification of Osteochondrodysplasias

I. Defects of the tubular (and flat) bones and axial skeleton
  A. Achondroplasia group
  B. Achondrogenesis
  C. Spondylodysplastic group (perinatally lethal)
  D. Metatropic dysplasia group
  E. Short rib dysplasia group (with or without polydactyly)
  F. Atelosteogenesis/diastrophic dysplasia group
  G. Kniest-Stickler dysplasia group
  H. Spondyloepiphyseal dysplasia congenita group
  I. Other spondyloepiphyseal/metaphyseal dysplasias
  J. Dysostosis multiplex group
  K. Spondylometaphyseal dysplasias
  L. Epiphyseal dysplasias
  M. Chondrodysplasia punctata (stippled epiphyses) group
  N. Metaphyseal dysplasias
  O. Brachyrachia (short spine dysplasia)
  P. Mesomelic dysplasias
  Q. Acromesomelic dysplasias
  R. Dysplasias with significant (but not exclusive) membranous bone involvement
  S. Bent bone dysplasia group
  T. Multiple dislocations with dysplasias
  U. Osteodysplastic primordial dwarfism group
  V. Dysplasias with increased bone density
  W. Dysplasias with defective mineralization
  X. Dysplasias with increased bone density
II. Disorganized development of cartilaginous and fibrous components of the skeleton
III. Idiopathic osteolysis

improves the rickets but may not correct growth.[875,879,880] Early initiation of this therapy in early infancy may lead to achievement of greater childhood and adult height.[877] There is no clear association between endogenous GH secretion, IGF1, or phosphate levels and height in this disorder.[881–883] Nevertheless, GH therapy in eight trials, including 83 patients, resulted in an enhancement of skeletal growth and improvement in BMD.[884–886] In 14 of those patients, treatment for 4 to 5 years resulted in a height gain of up to 1.2 SDS. However, a Cochrane review did not find conclusive evidence that GH treatment improved growth in patients with hypophosphatemic rickets.[887] One report cautioned that GH treatment appeared to exaggerate disproportionate truncal growth.[888]

Treatment with the FGF23 monoclonal antibody burosumab increased serum phosphate and improved linear growth rates after 64 weeks of treatment.[889] This therapy may supplant current therapies with phosphate supplementation and calcitriol.

Osteochondrodysplasias

The osteochondrodysplasias encompass a heterogeneous group of disorders characterized by intrinsic abnormalities of cartilage and bone.[890] These disorders include abnormalities in the size or shape of bones in the limbs, spine, or skull, often with abnormalities seen on radiographic evaluation. More than 100 osteochondrodysplasias have been identified based on physical characteristics and radiographic characteristics (Table 25.5).

Diagnosis of osteochondrodysplasias can be difficult, with clinical and radiologic evaluation central to the diagnosis. The family history is critical, although many cases are caused by de novo mutations, and this is generally the case in autosomal-dominant achondrodysplasia and hypochondrodysplasia. Measurement of body proportions should include arm span, sitting height, upper and lower body segments, and head circumference. Radiologic evaluation should be used to determine whether involvement is of the long bones, skull, or vertebrae and whether abnormalities are primarily at the epiphyses, metaphyses, or diaphyses. The osteochondrodysplasias most commonly encountered in endocrine practice are discussed in the following paragraphs.

Achondrodysplasia is the most common of the osteochondrodysplasias, with a frequency of 1 in 26,000 individuals. Characteristic abnormalities of the skeleton include megalocephaly, low nasal bridge, lumbar lordosis, short trident hand, and rhizomelia (shortness of the proximal legs and arms) with skin redundancy. Radiologic findings include small, cuboid-shaped vertebral bodies with short pedicles and progressive narrowing of the lumbar interpedicular distance. The small foramen magnum may lead to hydrocephalus, and spinal cord and root compression may result from kyphosis, stenosis of the spinal canal, or disk lesions.[891] Diminished growth velocity is present from infancy, although short stature may not be evident until after 2 years of age. Mean adult heights in males and females are 130 and 120 cm, respectively. GH secretion is comparable to that in normal subjects.

Achondrodysplasia is caused by mutations in the transmembrane domain of the FGF receptor 3 gene *(FGFR3)*.[892] It is transmitted in an autosomal dominant manner, but 80% to 90% of cases are caused by de novo mutations. Most of the cases are the result of activating mutations at nucleotide 1138 of the *FGFR3* gene, which creates new recognition sites for restriction enzymes, thus easing the molecular diagnosis. The mutation rate reported at this site is very high, and *FGFR3* has been labeled the most mutable gene in the genome. As a result of the upregulation of receptor activity, there is abnormal chondrogenesis and osteogenesis during endochondral ossification, leading to the typical phenotypic findings. The homogeneity of the mutation in achondroplasia probably explains the minimal heterogeneity in the phenotype. Infants homozygous for the mutation have severe disease, typically dying in infancy from respiratory insufficiency due to a small thorax.

Hypochondroplasia is also autosomal dominant, and 70% of affected individuals are heterozygous for mutations in the *FGFR3* gene, frequently at amino acid position 1620.[893] The facial features of achondroplasia are absent, and both short stature and rhizomelia are less pronounced. Adult heights are typically in the range of 120 to 150 cm. Poor growth may not be evident until after 2 years of age, but stature then deviates progressively from normal. Occasionally, the disproportionate short stature is not apparent until adulthood. Outward bowing of the legs may be accompanied by genu varum. On radiologic evaluation, lumbar interpedicular distances are diminished between L1 and L5, and there may be flaring of the pelvis and narrow sciatic notches. The diagnosis can be difficult to make, with mild variants of the syndrome difficult to distinguish from normal.

The short stature homeobox-containing (SHOX) gene is located in the pseudoautosomal region of distal Xp and Yp. Mutations or deletion of *SHOX* is associated with syndromes of poor growth and skeletal dysplasia, including Léri-Weill dyschondrosteosis (LWD), TS, and Langer mesomelic dysplasia (LMD).[894] Findings include short stature, Madelung deformity, increased carrying angle, tibial bowing, scoliosis, and high arched palate. The auxologic finding of relatively short limbs suggests a defect in *SHOX*, because the SHOX protein may affect cellular proliferation and apoptosis of chondrocytes at the growth plate.[895] Indeed, the skeletal manifestations have been associated with areas in which there is intrauterine expression of *SHOX*.[895] LWD is caused by homozygous gene

defects, whereas TS and LMD are caused by haploinsufficiency. The profound findings in LWD, compared with TS, may reflect the impact of pubertal estrogen exposure in LWD.[894]

Endochondral growth is regulated by multiple endocrine, paracrine, and autocrine factors, and many inborn errors have been identified. In the syndrome of acromesomelic dysplasia, growth is remarkably impaired, leading to adult heights that may be more than 5 SD below the mean. A homozygous mutation in the homodimeric transmembrane natriuretic peptide receptor B (NPR-B) that impairs binding of the ligand C-type natriuretic peptide has been found in some affected individuals.[896] Obligatory heterozygotes are significantly shorter than normal but have normal skeletal anatomy.[897] Therefore heterozygous mutations of NPR-B demonstrate that a subset of children with unexplained short stature may in fact have mild forms of osteochondrodysplasias.

Chromosomal Abnormalities

Abnormalities of autosomes or sex chromosomes can cause growth retardation without evidence of skeletal dysplasia, frequently with somatic abnormalities and developmental delay. In many cases, the precise cause of growth failure is not clear because the genetic defects do not affect known components of the GH-IGF1 system. Chromosomal lesions may directly influence normal tissue growth and development or indirectly modulate local responsiveness to IGF or other growth factors at the growth plate.

Down Syndrome

Trisomy 21, or Down syndrome, is probably the most common chromosomal disorder associated with growth retardation, affecting approximately 1 in 600 neonates. On average, newborns with Down syndrome have birth weights 500 g below normal and are 2 to 3 cm shorter than normal. Growth failure continues postnatally and is typically associated with delayed skeletal maturation and a delayed and incomplete pubertal growth spurt. Adult heights range from 135 to 170 cm in men and 127 to 158 cm in women.[14] The cause of growth failure in Down syndrome is unknown, and attempts to find underlying hormonal explanations for growth retardation have been unsuccessful. Growth retardation may be due to GH deficiency associated with hypothalamic dysfunction. A morphometric study of patients with Down syndrome revealed fewer neurons in the arcuate and ventromedial nuclei, which are areas responsible for GHRH neurosecretory function.[898] Fourteen children with Down syndrome (aged 10 months to 5 years, height SD scores [SDS] varying between −1.3 and −4.9) were studied and found to have a lack of response to levodopa and clonidine provocative testing, while responding to GH-releasing hormone (GHRH), indicating a likely hypothalamic deficiency.[899] Marginal levels of GH secretion and low-normal serum levels of IGF1 have been reported in patients with Down syndrome. Exogenous GH has been tried in some patients with Down syndrome and has produced increases in growth velocity, but the long-term effects on final adult height have not been studied. In addition, no improvements in gross motor or mental development were noted.[900,901] Hashimoto thyroiditis is common in individuals with Down syndrome and should be sought and treated promptly. Because of the concern for development of leukemia, which is more common in individuals with Down syndrome, GH use is generally not recommended.

Turner Syndrome

In girls with TS, short stature is the single most common feature, occurring more frequently than delayed puberty, cubitus valgus, or webbing of the neck.[902–904] Short stature occurs in 95% to 100% of girls with a 45,X karyotype.[905–908] Mean adult heights in the United States and Europe range from 142 to 146.8 cm, with important genetic and ethnic influences on growth of girls in different regions. Parental height correlates well with final adult height,[908,909] and a cross-cultural study in 15 countries demonstrated a very strong correlation between final height in TS and in the normal population, with an approximate 20-cm deficit.[905] Several distinct phases of growth have been identified in girls with TS[910,911]:

1. Mild IUGR with a mean birth weight of 2800 g and a mean birth length of 48.3 cm
2. Slow growth recognized during early infancy and reaching −3 SDS by 3 years of age[912]
3. Delayed onset of the "childhood" phase of growth[27,28,913] and progressive decline in height velocity from age 3 years until approximately 14 years, resulting in further deviation from normal height percentiles
4. A prolonged adolescent growth phase, characterized by a partial return toward normal height, followed by delayed epiphyseal fusion

These girls have many features of skeletal dysplasia, such as Madelung deformity and are haploinsufficient for the *SHOX* gene, which is located in the pseudoautosomal region of the short arm of the X chromosome.[914] When heights of girls with TS are compared with those of girls with LWD, which involves a *SHOX* deletion, it appears that the *SHOX* defect may account for about two-thirds of the height deficit in TS.[915] Girls with TS have normal GH and IGF levels during childhood; reports of low levels of GH or IGF, or both, in adolescents are likely due to low serum levels of gonadal steroids.[916] Multiple studies have shown that GH therapy is capable of accelerating short-term growth and increasing final adult height.[907,917,918] GH treatment in TS is discussed in detail later in the chapter.

Noonan Syndrome

Individuals with Noonan syndrome have postnatal growth failure, right-sided cardiac abnormalities (most often pulmonary valve abnormalities), webbing of the neck, low posterior hairline, ptosis, cubitus valgus, and malformed ears. Microphallus and cryptorchidism are common, and puberty may be delayed or incomplete. Cognitive delay of variable degrees is present in about 25% to 50% of patients. Although this disorder shares phenotypic features with TS, the two are clearly distinct.[919,920] In Noonan syndrome, the sex chromosomes are normal, and transmission is autosomal dominant, although about 50% of cases are sporadic. Noonan syndrome is caused by heterozygous activating mutations in the genes of proteins in the RAS-MAPK pathway, including *PTPN11, SOS1, RAF1, KRAS,* and *NRAS,* with approximately half of patients having mutations in *PTPN11*.[921,922] Through much of childhood, mean growth in length and weight is below the third percentile.[923–925] GH secretory abnormalities do not account for the short stature, although endogenous GH production may be slightly reduced.[925,926] The protein product of the *PTPN11* gene is the nonreceptor type 2 tyrosine phosphatase (SHP2). SHP2 dephosphorylates JAK2, suggesting that the growth failure of Noonan syndrome might be due to growth hormone resistance from the enhanced action of SHP2.[927–929] However, SHP2 is a positive regulator of the RAS-MAPK pathway, and the development of Noonan syndrome from activating mutations in other proteins in this pathway suggests that alterations in RAS-MAPK signaling are responsible for the phenotype. GH therapy has been used in the treatment of patients with Noonan syndrome who have short stature, as discussed in detail later.

Prader-Willi Syndrome

Prader-Willi syndrome (PWS), a neurogenetic developmental disorder, was initially described by Prader, Willi, and Labhart in 1956. This syndrome is characterized by obesity, hypotonia, hyperphagia, delayed motor skill development, short stature, mental retardation, hypothalamic dysfunction, and hypogonadism.[930]

PWS has an incidence of 1 in every 12,000 births, making it the most common syndrome causing obesity. PWS is caused by the absence of expression of genes on the paternally inherited chromosome 15 (q11–13). In about 70% to 75% of cases, PWS is due to deletion of the paternal allele; in 25% of cases, it is due to the presence of maternal uniparental disomy (UPD). Other rarer causes of PWS are due to translocations, molecular defects, or imprinting errors.[931,932] Although several genes and gene products of the PWS locus have been identified, the specific gene(s) responsible for its pathogenesis are not completely understood.[933,934]

In PWS, growth failure may be evident at birth but is more pronounced postnatally.[935] The hypothalamic dysfunction in PWS is associated with endocrinopathies that include short stature, hyperphagia and obesity with abnormal body composition, deficient growth hormone (GH) secretion, central hypothyroidism, and hypogonadism.[936–938]

Interestingly, a recent study showed that GH secretion in the patients with UPD was significantly lower than those with deletions.[939] Although infants with PWS typically have hypotonic and poor weight gain, the percentage of body fat is increased before the onset of hyperphagia and obesity.[940] This syndrome is discussed at length in a later section of this chapter.

Other Syndromes

Other syndromes associated with moderate to profound growth failure include Bloom syndrome, de Lange syndrome, leprechaunism, Ellis–van Creveld syndrome, Aarskog syndrome, Rubinstein-Taybi syndrome, mulibrey nanism, Dubowitz syndrome, progeria, Cockayne syndrome, and Johanson-Blizzard syndrome.[941]

Small for Gestational Age

Historically, infants born SGA have composed a heterogeneous group with birth weight or length below the 3rd, 5th, or 10th percentile for gestational age, depending on the study.[36] As the growth and metabolic consequences of being born SGA have been observed and characterized, studies have more consistently used the definition of SGA as birth weight or length (or both) at least 2 SD below the mean for gestational age (usually at or below the 2.3 percentile for a population). The term *IUGR* has been used interchangeably with SGA to describe these infants. However, it has been proposed that, because IUGR implies a known underlying pathologic process, that term should be reserved for infants whose abnormal prenatal growth has been confirmed by intrauterine growth assessments and whose growth restriction can be attributed to a specific cause.[942] The reason for abnormal fetal growth is unclear in up to 40% of cases[943]; known underlying reasons are listed in Table 25.6. Accurate assessment of an infant as SGA depends on accurate gestational dating and weight and length measurements, which can be difficult in both developed and developing countries.[944] However, recent technology has allowed ultrasound measurements of fetal size in utero to become increasingly more precise.[945]

Most SGA infants exhibit catch-up growth (as defined by a growth velocity greater than the median for chronologic age and gender) by 2 years of age. Catch-up growth occurs during the first 6 months of life in approximately 80% of infants born SGA.[37] Approximately 10% to 15% of infants born SGA exhibit slow, attenuated growth with persistent height deficits in childhood and adolescence. The remaining 5% to 10% exhibit a slower catch-up growth pattern, reaching heights 2 SD below the mean between 3 and 5 years of age. These estimations vary by study; in a population of severely affected infants with SGA who required care in a neonatal intensive care unit, 27% had not achieved catch-up growth by 6 years of age.[946]

Low-birth-weight premature babies who are appropriate for gestational age invariably experience catch-up growth in the first 2 years of life. Final adult height of all children born SGA is –0.8 to –0.9 SDS, a mean deficit of 3.6 to 4 cm when adjusted for family stature.[947] It has been estimated that the 10% to 15% of SGA children with short stature account for as much as 20% of all short children. In the United States, 2.3% of the population fitting the definition of SGA represents roughly an incidence of 1 in 43 neonates. Therefore SGA children have a fivefold to sevenfold greater possibility of short stature than AGA children.[36]

Normal fetal growth depends on a complex interplay of maternal and fetal genetic and external environmental influences. Abnormal intrauterine growth can result from pathologic processes in the fetus, the placenta, or the mother. Growth in length occurs early in fetal life, whereas weight gain occurs later in fetal life[948]; first-trimester growth failure has been closely associated with low birth weight and low-birth-weight percentile.[949] Because there is a differential effect on weight and length depending on the fetal period when the pathologic processes occur, IUGR has been subclassified into symmetric and asymmetric types. The

TABLE 25.6 Factors Associated With Small for Gestational Age (SGA) Births

I. Intrinsic Fetal Factors

A. Chromosomal disorders
   1. Mutations in genes encoding IGF1, IGF2, IGF1R, INS, KCNJ11, ABCC8, chromosome 6ICR, INSR (Donohue syndrome [leprechaun], Rabson-Mendenhall syndrome), PTF1A, IPF1, BLM (Bloom syndrome), FANC A-M (Fanconi syndrome), ACAN
B. Syndromes
   1. Russell-Silver syndrome
   2. Seckel syndrome
   3. Progeria
   4. Cockayne syndrome
   5. Rubinstein-Taybi syndrome

II. Placental Abnormalities

A. Abnormal implantation of the placenta
B. Placental vascular insufficiency; infarction
C. Vascular malformations

III. Maternal Disorders

A. Malnutrition
B. Constraints on uterine growth
C. Vascular disorders
   1. Hypertension
   2. Toxemia
   3. Severe diabetes mellitus
D. Uterine malformations
E. Drug ingestion
   1. Tobacco
   2. Alcohol
   3. Narcotics

symmetric type of IUGR results from an insult early in the pregnancy, often due to fetal genetic factors or syndromes, congenital infections, or toxic effects; asymmetric IUGR results from an insult occurring late in gestation, often due to fetoplacental insufficiency. Historically, it was thought that infants with symmetric IUGR do not experience catch-up growth, whereas those with asymmetric IUGR who have normal head circumference and length yet low birth weight usually experience catch-up growth postnatally.[948] However, studies have suggested that infants with asymmetric IUGR have worse perinatal outcomes than those with symmetric IUGR[950,951] and that both types of growth restrictions can arise in the second trimester of pregnancy.[952,953] Therefore the subclassification of IUGR, like the term *IUGR* itself, is controversial. A recent study found that 16% of dysmorphic children born SGA with postnatal growth retardation had pathologic genomic copy number variants.[954] In a recent study, 14% of children born SGA with advanced skeletal maturation had an ACAN gene mutation.[955]

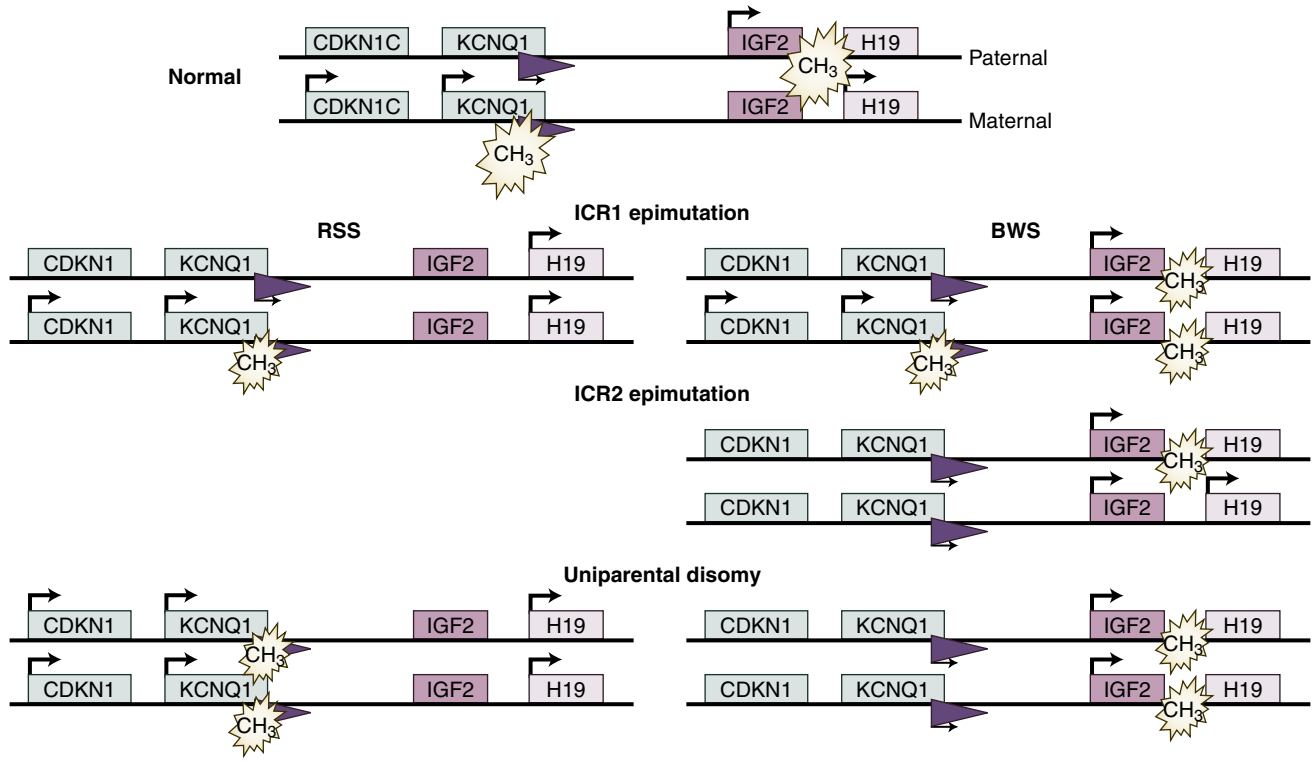
Endocrine-related causes account for a small fraction of the many contributors to fetal growth abnormalities, but hormonal disorders associated with fetal and neonatal growth restriction shed light on the endocrine mechanisms of growth in the fetus. Although GH plays a major role in postnatal growth, it has less of a role in fetal growth; infants with neonatal GHD are typically −0.5 to −1.5 SD below the mean in length and are heavy for this length.[338,956] An adequate nutrient supply is the main determinant of fetal growth, but growth factors such as insulin, IGF1, and IGF2 also play a role. IGF1 and IGF2 are the major hormonal regulators of fetal growth and can compensate for each other.[265,273,957–960] The growth-promoting effects of the IGFs are mediated by the type I or IGF1 receptor.[273] During fetal life, the IGF system operates largely independently of GH. Genetically modified mouse models have shown that approximately 70% of body size is due to IGF, half of which are mediated by GH-induced changes in IGF concentration and half due to direct IGF effects.[227] The direct effect of GH on body size is relatively minor. Neonates who are SGA exhibit hormonal patterns consistent with insensitivity to GH and IGF1 and insulin action. In neonates with IUGR, GH levels are elevated[961] and IGF1 and IGFBP3 levels are low.[151,961–964] IGFBP1 and IGFBP2 levels have been reported to be higher than in AGA infants,[291] a pattern seen in individuals with insulin resistance. Similar patterns are found in the first week of life after severe fetal malnutrition.[965] Exogenous GH treatment has little or no effect on growth, body composition, or energy expenditure[966,967] in the neonatal period. In most infants with SGA, normalization of GH, IGF1, and IGFBP3 levels occurs by 3 months of age, with normal response to GH stimulation testing in childhood.[968] In measurement of spontaneous GH secretion, high pulse frequency with attenuated pulse amplitude and elevated trough values of GH have been noted in SGA children.[969–971] Serum IGF1 levels in children with short stature born SGA are slightly but significantly lower than in children with catch-up growth.[968,969,971]

Defects of insulin secretion or action are associated with impaired fetal growth.[948] Congenital defects of insulin secretion such as glucokinase deficiency[972] or pancreatic agenesis[973] are associated with severe IUGR. Leprechaunism is caused by defects in the insulin receptor and is associated with severe insulin resistance and IUGR.[27] The initial case of a deletion of the IGF1 gene had profound intrauterine growth failure.[974,656] Polymorphisms of the *IGF1* gene have also been reported to be associated with IUGR.[974–976]

SGA children who have striking weight gain during the first several years of life can develop endocrine disorders later in childhood, including premature adrenarche, insulin resistance, polycystic ovary syndrome, and an attenuated growth spurt.[977] This subset of SGA children have increased risks of hypertension, maturity-onset diabetes, and cardiovascular disease later in life.[978–980] This is consistent with the Barker hypothesis, which states that fetal metabolic responses to a nutritionally hostile intrauterine environment may lead to inappropriate extrauterine consequences.[979,981] These problems do not appear to occur in SGA babies without catch-up growth, although insulin resistance has been described.[982] Whether being SGA is causally related to these disorders or is a symptom of an underlying inborn metabolic disorder is not yet known.

Russell-Silver syndrome (RSS) was independently described by Russell[983] and by Silver.[984] Findings include IUGR with postnatal growth failure, congenital hemihypertrophy, and small, triangular facies.[985] Other nonspecific findings include clinodactyly, delayed closure of the fontanel, delayed bone age, and precocious puberty.[986–988] Adults are short, with final heights approximately −4 SD below the mean.[985] The incidence is between 1 in 50,000 and 1 in 100,000 live births. Endogenous GH secretion in prepubertal RSS children is similar to that in other short IUGR children and less than in AGA short children.[969,989] Maternal uniparental disomy of chromosome 7 is present in 7% to 10% of cases.[985,990] Although there are a number of imprinted genes or factors involved in growth and development on chromosome 7, numerous studies have not detected any pathologic mutations in the candidate genes. A gene on chromosome 7p, *GRB10,* is involved in regulation of insulin and IGF1 receptor signaling and is mainly expressed from the maternal allele; loss of the maternal allele results in fetal and placental overgrowth.[990,991] Mutations that could cause overexpression of *GRB10* have not been found in patients with RSS.

A small number of patients with RSS have duplication of the maternal allele of the 11p15 region[992]; duplication of the paternal allele in this region is associated with BWS and overexpression of IGF2. The 11p15 region contains two imprinting control regions (ICR), ICR1 and ICR2 (Fig. 25.39). ICR1 comprises the *IGF2* and *H19* genes. The noncoding gene *H19* is downstream from *IGF2* and is oppositely imprinted, meaning that only the maternal allele is expressed and that the paternal allele is inactive. The promoters of the *IGF2* and *H19* genes share a set of enhancers that act on either gene. On the paternal allele, the *H19* promoter region is methylated and therefore inactivated.[244] Upstream from the *H19* and *IGF2* promoter region is a paternally methylated region that prevents binding of CTCF, allowing enhancers to act on the *IGF2* promoter to activate transcription.[245] On the maternal chromosome, this region is not methylated, allowing CTCF to bind and preventing transcription.[246] In this ICR1 region, mutations causing hypomethylation of the *H19* promoter region have been described in approximately 40% of patients with RSS.[993] Oppositely, hypermethylation of the ICR1 region has been associated with BWS. Disruption of ICR2 has been described in BWS (described later) but not in RSS. Reduced *IGF2* expression has been demonstrated in fibroblasts of patients with RSS in vitro,[993] but serum levels of IGF2 in patients with RSS are normal.[994] Mice with a null mutation of IGF2 have prenatal growth retardation but normal postnatal growth; how reduced expression of IGF2 contributes to postnatal growth failure in RSS has yet to be elucidated. A number of other rare genetic defects have also been associated with RSS, such that a known molecular abnormality may be identified in 60% of these individuals.[995]



• **Fig. 25.39** Schematic representation of the 11p15 region and the epigenetic mutations associated with Russell-Silver syndrome and Beckwith-Wiedemann syndrome. *Red and pink boxes* represent genes of imprinting control region 1 (1CR1), and *green boxes* represent genes of 1CR2. The *arrow* represents the presence of gene transcription. *Dark triangles* represent antisense transcripts that can repress transcription of *ICR2* genes. When methylated (represented by CH_3), the transcripts cannot be formed, allowing transcription of *ICR2* genes and reciprocal suppression of transcription of downstream genes (i.e., *IGF2*). (Adapted from Eggerman T. Silver-Russell and Beckwith-Wiedemann syndromes: opposite [epi]mutations in 11p15 result in opposite clinical pictures. *Horm Res.* 2009;71:S30–S35.)

Maternal and Placental Factors

Maternal factors and placental insufficiency can impair fetal growth and likely account for most cases of asymmetric IUGR. Maternal nutrition is an important contributor to fetal growth and to growth during the first year of life.[996] Fetal growth retardation may result from use of alcohol,[997] cocaine,[998] marijuana,[998] or tobacco[999] during pregnancy. The mechanisms for drug-induced fetal growth retardation are unclear but may include uterine vasoconstriction and vascular insufficiency, placental abruption, or premature rupture of membranes. The maternal hormonal milieu is affected by placental steroids and peptides. Maternal IGF1 affects placental function and may facilitate transport of nutrients to the fetus. Maternal IGF1 levels have been found to correlate with fetal growth.[1000,1001] Increased levels of free IGF1 are found during normal human pregnancy.[1002]

The placenta has multiple functions, including the transport of nutrients, oxygen, and waste and the production of hormones. It consumes oxygen and glucose brought by the uterine circulation. Placental GH affects maternal IGF production, which in turn affects placental function. A woman with GHD due to *PIT1* mutation exhibited normal levels of placental GH and IGF1, demonstrating the independent production of GH and IGF1 by the placenta.[1003] Human placental lactogen (hPL) is a major regulator of glucose, amino acid, and lipid metabolism in the mother, aiding in the mobilization of nutrients for transport to the fetus. Damage to the placenta resulting from vascular disease, infection, or intrinsic abnormalities of the syncytiotrophoblasts can impair these important functions. At times, examination of the placenta may yield causal information about fetal growth retardation.

An X-linked homeobox gene, *ESX1,* detected only in extraembryonic tissues and human testes, is a chromosomally imprinted regulator of placental morphogenesis.[1004–1006] Heterozygous and homozygous mutant mice are born 20% smaller than normal and have large edematous placentas.[1004] Vasculature is abnormal at the maternal-fetal interface, presumably causing the growth retardation.

Pathologic Basis of Excess Growth

Although by definition there are as many children with heights greater than 2 SD above the mean as those with heights less than 2 SD below the mean, tall stature as a chief complaint is encountered much less often in endocrine practice. Nevertheless, it is critical to identify those situations in which tall stature or an accelerated growth rate provides clues of an underlying disorder (Table 25.7).

Statural Overgrowth in the Fetus

Maternal diabetes mellitus is the most common cause of large-for-gestational-age (LGA) infants. LGA is defined as length or weight greater than the 90th percentile for gestational age. Even in the absence of clinical symptoms or family history, the birth of an excessively large infant should lead to evaluation for maternal or gestational diabetes. Two syndromes, Sotos syndrome and BWS, can also cause LGA infants.

TABLE 25.7 Differential Diagnosis of Statural Overgrowth

Fetal Overgrowth

Maternal diabetes mellitus
Cerebral gigantism (Sotos syndrome)
Weaver syndrome
Beckwith-Wiedemann syndrome
Other insulin-like growth factor 2 (IGF2) excess syndromes

Postnatal Overgrowth Leading to Childhood Tall Stature

Familial (constitutional) tall stature
Cerebral gigantism
Beckwith-Wiedemann syndrome
Exogenous obesity
Excess growth hormone (GH) secretion (pituitary adenoma with gigantism)
McCune-Albright syndrome or multiple endocrine neoplasia (MEN) associated with excess GH secretion
Precocious puberty
Marfan syndrome
Klinefelter syndrome (XXY karyotype)
Weaver syndrome
Fragile X syndrome
Homocystinuria
XYY karyotype
Hyperthyroidism

Postnatal Overgrowth Leading to Adult Tall Stature

Familial (constitutional) tall stature
Androgen or estrogen deficiency/estrogen resistance (in males)
Testicular feminization
Excess GH secretion
Marfan syndrome
Klinefelter syndrome (XXY karyotype)
XYY karyotype

Sotos Syndrome

Children with cerebral gigantism (Sotos syndrome) are typically above the 90th percentile for length and weight at birth.[1007–1009] Clinical features also include a prominent forehead; dolichocephaly; macrocephaly; high arched palate; hypertelorism with unusually slanting eyes; prominent ears, jaw, and chin; large hands and feet with thickened subcutaneous tissue; cognitive delay; and motor incoordination. Children continue to grow rapidly during early childhood, but puberty is usually early, with premature epiphyseal fusion. Therefore most children with Sotos syndrome have a final height within the normal range.[1009] GH secretion and serum IGF levels are normal, and no specific cause of the overgrowth has been identified. About 80% of patients have a loss-of-function mutation in the *NSD1* gene whose product is a nucleus-localized basic transcription factor.[1010]

Beckwith-Wiedemann Syndrome

BWS is the most common (1 of every 13,700 live births) of the overgrowth disorders, the group of disorders associated with excessive somatic and specific organ growth. It is characterized by fetal macrosomia with omphalocele[1011] and other clinical features secondary to organomegaly, such as macroglossia, renal medullary hyperplasia, and neonatal hypoglycemia due to islet cell hyperplasia.[1012] Excessive childhood growth ultimately leads to earlier puberty and early epiphyseal fusion with resultant normal adult height.[1013]

Various lines of evidence have shown that BWS is associated with loss of imprinting of the genes on chromosome 11p15.5, home of the *IGF2* gene (see Fig. 25.39). Under normal conditions, the paternally derived *IGF2* gene is expressed and the maternally transmitted gene is not active, as described in detail earlier in this chapter. BWS has been associated with uniparental disomy or duplication of the paternal 11p15 region and resultant overexpression of the *IGF2* gene.[1014] The ICR1 region of 11p15 contains the *IGF2* and *H19* genes; the noncoding gene *H19* is located downstream from *IGF2* and is oppositely imprinted. The promoters of the *IGF2* and *H19* genes share a set of enhancers that act on either gene. On the paternal allele, the *H19* promoter region is methylated and therefore inactivated.[244] Upstream from the *H19* and *IGF2* promoter region is a paternally methylated region that prevents binding of CTCF, allowing enhancers to act on the *IGF2* promoter to activate transcription.[245] On the maternal chromosome, this region is not methylated, allowing CTCF to bind and preventing transcription.[246] Hypermethylation of the *H19* promoter region with resultant loss of imprinting and biallelic expression of IGF2 has been associated with fewer than 10% of the cases of BWS.[1015,1016] Point mutations in the ICR1 region have been identified in patients with BWS. These mutations alter binding of OCT (octamer) transcription factors to the region leading to hypermethylation of the promoter.[1017] Hypomethylation of this region is associated with RSS, a syndrome associated with prenatal and postnatal growth failure (see earlier discussion).

ICR2, located 5′ of *ICR1*, contains the genes for cyclin-dependent kinase inhibitor 1C *(CDKN1C)* and potassium channel KQT family member 1 *(KCNQ1)*, among others that are methylated on the maternal allele. Associated with these two genes is an antisense transcript with paternal expression that may suppress transcription; it has been postulated that this cluster of genes is in "expression competition" with the *IGF2/H19* cluster.[1018] Up to 25% of familial cases of BWS are associated with mutations in the *CDKN1C* or *KCNQ1* gene,[1019] but there is debate about whether loss of imprinting of the *IGF2* gene convincingly occurs with most of the mutations. Four children with somatic overgrowth but not the diagnostic features of BWS had *IGF2* gene overexpression.[1020] Additionally, mutations in *GPC3,* a glypican gene that codes for an IGF2 neutralizing membrane receptor, cause the related Simpson-Golabi-Behmel overgrowth syndrome.[1021,1022]

Postnatal Statural Overgrowth

As in the case of the child with growth attenuation, crossing of height percentiles between infancy and the onset of puberty is an indication for further evaluation because it can indicate serious underlying pathology. As with short stature, children with tall stature must be evaluated in the context of familial growth and pubertal patterns.

Tall Stature

GH secretion and levels of IFG1 and IGFBP3 in familial tall stature are often in the upper-normal range.[1023] Tauber and colleagues[1024] divided 65 children with familial tall stature into a subset with high GH secretion rates and frequent secretory bursts and another subset with lower GH secretion and fewer episodic spikes. IGF1 levels were higher among those producing more GH and normal in the low-GH group. The authors postulated that both enhanced secretion of GH and greater efficiency of GH-mediated IGF1 production might be potential causes of familial tall stature.

Tall stature is also a characteristic of certain syndromes. Marfan syndrome, an autosomal dominant disorder of collagen

metabolism, is characterized by hyperextensible joints, dislocation of the lens, kyphoscoliosis, dissecting aortic aneurysm, and long, thin bones that result in arachnodactyly and moderately tall stature. It is caused by mutations in the fibrillin-1 gene *(FBN1)*. The abnormal FBN1 monomers from the mutated gene disrupt the normal aggregation of FBN1, impairing microfibril formation. Homocystinuria is an autosomal recessive disorder that phenotypically resembles Marfan syndrome, but patients also have cognitive disabilities. Tall stature has also been found in patients with familial ACTH resistance due to a defective ACTH receptor.[1025]

Dosage effects of the *SHOX* gene may result in tall stature.[1026] In females with three copies of the *SHOX* gene and gonadal dysgenesis, adult stature was +2 to +2.9 SDS.[1027] In women with the 47,XXX karyotype, mean final heights are 5 to 10 cm taller than population means, and men with the 47,XXY karyotype (Klinefelter syndrome) are about 3.5 cm taller than population means.[31,1028] Males with an XYY karyotype may also have moderately tall stature. In addition to SHOX effects, the variable degree of estrogen production in these syndromes may also influence skeletal maturation and final height.[26]

Failure to enter puberty and complete sexual maturation may result in sustained growth during adult life with ultimate tall stature and a characteristic eunuchoid habitus. The description of tall stature with open epiphyses resulting from mutation of the estrogen receptor or from aromatase deficiency[23,24] underscores the fundamental role of estrogen in promoting epiphyseal fusion and termination of normal skeletal growth.

Obesity

Obesity is frequently associated with rapid skeletal growth and early onset of puberty.[1029] Rapid early postnatal weight gain has been associated with taller stature at 8 years of age than that predicted from the midparental target height.[1030] Others have shown that early postnatal growth is associated with altered tempo of development, but sudden weight gain in middle childhood has little effect on height trajectory.[1031] Patients with obesity tend to have diminished overall GH production but normal to high GHBP, and IGF1 levels appear to be capable of maintaining adequate or enhanced linear growth velocity. Bone age is usually modestly accelerated, so that both puberty and epiphyseal fusion occur early and adult height is normal. This association between obesity and rapid growth is so characteristic that a child with obesity and short stature should always be evaluated for underlying pathology, such as hypothyroidism, GHD, Cushing syndrome, or PWS.

Tumors

Pituitary gigantism is a rare condition, analogous to acromegaly in the adult (see Chapter 9).[1032–1034] GH-secreting tumors of the pituitary are typically eosinophilic or chromophobic adenomas. Their cause is uncertain, although many result from somatic mutations that generate constitutively activated G proteins with reduced GTPase activity (see Chapter 9).[1035] The resulting increase in intracellular cAMP in the pituitary leads to increased GH secretion. Somatotropic tumors with excess GH secretion may occur in McCune-Albright syndrome, which is caused by mutations resulting in constitutive activation of G proteins.[1036,1037] GH-secreting tumors have also been reported in multiple endocrine neoplasia (MEN) and in association with neurofibromatosis and tuberous sclerosis.[1038]

GH excess that occurs before epiphyseal fusion results in rapid growth and attainment of adult heights above expected adult potential. When GH hypersecretion is accompanied by gonadotropin deficiency, accelerated linear growth may persist for decades, as was the case for the Alton giant, who reached a height of 280 cm by the time of his death in his 20s.[1039] Manifestations typical of acromegaly may also appear, such as soft tissue swelling; enlargement of the nose, ears, and jaw with coarsening of facial features; pronounced increases in hand and foot size; diaphoresis; galactorrhea; and menstrual irregularity.

Evaluation and Treatment of Growth Abnormalities

Clinical Evaluation of Growth Retardation

The most important parameter in assessing children with growth failure is careful clinical evaluation, including accurate serial assessment of height and height velocity. Consideration of a growth disorder is raised when a child's length or height falls below the normal range (<3rd percentile), the growth velocity is subnormal (indicated by length or height measurements that cross percentiles on the growth curve or by an annual growth velocity less than the 3rd percentile for age), or the child's height is below the range expected based on the parents' heights. To grow along the 3rd percentile for height, a child must maintain a height velocity at the 25th percentile for age.[1040] Therefore a height velocity consistently below the 25th percentile in a short child suggests abnormal growth. However, because of the greater error intrinsic to assessment of growth velocity compared with height velocity,[1041] a single height velocity measurement above the 25th percentile for age, even based on annual height data, cannot fully exclude a growth abnormality in a short child.[1041]

GH is relatively less important as a growth factor prenatally compared with its role postnatally. However, GH is not without impact on prenatal growth, so that although many of these children have lengths and weights within the normal range, the average size of infants with congenital GH deficiency is decreased, with a mean birth length of –1.3 SDS and a mean birth weight of –1.0 SDS.[1042] Growth velocity over the first year averages near –2 SD, so that over half of these infants have a length more than 2 SD below the mean at 1 year of age.[1042]

Table 25.8 provides an algorithm for evaluation of the child with growth failure. Although one-third of healthy infants will cross downward on the length percentile growth curve (discussed previously), this is normal only in relatively large infants born to small parents or in those infants who are following a growth pattern of CDGD (see later discussion). In other situations, the infant who is crossing downward on the length percentile curve should be investigated in the same way as other children with subnormal growth velocities.

History and Physical Examination

The many illness-related causes of diminished growth were discussed in earlier sections of this chapter. A growth pattern in which weight gain is impaired before linear growth or in which there is greater impairment of weight gain than of length/height gain suggests an impairment of nutrition, such as inadequate intake, malabsorption, or increased energy requirements. Nonhormonal causes of growth failure should be investigated based on data obtained from a careful history and physical examination. In addition, careful evaluation of the growth curve in the context of the family history may suggest a normal variant growth pattern,

TABLE 25.8 Clinical and Biochemical Evaluation of Growth Failure: Evaluating the GH-IGF1 Axis

Step 1: Defining the Risk of Disorders of the GH-IGF1 Axis

Auxologic abnormalities
- Severe short stature (height SDS <–3 SD)
- Severe growth deceleration
  - Height <–2 SD and height velocity <–1.0 SD over 2 years
  - Height <–1.5 SD and height velocity <–1.5 SD over 2 years

Risk factors
- History of a brain tumor, cranial irradiation, or other documented organic or congenital hypothalamic-pituitary abnormality
- Incidental finding of hypothalamic-pituitary abnormality on MRI
- If any of the above exists, proceed with step 2; if not, follow clinically and return to step 1 in 6 months

Step 2: Screening for Disorders of the GH-IGF1 Axis and Other Diseases

A. Order a laboratory panel, including a bone age, free T_4, TSH, chromosomes (in females), and nonendocrine tests; if indicated, refer back to primary care physician or treat diagnosed conditions as appropriate.
B. Order an IGF1 and IGFBP3 level.
   - If IGF1/IGFBP3 are both above the –1 SD, follow clinically and return to step 1 in 6 months.
   - If IGF1/IGFBP3 are both below –2 SD, proceed to step 4. If MRI is abnormal, GH provocative testing is optional.
   - Otherwise, proceed to step 3. If this is a patient with delayed adolescence, consider sex steroid treatment prior to step 3.

Step 3: Testing GH Secretion

This step can be bypassed if a clear GHD risk factor and severe IGF deficiency are identified.

Perform two of the following GH stimulation tests (if appropriate, estrogen prime) (see Table 25.11):
- Clonidine
- Arginine
- Insulin
- Glucagon
- L-Dopa
- Propranolol

If all GH levels are <7 μg/L, go to step 4.

If peak GH >15 μg/L, obtain GHBP; if GHBP <–2 SD, consider an IGF-generation test and, if abnormal, IGF treatment.

If peak GH >15 μg/L and GHBP is normal, follow clinically and return to step 1 in 6 months.

If peak GH is between 7 and 15 μg/L, return to step 1 in 6 months.

Step 4: Evaluating the Pituitary

Perform MRI, with particular emphasis on hypothalamic-pituitary anatomy.

Test HPA, if not already done (CRH stimulation or ITT), and teach cortisol supplementation as needed (must do this if MRI is abnormal).

Consider molecular evaluation of GH, GHR, or GHRHR and other potential genetic defects (see Fig. 25.2).

Step 5: Treating for Growth Promotion

Initiate GH treatment at appropriate dose levels.

If GHIS is suspected, consider IGF therapy, if available.

Regularly evaluate growth parameters, IGF1, and IGFBP3, as well as compliance and safety (see Table 25.14).

GH secretion should be retested, according to adult GH assessment protocols, at the end of growth.

CRH, Corticotropin-releasing hormone; *GH,* growth hormone; *GHD,* growth hormone deficiency; *GHIS,* growth hormone insensitivity; *GHR,* growth hormone receptor; *GHRHR,* growth hormone–releasing hormone receptor; *HPA,* hypothalamic-pituitary axis; *IGF1,* insulin-like growth factor 1; *IGFBP3,* IGF-binding protein 3; *ITT,* insulin tolerance test; *MRI,* magnetic resonance imaging; *SD,* standard deviation; *SDS,* standard deviation score; T_4, thyroxine; *TSH,* thyroid-stimulating hormone (thyrotropin).

TABLE 25.9 Key History and Physical Examination Findings That Suggest the Diagnosis of Growth Hormone Deficiency[a]

Findings That Suggest the Diagnosis of GHD

In the Neonate

Hypoglycemia
Prolonged jaundice
Hepatitis
Microphallus
Traumatic delivery

In a Child With Short Stature or Growth Failure

Cranial irradiation
Head trauma or central nervous system infection
Consanguinity or an affected family member
Craniofacial midline abnormalities

Findings That Support Immediate Investigation for GHD

In a Child With Short Stature

Signs indicative of an intracranial lesion
Neonatal symptoms and signs of GHD
Auxologic findings
- Severe short stature (<–3 SD)
- Height <–2 SD and height velocity over 1 year of <–1 SD
- A decrease in height SD of >0.5 over 1 year in children >2 years of age
- A height velocity <–2 SD over 1 year
- A height velocity >1.5 SD below the mean sustained velocity over 2 years

Signs of multiple pituitary hormone deficiency (MPHD)

[a]The Growth Hormone Research Society 2000 Criteria.

GHD, Growth hormone deficiency; *SD,* standard deviation.

From Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH research society. *J Clin Endocrinol Metab.* 2000;85:3990–3993.

such as crossing linear percentiles of infancy, familial short stature, or CDGD. In such cases, careful observation may be all that is required. One-third of all infants have growth parameters that cross percentiles downward on the growth curve, and 3% of all children have a length or height below the 3rd percentile. Most of these children have no disease or growth disorder and will demonstrate this by having a normal growth velocity on continued observation.

The physical examination should look for evidence of an underlying organ-specific or systemic disease. It should also evaluate for clues specific to growth disorders, such as findings suggestive of genetic disorders (e.g., Noonan syndrome, RSS, or TS). In addition, body proportions should be measured because skeletal disproportion suggests a skeletal dysplasia.

Findings on the history and examination may point to an increased likelihood of the presence of GHD (Table 25.9). Micropenis in a male newborn should always lead to an evaluation of the GH-IGF1 axis. Nystagmus, indicating neonatal blindness, suggests hypopituitarism due to its association with optic nerve hypoplasia in the syndrome of septo-optic dysplasia. A history of other midline defects, such as cleft lip and cleft palate,[1043] or a single central incisor increases concern for hypopituitarism. Unexplained neonatal hypoglycemia, hepatitis, or prolonged jaundice should prompt an evaluation of pituitary function. Older children with GHD have less impaired weight gain than height gain, resulting in

an increased weight for height; they are often described as having a "cherubic" appearance. Increased weight for height with growth failure is also characteristic of hypothyroidism. If the weight gain is dramatic, Cushing syndrome should be considered. (However, linear growth acceleration with excess weight gain is not consistent with Cushing syndrome.) Finally, GHD should be suspected in children with known or suspected CNS pathology (e.g., tumors, prior radiation therapy to the CNS, malformations, infection, trauma) or with documented deficiency of TSH, ACTH, antidiuretic hormone, or gonadotropin.

Laboratory Testing

If the history and physical examination do not suggest a specific disorder causing growth failure, and the growth pattern and family history do not provide sufficient reassurance that the growth is following a normal variant growth pattern, it is necessary to perform laboratory testing. In many cases, the testing does not identify an abnormality, and the child either is ultimately found to have a normal variant growth pattern or falls into the classification of ISS. The laboratory tests can be divided into those screening for disorders outside the GH-IGF1 axis and those evaluating that axis.

Screening Tests

Because a number of illnesses can cause growth failure either before or in the absence of other signs or symptoms, it is necessary to screen for these disorders in children with abnormal growth. A complete blood count looks for evidence of anemia, chronic infection, and inflammation. A complete blood chemistry panel provides evidence for silent renal disease (including renal tubular acidosis), liver disease, and disorders of calcium and phosphorus. The erythrocyte sedimentation rate is measured to look for evidence of disorders involving chronic inflammation, such as presymptomatic juvenile idiopathic arthritis and inflammatory bowel disease. A urinalysis is obtained to look for renal disease and chronic urinary tract infection. Tissue transglutaminase IgA (and total serum IgA) is measured to screen for celiac disease. In girls in whom no other explanation for short stature is found, a karyotype should be obtained to exclude TS. This is done even in the absence of other physical features of TS, because growth failure may be the only evident feature, particularly in cases of significant mosaicism.

Hypothyroidism should be screened for in children with growth failure. Because of the importance of thyroid hormone on brain development in infants, this possibility should be considered early in the evaluation of an infant with growth failure to correct identified hypothyroidism quickly. In addition, hypothyroidism results in lower serum IGF1 levels and decreases GH levels during provocative testing.[1044–1046] Therefore it is necessary to ensure that thyroid function is normal before evaluating for GHD. TSH is measured because it is the most sensitive indicator of primary hypothyroidism. However, because central hypothyroidism must also be considered as a cause for growth failure in children, the thyroxine level should also be measured.

Bone Age

After the neonatal period, a bone age determination can be useful in the evaluation of children with growth disorders. A radiograph of the left hand and wrist is commonly used for comparison with the published standards of Greulich and Pyle.[1047] An alternative method for assessing bone age from radiographs of the left hand involves a scoring system for developmentally identified stages of each of 20 individual bones,[1048] a technique that has been adapted for computerized assessment.[1049,1050] The left hand is used because radiographs of the entire skeleton would be tedious and expensive and would involve additional radiation exposure. However, the hand does not contribute to height, and accurate evaluation of growth potential may require radiographs of the legs and spine.

Although the bone age result does not identify a specific diagnosis in a child with a growth disorder, it can be used to classify the child's growth in relation to groups of diagnoses. Growth disorders caused by an underlying illness or hormone disorder (e.g., renal disease, malnutrition, glucocorticoid excess) are associated with a delayed bone age—that is, a bone age that is younger than the patient's chronologic age. Similarly, hypothyroidism and GHD are associated with a delayed bone age. If short stature is intrinsic to the condition, however, bone age is not delayed and is within the range of normal for the chronologic age. This is true for genetic disorders such as TS, Noonan syndrome, and RSS, and also for familial short stature. In CDGD, the bone age is delayed, consistent with the future delay in puberty and late epiphyseal fusion. Given the lack of precise diagnostic laboratory tests for GHD, a lack of bone age delay argues against this diagnosis. On the other hand, the fact that patients with CDGD have a delayed bone age does not help in the sometimes difficult discrimination between this condition and GHD.

A number of important caveats concerning bone age must be considered. Experience in the determination of bone age is essential to minimize intraobserver variance, and clinical studies involving bone age benefit from having a single reader perform all interpretations. The normal rate of skeletal maturation differs between boys and girls and among different ethnic groups. The standards of Greulich and Pyle are separable by sex but were developed in American white children between 1931 and 1942. Both those and the Tanner and Whitehouse standards are based on normal children[1051] and may not be applicable to children with skeletal dysplasias, endocrine abnormalities, or other forms of growth retardation or acceleration.

Prediction of Adult Height

The extent of skeletal maturation observed in an individual can be used to predict the ultimate height potential. Such predictions are based on the observation that the more delayed the bone age (relative to the chronologic age), the longer the time before epiphyseal fusion prevents further growth. The most commonly used method for height prediction, based on Greulich and Pyle's *Radiographic Atlas of Skeletal Development,*[1047] was developed by Bayley and Pinneau[1052] and relies on bone age, height, and a semiquantitative allowance for chronologic age (Table 25.10). The system of Tanner and colleagues[1048] uses measurements of height, bone age, chronologic age, and, during puberty, height and bone age increments during the previous year, as well as menarchal status. Roche, Wainer, and Thissen[1053] used the combination of height, bone age, chronologic age, midparental height, and weight (RWT method). Attempts have been made to calculate final height predictions without requiring the determination of skeletal age[1054] by using multiple regression analyses with available data such as height, weight, birth measurements, and midparental stature. These systems are, by nature, empiric and are not absolute predictors. Indeed, the 90% confidence intervals for the predictions are approximately ±6 cm at younger ages. The more advanced the bone age, the greater the accuracy of the

TABLE 25.10 Prediction of Adult Stature

	FRACTION OF ADULT HEIGHT ATTAINED AT EACH BONE AGE[a]					
	GIRLS			BOYS		
Bone Age (yr-mo)	Retarded	Average	Advanced	Retarded	Average	Advanced
6-0	0.733	0.720		0.680		
6-3	0.742	0.729		0.690		
6-6	0.751	0.738		0.700		
6-9	0.763	0.751		0.709		
7-0	0.770	0.757	0.712	0.718	0.695	0.670
7-3	0.779	0.765	0.722	0.728	0.702	0.676
7-6	0.788	0.772	0.732	0.738	0.709	0.683
7-9	0.797	0.782	0.742	0.747	0.716	0.689
8-0	0.804	0.790	0.750	0.756	0.723	0.696
8-3	0.813	0.801	0.760	0.765	0.731	0.703
8-6	0.823	0.810	0.771	0.773	0.739	0.709
8-9	0.836	0.821	0.784	0.779	0.746	0.715
9-0	0.841	0.827	0.790	0.786	0.752	0.720
9-3	0.851	0.836	0.800	0.794	0.761	0.728
9-6	0.858	0.844	0.809	0.800	0.769	0.734
9-9	0.866	0.853	0.819	0.807	0.777	0.741
10-0	0.874	0.862	0.828	0.812	0.784	0.747
10-3	0.884	0.874	0.841	0.816	0.791	0.753
10-6	0.896	0.884	0.856	0.819	0.795	0.758
10-9	0.907	0.896	0.870	0.821	0.800	0.763
11-0	0.918	0.906	0.883	0.823	0.804	0.767
11-3	0.922	0.910	0.887	0.827	0.812	0.776
11-6	0.926	0.914	0.891	0.832	0.818	0.786
11-9	0.929	0.918	0.897	0.839	0.827	0.800
12-0	0.932	0.922	0.901	0.845	0.834	0.809
12-3	0.942	0.932	0.913	0.852	0.843	0.818
12-6	0.949	0.941	0.924	0.860	0.853	0.828
12-9	0.957	0.950	0.935	0.869	0.863	0.839
13-0	0.964	0.958	0.945	0.880	0.876	0.850
13-3	0.971	0.967	0.955		0.890	0.863
13-6	0.977	0.974	0.963		0.902	0.875
13-9	0.981	0.978	0.968		0.914	0.890
14-0	0.983	0.980	0.972		0.927	0.905
14-3	0.986	0.983	0.977		0.938	0.918
14-6	0.989	0.986	0.980		0.948	0.930
14-9	0.992	0.988	0.983		0.958	0.943
15-0	0.994	0.990	0.986		0.968	0.958
15-3	0.995	0.991	0.988		0.973	0.967
15-6	0.996	0.993	0.990		0.976	0.971

TABLE 25.10 Prediction of Adult Stature—cont'd

	FRACTION OF ADULT HEIGHT ATTAINED AT EACH BONE AGE[a]					
	GIRLS			BOYS		
Bone Age (yr-mo)	Retarded	Average	Advanced	Retarded	Average	Advanced
15-9	0.997	0.994	0.992		0.980	0.976
16-0	0.998	0.996	0.993		0.982	0.980
16-3	0.999	0.996	0.994		0.985	0.983
16-6	0.999	0.997	0.995		0.987	0.985
16-9	0.9995	0.998	0.997		0.989	0.988
17-0	1.00	0.999	0.998		0.991	0.990
17-3					0.993	
17-6		0.9995	0.9995		0.994	
17-9					0.995	
18-0		1.00			0.996	
18-3					0.998	
18-6					1.00	

[a]The column headed "Retarded" is used when bone age is >1 year below chronologic age; the column headed "Advanced" is used when bone age is >1 year greater than chronologic age.

Table derived from Post EM, Richman RA. A condensed table for predicting adult stature. *J Pediatr*. 1981;989:440–442 based on the data of Bayley and Pinneau.[1052] Predicted final height is calculated by dividing the current height by the fraction of adult height achieved determined from the table.

adult height prediction because a more advanced bone age places a patient closer to his or her final height.

All methods of predicting adult height are based on data from normal children, and none has been documented to be accurate in children with growth abnormalities. For this kind of precision, it would be necessary to develop disease-specific atlases of skeletal maturation (e.g., for achondroplasia or TS). In addition, height predictions will clearly be inaccurate in predicting the final height in the case of a growth-impairing process that is not adequately treated. In addition, height predictions must be used with care in assessing height outcomes during treatment. For example, in patients with precocious puberty treated with GnRH agonists, height prediction is known to overpredict the actual final height.[1055] Thus height predictions should only be considered a reasonable estimate.

Tests of the GH-IGF1 Axis

In children with growth retardation in whom other causes have been excluded, the possibility of GHD should be considered. However, these tests can have poor specificity, so clinical assessment must always play an important part in the evaluation of abnormalities in the GH-IGF1 axis. For example, a child growing consistently just below the 3rd percentile for height, with a growth rate that is accordingly above the 25th percentile for age, is very unlikely to be GH deficient. Abnormal test results in this situation would most likely represent false-positive results.

Testing of the GH-IGF1 axis begins with "static" testing of GH function, measuring IGF1. It may also be helpful to measure IGFBP3. In some cases—if the clinical presentation is highly suggestive of GHD (see Table 25.9)[1056] and the IGF1 level (and the IGFBP3 level, if obtained) also indicates a high likelihood of GHD—it is appropriate to proceed directly to the dynamic tests of GH secretion. In most cases, however, unless there is an abnormality identified on the screening tests, it is appropriate to monitor the child's growth for a period of 6 to 12 months to accurately assess the child's growth rate. Then, based on the complete clinical picture, including the growth rate and the IGF1 level, the decision is made to proceed with dynamic testing of GH secretion or to continue monitoring the child's growth.

There is no test that definitively diagnoses GHD. Because there is no gold standard, it is impossible to precisely define the sensitivity or specificity of any test for GHD. Some information on specificity can be obtained by comparing the results with those obtained in normal children, although, for the more complicated tests, these data can be difficult to obtain in children. Sensitivity relies on comparing positive results from one test with those of another—for example, comparing low IGF1 concentrations to failed results on provocative GH tests. Poor sensitivity of IGF1 as an indicator of GHD has been based on results from children with normal IGF1 concentrations who have abnormal results on provocative GH tests.[1057] However, because of the known limited specificity of these provocative tests (discussed later), one cannot determine whether the discrepant results are due to the poor sensitivity of the IGF1 test or the poor specificity of the provocative GH test. Again, it is critical to interpret all results together and in the context of the clinical data.

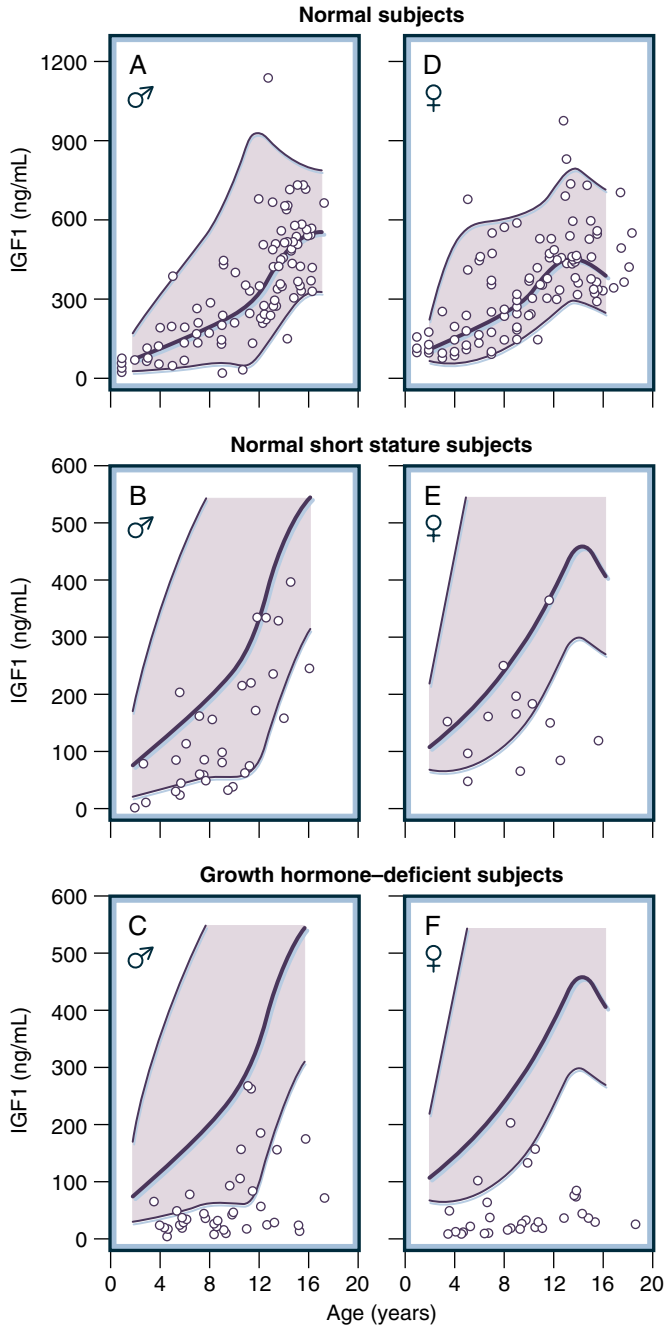
Insulin-Like Growth Factor 1

GHD is associated with low serum concentrations of IGF1 (Fig. 25.40). Unlike GH levels, which rise and fall with its pulsatile secretion, the IGF1 level in blood has minimal diurnal variation. The GH dependency of the IGFs was established in the initial

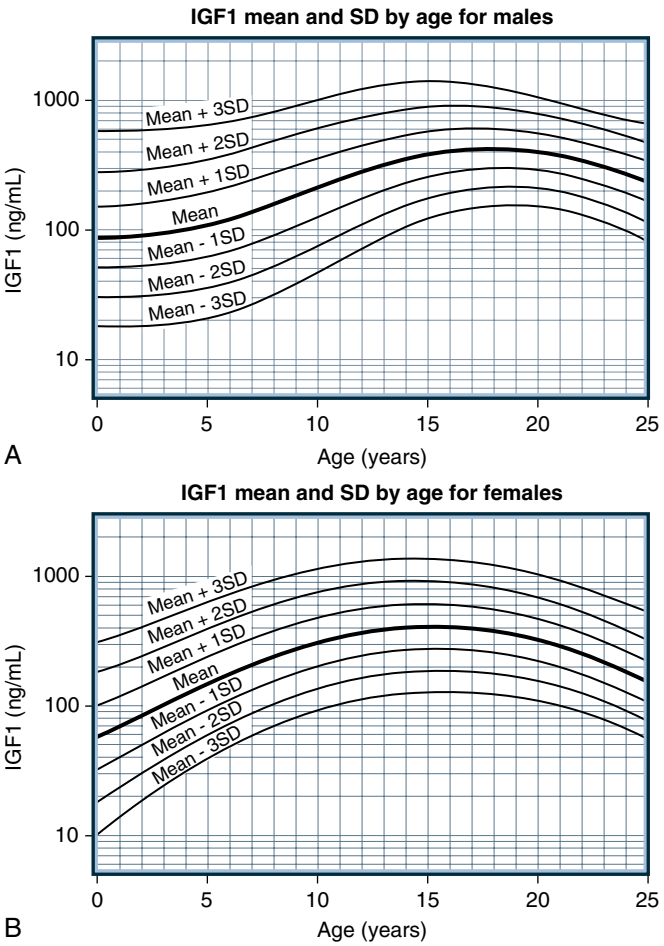
report from Salmon and Daughaday[164] and was further clarified with the development of sensitive and specific immunoassays that distinguish between IGF1 and IGF2.[1058] IGF1 levels are more GH dependent than IGF2 levels and are more likely to reflect subtle differences in GH secretory patterns. However, serum IGF1 levels are also influenced by age,[240,1059,1060] degree of sexual maturation, and nutritional status. Therefore IGF1 levels must be compared with age-specific ranges (Fig. 25.41) and with ranges defined by stage of sexual maturation. Some clinicians evaluate IGF1 results against the reference range based on bone age (rather than the chronologic age). This may improve the specificity of this test for GHD, although there are no data to address the validity of this approach.

IGF1 levels in normal children younger than 5 or 6 years of age are low. This leads to poor sensitivity of IGF1 levels for identifying GHD in young children. As few as 40% to 50% of young, short children with evidence of GHD on provocative tests have IGF1 levels below the lower level of the reference range.[1061,1062] IGF1 levels increase with age, resulting in better separation of IGF1 levels in GH-deficient children from those in normal children and a higher sensitivity of IGF1 levels for GHD. However, although sensitivities of 85% to 100% have been reported in some studies,[1062,1063] in others it has averaged approximately 70%.[1044,1063–1066] Again, however, the lack of a gold standard means that some of the false-negative findings obtained by measurement of IGF1 levels might rather represent false positives on provocative GH testing.

IGF1 levels also suffer from a lack of specificity in diagnosing GHD. In general, IGF1 levels have higher specificity in younger children, with declining specificity in older children. Juul and Skakkebaek[1066] found that IGF1 levels had a specificity for GHD of 98% in children younger than 10 years of age but a specificity



• **Fig. 25.40** Serum levels of insulin-like growth factor 1 (IGF1) in normal patients and in patients with growth disorders. *Circles* represent IGF1 levels in normal subjects (A and D), normal short stature subjects (B and E), and growth hormone–deficient subjects (C and F). The *lines* represent the 5th, 50th, 95th percentiles for log-normalized IGF1 levels in normal subjects. (From Rosenfeld RG, Wilson DM, Lee PD, et al. Insulin-like growth factors I and II in the evaluation of growth retardation. *J Pediatr.* 1986;109: 428–433.)



• **Fig. 25.41** Normal serum levels of insulin-like growth factor 1 (IGF1) for males (A) and females (B). *SD,* standard deviation. (Data courtesy Diagnostic Systems Laboratories, Inc., Webster, TX.)

of only 67% in those older than 10 years. Similarly, Cianfarani and colleagues[1061] found a specificity of 100% in children younger than 9 years of age that dropped to 76% in older children. Thus the overall specificity of a low IGF1 measurement may only be approximately 70%.[1044,1063,1064]

In addition to the specific challenges of accurately quantitating IGF1 levels in serum (see later discussion), the nutritional dependence of IGF1 levels significantly affects the accuracy of this test in evaluating for GHD. Even a few days of decreased caloric intake can lower IGF1 levels.[1044,1067] This probably contributes to the finding that IGF1 levels can vary by as much as 38% from day to day in a given patient.[1067,1068]

Early quantitation of IGFs used bioassays based on [^{35}S]-sulfate incorporation (hence "sulfation factor" as an early synonym for IGF/somatomedin C); on stimulation of the synthesis of DNA, RNA, or protein; or on glucose uptake.[1069,1070] Development of specific antibodies permitted the development of accurate and specific measurement of IGF1 and IGF2.[1058,1064,1071,1072] However, the presence of IGFBPs results in a significant technical challenge for the accurate quantitation of IGF1 (and IGF2) in serum.[620,1044,1069] Interference is a particular problem in conditions with a relatively high IGFBP/IGF peptide ratio and at the extremes of the assay (i.e., GHD, acromegaly). In uremia, IGFBPs artifactually lower IGF1 levels and increase IGF2 levels in assays that do not eliminate this interference.[749]

The most effective way to deal with IGFBPs is to separate them from IGF peptides by chromatography under acidic conditions.[1073] This labor-intensive procedure has occasionally been replaced by an acid ethanol extraction procedure.[1074] Although the latter method may be reasonably effective for most serum samples, it is problematic in conditions of high IGFBP/IGF peptide ratios, such as conditioned media from cell lines and sera from newborns or from subjects with GHD or uremia. A number of alternative approaches to addressing the interference from the IGFBs have been developed, including the use of tracers that do not bind to IGFBPs[1075] and "sandwich" assay methods.[1076] Automated IGF1 immunometric (IRMA) or immunochemiluminometric (ICMA) assays typically add an excess of IGF2 to the assay to displace the IGFBPs and use highly specific IGF1 antibodies.[1044,1069,1077] Although current IGF1 assays significantly minimize the interference from IGBPs, it is important that each assay develop reference ranges that match the clinical samples to be tested because ethnic variations and nutritional and environmental factors can affect "normal" serum IGF1 concentrations. However, even with assay-specific reference ranges, the variability in IGF1 assays can result in a lack of concordance across assays when levels are near the upper or lower edge of the reference range, potentially misclassifying the results as "normal" or "abormal."[1078]

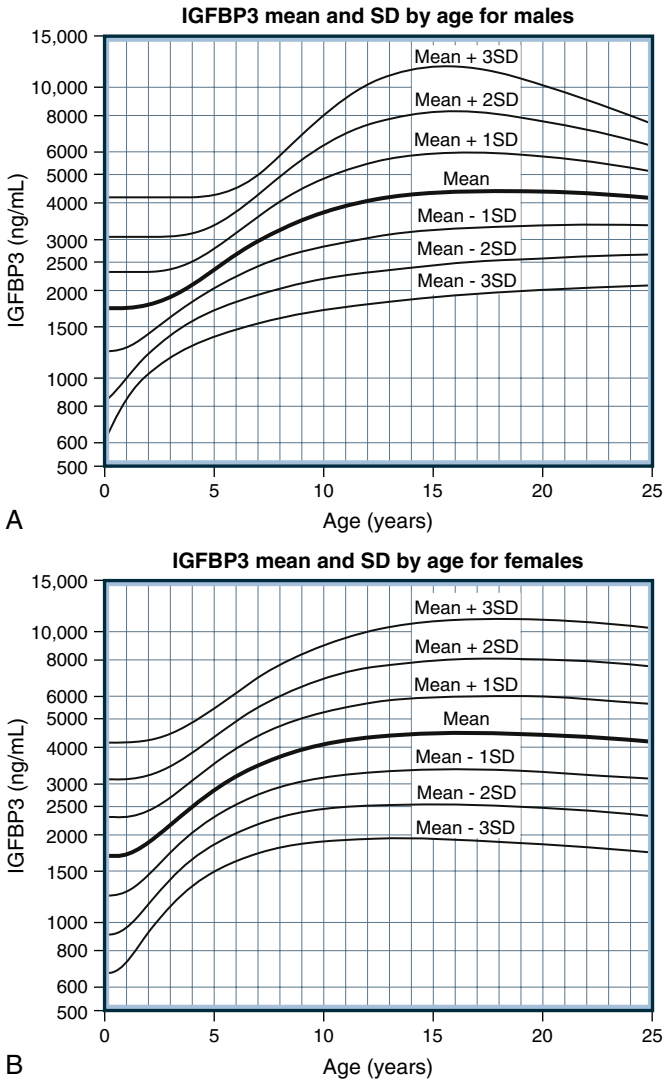
A number of assays have been developed that purport to measure "free" or "free dissociable" IGF1 as a means of assessing concentrations of IGF1 peptides that circulate unbound to IGFBPs.[1079] Both the accuracy and the physiologic relevance of these determinations remain open to debate.[240,1044,1079]

Insulin-Like Growth Factor–Binding Protein 3

Measurement of the serum level of IGBP3, the major serum carrier of IGF peptides, is a potential additional tool for evaluating GH function.[754,1080,1081] The advantages of assaying IGFBP3 concentration include the following:

1. IGFBP3 levels are GH dependent.
2. IGFBP3 levels are constant throughout the day.
3. The immunoassay of IGFBP3 is technically simple and does not require separation of the binding protein from IGF peptides.
4. Normal serum levels of IGFBP3 are high, typically in the range of 1 to 5 mg/L, so that assay sensitivity is not an issue. (The molar concentration of IGFBP3 approximates the sum of the molar concentration of IGF1 and IGF2.)
5. Serum IGFBP3 levels vary with age to a lesser degree than is the case for IGF1 (Fig. 25.42). Even in infants, serum IGFBP3 levels are sufficiently high to allow discrimination of low values from the normal range.
6. Serum IGFBP3 levels are less dependent on nutrition than serum IGF1, reflecting the "stabilizing" effect of IGF2 levels.

Like IGF1 levels, determination of the sensitivity and specificity of IGBP3 levels for identifying GHD suffers from the lack of a gold standard. With that limitation in mind, Blum and colleagues[754] initially found that low IGFBP3 levels had both high sensitivity (97%) and high specificity (95%) for GHD. Most subsequent studies confirmed the high specificity, with values generally greater than 80% to 90%.[1044,1061,1063,1065,1066] However, most



• **Fig. 25.42** Normal serum levels of insulin-like growth factor–binding protein 3 (IGFBP3) for males (A) and females (B). (Data courtesy Diagnostic Systems Laboratories, Inc., Webster, TX.)

studies have found that many children diagnosed with GHD on the basis of provocative GH testing actually have normal IGFBP levels, and the sensitivity of this test is less than 60%. In a study by Cianfarani and colleagues,[1061] only 7% (2/28) of children younger than 14 years of age with GHD had an IGFBP3 level more than 1 SD below the mean. Low IGFBP3 levels may be an indicator of more severe GHD. For example, in one study the sensitivity of the IGFBP3 assay was 93% if the peak GH was less than 5 μg/L on provocative testing, but when the peak GH was 5 to 10 μg/L, the sensitivity was only 43%.[1082]

Insulin-Like Growth Factor 2

IGF2 levels are higher than those of IGF1. Levels increase rapidly after birth, but thereafter IGF2 levels are less age dependent than those of IGF1. However, although GHD is associated with low IGF2 levels, IGF2 is much less GH dependent than IGF1 (Fig. 25.43). In two studies, IGF2 levels were more than 2 SD below the mean in only 21% and 31% of children defined as GH deficient based on provocative GH testing.[1061,1083]

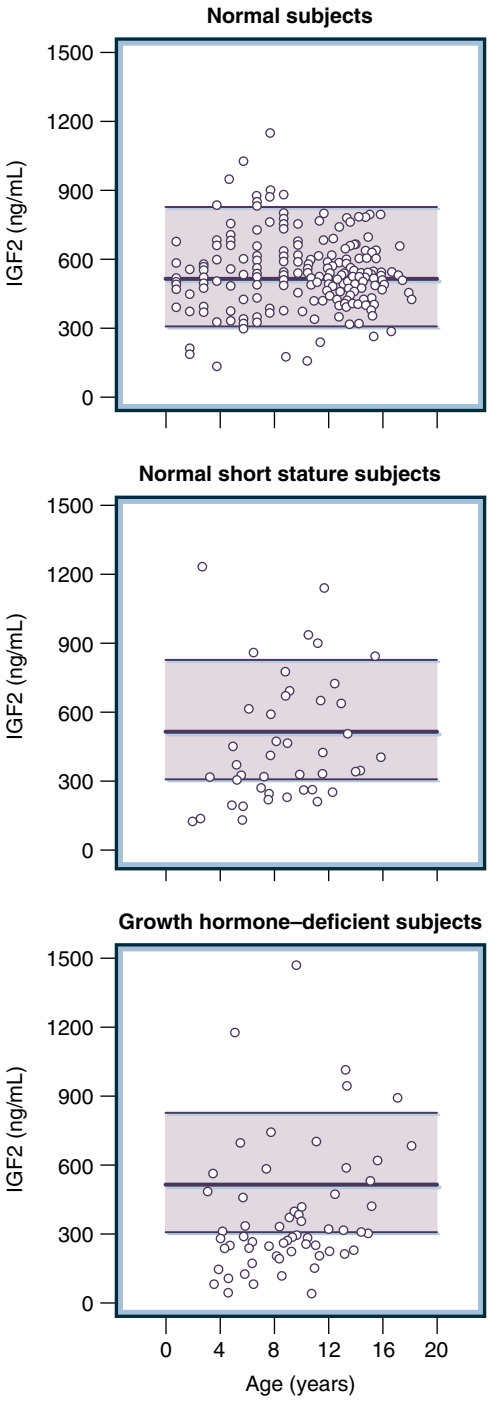
Rosenfeld and colleagues[1064] found low IGF2 levels in 52% of GH-deficient children and in 35% of normal short children. However, only 4% of GH-deficient children and 11% of normal short children had normal plasma levels of both IGF1 and IGF2, showing a similar sensitivity and specificity to combined IGF1/IGFBP3 testing.

Growth Hormone

Assessment of pituitary GH production is difficult because GH secretion is pulsatile, with the most consistent surges occurring at times of slow-wave electroencephalographic rhythms during stage 3 and stage 4 of sleep. Spontaneous GH secretion varies with gender, age, pubertal stage, and nutritional status, all of which must be factored into the evaluation of GH production.

Between normal pulses of GH secretion, serum GH levels are low (often <0.1 μg/L), below the limits of sensitivity of most conventional assays (typically <0.2 μg/L). Accordingly, measurement of random serum GH concentrations is almost useless in diagnosing GHD but may be useful in the diagnosis of GH insensitivity and GH excess. Measurement of GH "secretory reserve" relies on the use of physiologic or pharmacologic stimuli, and such provocative tests have formed the basis for diagnosis of GHD for more than 40 years.[1084,1085]

Assay Limitations. One of the biggest confounders in the evaluation of GH secretion is the variability of measured GH levels across different assays. Many studies have demonstrated as much as threefold variability in the measurement of serum GH levels among established laboratories.[1086,1087] A more recent study found an interassay coefficient of variation of 24.3% among nine assays for values within the range of 5 to 10 μg/L and a maximal difference for a given sample as high as 11.4 μg/L.[1088] This variability is explained, in part, by the presence of several molecular forms of GH in serum. Circulating GH consists of monomers of the two secreted GH isoforms (with ~43% existing as the monomeric 22-kDa isoform and 8% as the monomeric 20-kDa isoform), along with dimers and higher-order oligomers of the two isoforms, acetylated forms of GH, desamidated GH, and peptide fragments of GH.[1044] Therefore much of the variability in results is due to the use of different monoclonal or polyclonal antibodies, including variability in recognition of the different circulating forms of GH by these antibodies. Additionally, variations in the choice of standards, labeling techniques, and assay buffers



• **Fig. 25.43** Serum insulin-like growth factor 2 (IGF2) levels in normal patients and in patients with growth disorders. (From Rosenfeld RG, Wilson DM, Lee PD, et al. Insulin-like growth factors I and II in the evaluation of growth retardation. *J Pediatr.* 1986;109:428–433.)

(matrix) are also contributory.[1044] Consequently, a child who is determined to have GHD by one assay may be considered normal by another.[1088]

Provocative Tests. Because random GH levels cannot be used to diagnose GHD, evaluation of GH secretion requires that samples be obtained after an expected stimulation of GH secretion. Physiologic stimuli include fasting, sleep, and exercise, and pharmacologic stimuli include levodopa, clonidine, glucagon,

TABLE 25.11 Tests to Provoke Growth Hormone Secretion

Stimulus	Dosage	Times Samples Are Taken (min)	Comments
Sleep	Obtain sample from indwelling catheter	60–90 min after onset of sleep	
Exercise	Step climbing; exercise cycle for 10 minutes	0, 10, 20	Observe child closely when on the steps
Levodopa	<15 kg: 125 mg 10–30 kg: 250 mg >30 kg: 500 mg	0, 60, 90	Nausea, rarely emesis
Clonidine	0.15 mg/m^2	0, 30, 60, 90	Tiredness, postural hypotension
Arginine HCl (IV)	0.5 g/kg (max 30 g) 10% arginine HCl in 0.9% NaCl over 30 min	0, 15, 30, 45, 60	
Insulin (IV)[a]	0.05–0.1 unit/kg	0, 15, 30, 60, 75, 90, 120	Hypoglycemia, requires close supervision
Glucagon (IM)	0.03 mg/kg (max 1 mg)	0, 30, 60, 90, 120, 150, 180	Nausea, occasional emesis
GHRH (IV)[b]	1 μg/kg	0, 15, 30, 45, 60, 90, 120	Flushing, metallic taste

Patients must be euthyroid at the time of testing. Tests should be performed after an overnight fast. For prepubertal children, pretreating with sex hormones increases the specificity of the tests (see text).

[a]Insulin-induced hypoglycemia is a potential risk of this procedure, which is designed to lower the blood glucose by at least 50%. Documentation of appropriate lowering of blood glucose is recommended. If GHD is suspected, the lower dosage of insulin is usually administered, especially in infants. A solution of 10% dextrose in water ($D_{10}W$) and glucagon should be available.

[b]The cutoff used for tests involving GHRH is higher than that for other tests. (Maghnie M, Cavigioli F, Tinelli C, et al. GHRH plus arginine in the diagnosis of acquired GH deficiency of childhood-onset. *J Clin Endocrinol Metab*. 2002;87:2740–2744; Pandian R, Nakamoto JM. Rational use of the laboratory for childhood and adult growth hormone deficiency. *Clin Lab Med*. 2004;24:141–174.)

propranolol, arginine, and insulin. Standard provocative GH tests are summarized in Table 25.11. GH levels are measured on multiple specimens obtained after the stimulus. Failure to increase serum GH above a defined cutoff level is believed to indicate GHD.

Although provocative GH testing has been the foundation for the diagnosis of GHD since GH assays first became available, they have a number of weaknesses when used to identify GHD.[1084,1089,1090] Most important, there is no clear cutoff level that discriminates a normal response from a deficient response; second, these provocative tests have poor specificity.

Determination of the "Subnormal" Response to Provocative Tests. GH secretion has a continuous distribution; there is not a bimodal distribution of either spontaneous GH secretion or peak GH levels after provocative tests that would clearly separate normal from deficient secretion. The initial levels of GH during provocative testing that were used to define GHD were based on the study of patients with profound classic findings or organic destruction of the adenohypophysis.[1091] In addition, partly because of the limited supply of GH, one goal was to identify the most severely affected children.

Initially, a cutoff level of 2.5 μg/L was used; this was later increased to 5 μg/L and subsequently to 7 μg/L. After the development of recombinant DNA-derived GH eliminated the limits on GH supply, many pediatric endocrinologists considered a peak GH level lower than 10 μg/L to be indicative of GHD. The higher cutoff level can be partially justified by the desire to identify children with GHD that is less complete (i.e., partial GHD). Nonetheless, there have been few data validating the higher cutoff values.[1090,1092]

Further confounding the determination of an evidence-based cutoff level is the variability in GH levels measured across different assays (discussed earlier). Many new GH assays give results that are 33% to 50% lower than earlier assays, but there has not been a systematic reassessment of the "new normal" GH cutoff levels, nor has there been a critical evaluation by many endocrinologists of which assay their center might be using.[1093,1094] The same cutoff level has been applied without regard to the stimulus used. While insulin and arginine result in similar GH peaks, clonidine[1095] and GHRH[1096–1098] stimulate significantly higher levels of GH, thereby indicating a need for a higher cutoff value for such tests.

Specificity of Provocative Tests for Growth Hormone Deficiency. The data that are available suggest a low specificity for the provocative tests, with a substantial number of normal children having peak GH levels lower than 7 to 10 μg/L.[1089] Ghigo and colleagues[1097] studied 472 healthy, normal children, including 296 with normal stature and 177 with normal short stature. Excluding tests that used GHRH (which has generally not been used in the evaluation of GH function in children), they found that between 10% and 25% of their subjects had peak GH levels lower than 7 μg/L and 23% to 49% had peak levels lower than 10 μg/L. Similar results were found in other studies,[1099–1102] with the 5th percentile for peak GH response to most provovactive stimuli being less than 5 μg/L in normally growing children.[1103] Because of this poor specificity, failure on two provocative tests should be obtained before GHD is diagnosed on the basis of provocative testing. This approach significantly improves the specificity of provocative testing, although it remains imperfect. In the study of Ghigo and colleagues,[1097] two tests were performed on

78 children, and 10% had peak GH levels lower than 10 μg/L on both tests (2.6% had peak GH levels <7 μg/L on both tests).

The specificity of provocative GH tests can be increased by using a lower cutoff point to define a normal response. However, this is undesirable if it excludes individuals with less severe degrees of GHD. Because of the continuous nature of GH secretion across individuals and the lack of a gold standard test for GHD, the conflict between specificity and sensitivity cannot be completely resolved. However, two reports have examined multiple clinical and laboratory characteristics of short children who were divided into three groups based on their results on GH stimulation testing: a group whose results were low (<5 or <7 μg/L), fulfilling criteria for GHD; a group whose results were greater than 10 μg/L and therefore were believed not to have GHD; and a group whose results were intermediate between these high and low cutoff points. In both of these studies, the group with the lowest peak GH levels differed significantly from both the intermediate-level and the high-level GH groups on numerous measures.[1104,1105] However, the intermediate group was indistinguishable from the group without GHD, rather than having characteristics intermediate between the other two groups, as might be expected if they had a less severe degree of GHD. As noted earlier, 5 μg/L is the 5th percentile for GH levels on provocative testing of normally growing children; this same level of 5 μg/L identifies those children who will have the highest first-year growth response to GH treatment.[1103] Finally, a recent study using clonidine as the stimulus identified 3 μg/L (on an immunochemiluminescent assay) as the optimal cutoff to identify GHD in children and adolescents,[1106] while another identified cutoffs of 5.1 to 6.8 μg/L depending on the stimulus, with sensitivities of 88% to 93% and specificities of 92% to 97%.[1107]

Sex Hormone Priming. Serum GH levels rise during puberty, with GH secretion stimulated by the rise in estrogen produced from the ovary or from aromatization of testosterone.[152,1108] This same process results in higher peak GH levels during provocative testing in pubertal children (compared with prepubertal children)[1100,1109] and in children who have received treatment with estrogen or testosterone.[1057,1100,1109–1111] In a study by Marin and colleagues,[1100] 61% of normal prepubertal children and 44% of normal children in early puberty (Tanner stage II) had GH levels lower than 7 μg/L on provocative testing; based on these results (but not on their heights), they would have met criteria for a diagnosis of GHD. However, after 2 days of treatment with estrogen, 95% of these children had a peak GH level higher than 7 μg/L. Therefore the specificity of GH provocative tests can be improved by pretreating ("priming") the pediatric patients with exogenous gonadal steroids.[1112,1113] In a placebo-controlled comparison, the specificity of GH provocative tests (using 9 μg/L as a cutoff point on a polyclonal GH assay) increased from 80% to 98% after estrogen priming.[1057] In a study of 50 growth-retarded boys who had subnormal results on provocative GH tests without testosterone priming but normal results after priming, final height (without intervention) was greater than the midparental height, consistent with normal GH function in these children.[1114] The most recent guidelines from the Pediatric Endocrine Society recommends sex hormone priming in prepubertal boys older than 11 years and in prepubertal girls older than 10 years.[1103]

Another factor to consider when evaluating provocative GH testing is the impact of body weight on GH secretion. In both adults[144,1115–1117] and children,[1118,1119] obese individuals have decreased spontaneous and stimulated GH levels, compared with nonobese individuals. Even within the normal range, the BMI SDS is inversely associated with peak GH level on provocative tests in children.[1120] Therefore particular care must be taken when interpreting GH stimulation test results in obese individuals.

Although there are limitations to the information gained from provocative GH tests, they continue to be helpful in evaluating a child for GHD. The tests should be performed after an overnight fast, and the patient needs to be euthyroid at the time of testing. Testing should not be performed if the patient is taking supraphysiologic doses of glucocorticoid (i.e., >15 mg/m^2 per day of hydrocortisone or the equivalent of a synthetic glucocorticoid) because these drugs can suppress the GH response. The tests are generally safe, although appropriate precautions must be taken. Specifically, tests involving insulin administration carry the risk of hypoglycemia and seizures and should be performed only by experienced medical personnel and under appropriate patient supervision. Deaths have been reported from insulin-induced hypoglycemia and from its overly vigorous correction with parenteral glucose.[1121] The specificity of the tests can be increased by pretreating the child with estrogen or testosterone (e.g., 1–2 mg of micronized estradiol[1057] or 50–100 μg/day of ethinyl estradiol for 3 consecutive days before testing, or 100 mg of depot testosterone 3–7 days before testing) and by carefully selecting the lower limit for a normal response.

Tests of Spontaneous Growth Hormone Secretion. Another diagnostic approach to evaluate GH secretion involves measurement of spontaneous GH secretion. This can be done either by multiple sampling (every 5–30 minutes) over a period of 12 to 24 hours or by continuous blood withdrawal over 12 to 24 hours.[103,1122–1124] The former method allows one to evaluate and characterize GH pulsatility, whereas the latter only permits determination of the mean GH concentration. Both methods are subject to many of the same limitations as provocative GH testing. The problems of expense and discomfort are obvious, and, although it was thought that this approach might be more reproducible than provocative GH tests, variability remains a problem.[1095,1125,1126] The ability of such tests to discriminate between children with GHD and those with normal short stature is very limited because of significant overlap of each of the parameters measured between normal children and children with GHD. Rose and coworkers[1127] reported that measurement of spontaneous GH secretion identified only 57% of children with GHD as defined by provocative testing. Similarly, Lanes[1128] reported that one-fourth of normally growing children have low overnight GH levels, and a longitudinal study of normal boys through puberty demonstrated a wide intersubject variance, including many "low" 24-hour GH production rates in children with fully normal growth.[152,1129] Therefore measurement of spontaneous GH secretion does not appear to offer advantages over alternative means of evaluating GH function.

Summary. Despite the many problems associated with GH measurement methods, there continues to be value in determining GH secretory capacity in the diagnostic evaluation of a child with growth failure. Documentation of GH levels as being decreased, normal, or increased helps in discriminating among GHD, non–GH/IGF1-related growth failure (including ISS), and GH insensitivity. Results supporting the presence of GHD alert the clinician to the possibility of other pituitary deficiencies. The presence of pituitary dysfunction mandates clinical and radiologic evaluation for evidence of congenital or acquired structural defects of the hypothalamus or pituitary, including the possibility of intracranial tumors. Finally, documentation

of GHD, either alone or combined with other pituitary deficiencies, may warrant evaluation for molecular defects of GH production.

Growth Hormone–Binding Protein

Mutation of the GHR impairs GH signaling, resulting in GH insensitivity. The most severe mutations cause profound growth failure (Laron dwarfism). The extracellular portion of the GHR is cleaved from the remaining portion and circulates in the blood as GHBP, which can be measured in serum. Low levels, particularly undetectable levels, may be diagnostic of GH insensitivity due to GHR mutations.

GHBP levels are not low in all forms of GH insensitivity. The levels may be normal, or even increased, with mutations in the GHR that do not alter the GHBP portion of the protein (i.e., mutations in the transmembrane or intracellular domains) or with defects that are downstream of the GHR.

IGF1 and IGFBP Generation Tests

IGF1 and IGFBP3 generation tests are designed to evaluate for the presence of GH insensitivity.[1130] When patients with GH insensitivity are treated with GH for several days, the levels of IGF1 and IGFBP do not increase as they would in normal individuals.[1131,1132] Criteria for a response indicating GH insensitivity have included a rise in IGF1 of less than two times the intraassay variation (~10%)[617] or a failure to increase IGF1 by at least 15 μg/L.[750] Given the different protocols and cutoffs used to adequate define IGF1 generation, the recent Pediatric Endocrine Society guidelines suggest caution when using this diagnostic test and to combine it with other diagnostic tests, including genetic testing.[1103]

Interpretation of Tests

Neonate

GH levels in the neonate are much higher than levels seen after this period. Levels are highest in cord blood and within the first days of life.[1133] Cornblath and colleagues reported in 1965 an average GH concentration in cord blood of 66 μg/L; this fell over the first week to an average of 16 to 20 μg/L in infants 7 to 55 days of age.[1133] However, even under the assay conditions used, the range of levels reported included measurements as low as 1 μg/L. Subsequent studies confirmed the high GH levels in neonates, with many documenting cord blood levels in the range of 20 to 40 μg/L, although other studies using similar assays found levels of 13 to 18 μg/L.[1134] Other studies also confirmed that GH levels fall during the first week of life so that low basal values are seen by 1 to 2 months of age.[1134,1135] GH levels in preterm infants have generally been found to be even higher than those in full-term infants.[1133]

The neonatal period is one period during which random measurement of GH levels may be useful. However, low values can be found in healthy neonates.[1135] Therefore, although a high value can exclude GHD, a single low value is not diagnostic of GHD—except, perhaps, for the first few days of life in infants with a high probability of GHD.[1103] An IGFBP3 measurement is of value for the diagnosis of neonatal GHD and should be measured in infants with suspected GHD; IGF1 levels are rarely helpful.[1136]

Growth Hormone Deficiency

Both clinical and laboratory evaluation must be utilized when considering the diagnosis of GHD in a child being evaluated because of short stature or growth failure.[1056] As discussed earlier, there is no definitive test for the diagnosis for GHD. In addition, the laboratory tests for GHD have poor specificity and should be performed only in children who have a clinical presentation consistent with GHD. Short children who have well-documented normal height velocities usually do not require evaluation of GH function. Therefore the evaluation starts with identifying those children who may have GHD based on risk factors or growth parameters (see Tables 25.8 and 25.9).

In a child with a history and growth pattern that indicate a risk for GHD and in whom other causes for growth failure (including hypothyroidism) have been excluded, testing for GHD begins with measurement of the IGF1 level and proceeds to GH provocation tests in selected patients (see Table 25.8). In some cases, particularly in younger children, it may also be helpful to measure the IGFBP3 level. If IGHD is suspected, two GH provocation tests (given sequentially or on separate days) are required. A patient is diagnosed with classic GHD when the IGF1 (or IGFBP3) level is below the normal range (i.e., >2 SD below the mean for age and pubertal status) and peak GH levels on two provocative tests are below the cutoff value that supports a diagnosis of GHD. The diagnosis is more firmly established if the child is pretreated with estrogen or testosterone before the provocative GH tests. In some patients, provocative testing of GH levels is not required to make the diagnosis. This includes children with growth failure and a pituitary hormone deficiency other than growth hormone in the setting of a known risk of hypopituitarism, such as anatomic abnormalities of the pituitary (such as ectopic posterior pituitary and pituitary hypoplasia with an abnormal stalk), tumor, or irradiation.[1103] Similarly, a diagnosis of GHD due to congenital hypopituitarism can be diagnosed if random GH levels are below 5 ng/dL in the first week of life in an infant with hypoglycemia and either one other pituitary hormone deficiency or classic imaging findings (ectopic posterior pituitary and pituitary hypoplasia with abnormal stalk).[1103] In patients who have had cranial irradiation or malformations of the hypothalamic-pituitary unit, GHD may evolve over years, and its diagnosis may require serial testing.

Some patients with auxology suggestive of GHD may have IGF1 or IGFBP3 levels below the normal range on repeated tests but GH responses in provocation tests above the cutoff level. Such children do not have classic GHD but may have an abnormality of the GH-IGF1 axis. The child with a history of cranial irradiation, decreased height velocity, and reduced serum levels of IGF1 and IGFBP3 may have GHD (or GH insensitivity) even in the face of normal provocative tests.[1137] Other children may have GHD that is not supported by the results of nonphysiologic provocative tests (perhaps a milder degree of GHD than in those who fail the provocative tests), or they may have GH insensitivity (discussed later). Systemic disorders affecting the synthesis or action of IGF1 must again be considered and excluded.

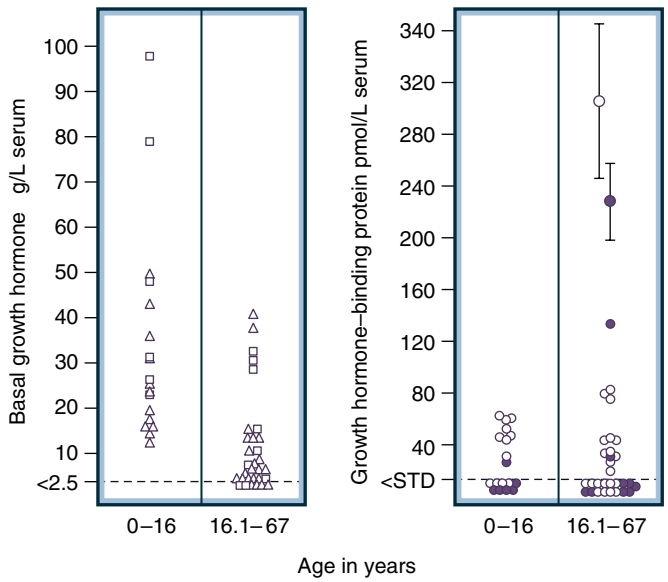
A difficult clinical situation to resolve is that of the short child with a persistently subnormal growth velocity whose IGF1 and IGFBP3 levels are normal. One can reasonably exclude consideration of GHD if the IGF1 level is higher than 1 SD below the normal mean. However, because up to 30% of children with GHD identified by GH stimulation testing have had IGF1 levels that were not low,[1044,1063–1066] it is appropriate to consider provocative GH testing in those children with persistent growth failure who have an IGF1 level between –1 SD and –2 SD for age and puberty status, with abnormal results on two tests indicating a diagnosis of GHD. In this situation in particular, it would be appropriate to pretreat the child with testosterone or estrogen to maximize the specificity of the provocative tests.

A cranial MRI scan with particular attention to the hypothalamic-pituitary region should be carried out in any child who is diagnosed as having GHD. In addition, documentation of abnormal pituitary GH secretion should prompt evaluation for other pituitary hormone deficiencies. Based on the clinical scenario, one may also consider molecular evaluation of GH, GHR, GHRHR, and other potential genetic defects.

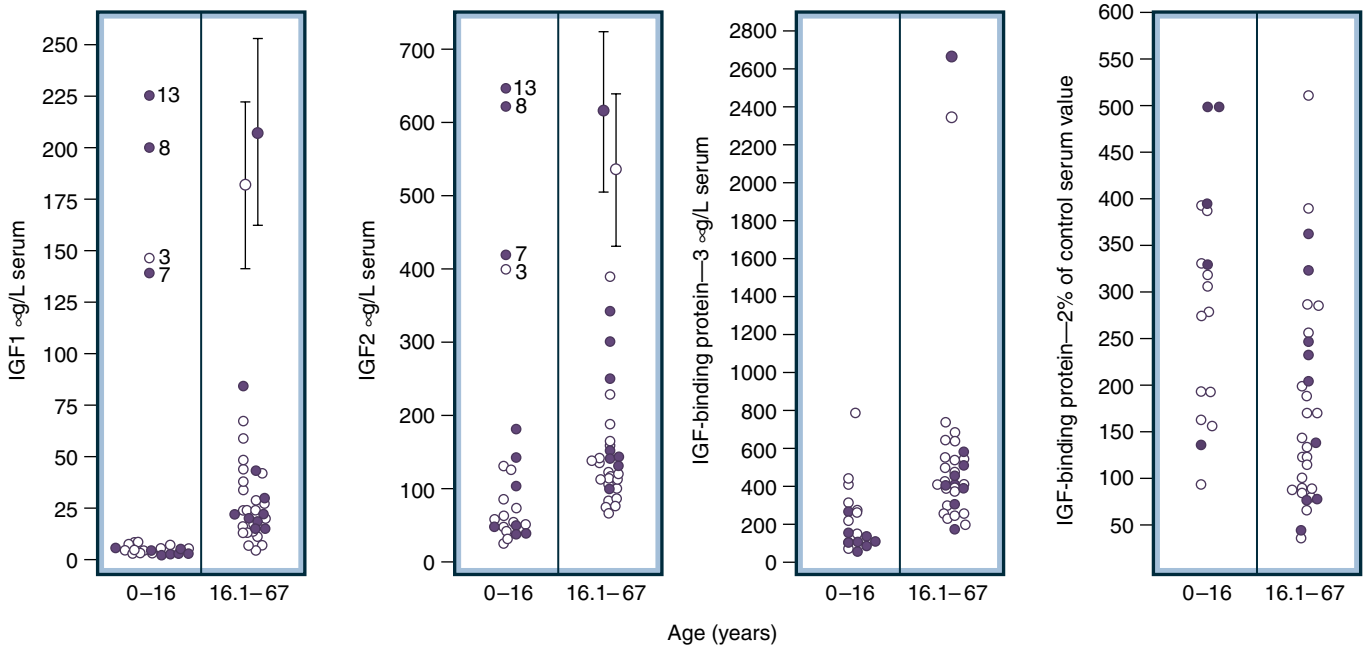
Growth Hormone Insensitivity

GH insensitivity is characterized by low serum IGF1 concentrations in the presence of normal (or increased) production of GH (Figs. 25.44 and 25.45). As discussed earlier, GH insensitivity can be caused by defects of the GHR (Laron syndrome), the GH signaling cascade, IGFBPs, IGF1, or the IGF1 receptor. GH insensitivity should be considered in a patient with growth failure who has a low IGF1 level but evidence of increased GH secretion based on increased basal or stimulated GH levels (e.g., basal GH levels >5 μg/L or stimulated levels >15 μg/L). This pattern is also seen with malnutrition, a form of physiologic GH insensitivity. If GH insensitivity is suspected, GHBP and ALS can be measured: Low values support the diagnosis of GH insensitivity due to mutations in the genes for the GHR or ALS, respectively. Growth attenuation with elevated IGF1 levels and IGFBP3 levels may indicate disruption in IGF1 bioavailability, as is seen in persons with mutation in the PAPP-A2 gene.[298]

The Pediatric Endocrine Society Guidelines suggest basing the diagnosis of primary IGF1 deficiency/GH insensitivity syndrome on a combination of factors, including testing of serum IGF1 and GHBP, exclusion of secondary IGF1 deficiency due to malnutrition, liver disease, of GH deficiency, and possibly IGF1 generation test.[1103] The IGF1 generation test has limitations and must be interpreted cautiously as there is limited normative data or standardization of such tests. Given the growing cadre of genes known to be involved in the GH-IGF1 growth cascade, genetic testing may play a vital role in diagnosis in the near future. However, there are currently few clinical laboratories offering mutation analysis of the genes involved in the GH-IGF1 growth cascade.



• **Fig. 25.44** GH assays were performed by radioimmunoassay *(squares)* and by immunoradiometric assay *(triangles)*. For GHBP levels, *open circles* represent females, and *closed circles* represent males. Standard error of the mean (±SD) for GHBP levels in adult Ecuadorian control subjects are shown. *STD,* Assay standard. (From Rosenfeld RG, Rosenbloom AL, Guevara-Acquirre J. Growth hormone [GH] resistance due to primary GH receptor deficiency. *Endocr Rev.* 1994;15:369–390.)



• **Fig. 25.45** Serum levels of insulin-like growth factors (IGF) and IGF-binding proteins in patients from Ecuador with growth hormone receptor deficiency. *Open circles* represent females, *closed circles* represent males. Control values for Ecuadorian adults are shown as mean ±SD for males and females, except for IGFBP3, for which large circles represent pooled male *(closed circle)* and female *(open circle)* control sera. Numbers adjacent to circles represent normal Ecuadorian children 3, 7, 8, and 13 years of age. (From Rosenfeld RG, Rosenbloom AL, Guevara-Acquirre J. Growth hormone [GH] resistance due to primary GH receptor deficiency. *Endocr Rev.* 1994;15:369–390.)

Additional considerations related to GH insensitivity include the following:

1. The presence of IUGR (in addition to severe postnatal growth attenuation) and developmental delay suggests *IGF1* gene deletions, bioinactive IGF1, or IGF type I receptor abnormalities.
2. Low GHBP suggests a defect in the extracellular domain of the GHR, but normal (or increased) GHBP may be seen in some patients with defects of the GHR or the GH signaling cascade.
3. An elevated IGF1 level (in a child with growth attenuation) may suggest a defect in the IGF type I receptor or defects in PAPP-A2.
4. IGFBP3 and ALS concentrations may be increased in patients with molecular defects of IGF1.
5. STAT5B defects should be suspected in a child with evidence of GH insensitivity who has evidence of immune deficiency; an elevated PRL level has also been reported in the presence of STAT5B mutation.[1138]

Constitutional Delay of Growth and Development

The term *constitutional delay of growth and development* describes children who have a normal variant of maturational tempo characterized by short stature with relatively normal growth rates during childhood, delayed puberty with a late and attenuated pubertal growth spurt, and attainment of a normal adult height that is also within the target height range based on parental heights (Table 25.12).[39,1139] In childhood, such patients have heights that are lower than expected based on parental heights. CDGD aggregates in families.[1140] The diagnosis of CDGD may be suspected in a child with short stature if one or both parents have a history of a late timing of puberty. Bone age is usually delayed, so the predicted final height is in the normal range and is within the child's target height range, although the correlation between predicted and final height is imperfect and must be viewed with caution.[44,1141,1142] The predicted final height, especially when the skeletal age is extremely delayed, is greater than that usually achieved but is difficult to reliably anticipate.[44,1142,1143] Although the findings described can lead to a presumptive diagnosis of CDGD, the diagnosis can only be made retrospectively, once the child demonstrates a late timing for puberty and completes his or her growth in the normal range and at a height consistent with the parents' heights.

Because of the estrogen stimulation associated GH secretion that occurs during puberty, children with CDGD can be expected to have decreased GH secretion and lower IGF1 levels compared with their pubertal, age-matched peers. However, IGF1 levels should be normal for pubertal stage, and GH levels on provocative tests should be normal after pretreatment with gonadal steroids.[1112,1143–1145] Overnight GH secretion is usually normal in these children when control groups are carefully matched.[1146]

It is often difficult to differentiate CDGD from GHD. Both groups of patients have height SDS below their target height SDS range, and both have delayed bone ages. As the child with CDGD enters the usual age for puberty, his or her growth rate can be too low to exclude GHD on that basis. (The growth rate normally declines throughout childhood until it increases at the time of the pubertal growth spurt; a child with CDGD and a delayed pubertal growth spurt may have an additional 2 or more years of declining growth velocity, which can result in growth velocities <4 cm/year.) If there is a clear family history of CDGD, the likelihood is high that the child also has CDGD, and careful continued observation may be all that is needed. An increase in growth velocity after treatment with a short course of sex hormone can be taken as evidence against the presence of GHD.[1147] In some cases, however, a laboratory investigation to exclude GHD is necessary. In such children, levels of IGF1 and IGFBP3 that are low for pubertal stage or skeletal age and a poor GH response to provocative testing after priming with gonadal steroids would raise concern for GHD and would mandate the appropriate investigation for underlying disease (e.g., intracranial tumor).

TABLE 25.12 Criteria for Presumptive Diagnosis of Constitutional Delay of Growth and Development

1. No history of systemic illness
2. Normal nutrition
3. Normal physical examination, including body proportions
4. Normal thyroid and growth hormone levels
5. Normal complete blood count, sedimentation rate, electrolytes, blood urea nitrogen
6. Height ≤3rd percentile but with annual growth rate >5th percentile for age
7. Delayed puberty: in males, failure to achieve Tanner G2 stage by age 13.8 years or P2 by 15.6 years; in females, failure to achieve Tanner B2 stage by age 13.3 years
8. Delayed bone age
9. Normal predicted adult height: in males, >163 cm (64 inches); in females, >150 cm (59 inches)

Genetic (Familial) Short Stature

The control of growth in childhood and the final height attained are polygenic in nature. Familial height affects the growth of an individual, and evaluation of a specific growth pattern must be placed in the context of familial growth and stature. As discussed at the beginning of this chapter, calculations can be made to determine whether a child's growth pattern is appropriate based on his or her parents' heights. As a general rule, a child who is growing at a rate that is inconsistent with that of siblings or parents warrants further evaluation.

Genetic short stature (GSS), also called familial short stature, is a normal growth pattern that describes the growth of healthy individuals who fall at the lower extreme of the distribution of height (i.e., below the 3rd percentile). Their height is appropriate for their genetic potential based on the parents' heights; that is, their height SDS is within the target height SDS range. Particularly when the midparental height is significantly above or below the mean, it is important to include adjustment for regression toward the mean when calculating this target height range.

Although the differentiation may not be complete, children with GSS are generally distinguished from children with CDGD: Those with GSS will have a final height that is below the 3rd percentile, whereas children with CDGD will achieve a final height in the normal range. The midparental height of children with GSS is lower than that of children with CDGD; often, height in both parents is below the 10th percentile. Growth in children with GSS is at or below the 3rd percentile, but the velocity is usually normal. The onset and progression of puberty are normal, so the skeletal age is concordant with chronologic age. Final heights in these individuals are in the target height range for the family.[43] By definition, the GH-IGF1 system is normal (as are all other systems).

Many diseases characterized by growth retardation are genetically transmitted, including GH insensitivity due to mutations

of the *GHR* gene; *GH* gene deletions; mutations of the *PROP1, POUF1,* or *SHOX* genes; pseudohypoparathyroidism; and some forms of hypothyroidism. Inherited nonendocrine diseases characterized by short stature include osteochondrodysplasias and dysmorphic syndromes associated with IUGR (both described earlier), inborn errors of metabolism, renal disease, and thalassemia. Identifying a patient's short stature as inherited does not, by itself, relieve the clinician of responsibility for determining the underlying cause of growth failure. Moreover, a parent's short stature may be the result of an uncharacterized genetic difference that has been transmitted to the child and is causing the child's short stature. Because height is normally distributed in the population, it can be arbitrary whether one characterizes such genetic differences as mutations or as allelic variants. However, the further the parents' and the child's heights are from the mean, the more reasonable it is to consider such genetic alterations as abnormal.

Idiopathic Short Stature

ISS is defined as "a condition in which the height of the individual is more than 2 SD below the corresponding mean height for a given age, sex and population group, and in whom no identifiable disorder is present."[1138] This definition includes children with CDGD, those with GSS, and short children who will not have delayed puberty and whose height is not consistent with parental heights. Therefore this definition includes both normal, healthy children (those with GSS and CDGD) and children who are presumed to have an unidentified disorder impairing their growth. It is not always a simple matter to distinguish among these possibilities: CDGD can be definitively diagnosed only at the completion of growth, and GSS does not exclude inherited disorders of growth.

Children with ISS may have undiagnosed disorders outside the GH-IGF1 axis (e.g., an uncharacterized chondrodystrophy) or they may have subtler disorders of the hypothalamic-pituitary-IGF axis than those identified by the currently available diagnostics tests.[640,1148] Because of the lack of a gold standard for the diagnosis of GHD, the distinction between isolated partial GHD and ISS is somewhat arbitrary, relying heavily on the results of the nonphysiologic provocative stimulation tests. The activity of different GH promoter haplotypes can differ as much as sixfold.[602,1149] Some children with ISS may have GH neurosecretory dysfunction that cannot be detected with current diagnostic tests.[1122,1123] Similarly, although the severe GH insensitivity of Laron syndrome can be identified by laboratory testing, partial GH insensitivity may be an unrecognized cause of ISS.[1150]

Heterozygous mutations in the GHR have been found in significant numbers of children with ISS.[639,642,1140] In heterozygotes, protein from the mutant allele may disrupt the normal dimerization and rotation that is needed for normal GHR activation, leading to diminished GH action and growth impairment.[215] In addition, GHBP expression may be decreased in patients with ISS, 20% of whom have serum levels of GHBP below the normal range.[1150–1152] Other potential causes for partial GH insensitivity in ISS include heterozygous mutations in other components of the growth system, a relatively greater preponderance of blockers of the GH-signaling cascade (e.g., enhanced intracellular phosphatase activity, production of such signaling factors as SOC2 and CIS), gene-mediated alterations in patterns of GH or IGF production, or other possibilities yet to be discovered.[1153]

Treatment of Growth Failure

When growth failure is the result of a chronic underlying disease, such as renal failure, CF, or malabsorption, therapy must first be directed at treatment of the underlying condition. Although growth acceleration may occur in such children with GH or IGF1 therapy, complete catch-up requires correction of the primary medical problem. If treatment of the underlying condition involves glucocorticoids, growth failure may be profound and is unlikely to be correctable until steroids are reduced or discontinued.

Correction of growth failure associated with chronic hypothyroidism requires appropriate thyroid replacement. As discussed earlier, thyroid therapy causes dramatic catch-up growth but also markedly accelerates skeletal maturation, potentially limiting adult height.

Treatment of Constitutional Delay

CDGD is a normal growth variant with delayed pubertal maturation and a normal adult height. Most subjects can be managed by careful evaluation to rule out other causes of abnormal growth or delayed puberty combined with appropriate explanation and counseling. A family history of CDGD is frequently a source of reassurance. The skeletal age and Bayley-Pinneau table are often helpful in explaining the potential for normal growth to the patient and parents. The predicted final height is usually greater than that achieved, especially when the skeletal age is extremely delayed, but this is difficult to reliably anticipate.[44,1141,1154]

On occasion, the stigmata of short stature and delayed maturation are psychologically disabling for a preadolescent or teenage patient. Some adolescents with delayed puberty have poor self-image and limited social involvement.[1155] In such patients and in some in whom pubertal delay is predicted based on the overall clinical picture, there may be a role for judicious short-term treatment with androgen.

Androgen (Oxandrolone and Testosterone)

Two aspects of CDGD in boys are addressed by androgen treatment: short stature, especially in boys 10 to 14 years of age, and delayed puberty after age 14. In the younger group, in whom CDGD is the presumed cause of short stature, the orally administered synthetic androgen oxandrolone has been used extensively to accelerate growth so that height increases into (or closer to) the normal range sooner than it would without treatment.[1156] In several controlled studies,[1139,1157–1161] oxandrolone therapy for 3 months to 4 years increased linear growth velocity by 3 to 5 cm/year without adverse effects and without decreasing either actual[1161–1163] or predicted[1158,1162,1164] final height. (This treatment does not increase the final height of these boys.) The growth-promoting effects of oxandrolone appear to be related to its androgenic and anabolic effects rather than to augmentation of the GH-IGF1 axis.[1165,1166] The lack of a measurable effect on the GH-IGF1 axis probably reflects the fact that oxandrolone cannot be aromatized to estrogen. Currently recommended treatment is 0.05 to 0.1 mg/kg orally per day.

Oxandrolone is a relatively weak androgen, and its use stimulates only minimal pubertal masculinization. In older boys in whom the delayed pubertal maturation is highly stressful and anxiety provoking, testosterone enanthate has been administered intramuscularly with success.[1156,1157,1167] Criteria for treatment in such adolescents should include (1) a minimum age of

14 years, (2) height below the 3rd percentile, (3) prepubertal or early Tanner G2 stage with an early-morning serum testosterone level of less than 3.5 nmol/L (<1 ng/mL), and (4) a poor self-image that does not respond to reassurance alone. Therapy consists of intramuscular testosterone enanthate, 50 to 100 mg every 3 to 4 weeks, for a total of four to six injections.[1155,1165,1167,1168] Patients typically show early secondary sex characteristics by the fourth injection and grow an average of 10 cm in the ensuing year. Testosterone enhances growth velocity by direct actions, increases GH production, and may have a direct effect on IGF1 secretion.[970,1108,1165,1169–1171] Brief testosterone regimens do not cause overly rapid skeletal maturation, compromise adult height, or suppress pubertal maturation.[1172] It is important to emphasize to the patient that he is normal, that therapy is short term and is designed to provide some pubertal development earlier than he would on his own, and that treatment will not increase his final adult height. In such situations, the combination of short-term androgen therapy, reassurance, and counseling can help boys with CDGD to cope with a difficult adolescence.

The availability of several new forms of testosterone, which are approved for adults with hypogonadism, provides adolescents with an opportunity for a choice among different androgen replacement therapies. Testosterone gel is painless and easy to apply and has proved popular since its release.[1173] However, there are concerns about environmental contamination, including reports of precocious puberty in children caused by topical testosterone use by an adult in the household.[1174–1176] If topical testosterone is prescribed, careful instruction must be given to avoid such inadvertent exposure of others. Testosterone patches also avoid the need for injections, but they are often poorly tolerated because of local skin reactions. The most recent testosterone products include a solution applied in the axilla and a nasal gel. Another disadvantage of these topical testosterone preparations and the transdermal patches is the need for daily application. The dosing of these alternative forms of therapy in children and adolescents has not been established, and care must be taken to avoid treating with too high a dose, which risks compromising final height.

Patients must be reevaluated to ensure that they enter "true" puberty. One year after testosterone treatment, boys should have testicular enlargement and a serum testosterone level in the pubertal range. If this is not the case, a diagnosis of hypothalamic-pituitary insufficiency or hypogonadotropic hypogonadism should be considered. Although the diagnosis of constitutional growth delay remains most likely in such patients, some eventually prove to be gonadotropin deficient, especially if they are still prepubertal late in adolescence.

Referrals for CDGD are more common in boys than girls. When CDGD is a problem in girls, short-term estrogen therapy may be used, but the acceleration of skeletal maturation is a greater hazard at doses that enhance growth velocity and sexual maturation.

Growth Hormone

The final height of a child with CDGD will be in the normal range and appropriate for the child's genetic potential. No treatment is needed for these children to achieve a normal height. However, as discussed earlier, the diagnosis of CDGD cannot be confirmed until a late puberty and normal height are achieved. Therefore it can be difficult in some cases to distinguish these children from children with ISS or GHD. In such cases, there may be uncertainty about whether the final height will be in the normal range, and treatment to try to increase final height may be considered. If laboratory studies support a diagnosis of GHD, then GH treatment would be appropriate. If there is no evidence of GHD, the consideration regarding treatment with GH would be the same as that regarding the use of GH to treat ISS (discussed later).

The Food and Drug Administration (FDA) indication for GH treatment includes in its definition of ISS the criterion that the child has "growth rate[s] unlikely to permit attainment of normal adult height." A child with CDGD, who is expected to achieve a normal adult height, would not fit within this definition. Nonetheless, partly because of the uncertainty of the diagnosis and the inability to perfectly predict final height, the database on outcomes of children treated with GH for ISS includes within it data on the treatment of children with CDGD. ISS GH treatment trials may include children with significant bone age delay,[1177,1178] at least some of whom have CDGD. For this reason, the outcome data for GH treatment of ISS (see later) can be taken as an indication of the expected outcome of GH treatment of CDGD. Moreover, because estimated final height gain with GH treatment of ISS is proportional to the bone age delay at the time of initiation of treatment,[1179] height gain with GH treatment of CDGD may be greater than that for treatment of ISS. However, a retrospective study specifically reporting the outcome of GH treatment in CDGD found no difference in adult height between those treated with GH and those receiving either no treatment or testosterone treatment.[1180]

Aromatase Inhibitor

In view of the important role of estrogen in the process of skeletal maturation, aromatase inhibitors could be used in conjunction with androgen therapy to prevent an acceleration of bone age and further enhance final adult height.[1181,1182] The one report of combined use of an aromatase inhibitor (letrozole) and testosterone did not provide a clear answer as to whether the addition of an aromatase inhibitor increased final height in boys with CDGD. Whereas the near-final height in the boys treated with letrozole plus testosterone was higher than that of boys treated with testosterone alone, the letrazole-treated boys were a year older at the time of these height measurements and had higher pretreatment and midparental heights.[1182] In addition, the boys treated with testosterone alone achieved a near-final height within the normal range (consistent with the diagnosis of CDGD). Given the lack of long-term safety data on the use of aromatase inhibitors in pubertal boys, there are insufficient data to suggest a role for aromatase inhibitors in CDGD.

Treatment of Growth Hormone Deficiency

Nomenclature and Potency Estimation

The nomenclature for the various biosynthetic GH preparations reflects the source and the chemical composition of the product. *Somatropin* refers to GH of the same amino acid sequence as that in naturally occurring human GH. Somatropin from human pituitary glands is abbreviated *GH* or *pit-GH;* somatropin of recombinant origin is termed *recombinant GH* or *rGH. Somatrem* refers to the methionine derivative of recombinant GH and is abbreviated *met-rGH.* Although the latter is a more antigenic preparation, that propensity is not clinically relevant; despite the presence of anti-GH antibodies, growth responses to met-rGH were similar to those in patients treated with rGH.[1183,1184] This derivative of GH is no longer available for use. In this discussion, we refer to the biosynthetic preparations as GH.

The biopotency of commercially available biosynthetic GH preparations, expressed as international units per milligram of the second WHO recombinant GH reference reagent for somatropin 98/574, is 3 IU/mg.[1185] It was necessary to standardize the early GH preparations by bioassay because of variable production techniques (e.g., extraction, column purification). The most common bioassays have been the hypophysectomized rat weight-gain assay, the tibial width assay, and the more sensitive Nb2 rat lymphoma proliferation assay.[1186–1188] With the availability of purified and essentially equivalent recombinant GH products, the requirement for bioassays has become an FDA requisite to substantiate biologic activity rather than to assess potential differences among preparations. The bioassays are likely to be replaced by in vitro binding assays using GHRs or GHBP derived from molecular techniques.

Historical Perspective

Because untreated patients with GHD have profound short stature (averaging almost –5 SDS[1189,1190]), the clinical urgency to use GH therapy as soon as it became available is understandable.[1092] The action of GH is highly species specific, and humans do not respond to animal-derived GH (except that from other primates).[1191] Human cadaver pituitary glands were for many years the only practical source of primate GH for treatment of GHD, and more than 27,000 children with GHD worldwide were treated with pit-GH.[1192] The limited supplies of pit-GH, the low doses used, and interrupted treatment regimens resulted in incomplete growth increments; usually, therapy was discontinued in boys who reached a height of 5 feet 5 inches and in girls who reached 5 feet. Nonetheless, this treatment did increase linear growth and in many patients enhanced final adult height. The dose-response relationship and the relation of age to GH response were recognized during this period.[1193]

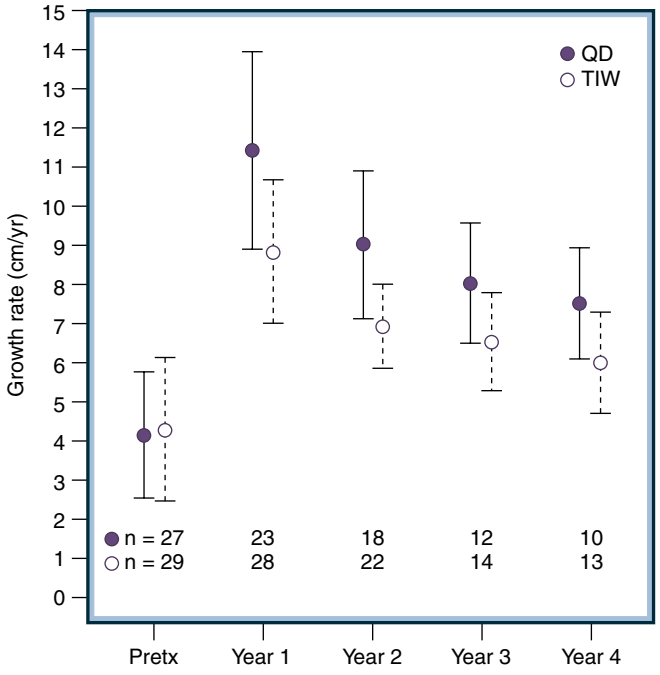
Distribution of pit-GH was halted in the United States and most of Europe in 1985 because of concern about a causal relationship with Creutzfeldt-Jakob disease (CJD), a rare and fatal spongiform encephalopathy that had been previously reported to be capable of iatrogenic transmission through human tissue. In North America and Europe, this disorder has an incidence of approximately 1 case per 1 million in the general population, and it is exceedingly rare before the age of 50 years. To date, more than 200 young adults who had received human cadaver pituitary products have been diagnosed with CJD, with the sad likelihood that all affected patients will die of the disease.[1194–1196] The onset of CJD has occurred 5 to 42 years after treatment, with a mean incubation of 17 years.[1196] No cases of CJD have been identified in Americans who began treatment after new methods of purifying the hormone came into use in the United States in 1977.

By the time the risks of pituitary-derived GH were discovered, biosynthetic GH was being tested for safety and efficacy.[1183,1197,1198] The original recombinant GH mimicked pit-GH in regard to both anabolic and metabolic actions and was scrupulously scrutinized for monoisomerism, antigenic bacterial products, and toxins of any sort. GH has universally replaced pit-GH as the accepted treatment for children with GHD.

Treatment Regimens

The recommended therapeutic starting dose of GH in children with GHD is 0.16 to 0.24 mg/kg body weight per week, administered in seven daily doses.[1103,1199] Alternative regimens include a 6-day/week and a 3-day/week schedule, with the same weekly dosage, but they are not as successful. In general, the growth response to GH is a function of the log-dose given, so increasing dosages further enhance growth velocities,[1192,1193,1200] but daily dosing may be the most important treatment parameter.[1201] Subcutaneous and intramuscular administration has equivalent growth-promoting activity[1202]; the former is now used exclusively. At this time, all of the commercially available GH preparations yield comparable growth outcomes.

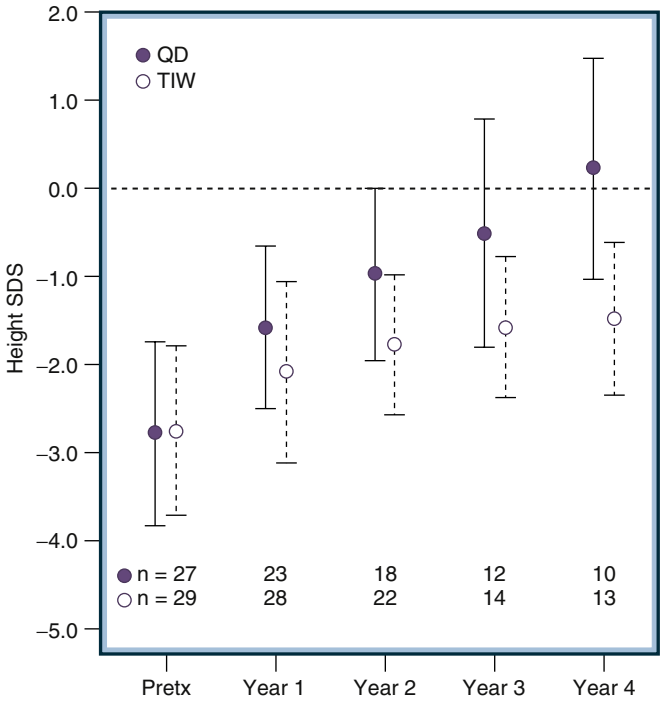
Growth responses to exogenous GH vary, depending on the frequency of administration, dosage, age (greater absolute gain in a younger child, although not necessarily greater growth velocity SDS), weight, GHR type and amount (as assessed by serum GHBP levels), and, perhaps, seasonality.[646,1203–1206] Nevertheless, the general regimen of daily GH at the recommended doses typically accelerates growth in a GH-deficient child from a pretreatment rate of 3 to 4 cm/year to 10 to 12 cm/year in year 1 of therapy to 7 to 9 cm/year in years 2 and 3. Progressive waning of GH efficacy occurs and is poorly understood. The importance of dosage frequency is illustrated (Figs. 25.46 and 25.47) by data from a carefully done assessment of growth responses in prepubertal naïve GH-deficient children randomly assigned to receive thrice-weekly or daily GH at the same total weekly dose (0.30 mg/kg per week).[1201] The mean total height gain during this period was 9.7 cm greater in the patients treated daily (38.4 vs. 28.7 cm, $p < 0.0002$), with similar increments in skeletal maturation and no acceleration of the onset of puberty. Mean height SDS at the end of 4 years was +0.2 or at the midpoint of normal for



• **Fig. 25.46** Annual growth velocity (mean ± standard deviation) for prepubertal patients with growth hormone (GH) deficiency before and during 4 years of GH treatment, contrasting results from daily *(QD)* versus thrice-weekly (TIW) injections. The mean annual growth velocity in the QD group was significantly greater than that in the TIW group during each year, although significance diminished from year 1 to year 4. (From MacGillivray MH, Baptista J, Johanson A. Outcome of a four-year randomized study of daily versus three times weekly somatropin treatment in prepubertal naive growth hormone deficient children. *J Clin Endocrinol Metab.* 1996;81:1806–1809. Reproduced by permission of M.H. MacGillivray.)

age. Studies using varying dosing of GH based on gender, growth responsivity, and growth factor concentrations have suggested the need for greater sophistication and individualization of the current treatment regimens.[1207,1208]

Sophisticated mathematical models[1200,1209] have examined many laboratory and auxologic parameters that influence response to GH therapy. Because age at onset of treatment is inversely correlated with growth responses, and because the smaller, lighter child requires less GH (with marked economic benefit), it is important to assess growth data in children treated early. In short-term studies of 134 patients[1210–1212] treated before age 3 years, marked early catch-up growth occurred, with a mean height gain of about 3 SDS by 4 years of therapy, allowing most children to reach their normal height range by middle childhood. Mean height in one study[1210] reached –0.4 SDS after 8 years of treatment. Near-adult height in 13 patients treated before 5 years of age[1213] did not differ significantly from the midparental target height (–0.9 vs. –0.7 SDS). In a group of 25 children treated before 12 months of age,[1214] adult height also matched the target height despite low dosage and less frequent administration. In an analysis of postmarketing data for development of a growth prediction model, a greater height gain per GH amount occurred in the very young children, but a seemingly lowered sensitivity to endogenous GH in early infancy adds complexity to interpretation of these data.[1215]



• **Fig. 25.47** Height standard deviation score (SDS) for prepubertal patients with growth hormone (GH) deficiency before and during 4 years of GH treatment, contrasting results (mean ± standard deviation) with daily (QD) versus thrice-weekly (TIW) injections. The mean SDS in the QD group was significantly greater throughout the treatment period. Younger patients had the greatest increase in height SDS, and the effect of age was more marked in the QD group. (From MacGillivray MH, Baptista J, Johanson A. Outcome of a four-year randomized study of daily versus three times weekly somatropin treatment in prepubertal naive growth hormone deficient children. *J Clin Endocrinol Metab.* 1996;81:1806–1809. Reproduced by permission of M.H. MacGillivray.)

Adult Height Outcomes

Treatment of growth-deficient children with pit-GH significantly increased their final heights, although the majority had final heights below –2 SD.[1216] This was likely due to the lower doses used, as well to treatment interruptions and the shorter duration compared to current treatment.

Patients treated with biosynthetic GH[1092,1213,1216–1225] have improved final adult height SDS, with average final height in more than 1400 patients approximating –1.3 SD. Data from the two largest databases,[1217,1219,1224–1226] representing the North American and European experiences as reported by pediatric endocrinologists, are shown in Table 25.13.

Despite the use of GH therapy, long-term studies still show that most patients fail to reach their genetic target heights. Evaluation of adult heights in 121 patients with childhood GHD treated in the Genentech GH research trials indicated a mean adult height in both male and female patients of –0.7 SDS, with 106 patients being within 2 SDS for normal adult Americans.[1222] Even in these closely monitored patients, however, a difference of –0.4 to –0.6 SDS from midparental target height still occurred. The achievement of the genetic target is possible, however: A Swedish subgroup (in the Kabi International Growth Study [KIGS] database) of consistently treated patients reached a median final height SDS of –0.32, which was equivalent to the midparental target height.[1226] A more recent study of the Swedish subgroup actually found a final height SDS greater than midparental target height by +0.2 (see Table 25.13).[1217]

Factors that have been found to correlate with enhanced adult height in GH-treated, GH-deficient children include baseline height, younger age at onset of treatment, longer treatment duration (especially during prepubertal years), and a greater growth velocity during the first year of treatment (Figs. 25.48 and 25.49).[1225] Increased height velocity and subsequent superior adult height outcome, although with considerable overlap, were demonstrated in children with GHD who carried one or both *GHRd3* alleles (i.e., with an exon 3 deletion),[646,1227,1228] although no difference in outcome was seen with GH treatment for ISS based on the GHRd3 allele polymorphism.[1229] Data from the National Cooperative Growth Study (NCGS) and KIGS showed that the height gained during puberty in patients with GHD was generally comparable to that in healthy children with delayed bone age.[1224,1230] As might be expected based on that observation, final height correlates with height at the onset of puberty in GHD patients.[1205,1221,1231–1233] Therefore every effort should be made to enhance growth before puberty, and delays in diagnosis and initiation of therapy probably contribute to the compromised adult heights still reported in many studies.

GH treatment of children who develop GHD as a result of cranial irradiation represents a special case when evaluating final height outcomes. In these children, who have received cranial irradiation for the treatment of malignancy, GH treatment is not initiated until there is no evidence of active tumor; this results in a delay between the diagnosis of GHD and the initiation of treatment. Final height is negatively correlated with the length of that lag time.[1234] In addition, those who have received spinal irradiation in addition to cranial irradiation have a lower final height due to impairment of spinal growth after irradiation.[1234,1235]

In an effort to increase final height in GHD patients, the use of high-dose GH during puberty has been studied, based on the rationale that GH secretion normally rises twofold to fourfold during the pubertal growth spurt, with dramatic concomitant

TABLE 25.13 Adult Height in Children With Growth Hormone Deficiency Treated With Biosynthetic Growth Hormone

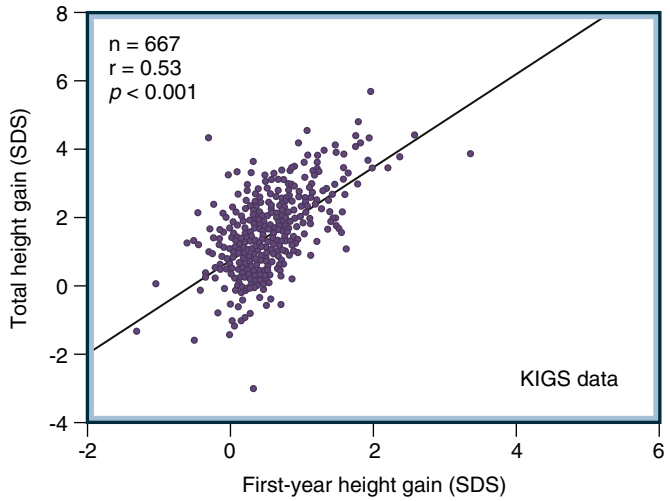
Study	Gender	N	GH Dose (mg/kg/wk)	Duration (yr)	Age (yr)	Height SDS	Change in Height SDS	Height vs. MPH
KIGS[a]								
	M	351	0.22	7.5	18.2	–0.8	+1.6	–0.2
	F	200	0.20	6.9	16.6	–1.0	+1.6	–0.5
KIGS (Sweden)[b]								
	M	294	0.23	8.4	18.5	–0.9	+1.8	+0.2
	F	107	0.23	8.5	17.4	–0.7	+2.1	+0.2
NCGS[c]								
	M	2095	0.28	5.2	18.2	–1.1	+1.4	–0.7
	F	1116	0.29	5.0	16.7	–1.3	+1.6	–0.9

F, Female; *GH,* growth hormone; *KIGS,* Pharmacia Kabi International Growth Study; *M,* male; *MPH,* midparental target height; *NCGS,* Genentech National Cooperative Growth Study; *SDS,* standard deviation score.

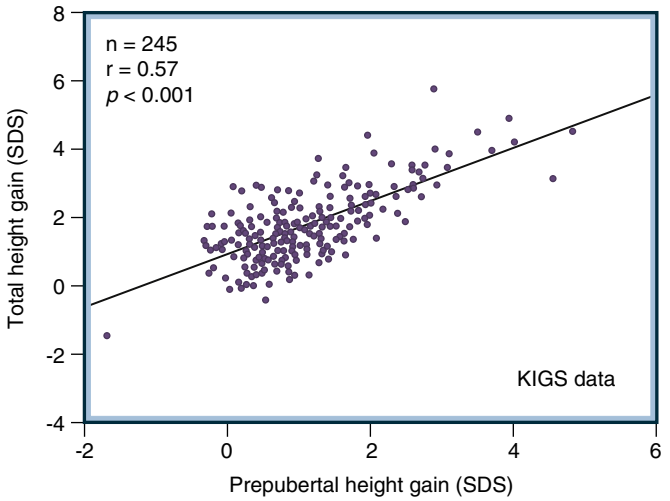
[a]Data from Reiter EO, Price DA, Wilton P, et al. Effect of growth hormone (GH) treatment on the near-final height of 1258 patients with idiopathic GH deficiency: analysis of a large international database. *J Clin Endocrinol Metab.* 2006;91:2047–2054.

[b]Data from Westphal O, Lindberg A, Swedish KIGS National Board. Final height in Swedish children with idiopathic growth hormone deficiency enrolled in KIGS treated optimally with growth hormone. *Acta Paediatr.* 2008;97:1698–1706.

[c]Data from August GP, Julius JR, Blethen SL. Adult height in children with growth hormone deficiency who are treated with biosynthetic growth hormone: the National Cooperative Growth Study experience. *Pediatrics.* 1998;102(2 Pt 3):512–516; updated NCGS data from Dana K, Baptista J, Blethen SL (personal communication, 2001).



• **Fig. 25.48** Relationship between first-year change in height standard deviation score (SDS) and total change in height SDS between start of growth hormone (GH) treatment and near-final height in children with idiopathic isolated GH deficiency. *KIGS,* Kabi International Growth Study database. (Modified with permission from Reiter EO, Price DA, Wilton P, et al. Effect of growth hormone [GH] treatment on the final height of 1258 patients with idiopathic GH deficiency: analysis of a large international database. *J Clin Endocrinol Metab.* 2006;91:2047–2054.)



• **Fig. 25.49** Relationship between prepubertal change in height standard deviation score (SDS) and total change in height SDS between start of growth hormone (GH) treatment and near-final height in children with idiopathic isolated GH deficiency. *KIGS,* Kabi International Growth Study database. (Modified with permission from Reiter EO, Price DA, Wilton P, et al. Effect of growth hormone [GH] treatment on the final height of 1258 patients with idiopathic GH deficiency: analysis of a large international database. *J Clin Endocrinol Metab.* 2006;91:2047–2054.)

increases in serum IGF1 levels, and the pubertal growth spurt normally accounts for approximately 17% of adult male height and 12% of adult female height. Earlier studies by Stanhope and associates[1236,1237] indicated that little difference in height gain could be observed when adolescent patients were treated with 30 versus 15 IU/m^2 per week of GH (~0.04 vs. 0.02 mg/kg per day). Mauras and colleagues[1238] evaluated higher pubertal GH doses (0.1 vs. 0.043 mg/kg per day) and found that the higher dosage resulted in a 4.6-cm increase in near-final height. Mean height SDS achieved in the group receiving 0.043 mg/kg per day of GH (as in the earlier report[1222]) was –0.7 ± 0.9, but in the group receiving 0.1 mg/kg per day, it was 0.0 ± 1.2. The higher GH dosage did not result in more rapid acceleration of skeletal maturation.

Another approach that has been taken to attempt to increase final height in GH-treated patients has been to adjust the dose of GH to reach a target IGF1 level rather than treating with a fixed, weight-based dose.[1239,1240] A study reported by Cohen and colleagues found that targeting a high IGF1 level (reaching a mean IGF1 SDS of approximately +1.5) resulted in an increase in growth rate compared with the two comparator groups, which had mean IGF1 SDS of approximately +1.[1239] It required a GH dose approximately three times higher to reach this IGF1 level (0.11 mg/kg per day vs. 0.041 and 0.033 mg/kg per day for the comparator groups).[1239] In the study reported by Marchisotti and colleagues,[1240] the difference in IGF1 levels between groups (+0.8 SDS vs. –0.3 SDS) was even greater than that in the report by Cohen and colleagues,[1239] but the Marchisotti study did not find a difference in growth rate between the two groups. The difference in GH dose was much smaller, however (0.038 vs. 0.30 mg/kg per day). Therefore, as would be expected, a higher GH dose results in a higher growth rate, but it is not yet known whether targeting a specific IGF1 level results in an increased final height; nor is it known whether these higher doses have adverse effects not seen with the more usual doses currently used. An interesting finding was the wide range of doses required to reach a given IGF1 level: The high-IGF1 targeted group required GH doses ranging from less than 0.025 mg/kg per day to greater than 0.25 mg/kg per day, whereas the low-IGF1 targeted group required doses ranging from less than 0.025 to 0.15 mg/kg per day.[1239]

The impact of treatments to alter sex hormone levels (e.g., GnRH agonists, aromatase inhibitors) with the aim of improving final height in GHD and other conditions is discussed later.

Benefits of GH Treatment Other Than Improved Growth

GH has effects beyond its impact on growth in childhood. Many non–growth-related benefits are discussed in the section on GH treatment in adulthood. There have been some data to suggest non–growth-related health benefits of GH treatment in childhood. A small study found treatment of GHD in childhood improved impaired cardiac function and structure identified in these subjects and improved body composition as seen with treatment of adult GHD.[1241]

Combined Pituitary Hormone Deficiencies

If GHD is part of a combined pituitary deficiency syndrome, it is necessary to address each endocrine deficiency, both for general medical reasons and to ensure maximal effect of GH therapy. TSH deficiency is often "unmasked" during the initial phase of therapy, and thyroid function should be assessed before the onset of therapy during the first 3 months of GH treatment[1242] and at least on an annual basis thereafter. The pituitary-adrenal axis can be evaluated during the insulin stimulation test in the workup for GHD or separately if GHD is identified with the use of a different provocative test. If ACTH secretion is impaired, patients may be placed on the lowest safe maintenance dose of glucocorticoids, certainly no more than 10 mg/m^2 per day of hydrocortisone and less if possible. Higher doses impair the growth response to GH therapy but should be given during times of stress. It is critical to monitor the long-term evolution of glucocorticoid deficiency, especially in those with *PROP1* mutations (discussed earlier). However, Lange and colleagues studied 24 adults with a prior history of idiopathic isolated childhood GHD and identified adrenal insufficiency in 10 of them, half of whom did not have evidence of ongoing GHD[1243]; this suggests a need to consider monitoring for impaired ACTH secretion even in those patients with presumed IGHD.

Gonadotropin deficiency may be evident in the infant with microphallus. This can be treated with three or four monthly injections of 25 mg of testosterone enanthate in the first months of life.[1244] Management at puberty can be more complicated, in that the physical and psychologic benefits of promoting sexual maturation must be balanced against the effects of epiphyseal fusion. When GH therapy is initiated in childhood and growth is normal before adolescence, it is appropriate to promote pubertal development with gonadal steroid replacement.

TABLE 25.14 Monitoring Growth Hormone Therapy

- Close follow-up with a pediatric endocrinologist every 3–6 months
- Determination of growth response (change in height SDS)
- Evaluation of compliance
- Screening for potential adverse effects
- Interval measurements of serum IGF1 and IGFBP3
  - Annual assessment of thyroid function
  - Consideration of dose adjustment based on IGF values, growth response, and comparison with growth prediction models
  - Periodic reevaluation of adrenal and thyroid function

IGBP, Insulin-like growth factor–binding protein; *IGF*, insulin-like growth factor; SDS, standard deviation score.

Monitoring Growth Hormone Therapy

Patients treated with GH should be seen every 3 to 6 months to monitor their response to treatment (Table 25.14). An increase in linear growth velocity should be seen within the first 6 months. Height SDS should increase at least 0.25 SDS in the first year.[1199] Treatment models have been developed that predict the expected growth rate in response to GH treatment.[1200,1209,1245–1247] A model[1200] explaining 61% of growth response variability in the first year of therapy includes inverse relationships with maximum GH response during provocative testing, age, and height SDS minus midparental height SDS and positive correlation with body weight SDS, GH dose, and birthweight SDS.[1248,1249] The single most important predictive factor for growth in years 2 through 4 is the first-year height velocity. These models can be used to quantify whether the individual patient is responding appropriately to GH treatment. If a patient's initial growth response is lower than predicted, the clinician should consider whether the diagnosis of GHD was correct, whether additional growth disorders (e.g., hypothyroidism) are present, and whether there is lack of compliance with the treatment.[1246]

It is appropriate to monitor the IGF1 levels after initiation of GH treatment and perhaps yearly thereafter.[1199,1250] A failure of IGF1 levels to increase into the normal range with treatment, together with an inadequate growth response, suggests compliance failure or the presence of GH insensitivity. Because of the association of elevated IGF1 levels with certain cancers (see later discussion), a reduction in GH dose should be considered for patients with serum IGF1 levels substantially above the normal range after the first 2 years of therapy.[1199] Whether the IGF1 level should be used to guide GH dosing awaits additional studies (see earlier discussion).

CPHD may evolve over several years, so children who are initially diagnosed with IGHD may develop CPHD. Thyroxine levels should be assessed after GH treatment has been initiated, and

annually thereafter, to identify the development of central hypothyroidism during treatment. Periodic reevaluation for ACTH deficiency should be performed. It is not necessary to routinely monitor fasting insulin and glucose levels, but if impaired glucose control is suspected, a fasting glucose level and glycosylated hemoglobin (HbA_{1c}) should be measured.

The growth response to GH typically attenuates after several years but should continue to be equal to or greater than the normal height velocity for age throughout treatment. Use of statistical growth treatment models can prove valuable in judging therapeutic efficacy.[1251–1253] A suboptimal response to GH can result from several causes: (1) poor compliance, (2) improper preparation of GH for administration or incorrect injection techniques, (3) subclinical hypothyroidism, (4) coexisting systemic disease, (5) excessive glucocorticoid therapy, (6) prior irradiation of the spine, (7) epiphyseal fusion, (8) anti-GH antibodies, or (9) incorrect diagnosis of GHD as the explanation for growth retardation (particularly in a patient with idiopathic IGHD and normal findings on MRI). Although 10% to 20% of recipients of recombinant GH develop anti-GH antibodies, growth failure is rarely caused by such antibodies, except in the case of individuals who have GHD as a result of GH gene deletion.[1254–1256]

Treatment During the Transition to Adulthood and in Adulthood

A growing challenge in the management of patients with GHD has been the issues surrounding their care after the growth process has ceased.[1257] This period, from the middle to late teenage years until the mid-20s, is a normal physiologic phase during which peak bone and muscle mass is achieved and the independence and self-sufficiency characteristics of adulthood are attained. It is also a time during which care of pediatric patients is transferred to endocrinologists who treat adults.

Clinical consequences of GHD in adults and the potential benefits of GH therapy in such patients have already been described.[1258,1259] Signs and symptoms of adult GHD include reduced lean body mass and musculature, increased body fat, reduced BMD, reduced exercise performance, and increased plasma cholesterol. Adults with GHD have a significantly increased risk of death from cardiovascular causes, a finding potentially linked to increased visceral adiposity and other cardiovascular risk factors.[1260] Adults with GHD have been found to have "impaired psychological well-being and quality of life."[1261] Several placebo-controlled studies have demonstrated that GH therapy for adult GHD results in marked alterations in body composition, fat distribution, bone density, and sense of well-being.[1259]

Given the metabolic derangements associated with untreated GHD, documentation of persistent GHD and continuation of GH treatment in late adolescence in the patient who shows persistent GHD is important. Among nearly 500 patients with IGHD, 207 (44%) had normal GH levels on provocative retesting.[1262,1263] In contrast, approximately 96% of patients with CPHD, with or without structural abnormalities of the hypothalamic-pituitary area, had sustained GHD.[1262,1263] The PES Guidelines on GH treatment recommend that persons with more than two pituitary hormone deficiencies be diagnosed with persistent GH deficiency.[1103] Persons with two or less pituitary hormone deficiencies, idiopathic GHD, idiopathic GHD with a small pituitary or isolated ectopic posterior pituitary, or persons postradiation require diagnostic evaluation for persistent GHD.

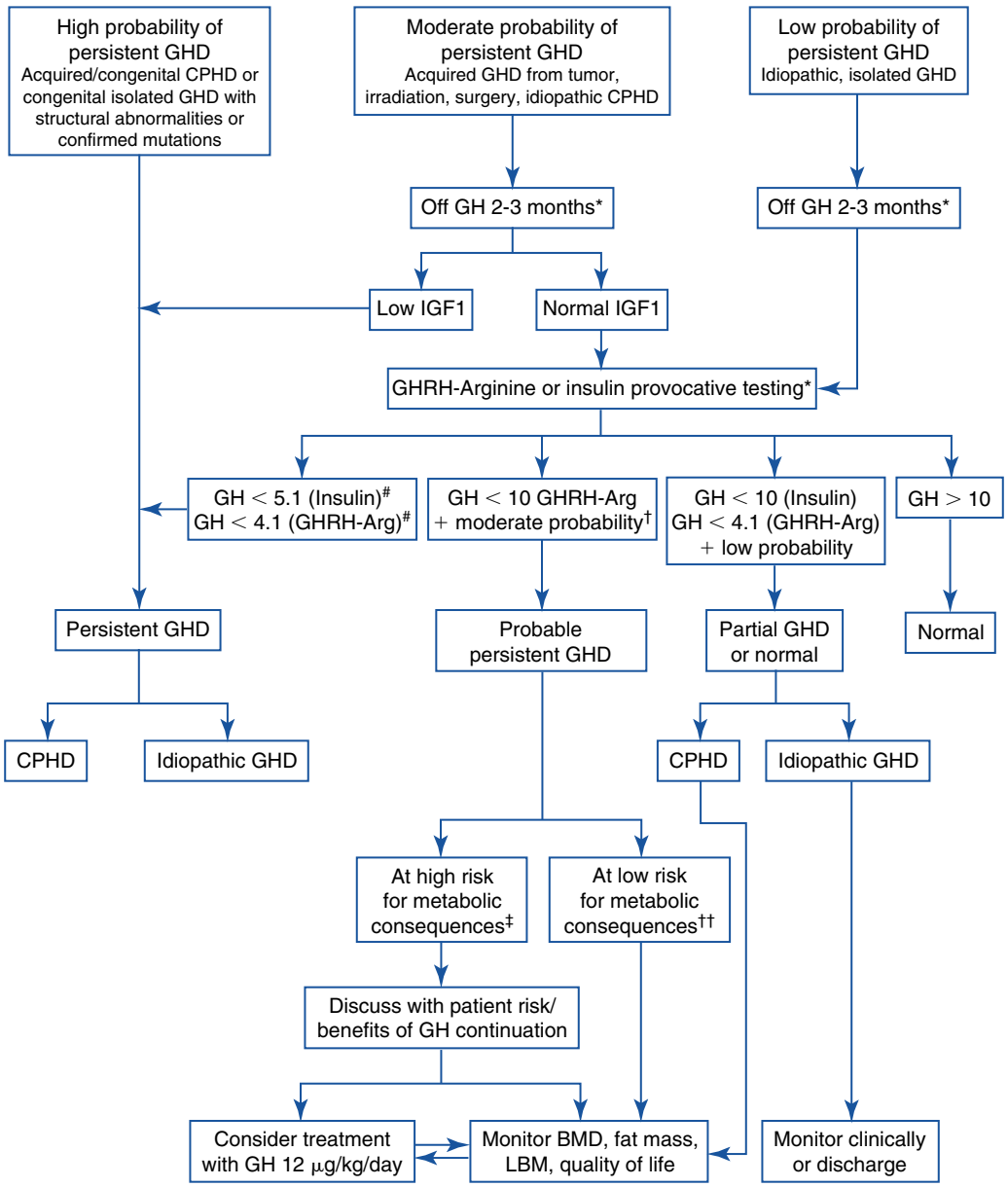
Many patients do not wish to continue the daily GH regimen, but the data support consideration for sustained treatment. After 1 to 2 years off GH therapy, IGF1 and IGFBP3 levels decrease substantially below baseline levels.[1258,1264,1265] Resumption of GH normalizes these levels, although there is a strong suggestion of a gender-based difference in GH requirements, with females needing higher GH doses.[1258,1264,1265] Loss of energy and strength may occur, and some quality-of-life data suggest age-specific psychologic issues in patients with untreated severe GHD during the transition to adulthood.[1266] Quality-of-life data with rigorous study designs are lacking in GH-treated childhood GHD.[1267] In untreated patients, total body fat and abdominal fat increase significantly and lean body mass is lost, compared with control subjects, comparable GH-treated patients, and patients who have reinstituted therapy.[1159,1268,1269]

Because bone mass accrual is not completed until the third decade, late adolescence is an important time for GH sufficiency, to prevent later osteopenia.[708,1257] There have been numerous GH treatment studies carried out in the transition age group to assess the impact on bone mineralization.[1265,1268,1270,1271] Differences in age at onset of retreatment, duration of therapy, GH dosage, and gender distribution have led to variations in results. In general, however, the data affirm the concept that reinstitution of treatment for 2 years in the transition period prevents deterioration in bone mineral density as experienced by untreated young adults with persistent GHD.

These studies support the continuation of GH treatment in late adolescence, albeit at lower doses than in childhood, to prevent the development of adverse cardiovascular risk, diminished bone mineralization, and a deterioration in quality of life. Whether the diversity of treatment and response data relates to the efficacy of the childhood GH therapeutic process is not clear, but the period of time off GH and the degree of persistent GHD would seem likely predictors of clinical status at the time of reinitiation of GH treatment.

After the adolescent with GHD has completed skeletal growth, the persistence of GHD is evaluated.[1103] Completion of skeletal growth is defined as growth rate less than 2 to 2.5 cm per year and bone age of 14 in females or 17 in males. GH at childhood doses should be discontinued, and if evaluation for persistent GHD is needed, GH therapy should be halted for 1 to 3 months, followed by a thorough reevaluation. A recommended algorithm for this evaluation is shown in Fig. 25.50.[1272]

As in childhood GHD, laboratory testing is imperfectly precise for diagnosis of ongoing GHD. Some patients have results that neither indicate nor eliminate ongoing GHD but are intermediate, suggesting either probable or partial GHD. If ongoing GHD is considered to be present or probable, assessment of body composition, BMD, and fasting lipid levels should be determined. The decision to reinitiate GH treatment is then based on a discussion with the patient and family regarding the risks and benefits in light of the laboratory test results and the risk for metabolic consequences (see Fig. 25.50).[1272] In addition, caution should be exercised in considering whether to continue GH therapy when there is a known risk of diabetes or malignancy. This is also an opportunity for a thorough clinical reassessment and a determination of the need for replacement of other hormones. The transition to adult GH replacement should be arranged as a close collaboration between endocrinologists who treat pediatric patients and those who treat adult patients and should include discussion with the patient and family.



• **Fig. 25.50** Algorithm for evaluating patients diagnosed with GHD during childhood at the completion of growth. *BMD,* bone mineral density; *CPHD,* combined pituitary hormone deficiencies; *GH,* growth hormone; *GHD,* growth hormone deficiency; *LBM,* lean body mass. (From Molitch ME, Clemmons DR, Malozowski S, et al. Evaluation and treatment of adult growth hormone deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2006;91:1621–1634; Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone [GH] deficiency in childhood and adolescence: summary statement of the GH Research Society. *J Clin Endocrinol Metab.* 2000;85:3990–3993. *, Based on consensus and clinical practice guidelines. #, Threshold values that achieve a sensitivity of 95% and specificity 91% to 92% for diagnosing GHD in adults; from Biller BMK, Samuels MH, Zagar A, et al. Sensitivity and specificity of six tests for the diagnosis of adult GH deficiency. *J Clin Endocrinol Metab.* 2002;87:2067–2079. †, Based upon data indicating that patients with irradiation-induced CPHD can have hypothalamic dysfunction causing GHD; from Darzy KH, Aimaretti G, Wieringa G, et al. The usefulness of the combined growth hormone [GH]-releasing hormone and arginine stimulation test in the diagnosis of radiation-induced GH deficiency is dependent on the post-irradiation time interval. *J Clin Endocrinol Metab.* 2003;88:95–102. ‡, Those at high risk for metabolic complications include patients with abnormal BMD, high fat mass, low LBM. ††, Those at low risk for metabolic complications include patients with normal BMD, fat mass, and LBM.)

Growth Hormone Treatment of Other Forms of Short Stature

Prader-Willi Syndrome

Growth failure due to PWS is an approved indication for hGH therapy in the United States and disordered body composition is an additional indication in Europe. In addition to promoting growth, hGH therapy improves body composition and physical strength, and increases energy expenditure.[1273–1276]

Guidelines for the clinical management of PWS were published after an international multidisciplinary expert meeting in 2013.[1277] Short stature due to GH insufficiency is almost always present in children with PWS. In a large cohort of 1135 children with PWS starting GH treatment, median height SDS was –2.2 (range, –4.1 to –0.3) at a median age of 6.4 years (range, 1.3 to 12.9 years).[1278] Growth standards for infants with PWS have been pubished.[1279]

Serum levels of IGF1 are reduced in most children with PWS.[938,1280–1282] Studies have shown that spontaneous GH secretion is reduced, and 70% of children with PWS have GH peaks of less than 10 μg/L during pharmacologic stimulation tests.[1273] Most experts agree that prior GH testing is not required before GH treatment is initiated. Numerous clinical trials have documented the efficacy of GH therapy to increase growth and improve body composition, energy expenditure, and strength and agility,[1283–1287] as well as increase growth and enhance gain of motor skills in infants and toddlers with PWS.[1288,1289]

In randomized, controlled studies there were significant increases in height and growth velocity and a significant decrease in percent body fat, as well as increased percentage of fat-free mass, improved muscle strength and agility, and increased fat oxidation during the first year of GH treatment.[1281,1290] Stabilization of these parameters occurs after the second year of therapy. Lean body mass increased significantly during the first 2 years in children with PWS who received GH treatment, compared with no treatment.[1220,1284] GH therapy for 2 additional years resulted in continued beneficial effects on body composition. When the dose of GH was reduced to 0.3 mg/m^2 per day, these improvements were not maintained.[1284] Beneficial effects continued after 8 years of GH treatment.[1287]

BMD improves in children with PWS treated with GH. In two German cohorts, the mean spontaneous adult heights were reported in one study as 162 cm in boys and 150 cm in girls[1291] and the other as 159 cm in boys and 149 cm in girls.[1292] In the KIGS database, 33 patients (21 boys and 12 girls) reached adult height, and two-thirds of them were above –2 SDS; the median adult height was –1 SDS after a mean duration of 8.4 years.[1293] Another study with 21 adults (13 males, 8 females) revealed a mean adult height of 0.3 SDS after a mean duration of 7.9 years of GH treatment.[1293] In this cohort, the strength and agility that were evident during the initial 2 years continued into adulthood. The patients also reported a higher quality of life and reduced depression.[1294] In 55 children monitored during 4 years of continuous GH treatment (1 mg/m^2 per day), body composition was significantly improved, mean height normalized, head circumference increased, and BMI significantly decreased. GH treatment had no adverse effects on bone maturation, blood pressure, glucose homeostasis, and serum lipids.[1295] Improved body composition and metabolic status were demonstrated in adults treated with GH in childhood and adolescence.[1296]

Before GH therapy is begun, screening for hypothyroidism is indicated because of the association with primary and central hypothyroidism.[1297,1298] Continued screening is also recommended. Treatment with GH beginning as early as 2 years of age is recommended, but there may be additional benefits to starting therapy between 6 and 12 months of age. Several studies have found improvements in motor development, muscle tone, head circumference, and, possibly, cognition and behavior.[1288,1299–1301]

A recent review documents the benefits of GH therapy, including an increase in female height, improved body composition, and increased exercise tolerance.[1302] Cognitive function was improved in children with treated GH.[1303]

Since October 2002, several reports of unexpected deaths in infants and children with PWS have been published.[1304–1306] Between 1985 and 2006, the NCGS monitored the safety and efficacy of recombinant human GH in 54,996 children. Two deaths were reported in patients with PWS.[1307] Although death from presumed obesity-induced hypoventilation or apneic events in PWS without GH treatment is well described, the occurrence of such deaths during GH administration raises the question of whether GH exacerbates this condition.[1308,1309] Tonsillar hypertrophy and fluid retention associated with GH therapy are potential risk factors. Most of the deaths, with or without GH treatment, were related to obesity or to a complicated course of a relatively mild respiratory tract infection, sleep apnea, adenoid or tonsillar hypertrophy (or both), hypoventilation, and aspiration. The obesity-hypoventilation syndrome is the more likely etiologic factor, suggesting that ventilatory and pulmonary function should be assessed with polysomnographic studies before and during GH treatment.[1308,1310]

In a review that included 64 children (42 boys and 22 girls, 28 receiving GH treatment), the highest death risk occurred during the first 9 months of GH treatment.[1306] Therefore it is recommended that GH treatment be started at a low dose (e.g., 0.25–0.30 mg/m^2 per day or 0.009–0.012 mg/kg per day) and increased during the first weeks and months to reach the standard replacement dosage of approximately 1 mg/m^2 per day or 0.24 mg/kg per week. Patients should be monitored for sleep apnea and IGF1 levels. The GH dose should be decreased if there is evidence of high IGF1 levels, especially if associated with edema, worsening or new onset of snoring, headache, or acromegalic clinical features. A longitudinal observational study evaluated 75 children with PWS and showed an increase in the apnea-hypoxia index (AHI) after GH treatment.[1311]

Five prospective studies have evaluated the effects of GH treatment on breathing disorders in PWS.[1285,1312–1314] Carbon dioxide responsiveness, resting ventilation, and airway occlusion pressure improved during 6 to 9 months of GH treatment,[1314] and the inspiratory and expiratory muscle strength improved during 12 months of GH treatment, compared with control subjects.[1312] In a double-blind, placebo-controlled, crossover study, AHI was found to decrease in 12 children with PWS, compared with control subjects, after 6 months of GH therapy although the difference was not statistically significant.[1285] Another study found a decrease in AHI in most of the adults and children studied after 6 weeks of GH therapy.[1315] A subset of patients had an increased AHI with more obstructive events, but most of these latter patients had upper respiratory tract infections and adenoid/tonsil hypertrophy, and two of them had high IGF1 levels. In another study in 35 prepubertal children with PWS, the AHI did not significantly change during 6 months of GH therapy.[1316] However, four of these children had an increase in the number of episodes

of obstructive apnea during an upper respiratory illness; these episodes were not present after recovery. Therefore it is recommended that obesity-related sleep and breathing problems be evaluated before and vigilantly monitored after GH treatment begins. Polysomnography and ear, nose, and throat evaluations should be performed as necessary. A longitudinal review of polysomnography data suggests that children younger than 2 years of age are most vulnerable to sleep-related disordered breathing (SRDB) after initiation of GH therapy.[1317]

A recent study[1318] showed that 60% of PWS patients had central adrenal insufficiency. This may explain the high rate of sudden death, especially during infection-related stress. The authors concluded that patients with PWS should be treated with hydrocortisone during stress until adrenal insufficiency can be ruled out.[1318,1319]

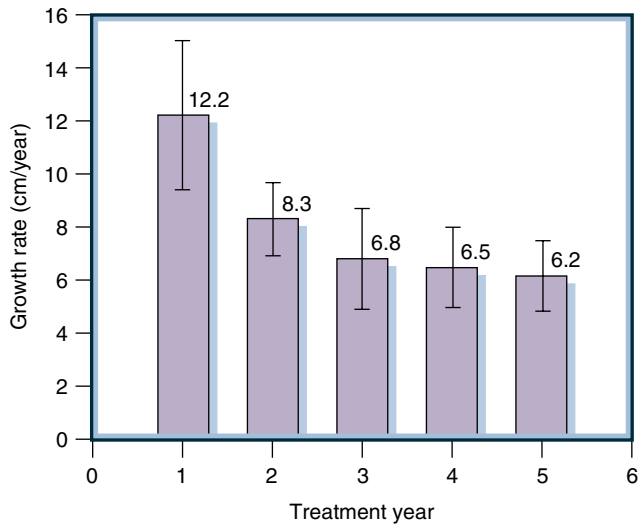
Between 30% and 70% of children with PWS have scoliosis.[1320–1325] Weight control is a vital part of its prevention and management. Therefore, before GH treatment is initiated, spinal radiographs and, if appropriate, orthopedic assessment are recommended. Reports of scoliosis worsening during GH treatment reflect the natural history of this condition rather than a side effect of treatment in most cases, and discontinuation of GH is not indicated.

In view of the childhood findings of low lean body mass and high fat content, osteopenia, and some degree of glucose intolerance ameliorated by GH, the issue of long-term therapy through adulthood must be considered and studied.[1326–1328] After growth is complete, attainment of a normal peak bone mass, continued improvement of muscle mass and strength, reduction of body fat, prevention of cardiovascular morbidity, and improvement in well-being and quality of life are potential benefits of continued GH therapy.[1329] Adult GHD and low IGF1 levels have been reported in patients with PWS.[1282,1330] Short-term GH treatment in GH-naïve adults with PWS has been reported to modestly improve body composition, cognition, motor performance, and social status.[1330] Further long-term studies are needed on adolescents with PWS transitioning to adult therapies.

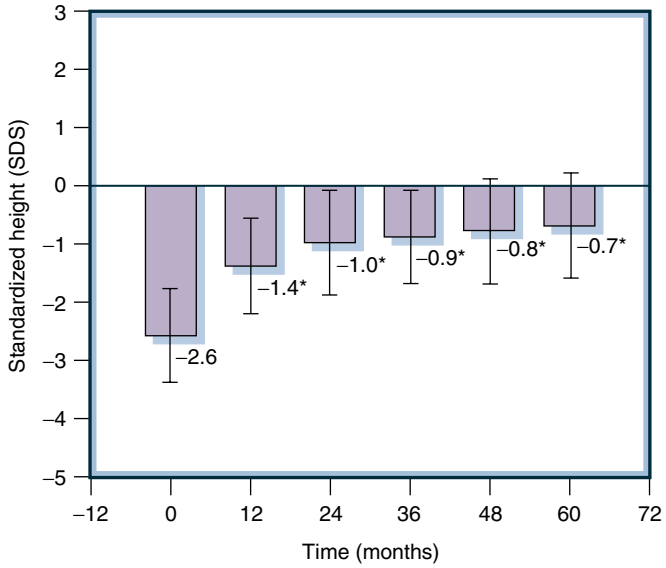
Chronic Renal Disease

Chronic renal disease (CRD) is regarded as an indication for the commencement of GH administration. Short stature is more severe in children with congenital renal disorders than in children with acquired renal diseases.[738,1331,1332] Even after renal transplantation, final height is below the lower limit of normal in about 50% of children.[1333] GH treatment is able to increase height velocity and height SDS[748,1334,1335] and significantly improves final height[1336–1338] in patients with CRD (Figs. 25.51 and 25.52). The therapy should be implemented if short stature persists for longer than 6 months and in subjects with marked deceleration of growth velocity, and it should be continued until transplantation is performed.[1339] Typical growth patterns for the year after GH initiation have been devised. A growth velocity less than –1 SDS for age (in a child without dialysis or transplant) is inadequate and indicates presence of confounding factors that require investigation.[1340]

GH accelerates growth in children with CRD, at least over 5 years of therapy.[1341–1344] Using a GH dosage of 0.05 mg/kg per day, Fine and colleagues[1334] reported a mean first-year growth rate of 10.7 cm in GH recipients and 6.5 cm in the placebo group; in the second year, GH-treated patients had a mean growth rate of 7.8 cm/year compared with 5.5 cm/year in placebo recipients,



• **Fig. 25.51** Annual growth velocity (mean ± standard deviation) in 20 growth-retarded prepubertal patients with chronic renal insufficiency who were treated with growth hormone. (From Fine RN, Kohaut E, Brown D, et al. Long-term treatment of growth retarded children with chronic renal insufficiency with recombinant human growth hormone. *Kidney Int.* 1996;49:781–785. Reproduced with permission of R.N. Fine.)



• **Fig. 25.52** Height standard deviation score (SDS) in 20 growth-retarded prepubertal patients with chronic renal insufficiency (mean ± standard deviation). Note that the basal height is outside the normal range (at –2.6 SDS), enters the normal range within 1 year of treatment initiation, and is not different from the mean by the fifth year of growth hormone therapy. (From Fine RN, Kohaut E, Brown D, et al. Long-term treatment of growth retarded children with chronic renal insufficiency with recombinant human growth hormone. *Kidney Int.* 1996;49:781–785. Reproduced with permission of R.N. Fine.)

resulting in an improvement of height SDS from –2.9 to –1.5. Twenty patients who were treated for 5 years reached a normal height SDS of –0.7, with a mean height increase of 40 cm.[1344] The youngest patients (<2.5 years of age) had the most impressive growth response to GH therapy (14.1 cm/year). Doses recommended in children with CRD are higher than in GHD patients (i.e., about 0.35 mg/kg per week) because growth patterns in these

patients are dose dependent. Deleterious effects on renal function and progression of osteodystrophy were not observed.[1345] This treatment regimen does not adversely affect renal graft function after transplantation, nor is there significant "catch-down" growth after the transplantation.[1346] The final height in 38 German children treated with GH for an average of 5.3 years was –1.6 ± 1.2 SDS, an increment of 1.4 SDS over the pretreatment baseline. The final height of an untreated control group was –2.1 ± 1.2, or 0.6 SDS below baseline.[1337]

More recently, adult heights of 178 French patients treated with GH were reported. Mean adult height was 162.2 cm in men and 152.9 cm in women, and 46% were more than –2 SDS for height. Adult height SDS was correlated with height SDS and spontaneous height velocity before treatment and with the effect of treatment. These adult heights were significantly better when compared with historic cohorts of patients not treated with GH.[1347]

In 2009, a review of longitudinal data from 7189 patients enrolled in the chronic renal insufficiency registry of the North American Pediatric Renal Trials and Collaborative Studies revealed that 827 patients (11.5%) had received GH. A total of 787 children with CRD who were previously naïve to GH treatment and received recombinant human GH for 1 to 4 years (median, 1.5 years) were paired with 787 control patients and monitored for 4 years. The GH-treated group had a significantly greater height velocity SDS than the control group at 2.5 years. Among 220 pairs evaluated at 2 years, the height SDS of the GH-treated group was 0.56 SDS higher than that of the control group (p <0.05). Treatment with GH had no significant impact on BMI or estimated glomerular filtration rate (GFR).[1348]

The magnitude of response to GH treatment depends on the GH dosage. A dose of 0.35 mg/kg per week appears to be optimal for short patients with CRD; half of this dose was less effective, and doubling did not significantly improve the response in double-blind studies.[1342] Long-term GH treatment has also been shown to be safe and effective for extremely short (–4.0 SDS) children with nephropathic cystinosis and should be considered if nutrition and cysteamine treatment do not prevent growth failure.[1332] Because children are often short at the time of renal transplantation, have hormone findings of relative GH insensitivity, and receive chronic prednisone therapy, GH is sometimes administered in the post-transplantation period. Data after 1 and 2 years of treatment of such children and adolescents[1349] indicated a large increment of growth velocity at year 1 and a smaller benefit at year 2. As with GH treatment of CRD, the pharmacologic regimen overcomes the relative GH insensitivity. Individual patients show a wide variation in response, and predictors include age, GFR, need for dialysis treatment, target height, and pretreatment growth rate.[1350]

A mathematic model for prediction of the individual response to GH in prepubertal children with CRD was developed from the KIGS.[11] Thirty-seven percent of the variation in the first-year growth response was explained by this model, with the greatest first-year response in younger children who had no weight reduction, no hereditary renal disorder, and high residual renal function. There was a small GH dose effect during the first treatment year.[11] Such models, using clinical variables, may allow individualized GH treatment decisions in children with CRD.

Considerable assessment must yet be undertaken to demonstrate whether there is increased growth over a longer term, that renal function does not deteriorate during therapy, and that the risk of rejection is not enhanced. GH treatment does not appear to cause an accelerated decline of allograft function[1351–1353] or changes in histopathologic findings.[1354] Use of nonsteroid-based immunosuppressive regimens may obviate the need for post-transplantation GH treatment.

A short-term study in chronic, well-nourished dialysis patients showed that combined administration of moderate doses of GH with recombinant human IGF1 (rhIGF1) had a complementary effect on protein metabolism.[1355] This approach is theoretically reasonable; however, the safety profile of this intervention is unknown.

Juvenile Idiopathic Arthritis

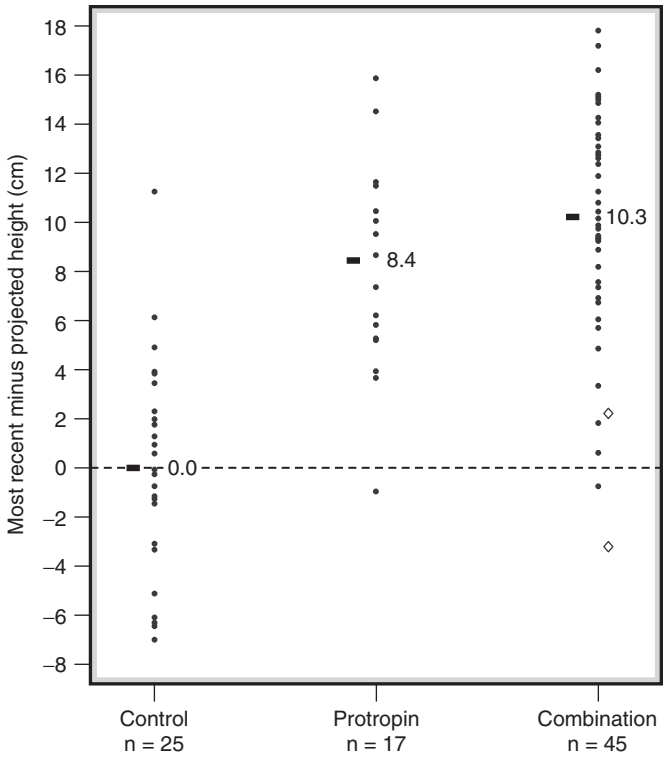
Juvenile idiopathic arthritis (JIA) is often complicated by growth deficiency. The decrease in linear growth usually correlates with the severity of the disease, although catch-up growth may not occur during remissions. The final height is less than –2 SDS in 11% of patients with polyarticular JIA and in 40% of those with systemic JIA.[1356] Serum GH levels are normal or low, usually with low plasma IGF1 levels.[1357] The pathogenesis of short stature is thought to be GH insensitivity associated with both the inflammatory process and glucocorticoid treatment.[1358] Several trials have been conducted to determine whether GH replacement therapy is effective in patients with JIA.[1359–1361] In trials of GH replacement, using GH dosages ranging from 0.1 to 0.46 mg/kg per week increased serum levels of IGF1 and linear growth velocity. However, the trials all had significant interindividual variability.[1362] GH therapy for up to 3 years was notable for a decrease in the loss of growth velocity associated with the active phase of JIA.[1363]

In a randomized, controlled trial of GH therapy (0.33 mg/kg per week for 4 years) in 31 children with JIA, a height increase of 1 SDS was found, compared with a decrease of 0.7 SDS in the untreated control group.[1361] The GH-treated patients had a significantly greater final height compared with untreated control patients (–1.6 ± 0.25 SDS vs. –3.4 ± 2.0 SDS; p <0.001).[1364] As expected, the severity of inflammation negatively affected the efficacy of GH therapy.[1361,1364] In a randomized trial of normal-height children with JIA treated with high-dose GH therapy (0.46 mg/kg per week) starting within 12 to 15 months after initiation of glucocorticoid therapy, the 3-year follow-up showed that the heights of GH-treated children remained normal (–1.1 SDS at baseline and –0.3 SDS after 3 years) while linear growth of the control patients declined from –1.0 SDS to –2.1 SDS.[1359] In the randomized, controlled trials published to date, patients were followed for up to 7 years, and no substantial differences were found in disease activity variables, including worsening of preexisting bone deformities, between GH-treated and control patients.[1358,1360,1361,1363]

In a longitudinal study of patients treated for a mean of greater than 5 years, GH increased and normalized final height and improved bone and muscle mass.[1365] Because chronic inflammation, glucocorticoid treatment, and GH therapy are all associated with decreased sensitivity to insulin, children with JIA are at risk for impaired glucose homeostasis.[328,1362,1366] Therefore monitoring of glucose tolerance using blood glucose, fasting insulin, and HbA_{1c} assays at intervals of 6 to 12 months is recommended. More intensive monitoring with oral glucose tolerance tests at baseline and then once yearly during treatment may be indicated.

Turner Syndrome

Patients with TS have a final height averaging 143 cm in the United States,[11,907] about 20 cm lower than the mean final height of normal women.[905] The goals of growth-promoting therapies are to attain



• **Fig. 25.53** Adult heights of patients with Turner syndrome treated with growth hormone (GH) or with a combination of GH plus oxandrolone and of historic control subjects, relative to each subject's projected adult height (indicated by the *dotted zero line*). The mean increments in relative adult height are indicated. The *diamonds* in the combination group indicate two subjects with poor compliance who terminated treatment early. (From Rosenfeld RG, Attie KM, Frane J, et al. Growth hormone therapy of Turner syndrome: beneficial effect on adult height. *J Pediatr.* 1998;132:319–324.)

a normal height for age as early as possible, to progress through puberty at a normal age, and to attain a normal adult height. A delay in diagnosis of TS is often the greatest obstacle for girls with TS to obtain a normal height. A 7-year delay in diagnosis was reported in one study in patients who averaged 2.9 SD below the mean in height and were below the 5th percentile for height for an average of 5.3 years.[1367] Growth should be assessed frequently after the diagnosis is made and plotted using TS-specific growth charts to monitor for associated growth issues and effectiveness of therapy.[11,912,1368]

Before the availability of recombinant GH, there were conflicting data concerning its efficacy in this disorder, but the ability of GH to accelerate growth has now been demonstrated in multiple reports. Growth responses are not affected by the karyotype. In 1983, a randomized, controlled North American study of treatment with GH (at a dose of 0.375 mg/kg per week) with or without added oxandrolone was initiated, with a mean age at onset of treatment of approximately 9 years.[1369] Analysis of all 62 girls enrolled in the study at near-final height showed a mean stature of 152.1 cm in the group treated with GH plus oxandrolone (a gain of 10.3 cm compared with the height predictions derived from Lyon and colleagues),[11] whereas girls receiving GH alone averaged 150.4 cm (a gain of 8.4 cm) (Fig. 25.53).[907] In another arm of this study, addition of estrogen to the GH regimen before age 15 years lowered the final height gain from 8.4 cm to 5.1 cm.[1370] In a reassessment of North American data in the NCGS, early initiation of GH treatment was shown to allow estrogen administration at a physiologic age without loss of adult height.[1371,1372]

Several other studies[917,1373] using higher doses of GH showed even greater gains in adult height outcomes. In a multicenter trial, Sas and coworkers used a maximum GH dose of approximately 0.63 mg/kg per week for 4.8 estrogen-free GH treatment years beginning at a mean age of 8.1 years, resulting in a gain of 16 cm over the modified Lyon and colleagues' projection.[11,917] In the same study, the group receiving a GH dose similar to that in the American studies achieved a height gain of 12.5 cm by age 16 with 4.8 estrogen-free GH treatment years starting at 7.9 years. In these girls, induction of puberty at a normal (not delayed) age was associated with these excellent height outcomes.[1374] Carel and colleagues,[1373] using 0.7 mg/kg per week in a group that received 5.1 estrogen-free GH treatment years beginning at 10.2 years, gained 10.6 cm over the projections of Lyon and colleagues.[11] The group receiving a conventional dose (0.3 mg/kg per week) gained only 5.2 cm with 3.0 estrogen-free GH treatment years starting at 11 years. Because girls with TS usually have a normal GH secretory pattern, provocative GH testing should be performed only in those whose growth is clearly abnormal relative to that expected for patients with TS, as determined by plotting lengths and heights on TS-specific growth curves.[11,910,914,1375]

Although it was well established that GH therapy was effective in increasing final adult height, the magnitude of the gain in height varied in the earlier studies, depending on study design and treatment parameters. In 2005, in the first randomized, controlled trial to follow GH-treated subjects with TS to final height, the Canadian Growth Hormone Advisory Committee corroborated the increases in adult stature reported by studies with historic control subjects.[907,1374,1376,1377] In the Canadian study, girls with TS (age 7–13 years) who were randomized to receive GH (0.3 mg/kg per week; maximum weekly dose, 15 mg) achieved a final adult stature 7.2 cm taller than that seen in the control group after an average of 5.7 years. Factors predictive of taller adult stature include a relatively tall height at initiation of therapy, tall parental heights, young age at initiation of therapy, a long duration of therapy, and a high GH dose.[917,1372,1378,1379]

Although the optimal age for initiation of GH treatment has not been established, preliminary data from the Toddler Turner Study, in which 88 girls between the ages of 9 months and 4 years were randomized to receive GH or no GH therapy, showed that GH therapy is effective beginning as early as 9 months, with a safety profile similar to that in older children with TS.[1380] Treatment with GH should be considered as soon as growth failure is evident. GH therapy in the United States is typically initiated at the FDA-approved dose of 0.375 mg/kg per week. The dose can be changed in response to the patient's growth response and IGF1 levels. Growth prediction models may be helpful in determining the potential effects of changes in dosing.[1378]

Studies have shown that higher doses produce a relatively small gain in final height, although there is no apparent increase in short-term adverse events.[1381] In a study by the Dutch Working Group, the mean gain in final height with 4 IU/m^2 per day (0.045 mg/kg per day), 6 IU/m^2 per day, and 8 IU/m^2 per day averaged 11.9 ± 3.6, 15.7 ± 3.5, and 16.9 ± 5.2 cm, respectively.[1374] However, when GH was given at the higher doses, IGF1 levels were often above the normal range, which theoretically could lead to long-term adverse effects.[1382]

In girls naïve to GH who are older than 9 years of age and in those with extreme short stature, consideration can be given to using higher doses of GH and adding a nonaromatizable anabolic steroid, such as oxandrolone.[907] The maximum suggested dose of oxandrolone is 0.05 mg/kg per day, because higher doses

have been associated with virilization and accelerated skeletal maturation. Liver enzymes should be monitored. Therapy may be continued until a satisfactory height has been attained (bone age >14 years) or until the yearly growth velocity falls to less than 2 cm/year. The children should be monitored at intervals of 3 to 6 months.[1383]

The substantial variations in GH-induced growth increments in these studies were presumably related to GH dose, duration of estrogen-free GH treatment years, and age at initiation of GH and estrogen administration, as well as the population and parental adult heights. Additionally, the *GHRd3* polymorphism has been reported to be associated with increased responsivity to GH in girls with TS.[1384] Transdermal or depot estrogen administration, as opposed to oral estrogen, may also contribute positively to growth outcomes.[1385,1386] In these higher-dose treatment studies, hyperinsulinism with presumed insulin resistance was evident but reversible.[1373,1387] Other data suggest that impaired insulin secretion may ultimately be the significant issue in patients with TS,[1388,1389] although long-term GH therapy has been well tolerated.

None of these studies were placebo-controlled to adult height, and some studies have yielded much poorer height outcomes,[918] so some uncertainty existed until the randomized, controlled, multicentered Canadian study, in which the mean height gain ascribed to GH treatment was 7.3 cm at 1 year after cessation of the treatment protocol. Estrogen was initiated at 13 years of age.[1376] Another temporally matched control study from Italy showed a gain of 8.1 cm.[1377] These studies corroborated and supported the information that had accumulated from historic data on natural growth in TS juxtaposed to the available GH treatment results. Such data, in aggregate, provides convincing support that GH can both accelerate growth velocity and increase adult height.

Recommendations include seeking the diagnosis vigorously at any age in every girl with otherwise unexplained short stature and initiating therapy at that young age (i.e., at diagnosis or in early childhood). One must bear in mind that growth velocity in girls with untreated TS can be slowed as early as ages 1 to 3 years old.[911,913] Treatment with the FDA-approved GH dosage of 0.375 mg/kg per week should be initiated, and compliance should be monitored with auxologic and IGF1 measurements; continued normal thyroid status should be assured. In overweight girls, the use of body weight for calculating dosage may result in the delivery of excess GH. Adult height was compared in two Dutch trials that used BSA-based dosing and five Swedish trials that used body weight (BW)–based dosing,[1390] finding that adult height was greater in girls treated with BSA-based dosage versus those using a BW-based dosing. A dose 0.375 mg GH/kg per week is approximately equivalent to 1.38 mg GH/m^2 per day.

Oxandrolone may be added to the regimen in the late-diagnosed girl. Initiation of estrogen therapy at 12 years of age, rather than at age 14 years and above, has recently been shown to improve adult height.[1374,1386]

A 2011 randomized, placebo-controlled study (double placebo, estrogen alone, GH alone, or GH and estrogen) that followed girls ages 5 to 12.5 to adult height showed that very low-dose ethinyl estradiol given prior to age 12 years, along with growth hormone treatment, had the greatest impact on increasing adult height.

The diminished areal bone density in TS is enhanced by GH, but estrogen therapy is needed to normalize volumetric density (i.e., not simply size).[1391] Absence of an adverse impact of GH on aortic diameters is reassuring, given the predisposition of such patients for dissecting aneurysms.[543] Use of statistical prediction models for long-term growth in TS may permit a more quantitative assessment of individual therapeutic efficacy.[1378]

Despite evidence for aggressive GH treatment of girls with TS to achieve adequate adult heights and relatively high quality-of-life assessments in adulthood,[1392,1393] health-related issues continue to imperil outcomes. Carel and colleagues[1394] reviewed health outcomes of 568 adult French women (in their mid-20s) with GH-treated TS who had a mean height of 150.9 cm (having gained about 9 cm over prediction) and found that neither height nor height gain was associated with quality-of-life scores. Rather, issues regarding cardiologic and otologic health concerns and delay of pubertal initiation beyond 15 years were of greater concern. In contrast, a long-term follow-up assessment of 49 women from the Netherlands who had reached a mean adult height of 160.7 cm (a gain of >15 cm), suggested that the high quality-of-life scores were related to height gain and adequate estrogenization.[1395]

The NCGS recently published data on the efficacy and safety of GH therapy in 5220 children with TS treated during the past 20 years. A total of 442 adverse events were reported for these patients, including 117 that were considered serious. Seven deaths occurred, including five from aortic dissection or rupture. The incidences of intracranial hypertension, slipped capital femoral epiphysis, scoliosis, and pancreatitis were increased compared with other patients in the registry who did not have TS. Ten new-onset malignancies occurred, including six in patients without known risk factors. The number of patients who developed IDDM also appeared to be increased. It is believed that these adverse events are likely to be unrelated to treatment with GH. The incidence of aortic dissection or rupture reflects the higher baseline risk in TS.[1396]

Two echocardiography studies reported normal left ventricular morphology and function in girls with GH-treated TS,[1397,1398] and two MRI studies found no deleterious effect of GH treatment on aortic diameter.[532,1383,1399,1400] Because GH treatment can alter craniofacial proportions, all girls with TS treated with GH should receive periodic orthodontic follow-up.[1401] These studies were not controlled, but their findings emphasize the broad range of health concerns in women with TS and affirm the need for multidisciplinary follow-up care.

Recommendations:

1. Early diagnosis and initiation of treatment.
2. GH therapy offered to all girls with TS with a subnormal predicted adult height.
3. Provocative testing of GH secretory status is not required since GH secretion is not impaired.
4. GH treatment at higher dosing than used for GH-deficient patients and the GH dose should be titrated to increase growth velocity. A GH dose of up to 67 mcg/kg per day has been approved by the USFDA, although this dose is higher than usually prescribed.
5. Initiate GH therapy at an earlier age, once decreasing height percentiles on the normal growth curve are noted (the current average age is around 9 years), since a longer time on therapy will result in improved adult height.
6. Therapy should be continued until an appropriate adult height is attained or the growth rate falls below 2 to 2.5 cm per year.
7. Early treatment with ultralow-dose estrogen may improve growth. Pubertal estradiol should begin at approximately 12 years of age. Oxandrolone at a dose of approximately 0.03 mg/kg per week may be considered in girls older than 8 years.

Small for Gestational Age

Studies using GH in short children born SGA have been hampered by the heterogeneity of this group of patients, whose poor

growth may reflect maternal factors, chromosomal disorders, dysmorphic syndromes (e.g., RSS, Dubowitz syndrome), toxins, and idiopathic factors.[1402] A 2001 consensus statement recommended that SGA be defined as a birth weight or length at least 2 SD below the mean.[1403] Almost 90% of infants born SGA undergo catch-up growth within the first 2 years of life, and those who do not would then be eligible for GH treatment.[37,38,947,989,1402,1404,1405] Based on these proportions, and because the prevalence of "short SGA" is 2 to 3 per 1000 children, between 1 in 300 and 1 in 500 children are eligible for GH treatment, as approved in the United States in 2001 and in Europe in 2003. It appears that GH mediates the postnatal catch-up growth.[1406] Children born SGA appear to grow at a low-normal growth velocity during childhood,[1407] but puberty tends to occur at a somewhat earlier age and to progress rapidly, resulting in decreased pubertal height gain and an adult height about 1 SD (~7 cm) below the mean[38,987,989,1408–1411] and about 4 cm below the midparental target height.[947]

The impaired postnatal growth observed in children born SGA is probably due to many factors, including generalized cellular hypoplasia,[1412] altered diurnal GH secretion patterns,[237,969,971,1407,1413] and, potentially, abnormalities in the GH-IGF axis resulting from GH sensitivity as determined by the presence or absence of exon 3 of the GHR.[1414–1416] Deletions and mutations of the *IGF1* gene have been reported in patients with profound IUGR, microcephaly, deafness, and postnatal growth failure.[974,660,1417] SGA patients have also been reported to have *IGF1* gene polymorphisms or missense mutations resulting in low serum IGF1 concentrations.[975,976,989,1416,1418,1419] Reduced levels of IGF2 expression, associated with hypomethylation of the telomeric domain of chromosome 11p15, were found in a cohort of SGA patients who had RSS.[993] Mutations in the type I IGF receptor gene (one compound heterozygous, one nonsense) were found in two patients with IUGR and poor postnatal growth (one with elevated IGF1).[663]

The low levels of IGF1 and IGFBP3 in many infants with IUGR, apparently related to fetal malnutrition, do not seem to predict the degree of subsequent growth impairment,[968] although continuing low levels are associated with poor catch-up growth.[1420] Short children born SGA make up a substantial portion of growth-retarded patients seen in pediatric endocrine practices.[947,989,1421] Because these children may have heights in the range seen in IGF deficiency syndrome, therapeutic attempts are appropriate, assuming that the insulin resistance noted in these thin, small children[982] does not become a clinical issue. Encouraging growth responses have been obtained with GH treatment.

The FDA approval of GH for the long-term treatment of growth failure in children born SGA who fail to manifest catch-up growth by age 2 was based on data obtained from four randomized, controlled, open-label, clinical trials that enrolled 209 patients between the ages of 2 and 8 years. Height velocity was determined after 1 year without treatment, and then patients were randomized to receive either GH treatment (34 or 69 mg/kg per day [0.24 or 0.48 mg/kg per week]) or no treatment for 2 years, after which a crossover design occurred. Children who received the higher GH dose had an increase of about 0.5 SDS in height after 2 years, compared with the children who received the lower dose, although both treated groups had significant increases in height velocity compared with untreated children (prescribing information for Genotropin somatropin of recombinant DNA origin for injection, Pfizer, Inc., New York, 2006). In Europe, the criteria for treatment differ from those in the United States: height SDS below –2.5, height velocity SDS below 0 during the previous year, and age greater than 4 years.

The data documenting efficacy in regard to adult height continues to be limited despite European regulatory authorities' requiring this evidence before approval. A meta-analysis of final height data for 56 children born SGA was performed at the request of European authorities and demonstrated a mean height increase of 1.9 SDS for subjects treated with GH at 34 mg/kg per day versus 2.2 SDS for 69 mg/kg per day. A meta-analysis of three randomized studies of 28 patients reported that GH treatment for 7 to 10 years, initiated at doses of 34 to 69 mg/kg per day, can be expected to increase adult height by about 1.0 to 1.4 SDS.[1422] A larger, randomized, controlled study of 91 GH-treated and 33 untreated French adolescents (mean age, 12 years) reported a lower intergroup difference of 0.6 SDS in adult height after 2.7 years of treatment.[1423]

The many studies documenting efficacy of GH therapy in thousands of children with SGA are far too numerous to report in detail here and have been reviewed elsewhere.[1422,1424,1425] Although results vary, it appears that GH treatment can be expected to result in a 1-cm increment in height gain each year. The factors that can increase the gain in height include a greater height deficit relative to the midparental height, a higher GH dose, initiation of therapy at an earlier age, longer treatment duration, and the proposed presence of the *GHRd3* allele.[1384,1403,1424,1426] The addition of a GnRH analogue was shown to improve adult height in a select group of children born SGA.[1427] In a prediction model derived from KIGS SGA data, Ranke and coworkers[1426] found that age and GH dose were strong predictors of initial growth but that growth achieved during the first year was a powerful predictor of later growth. Although from these studies it appears that relatively high doses may be required for the growth response (the FDA-approved dose is 70 μg/kg per day), initial GH dosing at 35 to 50 μg/kg per day is reasonable since the response in individual patients is highly variable. The dose may be increased if the growth rate is insufficient.

Routine measurement of serum IGF1 has been recommended for patients receiving GH as a safety marker; the rationale being that elevated levels of IGF1 may result in an increased risk of side effects and potential for future malignancy.[1199,1428,1429] However, there is no documented evidence of such a link, and some children with SGA may have evidence of IGF1 resistance, requiring higher serum IGF1 levels to optimize growth.[1430] Further, using IGF1 levels to inform GH dosing has been shown to be less efficacious than standard dosing.[1431] This is likely due to the wide range of "normal" IGF1 levels in children and that serum IGF1 levels are relatively poor indicators of IGF1 sensitivity.

Children born SGA, especially those who have rapid postnatal weight gain, have reduced insulin-mediated glucose uptake and are more likely to have insulin resistance.[982,1432–1437] However, there have been no solid data documenting an increased risk of non-IDDM.[1436] There have been reports of several children in GH registration studies who had mild, transient hyperglycemia (prescribing information for Genotropin somatropin of recombinant DNA origin for injection, Pfizer, Inc., New York, 2006). Insulin resistance during GH treatment in children born SGA has been reported[1381,1438,1439] and typically resolves after discontinuation of GH.[1440] In a large database of children treated with GH, no differences in glucose regulation were found in 1900 children born SGA compared with children with ISS.[1441] With 6000 patient-years of exposure, no cases of diabetes mellitus have been reported during GH treatment.[1441–1444]

The European product labeling for GH treatment of children born SGA reads, "The management of these patients should follow accepted clinical practice and include safety monitoring of fasting

insulin and blood glucose before treatment and annually during treatment."[1425] Since GH treatment of children who are SGA reduces insulin sensitivity but only rarely causes glucose intolerance, and insulin resistance returns to normal following cessation of GH therapy, there is currently no indication to routinely monitor metabolic parameters.[1445,1446] In addition to insulin resistance alone, children born SGA have an increased risk of metabolic syndrome.[1443,1447–1449] The current literature provides no information on whether GH treatment in childhood increases risks for adult metabolic disease. Long-term follow-up studies are needed to determine the metabolic risk associated with GH treatment.

Osteochondrodysplasias

GH therapy has been studied in several skeletal dysplasias. The largest published study in achondroplasia involved 40 children; during the first year of treatment, the height velocity increased from 3.8 to 6.6 cm per year, and in the second year it decreased to approximately 5 cm per year.[1450] A modest improvement was seen in the ratio of lower limb length to height. Although GH was well tolerated, atlantoaxial dislocation during GH therapy was reported in one patient. In another study, normal growth velocity was achieved for up to 6 years in 35 subjects, with a significant increment in height SDS for at least 4 years[1450,1451]; in that study, vertebral growth was disproportionately greater than limb growth.

Bridges and Brook[1452] reported on the effects of GH therapy in 27 patients with hypochondroplasia; response was maximal during the first year of treatment, but substantial benefit was seen through 4 years of treatment in pubertal subjects.

Experience with GH treatment is limited in other skeletal disorders, such as dyschondrosteosis, hereditary multiple exostoses, osteogenesis imperfecta, and Ellis–van Creveld syndrome.

SHOX Haploinsufficiency and Léri-Weill Syndrome. Patients with mutations or deletions of the *SHOX* gene have variable degrees of short stature with or without mesomelic skeletal dysplasia. If untreated, short patients with SHOX deficiency remain short in adulthood. The *SHOX* gene is located in the pseudoautosomal region 1 (PAR1) on the distal end of the X and Y chromosomes at Xp22.3 and Yp11.3.[1453] Because genes in PAR1 do not undergo X inactivation, normal individuals express two copies of the *SHOX* gene. This gene encodes a homeodomain transcription factor that is expressed during early fetal life in the growth plate and functions in the regulation of chondrocyte differentiation and proliferation.[895,1454] SHOX haploinsufficiency (or deficiency) is the primary cause of short stature in patients with LWD (see earlier discussion).[941,1455] *SHOX* mutations and deletions are also found in patients with short stature without clinical evidence of dyschondrosteosis.[1456]

Clinical manifestations of SHOX deficiency include bowing of the forearms and lower legs, cubitus valgus, Madelung deformity or partial dislocation of the ulna at the wrist and elbow, short fourth and fifth metacarpals, and a high-arched palate along with characteristic radiologic signs.[894,941,1455] In a randomized 2-year study of 52 prepubertal subjects with a *SHOX* gene defect, the first-year height velocity in the GH-treated group was significantly greater than in the untreated control group (mean ± standard error of the mean [SE], 8.7 ± 0.3 vs. 5.2 ± 0.2 cm/year; $p < 0.001$) and was similar to the first-year height velocity to GH-treated subjects with TS (8.9 ± 0.4 cm/year; $p < 0.592$). GH-treated subjects with SHOX deficiency also had significantly greater second-year height velocity (7.3 ± 0.2 vs. 5.4 ± 0.2 cm/year; $p < 0.001$), second-year height SDS (–2.1 ± 0.2 vs. –3.0 ± 0.2; $p < 0.001$), and second-year height gain (16.4 ± 0.4 vs. 10.5 ± 0.4 cm; $p < 0.001$) compared with untreated subjects.[1457] Data on 28 persons with SHOX deficiency who received GH for a mean of 4.5 years through to adult height indicate that adjusted final height was –2.1 SDS, similar to girls with TS enrolled in the study. Calculated height SDS gain was 1.3, again similar to girls with TS.[1458]

Turner Syndrome and Langer Mesomelic Dysplasia. Homozygous mutation of the *SHOX* gene results in the Langer type of mesomelic dwarfism (i.e., LMD). A child with LMD and TS was found to have a *SHOX* gene abnormality resulting from a downstream allele deletion in her normal X chromosome. Although there have been numerous studies documenting growth improvement with GH therapy in patients with TS[907,1373,1396] and in those with heterozygous *SHOX* gene deletions,[1455] only one case of growth response to GH therapy in the rare condition of LMD with TS has been found. Growth rates of 3.46, 3.87, 2.3, and 0.7 cm per year were observed in the first, second, third, and fourth years of GH therapy, respectively, and there was no clinical deterioration of the skeletal deformities in this patient. Because there was a failure to achieve growth improvement with GH therapy, the authors concluded that GH therapy was not beneficial in patients with the severe short stature caused by combined TS and LMD resulting from homozygous *SHOX* deficiency.[1459]

Noonan Syndrome

Since 1994, when a gene for Noonan syndrome was mapped to chromosome 12 (12q24.1) and a mutation in the protein tyrosine phosphatase nonreceptor type 11 *(PTPN11)* was identified and characterized in familial cases, at least three other gene mutations have been identified.[922,923,1460–1463] While contributing to the broad heterogeneity of NS, these mutations have not completely localized the cause of the short stature. However, several genotype-phenotype correlations are relevant to patient management. Patients with *PTPN11* mutations often have normal or elevated GH levels and low serum IGF1 concentrations.[928] The *PTPN11* genetically modified mouse model had a GH-stimulated increase in ERK activation, and growth was stimulated by inhibition of ERK1/2.[1464] Several studies have reported effects of GH on near-adult height in patients with Noonan syndrome,[1465–1469] but all involved small numbers of patients with varied enrollment ages, treatment durations, GH doses, and responses. The largest study of the response to GH in children with Noonan syndrome came from the NCGS, a postmarketing observational study of recombinant human GH treatment in children with various disorders. A total of 370 patients with Noonan syndrome (mean enrollment age, 11.6 years) received GH (mean dosage, 0.33 mg/kg per week) for a mean of 5.6 years. In the 65 patients with data to derive near-adult height, the mean gain above the projected height was 10.9 ± 4.9 cm in boys and 9.2 ± 4.0 cm in girls. Duration of prepubertal recombinant human GH therapy and height SDS at puberty were important contributors to the near-adult height. No new adverse events were observed. The authors suggested that greater growth optimization would be possible with earlier initiation of therapy.[1470] This height increase was similar to the change observed in patients with TS but significantly less in those with idiopathic GHD in the same study.

Most experience with GH treatment of short stature in Noonan syndrome has been limited to small, uncontrolled studies in which few patients reached final height.[920,1471,1472] The clinical diagnosis of a dysmorphic syndrome potentially makes the treatment groups heterogeneous, although identification of a mutant *PTNN11* gene[1473] helps with characterization. Overall, treatment results for 3 to 4 years are similar to those attained in patients with TS; mean

growth velocities improve by 2 to 4 cm per year (over baseline rates of ~4 cm/year) during the first 4 years of therapy, with patients gaining from about –3.5 to –1.7 SDS of stature without inordinate advancement of bone age.[919,920,929,1472] Those children with identifiable *PTPN11* mutations are reported to have a poorer response to GH treatment in terms of growth and IGF production, suggesting impaired efficiency of phosphorylation-dependent GH signaling pathways.[927–929] Although the initial anecdotal experience suggested progression of ventricular hypertrophic cardiomyopathy, this was not confirmed in larger, carefully monitored studies.[920,1474,1475] However, it has been suggested that children with Noonan syndrome receiving GH be monitored for hypertrophic cardiomyopathy and hematologic abnormalities.[921] Although neoplasia is a theoretic concern, there are no studies associating GH therapy with malignancy. A recent report of brain tumor growth after several years of GH therapy[1476] suggests caution when treating with GH.

Idiopathic Short Stature (Subtle Errors Throughout the Growth Axis)

Although children with ISS, by definition, are without an identifiable cause of growth retardation, the term clearly encompasses a heterogeneous group that may include children with constitutional growth delay, genetic short stature, or subtle defects of the GH-IGF axis. Even though they are often grouped in one category, children with ISS have been shown to have a broad range of provocative GH responses, ranging from normal to elevated, and a wide range of serum IGF concentrations, from normal to IGF deficient. This group may also include children with unidentified syndromes or unidentified chronic illnesses or endocrine disorders. Children with ISS may experience stressful behavioral circumstances, but studies suggest that the relationship of psychosocial problems to the short stature is variable.[1477–1479] Nonetheless, hormonal intervention to enhance growth, with the aim of diminishing such difficulties, has been used.

Although the specific etiologies are often unknown, GH treatment has been used widely. Important questions have been raised about the financial, ethical, and psychosocial impact of GH therapy in "normal" short children.[1474,1480,1481] Given the cost of GH, the financial implications of treating such children are considerable. The point is well taken that 5% of the population will always be below the 5th percentile, whether we treat with GH or not, and that focusing on short stature could potentially handicap an otherwise normal child psychologically and socially. No convincing data have been presented to show that GH treatment of short children definitively improves psychologic, social, or educational function.[1481–1483] A possible exception is the improved intellectual function in SGA children treated with GH.[1484] Furthermore, final adult height in the subset of children with CDGD (probably a common inclusion, although not ISS as defined in the FDA approval) may be adequate without any treatment.[41–43]

Finally, the known and unknown treatment risks of GH therapy in otherwise apparently normal children, even if exceedingly small, are a legitimate concern.[1485] The failure to report levels of IGF1, IGFBP3, and GHBP in many studies and differing interpretations of endogenous GH secretion studies (e.g., assay variance, control group size), as well as the heterogeneity of the patient groups, confounds assessment of response data. Nevertheless, it is clear that many children who do not meet conventional criteria for the diagnosis of GHD and who fall under the heading of ISS have as great a degree of growth retardation as children with bona fide GHD and might be considered suitable candidates for growth-promoting therapy.

Members of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Pediatric Endocrinology meeting in 2007 published a manuscript detailing their agreement on the evidence-based evaluation and management of ISS in children. They agreed that the primary goal of therapy should be the attainment of normal adult height. They also agreed that the expectation from patients and their families that taller stature is associated with positive changes in quality of life should be discouraged.[1480] Physicians should inform families about the available therapeutic options and provide a realistic expectation for height gain with therapy, including the fact that there may be variability in the outcome, and the psychologic counseling options should be discussed. In addition, the patient and family should be aware that therapy may be discontinued if the growth response is poor or if the child no longer provides assent. The physician is responsible for continued monitoring of efficacy and safety and should provide flexibility in treatment options.

The studies that ultimately led to approval of GH treatment in the United States included the long-term, randomized, double-blind, placebo-controlled trial at the National Institutes of Health by Leschek and associates[1178] and the randomized dosing trial of the European Idiopathic Short Stature Study Group.[1486] In the former trial, which used a less optimal regimen of 0.22 mg/kg per week given thrice weekly in subjects with a mean starting height of almost –3 SDS, the treated children had mean final height gain of 3.7 cm over the control group.[1178] In the latter randomized study, GH doses of 0.24 and 0.37 mg/kg per week, given as a regimen of six doses per week in children with a mean starting height of –3.0 to –3.4 SDS, the improvement in the higher-dose group exceeded that in the lower-dose group by 3.6 cm, with subjects achieving a final height of –1.12 SDS.[1486] When the data from the two trials were combined, there was a cumulative gain of 7.3 cm in the group treated with 0.37 mg/kg per week, compared with placebo.

In a meta-analysis looking at an aggregate group of 1089 children, there were four controlled trials that presented adult height data showing treatment benefits ranging from 0.54 to 0.84 SDS, corresponding to a mean effect on growth of 5 to 6 cm.[1487]

Concerns had been raised that GH treatment might accelerate pubertal onset and progression in children with ISS, resulting in failure to improve height SDS for bone age and offsetting the positive responses observed during the early years of GH treatment. In the European ISS study,[1488] there was no evidence of accelerated pubertal or skeletal maturation in the group receiving the higher dose, in contrast to data reported by Kamp and colleagues[1489] with a 30% greater GH dose. This hypothetical effect of advancing maturation has not been substantiated by additional studies.[1490–1493] Taken together, these data show that GH treatment of prepubertal children with ISS increases growth velocity and final height.

The auxologic criteria for GH therapy in the United States are based on FDA approval of GH treatment for children shorter than –2.25 SDS (1.2 percentile). Some believe that children should be treated if their height is below –2.0 SDS and more than 2.0 SDS below their midparental target height or if their predicted height is below –2.0 SDS (or both). Although there is no consensus on the age for initiating treatment, most studies of GH therapy in children with ISS have involved children from 5 years to early puberty. There are no accepted biochemical criteria for initiating GH treatment in ISS. The bone age is used to predict adult height, although in a longitudinal study of ISS subjects, bone age delay had an effect on the precision of the prediction. In children with

a bone age delay of about 2 years, the average adult height was almost equal to the predicted height; in children with no bone age delay, the adult height was greater than the initial predicted height; and if the bone age was delayed by more than 2 years, the adult height was significantly below the predicted adult height.[1494] The psychologic benefits of GH therapy in children with ISS are unproved,[1495] but medical and psychologic interventions should be considered for the child who seems to suffer from his or her short stature.

The clinical trials of the treatment of ISS with GH in the literature often did not contain long-term control groups, hence the results show variable growth responses.[1485,1496] Most short children treated with GH experience growth acceleration that is usually sustained over the first several years of therapy, with attenuation of the treatment response over time. In general, a slower pretreatment growth velocity and a higher weight-to-height ratio—factors suggestive of GHD and, to a lesser degree, bone age retardation—are associated with better early growth responses. Three thousand children were classified as having ISS in the KIGS database, with 153 having reached final height.[1491] GH treatment (0.2–0.25 mg/kg per week) resulted in achievement of target height in patients with familial short stature, although they continued to have short stature as adults (–1.7 SDS in males and –2.2 SDS in females), with a mean gain during therapy of 0.6 to 0.9 SDS. In children who did not have familial short stature, the mean final height was greater for males (–1.4 SDS), although not for females (–2.3 SDS), with average gains of 1.3 and 0.9 SDS, respectively. These attained heights were quite different from the midparental target heights, which were near 0 SDS. Hintz and colleagues[1177] assessed adult height in 80 North American children with ISS who were treated for up to 10 years at a GH dose of 0.3 mg/kg per week. At the conclusion of the study, the mean height SDS was –1.4 SDS with a gain of 1.3 SDS, results quite similar to those from the KIGS database. Although the Hintz study was not placebo controlled, the data were compared with predicted and actual final heights in two groups of untreated short children followed for similar periods. Compared to untreated children, treated boys achieved a mean of 9.2 cm and girls 5.7 cm stature above predicted heights.

Several additional studies have documented the increase in final height with GH therapy in children with ISS, although the responses have been highly variable and dose dependent. The mean increase in adult height in children with ISS is 3.5 to 7.5 cm (with an average duration of therapy of 4–7 years) when compared with historic control subjects,[1177,1497] with patients' pretreatment predicted adult heights, or with nontreatment or placebo control groups.[1178,1487] Multiple factors affect the growth response to GH, and the best response is seen in children who are younger or heavier, those receiving higher GH doses, and those who are shortest relative to their target height. Adult height outcome is influenced negatively by age at initiation of therapy and positively by midparental height, initial height, delayed bone age, and the magnitude of the first-year response to GH.[1498,1494] A 2-year study suggested that an increase in IGF1 correlates with height gain.[1178] Recently, a review of the literature on ISS was published, concluding that GH therapy increases height in some children; however, the cost-benefit ratio and long-term safety are concerning.[1499]

Studies using monotherapy with GnRH agonists have shown only a small and variable effect on final adult height, so GnRH agonist therapy is not recommended. Furthermore, GnRH agonists have been shown to have short-term adverse effects on BMD.[1500] However, combination therapy with a GnRH agonist and GH has potential value in increasing final height, but only with treatment lasting at least 3 years.[1501,1502] However, in a randomized study, children treated with a combination of GnRH agonist and GH and control subjects had no difference in adult height.[1502] A recent meta-analysis of eight selected studies showed a significant difference in adult height and predicted adult height comparing GnRHa and GnRHa/GH groups, but there was no significant difference in final height SDS and initial height SDS between the groups. The results suggest adding GH to GnRHa therapy may increase adult height in girls with early puberty. The study was limited by the small number of studies, its focus on early puberty in girls, and was not stratified to assess associations between patient age and predicted adult height at initiation of therapy and adult height.[1503]

Monitoring of GH treatment should include height, weight, pubertal development, and adverse effects (scoliosis, tonsillar hypertrophy, papilledema, and slipped capital femoral epiphysis) two to three times per year. After 1 year of therapy, the height velocity SDS and the change in height SDS should be calculated. The bone age may be obtained to reassess height prediction and if one is considering intervention to delay puberty. IGF1 levels may be helpful in guiding GH dose adjustment. Because elevated blood glucose levels in patients with GH-treated ISS have not been reported, routine monitoring of glucose metabolism is not advised.

The FDA-approved dose for GH in ISS is 0.3 to 0.37 mg/kg per week.[1504] The dose may be increased if the growth response is inadequate and compliance was assured. However, there are no data regarding the long-term safety of GH doses higher than 0.5 mg/kg per week in children with ISS. IGF1 levels may be helpful in assessing compliance and GH sensitivity. IGF1 levels that are elevated (>2.5 SDS) should prompt consideration of GH dose reduction. IGF-based dose adjustments in children with ISS increased short-term growth when higher IGF targets were selected, but long-term studies with respect to efficacy, safety, and cost effectiveness have not been done.[1489] If the growth rate continues to be inadequate, GH treatment should be stopped and alternative therapies considered. The duration of treatment is controversial, with consideration given for stopping treatment when near-adult height is achieved (height velocity <2 cm/year or bone age >16 years in boys or >14 years in girls) if height is in the normal adult range (above –2 SDS). Stopping therapy may be influenced by satisfaction of the patient and family with the resulting height, an ongoing cost-benefit analysis, or desire of the child to stop for other reasons (e.g., dissatisfaction with daily injections).

The possible side effects in GH-treated children with ISS are similar to those previously reported in children receiving GH therapy for other indications, with no long-term adverse effects documented.[1505,1506] Posttreatment surveillance with a focus on cancer prevalence and metabolic side effects is advisable.

The average cost of GH treatment of children with ISS is $10,000 to $20,000 per centimeter of growth improvement.[1497] The benefits are less clear,[1497] because it is unknown whether a gain in height improves quality of life. Therefore recommendations for treatment that increases adult height should be balanced against the high cost of these therapies.

Miscellaneous Causes of Growth Failure

In addition to the clinical conditions described earlier, GH has been used in the treatment of short stature associated with a variety of other conditions involving postnatal growth failure. In general, such trials have been uncontrolled and have not included sufficient numbers of subjects for efficacy to be evaluated. Examination of such treatments should be continued in the large international databases.

Down Syndrome. The encouraging results of GH trials in TS led to studies of GH therapy in children with Down syndrome. In several preliminary studies, GH accelerated growth in such patients, although ethical issues were raised concerning the appropriateness of such therapy.[901,1507–1509] In the uncontrolled NCGS experience, 23 children experienced a 1.3-SDS height gain over the first 4 years of GH therapy.[1471] No convincing data exist that GH improves neurologic or intellectual function in such patients. The increased risks of diabetes mellitus and leukemia in children with Down syndrome could be augmented by GH therapy.[1510] A review of GH therapy given to 15 children with Down syndrome reported an increase in mean height but no effect on mental development. They do not recommend GH therapy and call for further controlled studies.[900]

Normal Aging and Other Catabolic States. Detailed consideration of the potential use of GH in normal aging is beyond the scope of this chapter. The rationale for such therapy is based on the concept of the *somatopause,* a term that highlights the progressive decline in GH secretion after 30 years of age, as reflected in decreasing IGF1 levels. Aging can be viewed as a catabolic state, with the potential that GH therapy might reverse or retard the loss of muscle mass and strength and the decrease in bone density that occur with aging. There is no evidence that GH supplementation during aging improves muscle strength or quality of life.[1511]

Growth failure, often with impaired final adult height, is a characteristic clinical finding in endogenous or exogenous Cushing syndrome. Excess glucocorticoids cause a catabolic state characterized by increased proteolysis, decreased protein synthesis, lowered osteoblastic and increased osteolytic activity, and insulin resistance.[1254] GH treatment blunts some of these catabolic actions but increases the insulin resistance.[1255] Mauras and Beaufrere[862] showed that IGF1 therapy similarly induces an anabolic response, despite excess glucocorticoids, but does not cause insulin resistance. GH treatment after transplantation[1351–1353] and in other glucocorticoid-treated children[851] causes some height increments but does not produce as good a response as in individuals not taking glucocorticoids. GH does enhance bone formation and increases osteoblastic activity in such children.[1512] The marked increase in IGF1 levels during GH treatment may be sufficient to overcome the local insensitivity to IGF action.[300,756,757,1513]

GH therapy is also being investigated in catabolic states such as burns, tumor cachexia, major abdominal surgery, AIDS, sepsis, metabolic acidosis, and situations requiring total parenteral alimentation. GH should not be used in critically ill patients, because a randomized, controlled trial demonstrated increased morbidity and mortality rates with GH treatment of such patients.[1514] The FDA-approved indications for the use of GH for purposes other than stimulation of growth are (1) GHD in adults, (2) AIDS-associated wasting or cachexia, and (3) short-bowel syndrome requiring total parenteral nutrition.

Adverse Effects of Growth Hormone

Pituitary-derived human GH had an enviable safety record for a quarter of a century but proved to be an agent for transmission of the fatal spongiform encephalopathy, CJD.[1194–1196] Although pit-GH was removed from use in the United States in 1985, and later throughout the world, more than 200 patients with GH-derived CJD have been identified.[1194–1196] Although recombinant DNA–derived GH does not carry this risk, the experience with pit-GH serves as a grim reminder of the potential toxicity that can reside in products used for physiologic replacement.

Extensive experience with recombinant GH over nearly 30 years has been encouraging.[1092,1307,1430,1515–1517] Concerns have been raised about a number of potential complications, which clearly require continued follow-up and assessment. This evaluation has been facilitated by the extensive databases that have been established by GH manufacturers, for example, Genentech (NCGS)[1307] and Pharmacia (KIGS).[1442,1518] An important limitation of these registries, however, is that although they have provided valuable information about the risks of disease during GH use, they were not designed to provide data on adverse effects after discontinuation of GH use.

Development of Leukemia and Other Malignancies

A report in 1998 of five cases of leukemia that developed in GH-treated Japanese children raised concern that GH therapy could increase the risk of leukemia.[1519] One difficulty in assessing the role of GH treatment in this disorder is that many GH-deficient children have conditions that predispose to the development of leukemia, such as prior malignancy, a history of irradiation, or concurrent syndromes that themselves are associated with the development of leukemia (e.g., Bloom syndrome, Down syndrome, Fanconi anemia). After the initial concerns about the role of GH treatment in the development of leukemia, long-term data have shown that GH treatment does not increase the incidence of leukemia in children who are not already at an increased risk of leukemia,[1307,1520–1522] does not increase the risk of leukemia as a subsequent neoplasm in cancer survivors,[1523] and does not increase the rate of leukemia relapse.[1442,1520,1524] Although it is not possible to determine if GH treatment increases the incidence of leukemia in children at increased risk for reasons other than prior malignancy, the incidence of leukemia in these at-risk children does not appear to be out of line with the expected rate.[1521] Nonetheless, care should be used in prescribing GH therapy for children with a past history of leukemia or lymphoma or other disorders conveying an increased risk of leukemia.

With respect to malignancies other than leukemia, data from more than 88,000 GH-treated patients with more than 275,000 patient-years at risk did not reveal an increased risk for nonleukemic extracranial neoplasms.[1307,1452,1516] However, an increased risk of post-transplant lymphoproliferative disease (odds ratio 1.88; 95% confidence interval [CI] 1.00–3.55, $p = 0.05$) was found in children treated with GH before transplant for growth failure related to their end-stage renal disease.[1525] And a statistically nonsignificant increase in malignancies was found in GH-treated girls with TS (standardized incidence ratio compared with the age-matched general population of 2.1; 95% CI, 0.76–4.49).[1396]

Recurrence of Central Nervous System Tumors

Because many recipients of GH have acquired GHD due to CNS tumors or their treatment, the possibility of tumor recurrence with therapy is of obvious importance. Estimates of CNS tumor recurrence rates in non–GH-treated children and adolescents are difficult to obtain, bearing in mind the vast array of treatment programs used in the past four decades. In a total of 1083 patients compiled from 11 reports who were not treated with GH, 209 (19.3%) had recurrences.[1251,1524,1526–1534] Such data in a heterogeneous group, including patients with craniopharyngiomas, gliomas, ependymomas, medulloblastomas, and germ cell tumors, provide a background for assessing recurrence rates in GH-treated youth. Reports from nine centers, encompassing 390 patients, indicated recurrence in 64 patients (16.4%) at the time of publication,[1524,1526,1534–1539] which is not much different from the

recurrence rate observed in a much larger number of untreated patients. In a particularly well-done comparative study at three pediatric neuro-oncology centers comprising 1071 brain tumor patients, 180 patients were treated with GH for a mean of 6.4 years, and 31 of them were monitored for more than 10 years; the relative risk of recurrence or death was similar with and without GH treatment.[1538] In a study of 361 cancer survivors (including 172 brain tumor patients), disease recurrence of all cancers in GH-treated children did not differ from recurrence in those children not treated with GH.[1523] A single center study of 110 previously irradiated brain tumor patients found no difference in recurrence in GH-treated patients compared to matched control subjects after approximately 15 years of follow-up.[1540] Finally, extensive analysis of 4410 patients with a history of brain tumor or craniopharyngioma before GH therapy in the NCGS and KIGS databases[1442,1516,1541] showed a similar lack of increased tumor recurrence. In the NCGS series, recurrence rates of the most common CNS neoplasms, craniopharyngioma (6.4%), primary neuroectodermal tumors (medulloblastoma and ependymoma, 7.2%), and low-grade glioma (18.1%) were lower than or similar to those reported in non–GH-treated children.[1252,1524,1542]

Development of Subsequent Neoplasms

Children who have survived cancer are at an increased risk of developing a second neoplasm because of either an underlying genetic predisposition or the consequences of the treatment for the primary malignancy, including radiation therapy and treatment with alkylating agents. Because of the mitogenic and antiapoptotic effects of GH and IGF1, there is concern that GH treatment could increase the rate of subsequent neoplasms. This has been monitored through both the large NCGS and KIGS GH treatment databases, as well as through the Childhood Cancer Survivor Study (CCSS) group. The CCSS studies followed 361 GH-treated cancer survivors, and the initial report found an overall increased risk of 3.21 (95% CI, 1.88–5.46) for second neoplasms.[1523] Although the majority of these second neoplasms were meningiomas, there were three children who developed osteogenic sarcoma out of 122 leukemia/lymphoma survivors who had been treated with GH. A similar increased risk for second neoplasms, also largely meningiomas, was found in a study of 60 GH-treated adult patients.[1543] In 2500 GH-treated cancer survivors in the NCGS database, there was also an increased incidence in secondary malignances, predominantly CNS tumors (glioblastoma/glioma, astrocytoma, meningioma) and osteogenic sarcoma.[1307] However, the most recent follow-up of the CCSS found that the rate of meningioma in those children not treated with GH had risen over time to equal that of survivors treated with GH. Therefore the CCSS data found that the rate of subsequent CNS neoplasms was not increased by GH treatment,[1544] with an adjusted rate of 1.0 for any CNS secondary neoplasm, 0.8 for meningiomas, and 1.9 (95% CI, 0.7–4.8; $p = 0.21$) for gliomas. The equalization of the rate ratio for meningiomas with longer follow-up raised the possibility that GH treatment may accelerate the manifestation of these tumors, rather than increase the absolute rate. Another possible explanation, however, is that the GH-treated patients had more intense surveillance because of their GH treatment and were therefore identified at an earlier stage.

Pseudotumor Cerebri

Pseudotumor cerebri (idiopathic intracranial hypertension) has been reported in GH-treated patients.[1307,1442,1516,1545] The disorder may develop within months after treatment starts or as long as 5 years into the course; it appears to be more frequent in patients with renal failure than in those with GHD.[1307,1516] The mechanism for the effect is unclear but may reflect changes in fluid dynamics within the CNS. Pseudotumor has also been described after thyroid hormone replacement in patients with hypothyroidism. In any case, clinicians should be alert to complaints of headache, nausea, dizziness, ataxia, or visual changes. Significant fluid retention with edema or hypertension is rare.[1546] Because of the possible association of pseudopapilledema with GHD, perhaps representing a variant of optic nerve hypoplasia,[1547] careful ophthalmologic evaluation should be undertaken in patients with suspected GH therapy–associated pseudotumor cerebri to avoid overdiagnosis and invasive treatments.

Slipped Capital Femoral Epiphysis

Slipped capital femoral epiphysis is associated with both hypothyroidism and GHD. Whether GH therapy plays a role in this disorder has been difficult to determine, in part because the incidence of slipped capital femoral epiphysis varies with age, gender, race, and geographic locale. The reported incidence is between 2 and 142 cases per 100,000 population; the data in the KIGS and NCGS studies are in this range.[1307,1442,1516] Accordingly, although slipped capital femoral epiphysis cannot be attributed to GH therapy per se, complaints of hip and knee pain or limp should be evaluated carefully. The occurrence of such pain in a GH-treated child with an exceedingly rapid growth rate should lead to consideration of this diagnosis.

Scoliosis

Both progression of preexisting scoliosis and new-onset scoliosis have been described in children treated with GH.[1307] The numbers are extremely small, with only 238 reported cases among 54,996 patients in the NCGS database. There is specific concern for GH effects on scoliosis in TS and PWS patients, in whom the underlying rate of scoliosis is increased. Although the rate of scoliosis in GH-treated TS patients was higher than in other GH-treated patients, it remained rare, being reported in only 0.69% of patients.[1396] PWS by itself has a rate of scoliosis of 30% to 80%, and GH treatment does not appear to increase the rate or severity of scoliosis in PWS patients.[1325,1548]

Diabetes Mellitus

The association of GH treatment with insulin resistance is well documented.[1549] In addition, candidates for GH therapy include some who are known to have increased risk of type 2 diabetes mellitus (T2DM), including patients with TS, PWS, or those born IUGR. Data from the NCGS database estimated the incidence of T2DM in GH-treated patients as approximately 14 cases per 100,000 patient-years,[1307,1442] and data from KIGS and from GeNeSIS (Genetic and Neuroendocrinology of Short Stature International Study; another large pharmaceutical company–sponsored postmarketing surveillance database) found the incidence of T2DM in GH-treated children to be approximately 30 cases per 100,000 patient-years.[1550,1551] The incidence found in the KIGS and GeNeSIS studies was 6 to 6.5 times higher than the expected incidence.[1550,1551] Many of these children did have risk factors for T2DM, although not all of them were obese.[44,45,1516] In some, but not all, of the subjects who developed T2DM, there was resolution of the diabetes on discontinuation of GH treatment,[1520,1550,1551] likely highlighting the underlying risk of diabetes in these individuals and supporting a causative role of GH treatment in the development of diabetes. Therefore the reduction

of insulin sensitivity induced by GH is a concern that demands close assessment, particularly in high-risk patients such as those with PWS or TS or a history of IUGR. Reassuringly, however, the prevalence of diabetes in young adults who had previously been treated with GH in childhood was not increased in a large population-based study.[1552]

Miscellaneous Side Effects[1307,1442,1516]

Other potential side effects of GH therapy include prepubertal gynecomastia,[1553] pancreatitis,[1554] growth of nevi[1555,1556] (although typically without evidence of malignant degeneration[1520]), behavioral changes, worsening of neurofibromatosis, hypertrophy of tonsils and adenoids, and sleep apnea. GH treatment increases the conversion of cortisol to cortisone, which may trigger a need for glucocorticoid replacement therapy in those with subclinical central adrenal insufficiency.[1430] GH treatment also increases the conversion of thyroxine to triiodothyronine, which could either increase levothyroxine dose requirements in those treated for central hypothyroidism or unmask preexisting but undiagnosed central hypothyroidism.[1430] A report[1557] of reduced testicular volume and elevated gonadotropin levels in four young adult men previously treated with GH for ISS was not confirmed by a double-blind, placebo-controlled study,[1558] nor by the international databases.[1559] This list of side effects is only partial. It is best for the clinician to remember that GH and the peptide growth factors it regulates are potent mitogens with diverse metabolic and anabolic actions. All patients receiving GH treatment, even as replacement therapy, must be carefully monitored for side effects.

For the most part, the side effects of GH are minimal and rare. When they do occur, a careful history and physical examination are adequate to identify their presence. Management of these side effects may include transient reduction of dosage or temporary discontinuation of GH.[1056] In the absence of other risk factors, there is no evidence that the risk of leukemia, brain tumor recurrence, or slipped capital femoral epiphysis is increased in recipients of long-term GH treatment. Any patient receiving GH who has a second major medical condition (e.g., being a tumor survivor) should be followed up in conjunction with an appropriate specialist such as an oncologist or neurosurgeon. Whereas GH has been shown to increase the mortality rate of critically ill patients in intensive care units,[1514] there is no evidence that GH replacement therapy needs to be discontinued during intercurrent illness in children with GHD.

Recent in vitro studies have found that growth hormone could play a role in the progression of certain cancer types through the induction of an epithelial to mesenchymal transition.[1560] This effect would potentially increase the risk of cancer metastasis; therefore it strongly argues against GH treatment in patients with active malignancies.

While the impact of GH treatment on long-term mortality is complex (see upcoming discussion), there is no evidence that GH treatment in childhood increases the acute mortality risk.[1561]

The Question of Long-Term Cancer Risk

Several epidemiologic studies have suggested an association between high serum IGF1 levels and an increased incidence of malignancies.[1562–1564] Such studies have found an association of higher IGF1 levels with breast, prostate, and colon cancer.[1564–1566] In contrast, higher IGFBP3 levels have been associated with a decreased risk of prostate and breast cancer, although other studies found no association of IGFB3 levels and cancer[1564] or found a positive association.[1564,1566] Because GH increases production of both IGF1 and IGFBP3, the studies that found the highest association with cancer with high IGF1 and low IGFBP3 levels do not suggest a simple relationship between GH and cancer risk.

Epidemiologic studies assessing the risk of malignancy in patients with acromegaly found differing results, with some,[1566–1569] but not others,[1570,1571] identifying significant associations between acromegaly and colon cancer risk. The small size and uncontrolled retrospective nature of these studies and the multiple possible sources of bias make these reports difficult to interpret. The largest study to date, reviewing more than 1000 patients, indicated no overall increased cancer incidence in acromegaly.[1572] Although colon cancer risk was also not increased in that study, the mortality rate was higher, suggesting an effect of GH or IGF on established tumors.[1573] A prospective analysis of colon cancer and colonic polyps in acromegalics did not observe an association between these two diseases when using either autopsy series or prospective colonoscopy screening series for the control population.[1574] Acromegaly is associated with a marked increase in the incidence of benign hyperplasia in several organs, including colonic polyps.[1575] Such findings suggest that the GH-IGF1 axis may lead to symptomatic benign proliferative disease, which could be associated with symptoms, such as rectal bleeding, that would lead to a potential detection (or ascertainment) bias.

Children receiving GH do not appear to have a greater risk of de novo or recurrent tumors.[1092,1307,1442,1516,1576] A cohort of 1848 patients treated with GH in the United Kingdom was assessed after as long as 40 years and found to have increased rates of colorectal cancer and Hodgkin disease, but the tumor-associated deaths were so few that a single patient death would markedly alter the risk ratios.[1577,1578] No increased incidence of cancer was found in GH recipients among adults who were treated for GHD.[1579,1580] A large European study found no overall increase in cancer risk in adults who were treated with growth hormone as children and who did not have an apparent underlying cancer risk.[1576] However, that study did find an increased incidence of bone and bladder cancer, along with possible evidence of an increase in Hodgkin lymphoma risk in these adults. In addition, although not found in all studies,[1581] there have been some data suggesting an increased risk of cancer mortality after childhood GH treatment.[1576,1581,1582] One of these studies found an increased mortality rate from bone tumors,[1581] while a large European study found an increased cancer mortality risk associated with the daily GH dose in patients treated with GH after previous cancer.[1576] These reports represent imperfect, uncontrolled studies. The overall data do not indicate a clear association of GH therapy with the future development of neoplasms in the absence of other risk factors, but there remains some uncertainty over the risk of specific cancers and perhaps over cancer mortality risk.

The use of IGF1 and IGFBP3 in the monitoring of GH recipients, both adult and pediatric, has been recommended and endorsed by international bodies such as the Growth Hormone Research Society,[1055] the Drug and Therapeutics Committees of the Lawson Wilkins Pediatric Endocrine Society, and the European Society of Pediatric Endocrinology.[1577,1578] Until the issue of cancer risk in GH therapy is fully resolved, the prudent approach appears to be regular monitoring of both IGF1 and IGFBP3 and alteration of the GH dose to ensure that the theoretic risk profile induced by GH therapy is favorable. This can be done by avoiding the unlikely situation of a GH-treated patient with an IGF1 level at the upper end of the normal range and an IGFBP3 level at the lower end of the normal range.

Long-Term Mortality with GH Treatment

The effect of GH deficiency and GH treatment on overall mortality is complex. Subjects with untreated congenital GH deficiency from mutations in *PROP1* appear to have increased lifespan.[1564] In contrast, individuals with GH deficiency from *GH1* gene deletion have a decreased lifespan, with increased cardiovascular and infectious disease mortality rates,[1564] yet subjects with GH deficiency from *GHRH* mutations have no change in mortality rate compared with the local population.[1564,1583] The background mortality rates of the gene deletion and *GHRH* mutation ethnic populations were quite different, which may account for some of the mortality discrepancy. Untreated Laron patients have been found to have a marked decrease in cancer mortality rate.[1564] Mortality data on GH-treated Laron patients are not available. Mortality risk is increased in adults with untreated GH deficiency (due to increased mortality rate from cardiovascular disease) but also in adults with GH excess (due to an increase in cardiovascular, cerebrovascular, and respiratory deaths).[1564] In 2012, investigators reported on the all-cause mortality rate of French children treated with GH for isolated GH deficiency. GH treatment in this cohort was initiated between 1985 and 1996, with a mean age at follow-up of 28 years. They found an increase in all-cause mortality rate when compared to the French general population, with a standardized mortality ratio of 1.33 (95% CI 1.08–1.64).[1581] The diseases contributing to this increase in mortality rate were diseases of the circulatory system and subarachnoid or intracerebral hemorrhage. In response to this report, investigators looked at all-cause mortality rate in GH-treated children in Sweden, Belgium, and the Netherlands.[1582] These patients started treatment between 1985 and 1997 and had a mean age of 27 to 29 years at follow-up. In this cohort, no subjects died from cancer or cardiovascular disease. Another report also found no increase in mortality in 3847 adults in Sweden treated with GH in childhood for IGHD, ISS, or SGA.[1584] Thus the data do not give a clear answer to the long-term effects of GH treatment on longevity, including whether the impact would differ based on the indication for GH treatment.

IGF1 Treatment

In 2005, the FDA approved the use of rhIGF1 for "long term treatment of growth failure in children with severe primary IGF1 deficiency and in children with GH gene deletions who have developed neutralizing antibodies to GH. Severe primary IGF1 deficiency is defined by height SD score less than –3.0 and a basal IGF1 SD score less than –3.0 and normal or elevated GH. Severe primary IGF1 deficiency includes patients with mutations in the GHR, post-GHR receptor signaling pathway, and IGF1 gene defects" (Increlex [rhIGF1] prescribing information, 2009, Tercica, Inc.). Clinical trials to study the safety and efficacy of rhIGF1 therapy have been conducted mostly in individuals with proven mutations in the *GHR*; fewer than 10% of enrollees have had GH insensitivity due to GH antibodies. Because of the rarity of *GHR* mutations, the clinical trials have been small.

A number of short-term growth-related studies with subcutaneous IGF1 treatment at varied doses have been reported. Despite slight differences in IGF1 dosage and inclusion criteria, the short-term effects of IGF1 on growth in GH insensitivity were consistent: Growth increased from pretreatment rates of 3 to 5 cm per year to 8 to 11 cm per year on average. In five children with GHR deficiency, single daily doses of 150 μg/kg for 3 to 10 months resulted in growth acceleration to rates of 8.8 to 13.6 cm per year.[1585] Wilton[1253] reported collaborative data on the treatment of 30 patients, ages 3 to 23 years, with GH insensitivity due to GHR deficiency or IGHD type IA with anti-GH antibodies; the dose of IGF1 varied from 40 to 120 μg/kg twice daily. With the exception of the two oldest individuals, growth rates increased in Wilton's subjects by at least 2 cm per year. A mean increment of more than 4 cm per year in growth velocity was found in 11 prepubertal children treated with 80 μg/kg twice daily.[1585] This study also demonstrated a significant inverse relationship between growth response to exogenous IGF1 and severity of the GH insensitivity phenotype.

Longer-term studies of IGF1 treatment of GH insensitivity demonstrated a progressive waning effect of IGF1 on growth velocity.[1586] Data from 17 patients in a European collaborative trial who were treated for at least 4 years showed an increase in mean height SDS from –6.5 to –4.9, with two adolescents reaching the 3rd percentile. Children who received rhIGF1 for 2 years or less had a higher growth velocity than those children who received rhIGF1 for longer periods. In the longest treatment study of 58 children with GH receptor mutations, GH antibodies, or biochemical evidence of GH insensitivity without documented mutation,[1587] outcomes were similar to those in the European study, with an initial burst of growth (from 3.1 to 8.0 cm/year) followed by slowing to just above baseline (4.8 cm/year) by the fourth year of therapy. Height SDS improved from –6.5 to –5.2 after 5 to 6 years of therapy. Data on 21 of these patients at or near adult height with average treatment duration of 10 years ranged from –8.0 to –6.2 SDS, lower for those persons with GH antibodies as the underlying cause for their GH resistance.[1588]

These limited data indicate that rhIGF1 is effective in increasing statural growth, but the growth response is neither as robust nor as sustained as the growth response to GH among children with GHD. The suboptimal growth response to rhIGF1 compared with recombinant human GH has been attributed to (1) inability of rhIGF1 to increase IGFBP3 and ALS levels, leading to decreased delivery of IGF1 to target tissues; (2) lack of GH-induced proliferation of prechondrocytes in the growth plate; (3) absence of GH-induced local IGF1 at the growth plate; and (4) difficulty in using higher doses of IGF1 because of the risk of hypoglycemia.

IGF1 treatment administered to individuals with *IGF1* gene deletion[656] and post-GHR signaling defects with growth patterns was similar to those of children with GH insensitivity due to GHR deficiency. First-year results from an open-label trial of 124 children with low serum IGF1 and height SDS below –2 without biochemical evidence of GH insensitivity indicate that growth velocity increased for the year of therapy (from 5 to 7 cm/year).[1256]

The most common adverse effect of rhIGF1 is hypoglycemia. In some studies, it occurred in almost half of the patients with GH insensitivity treated with rhIGF1,[1587] usually within the first month of therapy. Importantly, individuals with GHR defects, the highest proportion of enrollees in these treatment studies, have baseline hypoglycemia. In a 6-month, placebo-controlled trial, 67% of children receiving placebo experienced hypoglycemia, compared with 85% of children receiving rhIGF1—a difference that was not statistically significant.[1589] In a study of 23 patients with GH insensitivity, hypoglycemia was noted before the start of treatment; after 3 months, 2.6% of glucose values in those receiving placebo were less than 50 mg/dL, compared with 5.5% of glucose values in those receiving rhIGF1.[1587] The hypoglycemia was not to be avoidable with adequate food intake. In children with low IGF1 but without GH insensitivity, 20% of subjects who received the higher IGF1 dose experienced symptoms attributed to hypoglycemia; documentation of hypoglycemia was only noted in one subject.[1256]

Patients with GH insensitivity treated with rhIGF1 have also experienced lymphoid tissue hypertrophy, encompassing tonsillar/adenoidal growth and associated snoring and sleep apnea, and thymic and splenic enlargement.[1587] The hypertrophy occurred in 22% of patients, with 10% of patients requiring tonsillectomy/adenoidectomy. Coarsening of the face was observed in patients of pubertal age.[1587] Studies have reported an increase in BMI with treatment and a twofold to threefold increase in body fat as assessed by dual-energy x-ray absorptiometry (DEXA).[1590] The study to adult height did not note this significant increase in body fat.[1588] Intracranial hypertension with associated papilledema has also been observed in as many as 5% of treated patients. In the study by Midyett and coworkers, 2 of 100 patients receiving IGF1 had documented intracranial hypertension.[1256] Anti-IGF1 antibodies develop in about half of treated patients during the first year, but they have no effect on the response.[1587] As with recombinant human GH treatment, an increased cancer risk with rhIGF1 treatment is an unknown but legitimate concern, considering the role of IGF1 in neoplasias and the increased cancer risk associated with hypersomatotropism.[1591]

Approval of rhIGF1 by the FDA and the European Agency for the Evaluation of Medical Products has ignited debate about expansion of the diagnosis of severe IGF1 deficiency and consequent expanded use of rhIGF1 beyond those patients with neutralizing GH antibodies or documented mutations in the *GHR*, GHR signaling pathways, or the IGF1 gene. Some advocate that individuals with ISS, low serum IGF1, or poor response to a GH trial may have unidentified subtle alterations in GHR signaling and could benefit from treatment with rhIGF1.[1592] Others express reservations about expanding the diagnosis of IGF1 deficiency and rhIGF1 use, postulating that the response of children with ISS to rhIGF1 may be minimal considering the lower long-term growth response of children with GHR defects treated with IGF1 compared with GH-deficient children treated with GH. Another theoretic concern regards negative feedback of rhIGF1 on GH secretion, which leads to diminished IGF1-independent effects of GH on growth.[1593]

Other Treatments to Promote Growth

GnRH Agonists

Increasing Adult Height of Children with Idiopathic Short Stature. The effect of GnRH agonist therapy on adult height has been studied in girls with ISS and normal puberty (8–10 years of age); there was a mean gain of 0 to 4.2 cm, compared with predicted height.[1485,1594–1605] In boys with rapidly progressing puberty, GnRH agonist therapy increased adult height compared with predicted height,[1606,1607] with mean gains of 4.4 to 10 cm in those receiving the combination therapy, compared with 0.5 to 6.1 cm in untreated control subjects.[1487,1608]

In these studies, the effects of GH cannot be separated definitively from those of the GnRH agonist. In two randomized studies of adopted girls with normal puberty, treatment with a GnRH agonist plus GH was compared with GnRH agonist alone, and a 3-cm height gain was demonstrated with the combination therapy.[1609,1610]

Disadvantages of the use of GnRH agonists in children with ISS include absence of pubertal growth acceleration, delayed puberty with potential psychosocial disadvantage, and decreased BMD. Long-term follow-up studies are lacking. A panel of experts concluded that GnRH agonist therapy alone in children with ISS and normally timed puberty is minimally effective in increasing adult height, may compromise BMD, and cannot be suggested for routine use.[1611] Combined GnRH agonist and GH therapy leads to a significant height gain but may have adverse effects. Routine use of GnRH agonists in children with ISS being treated with GH cannot be suggested (level of evidence, C-III).

Increasing Adult Height of Children Born SGA. Short children born SGA usually have a normal pubertal timing, although some have rapidly progressing puberty and may be treated with GH.[1445] Data on the additional effect of treatment with GnRH agonists are limited. Routine use of the combination of a GnRH agonist and GH in children born SGA cannot be suggested.

Increasing Adult Height of Children with Growth Hormone Deficiency. Some children with GHD are short at the start of puberty and are at risk for short adult stature. Retrospective studies evaluating the addition of GnRH agonists to GH therapy involved a limited number of subjects and provided controversial results.[1612,1613] Three prospective studies that reported near-adult or adult heights showed a height gain of –1 SDS.[1614–1616] Routine use of combined therapy with a GnRH agonist and GH in GH-deficient children with low predicted adult height at onset of puberty cannot be suggested (level of evidence, C-III).

Aromatase Inhibitors

Men with estrogen deficiency due to aromatase gene defects or estrogen resistance due to inactive estrogen receptors experience growth into the third decade, demonstrating the role of estrogen in growth plate fusion.[23,315] The aromatase enzyme catalyzes aromatization of testosterone and androstenedione to estradiol and estrone, respectively. Various studies have been conducted to explore the efficacy of the aromatase inhibitor class of drugs delaying growth plate fusion and increasing height in disorders associated with short stature in boys. Studies to date have been small, and many have used adjuvant treatments, including GH, GnRH agonist, or testosterone, so the singular effect of the aromatase inhibitor has been unclear. Additionally, most studies to date have been short term and have measured changes in predicted adult height, with only one study investigating the effect on final adult height.

Aromatase inhibitors were first tried in disorders of sex steroid excess and precocious puberty, with only modest, if any, effects on predicted adult height. Treatment of boys with familial male-limited precocious puberty with testolactone, a first-generation aromatase inhibitor, resulted in an improvement in predicted adult height only after 5 to 6 years of treatment.[1617] The improvement in predicted adult height after 6 years was robust, however, at 12.9 cm compared with untreated control subjects. In contrast, first-generation and second-generation aromatase inhibitors have not significantly affected predicted adult height in patients with McCune-Albright syndrome.[1618–1620] Final height data are not available.

Aromatase inhibitors have also been used in boys with CDGD. Available studies had a small number of subjects enrolled, and boys with CDGD who received the second-generation aromatase inhibitor letrozole, in combination with testosterone, for 12 months experienced an increase in predicted adult height of 5.1 cm compared with control subjects treated with testosterone alone.[1181] When followed to adult height, those boys who received letrozole in addition to testosterone achieved a final adult height 5.7 cm higher than that of boys receiving testosterone alone.[1182] In GH-deficient boys treated with GH, the addition of anastrozole for 3 years increased predicted adult height by 6.7 cm, compared with an increase of 1 cm in boys treated with GH alone.[1498] The results of these preliminary small trials indicate that aromatase

inhibitors may be able to increase adult height, but larger, longer trials are needed to determine the optimal conditions and duration of treatment to significantly increase adult height. In addition, longer follow-up will be needed to demonstrate the safety of such treatments in peripubertal and pubertal boys.

Given the observation of decreased BMD in males with defects in estrogen synthesis or action, the effects of short-term aromatase inhibitor therapy on BMD were investigated in these treatment studies. None of the studies found a difference in BMD as assessed by DEXA scans in patients who received aromatase inhibitor or placebo for up to 3 years.[1498,1621] However, mild vertebral abnormalities were noted in boys who received the aromatase inhibitor compared with placebo[1622]; it is unknown whether the deformities are due to treatment effects and whether they resolve upon longer duration letrozole therapy. In light of these effects, aromatase inhibitors must be used with caution. Clearly, longitudinal follow-up is needed to better characterize the safety and efficacy of aromatase inhibitors to promote growth.

Oxandrolone

Oxandrolone, an anabolic steroid, has been used to increase growth velocity in a number of disorders. Because it cannot be aromatized to estrogen, it should not accelerate skeletal maturation. In general, studies evaluating the effect of oxandrolone on growth have found that it increases growth velocity but is not associated with an increase in final height.

Clinical Trials of Efficacy. Numerous studies have investigated oxandrolone therapy in boys with CDGD. The studies have found that oxandrolone increases growth velocity in these boys.[1139,1158] The typical response is an increase in growth velocity from approximately 4 to 4.5 cm per year up to 8 to 9 cm per year. Although treatment does not decrease final height, as might occur with accelerated skeletal maturation from excessive sex hormone exposure, neither does it increase final height.[1158] Therefore oxandrolone can be used to accelerate the growth of boys with CDGD to allow them to increase their height sooner than they would naturally, but it does not increase their final adult height. There have been no trials comparing efficacy of oxandrolone versus testosterone treatment in boys with CDGD.

Oxandrolone has been studied in girls with TS, both as a single agent and as combined therapy with GH. As in boys with CDGD, oxandrolone increases the growth rate in girls with TS.[1623,1624] Although some studies found no effect on final height with oxandrolone treatment alone,[1625] others found a mean increase in final height of up to 5.2 cm with oxandrolone treatment.[1624] Results of three controlled trials testing the effects of combination therapy with GH versus GH alone on adult height were reviewed.[1626] The studies had different average age at treatment start and used different GH and oxandrolone doses.[1627–1629] In general, the studies reported a positive effect of oxandrolone when added to standard GH therapy, with the effect on adult height varying between 2.3 and 4.6 cm.

Side Effects. No significant side effects have been reported in boys treated with oxandrolone for CDGD. Although oxandrolone has significantly fewer androgenic effects than testosterone, mild virilization has been reported in girls taking oxandrolone, including clitoromegaly. This is less of a concern at lower doses.[1626] There are also reports of a delay in breast development that improves upon higher estrogen dosing. Hepatic dysfunction has been reported with oxandrolone treatment, manifested by alterations in HDL cholesterol, and thus monitoring of lipids is suggested.[1626]

Diagnosis and Treatment of Excess Growth and Tall Stature

Diagnosis

The normal distribution of height predicts that 2.5% of the population will be taller than 2 SD above the mean. The most common cause of tall stature is familial, and the diagnostic evaluation centers on distinguishing tall or constitutional stature from rare pathologic causes of tall stature. As with short stature, children with tall stature must be evaluated relative to familial growth patterns and parental target heights. When a family history of tall stature is available and the growth rate and physical examination findings are normal, support and reassurance are frequently all that is needed without further testing. A careful assessment of pubertal status and bone age facilitates prediction of adult height and discussions with the patient and family. If the history suggests an underlying disorder or growth rate is accelerated, additional testing should be done to investigate the GH-IGF1 axis, chromosomal disorders, puberty, or other rare causes.

In GH excess, serum IGF1 levels are elevated, although high IGF1 levels may be a normal manifestation of puberty. Basal serum GH levels may be normal to increased, but serum GH is not suppressed by administration of glucose (1.75 g/kg body weight up to a maximum of 100 g). Although abnormalities of the sella turcica can be evident on lateral skull films, the demonstration of increased GH-IGF secretion should lead to radiologic evaluation of the hypothalamus and pituitary by MRI or computed tomography.

Treatment

Definitive treatment of GH-secreting tumors requires surgical ablation, either transsphenoidally or with a more aggressive surgical approach if large. As described in Chapter 9, somatostatin analogues, dopamine agonists, and GH-receptor antagonists are important components of treatment programs for GH excess.

In the past, most patients treated for familial tall stature were females. The number of patients treated in the United States has fallen markedly over the past four decades as tall stature in girls has become increasingly acceptable socially and psychologically. Treatment regimens were generally with estrogen prior to pubertal onset to induce early epiphyseal maturation[1630] and considered girls with predicted heights greater than 183 cm (6 feet 0 inches). Treatment regimens varied considerably, and there are no randomized trials testing treatment effectiveness. Controversy surrounds the treatment of girls with tall stature, especially in light of long-term studies that raised the possibility of effects on fertility.[1631] One-fifth of pediatric endocrinologists reported use of estrogens for treatment of tall stature in 1999,[1632] a percentage that is likely diminishing. In males, androgens have been used to accelerate skeletal maturation via aromatization to estrogen, but virilization is rapid.

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The complete list of references is available online at ExpertConsult.com.

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26

Physiology and Disorders of Puberty

DENNIS M. STYNE

CHAPTER OUTLINE

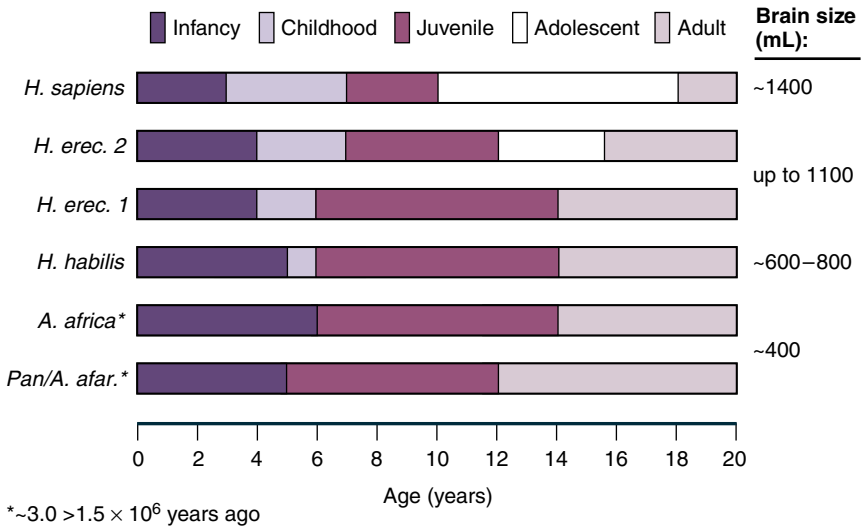
KEY POINTS

- Puberty is not a de novo event but rather a phase in the continuum of development of gonadal function and the ontogeny of the hypothalamic-pituitary-gonadal system from the fetus to full sexual maturation and fertility.
- During puberty, secondary sexual characteristics appear, and the pubertal growth spurt occurs, resulting in the striking sex dimorphism of mature individuals; fertility is achieved, and profound psychologic effects ensue.
- Gonadarche, or the awakening of gonadal sex steroid secretion, and adrenarche, or the awakening of adrenal androgen secretion, are separate processes that must be evaluated individually, as the customary chronologic relationship between the events may vary considerably among individuals.
- The age of onset of thelarche, or breast development, has decreased over the past several decades according to international data; the obesity epidemic may be one of the factors responsible for this secular trend toward earlier female development, although other factors are suspected.
- The discovery of new genes involved in regulation of the hypothalamic control of pubertal development greatly expands our understanding of the cause of delayed and precocious puberty, but the complex mechanisms that initiate normal pubertal development at a given age remain largely unknown.
- Evaluation of puberty in children and adolescents should include the use of highly specific and sensitive assays with pediatric standards; sex steroids should be measured with high-performance liquid chromatography tandem mass spectroscopy (HPLC-MS/MS) methods, unless painstaking extraction and manual immunoassays are invoked.
- The remarkable success in treatment of childhood neoplasia has led to secondary effects on pubertal development, either advancing or delaying the age of onset of puberty and often impairing subsequent fertility.

Puberty is not a de novo event but rather a phase in the continuum of development of gonadal function and the ontogeny of the hypothalamic-pituitary-gonadal system from the fetus to full sexual maturation and fertility. During puberty, secondary sexual characteristics appear, and the pubertal growth spurt occurs; this results in the striking sex dimorphism of mature individuals, and fertility is achieved.[1] These changes result from stimulation of the gonads by pituitary gonadotropins and a subsequent increase in gonadal steroid output. The term *puberty* is characteristically used to refer to the physical and endocrine changes of this period, while *adolescent* usually refers to the profound psychologic changes encountered during this time.

Puberty and Evolution

Human beings evolved into the most reproductively successful of mammals, and many anthropologists attributed this success to the prolonged pattern of human growth and development and to the delay in attaining full sexual maturity.[2] The human scheme of growth involves a childhood stage and a pubertal stage that includes a pubertal growth spurt (Fig. 26.1). Not even our closest biologic relative, the chimpanzee, which matures twice as rapidly as the human, unequivocally exhibits these two stages, and chimpanzees lack the unique human pubertal growth spurt.[3] Learning and practice of adult behaviors related to sex and childrearing, particularly provisioning children (not just infants) with food, which

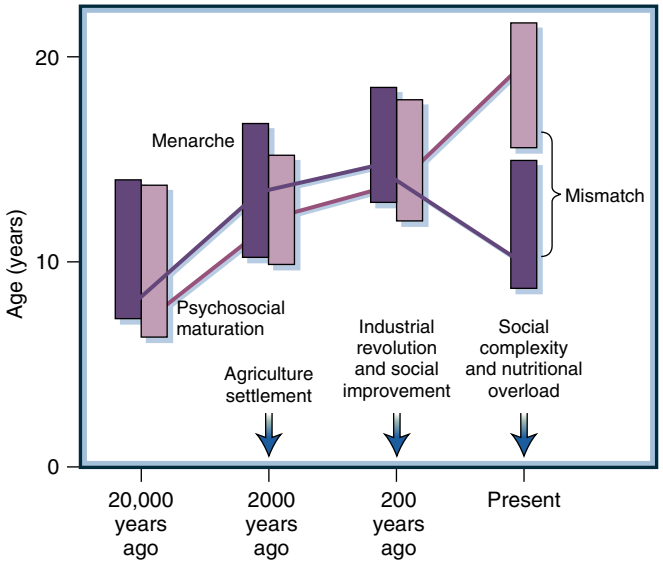


• **Fig. 26.1** Evolution of the human pattern of postnatal growth and development during the first 20 years of life. Specimens include A. afar, *Australopithecus afarensis*, a "bipedal chimpanzee"; A. africa, *Australopithecus africanus*; H. habilis, *Homo habilis*, the toolmaker; H. erec. 1, early *Homo erectus*; H. erec. 2, late *Homo erectus*; *H. sapiens*, *Homo sapiens*. The early hominid australopithecine specimens from South Africa date to about 3 to 1.5 million years ago. *H. afarensis*, although a hominid (family of all human species), retained many anatomic features of nonhominid species, such as an adult brain size of about 400 mL compared with *H. habilis* (650–800 mL), early *H. erectus* (850–900 mL), late *H. erectus* (up to 1100 mL), and modern *H. sapiens* (about 1400 mL). Infancy is the period when the mother's breast milk is the sole or most important source of nutrition, and in preindustrialized societies, it ends at about 36 months. Childhood is the period after weaning, when the child depends on others for food and protection; this period ends when the growth of the brain in weight is almost complete, at about age 7 years. The juvenile stage is defined as prepubertal individuals who are no longer dependent on their parents for survival. The adolescent stage, which begins with the onset of puberty, ends when adult height is attained (Moggi-Cecchi; Conroy and Kuykendall). The pattern in *A. afarensis* is no different from that of the chimpanzee *(Pan troglodytes)*. Notice the first appearance of the childhood stage in *H. habilis* (arising about 2 million years ago) and the first appearance of the adolescent stage in *H. erectus* 2 (about 500,000 years ago); *H. sapiens* arose about 120,000 to 150,000 years ago. (Modified from Bogin B. Growth and development: recent evolutionary and biocultural research. In: Boaz NT, Wolfe LD, eds. *Biological Anthropology: The State of the Science.* Bend, OR: International Institute for Human Evolutionary Research; 1995:49–70. Additional data from Moggi-Cecchi J. Questions of growth. *Nature.* 2001;414:595–597; Conroy GC, Kuykendall K. Paleopediatrics: or when did human infants really become human? *Am J Phys Anthropol.* 1995;98:121–131.)

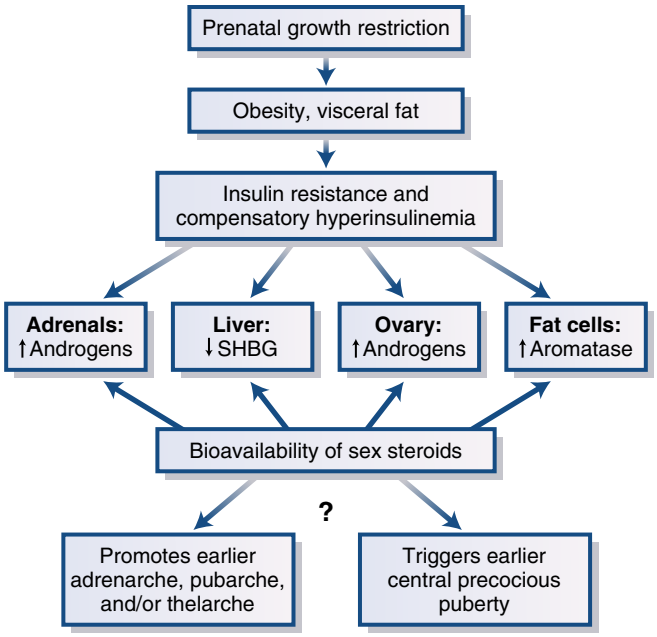
is unique to human beings, is considered a critical part of human success: "the building of a better, healthier body and the developing of greater biological, behavioral, and cultural resilience prior to sexual maturity that leads to greater adult health, fitness, and longevity."[2] Toolmaking preceded the evolutionary development of adolescence, suggesting that the evolution and value of human childhood and adolescence and this unique pattern of growth and development have had significant roles in the reproductive success of humans.

In the developed world, reproductive maturity occurs years earlier than psychosocial maturation, causing a mismatch between biologic stages and psychosocial expectations and roles (Fig. 26.2).[4] In past eras, such as the Neolithic, Greek, or Roman periods, there was not such a mismatch because menarche occurred at an age close to that of reproductive maturity.

With increased population, the advent of agriculture, and the growth of cities and later urban centers, menarche occurred later, and the complexity of life led to a delay in the attainment of an adult role in society. In Roman Britain, 2000 years ago, pubertal growth as an indication of puberty occurred at ages similar to those of today, but puberty progressed at a slower pace apparently leading to a later attainment of reproductive maturity.[5]



• **Fig. 26.2** The relationship between the likely range of ages of menarche *(purple)* and achievement of psychosocial maturity *(pink)* from 20,000 years ago to the current day. The mismatch in timing between these two processes is a novel phenomenon. (From Gluckman PD, Hanson MA. Evolution, development, and timing of puberty. *Trends Endocrinol Metab.* 2006;17:7–12.)



• **Fig. 26.3** Proposed endocrine pathways linking childhood obesity and insulin resistance to early pubertal onset and maturation. Childhood obesity and the predisposition to visceral adiposity after intrauterine growth restraint lead to insulin resistance and peripheral hyperinsulinemia. Insulin acts on various organs, including the adrenals, liver, ovary, and fat cells, to increase sex steroid bioavailability. Elevated circulating and tissue sex steroid levels in obese prepubertal children can have only mild local effects or activate early hypothalamic-pituitary puberty and early reproductive maturation. *SHBG,* sex hormone–binding globulin. (From Ahmed ML, Ong KK, Dunger DB. Childhood obesity and the timing of puberty. *Trends Endocrinol Metab.* 2009;20:237–242.)

In modern times, the age of menarche has decreased, but the age of social adulthood still occurs later, causing a discrepancy that probably has not occurred previously in human history. The study of human evolution adds to the understanding of many modern medical conditions as well as puberty, and the discipline of evolutionary biology is now recommended as a required premedical course by the American Association of Medical Colleges.[6,7]

Fetal Origins of Adult Disease

There are long-lasting effects of abnormalities in fetal and neonatal growth. As seen in longitudinal studies, low birth weight followed by rapid weight gain in infancy (i.e., catch-up growth) leads to tall childhood stature and early pubertal development. Poor prenatal nutrition and small-for-gestational-age (SGA) status tend to advance the age of puberty in girls and the age of adrenarche[8–10]; a secondary effect of increased postnatal nutrition, often leading to overweight or obesity, also lowers the age of puberty. Fig. 26.3 describes the relationship between excessive adipose tissue and early puberty. A prospective study demonstrated that a lower expected birth weight ratio (i.e., ratio of observed infant's birth weight to median birth weight appropriate for maternal age, weight, height, parity, infant sex, and gestational age) and a higher body mass index (BMI) at 8 years led to an earlier age of menarche.[11] Girls who are longer and lighter at birth and subsequently have greater BMI values at 8 years tend to have earlier menarche.[12] Rapid weight gain in the second to ninth months but not thereafter correlated with a greater BMI at 10 years and with earlier menarche in a longitudinal study.[13] On the other hand, higher birth weight is related to a later age of menarche, judging from data across several countries.[14] Large-for-gestational-age (LGA) infants tend to have an earlier age of takeoff of the pubertal growth spurt.[15] Many international studies find a relationship between low birth weight or catch-up growth and chronic diseases in adulthood; this phenomenon, the Barker hypothesis, is found in a wide range of populations.[16]

Birth weight and rate of postnatal growth—not prematurity alone—are inversely related to cardiovascular mortality risk and prevalence of insulin resistance syndrome (i.e., metabolic syndrome or syndrome X), which consists of hypertension, impaired glucose tolerance, and elevated triglyceride levels, among a growing list of other findings. This outcome is attributed to fetal and neonatal metabolic programming, in which early adjustments to enhance survival in difficult intrauterine circumstances set the stage for later disorders. Insulin resistance, which may be the basis for most of these complications or may be just one feature of the syndrome, may spare nutrients from use in muscle, leaving them available for the brain. This mechanism can minimize central nervous system (CNS) damage in the fetus during periods of malnutrition.

Determinants of the Age of Puberty and Menarche

Although historic records show that puberty occurs at an earlier age today, most evidence derives from reports of the age of menarche (Table 26.1).[17,18] Age of menarche is removed by several years from the first sign of secondary development in girls, and modern studies demonstrate correlation coefficients of only 0.37 between age of menarche and age of onset of puberty, suggesting both unique and similar factors exerting effects on these ages.[19] Changes in health and socioeconomic status in regions where data were collected during different decades lead to complexity in the interpretation of modern national data.

Age of menarche is determined in various ways. Recalled age of menarche is considered to be accurate within 1 year (in 90% of cases) up to 30 years after the event.[20] Contemporaneous recordings are performed with the probit method of asking for a response of "yes" or "no" to the question, "Are you menstruating?" However, the results are subject to social pressures of the culture and socioeconomic group considered.[21]

Physical examination with palpation of gonads or breasts by trained observers is the most accurate method of assessment of pubertal development. Detection of the onset of stage 2 breast development in an overweight girl on physical examination may be difficult even for a trained observer (although stage 3 usually is obvious). Visual observation of the stage of development in person (not by photographs) is one step removed from physical examination and palpation; errors in the evaluation of breast tissue in obese girls or the stage of testicular enlargement in boys may occur. Visual observation by multiple observers was used in the Third National Health and Nutrition Examination Survey (NHANES III) (and in the National Health Examination Survey [NHES] that occurred more than 20 years earlier), and visual observation is subject to interobserver variation. In a study by Pediatric Research in the Office Setting (PROS), a network fostered by the American Academy of Pediatrics, specially trained pediatricians, nurse practitioners, or physician assistants in 225 offices used palpation for 30% of the study population and visual inspection for all members of a convenience sample of 17,070 girls across the United States.[22]

TABLE 26.1 Comparison of Menarcheal Ages Reported by Various Studies

Study	Year	Plan	Evaluation	*n*	AGE AT MENARCHE (YR) Overall	W	B	M	Comments
Britain	1969	Long	Probit	192 (W)		13.5			
NHES III	1963-1970	Cross	Recalled	3272		Born 1940-1960: 12.8 Born 1890-1910: 13.5	12.52		
NHANES III	1988-1994	Cross	Yes/No Probit	330 (W) 419 (B) 419 (M)		12.7	12.3	12.5	Men. age black < white
NHANES III	1988-1994	Cross	Yes/No	2510	12.43				Men. age black < white
			Probit	710 (W) 917 (B) 883 (M)		12.6	12.06	12.3	
PROS	1992-1993	Cross	Status Quo Probit	17077 (W) 1638 (B)		12.9	12.16		Men. age black < white
NHLB Growth	1987-1997	Long	Recalled	1092 (W) 1164 (B)		12.7	12.1		Men. age black < white BMI inversely proportional to men. age
Bogalusa	1973-1974	Cross		5552		12.7	12.9		
	1992-1994	Cross				12.5	12.1		
	1973-1994	Long		2508		12.6	12.3		Men. age black < white BMI inversely proportional to men. age
NHES	1963-1970	Status Quo	Yes/No Status Quo	3272	12.75	12.8	12.48		
	1988-1994	Status Quo	Median Yes/N	1414	12.54	12.6	12.14		Men. age NHANES III < NHES Growing difference in men. age white-black BMI inversely proportional to men. age

B, Black; *BMI,* body mass index; *Cross,* cross sectional; *Long,* longitudinal; *M,* Mexican American; *men.,* menarcheal; *NHANES,* National Health and Nutrition Examination Surveys; *NHES,* National Health Examination Survey; *NHLB Growth,* National Heart, Lung, and Blood Institute Growth and Health Study; *PROS,* Pediatric Research in Office Settings; *W,* white.

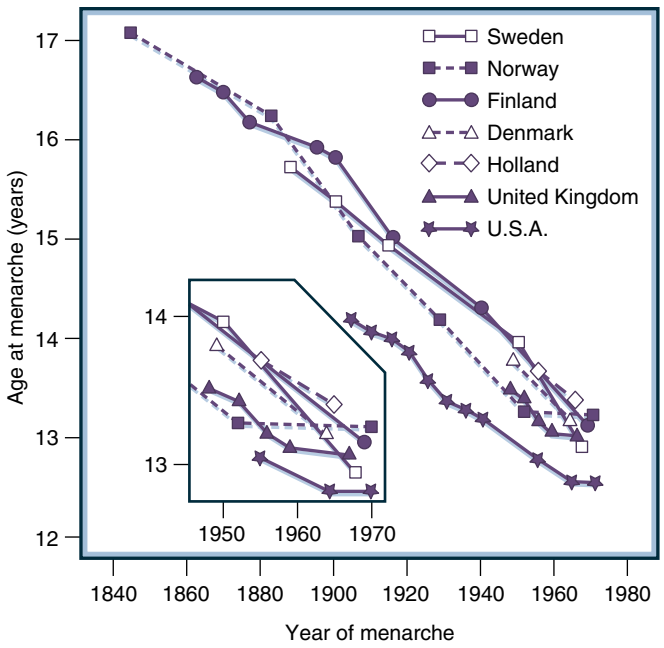
From Styne DM. Puberty, obesity and ethnicity. *Trends Endocrinol Metab.* 2004;15:472–478.

The availability of only limited numbers of trained personnel for examination or subjects' refusal of embarrassing examinations sometimes leads to the use of proxy measures. Photographs or drawings of pubertal development allow self-reporting or parental reporting of pubertal progress, but correlations range widely from 0.48 to 0.91 compared with physicians' examinations or visual observation of subjects.[23] The answers to self-assessment may be influenced by subjects' wishes to conform with their understanding of normal development and may be less accurate in some ethnic groups than others, similar to reported menarcheal age. Obese girls may overestimate breast developmental stage, and boys may overestimate pubic hair development. Individuals with learning disabilities, chronic diseases (e.g., cystic fibrosis, Crohn disease), or psychologic conditions (e.g., anorexia nervosa) may have less accurate pubertal staging.[24] Self-report is related to testosterone values for boys and girls and is said to be accurate enough if precision is not needed.[25] With all of its difficulties, physical examination remains the best method to accurately evaluate pubertal development, although self or parent report can likely determine prepuberty from puberty.[26,27]

The Secular Trend in Puberty and Menarche

Review of classic writings gives us insight into the changes in age of puberty and menarche through the epochs, although much of the data are inferred.[28,29]

During Paleolithic and Neolithic eras, menarche was estimated to occur between 7 and 13 years of age, equal to or earlier than



• **Fig. 26.4** The changes in age at menarche between 1840 and 1978 illustrate the advance in the age at menarche in Western Europe and the United States since 1840 and slowing of this trend since about 1965. (Modified from Tanner M, Eveleth PB. Variability between populations in growth and development at puberty. In: Berenberg SR, ed. *Puberty, Biologic and Psychosocial Components*. Leiden, The Netherlands: H.E. Stenfert Kroese; 1975:256–273.)

the present average. It was postulated that early menarche allows reproduction in the face of an early death. Writings during Greek, Roman, and Indian history suggest an age of menarche of 12 to 14 years and age of ejaculation in boys to be similar. Records from medieval Europe suggest a menarche at age 12 to 15 years. Up to the 20th century menarche age appeared to increase to 14 to 16 years.

The Developed World

The average age of menarche in industrialized European countries has decreased by 2 to 3 months per decade over the past 150 years; in the United States, the decrease has been approximately 2 to 3 months per decade during the past century[17,30] (Fig. 26.4). However, this secular trend slowed in developed countries such as the United States, Australia, and Western Europe between approximately 1940 and 1970, presumably due to improved socioeconomic status, better health, and the benefits of urbanization. There is a relatively small range of ages of menarche in the well-off developed world, where lower socioeconomic classes do not have an increased burden of disease or malnutrition. Chronic diseases previously increased the age of menarche, and delay in menarche is still associated with serious conditions (e.g., celiac disease, asthma) that are not adequately treated. The standard deviation of the mean age of menarche also decreased, suggesting a diminished number of those maturing very late, as might be found among disadvantaged people.[31] Teasing out the various factors involved in subtler secular trends will require further long-term study and newer methodologic approaches in areas where nutrition and health are optimal or close to it.[32] Remarkably, a reverse secular trend is reported in certain areas of Europe, leading to a later age of menarche. This has been attributed to a resurgence of physical and psychologic stress, as was seen in previous eras (e.g., World War II).[32]

There are cross-sectional and longitudinal data from the late 20th century demonstrating a resurgence of a secular trend in the United States, including ethnic influences.[33,34] The median age of menarche in the United States was 12.8 years according to the 1973 US National Center for Health Statistics National Health Education Study, and data from NHANES III (1988–1994) indicate the median age at that time was 12.43 years, 0.37 year earlier than in 1973.[35] During a 20-year period in the Bogalusa Heart Study, the median menarcheal age decreased by approximately 9.5 months among African-American girls compared with 2 months among white girls,[36] leading to a 4-month difference. Between the ages of 5 and 9 years, African-American girls in the Bogalusa study had taller stature and greater weight, factors that were predictive of menarche before age 11 years. African-American girls have advanced secondary sexual development compared with white American girls of the same age during the first three stages of puberty, and they have an advanced bone age; this may be related to the higher prevalence of obesity among African-American girls and to an ethnic-specific genetic influence.[37–39]

Obesity is defined as a BMI (calculated as weight in kilograms divided by height in meters squared) equal to or above the 95th percentile for age, and overweight is defined by the Centers for Disease Control and Prevention (CDC) as a BMI equal to or greater than the 85th percentile for age. Many studies have reported on the effect of the epidemic of childhood obesity and overweight on age of menarche.[40,41] Most cross-sectional and longitudinal studies found an inverse relationship between menarcheal age or other stages of puberty and BMI or other reflections of adiposity.[42,43] If the population in the 1970s had had the same range of BMI values as was found in the 1990s, the projected age of menarche would have been the same in the 1970s as it was in the 1990s.[44] Girls had a greater prevalence of overweight or obesity between the ages of 8 and 10 years if they were in puberty or had advanced breast development.[45] A study of the age at onset of puberty in US boys of various ethnic groups indicated an earlier age of onset between 6 months and 2 years depending upon indicator and ethnic group.[46] Except for a delay in pubertal development with BMI less than 15% there was no effect of overweight or obesity in these boys as was found in girls in other studies.

Longitudinal studies are limited in the United States but of great importance in the evaluation of a secular trend. The longitudinal National Heart, Lung, and Blood Institute (NHLBI) Growth and Health Study followed 1266 white and 1313 African-American girls from 9 or 10 years of age for 10 or more years. A recent report of a sample of 1239 girls from the study noted that 10.4% of white, 23.4% of black non-Hispanic, and 14.9% of Hispanic girls had attained breast stage 2 at 7 years; 18.3%, 42.9%, and 30.9%, respectively, attained breast stage 2 at 8 years, younger than data from 10 to 30 years ago. In a sample of 1155 girls, the mean age of menarche among whites was 12.7 years, and among African Americans it was 12.1 years; a direct relationship was found between weight and BMI and the age of menarche.[38] Of these girls, 51.6% started puberty with only one manifestation. Those who experienced breast development first (i.e., thelarche pattern), rather than the appearance of pubic hair (i.e., adrenarche pattern), had an earlier menarche (12.6 vs 13.1 years); this was associated with a greater BMI and body weight, which was not true for those girls manifesting the adrenarche pattern.[47] These findings support the analysis of NHANES III, in which girls with earlier breast development had greater BMI values at the time of menarche than those with adrenarche occurring first.[48]

African-American girls in the National Longitudinal Study of Adolescent Health (Add Health) were 1.55 times more likely than white girls to have menarche before 11 years[49] of age, and Mexican Americans were 1.76 times more likely to do so than whites. Asians were 1.65 more likely than whites to mature later than 14 years. Those undergoing early menarche were twice as likely to be overweight, and African-American girls had a 2.57-fold greater risk for overweight if they had menarche before they were 11 years old. Among early menarcheal African Americans, 57.5% had a BMI greater than the 85th percentile, and 32.5% had a BMI greater than the 95th percentile. A longitudinal study of 180 girls indicated earlier pubertal development in these cases: between the ages of 5 and 9 years with a higher percentage of body fat at 5 years; those with a higher percentage of body fat, higher BMI percentile, or larger waist circumference at 7 years; those with larger increases in the percentage of body fat from 5 to 9 years; and those with larger increases in waist circumference from 7 to 9 years.[50]

During the past 40 years, menarcheal age in the white subjects in the Fels Longitudinal Study has remained stable even as BMI has increased, with no relationship shown between the two. Subjects with early menarche had a tendency to increase BMI after menarche,[51] suggesting that increased weight appears to be a consequence rather than determinant of the age at menarche and that secular changes in BMI and in mean age at menarche may be independent phenomena. There was a rise in BMI, waist circumference, hip circumference, and serum levels of luteinizing hormone (LH), androstenedione, testosterone, and dehydroepiandrosterone sulfate (DHEAS) during the years immediately after menarche in a shorter longitudinal study.[52]

International data support the effects of BMI on puberty, but earlier onset of pubertal growth and a tall stature in childhood do not lead to a taller adult height.[53] Children studied longitudinally in Denmark showed earlier breast development (estimated mean age of 9.86 years compared to 10.88 years) and menarche (13.42 years compared to 13.13 years) in 2006 compared to 1991, which could not be explained by BMI or even by a difference in gonadotropin values suggesting other factors were operative.[54]

The Developing World

The interaction of socioeconomic conditions, nutrition, energy expenditure, states of health, and puberty is of particular importance in areas of the world where nutrition is suboptimal; where the most rapid improvement is found, such as in Oaxaca, Greenland, or South Korea, the age of menarche decreases more rapidly. Where standards of living do not change, the age of menarche is more stable. In South America and Africa, some rural children fare better and have earlier puberty and taller stature than urban children, demonstrating a trend of adverse health and nutritional conditions in crowded urban centers. Malnourished individuals have later age of menarche across the world.[55]

As a whole, these reports indicate that populations existing in the most difficult conditions who experience improvement in socioeconomic status demonstrate a greater decrease in the age of menarche. Once a minimum nutritional status or state of health is reached, the effects of socioeconomic status on the age of menarche are minimized or eliminated, but increased BMI can lower the age of menarche further. However, there are other factors, apparently environmental factors, that are postulated to affect the age of thelarche, which may not have as much effect on the age of menarche.

Factors Affecting the Age of Puberty and Menarche

The composition of diet and the number of calories in the diet may relate to menarche.[56] The prospective, longitudinal Harvard Longitudinal Studies of Child Health and Development found that girls had earlier menarche if they were taller and consumed more animal protein and less vegetable protein as early as 3 to 5 years old and that they had earlier peak growth velocity if they had higher dietary fat intake at 1 to 2 years old and higher animal protein intake at 6 to 8 years. Peak height velocity (PHV) increased, controlling for body size, if more calories and animal protein were consumed 2 years before peak growth. The correlation between higher animal protein intake and lower age of menarche is confirmed in Germany and Britain.[57,58] A positive relationship between high fiber intake and age of menarche held true in a comparison of 46 countries,[59] and a longitudinal study demonstrated a later onset of puberty and PHV in girls with higher isoflavone intakes, suggesting a lower risk for breast cancer with a high fiber prepubertal diet.[60] On the other hand, lifelong vegetarian dietary intake does not affect the age of menarche,[61] and a low-fat diet in otherwise healthy, prepubertal children 8 to 10 years old with elevated low-density lipoprotein (LDL) cholesterol produced no difference in age of menarche or pubertal progression.[62] Phytoestrogen (flavonol but not lignan) intake delays breast development, particularly in girls with lower BMI values.[63,64]

Another influence of the age of menarche is the maternal environment. Macrosomia due to maternal diabetes or obesity is associated with increased BMI in childhood, which itself is related to earlier puberty and menarche. Increased or decreased weight gain in pregnancy likewise decreases the age of menarche according to data from the Nurses' Health Study II.[65] Heavy maternal smoking of cigarettes but not of marijuana leads to earlier male puberty but not female puberty in offspring.[66] Maternal tea drinking but not coffee drinking was associated with a later age of menarche and puberty in their daughters. Daughters of mothers who were more physically active during pregnancy had a modest delay in menarche.[67]

Stress and Puberty

Life history theories aiming to explain influences on the age of puberty address energetics, stress suppression, psychosocial acceleration, paternal investment, and child development, each of which may have various effects on the timing and progression of pubertal development.[68] This approach acknowledges the fact that evolution optimizes allocation of limited resources to maximize fitness and allow reproductive success.[69]

Stress is variably reported to increase or decrease the age of menarche. The absence of a father or lower parental education increases the likelihood of early menarche; however, there is evidence that the exposure to paternal psychopathology that precedes the absence of the father might be causative in such situations.[70] War increases the age of voice breaking in boys (e.g., in Bach's choir during the War of Austrian Succession in 1727–1749), and the age of menarche increased during World War II and during more recent hostilities in the former Yugoslavia.[71] Sexual abuse is associated with earlier onset of puberty and earlier menarche compared with a control population in a large US national study, although it is difficult to eliminate the effect of family dysfunction in this situation as well.[72] Child abuse of various types is associated with delayed puberty and menarche in a large British study, and sexual abuse in this cohort led to both early and late menarche.[73]

Genetic Effects on Puberty and Menarche

There remains a difference in age at attainment of stages of puberty in different countries even with stability in socioeconomic factors; for example, Japanese boys undergo changes in testicular size about 1 year earlier than Swiss boys do.[74] When socioeconomic and environmental factors lead to good nutrition, general health, and infant care, 60% to 80% of the determination of the age at onset of puberty in normal children appears to be due to genetic causes.[31]

The important role played by genetic factors in the onset of puberty is illustrated by the similar age of menarche in members of an ethnic population and in parent-child and sibling or twin pairs.[75,76] The correlation between mother and daughter patterns should theoretically be equal to sister-sister age at menarche if only genetic factors are operative, but because sister-sister correlations are higher than mother-daughter correlations, environmental influences must provide an additional influence beyond genetic factors; the secular trend and nutritional factors may be invoked.

Concordance of ages of pubertal developmental stages and menarche is closer between monozygotic than dizygotic twins, supporting the influence of genetic factors. Monozygotic twins reared together have more similar ages of menarche than those reared apart, and dizygotic twins reared together are less similar than either of the monozygotic groups, pointing to environmental influences on genetic factors. Some twin research suggests that additive genetic factors account for 96% of the variance in the age of puberty in girls and 88% of the variance in boys (although other sources from the United States, Australia, Great Britain, Finland, and Norway have found genetic effects accounting for between 50% and 80% of the variance), with the remainder resulting from shared and nonshared environmental influences.[77,78]

A large study of 180 twins, 132 monozygotic and 48 dizygotic, showed a high level of hereditability of DHEAS sulfate levels and pubertal development with a higher estimate in monozygotic twins.[79]

Genome-wide association study (GWAS) techniques approach the greatly complex question of the genetic control of puberty and growth. Alleles near 6q21 (T) at rs7759938 *(LIN28B)* correlated with earlier puberty and shorter prepubertal childhood height, as well as age of menarche.[80,81] A meta-analysis of GWAS data for 17,510 women demonstrated the strongest signal related to age of menarche $\times 10^{-9}$, where the nearest genes include *TMEM38B, FKTN, FSD1L, TAL2*, and *ZNF462*, and the next strongest signal near the *LIN28B* gene (rs7759938; $p = 7.0 \times 10^{-9}$), which also influences adult height and cancer risk.[82] Studies of thousands of European individuals linked locus upstream of myocardin-like 2 *(MKL2)* ($p = 8.9 \times 10^{-9}$) with earlier puberty with reduced pubertal growth ($p = 4.6 \times 10^{-5}$) and short adult stature ($p = 7.5 \times 10^{-6}$) in both males and females.[83] Variants near *MAPK3, PXMP3*, and *VGLL3* linked taller prepubertal stature with earlier menarche, and a variant near *ADCY3-POMC* was associated with increased BMI, reduced pubertal growth, and earlier puberty.[83]

Gonadotropin-releasing hormone type I (GnRH-I) and its receptor (GnRHR) genes are only modestly related to the age of menarche.[84] The *LEP1875* and XbaI and PvuII polymorphisms of the estrogen receptor α (ERα) gene *(ESR1)* and maternal age at birth (i.e., >30 or <30 years) were associated with age of menarche.[85,86] High-activity *CYP17* alleles involved in estrogen formation and high-activity *CYP1A2* and *CYP1B1* alleles, whose gene products metabolize estradiol, are not associated with pubertal stage,[87] whereas the high-activity *CYP3A4** 1B/1B girls had an earlier age of onset of normal puberty alleles. The variant rs10235235 of *CYP3* is associated with a reduction in breast cancer risk for women who had their menarche age at 15 years or older but not for those with menarche younger than 11 years.[88] Girls with longer (>8) TAAAA repeats in their sex hormone–binding globulin gene *(SHBG)* have a later age of menarche than those with fewer repeats.[89] A GWAS study showed the common genetic variants contribute to early puberty in girls more than late puberty, and they contribute in boys to delayed puberty more than early puberty. It suggested that this may be an explanation why precocious puberty is more likely to be idiopathic than organic in girls and delayed puberty more likely to be constitutional in boys.[90] This study also found that early puberty regardless of BMI was associated with higher risks of breast, ovarian, and endometrial cancers in women.

Cancer and Age of Puberty

There is divergent data on puberty and prostatic cancer. The preceding study showed increased risk of prostate cancer in men with early puberty, which was confirmed in a study of 2927 cases of prostatic cancer that found that older onset of puberty led to a lower risk of prostate cancer, especially aggressive types, and another study of 757 men.[91] Conflicting results occurred in a study of 1088 cases of prostate cancer, which demonstrated an increased risk of 6% for each year of recalled delayed puberty with an odds ratio (OR) of 1.35 if puberty commenced after 15 years.[92]

There are genetic effects upon the age of onset of testicular growth in boys as well. Danish and Chilean boys had a later onset of puberty when they had genetic alteration in follicle-stimulating hormone (FSH) beta production (FSHβ c.-211G>T), which was further enhanced with a genetic variation in FSH receptor sensitivity (FSHR c.-29G>A).[93] There was a 1.5% to 1.7% variance explained by the genetic modifications in contrast to the effect of BMI, which explained 7.2% variance in Chilean boys and 17.2% variance in Denmark boys.

Other Factors

Seasonality of menarche was observed in a US cohort of 3000 college students; those born after 1970 had an earlier age of menarche and a more pronounced frequency peak in July. Factors hypothesized to contribute to seasonality of menarche include stress and the photoperiod.[94] In Peru, puberty begins at a later age, and pubertal development lasts longer at high altitudes than at low altitudes even when nutritional status is similar. There is a north-to-south decrease in the age of menarche in Europe[25] that may result from environmental factors or genetic influences.[31]

The Comorbid Conditions of Early Puberty

Many international studies show that earlier age at menarche is associated with a greater risk of development of breast cancer by a factor of 1.050 (95% confidence interval [CI] 1.044-1.057; $p < 0.0001$) for every year younger at menarche.[95] The risk of premenopausal breast cancer decreases 9% per year of delay in menarche, and the risk for postmenopausal breast cancer decreases 4%.[96] This trend in which a longer period of reproductive function increases the risk for breast cancer is more due to early menarche than to later menopause and has been found in other studies as well. In disease-discordant monozygotic twins, the one with cancer recalled puberty to be earlier; in disease-concordant

twins, the one with earlier menarche had the earlier diagnosis of breast cancer.[97] Women with breast cancer were taller and leaner in childhood and had increased height velocity at age 4 to 7 years and age 11 to 15 years; higher BMI increased this risk. These variables were particularly significant in women with early menarche (at age <12.5 years).[98] Remarkably, another study of 117,415 women found that increased birth weight, height at 8 years of age, peak growth at an early age, taller stature at 14 years of age, and low BMI at 14 years of age were independent risk factors for breast cancer, but there was no effect on age of menarche.[99] However, increased body weight at 8 years appeared to decrease the risk of breast cancer in another study of 90, in 50% of women.[100]

There is indirect evidence relating earlier menarche to increasing likelihood of hepatocellular carcinoma. On the other hand, later age of menarche (>14 years) is associated with an increased risk of glioma or non-Hodgkin lymphoma.[101,102]

Evidence from the Fels Longitudinal Study of white females revealed that girls with self-reported menarcheal age of less than 11.9 years (classified as early menarche; 23% of the sample) had adverse cardiovascular risk factors such as elevated blood pressure and glucose intolerance unrelated to body composition.[103] There was increased carotid artery intima media thickness in women with an age of menarche less than 11 years compared to those with the age of menarche over 11 years in 461 women in the Bogalusa Heart Study.[104]

National Trends in Pubertal Development

Limits of Normal Pubertal Development

The NHES enrolled subjects who were 12 years old; although it is useful for defining the upper limits of normal pubertal development, the survey is uninformative about the lower limits of the age at onset of puberty.[37,105] A longitudinal study (Tables 26.2 and 26.3) that enrolled 9.5-year-old white boys and girls added much to the determination of the mean age at attainment of stages of puberty[106]; however, it started too late to include normal children entering puberty at an earlier age. Two studies used data from NHANES III. One found onset of stage 2 breast development for African Americans, Mexican Americans, and whites to occur at 9.5, 9.8, and 10.3 years, respectively, and pubic hair stage 2 to occur at 9.5, 10.3, and 10.5 years, respectively, using a sample of 1623 girls.[107] The other study reported that the ages for onset of stage 2 breast development were 9.5 years for African Americans, 9.8 years for Mexican Americans, and 10.4 years for white Americans, whereas the onset of stage 2 pubic hair occurred at 10.4 years for white Americans, 9.4 years for African Americans, and 10.6 years for Mexican Americans in a sample of 2145 girls.[108]

Because the PROS started at 3 years of age but ended at 12 years of age, it excluded a proportion of normal girls who enter puberty at a later age, although the probit statistical method can estimate events even when only a portion of the population has achieved the event.[22] This study was criticized because the subjects from the convenience sample were not matched for the many factors considered in a national study such as NHANES. The standard deviation of the longitudinal study described earlier was low, 1.0 year or less in most cases, whereas the cross-sectional study PROS had a larger standard deviation of approximately 2 years. Any study of puberty will demonstrate a limit in the spread of the upper end of the age at onset of puberty curve, because rarely do individuals with the most severe constitutional delay of puberty (CDP) spontaneously enter puberty after 18 years of age. There may be a skewing of the normal age at onset of puberty to an earlier spread of ages.

A study of the age of pubertal stages in children in NHANES revealed that in those with normal BMI values, pubertal signs occurred before 8 years of age in fewer than 5% of the non-Hispanic white female population, although thelarche occurred before age 8 in 12% to 19% of normal-BMI, non-Hispanic black and Mexican-American girls; the 5th percentile for menarche was 0.8 year earlier for non-Hispanic black than non-Hispanic white subjects.[43] Although the appearance of pubic hair occurred in up to 3% of 8-year-old girls with normal BMI values in all ethnicities, it did occur significantly earlier in the minority groups. Girls with the higher BMI values had a significantly higher prevalence of thelarche between the ages of 8 and 9.6 years and pubarche from the ages of 8 to 10.2 years, compared with girls with normal BMI values. Menarche was significantly more likely to occur in younger girls with elevated BMI values. In boys with normal BMI values, pubic hair appeared in fewer than 2% before 10 years. The study authors concluded that pubertal development in girls with normal BMI values before 8 years of age is premature. These data support other analyses of NHANES III, in which the heaviest girls tended to have thelarche before pubarche and the heaviest boys had pubarche before gonadarche.[48]

The latest and largest multiracial and ethnic study of the age at onset of puberty in a convenience sample of 4131 American boys between the ages of 6 and 16 years found the mean ages for onset of Tanner 2 genital development for non-Hispanic white boys was 10.14, African-American boys was 9.14, and Hispanic boys was 10.04 years; and for stage 2 pubic hair, the ages were 11.47, 10.25, and 11.43 years, respectively. Mean ages for achieving testicular volumes of greater than 3 mL were 9.95 for white, 9.71 for African-American, and 9.63 for Hispanic boys; and for greater than 4 mL ages were 11.46, 11.75, and 11.29, respectively.[46]

Weight affects the onset of puberty in boys but not in a linear manner. Overweight boys have earlier puberty than normal weight boys, while obese boys start puberty later.[109]

Spanish investigators demonstrated that the earlier normal girls entered puberty, the longer the duration of puberty before menarche. In one of these studies, girls who started puberty at 9, 10, 11, 12, and 13 years of age experienced menarche 2.77, 2.27, 1.78, 1.44, and 0.65 years later, respectively, demonstrating a normalizing trend that keeps the age of menarche relatively stable in the group as a whole.[110] These data may suggest that earlier onset of the first stages of puberty may not exert major effects on the age of menarche. This contrasts with a suggestion of a decrease in the time required to transit puberty from start to end in Dutch and Swedish boys and girls.[31]

In sum, the data show that African-American girls develop before white girls, regardless of socioeconomic issues. There is evidence that rising BMI values in childhood are associated with earlier pubertal maturation, which may explain some of the increasing age differences between these ethnic groups. Environmental disruptors may also play a role. Although there is evidence for earlier menarche, a secular trend toward earlier puberty in girls in the absence of increased BMI cannot be supported from these data because of the different ages studied and lack of comparable studies over past decades.[34,111]

The United States is lacking a comprehensive, large, longitudinal study that would start early enough to include the youngest normal pubertal subjects and last long enough to include the oldest and that would be based on direct physical examination

TABLE 26.2 Descriptive Statistics for the Timing of Sexual Maturity Stages in Females

	BREAST STAGES			
	ONSET OF STAGE		MEAN AGE FOR STAGE	
Stage	Mean	SD	Mean	SD
Stage 2				
Roche et al (Ohio)	11.2	0.7	11.3	1.1
Herman-Giddens et al (United States)				
African American	8.9	1.9		
White	10.0	1.8		
Stage 3				
Roche et al (Ohio)	12.0	1.0	12.5	1.5
Herman-Giddens et al (United States)				
African American	10.2	1.4		
White	11.3	1.4		
Stage 4				
Roche et al (Ohio)	12.4	0.9		
	TANNER PUBIC HAIR			
Tanner Stage 2				
Roche et al (Ohio)	11.0	0.5		
Herman-Giddens et al (United States)				
African-American	8.8	2.0		
White	10.5	1.7		
Tanner Stage 3				
Roche et al (Ohio)	11.8	1.0		
Herman-Giddens et al (United States)				
African American	10.4	1.6		
White	11.5	1.2		
Tanner Stage 4				
Roche et al (Ohio)	12.4	0.8		
	MENARCHE			
Herman-Giddes et al (United States)				
African American	12.2	1.2		
White	12.9	1.2		

Percent Menstruating	At Age 11	AT AGE 12
African American	27.9%[a]	62.1%
White	13.4%[a]	35.2%

Onset of Axillary Hair (Stage 2)	
African American	10.1 ± 2.0
White	11.8 ± 1.9

[a]African-American girls enter puberty approximately 1 to 1.5 years earlier than white girls and begin menses 8.5 months earlier.

Data from Roche AF, Weilens R, Attie KM, et al. The timing of sexual maturation in a group of US white youths. *J Pediatr Endocrinol.* 1995;8:11–18; Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics.* 1997;99:505–512.

TABLE 26.3 Descriptive Statistics for the Timing of Sexual Maturity Stages in White Males (Ohio)

	AGE AT ONSET OF STAGE (YR)		AGE FOR STAGE (YR)	
Stage	Mean	SD	Mean	SD
Genital Stage				
2	11.2	0.7	11.3	1.0
3	12.1	0.8	12.6	1.0
4	13.5	0.7	14.5	1.1
5	14.3	1.1	—	—
Pubic Hair Stages				
2	11.2	0.8	11.3	0.9
3	12.1	1.0	12.4	1.0
4	13.4	0.9	13.7	0.9
5	14.3	0.8	14.8	1.0
6	15.3	0.8	—	—

From Roche AF, Wellens R, Attie KM, et al. The timing of sexual maturation in a group of US white youths. *J Pediatr Endocrinol.* 1995;8:11–18.

rather than observation. Such a study must be balanced in terms of ethnic groups, and the planners must use the predicted increase of certain ethnic populations in the United States to avoid the unfortunate position we now have as we look back and try to draw conclusions about secular trends without sufficient data from various ethnic groups.[34]

From all of the longitudinal and some of the cross-sectional data, we may consider the mean age at onset of puberty in boys to be 11 years, with the normal limits being 9 to 14 years.[106] It is possible that some normal boys, especially African-American boys, will enter puberty or adrenarche between 8 and 9 years of age. The influence of BMI must be taken into account. Obese boys characteristically enter puberty early, especially when there is an *MCR4* mutation, but obesity is also associated with delayed puberty.

Guidelines for the normal variation in pubertal development for girls in the United States are controversial. In the cross-sectional, convenience sample study, 3% of white girls had stage 2 breast development in their sixth year and 5% by the seventh year, whereas 6.4% of African Americans had stage 2 breast development by the sixth year and 15.4% by the seventh year. African-American girls have an earlier onset of pubertal development by about 1 year, even though their average age at menarche in the cross-sectional study was only 8.5 months different (12.2 years for African Americans and 12.9 for whites). We may combine these findings and set the normal range for age at puberty in white girls at 7 to 13 years and in African-American girls at 6 to 13 years. However, some girls in this early range may have a loss-of-function *MKRN3* mutation, possibly in a familial pattern; testing is available only in research laboratories at present but may be available for clinical diagnosis in the future.

These guidelines help the decision of which children with early onset of puberty are candidates for expensive diagnostic tests and for consideration of long-term therapy, because many of those children who appeared to have mild sexual precocity in years past may now be considered to represent a normal variation. We emphasize that family history, the rapidity of development of secondary sex characteristics, the rate of growth, and the presence or absence of CNS or other types of diseases must enter into the decision to evaluate a child. We recommended these ideas in previous editions of this textbook, and the Drug and Therapeutics and Executive Committees of the Lawson Wilkins Pediatric Endocrine Society supported such a revision of the lower limit of the normal age at onset of puberty to age 7 for white girls and age 6 for African-American girls, with no changes in the current guidelines for evaluating boys, which target those with signs of puberty developing before 9 years of age.[112]

Several studies indicate the likelihood of missing serious endocrine disorders if the new guidelines are followed.[113–115] With all of these studies, it may be inferred that if the examining physician looks for signs and symptoms of disease rather than just relying on the age criteria, less than 10% of true precocious puberty will be missed; of those cases, some (probably many) will be so mild as to not need intervention and may represent variations of normal. A multinational study from Europe suggested that valid indications for magnetic resonance imaging (MRI) of the CNS in the diagnosis of precocious puberty are puberty onset in girls before 6 years of age, in agreement with our recommendations, and an estradiol value higher than the 45th percentile (in the laboratory performing the diagnostic tests) for girls with central precocious puberty (CPP), a new criterion.[116]

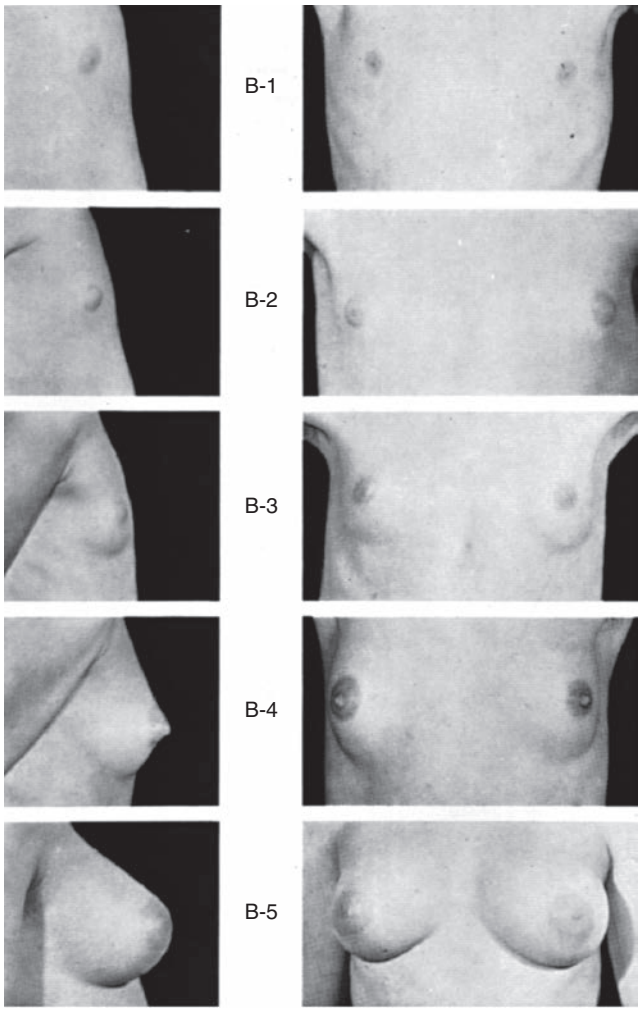
As noted, there are variations of pubertal development that do not demonstrate pubertal elevations of LH values. For instance, early breast development with bone age advancement and increased growth without elevation of LH may be described as "thelarche variant"[117] or "early normal variant puberty."[118] These classifications are not clearly explained in the literature. Obese girls may have local production of estrogen leading to thelarche without elevation of LH, but some studies, particularly from Denmark, demonstrate thelarche without elevation of LH in girls who do not have elevated BMI values.

Secondary Sexual Characteristics and Physical Changes of Puberty

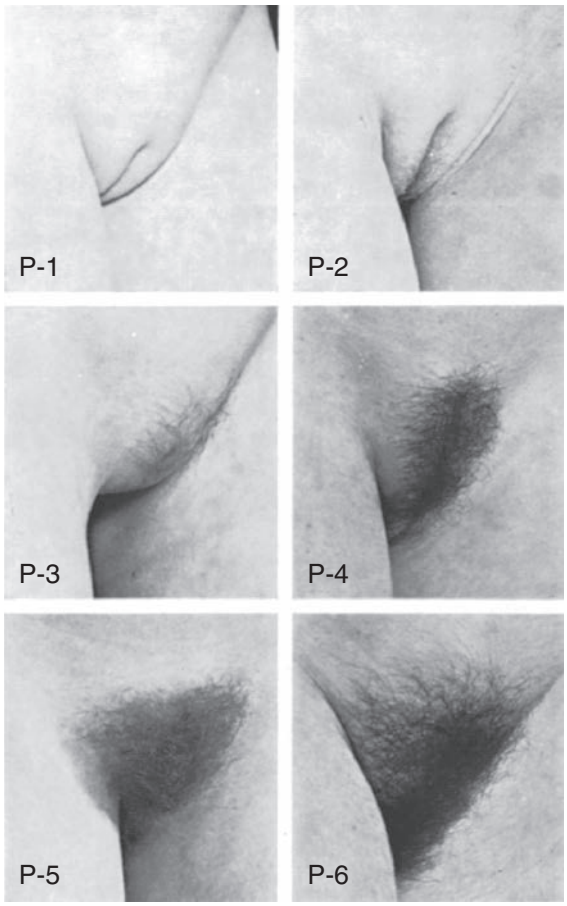
Female Development

Two distinct phenomena occur in the female. The development of the breast and its modified apocrine glands is primarily under the control of estrogens secreted by the ovaries (Fig. 26.5); the growth of pubic and axillary hair (Fig. 26.6) is mainly under the influence of androgens secreted by the adrenal cortex and the ovary. Breast cancer develops in rodents exposed to environmental toxins (e.g., endocrine disruptors) that alter normal mammary development, and this same relationship is postulated to occur in girls exposed to endocrine-disrupting chemicals, especially if development occurs early.[119] Aromatase is present in adipose tissue, and estrogen produced in excess adipose tissue may stimulate breast development at an earlier age in obese girls. Peripubertal obese girls also demonstrated elevated values of androgens, especially just before the onset of puberty and in the early stages of puberty, and there is also decreased LH secretion compared to nonobese girls.[120,121]

The five stages of breast development described by Tanner are the most widely used staging mechanism (see Fig. 26.5). Initial breast development may be unilateral for several months, causing unfounded concern by girls or parents. Needless surgical biopsies



• **Fig. 26.5** Stages of breast development according to Marshall and Tanner (1969). Stage 1: preadolescent; elevation of papilla only. Stage 2: breast bud stage; elevation of breast and papilla as a small mound, with enlargement of the areolar diameter. Stage 3: further enlargement of the breast and areola, with no separation of their contours. Stage 4: projection of the areola and papilla to form a secondary mound above the level of the breast. Stage 5: mature stage; projection of the papilla only, resulting from recession of the areola to the general contour of the breast. (Photographs from Van Wieringen JD, Wafelbakker F, Verbrugge HP, et al. *Growth Diagrams 1965 Netherlands: Second National Survey on 0-24 Year Olds.* Netherlands Institute for Preventative Medicine TNO. Groningen, The Netherlands: Wolters-Noordhoff; 1971. Additional data from Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child.* 1969;44:291–303.)



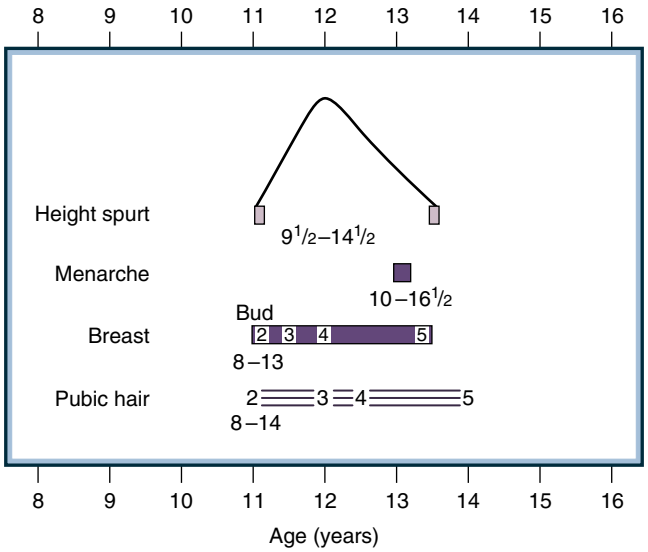
• **Fig. 26.6** Stages of female pubic hair development according to Marshall and Tanner (1969). Stage 1: preadolescent; the vellus over the pubes is not further developed than that over the anterior abdominal wall; there is no pubic hair. Stage 2: sparse growth of long, slightly pigmented, downy hair that is straight or only slightly curled, appearing chiefly along the labia. This stage is difficult to see on photographs. Stage 3: hair is considerably darker, coarser, and curlier. The hair spreads sparsely over the junction of the pubic region. Stage 4: hair is adult in type, but the area covered by it is still considerably smaller than in most adults. There is no spread to the medial surface of the thighs. Stage 5: hair is adult in quantity and type, distributed as an inverse triangle of the classic feminine pattern. The spread is to the medial surface of the thighs but not up the linea alba or elsewhere above the base of the inverse triangle. (Photographs from Van Wieringen JD, Wafelbakker F, Verbrugge HP, et al. *Growth Diagrams 1965 Netherlands: Second National Survey on 0-24 Year Olds.* Netherlands Institute for Preventative Medicine TNO. Groningen, The Netherlands: Wolters-Noordhoff; 1971. Additional data from Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child.* 1969;44:291–303.)

are carried out for this normal variation, and an ultrasound examination may preempt unfounded concerns about breast cancer. If concern arises about breast cancer during puberty (a rare event), ultrasound evaluation is suggested because of the dense nature of the tissue at this stage. Inherited or sporadic agenesis of the breast allows no glandular or fat enlargement, regardless of the level of estrogen stimulation. Virginal breast hypertrophy, an extreme and rapid increase in breast size at the onset of puberty, is rare but is attributed in part to increased sensitivity to estrogen action or to increased local estrogen synthesis and growth factors.

Changes in the diameter of the papilla of the nipple are sequential and are linked to stages of pubertal development.[122] Nipple papilla diameter (3–4 mm) does not increase much during pubic hair stage 1 to 3 or breast stage 1 to 3 but does increase after breast stage 3, providing an objective method of differentiating stage 4 from[123] stage 5 (final diameter, ~9 mm).

The stage of breast development usually progresses along with the stage of pubic hair development in normal girls, but because different endocrine organs control these two processes, discordance can occur. Therefore breast and pubic hair developmental stages should be classified separately for greatest accuracy (Fig. 26.7; also see Table 26.2).

A dulling and thickening of the vaginal mucosa from the prepubertal reddish, glistening appearance occurs as the lining cells cornify, changing the mucosa to a pink appearance, and the secretion of clear or whitish discharge increases in the



• **Fig. 26.7** The sequence of events at puberty in females. Diagram of the sequence of events at puberty in males. An average is represented in relation to the scale of ages; the range of ages within which some of the changes occur is indicated by the numbers below. The ages are from British girls 40 years in the past, so the sequence of changes, rather than the ages, is the important factor. (From Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child.* 1969;44:291–303.)

months before menarche as a result of estrogen action. Girls may notice light-colored discharge on their underwear at this stage. The vaginal pH decreases as menarche approaches due to the increase in lactic acid produced by lactobacilli in the vaginal flora. The length of the vagina increases from about 8 cm at onset of puberty to 11 cm at menarche. Thickening, protrusion, and rugation of the labia majora and minora occur. Fat is deposited in the area of the mons pubis, and the appearance of the labia majora becomes wrinkled. Occasionally, the labia minora may enlarge on one or both sides enough to suggest a tumor; childhood asymmetric labium majus enlargement is a disorder of prepuberty or early puberty.[124] The clitoris enlarges slightly, and the urethral opening becomes more prominent. Photographic atlases of normal female prepubertal genitalia are available and include standards for the variation in appearance of the hymenal opening; this information is invaluable in the evaluation of a victim of suspected child abuse.

Ovarian Development

The peak cohort of germ cells in the fetal ovary is attained at 16 to 20 weeks of gestation. Primordial follicles start to appear at 20 weeks of fetal life, and primary follicles soon follow; they constitute the lifelong store of follicles for the individual, which decreases with development and aging.[125] Follicle-stimulating hormone receptors have not been detected in midtrimester human fetal ovaries; fetal pituitary FSH is not required for proliferation of oogonia, oocyte differentiation, or formation of primordial follicles.[126] During fetal life and childhood, follicular growth to the large antral stage occurs, but before menarche all developing follicles are destined to undergo atresia (Fig. 26.8). Large preovulatory follicles are rarely present before puberty.

The ultrasound appearance of the prepubertal ovary changes with pulsatile gonadotropin secretion, and a multicystic appearance occurs with more than six follicles of at least 4 mm in diameter; this appearance differs from that found in the polycystic ovary syndrome (PCOS). During prepuberty, the ovarian volume is 0.2 to 1.6 mL on ultrasound scans, and after the onset of puberty the volume increases to 2.8 to 15 mL. Tall girls have greater ovarian volume than average-size girls.

The uterus grows until the age of 16 years under the influence of estradiol, progesterone, growth hormone (GH),[127] and insulin-like growth factor 1 (IGF1). Ultrasound studies (Fig. 26.9) show that the corpus of the uterus increases during pubertal progression, from an initial tubular shape to a bulbous structure; the length of the uterus increases from 2 to 3 cm to 5 to 8 cm, and the volume increases from 0.4 to 1.6 mL to 3 to 15 mL.[128] Decreased uterine size is found in patients with Turner syndrome, childhood exposure to radiotherapy, and abnormalities in *HOX* and *WNT* gene expression; maternal cigarette smoking can decrease uterine size at adolescence. Smaller uterine size is associated with an increased risk of miscarriage and failed implantation.

Uterine ultrasonography measurements are proposed to aid the clinician in differentiating premature thelarche from precocious puberty. The addition of color Doppler studies may improve accuracy of the diagnosis of precocious puberty and can differentiate the condition from premature thelarche. A Doppler study showed the lowest impedance of the uterine artery to be in girls with established CPP.[129]

Endometriosis is considered to be an estrogen-dependent process, although it has been reported in premenarcheal girls. It was suggested that this cause for chronic abdominal pain is more common than previously considered. One proposed explanation for early-onset endometriosis is that the condition results from müllerian rests.[130]

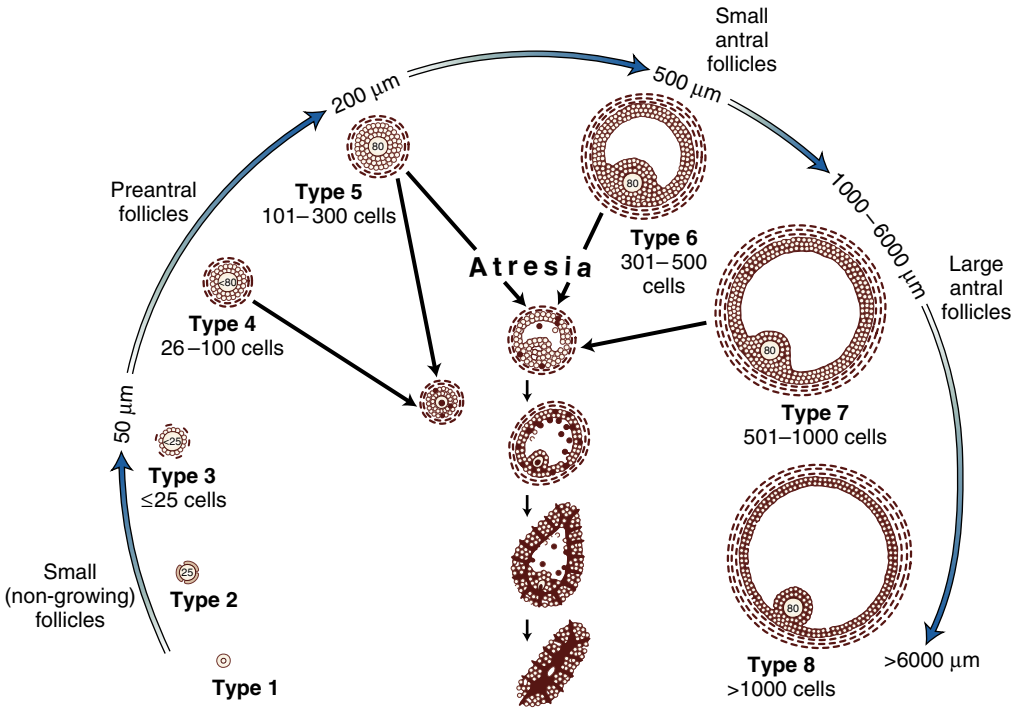
Menarche and Teenage Pregnancy

Menarche usually occurs in the 6 months preceding or following the epiphyseal fusion of the second and first distal phalanges and the appearance of the sesamoid bone; this corresponds to Tanner stage 4 in most cases. The 95th percentile for menarche is 14.5 years, although many textbooks define primary amenorrhea as absence of menses at 16 years. Reconsideration of the age at onset of female puberty may lead to a reconsideration of the definition of primary amenorrhea. Anovulatory cycles are common in the first years after menarche. There is a reported prevalence of 55% anovulation in the first 2 years after menarche that decreases to 20% anovulatory cycles by the fifth year; others have observed a lower number of ovulatory events shortly after menarche and 5 years after the event. With the high prevalence of PCOS, it is unclear how often delayed regularity is an early sign of PCOS or normal variation.

Male Development

The growth and maturation of the penis usually correlate closely with pubic hair development, because both features are under androgen control. However, the stages of pubic hair development and genital development should be determined independently, because discordant stages provide clues to potential disease states of the adrenal gland or testes (Figs. 26.10 and 26.11; also see Table 26.3).

Growth of the testes is usually the first sign of puberty in the male, and it begins approximately 6 months after the average chronologic age of initiation of breast development in girls (see Fig. 26.4). Pubertal testicular enlargement is indicated when the longitudinal measurement of a testis is greater than 2.5 cm (excluding the epididymis) or the volume is greater than 3 mL.



• **Fig. 26.8** Schematic representation of the growth of ovarian follicles during infancy and childhood. Type 1 (primordial follicle) and type 2 (primary follicle) are composed of a small oocyte and a few to a ring of flat granulosa cells. In the diplotene (nesting) stage of prophase, primary follicles are the predominant form of oocyte and constitute the reservoir of cells from which follicular growth occurs. Types 3 to 5 (preantral follicles) are follicles that have entered the growth phase; the oocyte is enlarging and is surrounded by a zona pellucida, and granulosa cells increase in number and differentiate. The growth of the oocyte is complete by the end of the preantral stage, and the increased follicular size is caused by follicular growth and fluid accumulation. Types 6 to 8 represent antral follicles (graafian follicles) and contain a fully grown oocyte, a large number of granulosa cells, a fluid-filled cavity, and a well-developed theca external to the basement membrane. Large preovulatory follicles are absent (10,000–15,000 µm). Follicular growth and atresia take place throughout childhood. All follicles that enter the growth phase become atretic, and this can occur at any stage in their development but mainly involves large antral follicles. (From Peters H, Byskov AG, Grinsted J. Follicular growth in fetal and prepubertal ovaries of humans and other primates. *Clin Endocrinol Metab.* 1978;7:469–485.)

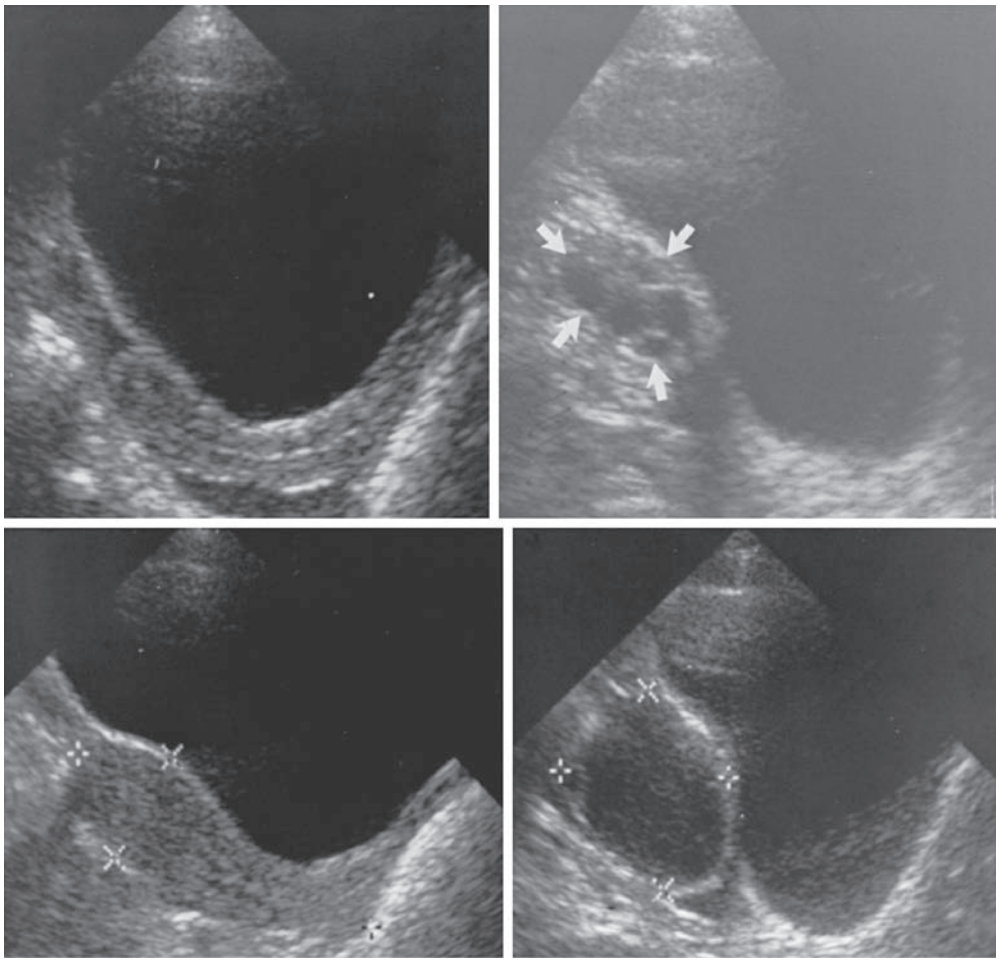
The testicular volume index ([length × width of right testis] + [length × width of left testis]/2) and testicular volume, measured by comparing the testes with ellipsoids of known volume (orchidometer), correlate with the stages of puberty.[131,132] A longitudinal study supported the utility of adding a stage 2a when testicular volume is 3 mL; further pubertal progression occurred within 6 months in 82% of boys who had reached this 3-mL phase[133] (Table 26.4). The most significant changes in serum testosterone and calculated free testosterone occur at the transitions of testicular volume between 1 and 2 mL, 2 and 3 mL, 6 and 8 mL, and 10 and 15 mL, suggesting the denotation of stages pre-1 (testis, 1 mL), pre-2 (testis, 2 mL), early (testis, 3–6 mL), middle (testis, 8–12 mL), late-1 (testis, 15–25 mL, the boy has not reached final height), and late-2 (testis, 15–25 mL, the boy has reached final height).[134] The right testis is normally larger than the left testis, and the left testis is located lower in the scrotum than the right testis.

The phallus should be measured while stretched and in the flaccid state because there is much variation among individuals in the length of the unstretched penis. The length of the erectile tissue (excluding the foreskin) increases from an average of 6.2 cm in the prepubertal state to 12.4 ± 2.7 cm in the white adult. Ethnic differences have been identified; the mean value in African-American men is 14.6 cm, and in Asians it is 10.6 cm.[135]

As in girls, the areolar diameter increases in boys during puberty, with a distinct separation between the sexes occurring in stage 4, when female areolar diameter increases much more than in the male. In gynecomastia, the areolar diameter increases to above-normal values.

Male Testicular Development in Puberty

The testes are active during the prepubertal period albeit at a lower level than during pubertal development.[136] During pubertal development, the testes increase in size, principally because of the growth of the seminiferous tubules associated with the onset of spermatogenetic activity and mitosis of Sertoli cells, and testosterone production increases (Fig. 26.12 and Table 26.5). The Sertoli cells are the major cell type in the seminiferous cords in prepuberty and early puberty, but in the adult, germ cells predominate. During progression through puberty, the Sertoli cells cease to undergo mitosis, differentiate into adult-type Sertoli cells, and form occlusive junctions with the development of the blood-testes barrier. Although Leydig cells are found in early gestation and during the neonatal period of testosterone secretion, the interstitial tissue is composed principally of undifferentiated mesenchyme-type cells during childhood. With pubertal development and rising serum LH levels, adult-type Leydig cells appear (Table 26.6). It is suggested that three phases of Leydig



• **Fig. 26.9** High-resolution pelvic ultrasonography. *Top left*, Prepubertal uterus. *Top right*, Prepubertal ovary demonstrating four small follicular cysts *(arrows)*. *Bottom left*, Pubertal postmenarchal uterus. *Bottom right*, Ovarian cyst in a girl with true precocious puberty.

cell maturation correspond with ages of increased testosterone production: 14 to 18 weeks of fetal life, 2 to 3 months after birth, and puberty through adulthood.[137] The seminal vesicle enlarges through childhood to puberty to hold 3.4 to 4.5 mL, or 70% of the seminal fluid. The mean blood flow in the testes increases to adult values (measured by Doppler sonography) in boys, with a testicular volume greater than 4 mL.

Testicular size is generally measured at the longest diameter excluding the epididymis or volume as determined by comparing the testis to a Prader orchidometer. New smoothed curves of testicular volume versus age are available as based on physical examination and ultrasonography in 769 healthy Dutch boys.[138]

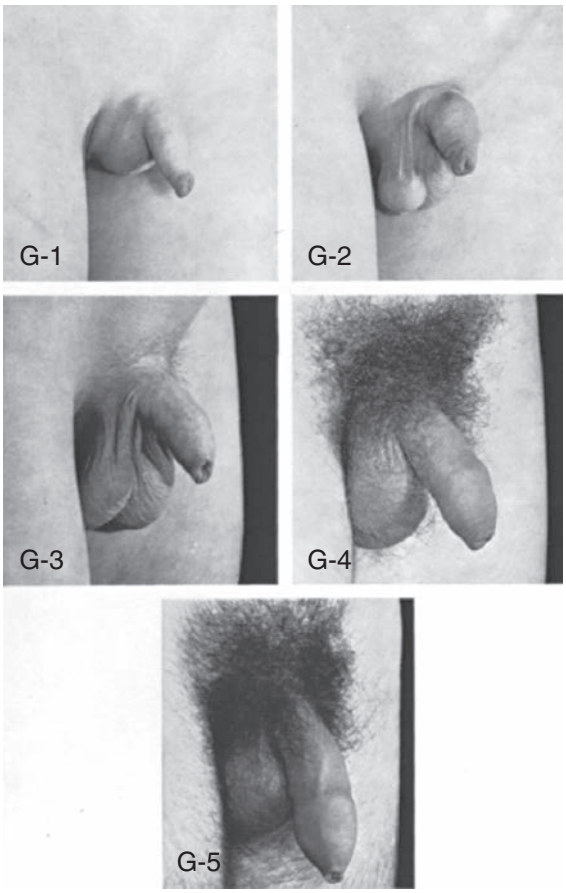
Spermatogenesis

The first histologic evidence of spermatogenesis appears between ages 11 and 15 years (Fig. 26.13; also see Figs. 26.6 and 26.9). Spermaturia may be the first sign of pubertal development, but the presence of sperm in urine is intermittent and therefore not a reliable indicator in all boys. Spermaturia is more prevalent in early puberty than in late puberty, suggesting that there may be a continuous flow of sperm through the urethra in early puberty but that ejaculation is necessary for sperm to appear in the urine in late puberty. Spermaturia in the first-morning urine specimen occurs at a mean chronologic age of 13.3 years and at a mean pubic hair stage 2 to 3 in one study (or 16 years in another study), but may be found in normal boys with bilateral testicular volumes of only 3 mL and no signs of puberty.[139] Normospermia (i.e., normal sperm concentration, morphologic appearance, and motility) is not present until a bone age of 17 years. The first conscious ejaculation occurs at a mean chronologic age of 13.5 years in normal boys and at a mean bone age of 13.5 years in boys with delayed puberty.[140] The age of the first ejaculation, spermarche, decreased in China between 1995 and 2010, and a higher BMI led to an earlier age of spermarche; this pattern mirrors to a degree the secular trend of menarche in girls.[141,142]

The potential for fertility is reached before an adult phenotype is attained, before adult plasma testosterone concentrations are reached, and before PHV occurs.

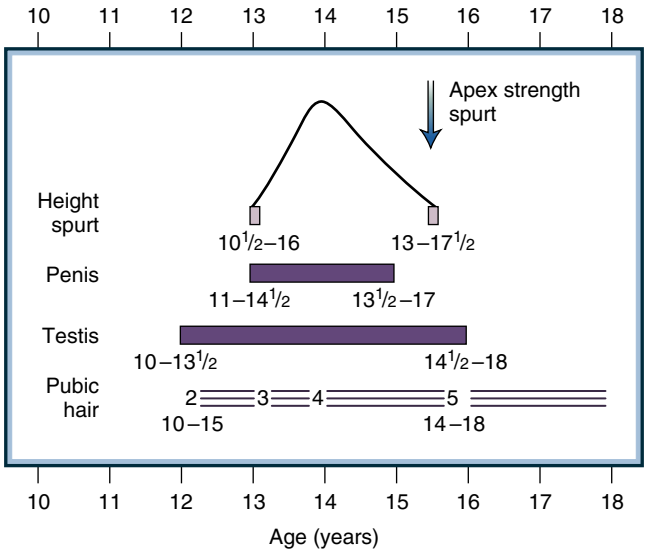
Other Physical and Biochemical Changes of Puberty

The gender difference in voice develops during puberty. In the peripubertal period, the length of the vocal cords in boys and girls is about 12 to 15 mm, of which the membranous portion is 7 to 8 mm.[143] In adult men, the vocal cords attain a length of 18 to 23 mm (membranous portion, 12–16 mm), whereas in women, the cords enlarge only slightly (13–18 mm). During puberty, the male larynx, cricothyroid cartilage, and laryngeal muscles enlarge,



• **Fig. 26.10** Stages of male genital development and pubic hair development according to Marshall and Tanner (1969). Genital development: stage 1: preadolescent. Testes, scrotum, and penis are about the same size and proportion as in early childhood. Stage 2: the scrotum and testes have enlarged; the scrotal skin shows a change in texture and some reddening. Stage 3: growth of the penis has occurred, at first mainly in length but with some increase in breadth; there is further growth of the testes and scrotum. Stage 4: the penis is further enlarged in length and breadth, along with development of the glans. The testes and scrotum are further enlarged. The scrotal skin has further darkened. Stage 5: genitalia are adult in size and shape. No further enlargement takes place after stage 5 is reached. Pubic hair development: stage 1: preadolescent; the vellus over the pubic region is not further developed than that over the abdominal wall; there is no pubic hair. Stage 2: sparse growth of long, slightly pigmented, downy hair that is straight or slightly curled, appearing chiefly at the base of the penis. Stage 3: hair is considerably darker, coarser, and curlier and spreads sparsely over the junction of the pubes. Stage 4: hair is adult in type, but the area it covers is still considerably smaller than in most adults. There is no spread to the medial surface of the thighs. Stage 5: hair is adult in quantity and type, distributed as an inverse triangle. The spread is to the medial surface of the thighs but not up the linea alba or elsewhere above the base of the inverse triangle. Most men will have further spread of the pubic hair. (Photographs from Van Wieringen JD, Wafelbakker F, Verbrugge HP, et al. *Growth Diagrams 1965 Netherlands: Second National Survey on 0-24 Year Olds.* Netherlands Institute for Preventative Medicine TNO. Groningen, The Netherlands: Wolters-Noordhoff; 1971. Additional data from Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. *Arch Dis Child.* 1969;44:291–303.)

leading to the appearance of an Adam's apple. The largest changes in singing and speaking frequencies occur between Tanner genital stages 3 and 4; breaking of the voice occurs at approximately 13 years, and the adult voice is achieved by about 15 years. Higher



• **Fig. 26.11** Diagram of the sequence of events at puberty in males. An average is represented in relation to the scale of ages, and the range of ages within which some of the changes occur is indicated by the numbers below. The ages are from British boys 40 years in the past, so the sequence of changes, rather than the ages, is the important factor. (From Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child.* 1970;45:13–23.)

BMI is associated with earlier voice breaking. Voice breaking is suggested to be a noninvasive method to monitor pubertal development in boys.[144]

Facial hair in boys is first apparent on the corners of the upper lip and the upper cheeks; it then spreads to the midline of the lower lip and finally to the sides and the lower border of the chin. The first stage of facial hair development usually occurs during pubic hair stage 3 (average age of 14.9 years in the United States), and the last stage occurs after pubic hair stage 5 and genital stage 5.

Axillary hair appears at approximately 14 years in boys. Ninety-three percent of African-American girls have axillary hair by age 12, in contrast to 68% of white girls.[22] Axillary sweat glands begin to function as the hair appears. The appearance of circumanal hair slightly precedes that of axillary hair in boys.

Comedones, acne, and seborrhea of the scalp appear as a result of the increased secretion of gonadal and adrenal sex steroids.[145] Early-onset acne correlates with the development of severe acne later in puberty. Acne vulgaris, the most prevalent skin disorder in adolescence, appears at a mean age of 12.2 ± a standard deviation (SD) of 1.4 years (range, 9–15 years) in boys and progresses with advancement through puberty. However, acne vulgaris can be the first notable sign of puberty in a girl, preceding pubic hair and breast development.[145] Analysis of the Nurses' Health Study II indicates that intake of milk and skim milk is related to the development of acne, an association suggested to reflect the hormone content of milk.[146] At late prepuberty, comedones are present in many boys, and 100% of boys have comedones by genital stage 5.

Facial morphologic appearance changes with pubertal development, leading to the mature appearance. The mandible and nose enlarge more in boys, but they and the maxilla, brow, frontal sinuses, and middle and posterior fossae enlarge in both sexes, mainly during the pubertal growth spurt. Children with isosexual precocity (ISP) have the facial appearance of older children, and

TABLE 26.4 Correlation of Testicular Volume (TV) With Stage of Pubertal Development

Parameter	PUBERTAL STAGE 1	2	3	4	5
TV Index[a]					
Burr et al and August et al	1.8	4.5	8.2	10.5	—
Volume (cm^3)					
Zachmann et al[b]	2.5	3.4	9.1	11.8	14
Waaler et al[c]	1.8	4.2	10.0	11.0	15
Waaler et al[d]	1.8	5.0	9.5	12.5	17

[a]TV index calculated as follows: [(length × width of right testis) + (length × width of left testis)] ÷ 2.

[b]Volume estimated by comparison with ellipsoid of known volume (orchidometer) that is equal to or smaller than the testes.

[c]Volume by comparison with orchidometer.

[d]Measurement with calipers and average volume of both testes calculated as follows: 0.52 × longitudinal axis × transverse axis.

Data from August GP, Grumbach, MM, Kaplan SL. Hormonal changes in puberty: correlation of plasma testosterone, LH, FSH, testicular size, and bone age with male pubertal development. *J Clin Endocrinol*. 1972;34:319–326. No abstract available; Burr IM, Sizonenko PC, Kaplan SL, et al. Hormonal changes in puberty: correlation of serum luteinizing hormone and follicle stimulating hormone with stages of puberty, testicular size, and bone age in normal boys. *Pediatr Res*. 1970;4:25–35. No abstract available; Waaler PE, Thorsen T, Stoa KF, et al. Studies in normal male puberty. *Acta Paediatr Scand Suppl*. 1974;249:1–36; Zachmann M, Prader A, Kind HP, et al. Testicular volume during adolescence. Cross-sectional and longitudinal studies. *Helv Paediatr Acta*. 1974;29:61–72.

individuals with delayed puberty have faces of younger children. There is a greater change in various measurements of the face compared with measurements of the skull, with the jaw showing the most increase.[147]

The size of the thyroid gland evaluated by ultrasonography normally increases roughly 40% to 50% with growth in height, weight, surface area, and fat-free mass during puberty but not with BMI.[148] This may lead an examiner to wrongly conclude there is a thyroid disorder due to the physiologic change. Lymphoid tissue growth reaches a maximum at about age 12 and thereafter decreases with pubertal progression.

A host of other physiologic and biochemical measurements change with the onset of puberty and must be interpreted in terms of the stage of pubertal development. Age-related standards should be used in all laboratories but often are not, and the interpreting clinician must turn to a textbook of pediatrics or *The Harriet Lane Handbook*. For example, hemoglobin levels increase at puberty in boys; the effect appears to be mediated by androgen, as treatment of boys with CDP with testosterone and letrozole (to block aromatization to estrogen) resulted in increased hemoglobin levels, even in the absence of a rise in IGF1.[149]

Adolescent Growth

Pubertal Growth Spurt

The pubertal growth spurt may be divided for purposes of comparison into three stages: the time of prespurt minimal growth velocity in peripuberty just before the spurt (takeoff velocity); the time of most rapid growth, or PHV; and the stage of decreased velocity and cessation of growth at epiphyseal fusion. The greatest postnatal growth occurs in infancy; growth decreases to the nadir known as the *minimal prespurt velocity,* the slowest period of growth in childhood, immediately before the pubertal growth spurt.

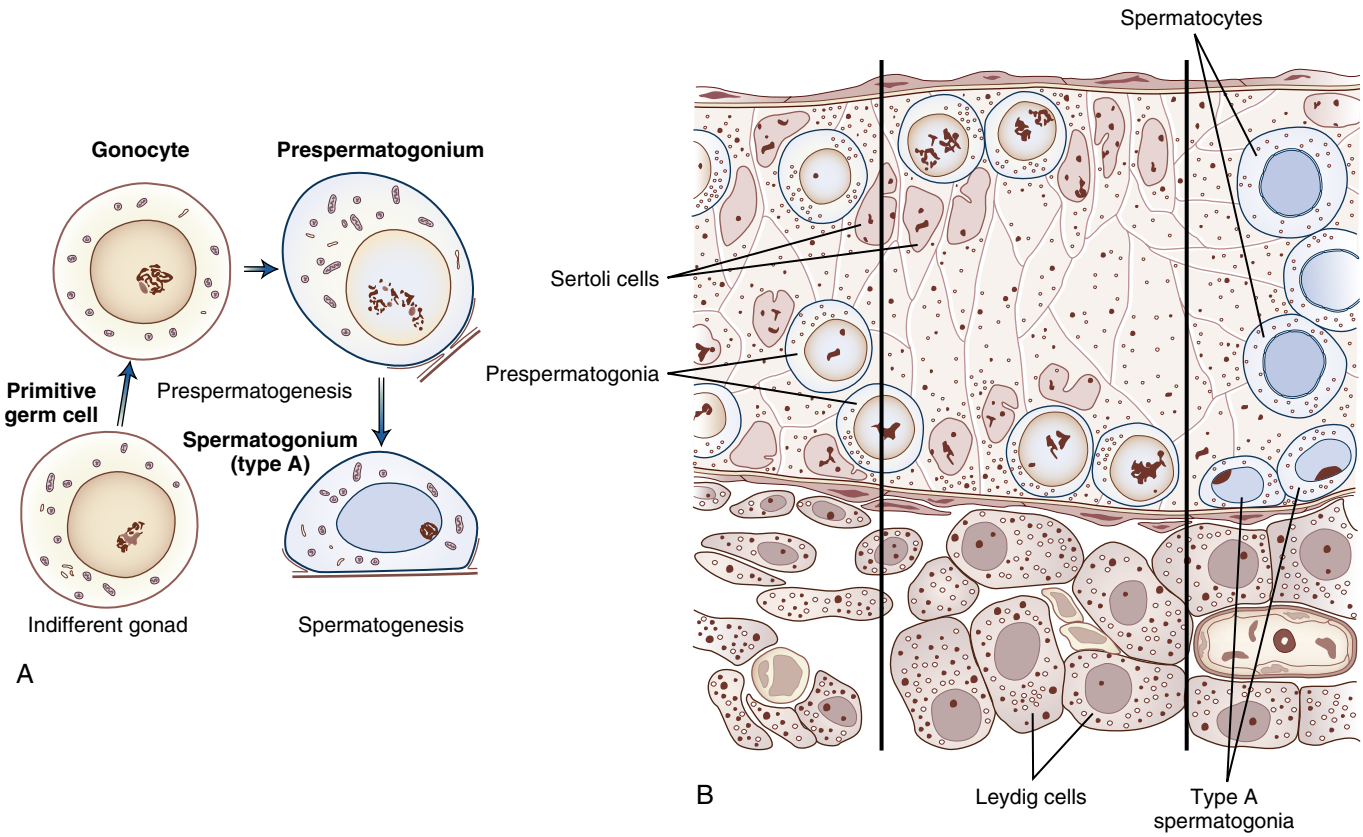
During puberty, boys and girls experience a growth velocity greater than at any postnatal age since infancy (but paling in comparison to fetal growth, when one fertilized cell grows to 7 pounds in 9 months). Boys reach PHV approximately 2 years later than girls and are taller at takeoff (Fig. 26.14); PHV occurs during stage 3 to 4 of puberty in most boys (see Fig. 26.11) and is completed by stage 5 in more than 95% of boys. Boys achieve a PHV of 9.5 cm per year at a mean of about 13.5 years, with a greater PHV in those who mature earlier than in those who mature later.[150] The pubertal growth spurt in girls (PHV in girls is ~8.3 cm/year at a mean chronologic age of 11.5 years) occurs between stages 2 and 3 (see Fig. 26.7). Boys grew a mean of 28 cm, and girls grew 25 cm between takeoff and cessation of growth in a study in the United Kingdom.[151] Although the 8 to 11 cm of increased height gained during the pubertal growth spurt in boys has been held mainly responsible for the difference in adult height between the sexes,[150] twin studies of bone morphologic appearance indicate that the difference in adult height results more from the later onset of pubertal growth in boys than a difference in growth rate between the genders. The difference in bone widths between boys and girls is in large part established before puberty.[152]

A mathematical model that attempts to define the various stages of the pubertal growth curve based on longitudinal data separates the infancy, childhood, and pubertal phases of growth and allows evaluation of growth despite variation in the age at onset of puberty. A slowly decelerating childhood component is the base, with a sigmoidal pubertal component added during secondary sexual development (see Fig. 26.14C). The infancy-childhood-puberty (ICP) model detected the onset of the pubertal growth spurt, predicted the actual magnitude of the pubertal growth spurt, and predicted adult height using only the age at onset of puberty and a measurement of height.[153] Tanner and Davies constructed growth curves for American children using data from the National Center for Health Statistics, and data calculated from theoretical growth curves can be adjusted for time of PHV.[154]

Daily, meticulous observations of girls during puberty over 120 to 150 days show stasis periods in each girl (three to seven events lasting between 7 and 22 consecutive days); steep changes in each girl (one to four episodes, with the sum of these steep changes calculated as a percentage of total growth during the study period ranging from 15.3–42.9%); and continuous growth the remainder of time, with no rhythms or cycles found.[155] Clinicians observe an integrated growth rate during puberty, rather than these various complex patterns occurring during shorter periods of observation.

In a large Swedish registry, faster linear growth during infancy and childhood was associated with earlier PHV during adolescence but less height gain between 8 and 18 years, although greater height and BMI at birth were associated with later PHV in adolescence and more height gain between the ages of 8 and 18 years.[156]

Because girls reach PHV about 1.3 years before menarche, there is limited growth potential after menarche; most girls grow only about 2.5 cm taller after menarche, although there is a variation from 1 to 7 cm. The ages at menarche, takeoff, and PHV are not good predictors of adult height because the duration of



• **Fig. 26.12** (A) The diagram shows the developmental stages of testicular germ cells based on electron microscopic findings in the rabbit. Notice the differences between prespermatogonium and spermatogonium. (B) The diagram shows maturation of testicular cell types in the rabbit from prepubertal appearance *(left)* to onset of spermatogenesis *(right)*. Interstitial cells undergo changes in shape, size, and arrangement in the process of Leydig cell differentiation. (From Gondos B. Testicular development. In: Johnson AD, Gomes WR, eds. *The Testis*, vol 4. New York, NY: Academic Press; 1977:1–37.)

TABLE 26.5 Mean Values of Various Body Measurements and Serum Hormone Levels by Pubertal Stage in Boys (Ohio)[a]

Variable	PS1	PS2a	PS2b	PS3	PS4	PS5
Age (yr)	11.44 (NS)	12.18	12.79	13.74	14.63	15.19
Height (cm)	144.2	149.8	154.6	162.3	169.9	173.3
Weight (kg)	38.18	41.65	47.27	54.67	61.11	66.88
Body mass index (kg/m^2)	18.1	18.4	19.5	20.6	21.0	22.2
Testosterone (nmol/L [ng/dL])						
Blacks	0.8 (23)	3.0 (86)	4.9 (141)	11.5 (331)	13.4 (338)	15.5 (449)
Whites	0.6 (16)	2.9 (83)	4.6 (132)	9.7 (281)	13.3 (383)	14.6 (422)
Free testosterone (pmol/L [ng/dL])	11 (0.33)	60 (1.74)	114 (3.28)	294 (8.49)	413 (11.9)	504 (14.5)
DHEAS (μmol/L [μg/dL])	2.71 (99.7)	3.31 (121.8)	4.04 (148.7)	4.75 (175.0)	5.08 (187.0)	5.89 (217.0)
TeBG (nmol/L)	34.6 (NS)	33.3 (NS)	28.4	21.5	14.4	10.7

[a]Subjects were 515 boys from Ohio, including 237 blacks and 278 whites, aged 10 to 15 years at intake, who were monitored every 6 months for 3 years. All values were significant by Duncan post hoc analysis at $p < 0.01$ except those marked *NS*. Pubertal stages were defined as follows: *PS1,* absence of pubic hair, testicular volume <3 mL; *PS2a,* absence of pubic hair, testicular volume ≥3 mL; *PS2b,* Tanner stage 2 pubic hair; *PS3* to *PS5,* Tanner pubic hair stages 3 to 5.

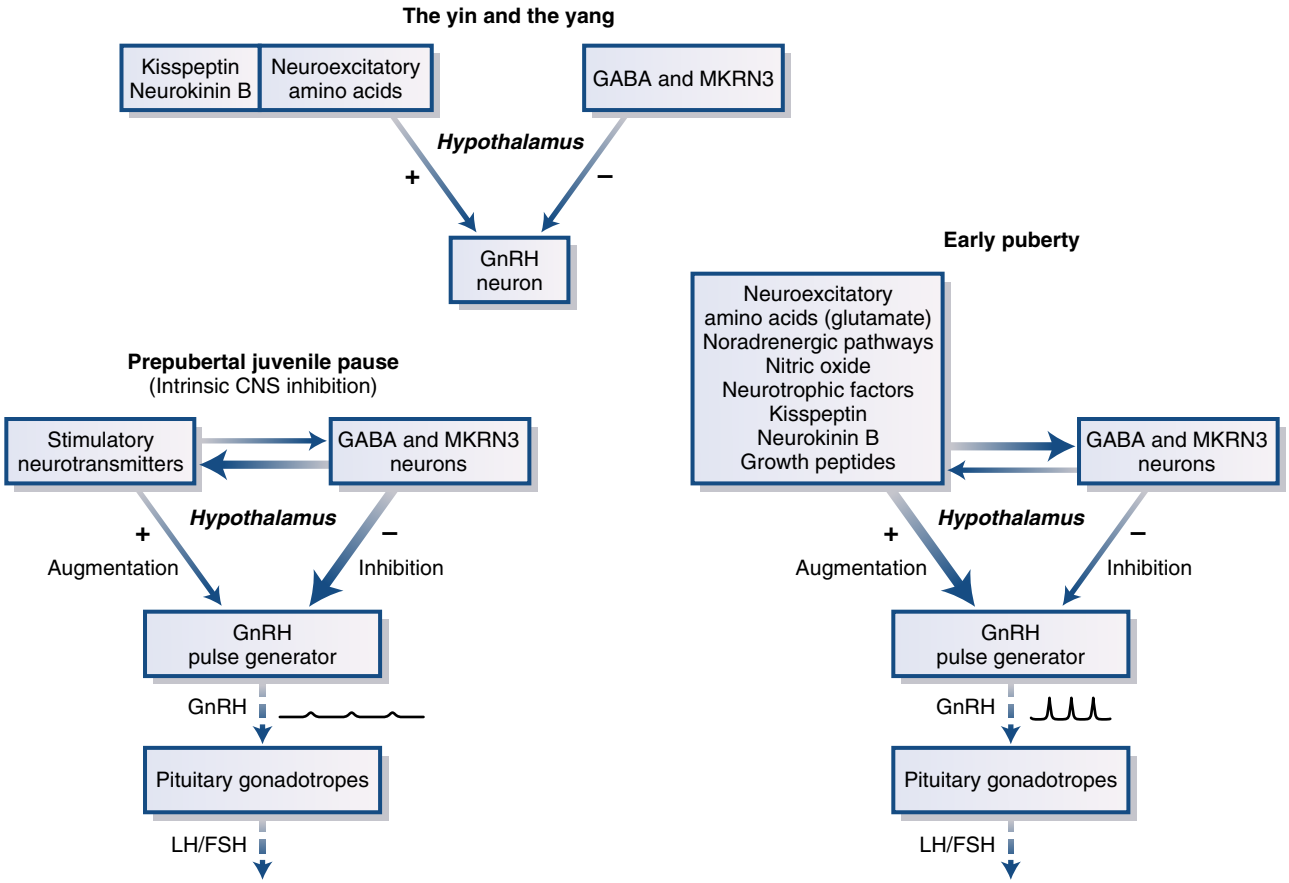
DHEAS, Dehydroepiandrosterone sulfate; *NS,* not significant; *TeBG,* testosterone-binding globulin.

Modified from Biro FM, Lucky AW, Huster GA, et al. Pubertal staging in boys. *J Pediatr.* 1995;127:40–46.

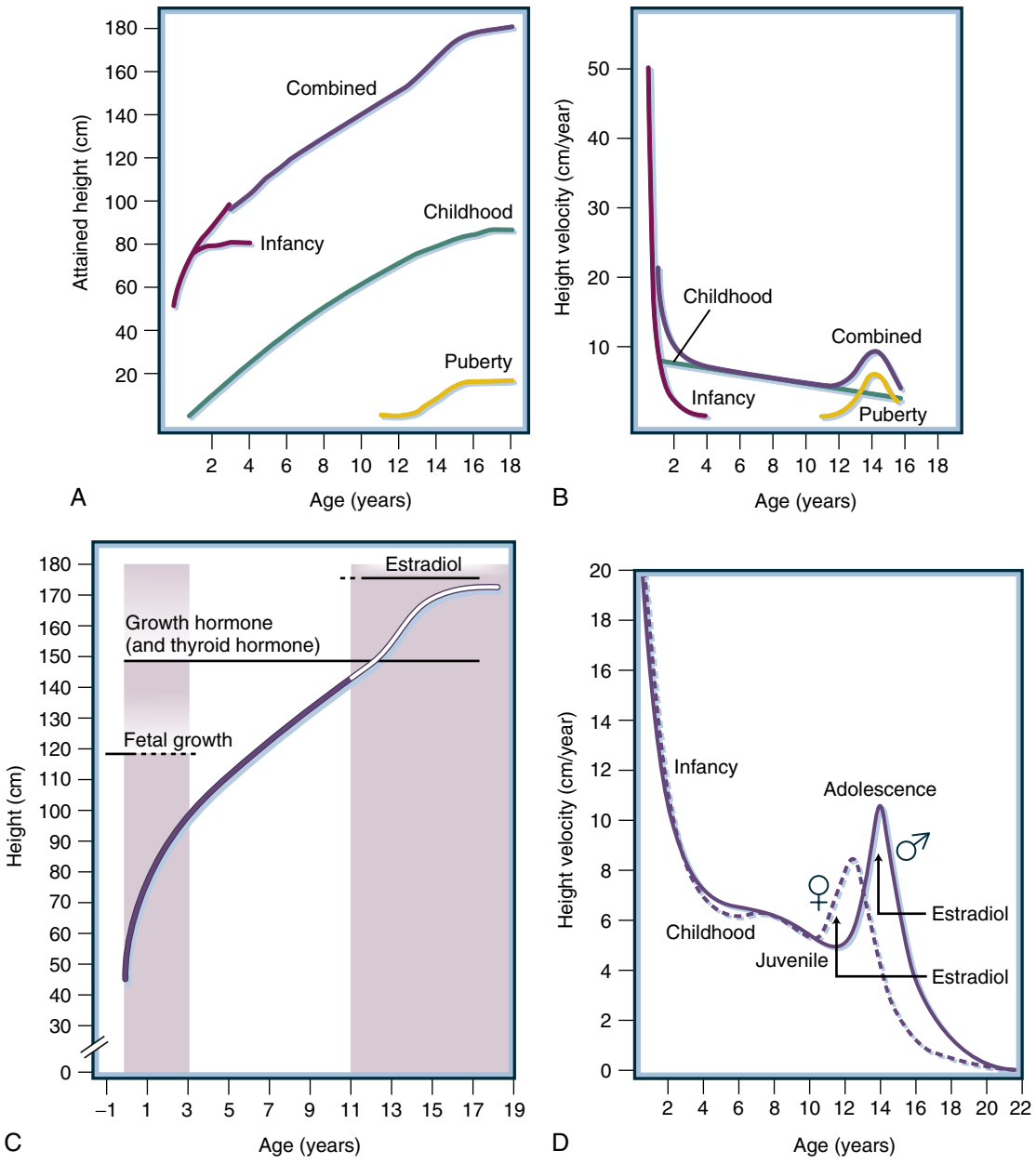
TABLE 26.6 Cellular Activity in Human Testis at Various Stages of Development

Stage	Germ Cells	Sertoli Cells	Leydig Cells
Prepubertal	Prespermatogenic cells are present	Predominant cells in seminiferous cords	Scattered, partially differentiated cells are present
Pubertal	Initiation of spermatogenesis	Increased complexity, formation of occlusive junctions	Fully differentiated cells appear
Adult	Active spermatogenesis, predominant cells	Individual cells associated with groups of germ cells	Groups of fully differentiated cells are present

From Gondos B, Kogan S. Testicular development during puberty. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:387–398.



• **Fig. 26.13** The yin and yang of the neuroendocrinology of the prepubertal juvenile pause and its intrinsic central inhibition of the gonadotropin-releasing hormone (GnRH) pulse generator and the reversal of this inhibition and termination of the juvenile pause, which leads to the onset of puberty. The GABAergic neuronal network and its neurotransmitter γ-aminobutyric acid (GABA) constitute the most ubiquitous inhibitory transmitter in the hypothalamus and the brain. During the prepubertal juvenile pause, this neurotransmitter system appears to play the major neural role in inhibiting the GnRH pulse generator. Suppression of GABA inhibition during this period promptly results in reactivation of the suppressed GnRH pulse generator in the rhesus monkey. With the approach of puberty, GABA inhibition of the GnRH pulse generator wanes, and its reactivation gradually occurs. This reactivation likely is augmented by stimulatory neurotransmitters (e.g., kisspeptin, excitatory amino acids), some of which depend on increased gonadal steroids for their activation, and by neurotrophic factors and growth peptides. A critical component of the reawakening of the GnRH neuronal network is the increase, independent of sex steroids, in KISS1 messenger ribonucleic acid (mRNA) expression in kisspeptinergic neurons in the medial basal hypothalamus and the secretion of kisspeptins, the cognate ligands for the kisspeptin receptor (KISS1R, formerly GPR54) on the surface of the GnRH neuron (Shahab et al). As a consequence, the amplitude and, to a lesser extent, the frequency of GnRH pulses increase, which leads to increased pulsatile secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and the activation of the ovary and testis. As shown experimentally in monkeys, the GnRH pulse generator can function in the absence of hypothalamic stimulatory factors. The nature of and factor(s) responsible for this transition from central inhibition and the postulated dominance of GABA in the release of inhibition and reactivation of the GnRH pulse generator are unknown. (Additional data from Shahab M, Mastronardi C, Seminara SB, et al. Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *Proc Natl Acad Sci U S A*. 2005;102:2129–2134.)



• **Fig. 26.14** The infancy-childhood-puberty (ICP) model of Karlberg for mean attained height (A) and height velocity (B) for boys. The mean value for each component (infancy, childhood, and puberty) and their sums (combined growth [A] and combined velocity [B]) are plotted. The growth curve for an individual represents the additive effect of the three biologic phases of the growth process (ICP). Karlberg has provided mathematical functions for each component of his model. *Infancy:* This component starts before birth and falls off by age 3 to 4 years. It can be described by the exponential function $y = a + b[1 - \exp(-ct)]$. Average total gain in height for Swedish boys is 79 cm (44% of final height) and for girls is 76.8 cm (46.2%). *Childhood:* This phase begins at the end of the first year of life and continues to mature height. A second-degree polynomial function describes this component: $y = a + bt + ct^2$. Average total gain in height for boys is 85.2 cm (47.4%) and for girls is 78.4 cm (47.3%). *Puberty:* The model for the pubertal growth spurt is a logistic function: $y = a/[1 + \exp(-b(t - t_v))]$. Average total gain in height for boys is 15.4 cm (8.6%) and for girls is 10.9 cm (6.5%); y designates attained height at time t in years from birth; a, b, and c are constants; t_v is the age at peak height velocity. (C) A schematic male growth chart shows the features of the ICP pattern overlaid and illustrates the predominant endocrine mechanisms controlling each phase of growth. The first *shaded area* emphasizes the decreasing velocity of infantile growth as the individual leaves the rapid growth phase of fetal life. The *open area* is the childhood phase, which continues and magnifies the decreased velocity of growth into a plateau of rather constant growth during childhood. These two phases depend largely on the effects of growth hormone (GH) and thyroid hormone, with no or little effect derived from gonadal steroids. In the next period of the pubertal growth spurt, gonadal steroids exert their direct and indirect effects. Gonadal steroids exert direct effects on the bone by stimulating the generation of insulin-like growth factor 1 (IGF1) and other growth factors locally, and they exert indirect effects by stimulating increased GH secretion, which exerts its own effects on bone and stimulates the production of IGF1. In the female, the major gonadal steroid involved in the pubertal growth spurt is estradiol, whereas in the male, testosterone and estradiol (arising mainly from the aromatization of testosterone) are the major gonadal steroids. (D) The adolescent growth spurt in girls and boys (growth velocity curves). Notice the later onset of the pubertal growth spurt in boys and the approximately 2-year difference in peak height velocity and the greater magnitude of peak height velocity compared with girls. The timing of the effects of estradiol is indicated. Progressive epiphyseal fusion terminates the growth spurt and leads to final or adult height. (A and B, modified from Karlberg J. On the construction of the infancy-childhood-puberty growth standard. *Acta Paediatr Scand Suppl.* 1989;356:26–37; C and D, from Grumbach MM. Estrogen, bone, growth, and sex: a sea change in conventional wisdom. *J Pediatr Endocrinol Metab.* 2000;13:S1439–S1455.)

pubertal growth is the more important determinant. Later onset of puberty and consequent increase in height at takeoff of the pubertal growth spurt can be balanced by a decrease in actual height achieved during PHV and result in no net change in adult height. However, early onset of puberty can diminish ultimate adult stature, prolonged delay of puberty can increase stature, and an older age at menarche leads to taller adult height in women. The age at PHV and the age at initiation of puberty correlate well with the rate of passage through the stages of pubertal development in normal children. Physical examination of a boy can reveal that he is likely to have significant growth left if he is in early puberty, whereas limited growth is likely in boys who are in late puberty.

Stature and the upper-to-lower (U/L) segment ratio, defined as the length from the top of the pubic ramus to the top of the head divided by the distance from the top of the pubic ramus to the sole of the foot, change markedly during the peripubertal and early pubertal periods because of the elongation of the extremities.[157] At birth, the U/L ratio is about 1.7; at 1 year, it is 1.4; at 10 years, it is 1.0 in a normal healthy individual. The legs begin to grow before the trunk, although late in puberty, during the growth spurt, growth of the legs is similar to growth of the upper torso.[158]

The mean U/L segment ratio of white adults is 0.92, and that of African-American adults is 0.85. There are no differences in U/L segment ratio between the sexes. In general, hypogonadal patients have delayed epiphyseal fusion and lack a pubertal growth spurt; therefore their extremities grow for a prolonged period, leading to a decreased U/L segment ratio and an increased span for height, a condition known as *eunuchoid proportions*. Eunuchoid proportions are found in subjects with defects in estrogen synthesis and estrogen receptor deficiency, but normal proportions occur in patients with complete androgen insensitivity syndrome, demonstrating the primary role of estrogen in mitigating or establishing these proportions.[159–161] The distal parts of the extremities, the hands and feet, grow before the proximal parts; a rapid increase in shoe size is a harbinger of the pubertal growth spurt. Boys with Klinefelter syndrome have long legs but not long arms, a physical feature that can assist diagnosis before the onset of puberty. The shoulders become wider in boys, and the hips enlarge more in girls. The female pelvic inlet widens, mainly because of growth of the os acetabuli. The size of the head approaches the adult size by age 10 years, and the brain reaches 95% of adult size by the onset of puberty.

Bone Age

Skeletal maturation is assessed by comparing radiographs of the hand, the knee, or the elbow with standards of maturation in a normal population.[162,163] Ossification centers appear in early life, the bones mature in shape and size and develop articulation of surfaces; ultimately, the epiphyses or growth plates fuse with their shafts in the process of epiphyseal fusion. Bone age, an index of physiologic maturation, does not have a well-defined relationship in normal children to the onset of puberty because it appears to be more variable than chronologic age.[164] In addition, the bone age evaluator must have experience with the method or an erroneous reading may occur; smaller hospitals/radiology offices will not likely have such experience. However, bone age is still used for predicting the age of menarche, and in boys, the onset of normal, premature, and delayed puberty in a general sense.

A difference between bone age and chronologic age must exceed 2 SDs to be of biologic significance. Standard deviations may range from a few months in infancy to 1 year in later adolescence; a 2-year variation of bone age from chronologic age is within normal limits in teenage years. As commonly estimated, bone age is imprecise and is a qualitative rather than a quantitative measure.[165] The development of techniques for scanning radiographs coupled with computer analysis is now approved in Europe and may improve accuracy of assessment.[166]

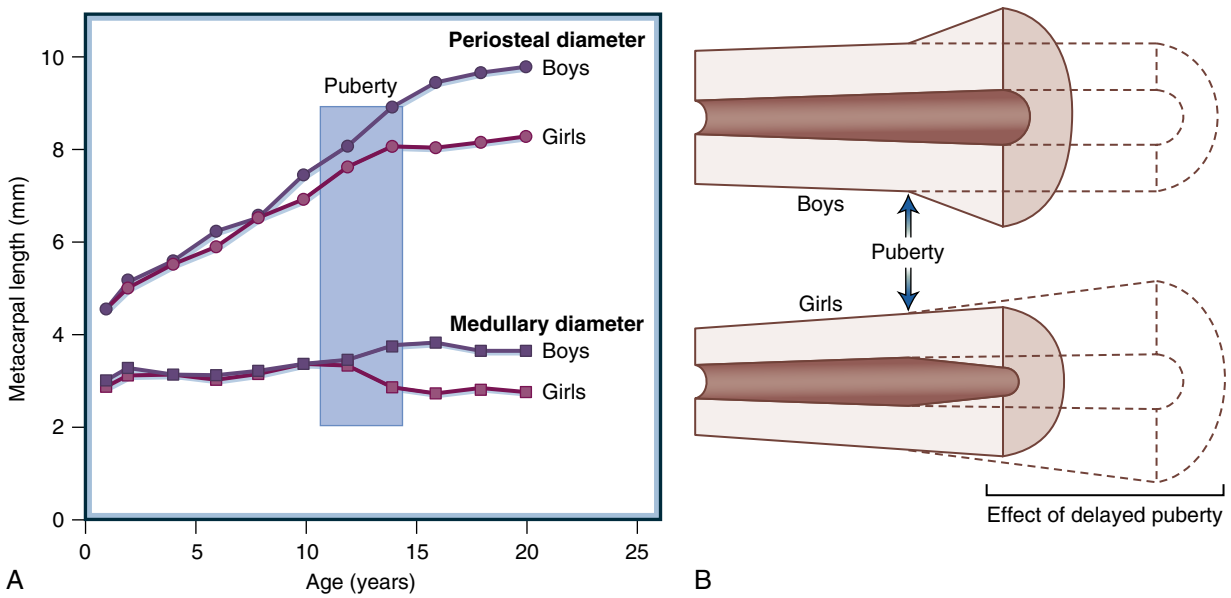
African-American children have reportedly had slightly more advanced bone ages than white children of the same chronologic age,[167] but recent evidence suggests that Asian and Hispanic children have more advanced bone ages than African Americans or Caucasians at the same chronologic age.[168]

Bone age, height, and chronologic age can be used for prediction of adult height from the Bayley-Pinneau tables[169] or by the use of the Roche-Wainer-Thissen,[170] Khamis-Roche[171] (for healthy whites only), or Tanner-Whitehouse techniques TW2[158] and TW3.[172] Skeletal maturation is more advanced in girls than in boys of the same chronologic age because the early pubertal bone ages of 11 years in girls and 13 years in boys are equivalent stages of bone maturation by the hand-wrist method. There are reportedly considerable variations in height prediction between the methods, so the subjective manner of prediction must be borne in mind.[173]

Skeletal Density

Prevailing views locate the determinants of adult bone density in large part in genetic tendencies and the appropriate acquisition of bone mineral in childhood during growth. Osteoporosis and osteopenia are important conditions of the adult that are held to have antecedents in youth, and increasing interest focuses on bone health in children and adolescents, including the effects of age of menarche, nutrition, exercise, and genetics on normal skeletal development.[174,175] A relationship of bone density exists between generations if the effects of age and puberty are eliminated, as 60% to 80% of variance in peak bone mass is attributed to genetic factors.[176] Clinical studies and animal models lead to the conclusion that distant-past patterns of bone growth are less important than recent conditions and that childhood bone growth does not exert a strong effect on adult bone density.[177] Thus even with poor diet in childhood and adolescence, improvement in bone density may be possible later in life.

Areal bone mineral density (BMD) represents a two-dimensional image and is a function of the size of bone; this is the measurement most often available clinically with commercial dual-energy x-ray absorptiometry (DXA) devices. Smaller bones attenuate the radiation beam less than larger bones, and this factor must be considered in the interpretation when measuring bone density using commercial DXA devices. BMD of the total body, lumbar spine, and femoral neck measured by DXA increases at a mean annualized rate of 0.047 g/cm^2 for boys and 0.039 g/cm^2 for girls (Fig. 26.15A). Longitudinal studies of total-body DXA assessments indicate that boys accumulate 407 g per year and girls 322 g per year of mineral (i.e., 359 mg/day for boys and 284 mg/day for girls); 26% of adult calcium is laid down during the adolescent years of peak calcium accretion—14 years (mean) for boys and 12.5 years for girls.[178] BMD approaches a maximum accretion in girls by the age of 16 years, and in boys by about 17 years, with the difference in timing related to the disparity in PHV; the rate then decreases, reaching a plateau in the third decade of life.[179]



• **Fig. 26.15** (A) Periosteal diameter of the metacarpal bones does not differ before puberty in boys and girls. During puberty, the periosteal diameter expands in boys and ceases to expand in girls, whereas medullary diameter remains fairly constant in boys throughout growth but contracts in girls. (B) In boys, delayed puberty may reduce periosteal apposition, leaving a smaller bone with a thinner cortex but normal medullary diameter *(top)*. In girls, delayed puberty may result in reduced endocortical apposition, leaving a normal or larger bone (if periosteal apposition continues in the absence of the inhibitory effect of estrogen) with a thinner cortex and larger medullary diameter *(bottom)*. (From Seeman E. Pathogenesis of bone fragility in women and men. *Lancet.* 2002;359:1841–1850.)

Quantitative computed tomography (CT) is at present a research technique but provides important information about the material aspects of bone.[180] Although quantitative CT demonstrates an increase in the cortical bone density of the lumbar spine with age, less increase in cancellous bone density with age occurs until the later stages of puberty.

Increased BMD correlates well with height, weight (a main determinant of bone density in adolescent and postpubertal females), age, pubertal development, and BMI but has less relationship with serum IGF1 levels. Increased bone density in a longitudinal study of 227 girls was associated with an earlier onset of puberty.[181]

Some consider that the concept of age at peak bone density attainment is too simplistic and prefer to consider the strength of the bone and its geometry.[182]

Volumetric bone density (bone mineral apparent density [BMAD]) represents the amount of bone within the periosteal envelope and is of more physiologic importance, because it does not rely on the size of the bone that is changing during growth, particularly pubertal growth (see Fig. 26.15A). Volumetric bone density grows in a region-specific pattern, and conditions in childhood and adolescence that affect the accrual of bone mineral have different effects based on the length of time the affected bone has left to achieve its maximum bone mineral content (BMC); deficits may occur in limb dimensions (prepuberty), spine dimensions (early puberty), or volumetric bone density by interference with mineral accrual (late puberty).[183]

Calculations for BMAD are made as follows:

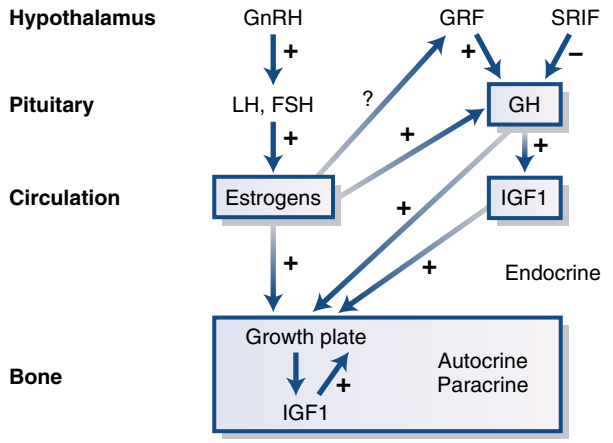
$$\text{BMAD} = \text{BMC} \div \text{the area of the region under consideration}$$

The patterns of areal or volumetric bone density differ during development.[184]

DXA device manufacturers do not always provide standards for children and adolescents. Children are often referred for evaluation of osteoporosis because their DXA results are compared with young adult values, although they have not come close to reaching maximal bone density characteristic of young adults. Bone reaches its adult size and PHV occurs before maximal BMC is reached[185]; these factors may result in a period of increased fragility and susceptibility to trauma characteristic of adolescence. Lean body mass (LBM) is related to skeletal density (stronger in boys than girls) and fat mass and skeletal density (stronger in girls than in boys).[186] Standards are available to interpret BMC in terms of LBM, which appears to be better related to normal bone growth than to chronologic age; muscle stress is an important factor in the development of bone.[187] Normative data for pediatric DXA studies are available from various centers.[179,187,188]

Quantitative ultrasound standards are available for children and adolescents.[189] Because they do not require radiation exposure, they may achieve wider use in childhood.

Seeman presented what he considers to be two fallacies in the interpretation of densitometry.[184] The first is the concept that volumetric BMD increases during growth. It does not. Growth builds a bigger, not denser, skeleton. Second is the idea that peak volumetric BMD is higher in men than in women. It is not. Bone size is greater and is underestimated in patients with larger bones than controls. The misconceptions occur because the result of areal bone density is the BMC per unit projected bone area of bone in the coronal plane, or an areal BMC (g/cm^2). Too often, the "areal" element is deleted, and "content" is replaced by density, so that BMC per unit projected area is



• **Fig. 26.16** Interactions of the major growth-promoting hormones during puberty. Plus (+) indicates stimulatory action; minus (–) indicates inhibitory action. Circulating insulin-like growth factor 1 (IGF1) arises mainly from liver, but other tissues also contribute (i.e., endocrine action). Growth hormone (GH) and gonadal steroids have a direct stimulatory effect on the generation of IGF1 (i.e., paracrine action) locally in bone and cartilage cells. For simplification, the feedback loops for IGF1 and gonadal steroids on the hypothalamic pituitary unit are omitted. *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *GRF,* growth hormone–releasing factor; *LH,* luteinizing hormone; *SRIF,* somatotropin release–inhibiting factor.

called BMD, even though volumetric bone density is the desired measurement. Although BMC may normally be higher in boys than in girls and rises with development, volumetric bone density of the long bones is identical in boys and girls. In contrast to the long bones, volumetric bone density increases at the spine in both sexes.[179,183,184]

The increase in BMD during the prepubertal and pubertal years reflects the increase in the size of the long bones. Legs grow more rapidly than the trunk in prepubertal girls, but during puberty there is more truncal growth. Boys develop greater bone size due to increased periosteal apposition (increasing bone strength) and endosteal resorption compared with girls; girls add bone on the endocortical surface, which may serve as a reservoir for calcium for later lactation and pregnancy.[190]

Birth weight, weight gain during infancy, and weight gain during the years 9 through 12 influence bone mass achieved at 21 years.[191] The BMD at the beginning of puberty predicted the peak bone mass at sexual maturity and appeared to predict the likelihood of osteoporosis as an adult in longitudinal studies, suggesting a method of identification of those most in need of intervention[192] (Fig. 26.16). The mechanostat concept posits that developmental changes in bone strength result from the increasing loads imposed by larger muscle forces, which stimulate bone mineral acquisition. In a longitudinal study, a rise in LBM occurred before peak BMC, and fat mass later exerted more influence.[193] Increased physical activity is generally beneficial for bone health, but excessive running, gymnastics, and cheerleading are progressively more likely to lead to stress fractures,[194] and excessive exercise can lead to the female athletic triad (discussed later). Femoral head strength increases markedly during puberty, and the femoral neck increases in density more with impact load sports such as running (compared with active load sports such as swimming); only 3 to 12 minutes of daily exercise increases femoral bone density in early pubertal children,[195] with greater increases occurring during puberty.

Prepubertal girls engaged in gymnastics have increased bone density in the limbs that are more often used, and this occurs in a dose-response manner.[196] A longitudinal study of gymnasts and their mothers found that these effects do not mainly result from genetic influences. Female adolescent athletes have increased bone density, although the effects last only as long as the activity continues.

Calcium intake during puberty has been documented to affect bone density later in life in most studies,[176] but the effect of increased ingestion of calcium may last only as long as the calcium is administered. Pubertal girls are estimated to get well below the recommended intake levels, and even recommended levels may be too low for optimal mineralization. Two cups of milk or equivalent is shown to provide adequate calcium intake for children 8 to 16 years of age.[197] Children who avoid dairy products and are without calcium supplementation have an increased prevalence of fractures in the prepubertal period, even with minor trauma.[198] Early pubertal girls cannot increase gastrointestinal calcium absorption enough to compensate for a poor diet, as older individuals may. African-American children retain more calcium than white children do, and the bone structure is thicker in African-American children; the difference in vertebral bone density between ethnic groups appears to develop by late puberty. Randomized, placebo-controlled clinical trials between 1985 and 2005 that enrolled normal children for at least 3 months revealed a small effect of calcium supplementation in the upper limb, but the increase in BMD was not thought to influence the likelihood of a fracture later in life.[199] However, the lack of effects applies only to normal children, and studies of subjects with disorders of puberty that affect bone development may reveal other findings. Remarkably, calcium intake is directly related to the rate of bone age advancement to a degree.[200] Increased sodium intake at the expense of calcium intake adversely affects bone accretion. Adequate zinc intake is another factor related to BMD in puberty.

Vitamin D status is a concern, because 32% of girls with low calcium intake were also vitamin D deficient and had elevated serum concentrations of parathyroid hormone (PTH) and thyroid hormone receptor–associated protein 5b (TRAP5b), as well as significantly lower cortical volumetric BMD of the distal radius and tibia shaft, and another 46% had low-normal concentrations of vitamin D.[201] Deficient calcium and vitamin D can lead to secondary hyperparathyroidism in adolescence.

Girls with heterozygote ERα genotype (Pp) and high levels of physical activity had significantly higher bone mass, higher BMD, and thicker cortex at loaded bone sites (compared with the distal radius, which is not a weight-bearing bone) than their counterparts[202] with low physical activity. These results suggest that high physical activity benefits those with heterozygous ER genotypes, and the less favorable Pp genotype may be compensated by increasing the amount of leisure-time physical activity at early puberty.

Studies of male athletes are less common than those of girls, but athletic boys age 16 to 19 years can still gain more bone mass in the spine and femora than nonathletic control subjects.[203] Abnormalities of puberty impair bone accretion in both sexes and are mainly consequences of estrogen deficiency due to decreased secretion or peripheral aromatization of androgens (see Fig. 26.15B). A 1.9-year increase in mean age at menarche in young women was associated with lower radial areal BMD T-scores; lower trabecular number, thickness, and spacing; and cortical thickness without a reduction in cross-sectional area, a finding compatible with less endocortical accrual and a possible explanation of

how late menarche is a risk factor for forearm osteoporosis.[204] Urinary adrenal hormone metabolites are related to the achievement of increased proximal radial diaphyseal bone strength; the level of urinary androstenediol at about 8 years of age is an early predictor of diaphyseal bone strength in late puberty (about 16 years of age).[205] Peripheral conversion of adrenarchal dehydroepiandrosterone (DHEA) by 17β-hydroxysteroid dehydrogenase (17βHSD) to androstenediol may be associated with radial bone accretion during growth.

Testosterone administration to normal prepubertal boys increases calcium retention and bone growth and also increases bone density in adolescents with CDP, testosterone-deficient Klinefelter syndrome, or male hypogonadotropic hypogonadism. Bone density is increased in females with excess androgens, whereas girls with anorexia nervosa, hypothalamic amenorrhea, or ovarian failure have decreased bone density.

Serum levels of inorganic phosphate, alkaline phosphatase, serum osteocalcin (Gla-protein level), collagen type I cross-linked N-terminal telopeptides (NTX),[206] and procollagen type I C-terminal propeptide (PICP); the cross-linked C-terminal telopeptide of type I collagen (ICTP), procollagen type III N-terminal propeptide (P3NP), and tartrate-resistant acid phosphatase isoform 5b; and urinary pyridinoline, deoxypyridinoline, and galactosyl-hydroxy-lysine excretion reflect the increased osteoblastic activity and growth rate during childhood and pubertal growth in both sexes, with values reaching a peak at midpuberty and decreasing thereafter.[207]

Body Composition

Just as endocrine changes bring about remarkable changes in secondary sexual development and growth, body composition is dramatically affected. GH and gonadal steroids play major roles in this process.[208] LBM, skeletal mass, and body fat are equal in prepubertal boys and girls, but by maturity, men have 1.5 times the LBM and almost 1.5 times the skeletal mass of women, whereas women have twice as much body fat (25%) as men (13%), producing a gynecoid (woman-like) or android (man-like) appearance.[209] LBM increases by the age of 6 years in girls and 9.5 years in boys. Boys acquire fat-free mass more quickly and for a longer period than girls during puberty; stability is attained by 15 to 16 years in girls and 2 to 3 years later in boys.[150] Fat mass increases in girls at an average rate of 1.14 kg per year, but the fat mass does not change in boys during the pubertal years, leading to the greater fat value in girls than boys with age.[150]

The generalized distribution of fat in males (central fat, apple shaped, android), which is different from that in females (lower body fat predominance, pear shaped, gynecoid), develops largely during puberty as males become more android than they were in prepuberty, although girls start and remain gynecoid. There are ethnic differences in the pattern of change, and Asians have the most significant changes.[210]

A strength spurt occurs during puberty after the pubertal growth spurt. Muscle mass is 54% of body weight in adolescent boys and 42% of body weight in adolescent girls, with the difference partly due to the presence of more muscle cells and larger muscle cells in men. There is little gender difference before 8 years of age, but by 14 years, boys usually have developed greater lean leg mass and greater power than girls.[211]

Obesity, Puberty, and the Metabolic Syndrome

Between NHANES II and NHANES III, a period of roughly 20 years, there was more than a tripling of the prevalence of children and adolescents equal to or above the 95th percentile in weight (denoted by the CDC as overweight), to a prevalence of 17.1%, and a 50% increase in those equal to or above the 85th percentile (denoted as obese).[212]

Weight is not an adequate reflection of body fat. The BMI is now invoked in describing the shape of the body in age-adjusted terms and is used as a better, if imperfect, reflection of body fat in childhood and adolescence.[40] BMI changes with age, and there is no specific number indicating normal or abnormal BMI at all stages of development, as there is for adults. Reference charts of BMI compared with age and gender between the 3rd and 97th percentiles are available online for interpretation of BMI values.[213] There are racial differences in the correlation of BMI with the amount of body fat, which decreases its accuracy.

Increased visceral fat (i.e., intra-abdominal adipose tissue [IAAT]) in obese teenagers is associated with hypertriglyceridemia, decreased high-density lipoprotein (HDL) cholesterol, and small, dense, cholesterol-laden very low-density lipoprotein (VLDL) particles. Subcutaneous fat is associated with large, lipid-laden VLDL particles, which are removed directly from the circulation and pose less risk. The subcutaneous adipose tissue that leads to visibly different body forms is only an imperfect reflection of this internal distribution of fat cells, because increased IAAT may cause metabolic derangements without increasing total-body fat. Studies support the role of increased intra-abdominal visceral fat in children as a cause of insulin resistance and dyslipidemia, with small adipocytes demonstrating limited storage ability, leading to increased ectopic fat deposition in myocytes and hepatocytes.[214,215] Waist-to-hip ratios may not reflect IAAT in children and adolescents, because subcutaneous abdominal fat may be low despite increased visceral fat.[214] Although there is interest in waist circumference in children as a reflection of IAAT and standards are available, there is question if the waist circumference adds to the determination of IAAT more than BMI alone.[216]

DXA is used to determine the percentage of body fat, water, and bone mineral with great accuracy but cannot differentiate visceral from subcutaneous adipose tissue. CT was used until the validation of MRI to determine intra-abdominal and subcutaneous fat distribution without the use of radiation. Excessive body fat during childhood and adolescence has significant medical effects early and later in life. Obesity, glucose intolerance, and hypertension in childhood are strongly associated with increased rates of premature death among Native Americans, but childhood hypercholesterolemia is not.[217]

Serum Lipids in Normal Puberty and in Obesity and the Metabolic Syndrome

In normal puberty, testosterone increases serum levels of LDL cholesterol and decreases HDL cholesterol concentrations, accounting for the adverse LDL to HDL ratio in men compared with women.[218] Postheparin hepatic lipase activity is increased by exogenous androgens (and decreased by estrogens), accounting for the decrease in HDL after androgen treatment or after a rise in endogenous androgen secretion. Standards for lipid levels in childhood differ from adult values.[219]

The epidemic of obesity has led to the advent of the metabolic syndrome in youth. Diagnosis of metabolic syndrome varies among studies, and a generally accepted definition is needed.[220] Elevated cholesterol levels in children and adolescents track to adult values in longitudinal studies. Although familial hypercholesterolemia leads to carotid intimal plaques by puberty, random autopsies demonstrate macroscopic or microscopic evidence

of arteriosclerosis in normal youth without familial hypercholesterolemia, and the tendency is increased by obesity. By 15 to 19 years, 2% of autopsied males had advanced (American Heart Association grade 4 or 5) atherosclerotic coronary artery lesions associated with increased serum cholesterol, obesity, and hypertension.[221]

Insulin and Insulin Resistance

Insulin resistance is a hallmark of obesity and is thought to be the cause of or an associated factor in the metabolic syndrome associated with cardiac disease.[222] Euglycemic clamp techniques are the gold standard for measurement of insulin resistance. Fasting insulin concentrations offer little insight into insulin resistance in an individual, and equations such as the homeostatic model assessment (HOMA) that are based on fasting insulin levels offer little more; however, fasting insulin values are used in epidemiologic studies and offer more useful information.[223] The fasting insulin concentration increases twofold to threefold with PHV, insulin secretion after a glucose load increases over prepubertal levels, and insulin-mediated glucose disposal in peripheral tissues decreases in the hyperinsulinemic euglycemic clamp or the minimal model frequently sampled intravenous glucose tolerance test (IVGTT), showing increased insulin resistance during normal puberty. Insulin sensitivity is inversely related to pubertal stage and BMI.

The response of insulin to an oral glucose tolerance test is greater in African-American subjects than in white subjects at all stages of pubertal development; this ethnic difference in insulin resistance is suggested as a cause for the increased incidence of type 2 diabetes among African-American adults compared with white adults and appears to offer a similar explanation of the ethnic disparity in youth, with white teenagers having greater insulin sensitivity than African-American or Hispanic youth.[224,225] Insulin resistance occurs early in the course of Turner syndrome and thalassemia major, but even in Turner syndrome, in which there is an underlying increase in insulin resistance, there seems to be low or no risk of these conditions developing with GH treatment.

With the increased prevalence of type 2 diabetes (non–insulin-dependent diabetes mellitus [NIDDM]), proposed screening criteria are being evaluated. Currently, children with a BMI higher than the 85th percentile should be screened if they (1) have a family history of type 2 diabetes, (2) have signs of insulin resistance (e.g., acanthosis nigricans, functional ovarian hyperandrogenism, hypertension, dyslipidemia), or (3) belong to one of several specific ethnic groups (e.g., African American, Native American, Hispanic American, Asian American). If a fasting plasma glucose level is higher than 126 mg/dL or a 2-hour postprandial value is higher than 200 mg/dL, or if there are symptoms such as weight loss, polyuria, or polydipsia and a casual plasma glucose level higher than 200 mg/dL, the diagnosis of diabetes is likely, and determination of the type of diabetes (type 1 or 2) is appropriate.

Patients with type 1 diabetes (insulin-dependent diabetes mellitus [IDDM]) usually require an increase in the dose of insulin for euglycemic control at puberty. The cause of insulin resistance has been attributed in part to increased fat oxidation at puberty, which correlates with rising serum IGF1 levels and may be linked to increased GH secretion. However, there is no evidence that GH treatment alone increases the likelihood of development of type 2 diabetes or impaired glucose tolerance. Weight gain increases in children with type 1 diabetes during puberty, leading to a higher incidence of obesity in children with IDDM than would be expected from family patterns. Some adolescents with IDDM, predominantly girls, reduce their insulin use to lose weight, with dire consequences. Retinopathy due to IDDM characteristically appears in the teenage years or later, but duration and control of diabetes in the prepubertal years are contributing factors. The American Diabetes Association recommends screening for microalbuminuria, an indicator of the development of diabetic nephropathy during puberty.

A normal individual adapts to the changes in the physiologic rise in pubertal insulin resistance, but an individual at genetic risk for type 2 diabetes, with the accompanying defect in pancreatic beta-cell function,[226] may not adapt to the insulin resistance and, with the additional insulin resistance characteristic of obesity, may develop clinical type 2 diabetes during the pubertal years or earlier. Type 2 diabetes in children or adolescents should not be confused with the various forms of monogenetic diabetes (previously called maturity-onset diabetes of the young [MODY]), which are inherited as autosomal dominant traits.[227]

Although PCOS is common, several rare syndromes of severe insulin resistance combine hyperglycemia and virilization.[228] The Kahn type A syndrome features include a lean, muscular adolescent female phenotype with acanthosis nigricans, hirsutism, oligomenorrhea or amenorrhea, and ovarian hyperthecosis and stromal hyperplasia associated with abnormalities of the insulin receptor gene. Hyperandrogenism, insulin resistance, acanthosis nigricans (HAIR-AN) syndrome, and PCOS are less severe than Kahn type A and usually manifest in adolescent females. Persons with Rabson-Mendenhall syndrome have severe insulin resistance (possibly leading to diabetic ketoacidosis), dysmorphic facies, acanthosis nigricans, thickened nails, hirsutism, dental dysplasia, abdominal distention, and phallic or clitoral enlargement. The Rabson-Mendenhall syndrome, similar to the Donahue (leprechaunism) syndrome, which shares some of these features, is caused by homozygous or compound heterozygote defects in the insulin receptor gene. Kahn type B syndrome is caused by inhibitory or stimulatory antibodies to the insulin receptor and sometimes occurs with acanthosis nigricans and ovarian hyperandrogenism. This syndrome can occur with ataxia-telangiectasia syndrome or in otherwise normal adolescents. Individuals with the Berardinelli-Seip syndrome combine lipodystrophy and severe insulin resistance and complete or partial absence of subcutaneous fat with increased growth and skeletal maturation, muscle hypertrophy, acanthosis nigricans, hypertrichosis, organomegaly, and mild hypertrophy of the external genitalia.

Most of these NIDDM syndromes can be treated with oral hypoglycemic agents initially; progression of the disorder may require the use of insulin. Several girls with these syndromes of insulin resistance have been described to have low serum gonadotropin values during puberty without response to GnRH but with enlarged ovaries, suggesting a direct role for insulin in stimulating the growth of the ovary. The hypoleptinemic state found in various degrees of lipodystrophy does not appear to affect pubertal progression, but administration of leptin has led to resumption of menstrual periods in some females and adjustment of testosterone production toward normal levels in males.[229]

Blood Pressure

Blood pressure is related to the age, gender, and height of the child using appropriate standards.[230] Blood pressure increases with pubertal maturation, related to increased stature and synchronized with the pubertal growth spurt, suggesting some

relationship in the control of the two processes.[231] Hypertension is becoming common in puberty as a comorbid condition of obesity. Increased blood pressure at puberty depends on BMI and height, factors that are interrelated. Blood pressure in childhood and adolescence is predictive of adult blood pressure (tracking). Blood pressure rises in African-American children at lower BMIs than in white children, making the problem worse in the African-American population. In sexual precocity, blood pressure rises above prepubertal levels to values commensurate with body size and BMI.

Central Nervous System Anatomy, Function, Psychology, and Electroencephalographic Rhythm in Puberty

Brain anatomy and function change substantially during late childhood and adolescence (Fig. 26.17). Puberty is the time of appearance of the ability to solve complex problems in a mature manner. An increase in cortical metabolic rate in infancy is followed by a late childhood decline to adult levels; this decline ceases by the end of the second decade. The prefrontal association cortex, an area of the brain that is concerned with forward planning and regulatory control of emotional behavior, continues to develop until the age of 20 to 25 years.[232] Stress at various stages of development, even in early childhood, may cause psychologic manifestations during puberty.

Behavior or psychopathology that becomes evident at this time has its basis in these changes and exposures dating from early life and the prenatal period, all interacting against a genetic background. The anatomic changes revealed by functional MRI studies of the prefrontal cortex, an area that is involved with emotional regulation and planning, occur during a time of physical maturation and are likely to relate to many of the characteristic behavior changes of puberty. The volume of white matter increases linearly between 4 and 22 years of age owing to an increase in myelination during development.[233] A reduction in cortical synaptic density and neuronal density, analogous to programmed cell death, occurs between 2 and 16 years of age, and this pruning of synapses appears to be linked to improved memory. This change in gray matter follows an inverted U-shaped curve of increase from the age of 6 years. Longitudinal studies using dynamic mapping of human cortical development demonstrate that higher order association cortices (e.g., those involved in executive function, attention, and motor coordination) mature after lower-order somatosensory, motor, and visual cortices mature, and those areas that are phylogenetically older mature before newer ones.[234,235] Intrauterine excess of androgens leads to enlargement of the amygdala, and girls reach greater mass of gray matter 2 years before boys during puberty, demonstrating aspects of the effects of sex steroids on brain growth and remodeling in human beings. Gray matter volume, at least in girls, appears to be related to a pubertal increase in estradiol[236] levels. Increased LH levels are related to areas of increased white matter density, including the cingulum, middle temporal gyrus, and splenium of the corpus callosum; there is a genetic overlay to this relationship. Brain plasticity decreases during puberty. Examples include the inability to learn to speak a foreign language without an accent after puberty and recovery of a child from the effects of a CNS injury that in an older teenager or an adult might have led to aphasia. Loss of plasticity may be maladaptive to our rapidly changing world and extended life span compared with prehistoric times.[237] Plasticity allowed developmental learning before puberty, but lack of plasticity and a standard response to conditions in the adult allowed success in that static environment of the past.

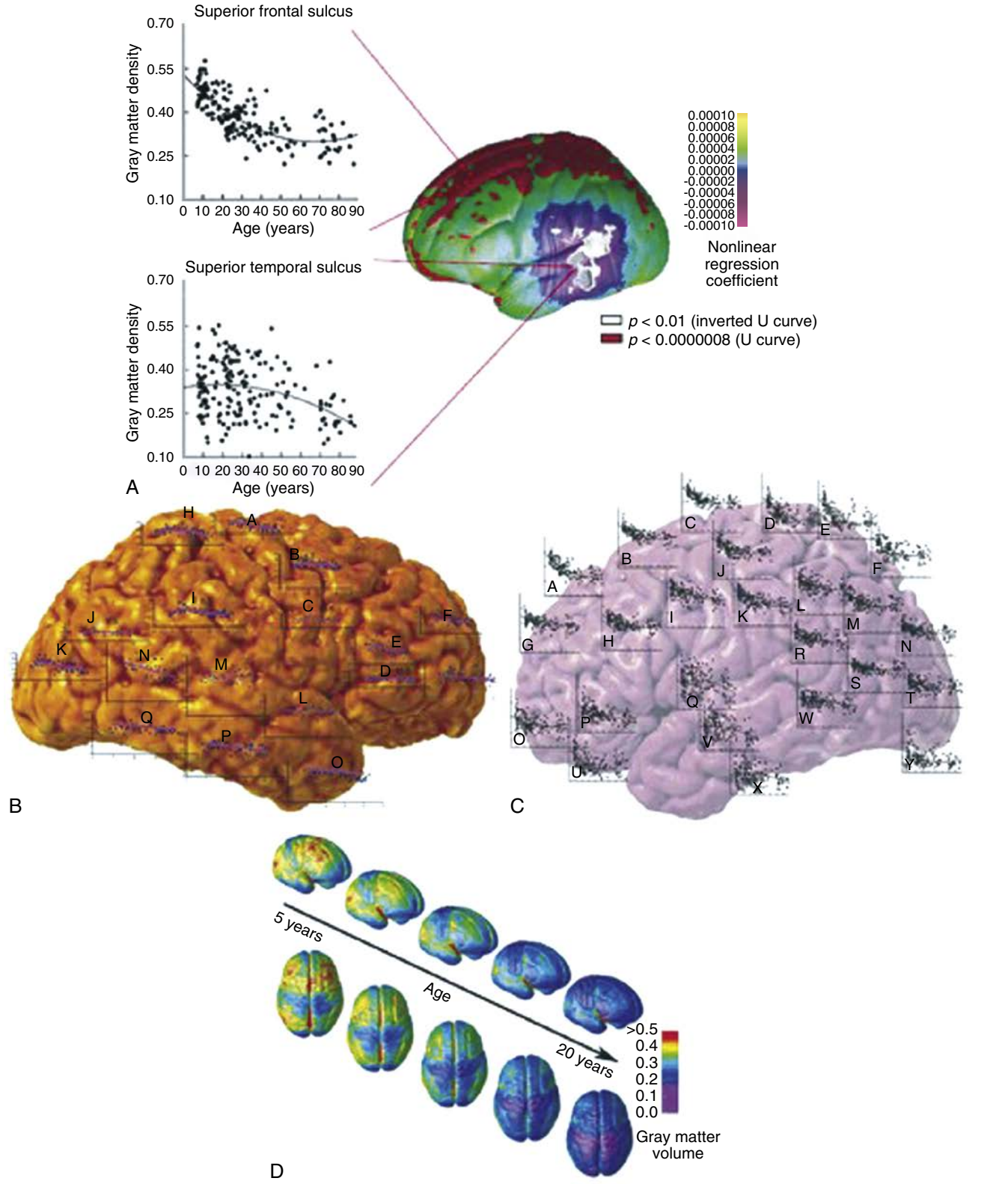
Mania, depression, obsessive-compulsive disorders, and schizophrenia are more common after puberty. They are postulated to be related to alterations of the normal changes in brain architecture and function that occur during puberty.

Sleep Patterns in Puberty

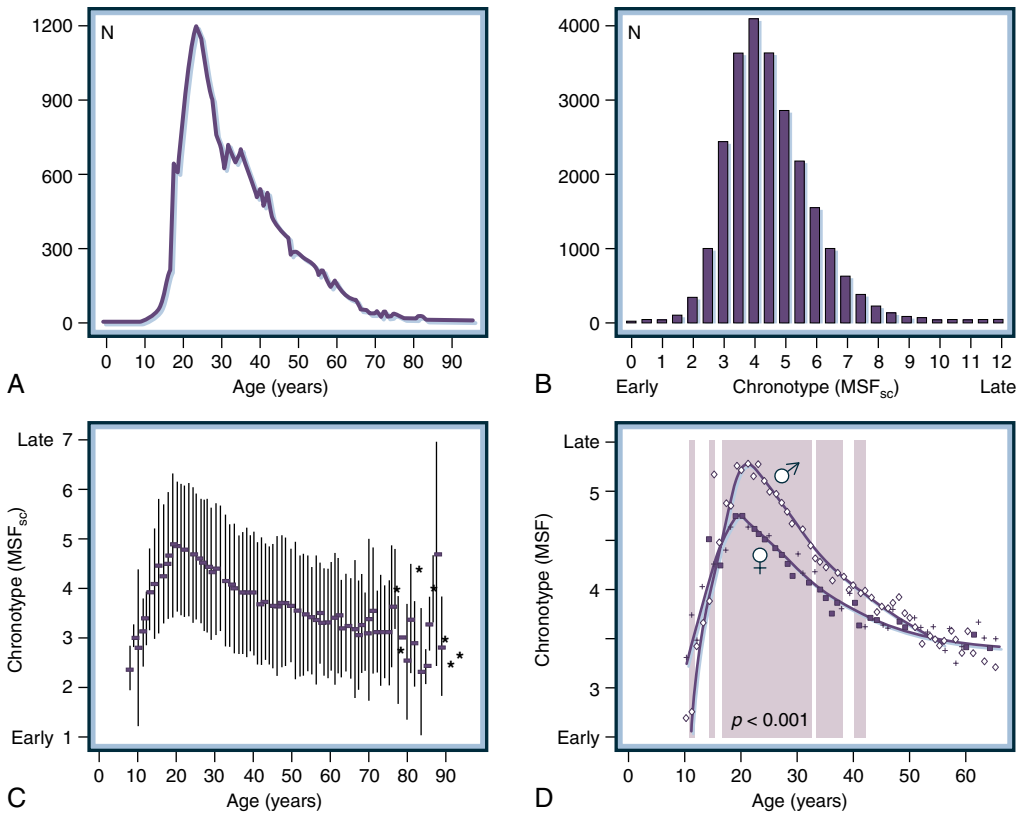
Increased sleep is characteristic of the period of growth and development across species. Because sleep is a time of vulnerability, threats leading to stress are antithetical to normal sleep, and a feeling of safety is thought to be necessary to allow sleep to proceed normally as the adolescent is preparing for independence and increased self-care in a possibly hostile world. Rising complexity of brain function during puberty is reflected in increases in the amplitude and frequency of delta waves (0–3-Hz electroencephalographic waves) found during deep sleep, which appears more related to age than to pubertal development or growth.[238] The function of deep sleep (i.e., slow-wave or non–rapid eye movement sleep) is thought to be restorative to learning and other activities of the waking state, and the most restorative portion is high-amplitude delta-wave sleep. During adolescence, the time spent in deep (stage 4) sleep declines by 40% to 50%, and increased (19.7%) stage 2 sleep occurs with pubertal development. The decline of slow-wave sleep during adolescence may reflect developmental changes of the brain.

When an individual is allowed to "run free," the period is entrained (synchronized) close to the earth's 24-hour light-dark cycle. Because human beings had little to do after dark, evolution favored an early bedtime, but within this schedule, developmental changes occur. One-year-old infants sleep an average of 11 hours per day, and by age 18, if circumstances permit, the mean is 8 hours. Older people have earlier waking times and rate themselves as more morning-like than adolescents or young adults; because children are also morning-like, there is an inverted U-shaped curve of preferred times of awakening across the span of development.[239] This change to evening from morning alertness during puberty appears to be related to biologic factors, in contrast to social factors; in the past, social factors were thought to be more important.

Without the pressure of work or school, adolescents would stay up longer and awaken hours later than a normal weekday schedule would dictate, a schedule far different from the one they followed at a younger age. Adolescents with early school starts awaken earlier than those with later school starts, but they do not change the time they go to sleep, leading to great variation in the amount of sleep attained. Data from the Add Health study showed decreased self-reported sleep duration during self-reported pubertal development, with girls reporting more problems with sleep (e.g., insomnia, insufficient sleep, awakening tired) as puberty progressed but with no such relationship seen in boys.[240] There is an increase in daytime sleepiness during adolescence, particularly during midpuberty up to stage 3 to 4, even if total sleep time is held constant during longitudinal multiple-year studies.[241] With voluntary sleep deprivation (e.g., with late-night homework habits), sleepiness can reach levels seen in narcolepsy and sleep apnea. Adolescents adapt more poorly to changes in sleep patterns than other age groups;



• **Fig. 26.17** Mapping brain change over time. Brain changes in development can be identified by fitting time-dependent statistical models to data collected from subjects cross sectionally (i.e., across a group of subjects at a particular time) or longitudinally (i.e., following individual subjects as they aged), or both. Measurements such as cortical thickness are then plotted onto the cortex using a color code. Trajectory of gray matter loss over the human life span is based on a cohort of 176 subjects between 7 and 78 years of age.[13] (A) represents a region in which the gray matter density decreases rapidly during adolescence (i.e., superior frontal sulcus in which the decrease in gray matter is described by a quadratic equation represented by an inverted U-shaped curve), or follows a more steadily declining time course during the life span (i.e., superior temporal sulcus in which the decrease in gray matter is described by a quadratic equation represented by a U-shaped curve). (B, C) plots superimposed on the brain show how gray matter density decreases for particular regions with age, with the regions denoted by different letters. Brain maturation and change in gray matter density is mapped by year of age in (D) with fractional change in gray matter shown by color coding (C, D). (From Sowell ER, Peterson BS, Thompson PM, et al. Mapping cortical change across the human life span. *Nat Neurosci.* 2003;6:309–315; Toga AW, Thompson PM, Sowell ER. Mapping brain maturation. *Trends Neurosci.* 2006;29:146–159.)



• **Fig. 26.18** Assessment of chronotype (time of awakening in the absence of external cues) using the MCTQ database (N ≈ 25,000). Age distribution within the database (A). Distribution of chronotypes (B). Age-dependent changes (C) in average chronotype (± SD [standard deviation]) are highly systematic (except for the age groups of 19, 21, 22, and 23, all other age-dependent averages ± SD are significantly different from that of age group 20; t-test, $p < 0.001$). Age-dependent changes of the chronotype (D) are different for males and females (*filled circles and black line*: females; *open circles* and *gray line*: males). Gray areas indicate significant male-female differences (t-test, $p < 0.001$). (From Roenneberg T, Kuehnle T, Pramstaller PP, et al. A marker for the end of adolescence. *Curr Biol.* 2004;14:R1038–R1039.)

this is manifested in the difference in hours awake between the school week and the weekend. Adolescents are able to shift to a later schedule more easily than to an earlier schedule. When self-selected bedtimes are late during summer but have to be changed to allow for school attendance, the adjustment is particularly lengthy and difficult.

After study of 27,000 individuals, it was proposed that the point of inflection from evening alertness during adolescence (after a morning-like pattern in childhood) to morning alertness in adulthood might be used as a marker of the end of adolescence, a sign that developmental remodeling of brain pathways is completed.[242] The age of this inflection point is about 20.9 years in males and 19.5 years in females, who have an earlier change in this and other aspects of puberty than males (Fig. 26.18).

Characteristics of Adolescence

Most of this chapter deals with the biochemical and physical changes of the period we denote as puberty, but there are also profound psychosocial changes during this period, usually denoted as adolescence. Although the attainment of an adult role in society occurs within a few years after achievement of reproductive maturity in non-Western societies, the more technologically advanced the society, the more protracted the time allowed for adolescent psychosocial development. The prolonged period of adolescence in current society, ranging from 11 to 20 years in the United States, arose recently in human history, beginning no more than 100 years ago in Western society.

As expressed by Remschmidt, the most important psychologic and psychosocial changes in adolescence are the emergence of abstract thinking, the growing ability to absorb the perspectives or viewpoints of others, an increased capacity for introspection, the development of personal and sexual identity, the establishment of a system of values, increasing autonomy from family and personal independence, greater importance of peer relationships of sometimes subcultural quality, and the emergence of skills and coping strategies to overcome problems and crises.[243]

Adolescence may be divided into three periods (early, middle, and late) by chronologic age. However, these periods may be reached at different maturational ages, because rates of physiologic maturation differ among individuals in these age groups.[244]

Early adolescence (age 11–15 years) encompasses most of the physical changes of puberty and in American society includes a profound social change from the sheltered, single-classroom environment of elementary school to the multiple-classroom and multiple-teacher experience of middle school or junior high school. The individual develops a maturing, but not mature, capacity for abstract thought and decision-making processes in contrast to the concrete reasoning of childhood.

Middle adolescence (ages 15–17 years) is the period of the high school years, a calmer period than early adolescence. The school experience is not a striking change, and many of the most

prominent biologic and physical changes of puberty have been accomplished. There is acceptance of some increased autonomy (e.g., driver's permit and license are allowed), but the individual still lives at home. The individual emotionally moves away from the family and is less influenced by his or her peer group than are early adolescents; friendships assume an increasingly important role.

Late adolescence starts at the senior year of high school and is the age of acceptance of adult roles in work, family, and community. If the individual attends college, this stage is prolonged.

Behavior and Normal Puberty

Almost 100 years ago, G. Stanley Hall, without using what would be considered contemporary research techniques, characterized the maturing child as experiencing *Sturm und Drang* (i.e., storm and stress), which is normally restrained by cultural influences.[245] Contrary to this view, most recent empiric studies describe adolescent development as a continuous, adaptive phase of emotional growth characterized more by stability than disorder and more by harmonious relationships between generations than conflict. Although mood changes are normally more rapid (occurring over hours or days) and more clearly marked in teenagers than in adults, these shifts must be differentiated from long-standing mood and behavior changes associated with serious psychopathology.

Turmoil, or truly tumultuous behavior, in adolescence is not a normal phase but may reflect psychopathology that requires diagnosis and treatment. It is often misdiagnosed as a temporary problem of adjustment reactions of adolescence. When the conditions extend from adolescence to the adult stage, they are more severe. A 4-year longitudinal study of normal first-year US high school students showed that 25% experienced continuous growth, characterized by smooth, well-adjusted functioning despite stressful situations; 34% experienced surgent growth, demonstrating good adaptation in general and short periods of difficulty and distress after some stressful situations. Twenty-one percent were judged to be in turmoil, characterized by mood swings, anxiety, and depression; these teenagers mainly came from homes characterized by conflict, familial mental illness, and socioeconomic distress.[246] Many with adolescent turmoil had not "grown out of it" when studied 5 years later and eventually were diagnosed with unipolar and bipolar depressive disorders. It may be concluded that 80% to 90% of adolescents do well psychologically during puberty and are happy individuals, but 10% to 20% have significant difficulties.

Mood and Self-Image in Puberty

Young girls at the beginning of puberty frequently exhibit a negative self-image but a positive body image; positive peer relationships and superior adjustment improvement are observed with continued breast development. Mood in adolescence is not closely related to stage of puberty, but a significant curvilinear trend is seen for depressive affect (i.e., an increase followed by a decrease), for impulse control (a decrease followed by an increase), according to the level of serum estradiol. These data suggest that hormonal changes may be more important than physical changes as determinants of certain mood and behavior patterns during adolescence.

Behavior in Variations of the Normal Age at Onset of Puberty

Within the normal limits of pubertal development, early-maturing girls and late-maturing boys have the greatest prevalence of adjustment reactions in puberty and thereafter. Boys and girls who mature earlier are at increased risk for abuse.[247]

Early-developing boys are perceived to be more mature, attractive, and smart and are given more leadership roles; late-developing boys are more insecure, more susceptible to lower levels of self-esteem and body image, and more vulnerable to peer pressure, especially in working-class and minority groups. Most of these difficulties of late maturation focus on the decreased height of the individual rather than the lack of sexual development. Social maturation lags even after androgen treatment in patients with severe CDP. Delayed social maturation may put boys at risk for missing educational opportunities.

In contrast, early-maturing girls tend to experience more difficulty, especially in the junior high school setting, where they may attract the attention of older, more mature boys and have a higher prevalence of internalizing symptoms and disorders. They are more often the target of bullying but also more often the perpetrator of bullying than average-maturing girls.[248]

Early puberty may lead to negative body image in girls, compared with boys, in whom the effect is positive. Early maturation may increase a propensity to violent behavior, which is fostered by living in a disadvantaged neighborhood. Early pubertal maturation in girls may be related to a small intelligence quotient (IQ) advantage over late-maturing girls. Late-maturing girls are often more comfortable, remaining with the support of their families longer, and they are less often brought to medical attention than late-maturing boys. Early-maturing and late-maturing girls have a tendency to engage in health-risking behaviors involving strategies to lose weight, strategies to increase muscle, disordered eating, use of food supplements and steroids, and exercise dependence—tendencies not found in boys at the same developmental stages.

Risk-Taking Behavior

Adolescents who function at lower levels of cognitive complexity or concrete thinking and have an early onset of puberty demonstrate an increase in risk-taking behavior. The age at onset of cigarette smoking and alcohol use is proportional to the age at onset of puberty in girls: early-maturing girls partake earlier, and boys may follow the same pattern.

Sexuality During Puberty

The social pressures are more mixed in their messages to girls, both encouraging sexuality and restricting it in a way more disparate than that encountered by boys. The earlier onset of puberty today compared with previous centuries has had a profound effect on societal norms of sexual behavior.[4] For example, early onset of puberty is associated with earlier onset of sexual intercourse.[249]

The percentage of adolescents who have had sex rises from less than 2% by 12 years of age, 16% by age 15, 33% by 16, 48% by 17, 61% by 18, and 71% by 19-year-olds for either sex. Teenagers are waiting longer to have sex than they did in the recent past. Factors such as the onset of puberty, weak self-concept, having tried smoking or drinking, and not being overweight were significantly associated with early sexual activity in girls. For boys, older age, a poor relationship with parents, low household income, and having tried smoking were factors significantly associated with sexual activity. Add Health revealed that adolescents at the upper and lower ends of the intelligence distribution were less likely to have sex.

Fertility is reached well before adult phenotype is acquired in boys and girls. The number of pregnancies for US teenagers 15

to 19 years old decreased almost 50% since 1991 and 22% since 2009; it was 31.3 per 1000 women in 2011.[250] However, there is an ethnic-specific difference as the rate for Hispanic was 49.6 per 1000, non-Hispanic black was 47.3, American Indian/Alaska Native (AIAN) was 36.1, non-Hispanic white was 21.7, and American Pacific Islanders (API) was 10.2. The decline is attributed to pregnancy prevention messages, and there is evidence of increased use of contraception.

Sexuality appears to be correlated with testosterone production in boys in some studies, but in others it appears to be modified by the social effects of pubertal maturation. Religious activity may decrease the likelihood of sexual activity.

A randomized, double-blind, placebo-controlled, crossover clinical trial of boys and girls with delayed puberty addressing the effects of administration of oral conjugated estrogen to girls and testosterone enanthate to boys at three dose levels that were intended to simulate early, middle, and late pubertal levels demonstrated modest or no effects on sexual behavior. Boys had increased nocturnal emission and touching behaviors at the middle and high doses but no other effects. Girls demonstrated a significant increase in necking related to the administration of estrogen only at the late pubertal dose and no other effects.

Hormonal and Metabolic Changes in Puberty

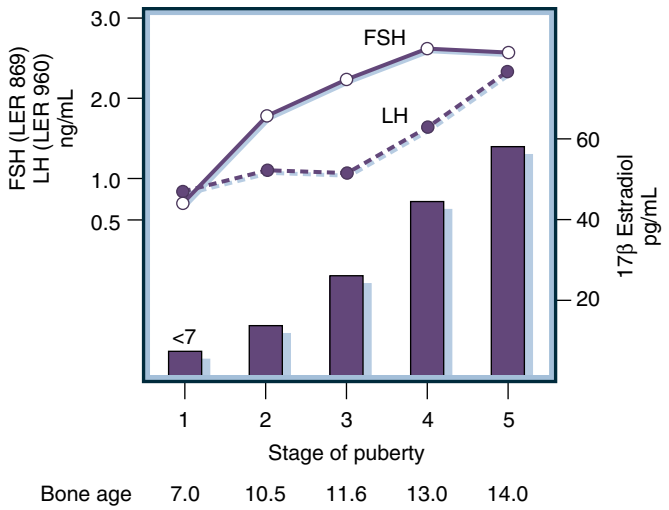
Increased amplitude and alterations of the patterns of GnRH secretion at puberty initiate and regulate the sequential increases in secretion of pituitary gonadotropins and gonadal steroids that culminate in fertility.

Gonadotropins

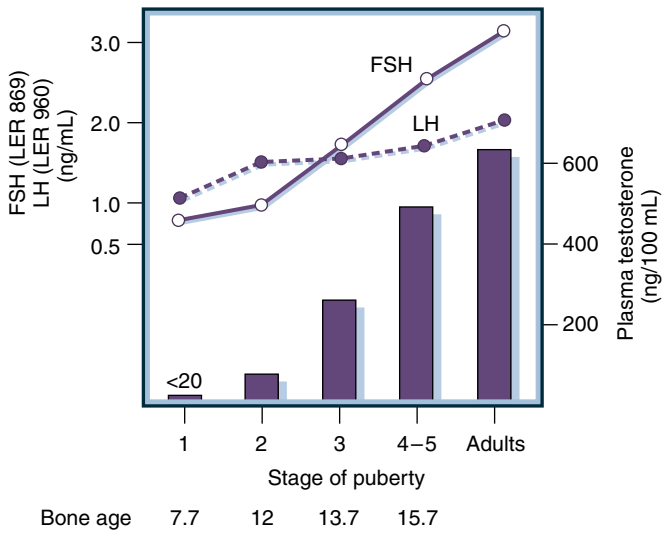
Because of the pulsatile secretion of GnRH, gonadotropin secretion is also episodic. The newer immunometric supersensitive assays allow accurate measurement in small pediatric samples. The results are lower than previously reported by the older assays.

During the first 2 years after birth, plasma levels of LH and FSH rise intermittently to adult values (the "mini puberty of infancy") and occasionally higher but then remain low until puberty. In healthy Danish girls below 3 years of age LH increases up to 9.2 IU/L after GnRH test with lower values after 3 years.[251] Ultrasensitive assays and third-generation assays for LH and FSH confirm earlier evidence of pulsatile secretion of the gonadotropins in prepuberty and indicate that the basal immunoreactive levels of LH are much lower than previously reported.[252,253] The serum FSH level is higher than the LH level in prepubertal boys and girls.[254] There is a more striking rise in serum LH amplitude by at least 1 year before the onset of puberty (i.e., the peripubertal period immediately preceding the appearance of signs of sexual maturation) that reaches an early plateau, whereas FSH rises more consistently through male puberty rather than before, with increased pulse amplitude. An increased amplitude of LH and FSH secretion occurs at night in prepubertal boys and girls by 5 years of age; the amplitude and frequency of such peaks increase, and daytime secretion increases with the progression of pubertal development.

In girls, FSH levels rise during the early stages of puberty, and LH levels tend to rise in the later stages; from beginning to late puberty, the LH concentration rises more than 100-fold (Figs. 26.19 and 26.20). Disorderly patterns of secretion of LH, but not FSH, were noted just before the onset of puberty; this was followed by, first, increased orderliness in early puberty and,



• **Fig. 26.19** Mean plasma estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) concentrations in prepubertal and pubertal females by pubertal stage of maturation (1, prepubertal; 5, menstruating adolescents) and the mean bone age for each stage. Single daytime values of gonadotropins have limited usefulness because of pulsatility of gonadotropin release and the increased amplitude of LH pulses during sleep throughout puberty. The gonadal steroid values, however, are useful in determining the stage of pubertal development. To convert FSH values (LER-869) to international units per liter, multiply by 8.4. To convert LH values (LER-960) to international units per liter, multiply by 3.8. To convert estradiol values to picomoles per liter, multiply by 3.671. (From Grumbach MM. Onset of puberty. In: Berenberg SR, ed. *Puberty, Biologic and Social Components*. Leiden, The Netherlands: H.E. Stenfert Kroese; 1975:1–21. Reprinted by permission of Kluwer Academic Publishers.)



• **Fig. 26.20** Mean plasma testosterone (after solvent extraction and chromatography) and gonadotropin levels in normal boys by stage of maturation (1, prepubertal) and mean bone age for each stage (see Fig. 26.19). To convert testosterone values to nanomoles per liter, multiply by 0.03467. (From Grumbach MM. Onset of puberty. In: Berenberg SR, ed. *Puberty, Biologic and Social Components*. Leiden, The Netherlands: H.E. Stenfert Kroese; 1975:1–21. Reprinted by permission of Kluwer Academic Publishers.)

then, increased disorderliness again in later puberty. This suggests that a more integrated feedback system operates in early puberty and is then followed by less stability.[255]

Doses of exogenous GnRH that are relatively ineffective in stimulating gonadotropin or gonadal steroid secretion before puberty become effective with the onset of puberty; an amplification occurs in the hypothalamic-pituitary-gonadal axis with progression of puberty.[256] Although the GnRH or GnRH agonist test usually requires multiple sampling after the administration of GnRH, a single determination at 60 or 24 minutes now suffices with the new, sensitive assays.[257–260] The basal values of serum LH and FSH measured in modern supersensitive assays are reported to predict the onset of pubertal development as well as GnRH testing does; a value of serum LH greater than 4 mIU/mL measured by immunochemiluminometric assay (ICMA) is consistent with the onset of puberty. Moreover, the use of these ultrasensitive assays to determine concentrations of LH and FSH in urine reveals a pattern of a 5-fold rise in urinary FSH in boys and girls, with a 50-fold rise in urinary LH in boys and a 100-fold rise in girls during puberty.[261]

In addition to the well-defined quantitative changes that occur in the pattern of FSH and LH in the pituitary gland, serum, and urine during development, qualitative changes occur. The pattern of glycosylation of the α-subunit and β-subunit of the gonadotropins is influenced by maturation, GnRH secretion, and the effects of gonadal steroids on the pituitary gonadotrophs. Variation in glycosylation that affects the size and charge of the hormone is the principal cause of the heterogeneity of FSH and LH and the large number of isoforms that varies according to the more acidic or more basic charge.[262] This pleomorphism has an important effect on biologic half-life and biologic activity and provides an additional mechanism of regulating the biologic activity of the gonadotropins. Discrepancies between serum bioactivity and immunoactivity of LH during pubertal development have been reported by some but not all researchers. However, a change in the isoforms of FSH released during puberty may favor the secretion of increased bioactive FSH, which may favor reproductive development.[262]

Gonadal Steroids

Only recently has it been appreciated that many actions on linear skeletal growth, skeletal maturation, and accretion of bone mass thought to be due to testosterone in the male are mainly attributable to its peripheral aromatization to estrogen (Table 26.7).

Testosterone

The Leydig cells of the testes produce testosterone and, in lesser amounts, androstenedione, α5-androstenediol, dihydrotestosterone, and estradiol, although a small amount of testosterone is derived from extraglandular conversion of androstenedione secreted by the testes and the adrenal. In the female, extraglandular conversion of ovarian and adrenal androstenedione accounts for almost all of the circulating testosterone.

Previous methods of determination of low levels of sex steroids have been demonstrated to be inaccurate.[263,264] This insensitivity is mainly due to the presence of interfering substances and relative insensitivity of antibodies used in assays. Now, larger national laboratories are beginning to use HPLC-MS/MS, which allows the accurate measurement of extremely low values present in pediatric samples.[265] These newer techniques may lead to revision of some of the results (described here) obtained by older methods. Measurement of testosterone and estradiol by HPLC-MS/MS in laboratories with pediatric standards is the preferred method to measure gonadal steroids to investigate disorders of puberty.

Prepubertal boys and girls have plasma testosterone concentrations of less than 0.3 nmol/L except during the first 3 to 5 months of infancy in the male, when pubertal levels are found. Nighttime elevations of serum testosterone levels are detectable in the male by 5 years of age, before the onset of physical signs of puberty, and increase during early puberty after the appearance of sleep-entrained secretion of LH and increased pituitary sensitivity to GnRH. The 60-minute lag between the peak of LH and the increase in testosterone is presumably due to synthesis and secretion of the steroid. In the daytime, increases in testosterone levels are detectable after the testis volume is greater than 4 mL, with a consistent increase throughout puberty. The steepest increment in testosterone concentration occurs between pubertal stages 2 and 3 in males (see Fig. 26.20 and Table 26.5). The ratio of testosterone to epitestosterone in the urine, which is used to evaluate doping of athletes, may be elevated normally during puberty.

TABLE 26.7 Early Clinical Clues to the Effect of Estrogen on Growth and Skeletal Maturation in the Male

Complete androgen insensitivity (resistance) syndrome (Zachmann M, Prader A, Sobel EH, et al. Pubertal growth in patients with androgen insensitivity: indirect evidence for the importance of estrogen in pubertal growth of girls. *J Pediatr.* 1986;108:694–697.)
Short-term estradiol administration increased rate of ulnar growth in prepubertal boys (Caruso-Nicoletti M, Cassorla FG, Skerda MC, et al. Short term, low dose estradiol accelerates ulnar growth in boys. *J Clin Endocrinol Metab.* 1985;61:896–898.)
Aromatase inhibitor decreased rapid growth and skeletal maturation in testotoxicosis, whereas antiandrogen had no effect on skeletal maturation (Laue L, Jones J, Barnes K, et al. Treatment of familial male precocious puberty with spironolactone and deslorelin. *J Clin Endocrinol Metab.* 1993;76:151–155.)
Aromatase excess syndrome in boys associated with increased rate of growth and skeletal maturation, elevated plasma estrogen concentrations, but prepubertal testosterone values (Stratakis CA, Vottero A, Brodie A, et al. The aromatase excess syndrome is associated with feminization of both sexes and autosomal dominant transmission of aberrant P450 aromatase gene transcription. *J Clin Endocrinol Metab.* 1998;83:1349–1357.)
Estrogen-secreting tumors: adrenal and testicular neoplasms (especially Peutz-Jeghers syndrome) (Bulun SE, Rosenthal IM, Brodie AM, et al. Use of tissue-specific promoters in the regulation of aromatase cytochrome P450 gene expression in human testicular and ovarian sex cord tumors, as well as in normal fetal and adult gonads. *J Clin Endocrinol Metab.* 1993;77:1616–1621.)

From Grumbach MM. Estrogen, bone, growth, and sex: a sea change in conventional wisdom *J Pediatr Endocrinol Metab.* 2000;13:S1439–S1455.

Free testosterone measurements may be determined by dialysis or by calculation using testosterone values and available protein binding sites; low accuracy of the testosterone assay can make free testosterone measurements inaccurate.[265] Nonetheless, if the total testosterone concentration on which the free testosterone level is based is measured by a highly specific assay, free or bioavailable testosterone determinations are helpful in evaluation of PCOS or nonclassic congenital adrenal hyperplasia (CAH) in girls.

A sensitive mammalian cell recombinant bioassay for androgen bioactivity strongly correlates with serum immunoreactive testosterone concentration and demonstrates a rise with pubertal development in concert with progression of pubic hair and penile development in patients with CDP.[266] In contrast to this specific bioassay, a novel, highly sensitive transcriptional androgen receptor (AR)–mediated bioassay system demonstrated higher circulating values of bioactive androgen in menopausal women and may be directed toward children in the future.[267]

The values of sex steroids measured in saliva are much lower than in the serum, but trauma (even tooth brushing) that leads to blood in the specimen can influence the results, and the accuracy of the basic assay is critical (see earlier discussion).[265] The steroid level in saliva is not a direct representation of free steroid in the serum, as is often claimed. Testosterone in saliva is said in some reports to correlate well with serum levels of testosterone in normal subjects and in patients with chronic disease (e.g., cystic fibrosis). Salivary progesterone is said to rise with the progression of puberty. Salivary DHEA is higher after the onset of puberty than before. Salivary steroid measurements, if accurate, can increase the ability of investigators to address the relationship between development and behavior in a noninvasive manner, but it may take the use of LC/MS-MS in salivary assays to achieve such accuracy.

Estrogens

In the female, estradiol is secreted principally (90%) by the ovary; a small fraction of circulating estradiol arises from the extraglandular conversion of testosterone and androstenedione. In the male, approximately 75% of estradiol is derived from extraglandular aromatization of testosterone and (indirectly) androstenedione, and 25% is from testicular secretion.

In the fetus and at term, estrogen is high due to conversion of fetal and maternal adrenal C19-steroids to estrogen by the placenta, but they drop precipitously during the first few days of life. Plasma estradiol levels are so low in prepuberty that detection by standard immunoassays is difficult, but a rise through puberty and a diurnal rhythm is seen with a sensitive radioimmunoassay (Table 26.8; see Fig. 26.19). Estrone levels rise early and reach a plateau by midpuberty. A highly sensitive bioassay demonstrated higher estradiol concentrations in girls than in boys before puberty, with a rise through puberty until the pubertal growth spurt and a decrease thereafter. Today, estrogen measurements by high-performance liquid chromatography (HPLC) tandem mass spectroscopy is preferred. There is a significant correlation between peak growth velocity and the rise in estradiol concentration; the rise is earlier in girls than in boys, but bioactive estradiol levels are equivalent at peak growth velocity.[160,268] The higher estrogen levels in girls may be an important factor in the more advanced levels of skeletal maturation in girls compared with boys and may play a part in their earlier onset of sexual maturation. A human cell bioassay measuring total estrogenic bioactivity (rather than estradiol alone) in children has an extremely sensitive detection limit of less than 1 pg/mL.[269]

The daily peak of estradiol in early pubertal girls occurs about 6 to 9 hours after the peak of serum LH detected during the night, apparently related to time required for synthesis. In all stages of puberty, boys have higher concentrations of estrone than estradiol, and levels of both estrogens are lower than those measured in girls at comparable stages.

Protein Products of the Gonads

Inhibin, Activin, and Follistatin

Inhibin, a heterodimeric glycoprotein product of the Sertoli cell of the testes and the ovarian granulosa cell (as well as the placenta and other tissues), exerts negative feedback action on the secretion of FSH from the pituitary. Inhibin is composed of an α-subunit and one of two β-subunits, β_A or β_B, which, respectively, form inhibin A or inhibin B, dimers with apparently identical function. Inhibin is a member of the transforming growth factor-β (TGFβ) superfamily that includes antimüllerian hormone (AMH) and the dimers of two inhibin subunits, activin A and activin B, which stimulate the release of FSH from pituitary cells.[270] Synthesis and secretion of gonadal inhibin are induced by FSH. Inhibin plays a role in the feedback regulation of FSH secretion during puberty in males and females.

Like inhibin, follistatin inhibits, whereas activin stimulates, FSHβ-subunit expression and therefore affects FSH biosynthesis and secretion. Inhibin may also be an inhibitor of LH release in the follicular phase.[271] These hormones are synthesized in a variety of tissues in addition to the gonads and have diverse activities apart from those on the reproductive apparatus.

Two distinct binding proteins for inhibin and activin are present in the circulation, the gonads, and other tissues: α_2-macroglobulin, a high-capacity, low-affinity binding protein; and follistatin, a glycosylated, single-peptide chain that functions as a high-affinity binding protein and as a regulator of activin bioactivity (e.g., in the pituitary gland, a site of synthesis of activin and follistatin).

During pregnancy, the placenta secretes inhibin A, and the fetal membranes secrete inhibin A and inhibin B, but for at least the first 20 weeks of gestation, only inhibin A is detected in maternal serum. In umbilical cord serum from term female newborn infants, no inhibin dimer was detected, whereas cord serum from male newborns contained inhibin B, the only inhibin detected in adult males.[272] In the human fetal testis, α-subunit and β_B-subunit (but not β_A-subunit) were present in Sertoli and Leydig cells at 16 weeks of gestation; by 24 weeks of gestation, immunoexpression of both subunits was greater in the Sertoli cells. Postnatally the expression of both subunits was decreased by 4 months of age. Inhibin subunits were not detected in the fetal ovary, nor was immunoreactive follistatin present in fetal or neonatal gonads.[273]

In large, cross-sectional studies using highly specific inhibin B and inhibin A immunoassays that correlate with the bioactivity of inhibin and distinguish inhibin B from inhibin A, the mean concentration of serum inhibin B in males increased between prepuberty (a stage when it is higher than the undetectable levels in castrated men)[274] and the first stage of puberty; when the strong

TABLE 26.8 Plasma Gonadal Steroid Values in Children

Steroid and Assay	Age	NORMAL VALUES		Sample Volume (Pediatric Minimums)
		Males	Females	
Testosterone, by LC-MS/MS (ng/dL)	Term infant	75-400	20-64	0.18 mL serum (Quest) 0.5 mL serum (Esoterix) 0.15 mL serum (ARUP)
	1-7 mo	Levels decrease rapidly in first week to 20-50, then increase to 60-400 between 20 and 60 days; levels then decline to prepubertal range by 7 mo	Levels decrease during the first month to <10 and remain at that level until puberty	
	7-12 mo	<16	<11	
	Tanner stage I	<16	<16	
	Tanner stage II	<167	<40	
	Tanner stage III	7-762[a]	<60	
	Tanner stage IV	25-912	<62	
	Tanner stage V	110-975	<68[a]	
Androstenedione, by RIA after extraction (ng/dL)	Term infant	20-290; levels decrease to 10-80 after 1 week	20-290 ng/dL; levels decrease to 10-80 after 1 week	0.25 mL serum (Esoterix) 0.5 mL serum (Quest)
	1-11 mo	6-68	6-68	
	Prepubertal	8-50	8-50	
	Tanner I	8-50	8-50	
	Tanner II	31-65	42-100	
	Tanner III	50-100	80-190	
	Tanner IV	48-140	77-225	
	Tanner V	65-210	80-240	
DHT, by extraction chromatography, RIA (ng/dL)	1-6 mo	12-85	<5	0.5 mL serum (Esoterix) 1.1 mL serum (Quest)
	Prepubertal	<5	<5	
	Tanner II-III	3-33	5-19	
	Tanner IV-V	22-75	3-30	
Estradiol, by LC-MS/MS (ng/dL)	Newborn	Levels are markedly elevated and fall during first week to <1.5	Levels are markedly elevated and fall during first week to <1.5	1.2 mL serum (Esoterix)
	1-11 mo	Levels increase to 1-3.2 between 1 and 2 mo, then decrease to <1.5 by 6 mo	Levels increase to 0.5-5 between 1 and 2 mo, then decrease to <1.5 during the first year	
	Prepubertal	<1.5	<1.5	
	Tanner I	0.5-1.1	0.5-2	
	Tanner II	0.5-1.6	1-2.4	
	Tanner III	0.5-2.5	0.7-6	
	Tanner IV	1-3.6	2.1-8.5	
	Tanner V	1-3.6	3.4-17	
Estradiol, by extraction chromatography, RIA (ng/dL)	Tanner stage I	0.3-1.5	0.5-1	0.6 mL (Quest)
	Tanner stage II	0.3-1	0.5-11.5	
	Tanner stage III	0.5-1.5	0.5-18	
	Tanner stage IV	0.3-4	2.5-34.5	
	Tanner stage V	1.5-4.5	2.5-41	

TABLE 26.8 Plasma Gonadal Steroid Values in Children—cont'd

Steroid and Assay	Age	NORMAL VALUES Males	NORMAL VALUES Females	Sample Volume (Pediatric Minimums)
Estradiol, chemiluminescent immunoassay (ng/dL)	0-8	0.7-0.8	0.7-1.4	0.2 mL serum (ARUP)
	9-10	0.7-1.1	0.7-3.2	
	11-12	0.7-2.2	0.7-3.8	
	13-14	0.7-2.4	1-9.1	
	15-16	1.1-3.3	1.7-18.1	
	17-40	1.8-6.7	2.3-17.0	
Extrone, by LC-MS/MS (ng/dL)		Levels are markedly elevated at birth, then decrease during first week to <1.5	Levels are markedly elevated at birth, then decrease during first week to <1.5	1.2 mL serum (Esoterix)
	Prepubertal	<1.5	<1.5	
	Tanner I	0.5-1.7	0.4-2.9	
	Tanner II	1.0-2.5	1-3.3	
	Tanner III	1.5-2.5	1.5-4.3	
	Tanner IV	1.5-4.5	1.6-7.7	
	Tanner V	2-4.5	2.9-10.5	

[a]Because this chart combines values from different laboratories, the ranges are larger in the aggregate than would be found in the specific laboratories' standards. Please consult the laboratory being used to interpret results for clinical decisions.

DHT, Dihydrotestosterone; *LC-MS/MS,* liquid chromatography tandem mass spectrometry; *RIA,* radioimmunoassay.

From Albrecht L, Styne D. Laboratory testing of gonadal steroids in children. *Pediatr Endocrinol Rev.* 2007;5:S599–S607.

correlation with chronologic age was taken into account, a correlation with LH and testosterone values remained. From genital stage 2 of puberty on, inhibin B levels were relatively constant, despite a rise in the mean concentration of serum FSH between stages 2 and 3, after which the FSH value was relatively unchanged. The rise in inhibin B is mirrored by a drop in AMH in early puberty, apparently reflecting Sertoli cell maturation.[275] By genital stage 3, a negative partial correlation between inhibin B and FSH was found that persisted as puberty advanced, and by genital stage 4, there was a clear negative correlation of inhibin with serum FSH. Dimeric inhibin B rises twice during development, reflecting the two periods of Sertoli cell proliferation in infancy and in early puberty, whereas an inverse relationship between inhibin and FSH is seen at midpuberty and thereafter, indicating the development of negative feedback inhibition. In the early stages of puberty, inhibin B values are closely related to LH and testosterone levels, but in stage 3, when inhibin B values peak, this relationship is lost, and inhibin B values become more closely related to FSH levels.[276]

Serum levels of inhibin A and B increase early in puberty in girls, although there are individual increases in the prepubertal period directly related to FSH levels, demonstrating sporadic follicular development in the infant and child due to FSH stimulation. Inhibin B is predominant in the follicular phase, as is inhibin A during the luteal phase. Inhibin A and inhibin B peak in midpuberty, and inhibin B is thereafter decreased. During the early stages of puberty, inhibin B values are related to estradiol and FSH values, but these relationships diminish with the progression of puberty.[277] Although there is no significant change in activin during female puberty, follistatin decreases from a midpuberty peak to later values that fall below prepubertal values.

Serum values of FSH-regulating proteins follow circadian patterns, with higher values of LH and FSH overnight, just after a nadir of inhibin B. Follistatin concentrations were found to reach their greatest value in early morning, and activin A concentrations declined coincident with the nighttime increase in FSH levels in pubertal girls.[278] Diurnal variation of inhibin B in boys in the peripubertal or early pubertal period demonstrates a fall in inhibin during the night as LH and, subsequently, testosterone rise, showing the negative feedback effect of testosterone on inhibin B secretion.[279] Recombinant FSH treatment, which raises testosterone secretion, suppresses inhibin B, demonstrating the ability of testosterone to negatively influence inhibin B secretion.[280] Administration of a GnRH agonist led to an increase in the FSH level by 30 minutes as well as an increase in the inhibin B level in girls older than 5 years of age by 8 hours and in boys by 20 hours.[281] The baseline inhibin B concentration was greater in boys than in girls, baseline activin A concentrations were greater in girls, and activin did not change with GnRH administration. Testosterone administration to boys in Tanner stage 2 led to decreased FSH and LH, increased activin, and decreased inhibin B levels but caused no change in follistatin. Estradiol administered to girls in Tanner stage 1 or 2 led to decreased LH and

FSH and increased activin A levels but no change in inhibin B or follistatin concentrations, whereas administration of estradiol to girls with Tuner syndrome led to decreased serum levels of FSH, although the effectively nondetectable levels of activin and inhibin did not change.[282]

A low concentration of inhibin B in men and pubertal boys is an indicator of impaired seminiferous tubule function.[283] Early pubertal boys with testicular defects have higher FSH concentrations and low inhibin levels. Inhibin B is the form most closely related to testicular function, and it is absent in orchidectomized men. Inhibin B is related to Sertoli cell function in prepuberty, but a developmental change occurs during puberty so that later in life, inhibin B concentration is related to spermatogenesis. Prepubertal boys with the Sertoli cell–only syndrome had normal inhibin B levels, whereas postpubertal affected boys and men with Sertoli cell–only syndrome and early-stage spermatogenic arrest had undetectable or low levels of inhibin B, and those with late-stage spermatogenic arrest or obstructive azoospermia had normal or near-normal levels of serum inhibin B.[274] In prepuberty, the α and the β_B inhibin subunits are expressed in Sertoli cells, but during puberty and in men, fully differentiated Sertoli cells express only the α-subunit; the β-subunit is expressed in germ cells. Inhibin B in the adult appears to be a product of germ and Sertoli cells. In prepubertal boys, basal plasma inhibin B concentrations have a high correlation with the incremental testosterone response to administration of human chorionic gonadotropin (hCG), and they provide a useful assessment of the presence of testes and their function.[284]

Antimüllerian Hormone

AMH (also called müllerian inhibiting substance [MIS] or factor [MIF]), a 14-kDa homodimeric glycoprotein that is structurally related to the subunit of inhibin and TGFβ, is produced by the Sertoli cells of the fetal testis after 7 weeks and the prepubertal testis and causes the regression of the müllerian ducts in boys during early fetal development and later in gestation by granulosa cells of the fetal ovary. AMH production is first stimulated by SOX9, SF1, GATA4, and WT1 before FSH rise but later is stimulated by FSH.[285] Immunoassayable concentrations of AMH values rise from birth to relatively high levels during the first year of life in males, decrease by age 10 years, and decrease further during puberty.[286] Newborn females have low or nondetectable serum levels of AMH, which rise only slightly thereafter; serum AMH concentrations are virtually nondetectable in most girls just before puberty. Serum levels of AMH and of estradiol after GnRH analogue stimulation are increased in low-birth-weight and high-birth-weight female infants, suggesting altered follicular development.[287] Increased poststimulation FSH levels and low adiponectin concentrations are observed only in high-birth-weight infants, indicating that altered ovarian function occurs by a different mechanism than that found in low-birth-weight infants.

Serum levels of AMH and inhibin B are inversely related to androgen concentrations in pubertal boys,[275] and values in boys with CPP are appropriate for pubertal stage rather than chronologic age.[286] Elevated serum AMH concentrations occur in androgen resistance. Treatment with recombinant FSH and hCG in hypogonadotropic hypogonadism increases testosterone and decreases the elevated levels of serum AMH (due to immature Sertoli cells) and inhibin B, further demonstrating this relationship.[280] AMH is slightly higher in boys with delayed puberty than in pubertal age-matched control subjects and lower in those with testicular dysgenesis associated with impaired virilization than in normal boys. Boys with isolated cryptorchidism have normal values of AMH, and AMH and inhibin B are absent in anorchia, allowing a differential diagnosis during the first month after birth.[288] Dysgenetic testes secrete only low serum AMH levels; the testosterone response to hCG indicates the presence of testicular tissue.[289]

AMH is elevated in girls with PCOS and in girls with oligomenorrhea without classic AMH. This finding suggests that oligomenorrheic adolescents may have increased antral follicle number, similar to that observed in girls with PCOS.[290] AMH is a useful gonadal tumor marker because values are elevated in males with primitive Sertoli-like tumors and in girls and women with granulosa cell tumors.

Adrenal Androgens

A progressive increase in plasma levels of Δ^5-steroids, DHEA, and DHEAS in boys and girls begins before age 8 (skeletal age of 6–8 years) and continues through early adulthood (Table 26.9). The increase in the secretion of adrenal androgen and its precursors is known as *adrenarche,* and the appearance of pubic hair caused by adrenarche is known as *pubarche*. Plasma DHEA levels have a diurnal rhythm similar to that of cortisol, but plasma levels of DHEAS show less variation and are a useful biochemical marker of adrenarche.

TABLE 26.9 Mean Serum Concentrations (mmol/L [ng/mL]) of DHEAS During Childhood

	6-8 yr	8-10 yr	10-12 yr	12-14 yr	14-16 yr	16-20 yr
By Chronologic Age						
Boys	0.5 (188)	1.6 (586)	3.4 (1260)	3.6 (1330)	7.2 (2640)	7.2 (2640)
Girls	0.8 (306)	3.2 (1170)	3.1 (1130)	4.6 (1690)	6.9 (2540)	6.3 (2320)
By Bone Age						
Boys	0.98 (360)	1.6 (574)	3.4 (1250)	5.8 (2150)	10.9 (4030)	
Girls	0.73 (276)	3.1 (1130)	4.33 (1560)	7.1 (2610)	3.9 (1450)	

DHEAS, Dehydroepiandrosterone sulfate.

Modified from Reiter EO, Fuldauer VG, Root AW. Secretion of the adrenal androgen, dehydroepiandrosterone sulfate, during normal infancy, childhood, and adolescence, in sick infants, and in children with endocrinologic abnormalities. *J Pediatr*. 1977;90:766–770.

DHEA is the predominant precursor to more potent androgens in females, but DHEAS cannot be converted. DHEAS is produced from DHEA by the action of the sulfotransferase enzyme SULT2A1, mainly in the adrenal glands and the liver. The sulfate donor phosphoadenosine phosphosulfate (PAPS) is required by SULT2A1, and in human beings, PAPS is synthesized by the two isoforms of PAPS synthase, PAPSS1 and PAPSS2.[291]

Testosterone-Binding Globulin

Between 97% and 99% of circulating testosterone and estradiol is reversibly bound to testosterone-binding globulin (TeBG) (or sex steroid–binding globulin [SHBG]); prepubertal levels of TeBG are approximately equal in boys and girls, but a decrease in TeBG level occurs with advancing prepubertal age and the concomitant increase in the plasma gonadal steroid levels.[292] At puberty, there is a small decrease in TeBG levels in girls; as a consequence of testosterone, there is a greater decrease in boys, although the drop observed in normal boys is attenuated by treatment with tamoxifen, even with advancing pubertal development. The rise in adrenal androgen levels at adrenarche may explain the early drop in TeBG levels, which allows more circulating free hormone at a given concentration of testosterone. Although the plasma concentration of testosterone is 20 times greater in men than in women, the concentration of free testosterone is 40 times greater. Boys with hypogonadotropic hypogonadism and patients with the androgen resistance syndrome show the same characteristic fall in TeBG levels at puberty, but values are intermediate between those of normal adult males and females.

Prolactin

Prolactin levels rise in girls during puberty. Prepubertal mean (± standard error) plasma prolactin concentrations are less than 10 μg/L in boys and 3 to 12 μg/L in girls. Late pubertal girls and adult women have higher concentrations of prolactin (3–20 μg/L and 3–20 μg/L, respectively), whereas the mean concentration in adult men is 2 to 18 μg/L.[293] This sex difference is probably a consequence of the higher estradiol levels during puberty in girls and in women.

Insulin-Like 3 Protein

During puberty, serum levels of insulin-like 3 (INSL3), a protein produced by the Leydig cells, rise in normal boys under LH stimulation and in those in whom increased secretion is induced by letrozole treatment.[294,295] Values do not increase in Klinefelter syndrome, in which the initial rise levels off during midpuberty.[295] INSL3 is said to be as sensitive as testosterone as an indication of Leydig cell function.[296]

Prostate-Specific Antigen

Prostate-specific antigen (PSA) is detectable in male and female cord blood and in the serum of infants, but PSA concentrations decrease to undetectable levels during childhood. PSA concentrations rise to the measurable range with the onset of puberty in the male and correlate with the progression of pubertal stage, the size of the testes, serum LH and testosterone concentrations, and, presumably, the size of the prostate.[297,298] PSA values are increased to the pubertal range in boys with idiopathic CPP, and they decrease with GnRH agonist treatment.

Hormonal Control of the Pubertal Growth Spurt

Postnatal growth follows a specific pattern: An extremely high growth rate just after birth is followed by a deceleration that continues until 3 years of age; next, there is a slower phase of deceleration until puberty. The subsequent pubertal growth spurt, the second greatest period of postnatal growth, is followed by maturation of the spine and long bones until adult height is reached.[151] Many factors influence the growth plate.[299] The adolescent growth spurt in normal girls and boys depends on estradiol and GH levels, among other factors.

Hormonal control of the pubertal growth spurt is complex (see Figs. 26.15 and 26.16). GH is involved in increasing growth at puberty through stimulation of IGF1 production. Gonadal steroids have two effects on pubertal growth: (1) induction of an increase in GH secretion, with a consequent increase in IGF1 production, thereby indirectly stimulating pubertal growth; (2) a direct effect on cartilage and bone through stimulation of local production of IGF1 and other local factors.[160,300]

Gonadal Steroids[159,160]

In the developing human skeleton, gonadal steroids have growth-promoting and maturational effects on chondrocytes, osteoblasts, and other bone constituents.[159,160] This action, which eventually leads to epiphyseal fusion and the cessation of longitudinal growth in boys and girls, is mediated mainly by estrogen that is directly secreted (in girls) or arises from the conversion of testosterone and androstenedione to estrogen in peripheral tissues by aromatase (see Table 26.8). Detection of estrogen resistance resulting from a null mutation in the gene encoding the estrogen receptor and from derangements in the *CYP19A1* gene, leading to severe cytochrome P450 aromatase deficiency, has highlighted the cardinal role of estradiol (but not testosterone) in both boys and girls in the pubertal growth spurt, completion of epiphyseal maturation, and normal skeletal proportions and mineralization. Individuals with a mutation in the ERα gene *(ESR1)* or the *CYP19A1* gene encoding aromatase continue to grow, lack a pubertal growth spurt, and have open epiphyses and osteopenia.[301–303] Estrogen treatment of men with aromatase deficiency leads to epiphyseal closure, cessation of growth, and a striking increase in bone mass.[304–306] Patients with aromatase excess, who produce excess estrogen, have advanced skeletal maturation and rapid growth and ultimately reach short adult stature.[307]

Although estradiol secreted by the ovary has long been recognized as the major sex steroid responsible for the pubertal growth spurt, skeletal maturation, and bone mineral accrual in females, until the detection of the rare human genetic defects in estrogen synthesis or action, conventional wisdom dictated that testosterone mediated these maturational changes during puberty in males. Now it is known that estrogen (not androgen) is the critical sex hormone in males and females in the pubertal growth spurt, skeletal maturation, accrual of peak bone mass, and maintenance of bone mass in the adult. Estrogen stimulates chondrogenesis in the epiphyseal growth plate, increasing pubertal linear growth.[270] At puberty, estrogen promotes skeletal maturation and the gradual progressive closure of the epiphyseal growth plate.[159] The use of a supersensitive assay for plasma estradiol in prepubertal and pubertal boys revealed a high positive correlation between estradiol concentrations and peak growth velocity (but not serum GH level), which was greatest about 3 years after the onset of puberty,[268]

further implicating estrogen in the pubertal growth spurt and skeletal maturation of boys and girls.

There are estrogen receptors, both ERα and ERβ, in the growth plate chondrocytes.[299] Histologic studies of the bone and cartilage of rodents treated with corticosteroids or estrogen and clinical evaluations of children with precocious puberty support the theory that senescence of the growth plate occurs because of estrogen exposure in precocious puberty, causing decreased growth during treatment with GnRH agonists.[308]

The high rate of bone turnover in early puberty followed by a decrease in periosteal apposition and endosteal resorption within cortical bone and decreased bone remodeling within cortical and cancellous bone mediated by apoptosis of chondrocytes in the growth plate and osteoclasts within cortical and cancellous bone is mediated in part by estrogen. This leads to a reduction in bone turnover markers at menarche, reflecting the closure of the epiphyseal growth plates.[309]

Girls with Turner syndrome without estrogen exposure retain elevated markers of bone turnover. Prepubertal girls with Turner syndrome tend to lose bone, but that ceases when estrogen therapy begins.[310] During puberty and into the third decade, estrogen has an anabolic effect on the osteoblast and an apoptotic effect on the osteoclast, increasing bone mineral acquisition in the axial and appendicular skeletons. Evolutionary theory suggests that positive effects of estrogen on bone density, added to mechanical loading, allow women to carry increased weight for pregnancy and lactation; this process is unnecessary after reproduction, and osteoporosis becomes more common at menopause.[311]

Testosterone may also have a direct action on bone in the human male, because ARs are found in human tibial growth plates in osteoblasts and chondrocytes, osteocytes, mononuclear cells, and endothelial cells of blood vessels in the bone marrow.[312] Androgens that cannot be aromatized to estrogen still cause an increase in growth rate, presumably due to interaction with these receptors. The greater increase in periosteal bone deposition, the resultant thickening of cortical bone and greater bone strength, and the greater bone dimensions in boys probably result from direct effects of testosterone. Androgens may protect men against osteoporosis by maintenance of cancellous bone mass and expansion of cortical bone.

A pubertal growth spurt leading to adult height close to that of genotypic men occurs in individuals with the complete form of androgen resistance, demonstrating the critical role of estrogen rather than androgen in the adolescent growth spurt in boys. A modest decrease in BMD Z-scores occurs in the spine but not in the hip based on age-specific female standard values, but the reductions are greater when male standards are used. Affected women have an increased prevalence of fractures, even with estrogen replacement. This suggests that lack of a direct effect of testosterone on the skeleton, especially the spine, has a part in the defects in bone mineralization observed in women with complete androgen insensitivity[313] (see Table 26.8).

Growth Hormone and Growth Factors

GH secretion approximately doubles during puberty in boys and girls in the basal state or after stimulation but decreases after pubertal development. Remarkably, peak values after hexarelin, a 6–amino acid GH-releasing peptide (or GH secretagogue) stimulates as much GH secretion in prepuberty as in puberty. The greater elevation in girls starts at an earlier age and pubertal stage than in boys due to the earlier onset of puberty in girls. GH secretion increases coincident with the onset of breast development (Tanner stage 2) and is maximal at Tanner stage 3 to 4 breast development; in boys, GH rises later and peaks at stage 4 genital development. GH secretion and IGF1 levels decrease after late puberty in both sexes. Adolescents of normal height have an inverse relationship between weight and GH levels. Increased GH pulse amplitude and content of GH secreted per pulse (but not frequency, metabolic clearance rate, or intersecretory burst interval and half-life of GH) in the basal state are mainly responsible for the augmented GH levels.[314]

The increase in estradiol at puberty, which in boys results from testicular secretion and extraglandular synthesis from testosterone and androstenedione and in girls from secretion by the ovaries, is the principal mediator of the increase in pulse amplitude and amount of GH secreted per pulse. Administration of exogenous androgens in delayed puberty raises GH secretion. Transdermal application of testosterone increases spontaneous GH secretion overnight independent of growth hormone–releasing hormone (GHRH), because infusion of GnRH antagonist does not affect this phenomenon.[315] The effect of testosterone is mediated mainly through its conversion to estradiol, because treatment of late pubertal boys with tamoxifen, an estrogen receptor blocker, causes smaller GH secretory peaks and fewer GH secretory episodes. Exogenous estrogen increases the peak GH reached after insulin-induced hypoglycemia, exercise, and arginine, a priming effect that is used in clinical practice, because estrogen administered before a provocative test in prepubertal subjects increases the GH response. Androgens that cannot be aromatized to estrogen (e.g., oxandrolone, dihydrotestosterone) have less effect on GH secretion; however, androgen blockade with flutamide increases GH secretion. Dihydrotestosterone, which is not aromatized to estrogen, does not increase GH secretion or the plasma concentration of IGF1 and may decrease the integrated GH secretion, but it still stimulates increased growth rate, suggesting a possible direct effect of androgen on pubertal growth independent of GH or estradiol.[300] Increased GH secretion also occurs in sexual precocity. GH secretion decreases with the fall in gonadal steroid levels after treatment of CPP with potent GnRH agonists.[316]

GH deficiency or GH resistance causes an attenuated pubertal growth spurt, indicating the importance of GH and IGF1 in this process. Severe primary or secondary hypogonadism leads to a minimal or absent growth spurt, demonstrating the primary role of gonadal steroids in pubertal growth. Hypopituitary patients deficient in GH and gonadotropins do not have an adolescent growth spurt when GH alone is replaced; gonadal steroids must also be given, substantiating the interaction of GH and gonadal steroids in the pubertal growth spurt. In normal puberty, neither the magnitude of the increase in GH secretion nor the concentration of plasma IGF1 correlates with the PHV of the pubertal growth spurt. Although a threshold level of GH secretion is necessary, the extent of the growth spurt correlates with gonadal sex steroid secretion. Individuals with both CPP and GH deficiency (usually as a consequence of cranial irradiation for a brain tumor) have a growth spurt clinically indistinguishable from that of CPP and normal GH secretion.[300] After treatment with a GnRH agonist for sexual precocity, growth velocity in patients with GH deficiency and CPP is decreased and pubertal progression is suppressed, illustrating the direct effect of gonadal steroids, principally estradiol, on the pubertal growth spurt.

Urinary GH excretion reflects serum levels and changes occurring with pubertal development. A peak is reached at pubertal stage 3 to 4. The level is higher in boys than in girls.

Growth Hormone–Binding Protein

Growth hormone–binding protein (GHBP) has the same amino acid sequence as the extracellular component of the GH receptor (GHR), and serum concentrations are directly related to the number of GHRs. In normal children, the plasma GHBP level is inversely related to 24-hour GH secretion. The serum GHBP level rises early in childhood and also through puberty in some cross-sectional studies, but not in others. Because plasma GHBP does not change appreciably with the onset of puberty, at the time of the pubertal growth spurt there is a relative increase in unbound (free) GH in relation to GH bound to GHBP. GHBP is related to adiposity, and it may be this factor that accounts for the increased levels of GHBP in girls compared with boys, the rise in GHBP in girls with precocious puberty, and the negative influence of testosterone on GHBP levels.[317]

Insulin-Like Growth Factor Type 1

Concentrations of IGF1 rise during puberty to levels higher than those of prepuberty or adulthood; they remain elevated past the time of PHV, with a peak attained 1 or 2 years after the pubertal growth spurt (later in boys than in girls) and then fall to normal adult levels.[316,318] The pattern of the GH-dependent serum levels of IGF-binding protein 3 (IGFBP3) in pubertal development is similar to that of serum[319] IGF1. However, serum IGFBP3 concentrations correlate with BMI even though IGF1 does not. Measurement of free IGF1 shows the same pattern of change with development as does that of total IGF1, a slow rise in serum free IGF1 in prepuberty followed by a steeper rise during puberty. A decrease of free IGF1 is associated with age in the later stages of puberty.[319,320] The increase in the serum ratio of IGF1 to IGFBP3 at the time of the pubertal growth spurt appears to result from production, because proteolysis of IGFBP3 does not change in puberty in normal children. The testosterone level in boys and the estradiol level in girls correlate with the rise in IGF1 concentration, but gonadal steroids are not the direct cause of the increase in circulating IGF1 levels; rather, GH secretion approximately doubles during puberty owing to the effect of estrogen causing augmented release of GH.

Plasma IGF1 concentrations are high for chronologic age in sexual precocity and low in delayed puberty. Estrogen mediates the pubertal increase in IGF1 concentration through increased secretion of GH, with an additional effect through the gonadal steroid–induced local generation of IGF1 in cartilage and bone. Treatment with GnRH agonist in a 16-year-old boy with a homozygous mutation in the WSXWS-like motif of the human GHR causing Laron syndrome led to a further decrease in the already low serum levels of IGF1 and IGFBP3, which did not reverse with dihydrotestosterone treatment, suggesting a direct effect of estradiol on IGF1 production.[299,321] Children with CPP treated with a GnRH agonist showed suppression of the untreated elevated serum GH concentrations and a decrease in plasma IGF1 concentrations, although not to prepubertal values, supporting the concept that GH is the major (but not the only) factor that raises circulating IGF1 levels in puberty.[316]

A confounding factor is the relative roles of hepatic-generated circulating IGF1 (i.e., endocrine role) and of locally produced IGF1 (i.e., paracrine/autocrine role) in linear growth. For example, mice with a totally deleted hepatic IGF1 gene have strikingly reduced circulating levels of IGF1 but normal postnatal body and bone growth.[322]

GH stimulates local IGF1 production in resting zone chondrocytes, located at the epiphyseal end of the growth plate in the area known as the reserve zone or stem cell zone, through GHRs in the chondrocytes. This IGF1 production stimulates, through autocrine and paracrine effects, the clonal expansion of proliferating chondrocytes derived from the resting chondrocyte or germ cells. GH and IGF1 can reduce the stem cell cycle time, proliferating cell cycle time and duration of the hypertrophic phase, a phase that leads to apoptosis, leaving the cells serving as a scaffold for the mineralization and production of new bone.

Other Hormones

There are glucocorticoid receptors in human growth plates, mostly in hypertrophic chondrocytes. However, children with chronic adrenal insufficiency who receive appropriate replacement therapy have a normal pubertal growth spurt despite deficient adrenal androgen secretion, indicating a minimal impact of these adrenal androgens on normal growth at puberty.[290]

Hypothyroid subjects lack a pubertal growth spurt even when the disorder is accompanied by sexual precocity.[323] Thyroid hormone has a permissive role in the pubertal growth spurt but is a requisite for normal growth. Hypothyroidism decreases GH secretion and affects growth indirectly. However, thyroid hormone also interacts with the thyroid hormone receptors α1 and β, whose proteins are found in early proliferating chondrocytes of the human growth plate and in the messenger ribonucleic acid (mRNA) found in other developing stages of chondrocytes and osteoblasts. Thyroid hormones also interact with the local effects of IGF1 and GH at the growth plate.[299] A longitudinal cohort study of 323 children demonstrated that higher prepubertal free T_4 (FT_4) and total T_4 (TT_4) are associated with earlier pubarche.[324]

Central Nervous System and Puberty

Two independent but associated processes (controlled by different mechanisms but closely linked temporally) are involved in the increased secretion of sex steroids in the peripubertal and pubertal periods. In the first process, adrenarche, the increase in adrenal androgen secretion[301,325] precedes by approximately 2 years the second process, gonadarche, which is a consequence of the pubertal reactivation of the hypothalamic-pituitary gonadotropin-gonadal apparatus.[290,325,326]

The onset of puberty is a consequence of maturational changes, including the development of secondary sexual characteristics, the adolescent growth spurt, the attainment of fertility, and psychosocial changes, all emanating from the disinhibition or reaugmentation of the hypothalamic GnRH pulse generator and gonadotropin secretion, causing an increase in gonadal steroid secretion[256,327] (Table 26.10). The events characterizing the development of gonadal function can be viewed as a continuum extending from sexual differentiation and the ontogenesis of the hypothalamic-pituitary gonadotropin-gonadal system during fetal life and early infancy,[126,254,326,327] through a juvenile pause (in which the system is suppressed to a low level of activity,[326] discussed later), to the attainment of full sexual maturation and fertility during puberty, leading to the ability to procreate (Fig. 26.21). In this light, puberty does not represent the initiation or first occurrence of pulsatile secretion of GnRH or pituitary gonadotropins but the reactivation or disinhibition of GnRH neurosecretory neurons in the medial basal hypothalamus and the endogenous, apparently self-sustaining oscillatory secretion of GnRH after the period of quiescent activity during childhood. An increase in the pulsatile

TABLE 26.10 Hypothesis of the Control of the Onset of Human Puberty

1. *Central dogma:* The CNS exercises the only major restraint on the onset of puberty. The neuroendocrine control of puberty is mediated by the hypothalamic GnRH-secreting neurosecretory neurons in the medial basal hypothalamus, which act as an endogenous pulse generator (oscillator).
2. The development of reproductive function is a continuum extending from sexual differentiation and the ontogeny of the hypothalamic-pituitary-gonadal system in the fetus to the attainment of full sexual maturation and fertility.
3. In the prepubertal child the GnRH pulse generator, operative in the fetus and infant, functions at a low level of activity (the juvenile pause) because of steroid-independent and steroid-dependent inhibitory mechanisms.
4. Puberty represents the *reactivation* (disinhibition) of the CNS suppressed GnRH pulse generator characteristic of late infancy and childhood, leading to increased amplitude and frequency of GnRH pulsatile discharges, to increased stimulation of the pituitary gonadotropes, and finally to gonadal maturation. Hormonally, puberty is initiated by the recrudescence of augmented pulsatile GnRH and gonadotropin secretion, mainly at night.

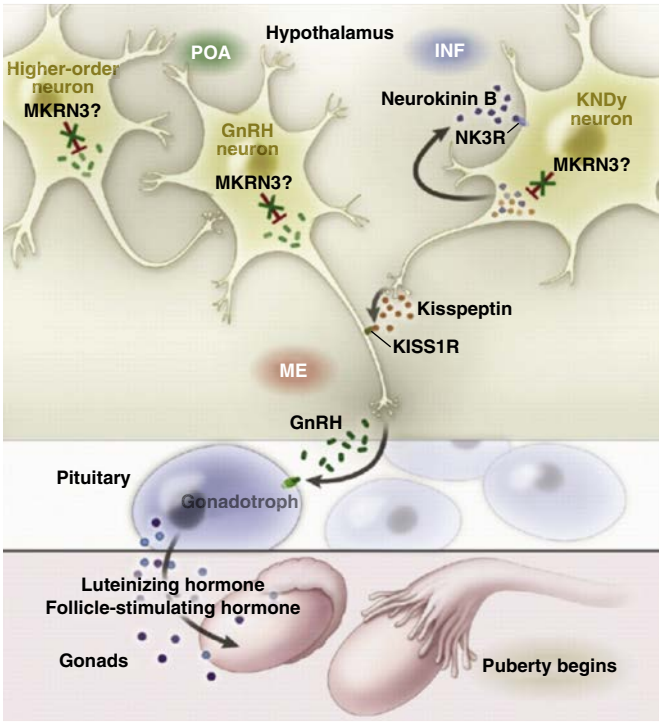
CNS, Central nervous system; *GnRH,* gonadotropin-releasing hormone.

From Grumbach MM, Kaplan SL. The neuroendocrinology of human puberty: an ontogenetic perspective. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins, 1990:1–68. © 1990, the Williams & Wilkins Co., Baltimore.

release of GnRH heralds the onset of puberty in primates and other mammals.[126,327,328] The CNS, and not the hypothalamic GnRH pulse generator, pituitary gland, gonads, or gonadal steroid target tissues, restrains activation of the hypothalamic-pituitary-gonadal system during the prepubertal years.

Certain CNS lesions involving the hypothalamus and nearby structures can advance or delay the onset of human puberty.[256,326] CPP, including cyclic ovulation in girls and spermatogenesis in boys, can result from a variety of CNS disorders. Several regulatory systems control puberty (Fig. 26.22):

1. In primates, the neural component controlling gonadotropin secretion resides in the medial basal hypothalamus, including the arcuate region. There are about 1500 to 2000 transducer GnRH neurosecretory neurons, which are not segregated into a specific nucleus but are functionally interconnected. These GnRH neurons comprise the GnRH pulse generator, which drives and controls the pituitary gonadal components, stimulates the release of LH and FSH, and translates neural signals into a periodic, oscillatory chemical signal, GnRH, in a coordinated manner. These pulses appear to be generated by a propagated depolarization, the firing of action potentials in individual cells, and the resulting influx of calcium through L-type calcium channels.[329]
2. In response to the GnRH rhythmic signal, the pituitary gonadotrophs, which contain the seven-transmembrane domain G_s-coupled LH/hCG receptors (LHCGRs),[330] release LH and FSH in a pulsatile manner. Each LH and FSH pulse is induced by a pulse of GnRH.



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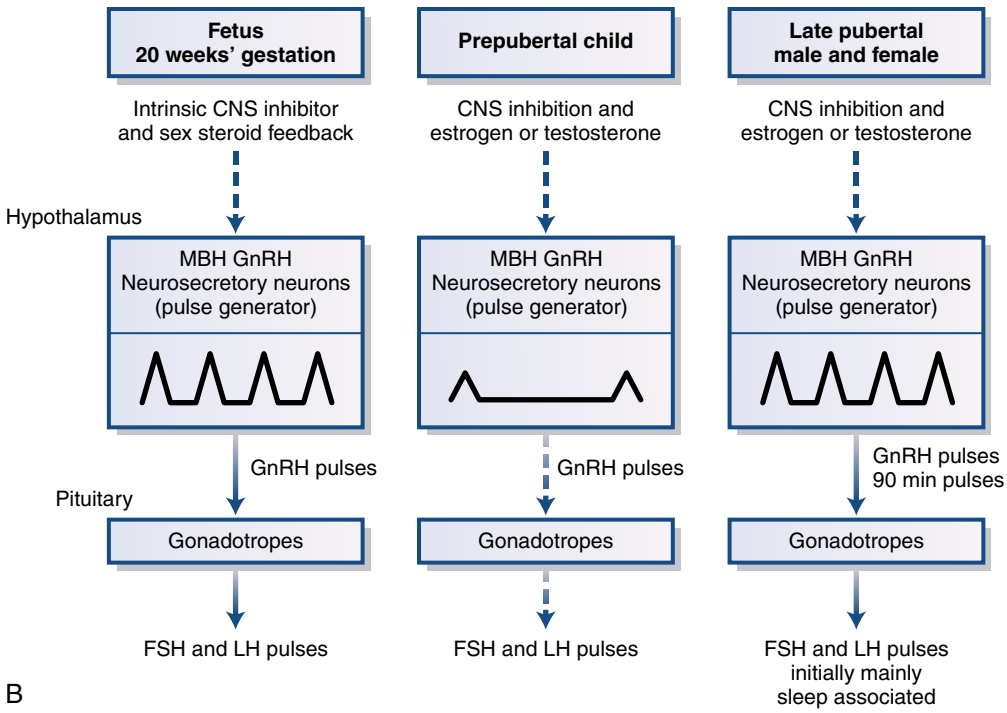
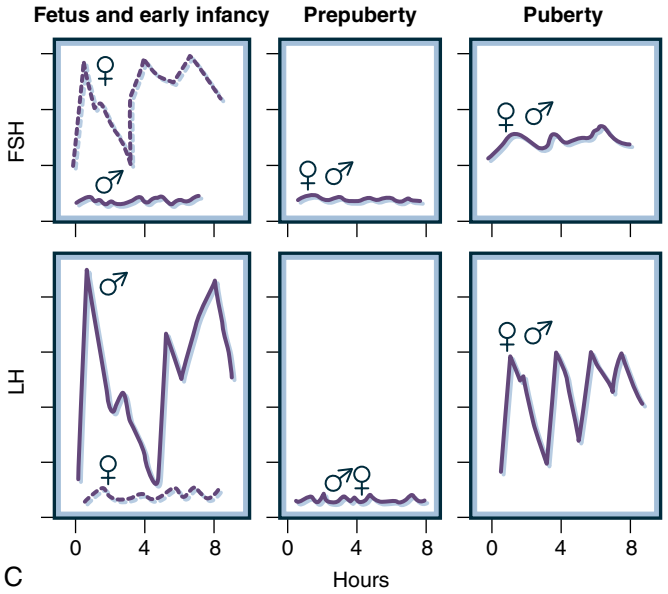
• **Fig. 26.21** Timing of puberty. A pivotal event in the onset of puberty in mammals is the resumption of pulsatile release of gonadotropin-releasing hormone (GnRH) from neurons of the hypothalamus. Known influences on the timing of the onset of puberty in mammals include the photoperiod, leptin levels, and the increased expression of neurokinin. (A) Kisspeptin, and their receptors (NK3R and KISS1R, respectively). Abreu and associates[427] implicate MKRN3, a protein that is believed to mediate ubiquitination, in puberty onset. In contrast with kisspeptin and neurokinin B, which stimulate the commencement of puberty, MKRN3 seems to inhibit puberty: Abreu and associates[427] show that mutations in MKRN3 predicted to cause loss of function of the protein cause central precocious puberty. *INF,* infundibular nucleus; *KNDy,* kisspeptin–neurokinin B–dynorphin; *MBH,* medial basal hypothalamus; *ME,* median eminence; *POA,* preoptic area.

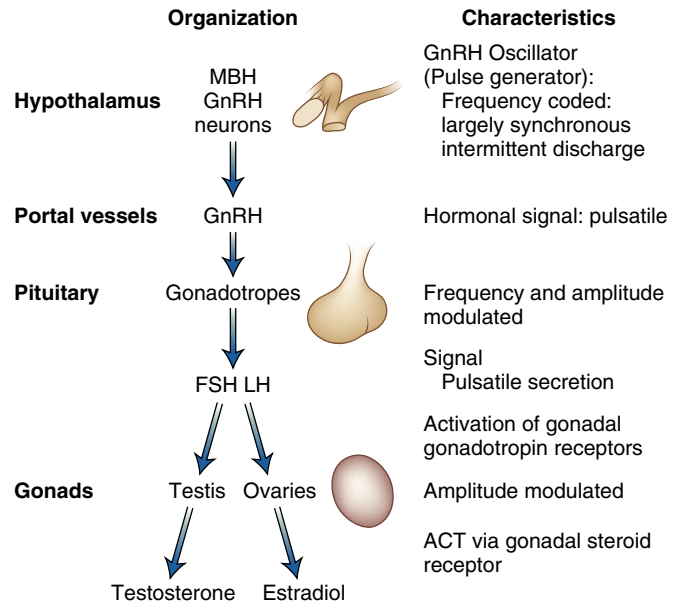




• **Fig. 26.21, cont'd** (B) Postulated ontogeny of the dual mechanism for the inhibition of puberty. *Dashed arrows* indicate inhibition. Notice the action of both components during the juvenile pause (i.e., prepuberty) (see Fig. 26.45 for the relative roles of these two mechanisms during development). (C) Change in the pattern of pulsatile follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion in the fetus and early infancy, prepuberty, and puberty (data from Waldhauser et al). Notice the pulsatile secretion in the fetus and infant and the striking difference in the amplitude of FSH and LH pulses between male and female infants. After infancy, the amplitude and frequency of gonadotropin pulses decrease greatly for almost a decade (i.e., juvenile pause) until the onset of puberty. (A, from Hughes IA. Releasing the brake on puberty. *N Engl J Med.* 2013;368:2513–2515; B and C, modified from Grumbach MM, Kaplan SL. The neuroendocrinology of human puberty: an ontogenetic perspective. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:1–68. Additional data from Waldhauser F, Weissenbacher G, Frisch H, et al. Pulsatile secretion of gonadotropins in early infancy. *Eur J Pediatr*. 1981;137:71–74.)

3. The gonads, which are modulated primarily by the amplitude of the gonadotropin pulse, transmit the episodic gonadotropin signal into pulsatile secretion of gonadal steroids.[331]

This control mechanism is common to all mammalian species. At the last two levels—the pituitary gland and the gonad—the target cells contain receptors for the peptide hormones that mediate the cellular response to the signal.[330,331] Diverse adaptive mechanisms and strategies have evolved among species and between the sexes that influence the biology and timing of puberty. Photoperiodicity and seasonal breeding, biologic clocks, and pheromones are integral parts of the pubertal process in some species but not humans. The most enlightening studies on the neuroendocrinology of human puberty have emerged from studies of humans and nonhuman primates.[328,332]



• **Fig. 26.22** Organization and characteristics of the hypothalamic-pituitary gonadotroph-gonadal system. The medial basal hypothalamus (MBH) contains the transducer gonadotropin-releasing hormone (GnRH) neurosecretory neurons. These neurons translate neural signals into a periodic, oscillatory chemical signal, GnRH. This MBH complex functions as a GnRH pulse generator (oscillator), which is frequency coded and releases GnRH from its axon terminals at the median eminence as a largely synchronous, intermittent discharge into the primary capillary plexus of the hypothalamic-hypophyseal portal circulation. The GnRH pulse generator is influenced by biogenic amine neurotransmitters, peptidergic neuromodulators, neuroexcitatory amino acids, and neural pathways. During the follicular phase in women and men, a GnRH pulse (estimated indirectly by monitoring luteinizing hormone [LH] pulses in peripheral blood) occurs approximately every 90 to 120 minutes throughout the day. Changes in the frequency and probably in the amplitude of the GnRH secretory episodes modulate the pattern of LH and follicle-stimulating hormone (FSH). The major site of action of testosterone and progesterone is on the GnRH pulse generator, because these two classes of steroids decrease LH pulse frequency, but a pituitary site of action has also been described. Estrogens have major direct inhibitory and stimulatory effects on the GnRH-primed pituitary gonadotroph; the inhibitory or negative feedback action is associated with a decrease in the frequency and the amplitude of pituitary LH secretion. Evidence supports the negative and positive feedback action of estrogen on the GnRH pulse generator. Inhibin has a direct inhibitory effect on the pituitary gland and the secretion of FSH. The secretion of gonadal steroids by the gonads is controlled mainly by the amplitude of the gonadotropin signal. (Modified from Grumbach MM, Kaplan SL. The neuroendocrinology of human puberty: an ontogenetic perspective. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:1–68.)

Pattern of Gonadotropin Secretion

Tonic Secretion

Tonic, or basal, secretion is regulated by a negative, or inhibitory, feedback mechanism in which changes in the concentration of circulating gonadal steroids and inhibin result in reciprocal changes in the secretion of pituitary gonadotropins. This is the pattern of secretion in the male and one of the control mechanisms in the female. Clinical studies reveal that testosterone and estradiol in the male have independent effects on LH secretion. Inhibition of LH by testosterone requires aromatization for its pituitary but not its hypothalamic effects, and estradiol-induced negative feedback on LH occurs at the level of the hypothalamus.[333]

In the female, cyclic secretion involves a positive, or stimulatory, feedback mechanism in which an increase in circulating estrogens, to a critical level and of sufficient duration, initiates the synchronous release of LH and FSH (i.e., preovulatory LH surge) that is characteristic of the normal adult woman before menopause.

Pulsatile Secretion

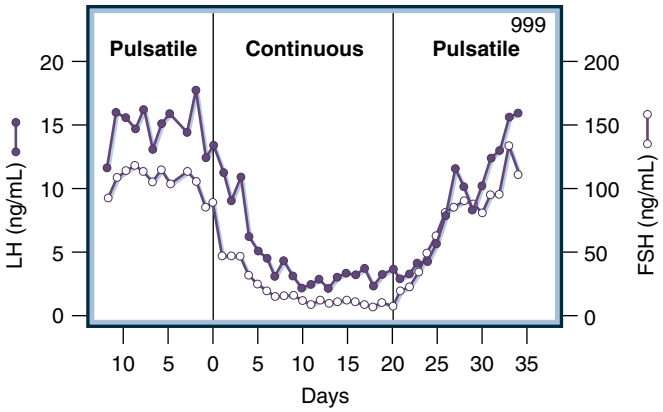
The GnRH Pulse Generator

GnRH. Generation of the GnRH pulse is an intrinsic property of the GnRH neurosecretory neuronal network, and other factors modulate the fundamental autorhythmicity of the GnRH neuron, including the downstream effects of cyclic adenosine monophosphate (cAMP)–gated cation channels on the regulation of pulsatility by cAMP.[334] The immortalized GnRH neurosecretory neuronal cell line and cultured monkey GnRH-I neurons exhibit spontaneous pulsatile release of GnRH at a frequency similar to that observed in vivo. Patch-clamped primary GnRH neurons show disordered patterns of release that are sensitive to increased extracellular potassium; firing activity can be stimulated by exposure to estrogen in a manner that appears to function through the estrogen receptor.[335]

The secretion of FSH and LH is always pulsatile or episodic, regardless of developmental stage, due to the pulsatility of the GnRH pulse generator. The pulsatile secretion of immunoreactive FSH in normal adults is less prominent than that of LH; this is attributed in part to the longer half-life of FSH compared with LH, to differences in the factors that modulate the action of GnRH on FSH and LH release by the gonadotrophs (especially gonadal steroids, inhibin, and possibly activin and follistatin), and to intrinsic differences in the secretory pattern of the two gonadotropins. For example, a change in the frequency of GnRH pulses can modify the ratio of FSH to LH released; midfollicular-phase concentrations of estradiol and adult male concentrations of plasma testosterone have a greater inhibitory effect on the response of FSH to pulsatile injections of GnRH, compared with that of LH.

Intermittent or pulsatile administration (e.g., GnRH, 1 μg/minute for 6 minute/hour) induces pulsatile release of LH and FSH in adult monkeys in which hypothalamic lesions have obliterated the arcuate nucleus region and eliminated endogenous GnRH secretion.[336] Continuous infusion of GnRH inhibits gonadotropin secretion because of desensitization of GnRH receptors on the gonadotroph. Pulsatile GnRH administration reestablished gonadotropin secretion in animals in which gonadotropin secretion was suppressed by the continuous infusion of GnRH (Fig. 26.23). The GnRH signal to the pituitary gonadotrophs of the adult is frequency coded.

The GnRH neurosecretory neurons of the hypothalamic GnRH pulse generator that arise in the olfactory placode exhibit spontaneous autorhythmicity and function intrinsically as a neuronal oscillator for entrainment of the repetitive release of GnRH. The autorhythmicity in the GnRH neurosecretory neurons involves cAMP and cyclic nucleotide-gated cation channels associated with oscillatory increases in intracellular calcium ions (Ca^{2+}), a hallmark of neurosecretion and gap junctional communication.[337] Moreover, the immortalized GnRH neuronal cell line contains neuronal nitric oxide (NO) synthase, and NO generated by GnRH neurons may act as an intercellular or intracellular



• **Fig. 26.23** The Knobil paradigm. Effect of pulsatile administration of gonadotropin-releasing hormone (GnRH) in contrast to continuous infusion of GnRH in adult oophorectomized rhesus monkeys in which gonadotropin secretion has been abolished by lesions that ablated the medial basal hypothalamic GnRH pulse generator. Notice the high concentrations of plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in monkeys given one GnRH pulse per hour, the suppression of gonadotropin secretion by continuous infusion of GnRH even though the total dose of GnRH was the same, and the restoration of FSH and LH secretion when the pulsatile mode of GnRH administration was reinitiated. (From Belchetz PE, Plant TM, Nakai Y, et al. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin releasing hormone. *Science.* 1978;202:631–633.)

messenger.[338] GnRH acting as an autocrine factor may play a role in the synchronization mechanism. GnRH is synthesized in these neurons and released episodically from axon terminals at the median eminence into the primary plexus of the hypothalamic-hypophyseal portal circulation; it is then transported by the portal vessels to the anterior pituitary gland to produce the pulsatile LH and FSH secretion. Remarkably, GnRH neurons exhibit characteristics of both axons and dendrites and are known as *dendrons*.[339] Ionotropic γ-aminobutyric acid (GABA) and glutamate receptors on the dendrons can be stimulated to depolarize the cells to produce action potentials.

Gonadotropin-Inhibitory Hormone. Gonadotropin-inhibitory hormone (GnIH), a peptide first described in quail but now homologues of GnIH known in most animals, is a member of the RFamide family that has an RFamide (Arg-Phe-NH2) motif at the C-terminus.[340] The family is called *RFamide–related peptides* (RFRPs); kisspeptin is one of the neurotransmitters belonging to this family. The human homologue is known as *RFRP3* and is coded for by the *NPVF* gene. The mammalian receptor for RFP3 is GPR147, a seven-transmembrane domain G protein–coupled receptor.[341] RFP3 and its receptor are found in the human dorsomedial hypothalamus with axons terminating both on the GnRH neuron and the median eminence, suggesting a role on the hypothalamus and the pituitary in human beings. Surprisingly, the first survey of isolated hypogonadotropic hypogonadism (IHH) and CPP in a well-characterized cohort of patients noted a 3-nucleotide in-frame deletion in the *NPVF* gene (p.I71K72), associated with a reduced risk for the occurrence of CPP.[342] Further study is needed to determine if RFP3 may play a role in the treatment of disorders of puberty.

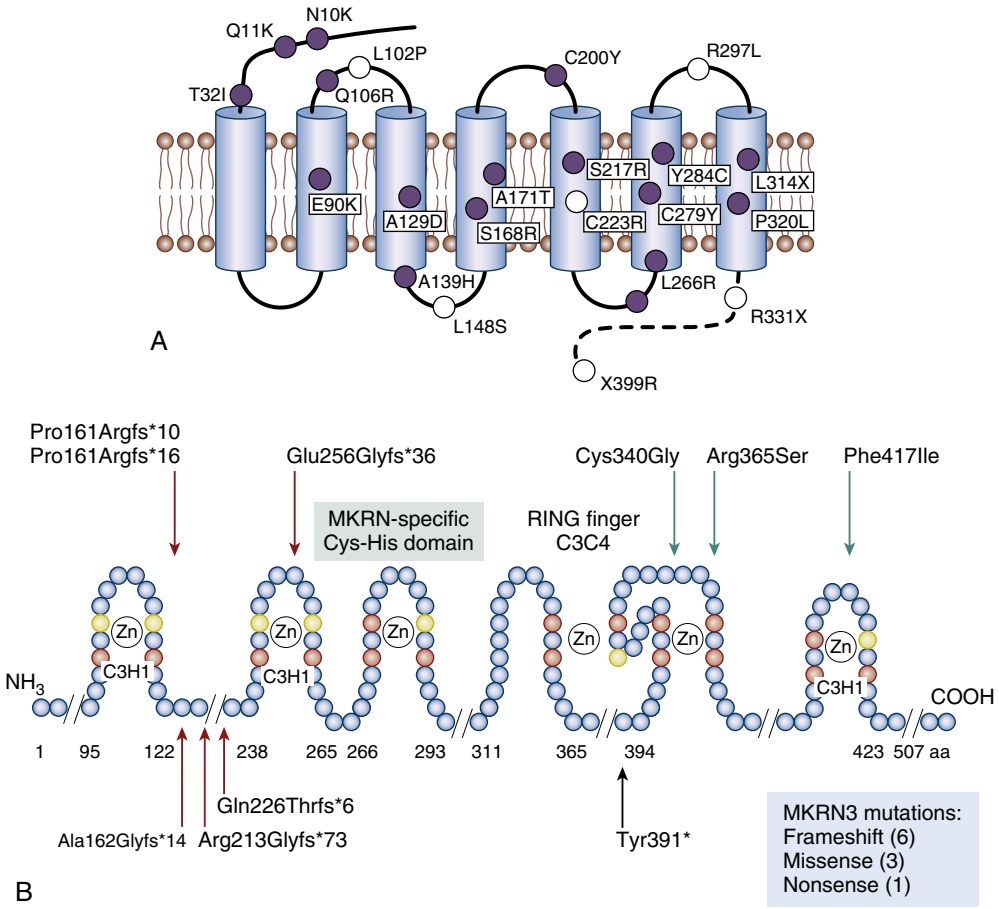
Kisspeptins and KISS1R. Kisspeptins and their receptors (KISS1R or GRP54) in the CNS hypothalamic-pituitary-gonadal axis play a major role in reproduction (Figs. 26.24 and 26.25; see Fig. 26.21).[343,344] *KISS1* is a human metastasis suppressor gene at gene map locus 19p13.3, and *KISS1* mRNA is found in placenta, testes, pancreas, liver, small intestine, and the brain, mainly in the hypothalamus and basal ganglion.[345,346] *KISS1* mRNA is present in the primate in the medial arcuate nucleus only, but in the mouse it occurs in the arcuate, periventricular, and anteroventral periventricular (AVPV) nuclei, regions important in reproductive function.[347,348] While Kiss neurons do not affect the migration of GnRh neurons from the olfactory placode, under the influence of steroids they connect to the GnRH neurons once they reach the hypothalamus. The product of the *KISS1* gene is a 145–amino acid peptide, but the cleaved and secreted product is a 54–amino acid protein known as *metastin* or *kisspeptin,* which binds to an endogenous receptor.

The KISS1 receptor, KISS1R (formerly called GPR54), is a G protein–coupled receptor found in the brain, mainly in the hypothalamus and basal ganglia, and in the placenta, from which it was first isolated and sequenced. KISS1R is coexpressed within GnRH neurons in the rat, in the medial and lateral sections of the arcuate nucleus and the ventral aspect of the ventromedial hypothalamus in mice, and in primates.[347,349] Although the expression of KISS1R mRNA does not increase with development in the mouse, kisspeptin mRNA increases dramatically in the AVPV nucleus, and the number of receptors responsive to kisspeptin increase with development.[350] Activation of GnRH neurons by kisspeptin at puberty in the mouse reflects a dual process involving an increase in kisspeptin input from the AVPV and a post-transcriptional change in KISS1R signaling within the GnRH neuron. Increases in kisspeptin lead to increased GnRH release by means of an interneuron, rather than acting directly on the GnRH-secreting neurons.[351] It is released in a pulsatile manner as is GNRH.

Mice transfected with mutant KISS1R genes exhibited hypogonadotropic hypogonadism, although they had normal content of GnRH in their hypothalamus and were responsive to GnRH or gonadotropin administration, suggesting normal function of the gonadotroph GnRH receptors and the gonadal LH and FSH receptors despite the mutation.[352] The intact mouse also releases significant LH boluses after kisspeptin administration, an effect that is abolished in the $KISS1R^{-/-}$ mouse, which lacks the receptor.[349]

Underfed prepubertal mice have decreased hypothalamic KISS1, but kisspeptin administration leads to increases in KISS1R mRNA, which in turn leads to increased in vivo LH secretion and in vitro GnRH secretion.[353] Chronic kisspeptin administration to these underfed mice restores vaginal opening and enhances gonadotropin and estrogen responses.

Administration of kisspeptin into the rostral preoptic area (RPOA), medial preoptic area (MPOA), paraventricular nuclei (PVN), and arcuate nuclei of the hypothalamus of male adult rats increased plasma LH and testosterone substantially,[354] and intracerebral kisspeptin administration stimulated the release of FSH, albeit at a far higher dose than needed to stimulate LH release.[355,356] Because the release of FSH is abolished with blockade of GnRH, GnRH modulates the central actions of kisspeptin in the rodent. In the rat, the mRNA for kisspeptin and its receptor increases at puberty, and administration of intracerebral injection of kisspeptin in prepubertal female rats caused large peaks of LH and advanced vaginal opening as a sign of pubertal development and premature ovulation.[357,358] The ovulation elicited by peripheral kisspeptin in the prepubertal female rat is abolished by blockade of GnRH.[359]

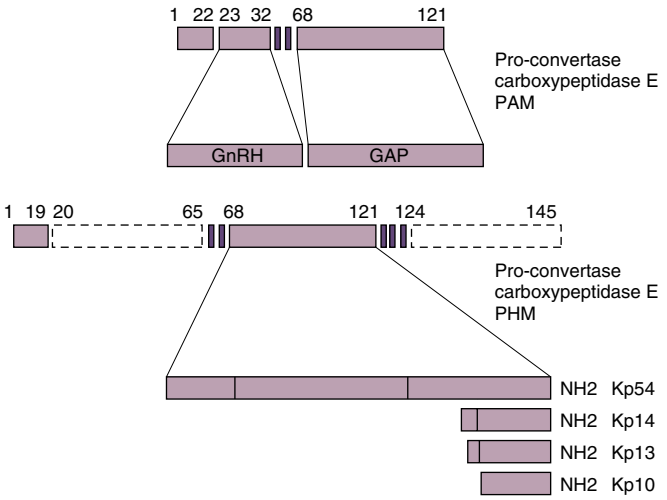


• **Fig. 26.24** (A) Inactivating mutations of the gonadotropin-releasing hormone receptor GnRHR *(closed circles)* and the kisspeptin receptor (KISS1R, formerly GPR54) *(open circles)* were identified in patients with isolated hypogonadotropic hypogonadism. The *dashed line* indicates the intracellular domain of KISS1R. The seven-transmembrane G protein–coupled receptor model is used for illustration of both receptors. (B) Schematic illustration of mutations found in the *MKRN3* gene causing precocious puberty. The amino acid positions in the protein are noted by the numbers and frameshift mutations are indicated by *red arrows*, a nonsense mutation is indicated by a *black arrow*, and the three missense mutations are indicated by *green arrows*. (A, from de Roux N. GnRH receptor and GPR54 inactivation in isolated gonadotropic deficiency. *Best Pract Res Clin Endocrinol Metab.* 2006;20:515–528; B, from Macedo DB, Brito VN, Latronico AC. New causes of central precocious puberty: the role of the genetic factors. *Neuroendocrinology.* 2014;100:1–8.)

KISS1 and KISS1R mRNA expression is found in the posterior two-thirds of the arcuate nucleus of the monkey.[360] Kisspeptin-containing axons make only infrequent contacts with GnRH neurons in the medial basal hypothalamus, whereas in the median eminence, kisspeptin and GnRH axons were found extensively, and intimate association with GnRH contacts on kisspeptin perikarya and dendrites was observed. Nonsynaptic pathways of communication in the median eminence may offer a possible mechanism of kisspeptin regulation of GnRH release and provide an anatomic basis for reciprocal control of kisspeptin neuronal activity by GnRH.[361] Although KISS1 increases with puberty in intact male and female monkeys, KISS1R mRNA levels increase in intact females but not in agonadal male monkeys. Administration of KISS1 through intracerebral catheters to GnRH-primed juvenile female rhesus monkeys stimulates GnRH release, but this release is abolished by infusion of GnRH antagonist. These findings have led to the postulation that KISS1 signaling through KISS1R of primate hypothalamus may be activated at the end of the juvenile pause and may contribute to the pubertal resurgence of pulsatile GnRH release at puberty.[347]

Short-term administration of kisspeptin raises gonadotropin secretion in men and nonhuman primates (Fig. 26.26A). Just as continuous infusion of GnRH suppresses GnRH release, continuous infusion of kisspeptin decreased the response of gonadotrophs in the agonadal male monkey to boluses of kisspeptin. However, the release of FSH and LH after a bolus of *N*-methyl-D-aspartate (NMDA) or GnRH was maintained demonstrated that the desensitization of the KISS1Rs was selective for kisspeptin administration[362] (see Fig. 26.26B). This is in contrast to the finding that continuous infusion of kisspeptin in men and a woman with *TAC3* mutations and hypogonadotropic hypogonadism experienced renewed pulsatile release of gonadotropins, and the men had increased secretion of inhibin B.[363] Treatment with kisspeptin for a variety of disorders of puberty and reproduction appears promising, but the pattern of optimal administration is not yet clear.

Administration of testosterone to castrated young male monkeys led to decreased kisspeptin mRNA in the mediobasal hypothalamus but not in the preoptic area. There was no change in KISS1R expression. This suggests that feedback inhibition of



• **Fig. 26.25** Post-translational maturation of gonadotropin-releasing hormone (GnRH) (*top*) and kisspeptins (Kp). Doublets of basic residues and glycine are indicated by *shaded vertical bars*. Enzymes involved in the normal maturation are indicated. *GAP,* GnRH-associated peptide; *PAM*, peptidyl glycine α-amidating monooxygenase; *PHM*, peptidyl α-hydroxylating monooxygenase. (From de Roux N. GnRH receptor and GPR54 inactivation in isolated gonadotropic deficiency. *Best Pract Res Clin Endocrinol Metab.* 2006;20:515–528.)

gonadotropin secretion by testosterone is mediated by kisspeptin upstream of the GnRH network.[364]

KISS1R mRNA is expressed in the pituitary gland, and there is evidence that kisspeptin can act directly on the gonadotroph to prompt LH secretion.[355] In sheep, kisspeptin colocalizes to a high proportion of GnRH receptor cells in the preoptic area as well as various neuronal fibers within the external neurosecretory zone of the median eminence. This raises the possibility that both kisspeptin and GnRH are secreted into the pituitary portal system to affect the pituitary gland.[365] There are also single-labeling KISSP1R cells in the preoptic area, and the number rises with ovariectomy.

In addition to neurons that specifically secrete kisspepetin, other infundibular nucleus neurons in the human being that coexpress kisspeptin and neurokinin B as well as B-dynorphin (an opioid inhibitor) are known as *kisspeptin-neurokinin* (KNDy) neurons.[366] KNDy cells have receptors for neurokinin B and B-dynorphin axons and connect with their own KNDy cell and other KNDy cells as well as GnRH neurons. It appears that the stimulatory role of neurokinin B and the inhibitory action of dynorphin autosynaptically coordinate the pulsatile release of kisspeptin, which in turn stimulates the pulsatile release of GnRH.[366] There are no steroid receptors on GnRH neurons, but there are steroid receptors on kisspeptin and KNDy neurons, which appear to modulate both the feedback inhibition and positive feedback effects of estrogen on gonadotropin secretion.

Spontaneous mutations in the KISS1/KISS1R axis in human beings are rare but instructive in elucidating the role of KISS1 in pubertal development; hypogonadotropic hypogonadism and CPP occur with different mutations of KISS1R (see later). Plasma concentrations of kisspeptin are higher in children than adults, and preliminary studies indicate that a rise occurs at puberty in boys and girls.[367] These cases, augmented with studies of various animal species, demonstrate that kisspeptin acting through the KISS1R is an afferent influence that stimulates GnRH secretion.

With the demonstrated importance of KISS/KISSR in human reproduction, it is interesting to note that in mice alterative pathways operate. Thus elimination of KISS signaling by knockout of *KISS* (5% remained) and of *KISSR* genes (90% remained) did not eliminate puberty or fertility in developing female mice, although acute ablation of the axis in adult mice did eliminate fertility.[368] Alternative studies demonstrated that retention of 5% of the KISS/KISSR axis in male mice allowed fertility but conversely demonstrated decreased fertility in female mice.[368] The discrepancy in the female mouse results may be due to variations in techniques used in the studies but is not yet resolved. However, it does appear clear that there are far more KISS or KISSR neurons than are needed for fertility and that redundancy is invoked to ensure the vital role of reproduction can occur.

Ontogeny

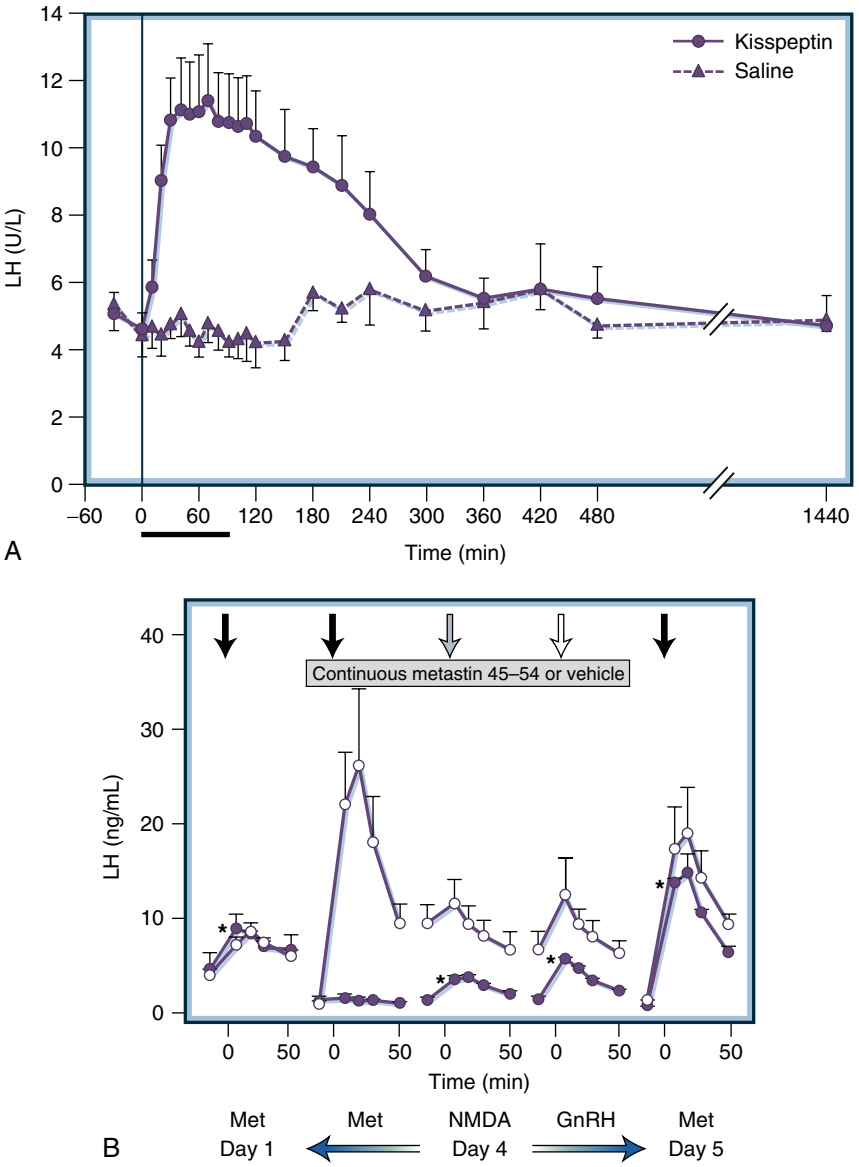
In all vertebrates examined, GnRH neurons arise in the embryo from the epithelium of the olfactory placode and migrate in a rostrocaudal direction by an ordered spatiotemporal course along the pathway of the nervus terminalis-vomeronasal complex to the forebrain.[369] The terminalis-vomeronasal complex also originates in the olfactory placode and forms a connection between the nasal septum and the forebrain. This contrasts to the pattern of growth hormone–releasing factor (GRF), thyrotropin-releasing factor (TRF), or corticotropin-releasing factor (CRF) neurosecretory neurons, which originate from ventricular zones within the embryonic forebrain.[370]

The GnRH green fluorescent protein model demonstrates an increase in dendritic and somal spines in adult mice compared with juveniles, suggesting an increase in direct excitatory inputs to GnRH neurons and increased glutamatergic stimulation of GnRH neurons across the time of puberty.[371] Embryonic GnRH neurons of the olfactory placode and the hypothalamus coexpress mRNAs for GnRH and the type 1 GnRH receptor. These neurons demonstrate spontaneous electrical pulsatile activity, which can be stimulated by GnRH agonist and abolished by GnRH antagonist in the same pattern as GnRH pulses, all in a calcium-dependent manner (i.e., the intracellular calcium responses are stimulated by the agonist and inhibited by the antagonist).[353]

Human Fetus

GnRH immunoreactivity was observed in the epithelium of the medial aspect of the olfactory placode of the normal human fetus by 42 days of gestation but not at 28 to 32 days[369] (Table 26.11). No GnRH neurosecretory neurons were found in the brain, including the hypothalamus, of a 19-week gestational male human fetus with Kallmann syndrome,[372] and the olfactory bulbs were absent. However, dense clusters of GnRH cells and fibers were present in the nose, including the nasal septum and cribriform plate, and within the dural layers of the meninges under the forebrain. The GnRH neurosecretory neurons migrate from the olfactory placode, where they arise outside of the central nervous system from neural crest and ectodermal progenitors, to the hypothalamus in normal humans and other mammals.[373] As they reach the hypothalamus they detach from the olfactory axons that guided them there and some end at the median eminence where they can release their products into the hypothalamic pituitary portal system.

Numerous genes are involved in the migration of the gonadotropin-releasing hormone neurons. They include fibroblast growth factor 8 (FGF8), chromodomain helicase DNA-binding protein 7 (CHD7), and sex-determining region Y-box 10 (SOX10). Other



• **Fig. 26.26** (A) Effects of kisspeptin-54 (4 pmol/kg/min) or saline infusion in male volunteers (n = 6) on mean plasma. Each volunteer received both kisspeptin-54 and saline infusions and acted as their own controls. (B) Effect of single sequential boluses of human metastin 45-54 (Met-Kisspeptin 10) *(black arrow)*, *N*-methyl-D-aspartate (NMDA) *(gray arrow)*, and gonadotropin-releasing hormone (GnRH) *(white arrow)* on plasma luteinizing hormone (LH) concentrations (mean ± SEM [standard error of the mean]) during the last 3 hours of the 98-hour intravenous infusion *(shaded box)* of human metastin 45-54 at a dose of 100 μg/hour *(closed circles)* or vehicle compared with the LH response to the same bolus of human metastin 45-54 1 hour before (day 1) and 21 hours after (day 5) the termination of continuous human metastin 45-54 or vehicle infusion. Infusion of human metastin 45-54 *(asterisk)* was significantly different ($p < 0.05$) from the preinjection mean; n = 3. (A, from Dhillo WS, Chaudhri OB, Patterson M, et al. Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *J Clin Endocrinol Metab.* 2005;90:6609–6615; B, from Seminara SB, Dipietro MJ, Ramaswamy S, et al. Continuous human metastin 45-54 infusion desensitizes G protein–coupled receptor 54-induced gonadotropin-releasing hormone release monitored indirectly in the juvenile male rhesus monkey *(Macaca mulatta)*: a finding with therapeutic implications. *Endocrinology.* 2006;147:2122–2126.)

genes influencing the migration are the Kallmann protein (now called anosmin-1 with gene *ANOS1*) as well as prokineticin-2 and prokineticin receptor 2 (PROK2 and PROKR2), WD repeat domain 11 (WDR11), semaphorin-3A (SEMA3A), FEZ family zinc finger 1 (FEZF1), NELF,[374] *DAX1* (or *NR0B1*), *LEP*, *LEPR*, *KISS1*, *KISS1R*, *TAC3*, *TACR3*, and *GNRH1*.

The number of the GnRH neurons and the GnRH mRNA levels in nonhuman primates and mice do not appear to change during pubertal development. The ability of the GnRH neuron to respond to electrical or neurochemical (e.g., glutamatergic, kisspeptinergic) stimuli does not change with pubertal development.[375]

TABLE 26.11 Early Development of the Human Fetal Pituitary and Hypothalamus

Gestational Age (wk)	Hypothalamus	Pituitary	Portal Circulation
3	Forebrain appears		
4		Rathke pouch in contact with stomodeum	
5	Diencephalon differentiated	Rathke pouch separated from stomodeum and in contact with infundibulum; pituitary in culture can secrete corticotropin, prolactin, GH, FSH	
6	Premammillary preoptic nucleus; GnRH detected	Intermediate-lobe primordia; cell cords penetrate mesenchyme around Rathke pouch	
7	Arcuate, supraoptic nucleus	Sphenoidal plate forms	
8	Median eminence differentiated: TRH detected	Basophils appear	Capillaries in mesenchyme
9	Paraventricular nucleus; dorsal medial nucleus	Pars tuberalis formed: β-endorphin detected[a]	
10	Serotonin and norepinephrine detected[a]	Acidophils appear	
11	Mammillary nucleus; primary (hypothalamic) portal plexus present; β-endorphin and opioidergic neurons detected[a]	Secondary (pituitary) portal plexus present; catecholamines detected by IF	Functional hypothalamic hypophyseal portal system
12	Dopamine present		
13	Corticotropin-releasing hormone detected[a]	α-Melanocyte-stimulating hormone detected	
14	Fully differentiated hypothalamus	Adult form of hypophysis developed	

[a]Hormone is detected at this gestational age but may be present earlier.

FSH, Follicle-stimulating hormone; *GH,* growth hormone; *GnRH,* gonadotropin-releasing hormone; *IF,* immunofluorescence; *TRH,* thyrotropin-releasing hormone.

Modified from Gluckman P, Grumbach MM, Kaplan SL. The human fetal hypothalamus and pituitary gland. In: Tulchinsky D, Ryan KJ, eds. *Maternal-Fetal Endocrinology.* Philadelphia: WB Saunders, 1980:196–232.

The pituitary gland develops from cells originating in Rathke pouch and ultimately contains five different cell types. Development of the pituitary gland is influenced by multiple genes, including *HESX1, LHX3, LHX4, POU1F1, PROP1, SIX6, OTX2, PITX1, PITX1, PTX2, GLI2, GLI1,* and *SOX3*.[376]

Gonadotropin cells are affected by *TBX19* (or *TPIT*), *GATA2*, and *SF1* (or *NR5A1*). Luteinizing hormone can be detected in human fetal pituitary glands from gestational week 12; follicle-stimulating hormone can be detected by week 14.[254]

GnRH has been detected in human embryonic brain extracts by 4.5 weeks and in the fetal hypothalamus by 6 weeks (see Table 26.11); the fetal pituitary gonadotrophs are responsive to GnRH. The hypothalamic-hypophyseal portal system is functional by 11.5 weeks of gestation, and by 16 weeks, axon fibers that contain GnRH are present in the median eminence and terminate in contact with capillaries of the portal system.[126,254,326,327] The available data are consistent with the development of a human fetal hypothalamic GnRH pulse generator by at least the end of the first trimester.[127,327]

The adreno-gonadal primordium gives rise to the testes, which can be detected by 7 weeks of gestation. Sertoli cells under the influence of FSH after the first few weeks of gestation proliferate and produce antimüllerian hormone as well as inhibin B, which rises by midgestation. Leydig cells differentiate 1 week later than Sertoli cells and initially secrete testosterone under the influence of chorionic gonadotropin during the first third of gestation. By the second third of gestation, LH is the major driving force toward Leydig cells producing testosterone. Both human chorionic gonadotropin and LH utilize the LH-CG receptor, which when stimulated causes Leydig cell proliferation, testosterone secretion, and secretion of INSL3.

The human fetal gonad is affected by placental gonadotropins and by fetal pituitary FSH and LH.[327] The placental gonadotropin hCG may play an important role in the secretion of testosterone by the Leydig cells of the fetal testes during masculinization of the wolffian ducts and the external genitalia at 8 to 13 weeks of gestation. However, it is uncertain whether functional hCG/LH and FSH receptors are present in the fetal testis by 12 weeks of gestation and whether the early fetal testis responds to hCG. Fetal Leydig cells are a unique population of Leydig cells limited to the fetus and infant, which regress to be followed by the differentiation of adult-type Leydig cells in the peripubertal period.[377] Fetal testosterone and hCG levels, but not LH levels, were decreased in fetuses studied after elective second-trimester abortions between 11 and 19 weeks of gestation, with no significant change in testicular responsiveness. The proportion of nonfunctional LH-CGR transcripts in fetal testes was 2.3-fold lower than in adults, so available ligand may exert a greater effect. Fetal hCG was reduced, and the ratio of inactive to active LH-CGR isoforms was lowered by maternal smoking. Second-trimester fetal testosterone levels appear to decrease due to decreasing maternal hCG because Leydig cell LH/hCG responsiveness remains constant. Even with the decrease of fetal hCG caused by maternal cigarette smoking, because the ratio of inactive to active LHCGR isoforms is reduced, testosterone remains normal due to fetal gonadotropin stimulation.

Compared with the adult type, fetal Leydig cells form tightly opposed clusters joined by gap junctions and lack Reinke crystals; they are resistant to hCG/LH-induced desensitization (hCG/LH produce upregulation of LH-CGRs), and they contain little aromatase activity and few estradiol receptors.

In contrast to the fetal testis, FSH receptors in the fetal ovary appear only late in the second trimester, well after completion of male phenotypic differentiation, demonstrating a sex difference in the stage of gestation at which fetal pituitary gonadotropins have an important effect on the development of the fetal gonad. In the anencephalic fetus (which is deficient in hypothalamic GHRH, resulting in deficiency of pituitary gonadotropins), the testes appear hypoplastic by early in the third trimester; however, the ovaries in this disorder are normal until at least 32 weeks of gestation.[126,327] Androgen receptor mRNA expression is lower, and AMH mRNA expression is higher in fetal testicular Sertoli cells than in adult testes. This may explain the failure of testicular testosterone to support spermatogenesis and to suppress AMH at that stage; patients with androgen insensitivity syndrome also have an increase in circulating testosterone and AMH and have combined gonadotropin stimulation that is consistent with a failure of testosterone to repress AMH in the absence of AR signaling.[378] AR was expressed in peritubular and Leydig cells.

The human fetal pituitary gland contains FSH and LH by 10 weeks, secretion begins by 11 to 12 weeks, and the gonadotropin content increases until approximately 25 to 29 weeks of gestation.[126,254,327] Fetal serum LH and FSH concentrations rise to peak levels by midgestation and then decrease to low values in umbilical venous blood at term. The serum concentrations of FSH and LH and of bioactive FSH at 17 to 24 weeks of gestation are strikingly higher in female than in male fetuses, and in both sexes, they decrease remarkably between 25 and 40 weeks of gestation. Mean FSH and LH concentrations are elevated at the beginning of the third trimester and decrease with advancing gestational age to undetectable values in term fetuses. Mean FSH values are higher in female fetuses between 26 and 36 weeks, whereas the mean LH level is higher in males. In the ovine fetus, LH and FSH are secreted in a pulsatile manner in response to the episodic secretion of fetal hypothalamic GnRH; human fetal pituitary gonadotropins are probably released in the same mode. The mean FSH and LH content of fetal pituitary glands is greater in female than in male fetuses at midgestation. This difference has been ascribed to the higher concentration of plasma testosterone between 11 and 24 weeks in the male fetus (the only major difference in gonadal steroids between the male and female fetus) and to fetal testicular inhibin.[126,327] The decrease in serum FSH and LH concentrations during late gestation and near term is attributed to maturation of the negative feedback mechanism, the development of gonadal steroid receptors in the hypothalamic-pituitary unit,[254] and the effect of inhibin.[126,327]

In vitro studies indicate that the human fetal pituitary gland is responsive to GnRH as early as 10 weeks of gestation; the GnRH-stimulated release of LH is greater in second-trimester fetal pituitary cells cultured from females than in those cultured from males and is augmented by estradiol in both sexes.[354] In vivo studies during middle and late gestation demonstrate the stimulating action of exogenous GnRH on fetal FSH and LH release by 16 weeks, with a striking sex difference in the FSH response and a fall in responsiveness to GnRH in late gestation. Anencephalic infants and some infants with neonatal hypothalamic hypopituitarism have an absent or diminished gonadotropin response to GnRH,[126,254,327] in contrast to the brisk increase demonstrated in the normal infant.

A pattern of increasing synthesis and secretion of FSH and LH leading to peak serum concentrations at castrate levels—probably the result of relatively autonomous, unrestrained activity of the fetal hypothalamic GnRH pulse generator and subsequent stimulation of the fetal gonadotrophs by GnRH—is followed by a decline after midgestation that persists to term, probably due to maturation of the negative feedback mechanism and increasing sensitivity of the GnRH pulse generator to the inhibitory effects of the high concentration of sex steroids (estrogens and progesterone from the placenta and, in the male, testosterone from the fetal testes) in the fetal circulation; in the male fetus, there is also a contributory effect on the decrease in FSH by testicular inhibin in late gestation. The increasing CNS control of gonadotropin secretion seems to require the maturation of gonadal steroid receptors (intracellular or on the cell surface, or both) in the fetal hypothalamus and in the pituitary gonadotrophs.[254]

Sheep Fetus

In fetal sheep, the hypothalamus secretes GnRH in a pulsatile manner.[326] By 0.6 gestation, the secretion of fetal LH and FSH is pulsatile, mediated by the hypothalamic GnRH pulse generator.[326] A sex difference in gonadotropin secretion occurs in ovine and human fetuses, and orchiectomy (but not oophorectomy) in the ovine fetus leads to increased pulsatile secretion of LH and, to a lesser degree, of FSH.[379] Opioidergic neurons have a tonic suppressive effect on the pulsatile release of GnRH in the ovine fetus,[380] and the excitatory amino acid analogue NMDA evokes an LH pulse mediated by GnRH.[381] The excitatory amino acids, glutamate and aspartate, can stimulate the GnRH pulse generator directly or indirectly. Glutamate is present in abundance in the hypothalamus and is released from glutamatergic neurons by exocytosis in an adenosine triphosphate–dependent and calcium-dependent process. FSH stimulates inhibin synthesis by the ovine fetal testis and ovary, and administration of an inhibin-rich extract inhibits fetal FSH but not LH secretion, evidence of the functional capacity of the FSH-fetal gonadal inhibin feedback system.[382] These observations provide support for the central role of this process in the regulation of the CNS in the hypothalamic GnRH-pituitary gonadotropin unit.

Human Neonate and Infant[291,326]

In both sexes, the concentration of plasma FSH and LH is low in cord blood as a consequence of the inhibitory effect of the high levels of placental-derived estrogens, but within a few minutes after birth, the concentration of LH increases abruptly in peripheral blood (about 10-fold) in the male neonate, but not in the female; this is followed by an increase in serum testosterone concentration during the first 3 hours that persists for 12 hours or longer.[326,383] After the fall in circulating levels of steroids of placental origin (especially estrogens) during the first few days after birth, the serum concentrations of FSH and LH increase and exhibit a pulsatile pattern with wide perturbations during the first few months of life. FSH pulse amplitude is much greater in the female infant and is associated with a larger FSH response to GnRH throughout childhood; LH pulses are of greater magnitude in the male. This striking sex difference also is present in agonadal male and female infants and in infant rhesus monkeys.[326,384,385] This sex difference

may in part be related to the effect of testosterone in the male fetus on the development and function of the hypothalamic-pituitary apparatus.[126,327]

The high gonadotropin concentrations are associated with a proliferation of Sertoli cells and gonocytes (and their transformation into spermatogonia) and with a transient second wave of differentiation of fetal-type Leydig cells and increased serum testosterone levels in male infants during the first few postnatal months and increased estradiol levels intermittently during the first year of life and part of the second year in females.[377] The mean FSH concentration is higher in females than in males during the first few years of life. By approximately 6 months of age for boys and 2 to 3 years for girls, the concentration of plasma gonadotropins decreases to the low levels that are present until the onset of puberty (earlier in boys than girls) in the juvenile pause.

The neonatal-to-midinfancy surge in pulsatile gonadotropin secretion, sex hormones, and inhibin—the postnatal surge or minipuberty of infancy—is attributable to an increase in GnRH pulse amplitude and is associated in the male infant with the following[327]:

1. Increase in testicular volume (by direct measurement) due to increased seminiferous tubule length (about a sixfold increase in year 1)
2. Rapid expansion of the Sertoli cell population (which makes up 85–95% of seminiferous tubular cell mass)
3. High concentration of circulating inhibin B (low in hypogonadotropic hypogonadism)
4. Sertoli cell number, including postnatal proliferation, as a determinant of spermatogenic function

The increase in circulating testosterone in the normal male infant may lead to facial comedones and even to acneiform lesions, and the increase in gonadotropins may lead to a transient increase in testicular size, but there may be subtler changes. A detailed study of 18,570 infants in Finland (51% were boys) demonstrated more rapid growth in boys and girls during minipuberty with the increase in the rate of growth in boys compared to girls being similar to that noted during pubertal growth.[386]

The minipuberty of infancy offers an excellent time to evaluate children who have features that have been described as red flags of congenital hypogonadotropic hypogonadism.[387] Missing an opportunity to measure testosterone in a sensitive pediatric assay at this age may delay diagnosis of hypogonadism until well past the pubertal period. An LH and testosterone surge is absent in this period in those with the complete androgen insensitivity syndrome.

The postnatal surge apparently is not essential for masculine-typical psychosexual development. The brain in patients with congenital hypogonadotropic hypogonadism, including Kallmann syndrome, is masculinized by testosterone therapy at puberty despite the lack of an infantile surge in gonadotropins and testosterone.

The transient postnatal to midinfancy function of the GnRH pulse generator in the male infant may be related to future spermatogenic function and fertility.[388,389] Sertoli cells and germ cells proliferate for about 100 days after birth (indicating mitotic activity and the transformation of gonocytes into adult dark [Ad] spermatogonia, the stem cells for spermatogenesis), with a subsequent decrease, mainly by apoptosis, after about 6 months of age, coincident with the waning of gonadotropins and testosterone.

Neural Control

Maturation of the CNS is the outcome or consequence of the totality of environmental and genetic factors that retard or accelerate the onset of puberty. It is a provocative but unproven hypothesis that a metabolic signal related to body composition is an important factor in the maturation or activation of the hypothalamic GnRH pulse generator and not a result of the early hormonal and body composition changes in human puberty. In either event, clinical and experimental data support the contention that the factors influencing the timing of puberty are expressed finally through CNS regulation of the onset of puberty.[256,326] In humans, the pineal gland and melatonin do not appear to have a major effect on this control system.[326,390]

Timing and Onset of Puberty

Genetic Neural Control

Because many levels control the onset of pubertal development, a systems biology approach holds promise in characterizing the complex components of this neural and neuroendocrine network. It appears that normal and some types of abnormal puberty are under polygenic control. Although the increased pulsatile release of GnRH is most frequently considered, this change is caused by a balance in the inhibitory and excitatory factors through coordinated changes in transsynaptic and glial-neuronal communication, increased stimulatory factors (most prominently glutamate and kisspeptin), and decreased inhibitory tone, mostly through GABAergic neurons (i.e., those secreting γ-aminobutyric acid), opioidergic neurons, and MKRN3 expression all controlled by gene expression. Glial cells affect GnRH secretion through growth factor–dependent cell-cell signaling coordinated by numerous unrelated genes. A second level of genes is postulated to control cell-cell interaction. The third highest level of control occurs through transcriptional regulation of the subordinate genes by other higher level genes that maintain the function and integration of the network.

The activation of GnRH release at puberty involves many different cellular phenotypes and intracellular and cell-cell signaling molecules, which is controlled by a highly coordinated and interactive regulatory system involving hundreds of gene products; a systems biology approach is proposed to explain how these networks are organized in a hierarchical fashion.[391] This theory states that there are transcriptional regulators that direct expression of downstream subordinate genes to allow stability and to coordinate the cellular networks involved in controlling the onset of puberty. Epigenetic mechanisms sensitive to external inputs such as nutrition or endocrine disruptors are posited to integrate the response of these gene networks.[392] Endocrine disrupting chemicals discussed elsewhere in this chapter appear to exert their effect through epigenetic mechanisms.

Although the action of multiple genes (i.e., quantitative or polygenic inheritance) on the time of onset of puberty (e.g., on stature) has long been recognized, little is known about the gene loci involved in this complex quantitative trait or the effect of gene interactions on this paradigm of complex traits.[393] Genetic factors are estimated to account for 50% to 80% of the variation in the onset of normal puberty. These complex traits have been analyzed by linkage analyses (in which quantitative trait loci have been shown to relate to the age of menarche) and by large-scale haplotype-based association studies. Pedigree analyses have revealed relative risks for delay of puberty in kindreds with histories of CDP compared with those without such a history; for

example, for first-degree relatives, the risk of a 2-SD delay in the onset of puberty is 4.8. There are clear genetic influences on the time of onset of puberty in monogenetic disorders, such as *KAL1, KISS, KISS1R (GRP54)*, *MKRN3,* and mutations and other influences, that can prevent or advance pubertal development (see earlier discussion of genomic effects on the onset of puberty and later discussion of specific genomic conditions).

Nutrition and Metabolic Control

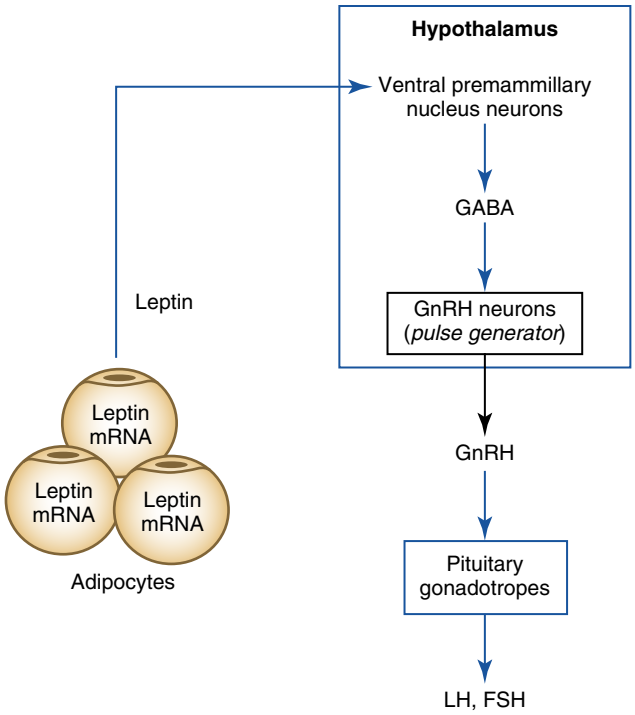
The genetic effects on the time of onset of puberty and its course are influenced by environmental factors (e.g., socioeconomic factors, nutrition, general health, geography) operating through the CNS. It has long been postulated that some alteration of body metabolism linked to energy metabolism may affect the CNS restraints on pubertal onset and progression, because of the earlier age of menarche in moderately obese girls, delayed menarche in states of malnutrition and chronic disease and with early rigorous athletic or ballet training, and changes in gonadotropin secretion and amenorrhea in girls with anorexia nervosa, voluntary weight loss, and strenuous physical conditioning.

An invariant mean weight (48 kg) for initiation of the pubertal spurt in weight, the maximal rate of weight gain, and menarche in healthy girls regardless of chronologic age was proposed in the 1970s, but the concept generated controversy and criticism, in part because the empiric estimations and the equations used to determine fat mass were challenged and because no direct measurements supported the theory.[394] A more recent, 5-year longitudinal study of 469 girls revealed that there was a similar percentage of body fat associated with the onset of puberty, although there was a spread of chronologic ages at onset.[395] Body composition in the 2 years before puberty had a modest impact on the age of pubertal growth, but higher fat mass led to more rapid progress through pubertal stages.[396]

The relationships of adipose tissue mass, fat metabolism, and energy balance to reproduction were illuminated by the discovery of the genes encoding leptin, an adipocyte satiety factor,[397,398] and its receptor.[399] Considerable interest has focused on the potential role of leptin in the control of the onset of puberty—from a proposal that it was an essential, if not a key, factor in triggering the onset of puberty to one in which it had a more subsidiary role. Leptin is a well-established afferent satiety factor in humans; it acts on the hypothalamus, including nuclei controlling appetite, to suppress appetite. Leptin reflects body fat and therefore energy stores and has an important role in the control of body weight and the regulation of metabolism.

Ob/ob mice (which lack leptin) and db/db mice (which lack leptin receptors) are obese and exhibit hypogonadotropic hypogonadism, providing evidence for an important role of leptin in reproduction. Administration of recombinant leptin to hypogonadal ob/ob mice and to rats experiencing pubertal delay associated with food restriction in the rat partially reverses the hypogonadism. However, leptin administration to normal prepubertal rats did not advance the time of onset of puberty. A critical threshold level of leptin was necessary for puberty to begin and advance, but leptin alone (as in administration to normal rodents) was insufficient to promote puberty; it was but one among several permissive factors.[400]

The site of action of leptin in its effect on the hypothalamic GnRH-pituitary gonadotropin apparatus was clarified by experiments in the mouse.[401] Even though leptin receptors (LepR) were reported to be expressed in immortalized mouse GnRH (GT1) cells, leptin receptors are not expressed on GnRH neurons in vivo.[402] Mice with selective deletion of the long form (signaling) of the leptin receptor underwent normal puberty and fertility. Kiss1 neurons express the leptin receptor (Ob-Rb isoform), and the suggestion was advanced that the effect of leptin on puberty onset and fertility was mediated through the Kiss1/Kiss1n complex. Mice with selective deletion of the leptin receptor from Kiss1 neurons underwent normal puberty and fertility, strongly suggesting that the action of leptin on the hypothalamic GnRH-pituitary gonadotropin system is not mediated through Kiss1 neurons.[403,404] The leptin receptor is expressed at a high level in ventral premammillary neurons, which express the excitatory neurotransmitter glutamate, which stimulates either GnRH neurons or GnRH terminals in the median eminence or both sites. These observations dissociate the action of leptin on reproductive function and on nutrition (Fig. 26.27).



• **Fig. 26.27** The leptin receptor is expressed in ventral premammillary neurons (PMV). Leptin secreted by adipocytes evokes release of the excitatory neurotransmitter glutamate from the PMV leading to activation of gonadotropin-releasing hormone (GnRH) neurons. Leptin functions as a permissive factor, not a trigger, in the onset of human puberty. In rodents, leptin advances the onset of puberty and plays a key role in initiating puberty and infertility. The hypothalamic pathway that regulates energy expenditure and food intake by leptin is independent of the effect of leptin on reproduction. *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone; *mRNA,* messenger ribonucleic acid.

Leptin levels in the male rhesus monkey were similar during the advancement of prepuberty to puberty. In peripubertal rhesus monkeys age 3 to 5 years and fasted for 2 days, the administration of leptin prevented the decrease in plasma gonadotropins detected in the untreated animals. Continuous infusion of leptin into the lateral ventricle of agonadal male monkeys failed to evoke an increase in GnRH on gonadotropin secretion.[332]

Is leptin the peripheral, somatic trigger for the onset of puberty to the CNS, or does it have a permissive role, signaling the hypothalamus and the GnRH pulse generator that a critical energy store has been attained? A longitudinal study of serum leptin levels in prepubertal and pubertal boys and girls showed leptin

to increase gradually during the prepubertal years, with similar levels in the two sexes.[405] During puberty, leptin continued to rise in girls, whereas in boys, the leptin mean levels peaked at Tanner stage 2 and decreased to prepubertal concentrations by genital stage 5.[406] This decrease is attributed to the effect of testosterone on leptin secretion.[407] Adipose tissue mass, percentage of body fat, and age correlated with leptin levels, among other variables,[405,408] but there was no correlation between 24-hour serum estradiol and leptin concentrations in nonobese and obese prepubertal and early pubertal girls. Serum leptin levels rose after administration of pulsatile GnRH administration for 36 hours to children with delayed puberty but not after a single dose of buserelin, a GnRH agonist, suggesting that, rather than puberty being triggered by leptin, pubertal increase in GnRH pulsatility increases leptin.[409]

Leptin circulates in both a free form and a high-molecular-weight, bound form. Leptin-binding activity in serum is highest in childhood and decreases to relatively low levels during puberty. Free leptin is postulated to have more relevance to reproductive development than total leptin measured in the circulation; the soluble leptin receptor appears to be higher in males than in females and is inversely related to leptin levels later in development in females.[410] A study of a 132 monozygotic female twin pairs and 48 dizygotic female twin pairs demonstrated a rise in leptin throughout puberty and a decrease in the soluble leptin receptor between stages 1 and 2, leading to a rise in the free leptin index between stages 1 and 2; there was greater heritability for the soluble leptin receptor than leptin,[411] and there was also high heritability of free IGF1 values. There is no known relationship between leptin and leptin receptor gene polymorphism and CDP; however, the presence of a short allele of leptin was associated with heavier weight, whereas those who were thin, had significant bone age delay, and had increased frequency of parental pubertal delay were less likely to have this leptin short allele.[412]

As in the ob/ob mouse, human beings with a homozygous mutation in the leptin gene[413] or the leptin receptor[414] have morbid obesity and a striking delay in puberty because of hypogonadotropic hypogonadism. In a pedigree affected by a stop codon mutation in the gene encoding leptin, a 23-year-old man failed to attain puberty because of hypogonadotropic hypogonadism, and two affected women were prepubertal and amenorrheic, one until 29 years of age, after which she began to have irregular, scanty periods, and the other until age 36 years, at which time she began to menstruate monthly. A 9-year-old girl with a bone age of 13 years who was affected with congenital leptin deficiency lost weight and had an early pubertal pattern of LH release in response to the administration of GnRH after treatment with recombinant leptin.[415] A 4-year-old affected relative of this 9-year-old girl benefited from the metabolic improvement that occurred with leptin administration but did not undergo early pubertal development, indicating the permissive nature of leptin on puberty.

Boys with CDP have lower mean levels of leptin than expected and can enter puberty without an increase in circulating leptin. Two women with congenital lipoatrophic diabetes (Berardinelli-Seip syndrome), which is associated with absence of subcutaneous and visceral adipose tissue, did not have a delay in menarche despite severe hypoleptinemia, and one of the women had three unaffected children.[416] Severe leptin deficiency causes hypogonadotropic hypogonadism, suggesting that a critical level of leptin and a leptin signal are required to achieve puberty, but a rise in leptin is not required to trigger puberty. In summary, leptin is a permissive factor (tonic mediator) and not a trigger (phasic mediator) in the onset of human puberty (Table 26.12).

TABLE 26.12 Leptin and Puberty: A Permissive Factor, Not a Trigger for the Onset of Puberty

Pro-Trigger Evidence

Congenital leptin deficiency or congenital leptin resistance related to mutations is associated with delayed puberty and gonadotropin deficiency, evidence that the virtual absence of leptin or the leptin signal leads to severe hypogonadotropic hypogonadism.

In congenital leptin deficiency, administration of leptin led to a reduction in weight and an early pubertal pattern of luteinizing hormone release in an affected prepubertal girl.

Pro-Permissive Evidence

A sharp rise in circulating leptin does not occur at the onset of puberty.

In prepubertal and early pubertal girls, the rise in serum leptin did not correlate with the increase in serum estradiol.

In constitutional delay in growth and adolescence, an increase in prepubertal leptin levels is not essential for the onset of puberty.

In congenital lipoatrophic diabetes, despite the absence of subcutaneous and visceral adipose tissue and, as a consequence, severe hypoleptinemia, puberty can occur at the usual age and fertility is reported.

Supportive experimental data exist in rodents, sheep, and nonhuman primates.

From Grumbach MM. The neuroendocrinology of human puberty revisited. *Horm Res.* 2002;57:S2–S14.

In a longitudinal study of boys leading up to an increase in morning salivary testosterone concentrations, they had a relatively constant ratio of basal metabolic rate (BMR) to LBM but an increase in the ratio of BMR to total daily energy expenditure. A subtle energy-dependent process is in play, possibly related to an increase in brain BMR as a secondary phenomenon at the initiation of puberty or indicating that a central rise in BMR is a signal for the onset of puberty.

Ghrelin is the natural ligand for the GH secretagogue receptor but also serves as an orexigenic signal that is increased after food deprivation; ghrelin is usually negatively correlated with BMI. Ghrelin administration delays pubertal development in rats because of gonadal and central effects, suggesting a link between malnutrition and the decrease in reproductive development or function.[417]

Adiponectin is an adipocytokine produced in fat cells that has antidiabetic, antiatherogenic, and anti-inflammatory effects. Adiponectin decreases in the face of excess fat mass in obesity and is suppressed by rising testosterone and DHEAS levels. The concentration of adiponectin falls during pubertal development in males but remains rather stable in females with advancing Tanner stage.[418]

Resistin is an adipocytokine belonging to the resistin-like molecular family of cysteine-rich molecules (RELMs). Values increase with pubertal development in boys, and this appears to be true in girls as well, although the evidence is weaker. Because resistin serum levels were elevated in mouse models of obesity, it was considered to be a potential link between insulin resistance and obesity, but serum resistin levels appear to relate more to pubertal development than to insulin resistance.[419]

TABLE 26.13 Potential Components of the Intrinsic Central Nervous System Inhibitory Mechanism ("Juvenile Pause")

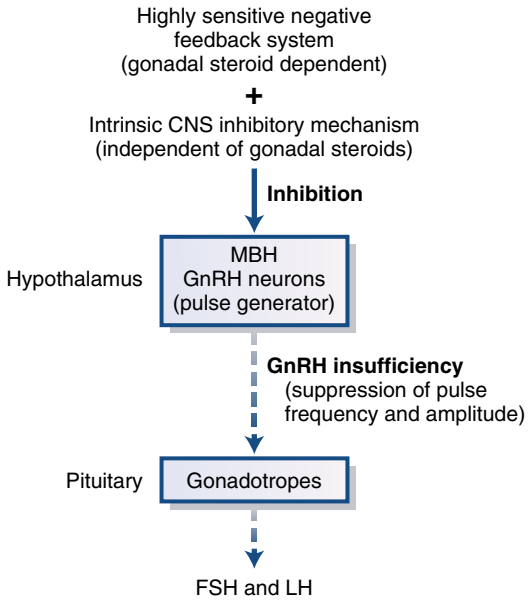
I. Inhibitory

A. Inhibitory central neurotransmitter-neuromodulatory pathways
   1. γ-Aminobutyric acid (the main inhibitory factor)
   2. Endogenous opioid peptides

II. Stimulatory

A. Stimulatory central neurotransmitter-neuromodulatory pathways
   1. Excitatory amino acids
   2. Calcium-mobilizing agonists
   3. Noradrenergic
   4. Dopaminergic
   5. Neuropeptide Y
   6. Nitric oxide
   7. Prostaglandins (PGE_2)

B. Other brain peptides
   1. Neurotrophic and growth peptides
   2. Activin A
   3. Endothelins 1, 2, 3



• **Fig. 26.28** Postulated dual mechanism of restraint of puberty involves gonadal steroid-dependent and gonadal steroid-independent processes (i.e., intrinsic central nervous system [CNS] inhibitory mechanism). *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone; *MBH,* medial basal hypothalamus. (Modified from Grumbach MM, Kaplan SL. The neuroendocrinology of human puberty: an ontogenetic perspective. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:1–68.)

Mechanisms of Control

Diverse strategies and adaptive mechanisms have evolved to control puberty in different species.[332,385] In rodents, exteroceptive factors and cues, including light, olfaction, and pheromones, have an important influence by way of the CNS on gonadotropin secretion. In seasonal-breeding species such as sheep, the length of the light-dark cycle is critical, and the pattern of gonadotropin secretion is different. In contrast, male and female primates exhibit an estrogen-provoked LH surge.

In humans and nonhuman primates, after the initial development and function in the fetus, the infantile surge of increased LH and FSH secretion occurs; this is followed by a decade of suppression (but not absence) of activity of the hypothalamic GnRH pulse generator and the resulting quiescence of the pituitary gonadotropin-gonadal axis, known as the prepubertal period or juvenile pause (Table 26.13).[126,326,332,385] Then there is gradual disinhibition and reactivation, mainly at night during late childhood,[326,332,385,420] and, finally, the increased amplitude of the GnRH pulses, reflected in the progressively increased and changing pattern of circulating LH pulses that occurs with the approach of and during puberty. Two interacting mechanisms have been proposed to explain the juvenile pause[326,420] (Fig. 26.28).

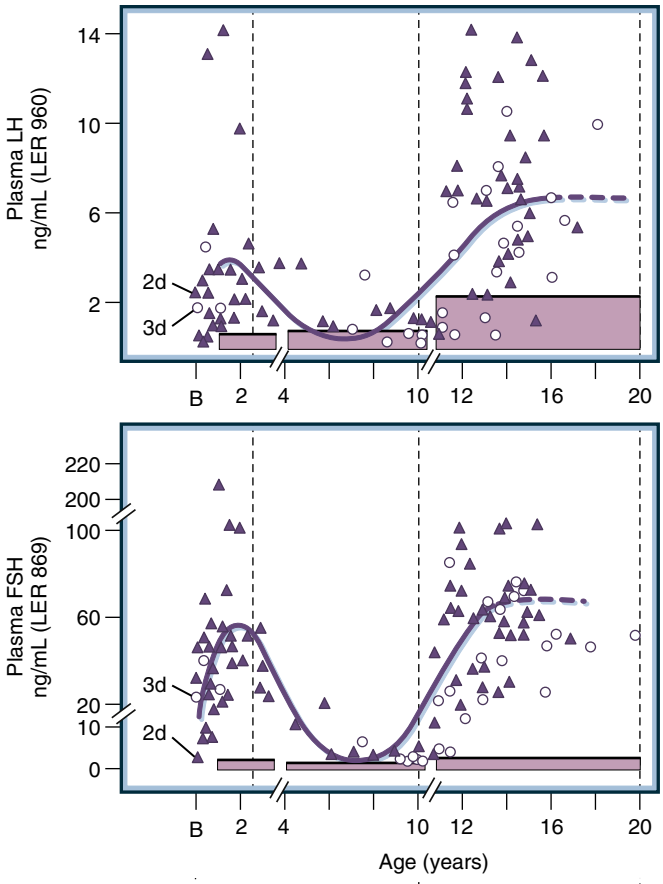
Gonadal Steroid–Dependent Negative Feedback Mechanism

There are three lines of evidence for an operative negative feedback mechanism in prepubertal[256,326] children[258,327]:

1. The pituitary of the prepubertal child secretes small amounts of FSH and LH, showing a low level of activity of the hypothalamic-pituitary-gonadal complex.
2. In agonadal infants and prepubertal children (e.g., in Turner syndrome), secretion of FSH and, to a lesser degree, LH is increased, suggesting that even low levels of hormones secreted by the normal prepubertal gonad inhibit gonadotropin secretion by a sensitive, functional, tonic, negative feedback mechanism[256,326,420] (Fig. 26.29).
3. The low level of gonadotropin secretion in childhood is shut off by administration of small amounts of gonadal steroids, showing that the hypothalamic-pituitary gonadotropin unit is highly sensitive (approximately 6–15 times more sensitive than in the adult) to the feedback effect of gonadal steroids.[258,327]

Gonadal Steroid–Independent (Intrinsic) Central Nervous System Inhibitory Mechanism

The diphasic pattern of basal and GnRH-induced FSH and LH secretion from infancy to adulthood is similar in normal individuals and in agonadal patients, but in the latter, gonadotropin concentrations are higher, except during the middle childhood nadir.[392,393,420] The high plasma concentrations of FSH and LH in agonadal children between infancy and about 4 years of age and the increased gonadotropin reserve reflect the absence of gonadal steroid inhibition (see Fig. 26.29) of the hypothalamic-pituitary unit by the low plasma levels of gonadal steroids.[326,327] However, the striking fall in gonadotropin secretion between the ages of 4 and 11 years suggests the presence of a CNS inhibitory mechanism that restrains the hypothalamic GnRH pulse generator, independent of gonadal steroid secretion. The resulting fall in gonadotropin secretion in agonadal children does not result from gonadal steroid feedback (because functional gonads are lacking) nor from increased secretion of adrenal steroids (because concentrations are low, and glucocorticoid suppression of the adrenal does not augment the concentration of circulating gonadotropins).[326,329,327] A CNS steroid-independent inhibitory mechanism for suppression of the hypothalamic GnRH pulse generator seems to be the dominant factor in restraint of puberty between[326,421] the ages of 4 and 11 years,[327,394] and a gradual loss of this intrinsic CNS inhibitory mechanism leads to disinhibition or reactivation of the GnRH pulse generator at puberty.



	Juvenile pause		
Gonadal steroid negative feedback	3+ Dom	±→1+	3+ Dominant
Intrinsic CNS inhibition	?	3+ Dominant	±→?

• **Fig. 26.29** Interaction of the negative feedback mechanism and the putative intrinsic central nervous system (CNS) inhibitory mechanism in restraining puberty as extrapolated from the pattern of change in the concentrations of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in agonadal infants, children, and adolescents. *Triangles* designate patients with the 45,X karyotype. *Circles* indicate Turner syndrome patients with X chromosome mosaicism or structural abnormalities of the X chromosome, or both. Notice the values for the 2-day-old and 3-day-old infants. The *solid line* represents a regression line of best fit. The *hatched area* indicates the mean plasma values for normal females. To convert FSH values to international units per liter, multiply by 8.4. For about the first 3 years of life, the sensitive gonadal steroid negative feedback mechanism has a dominant role in restraining gonadotropin secretion, as exemplified by the high gonadotropin concentrations in this age group in the absence of gonads (and gonadal steroid feedback). A major role of the intrinsic CNS inhibitory mechanism in this age group is unlikely in light of the rise in gonadotropins to castrate levels in the absence of functional gonads. From 4 to 6 years of age, the postulated intrinsic CNS inhibitory mechanism is dominant, as indicated by the fall in FSH and LH concentrations in the absence of gonads. Even in this age group, the augmented gonadotropin response evoked by gonadotropin-releasing hormone (GnRH) and the slightly higher mean basal gonadotropin concentrations in agonadal individuals support a role, although a subsidiary one, for gonadal steroid negative feedback in the suppression of gonadotropin secretion during this period of the juvenile pause. The investigators suggested that the intrinsic CNS inhibitory mechanism suppresses the functional GnRH pulse generator. After about 10 years of age, the CNS inhibition gradually wanes, resulting in disinhibition of the GnRH pulse generator. The gonadal steroid negative feedback mechanism with an adult-type set-point and inhibin plays a dominant role in regulating the GnRH pulse generator and pituitary gonadotropin system. For conversion to SI units, see Fig. 26.19. (Modified from Grumbach MM, Kaplan SL. The neuroendocrinology of human puberty: an ontogenetic perspective. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:1–68; Conte FA, Grumbach MM, Kaplan SL. A diphasic pattern of gonadotropin secretion in patients with the syndrome of gonadal dysgenesis. *J Clin Endocrinol Metab.* 1975;40:670–674. Copyright by The Endocrine Society.)

Interaction of the Negative Feedback Mechanism and the Intrinsic Central Nervous System Inhibitory Mechanism

The negative feedback mechanism and the intrinsic CNS inhibitory mechanism appear to interact in restraining puberty (see Fig. 26.29). During the first 2 to 3 years of life, the gonadal-steroid negative feedback mechanism seems to be dominant, but beginning at about 3 years of age, the intrinsic CNS inhibitory mechanism becomes dominant and remains so during the rest of the juvenile pause, as evidenced by the fall in FSH and LH levels between the ages of 3 and 10 years despite the lack of functional gonads. The negative feedback mechanism remains operative during the juvenile pause; agonadal patients in this age group have higher mean plasma FSH levels than normal prepubertal children and a greater FSH and LH response to the acute administration of GnRH.[392,393,420] As puberty approaches, the CNS inhibitory mechanism gradually wanes, initially during nighttime sleep, and the hypothalamic GnRH pulse generator becomes less sensitive to gonadal steroid negative feedback (see Fig. 26.29).[326,327] After the onset of puberty, gonadal-steroid negative feedback becomes the dominant mechanism in restraining gonadotropin secretion (along with inhibin), as reflected in the increased gonadotropin concentrations that are characteristic of the adolescent with severe primary hypogonadism.[332] The postulated ontogeny of this dual mechanism of restraint of puberty is illustrated in Fig. 26.29.

Potential Components of the Intrinsic Central Nervous System Inhibitory Mechanism

The intrinsic CNS inhibitory mechanism has long remained elusive.[385] In the rhesus monkey, despite the damping of the GnRH pulse generator during the juvenile pause, the content of hypothalamic GnRH and GnRH mRNA during this phase is similar to that in the infant or the adult monkey. Low-amplitude LH and FSH pulses are detectable by sensitive and specific immunoradiometric assays in the juvenile pause, demonstrating a low level of activity of the GnRH pulse generator.[329,385] The end of the juvenile pause is marked by an increase in LH pulse amplitude that is most evident during the early hours of sleep.

Children with CPP associated with posterior hypothalamic neoplasms (usually a pilocytic astrocytoma), irradiation of the CNS, midline CNS developmental abnormalities such as septo-optic dysplasia with deficiency of one or more pituitary hormones, or other CNS lesions provide indirect evidence for an inhibitory neural component located in or projecting through the posterior hypothalamus. These lesions compromise the neural pathway, which inhibits the hypothalamic GnRH pulse generator and results in its disinhibition and activation leading to CPP.[326] For example, a suprasellar arachnoid cyst can cause CPP by compressing and distorting the hypothalamus,[326] but the puberty is reversed with regression of the hormonal and physical features of puberty after decompression of the cyst due to reversal of the disinhibition of the CNS inhibitory mechanism of the posterior pituitary (Fig. 26.30). Precocious sexual maturation can be induced in the juvenile female rhesus monkey by posterior hypothalamic lesions; such lesions advance the age at onset of a pubertal increase in LH secretion and the time of the first positive feedback effects of estrogen.[385]

The GnRH-secreting hypothalamic hamartoma, a heterotypic mass of nervous tissue that contains GnRH neurosecretory neurons attached to the tuber cinereum or the floor of the third ventricle, can cause CPP.[422,423] The GnRH neurons within the hamartoma with their axon fibers projecting to the median eminence secrete GnRH in pulsatile fashion. We consider the hypothalamic hamartoma to be an ectopic GnRH pulse generator that functions independently of the CNS inhibitory mechanism, which normally restrains the hypothalamic GnRH pulse generator (Fig. 26.31).[326,423] An analogy can be drawn between the GnRH-secreting hypothalamic hamartoma and the rescue of fertility in GnRH-deficient hypogonadal (hyp/hyg) mice by transplantation of fetal or neonatal hypothalamic tissue into the third ventricle. Some rare, large hypothalamic hamartomas that cause CPP contain TGFα, an astroglia-derived growth factor, with few or no GnRH neurosecretory neurons, raising the possibility that the secretion of TGFα may interact directly or indirectly to stimulate GnRH release.

The recent discovery of families with precocious puberty associated with loss of function mutations in the *MKRN3* gene has led to important insights into the CNS "brake" that intrinsically restrains puberty.[424,425] *MKRN3* decreases in mice at the same time that KISS and neurokinin B rise to promote puberty.

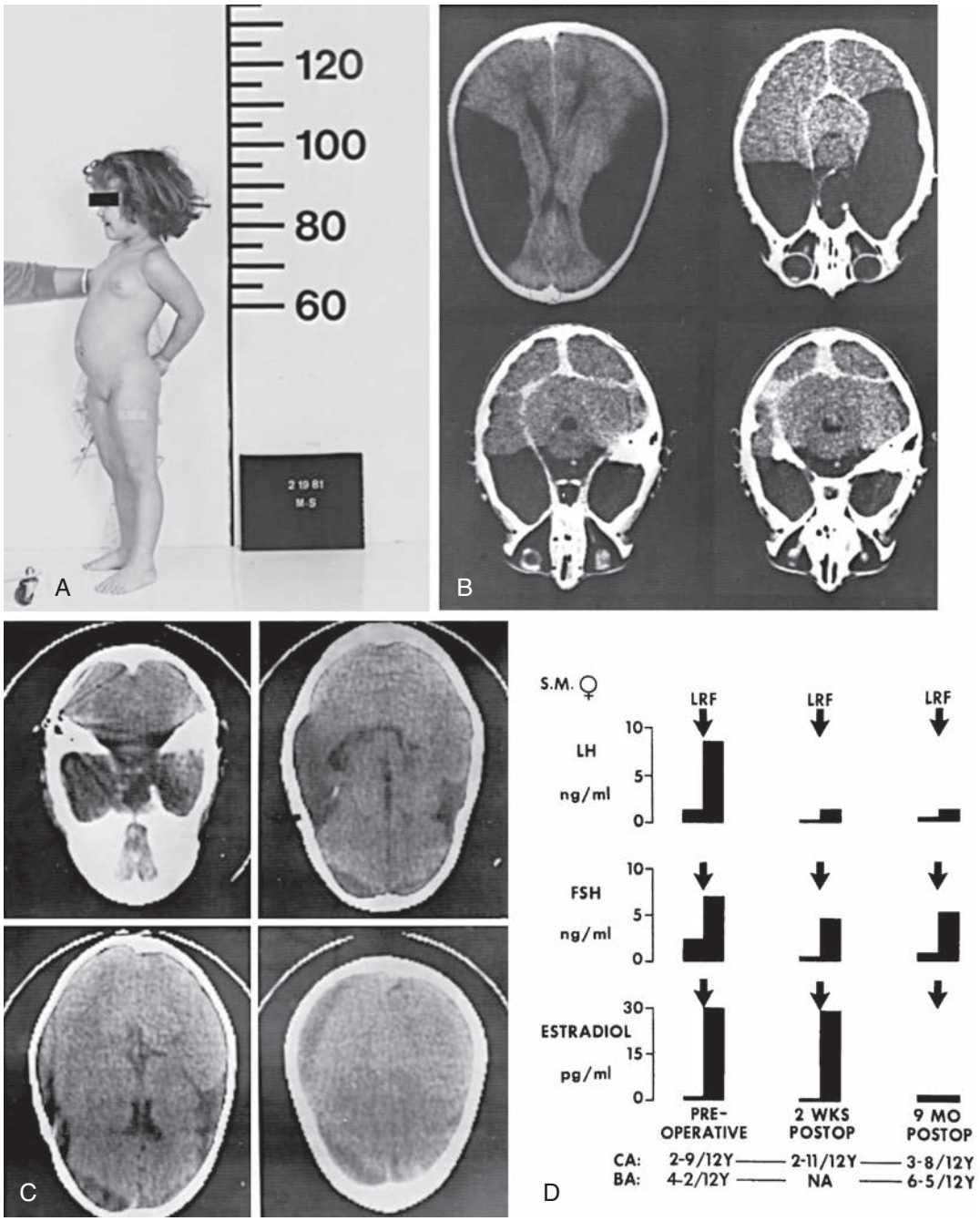
MKRN3 is imprinted as the maternal allele is methylated and suppressed in normal individuals and only the paternal gene functions; with the mutation in the paternal *MKRN3* there is no longer function. *MKRN3* encodes makorin ring finger protein 3, which is involved with ubiquitination and cell signaling, and is expressed in the arcuate nucleus, a location where other genes involved with puberty are found. MKRN3 concentrations in blood decrease in both boys and girls prior to the onset of puberty.[426,427,984]

At present, more than 10 different loss-of-function mutations of *MKRN3* have been described in CPP, including eight frameshift defects, three missense mutations, one nonsense mutation, and a most recent heterozygous deletion in the MKRN3 promoter region[428,429] (see Fig. 26.24B).

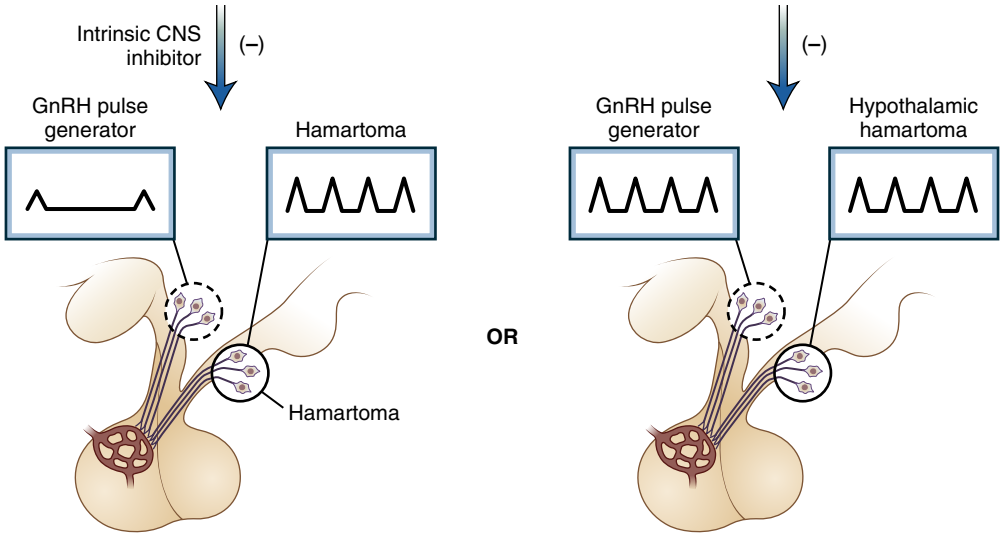
The following affect the hypothalamic GnRH pulse generator: noradrenergic, dopaminergic, serotoninergic, and opioidergic pathways; inhibitory neurotransmitters (e.g., GABA); excitatory amino acids (e.g., glutamic acid, aspartic acid); nitrergic transmitters; other brain peptides, including neurotrophic and growth factors; and corticotropin-releasing hormone (CRH) (see Table 26.13). Melatonin is not a critical restraining factor in primates, nor are endogenous opioid peptides.[326,330,384]

The GnRH pulse generator is inhibited by GABA (the most important inhibitory neurotransmitter in the primate brain) and GABAergic neurons during prepuberty, but exogenous administration of GABA in prepuberty is ineffective because of the high local endogenous GABA levels[328,329] (Fig. 26.32). Both GAD65 and GAD67 forms of glutamic acid decarboxylase (GAD), the enzyme that catalyzes the conversion of glutamate to GABA, are present in the mediobasal hypothalamus, the site of the GnRH pulse generator. Antisense oligodeoxynucleotides for GAD67 and GAD65 mRNAs infused into the stalk median eminence of prepubertal monkeys induced a striking increase in GnRH release, whereas nonsense D-oligos did not. These studies provided additional support for GABA arising from interneurons as the intrinsic CNS inhibitor during the juvenile pause of prepuberty.

GABA acting through $GABA_A$ and $GABA_B$ receptors affects GnRH secretion in the perifused mouse GT1 GnRH-releasing neuronal cell line. Stimulation of $GABA_B$ receptors also inhibits kisspeptin secretion in the adult mouse.[430] Conversely, chronic, repetitive administration of bicuculline, a GABA inhibitor, into the base of the third ventricle of a prepubertal monkey caused premature menarche and the onset of the first ovulation.[328] Although GABA is inhibitory in the juvenile and adult brain, it is excitatory early in brain development through the postnatal period.[431]



• **Fig. 26.30** (A) True precocious puberty in a 2.75-year-old girl is caused by a large, bilateral, congenital suprasellar arachnoid cyst. Signs of sexual precocity were observed during the preceding year. The head circumference was +5 SD above the mean value for age, and frontal bossing was present. Breasts were Tanner stage 3. The serum estradiol level was 26 pg/mL, the estrone level was 38 pg/mL, and the dehydroepiandrosterone sulfate (DHEAS) level was less than 3 μg/dL. The serum luteinizing hormone (LH) concentration rose from 1.4 to 8.7 ng/mL (LER-960) after intravenous administration of gonadotropin-releasing hormone (GnRH), which constitutes a pubertal response. Bone age was 3.5 years. Pelvic ultrasonography showed pubertal-size uterus and ovaries. To convert estrone values to picomoles per liter, multiply by 3.699. To convert DHEAS values to micromoles per liter, multiply by 0.02714. For other conversions, see Fig. 26.19. (B) Cranial computed tomography (CT) scans show a low-density fluid collection in the middle cranial fossa, thinning of the cortex, and striking compression of the lateral and third ventricles. (C) Cranial CT scans 8 months later, after decompression of the arachnoid cyst and creation of a communication between the cyst and the basal cerebrospinal fluid cisterns and a cystoperitoneal shunt. Notice the striking decrease in size of the fluid collections and expansion of the cerebral cortex. (D) Basal and peak LH and follicle-stimulating hormone (FSH) concentrations after GnRH administration in SM and serum estradiol values before surgical decompression and 2 weeks and 9 months after surgical decompression of the arachnoid cyst. Notice the prepubertal LH response to GnRH and fall in serum estradiol level by 9 months after surgery. The bone age had increased by 3 years over an 11-month period, but the velocity returned to normal. The patient remained prepubertal during follow-up. *LRF,* luteinizing hormone-releasing factor; *SD,* standard deviation. (From Grumbach MM, Kaplan SL. The neuroendocrinology of human puberty: an ontogenetic perspective. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:1–68.)



• **Fig. 26.31** Hypothalamic hamartoma as an ectopic gonadotropin-releasing hormone (GnRH) pulse generator that escapes the intrinsic central nervous system (CNS) inhibitory mechanism and results in true precocious puberty. Two possible mechanisms are proposed. *Left,* The GnRH neurosecretory neurons in the hamartoma are functioning as a GnRH pulse generator without activation of the suppressed, normally located GnRH pulse generator. *Right*, The hamartoma acts as an ectopic GnRH pulse generator but communicates with and activates (possibly through axonic connections or by GnRH itself) the normally located hypothalamic GnRH pulse generator, which then functions synchronously with the hamartoma.

The switch from dominance of the gonadal steroid-dependent negative feedback mechanism in infancy and early childhood to dominance of the intrinsic CNS inhibitory mechanism may be associated with the developmental switch of GABAergic synaptic transmission from excitatory to inhibitory.

The onset of puberty in the rhesus monkey is characterized by a decrease in GABAergic (and possibly neuropeptide Y [NPY]) inhibition of the hypothalamic GnRH pulse generator and increased release of glutamate,[329] the major excitatory amino acid neurotransmitter in the hypothalamus.[328] The sensitivity to the stimulatory glutamatergic input into the GnRH pulse generator increases strikingly after the onset of puberty, but it is the reduction in GABAergic inhibition that is the critical factor in disinhibition of the GnRH pulse generator.[329]

A persistent question has been how a single central signal can activate GnRH neurons to cause LH release and bring about ovulation by simultaneous suppression of GABA and stimulation of glutamate release, both of which converge in the AVPV nucleus. Most neurons in the AVPV of female rats express both vesicular glutamate transporter 2 (VGLUT2), a marker of hypothalamic glutamatergic neurons, and GAD and vesicular GABA transporter (VGAT), markers of GABAergic neurons. These dual-phenotype neurons are twice as prevalent in females than in males and are the main targets of the E2 binding site in the region.[432] Moreover, dual-phenotype synaptic terminals contact GnRH neurons, and at the time of the surge, VGAT-containing vesicles decrease and VGLUT2-containing vesicles increase in these terminals. Dual-phenotype GABA/glutamate neurons may act as central transducers of hormonal and neural signals to GnRH neurons to simultaneously decrease GABA and increase glutamate release.

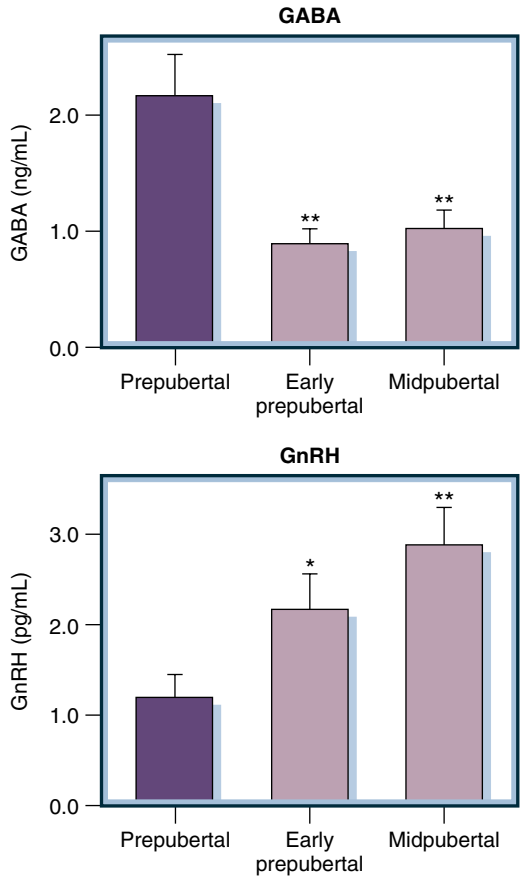
NELL2, a protein containing epidermal growth factor (EGF)–like repeats, is selectively expressed in the glutamatergic neurons containing VGLUT1 and in those expressing VGLUT2 in the postnatal rodent brain. NELL2 mRNA abundance increases selectively in the medial basal hypothalamus of the female rat, reaching a peak at the end of the juvenile period, and declines at the time of puberty with less change observed in the preoptic area. Intraventricular administration of antisense oligodeoxynucleotides to NELL2 reduced GnRH release from the medial basal hypothalamus and delayed the initiation of female puberty. Therefore NELL2 plays an important role in glutamate-dependent processes of neuroendocrine regulation in puberty.[433]

Excitatory NMDA stimulates LH release in neonatal and adult rats, fetal sheep, and prepubertal and adult rhesus monkeys, and its receptors are widely distributed throughout the CNS, including the hypothalamus. NMDA evoked GnRH secretion from rat hypothalamic explants from a GnRH neuronal cell line and acutely stimulated the GnRH pulse generator in the ovine fetus but did not have a direct effect on pituitary gonadotrophs.[332,381,385,434]

Immortalized GnRH neurons contain inotropic NMDA receptors that mediate the release of GnRH by NMDA.[404] The prepubertal male rhesus monkey may be forced to enter puberty by repetitive intravenous administration of NMDA; in the prepubertal and pubertal female rhesus monkey, NMDA administered centrally and peripherally induced the release of GnRH.[435]

NPY has been suggested as a component of the central restraint mechanism in the male rhesus monkey. A small study of girls demonstrated higher NPY concentrations in those with CDP than in those with normal onset of puberty, supporting this relationship.[332,436]

These observations provide additional evidence that the hypothalamic GnRH neurosecretory neuron is not a limiting factor in puberty, as is the GnRH pulse generator; the anterior pituitary gland, gonads, and gonadal steroid end organs are functionally intact in the fetus and prepubertally and can be fully activated by the appropriate stimulus. CNS restraint of puberty lies therefore above the level of the autorhythmic GnRH neurosecretory neurons in the hypothalamus. Fig. 26.13 contrasts the direct and indirect effects of the GABA inhibitory and excitatory amino acid stimulatory neurotransmitters (as represented by NMDA and



• **Fig. 26.32** The striking developmental changes in γ-aminobutyric acid (GABA) and gonadotropin-releasing hormone (GnRH) release between the prepubertal and the pubertal rhesus monkey as measured in 10-minute perfusate samples from the stalk of the median eminence. Multiple samples were obtained from each animal. Mean ± SEM (standard error of the mean); $^{**}p < 0.01$; $^{*}p < 0.05$ vs prepubertal monkeys. (From Mitsushima D, Hei DL, Terasawa E. γ-Aminobutyric acid is an inhibitory neurotransmitter restricting the release of luteinizing hormone-releasing hormone before the onset of puberty. *Proc Natl Acad Sci U S A.* 1994;91:395–399.)

other glutamate receptors) on GnRH release. In the primate, the GABA hypothalamic neural network seems to be the major component of the intrinsic CNS inhibitory mechanism during the juvenile pause.

Sleep-Associated Luteinizing Hormone Release and Onset of Puberty

In sensitive radioimmunoassays, a diurnal rhythm of serum LH, FSH, and testosterone is already demonstrable in short but otherwise normal girls age 5 to 6 years, demonstrating that preparation for the changes of puberty starts long before the physical features and classic endocrine markers of puberty appear.[252,326] Although adult men and women during most phases of the menstrual cycle have little difference in the amplitude or frequency of LH pulses over a 24-hour period, sleep-associated pulsatile release of LH is prominent in early and midpuberty; only in late puberty are prominent LH-secretory episodes detected during the day, but they are still less than during sleep until the adult pattern is achieved. Augmented LH release during sleep leads to a rise in the plasma concentration of testosterone at night in boys, in children with CPP, in glucocorticoid-treated children with CAH who have an advanced bone age and early onset of true puberty, and in agonadal patients during the pubertal age period, suggesting that it does not depend on gonadal function. There is significantly increased excretion of urinary LH in prepubertal children at night compared with the day.

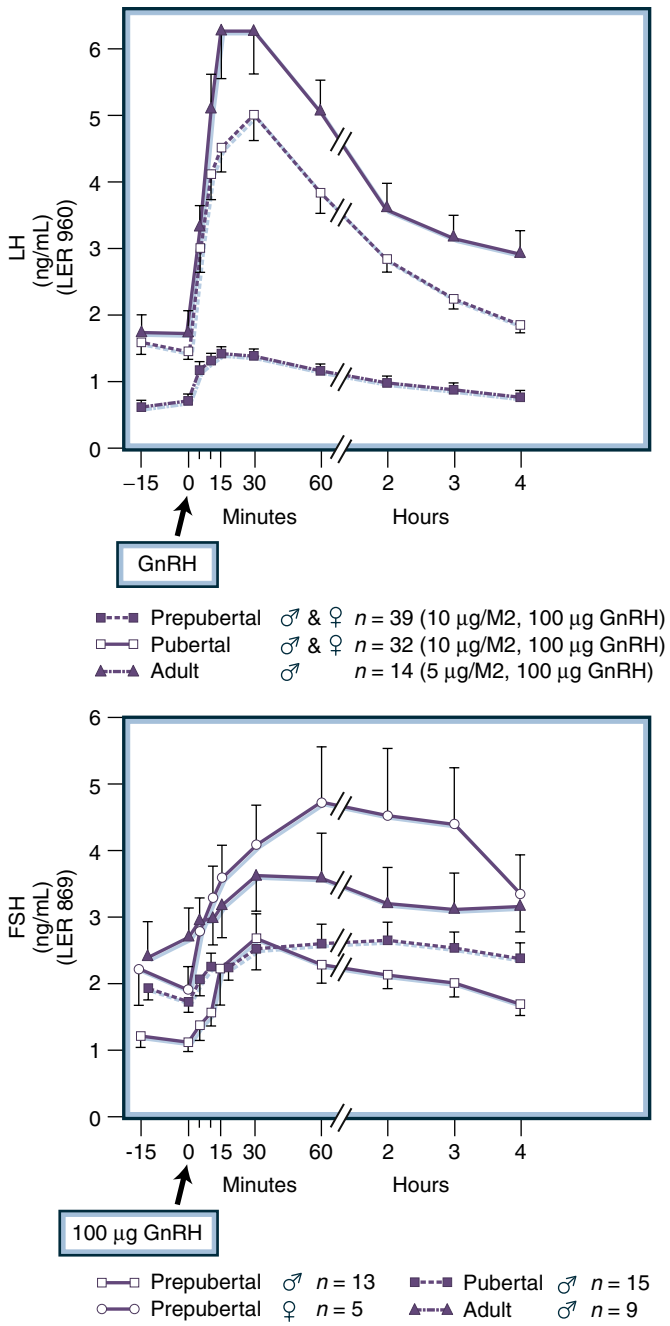
Sleep-enhanced LH secretion can be viewed as a maturational phenomenon related to changes in the CNS and in the hypothalamic restraint of GnRH release. Episodic release of gonadotropins is suppressed by anti-GnRH antibodies and by the administration of gonadal steroids or certain catecholaminergic agonists and antagonists and is augmented by the opioid antagonist naloxone. Naloxone does not alter the testosterone-mediated suppression of LH, nor does it alter the testosterone effect on LH pulsatility in boys in early to middle puberty. We have suggested that an increase in endogenous GnRH secretion at puberty has a priming effect on the gonadotroph and leads to increased sensitivity of the pituitary to endogenous or exogenous GnRH.[256] In monkeys, a striking increase in pulse amplitude and a lesser increase in pulse frequency occurs between prepuberty and puberty.[328,332] Sleep-associated LH release in the peripubertal period correlates with increased sensitivity of the pituitary gonadotrophs to administration of GnRH in the peripubertal period and in puberty and is an indication that the hypothalamic GnRH pulse generator initially is less inhibited during sleep, even in prepubertal children.

Pituitary and Gonadal Sensitivity to Tropic Stimuli

Endogenous GnRH secretion is estimated indirectly and qualitatively by determining the pulsatile pattern of LH and by the gonadotropin response to exogenous GnRH at different stages[425] and in disorders of the hypothalamic-pituitary-gonadal system. The release of LH after administration of GnRH is minimal in prepubertal children beyond infancy, increases during the peripubertal period and puberty (Fig. 26.33), and is still greater in adults (depending on the phase of the menstrual cycle in women).[256] These results support the concept that the prepubertal state is characterized by functional GnRH deficiency. FSH release after the administration of GnRH is comparable in prepubertal, pubertal, and adult males, indicating similar pituitary sensitivity to GnRH, but females release more FSH than males at all stages of sexual maturation. There is a striking reversal of the FSH/LH ratio after administration of GnRH to males or females between prepuberty and puberty (see Fig. 26.33).

These observations suggest a striking change in pituitary sensitivity to GnRH in prepubertal and pubertal individuals and indicate a sex difference in the dynamic reserve of pituitary FSH because the pituitary gonadotrophs of prepubertal females are more sensitive to GnRH than those of prepubertal males, even though the concentration of circulating gonadal steroids is very low in both sexes at that stage of maturation.[256] Prepubertal girls have a larger readily releasable pool of pituitary FSH than prepubertal or pubertal males, possibly related in part to the higher concentration of inhibin B in prepubertal boys (see Fig. 26.33). These may be factors in the higher frequency of idiopathic CPP in girls and in the occurrence of premature thelarche.[437] The available data are consistent with the hypothesis that less GnRH is required for FSH than for LH release.

This change in responsiveness of the gonadotrophs is apparently mediated by increased pulsatile secretion of GnRH[256,326]; the increased LH response to synthetic GnRH is one of the earliest hormonal markers of puberty onset. Studies of the effects of acute and chronic administration of synthetic GnRH in hypergonadotropic hypogonadism, hypogonadotropic hypogonadism, constitutionally delayed growth and adolescence, and idiopathic



• **Fig. 26.33** Changes in plasma luteinizing hormone (LH) *(top)* and follicle-stimulating hormone (FSH) *(bottom)* levels in prepubertal, pubertal, and adult individuals. Notice the limited LH response in prepubertal children compared with that of pubertal and adult subjects. The FSH response to gonadotropin-releasing hormone (GnRH) is similar in prepubertal, pubertal, and adult males. In females, the FSH response is significantly greater than that of prepubertal, pubertal, or adult males. For conversion to SI units, see Fig. 26.19. (Modified from Grumbach MM, Roth JC, Kaplan SL, et al. Hypothalamic pituitary regulation of puberty in man: evidence and concepts derived from clinical research. In: Grumbach MM, Grave GD, Mayer FE, eds. *Control of the Onset of Puberty.* New York, NY: John Wiley & Sons; 1974:115–166.)

precocious puberty indicate that the degree of previous exposure of gonadotrophs to endogenous GnRH appears to affect both the magnitude and the quality of LH responses—a self-priming phenomenon.[256] With the approach of puberty, the derepression of the hypothalamic GnRH pulse generator and the increased pulsatile secretion of GnRH augment pituitary sensitivity to GnRH and enlarge the reserve of LH. Reduction in the frequency of exogenous GnRH pulses (from one per hour to one every 3 hours) in adult rhesus monkeys with ablative hypothalamic lesions that eliminated endogenous GnRH secretion increased the FSH/LH ratio, suggesting that GnRH pulse frequency is one factor affecting relative secretion of FSH and LH. Inhibin and endogenous gonadal steroids may also affect this ratio through action on the hypothalamus or the pituitary or both.

Pulsatile administration of GnRH to prepubertal monkeys promptly initiates puberty (and ovulatory menstrual cycles in females) and restores complete gonadal function in adult monkeys with hypothalamic lesions.[332,438] Similar studies in humans yielded comparable results for prepubertal children, patients with anorexia nervosa, and adults with hypothalamic-hypogonadotropic hypogonadism.[439] These results provide further support for reactivation of the hypothalamic GnRH pulse generator as the first hormonal change in the onset of puberty.

Responsiveness of the gonads to gonadotropins increases during puberty. For example, the augmented testosterone secretion in response to administration of hCG at puberty in boys is probably a consequence of the priming effect of the increase in endogenous secretion of LH (in the presence of FSH) on the Leydig cells.

Maturation of Positive Feedback Mechanism

Estrogen exerts suppressive effects from late fetal life to peripuberty, when the positive action of endogenous (or exogenous) estradiol on gonadotropin release is not demonstrated.[256,326] A positive feedback effect, which is required for ovulation, is a late maturational event in puberty and probably does not occur before midpuberty in normal girls. The positive feedback effect requires an increased concentration of plasma estradiol for a sufficient length of time during the latter part of the follicular phase in later pubertal and adult women.[336]

Among the requirements for the positive feedback action of estradiol on gonadotropin release at puberty are ovarian follicles that are primed by FSH to secrete sufficient estradiol to reach and maintain a critical level in the circulation, a pituitary gland that is sensitized to GnRH and contains a large enough pool of releasable LH to support an LH surge, and sufficient GnRH stores for the GnRH neurosecretory neurons to respond with an acute increase in GnRH release in addition to the usual adult pattern of pulsatile GnRH secretion (this last requirement is controversial in humans but not in lower animals).[256]

Estrogen exerts effects at the anterior pituitary and the hypothalamus.[440] In the rhesus monkey, positive and negative feedback can occur in adult ovariectomized females in whom the medial basal hypothalamus is surgically disconnected from the remainder of the CNS.[332] In monkeys with hypothalamic lesions, unvarying, intermittent GnRH administration leads to sufficient estradiol release from the ovary to induce an ovulatory LH surge in the absence of an increase in the dose of the GnRH pulses.[336,438] Estradiol has a positive feedback effect directly on the pituitary gland in normal women, and prolonged administration of estradiol is accompanied by an augmented LH response to GnRH administration. The fact that the major positive feedback action on the pituitary gland is demonstrable in the absence of an increase in pulsatile GnRH secretion suggests that the failure to elicit positive feedback action with administration of estradiol to prepubertal girls may be related to the inadequate GnRH pulses or insufficient LH reserve or both.

Gonadotropin cyclicity and estradiol-induced positive feedback can be demonstrated by midpuberty and before menarche but may be insufficient to induce an ovulatory LH surge even when there is an adequate pituitary store of readily releasable LH and FSH.[1,256] The ovary does not secrete estradiol at a high level or long enough to induce an ovulatory LH surge. We visualize the process leading to ovulation as a gradual one in which the ovary (i.e., the zeitgeber for ovulation[336]) and the hypothalamic-pituitary-gonadal complex become progressively more integrated and synchronous until an ovary primed for ovulation secretes sufficient estradiol to induce an ovulatory LH surge.

As many as 55% to 90% of cycles are anovulatory during the first 2 years after menarche, but the proportion decreases to less than 20% of cycles by 5 years after menarche.[441] The mechanism of ovulation seems unstable and immature, and it does not appear to have attained the fine tuning and synchronization that are requisite for maintenance of regular ovulatory cycles. However, the prevalence of PCOS adds to the irregularity of menses and anovulation in puberty.

Overview of Current Concept

Puberty is not an immutable process; it can be arrested or reversed. Environmental factors and certain disorders that affect the onset or progression of puberty mediate their effects by direct or indirect suppression of the hypothalamic GnRH pulse generator and its periodic oscillatory signal, GnRH. Table 26.14 lists some of these factors.

Adrenal Androgens and Adrenarche

Speculation has focused on the mechanism of adrenarche (the adrenal component of pubertal maturation), the fact that adrenarche occurs earlier than gonadarche (the maturation of the hypothalamic-pituitary-gonadal system), and the interaction between adrenal and gonadal hormones at puberty.[290,325,442]

Nature and Regulation of Adrenal Androgens

The major adrenal androgen precursors secreted by the adrenal cortex are DHEA, DHEAS, and androstenedione, which can undergo extraglandular metabolism to produce physiologically active testosterone and estradiol[159]; however, adrenal androgens do not directly activate the AR. DHEA and especially DHEAS (which binds avidly to serum proteins, particularly albumin) are useful biochemical markers of adrenal androgen secretion and the onset of adrenarche. Androstenedione is the major androgen secreted by the ovary during and after puberty, and it is more readily converted to potent androgens than DHEA or DHEAS.

Cross-sectional and longitudinal studies have demonstrated a progressive increase in the plasma concentration of DHEA and DHEAS in boys and girls starting by 3 years of age and becomes more noticeable approximately 2 years before the increase in gonadotropin and gonadal steroid secretion that continues through puberty (13–15 years),[290,325,443,444] reaches a peak at age 20 to 30 years, and then gradually decreases (Fig. 26.34).[442] The increase is not associated with increased sensitivity of the pituitary gonadotrophs to GnRH[430] or with sleep-associated LH secretion and occurs at an age when the hypothalamic-pituitary-gonadal complex is functioning at a low level.[290,437] The importance of adrenarche is a matter of long-term debate. DHEA is a neurosteroid with parallel patterns of increase along with cortical maturation from approximately age 6 years to the middle 20s, suggesting that adrenarche affects brain development. DHEAS may increase activity of the amygdala and hippocampus and promote synaptogenesis within the cortex, with effects on fearfulness,

TABLE 26.14 Postulated Ontogeny of the Hypothalamic-Pituitary-Gonadal Circuit

Fetus

- Medial basal hypothalamic GnRH neurosecretory neurons (pulse generator) operative by 80 days of gestation
- Pulsatile secretion of FSH and LH by 80 days of gestation
- Initially unrestrained secretion of GnRH (100–150 days of gestation)
- Maturation of negative gonadal steroid feedback mechanism by 150 days of gestation—sex difference
- Low level of GnRH secretion at term

Early Infancy

- Hypothalamic GnRH pulse generator highly functional after 12 days of age
- Prominent FSH and LH episodic discharges until approximately age 6 mo in males and 18 mo in females, with transient increases in plasma levels of testosterone in males and estradiol in females

Late Infancy and Childhood

- Intrinsic CNS inhibition of hypothalamic GnRH pulse generator operative; predominant mechanism in childhood; maximal sensitivity by approximately 4 years of age
- Negative feedback control of FSH and LH secretion highly sensitive to gonadal steroids (low set-point)
- GnRH pulse generator inhibited; low amplitude and frequency of GnRH discharges
- Low secretion of FSH, LH, and gonadal steroids

Late Prepubertal Period

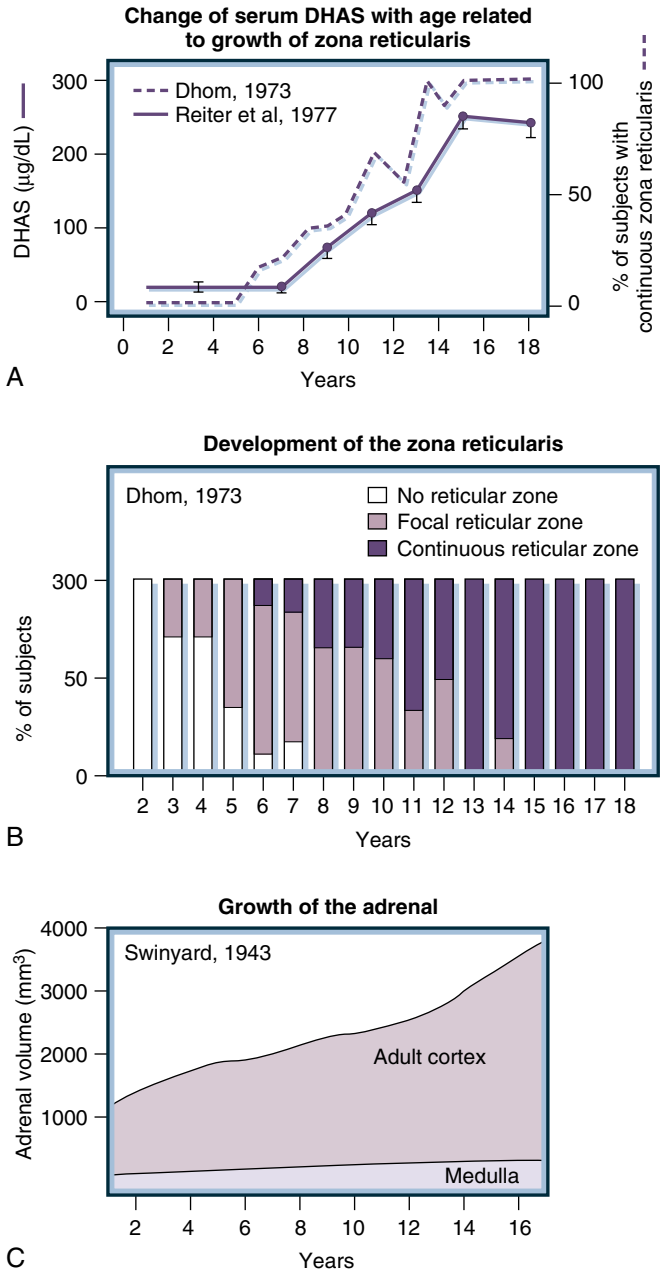
- Decreasing effectiveness of intrinsic CNS inhibitory influences and decreasing sensitivity of hypothalamic-pituitary unit to gonadal steroids (increased set-point)
- Increased amplitude and frequency of GnRH pulses, initially most prominent with sleep (nocturnal)
- Increased sensitivity of gonadotrophs to GnRH
- Increased secretion of FSH and LH
- Increased responsiveness of gonad to FSH and LH
- Increased secretion of gonadal hormones

Puberty

- Further decrease in CNS restraint of hypothalamic GnRH pulse generator and in the sensitivity of negative feedback mechanism to gonadal steroids
- Prominent sleep-associated increase in episodic secretion of GnRH gradually changes to adult pattern of pulses about every 90 min
- Pulsatile secretion of LH follows pattern of GnRH pulses
- Progressive development of secondary sexual characteristics
- Spermatogenesis in males
- Middle to late puberty—operative positive feedback mechanism and capacity to exhibit an estrogen-induced LH surge
- Ovulation in females

CNS, Central nervous system; *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone.

Modified from Grumbach MM, Roth JC, Kaplan SL, et al. Hypothalamic-pituitary regulation of puberty in man: evidence and concepts derived from clinical research. In: Grumbach MM, Grave GD, Mayer FE, eds. *Control of the Onset of Puberty.* New York: John Wiley & Sons; 1974:115–166.



• **Fig. 26.34** Relation of plasma dehydroepiandrosterone sulfate (DHEAS [DHAS]) levels to growth of the zona reticularis and increase in adrenal volume with age. (A) The close correlation between the development of the zona reticularis and the increase in the plasma DHEAS level. (B) The age at which focal islands of reticular tissue or a continuous reticular zone was found in a series of patients with sudden death who had not had an antecedent illness. (C) The increase in adrenal volume at the time of puberty. For conversion to SI units, see Fig. 26.19. (From Grumbach MM, Richards HE, Conte FA, et al. Clinical disorders of adrenal function and puberty: assessment of the role of the adrenal cortex and abnormal puberty in man and evidence for an ACTH-like pituitary adrenal androgen stimulating hormone. In: James VHT, Serio M, Giusti G, et al, eds. *The Endocrine Function of the Human Adrenal Cortex.* New York, NY: Academic Press; 1978:583–612.)

anxiety, and memory that increase social interaction with unfamiliar individuals and shape cognitive development.[445]

Associated with the increase in adrenal secretion of DHEA and DHEAS (and independent of a change in the secretion of cortisol or aldosterone) is the appearance and growth of the zona reticularis (i.e., the principal source of DHEA and DHEAS) that occurs coincidently with adrenarche (Fig. 26.35).

In contrast to the zona glomerulosa and fasciculata, four main features distinguish the zona reticularis:

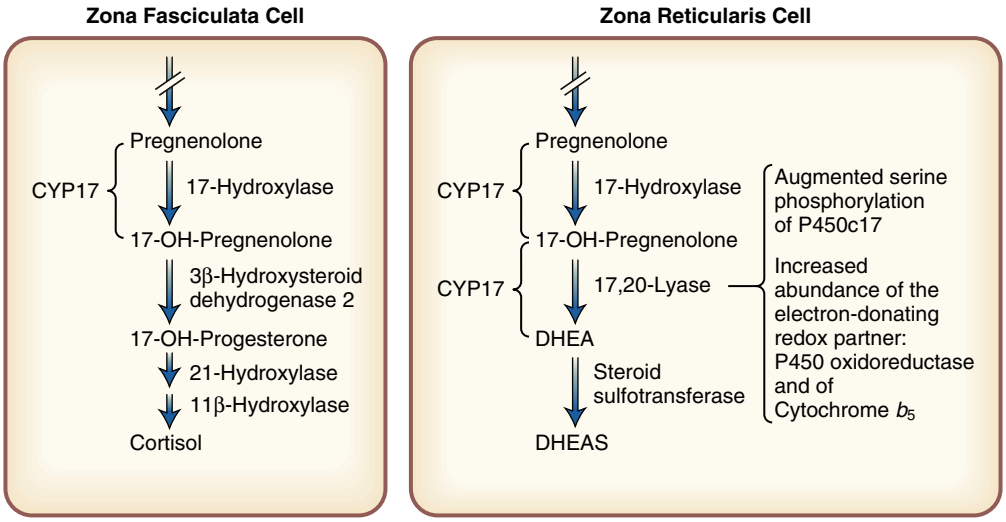
1. There is a low level of expression of 3βHSD/$\Delta^{4,5}$-isomerase type 2 and CYP21 mRNAs and enzyme activities.
2. Abundant DHEA (hydroxysteroid) sulfotransferase activity is found.
3. There is a relative increase in 17,20-lyase vs 17α-hydroxylase activity of CYP17, the enzyme that catalyzes both activities. These characteristics are shared by the fetal zone of the fetal adrenal cortex.
4. There is expression of major histocompatibility complex (MHC) class II (HLA-DR) antigens, which are not expressed in the fetal zone of the fetal adrenal cortex.

In contrast to the zona fasciculata, the zona reticularis has an increased ratio of 17,20-lyase to 17α-hydroxylase. An Arg347Ala mutation in human CYP17 resulted in strikingly decreased 17,20-lyase activity but retention of 17α-hydroxylase activity. Two XY phenotypic females with hypergonadotropic hypogonadism and normal mineralocorticoid and glucocorticoid function had isolated 17,20-lyase deficiency due to homozygous mutations at the Arg347 or the Arg358 residue in CYP17.

In contrast to these observations of loss of 17,20-lyase activity with retention of 17α-hydroxylase activity, the ratio of human 17,20-lyase to 17α-hydroxylase activities was increased by increased phosphorylation of serine and threonine residues on the CYP17 enzyme and by the increased abundance of redox partners such as cytochrome P450 oxidoreductase and by cytochrome b_5, which preferentially promotes 17,20-lyase activity by allosterically affecting the interaction between CYP17 and P450 oxidoreductase.[446] These studies provided a provisional hypothesis of the mechanisms that appear to be involved in the relatively increased 17,20-lyase activity of the zona reticularis, although not its regulation (see Fig. 26.35).

Regulation of adrenal androgen secretion in the zona reticularis is postulated to be based on a dual-control mechanism. First, corticotropin (adrenocorticotropic hormone [ACTH]) is obligatory, as evidenced by the findings in cases of ACTH deficiency or resistance. Second, the mechanism requires the action of an unidentified adrenal androgen-stimulating factor, possibly pituitary in origin or from a nonadrenal source or an intra-adrenal event.[301] This concept is illustrated in Fig. 26.36.[443]

CRH has been advanced as an adrenal androgen secretagogue that has stimulatory action on the zona reticularis. The intravenous infusion of human CRH into dexamethasone-suppressed young men increased DHEA, DHEAS, and androstenedione secretion within 3 hours. Similar results were obtained in adolescent girls with hyperandrogenism and a history of premature adrenarche. CRH directly stimulates DHEAS secretion and the expression of CYP17 by the fetal adrenal cortical cells.[447] Leptin in vitro vigorously stimulates 17,20-lyase activity and transiently stimulates 17α-hydroxylase activity of the microsomal enzyme CYP17, implying a role in adrenarche,[448] but no clinical evidence suggests a pivotal role of leptin in adrenarche. Therefore a distinct hormone or factor that in addition to ACTH stimulates the zona reticularis and adrenal androgen secretion has not been isolated, and the mechanism regulating adrenarche remains unknown.[325]



• **Fig. 26.35** Adrenarche and the zona reticularis. The rise in circulating dehydroepiandrosterone sulfate (DHEAS) levels is the biochemical hallmark of adrenarche. The diagram compares and contrasts the major steroidogenic pathway in the zona fasciculata with that in the zona reticularis. In contrast to the zona fasciculata, the expression of 3β-hydroxysteroid, $\Delta^{4,5}$ isomerase type 2 messenger ribonucleic acid (mRNA), and its activity (the enzyme that irreversibly traps Δ^5 precursors into Δ^4 steroids) is very low in the zona reticularis, whereas the expression of and activity of steroid sulfotransferase is high. A single gene, *CYP17* (now designated *CYP17A1*), encodes a single enzyme that has 17α-hydroxylase and 17,20-lyase activity, but the ratio of 17,20-lyase to 17α-hydroxylase activity is relatively high in the zona reticularis compared with that in the zona fasciculata. Some of the factors that seem to amplify the increased 17,20-lyase activity of *CYP17* are the augmented serine phosphorylation of the enzyme and the apparent increased abundance of the electron-donating redox partner, including P450 reductase and of cytochrome b_5.

A distinct adrenal androgen-stimulating factor, whether of pituitary, intra-adrenal, or other origin, may explain the following observations[301]:

1. The spurt in adrenal growth and the differentiation and growth of the zona reticularis at adrenarche occur independently of an increase in ACTH or cortisol secretion but correlate with the increase in plasma levels of DHEAS (see Fig. 26.35).
2. Cortisol and adrenal androgen secretions vary independently with age, during normal and premature adrenarche, and in Cushing disease, starvation, malnutrition, anorexia nervosa, and chronic disease.
3. Unlike cortisol secretion, the secretion of DHEA and DHEAS in response to ACTH administration varies with age.
4. Dissociation of adrenarche and gonadarche occurs in a variety of disorders of sexual maturation (see Fig. 26.36), including premature adrenarche (i.e., onset of pubic or axillary hair before 8 years of age), chronic adrenal insufficiency, CPP (when the onset is before age 6 years), primary hypogonadism, isolated gonadotropin deficiency, and anorexia nervosa.[442]

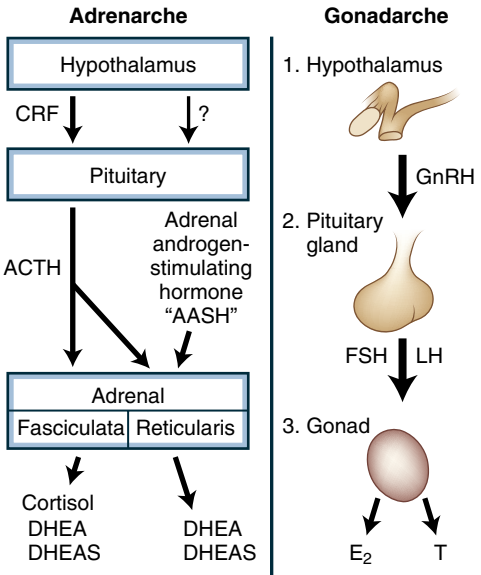
A longitudinal study of 42 children demonstrated that an increase in BMI (but not the value itself at any age) was related to the rise in urinary excretion of DHEAS, suggesting that a change in nutritional status is one physiologic regulator of adrenarche.[444,449]

The increase in DHEA before and at adrenarche might suggest a similarity to the actions of the fetal adrenal zone; indeed the histologic finding and biochemistry are similar in both the fetal adrenal zone and the zona reticularis. However, the fetal adrenal zone undergoes involution at birth; in addition biomarkers such as enzyme AKR1C3 (17β-hydroxysteroid dehydrogenase type 5 [17βHSD5]) are higher in the zona reticularis than the fetal adrenal zone.[325]

Adrenal Androgens and Puberty

The earlier onset of adrenarche than gonadarche and the contribution of adrenal androgens to the growth of pubic and axillary hair have led some to suggest that adrenal androgens in normal children are an important factor in the onset of puberty and the maturation of the hypothalamic-pituitary-gonadal complex.

Although CPP may occur when the prepubertal child has previously been exposed to excessive levels of androgens from an endogenous or exogenous source (e.g., after the initiation of glucocorticoid therapy in congenital virilizing adrenal hyperplasia or after removal of a sex steroid–secreting adrenal or gonadal neoplasm[290,450]), there is little evidence that adrenal androgens play an important qualitative or rate-limiting role in the onset of puberty in normal children.[290] Most patients with premature adrenarche, who secrete excessive amounts of adrenal androgens for their age, enter puberty and experience menarche within the normal age range.[290] Moreover, prepubertal children who have congenital or acquired chronic adrenal insufficiency (i.e., Addison disease) and consequently have deficient or absent adrenal androgen secretion usually have a normal onset and normal progression through puberty when given appropriate glucocorticoid and mineralocorticoid replacement therapy.[290] Studies of children with chronic adrenal insufficiency, isolated gonadotropin deficiency, hypergonadotropic hypogonadism, or androgen resistance suggest that adrenal androgens in girls and boys are not essential for the adolescent growth spurt, whereas gonadal steroids secreted by the testis and ovary are essential and act in concert with GH.[290] The transient increase in height velocity (about 1.5 cm/year in both sexes) that occurs in middle childhood (6–7 years) and lasts about 2 years terminates while the serum DHEAS level continues



Premature adrenarche	+	–
Primary adrenal insufficiency	–	+
Idiopathic precocious puberty (onset <6 yrs)	–	+
Idiopathic precocious puberty (onset >6 yrs)	+	+
Syndrome of gonadal dysgenesis	+	–
Isolated gonadotropin deficiency	+	–
Constitutional delayed adolescence	–	–

• **Fig. 26.36** Hypothesis of the control of pituitary adrenal androgen secretion by a putative separate adrenal androgen-stimulating hormone acting on a corticotropin (ACTH)–primed adrenal cortex. Although this diagram suggests that adrenal androgen-stimulating hormone (AASH) arises from the pituitary gland, a distinct pituitary factor with AASH activity has not been isolated; an extrapituitary factor is not excluded. The lower part of the diagram shows the relationship of adrenarche to gonadarche, including dissociation in various clinical disorders of sexual development (+, present; –, absent). *CRF,* corticotropin-releasing factor; *DHEA,* dehydroepiandrosterone; *DHEAS,* DHEA sulfate; *E_2,* estradiol; *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *T,* testosterone. (Modified from Sklar CA, Kaplan SL, Grumbach MM. Evidence for dissociation between adrenarche and gonadarche: studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotropin deficiency, and constitutionally delayed puberty. *J Clin Endocrinol Metab.* 1980;51:548–556. Copyright by The Endocrine Society.)

to increase. This increase in height velocity is related to the cyclic pattern of prepubertal growth and to genetic regulation of growth rather than to an increase in adrenal androgen or GH secretion.[451]

Disorders of Puberty

Delayed Puberty and Sexual Infantilism

The upper limits of the normal age of onset of puberty are 2 SD above the mean and are 14 years for boys (although some sources say 13.5 years) and 13 years for girls (Table 26.15). Functionally, delayed puberty can be divided into disorders that affect the operation of the GnRH pulse generator, the pituitary gland, or the gonad.

Idiopathic or Constitutional Delay in Growth and Puberty

Otherwise healthy girls who spontaneously enter puberty after the age of 13 years and boys who begin after 14 years may have constitutional delay in growth and adolescence (CDP), the most common diagnosis for delayed puberty. Affected individuals usually are short (2 SD below the mean value of height for age) at evaluation and have been shorter than their classmates for years, although growth velocity and height are usually appropriate for bone age (Fig. 26.37 and Table 26.16). Family history in as many as 77% of cases reveals a mother who had delayed menarche or a father (or sibling) who entered puberty late (i.e., age 14–18 years), and the pattern in some cases suggested dominant inheritance with incomplete penetrance.[452–454]

Recent GWAS studies demonstrate a low prevalence of genes found in CHH in CDP[455,456]; remarkably, genes usually in CHH were found in 17% of controls. However, there was only 2% oligogenicity in controls compared to 15% oligogenicity in CHH subjects, demonstrating the importance of this genetic phenomenon (oligogenicity is discussed later). Recently mutations in immunoglobulin superfamily member 10 (IGSF10) were found in males with CDP.[457] This gene is important in the earliest stage of GnRH neuron migration from the nasal mesenchyme to the border with the telencephalon in mice; mutations in this gene appear to inhibit the normal migration of the neurons. While the IGSF10 gene is not solely responsible for IHH, it appears to play a role in partial HH found in hypothalamic amenorrhea. Finding mutations in CDP may demonstrate a relationship with IHH.

Constitutional delay in development is physiologic immaturity with a slow tempo of maturation; full sexual maturity will be reached, but the process takes longer than usual but almost always occurs by 18 years. Because of delay in reactivation of the GnRH pulse generator, there is functional deficiency of GnRH for chronologic age but not for the stage of physiologic development. Adrenarche and gonadarche occur later in individuals with constitutional (idiopathic) delay in growth and adolescence,[452] whereas adrenarche reportedly occurs at a normal age in patients with isolated gonadotropin deficiency.[442] Bone age is delayed at presentation, but after a bone age of approximately 12 to 14 years for boys or 11 to 13 years for girls is achieved, sexual maturation begins (although bone age is variable and not a reliable indicator). Patients achieve sexual maturation by 18 years of age.

Most of these patients with CDP are thin, but 25% are above the 85th percentile in BMI for age, and the bone age in these heavier boys is less delayed than in classic thin patients with congenital disorders of glycosylation (CDG) (i.e., they tend to achieve taller adult stature[452]). Prepubertal boys who meet the criteria for diagnosis with delayed bone age have greater increased total energy expenditure (TEE) per kilogram fat-free mass (FFM) and increased nutritional needs possibly due to an alteration in mitochondrial metabolism or increased nonexercise activity thermogenesis (NEAT); increased nutrition increased energy intake but TEE rose as well, continuing the need for high energy intake.[458,459] There is no impairment of olfaction, as in Kallmann syndrome, and undescended testes occur in the rate found in the general population. Plasma gonadal steroid levels are low in sensitive pediatric assays at the time of presentation, but as bone age advances, the serum gonadotropin concentration and the amplitude of LH

TABLE 26.15 Classification of Delayed Puberty and Sexual Infantilism

IDIOPATHIC (CONSTITUTIONAL) DELAY IN GROWTH AND PUBERTY (DELAYED ACTIVATION OF HYPOTHALAMIC LRF PULSE GENERATOR)

HYPOGONADOTROPIC HYPOGONADISM: SEXUAL INFANTILISM RELATED TO GONADOTROPIN DEFICIENCY

CNS Disorders

Tumors

Craniopharyngiomas
Germinomas
Other germ cell tumors
Hypothalamic and optic gliomas
Astrocytomas
Pituitary tumors (including MEN1, prolactinoma)

Other Causes

Langerhans histiocytosis
Postinfectious lesions of the CNS
Vascular abnormalities of the CNS
Radiation therapy
Congenital malformations especially associated
  with craniofacial anomalies
Head trauma
Lymphocytic hypophysitis

Isolated Gonadotropin Deficiency

Kallmann syndrome
  With hyposmia or anosmia
  Without anosmia
LHRH receptor mutation
Congenital adrenal hypoplasia (*DAX1* mutation)
Isolated LH deficiency
Isolated FSH deficiency
Prohormone convertase 1 deficiency (PCI)

Idiopathic and Genetic Forms of Multiple Pituitary Hormone Deficiencies Including PROP1 Mutation

Miscellaneous Disorders

Prader-Willi syndrome
Laurence-Moon and Bardet-Biedl syndromes
Functional gonadotropin deficiency
  Chronic systemic disease and malnutrition
  Sickle cell disease
  Cystic fibrosis
  Acquired immunodeficiency syndrome (AIDS)
  Chronic gastroenteric disease
  Chronic renal disease
  Malnutrition
  Anorexia nervosa
  Bulimia
  Psychogenic amenorrhea
  Impaired puberty and delayed menarche in female athletes and ballet dancers (exercise amenorrhea)
  Hypothyroidism
  Diabetes mellitus
  Cushing disease
  Hyperprolactinemia
  Marijuana use
  Gaucher disease

HYPERGONADOTROPIC HYPOGONADISM

Males

The syndrome of seminiferous tubular dysgenesis and its variants (Klinefelter syndrome)
Other forms of primary testicular failure
  Chemotherapy
  Radiation therapy
  Testicular steroid biosynthetic defects
  Sertoli-only syndrome
  LH receptor mutation
  Anorchia and cryptorchidism
Trauma/surgery

Females

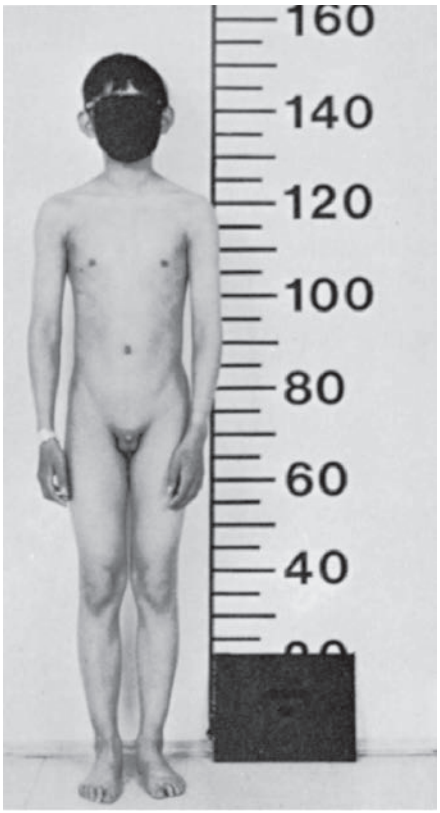
The syndrome of gonadal dysgenesis (Turner syndrome) and its variants
XX and XY gonadal dysgenesis
  Familial and sporadic XX gonadal dysgenesis and its variants
  Familial and sporadic XY gonadal dysgenesis and its variants
Aromatase deficiency
Other forms of primary ovarian failure
  Premature menopause
  Radiation therapy
  Chemotherapy
  Autoimmune oophoritis
  Galactosemia
  Glycoprotein syndrome type 1
  Resistant ovary
  FSH receptor mutation
  LH/hCG resistance
  Polycystic ovarian disease
  Trauma/surgery
  Noonan or pseudo-Turner syndrome
  Ovarian steroid biosynthetic defects

CNS, Central nervous system; *hCG*, human chorionic gonadotropin; *LHRH*, luteinizing hormone–releasing hormone; *LRF*, luteinizing hormone–releasing factor; *MEN*, multiple endocrine neoplasia.

pulses increase (initially at night); the basal serum gonadotropin concentrations measured by third-generation assays and the LH response to GnRH or GnRH agonists reflect maturation of the hypothalamic-pituitary system.

The first signs of secondary sexual development occur within 1 year after LH rises to pubertal levels in response to administration of intravenous synthetic GnRH or subcutaneous GnRH agonist or within 1 year after gonadotropin and testosterone or estradiol concentrations begin to increase spontaneously above prepubertal values.[256,326] An 8 AM serum testosterone value of 0.7 nmol/L (20 ng/dL) heralds the development of phenotypic puberty in boys within 12 to 15 months.[460]

CDP is more common in boys and may be the opposite of idiopathic CPP, a condition that is many times more common in girls. Familial short stature is a physiologic variant of growth in which the velocity of development and bone age are normal but stature is decreased, in contrast to CDP, which is a disorder of tempo that secondarily impairs growth. The combination of CDP and familial short stature leads to conspicuous shortness during adolescence, especially when other children increase their growth velocity,



• **Fig. 26.37** A boy age 16 years and 2 months has constitutional delay in growth and puberty. His height is 149.5 cm (4 SD [standard deviation] below the mean value for age); upper-to-lower body ratio is 1.1 (retarded for age); phallus is 6.0 × 1.6 cm; testes are 2.5 × 1.4 cm; and the scrotum showed early thinning. At a chronologic age of 15 years and 4 months, the bone age was 11 years and the sella turcica was normal. The plasma concentration of luteinizing hormone (LH) was 0.7 ng/mL (LER-960); the concentration of follicle-stimulating hormone (FSH) was 0.5 ng/mL (LER-869). On gonadotropin-releasing hormone (GnRH) testing, the plasma concentration of LH increased to 2.2 ng/mL (an increment of 1.5 ng/mL), and the testosterone level rose from 52 to 77 ng/dL. The testes subsequently spontaneously enlarged, and the patient progressed through puberty. For conversion to SI units, see Figs. 26.19 and 26.20. (From Styne DM, Grumbach MM. Puberty in the male and female: its physiology and disorders. In: Yen SCC, Jaffe RB, eds. *Reproductive Endocrinology*. 2nd ed. Philadelphia, PA: WB Saunders; 1986:313–384.)

and referrals occur more often with this combination than with either condition alone. Because no single test reliably distinguishes between CDP and IHH, watchful waiting is usually in order.

The growth rate before the actual onset of puberty in constitutional delay is often suboptimal for chronologic age, but growth velocity usually increases to normal levels after puberty begins.[461] Affected boys seem to be more distressed by short stature than by delay in sexual development.

GH release in the basal state and in response to GH secretagogues, including the administration of GHRH, is low for age and may be decreased further in children with CDP, but the amplitude of GH secretion and the GH response to GHRH is greater after administration of exogenous (aromatizable) androgens or estrogens. Therefore CDP may constitute a temporary state of functional GH insufficiency for chronologic age but not for bone age. Patients with CDP are sometimes mistakenly diagnosed with growth hormone deficiency. IGF1 interacts with gonadotropins in the ovary and testis, and the relatively low secretion of GH (and presumably intragonadal IGF1) in CDP may impair the gonadal response to gonadotropins.

TABLE 26.16 Constitutional Delay in Growth and Adolescence

A variation of normal
Males more often seek assistance
Family history of delayed menarche or delayed secondary sexual characteristics
Height is often below the 5th percentile, but growth rate is normal for skeletal age
Onset of adrenarche is delayed
The combination of genetic short stature and constitutional delay leads to more profound short stature
Final height is less than predicted

Patients with constitutional delay in adolescence and growth often do not reach their predicted height. When the genetic tendency for growth is greater, subjects with CDP reach taller adult stature, but the patients most likely to be referred are those who combine genetic short stature with CDP. Girls with CDP have a mean deficit in adult height of 2.4 cm below the mean predicted height, although the range of adult height varies about 10 cm above or below predictions. The magnitude of the catch-up in linear growth during puberty in boys is a major determinant of adult height. Heavier individuals with CDP reach greater height than those who are thinner.[452] Adult height is lower if affected individuals show decreased height standard deviation scores (SDS) in earlier life.[462,463]

Because 15% to 20% of adult height is gained during puberty, many approaches aim to increase stature in otherwise normal, short children. Delaying the onset or progression with puberty by the use of GnRH agonists was suggested by some, but a decrease in bone density 1 year after cessation led to warnings that routine administration of this treatment carries substantial risk.[464] The additional psychologic risk of delaying puberty in otherwise normal children must also be considered, and this treatment is neither established nor recommended.[465]

The US Food and Drug Administration (FDA) approved GH treatment for children who are predicted to reach an adult height less than the 1st percentile (160 cm), which includes some children with CDP (but it is not specifically approved for CDP itself), and some studies reported an increase in adult height with this treatment. Because endogenous GH level rises during pubertal development, the FDA approved GH therapy in larger doses for subjects with GH deficiency during puberty. However, there are only moderate effects of increased GH doses during puberty on adult height. Male gender has a positive effect and age at onset of puberty has a negative effect on this increased dose on adult height.[466]

The cost of GH for treatment of non–GH-deficient short stature is exceptionally high: $14,000 per cm or $35,000 per inch gained.[467] Payers are reluctant in many cases to cover the cost of the GH therapy for those without confirmed GH deficiency. There are few controlled studies of adult height, but some available results demonstrate an increase in adult height of several centimeters; more studies are strongly recommended to better determine the efficacy of this treatment in short, normal children with CDP.[468]

The combination of GnRH agonist therapy with GH treatment in attempts to increase adult height in children who were

normal except for genetic short stature and in SGA children led to inconclusive results or to increased predicted or near-final height, which does not necessarily translate into increased adult height. This approach to treatment remains experimental. Review of a large database from a postmarketing survey did not support the efficacy of this approach,[469] and there was no good follow-up evaluation of adult height. This combination therapy cannot be supported by substantial evidence.[470]

When the critical role of estradiol in skeletal maturation was appreciated, treatment with a potent aromatase inhibitor to increase adult height by inhibiting skeletal maturation[159,160,303] aroused interest. A double-blind, randomized, placebo-controlled study enrolled boys with CDP who were treated with 6 months of monthly testosterone or testosterone plus an added 12-month trial of daily oral letrozole (a potent fourth-generation aromatase inhibitor). The results revealed a mean increase in predicted adult height of 5.1 cm in the letrozole plus testosterone group; a subsequent study supported these promising effects on increasing the time of pubertal growth height without affecting the development of male secondary sex characteristics, but there are few data on actual adult height.[471] The boys in the group treated with testosterone and letrozole developed increased bioactive testosterone, analyzed by a cellular assay, compared with control boys.[266] Serum testosterone measured by HPLC-MS/MS can reach levels of more than 1000 ng/dL. However, markers of bone turnover decrease, and vertebral abnormalities may develop with the use of these agents in idiopathic short stature.[472] A 1-year study of the aromatase inhibitor anastrozole plus GH, compared with GH treatment alone, in boys demonstrated no ill effects on body composition, plasma lipids, bone metabolism, or the tempo of puberty (although estrogen decreased, as expected, with the use of anastrozole), and the predicted height was increased.[473] This treatment has not been supported by long-term studies observing patients up to adult height. Concerns about possible effects on bone density and morphology must be addressed, probably until the patients are at least 20 to 25 years old, as well as a decrease in serum HDL cholesterol levels and increase of erythrocytosis before this off-label therapy can routinely be recommended.[474,475]

Hypogonadotropic Hypogonadism: Sexual Infantilism Related to Gonadotropin Deficiency

Insufficient pulsatile secretion of GnRH and the resulting FSH and LH deficiency lead to delayed sexual maturation, which may be permanent. The phenotype in hypogonadotropic hypogonadism can vary from severe sexual infantilism to apparent CDP. Both conditions may be found in the same family, or hypogonadotropic hypogonadism may first appear to be CDP (e.g., a homozygous R262Q mutation in GNRHR presented with constitutional delay but later was associated with oligospermia).[476] There may be an absolute or relative quantitative deficiency of pulsatile GnRH, or the deficiency may be qualitative, especially in females; it may involve abnormalities in amplitude or frequency of GnRH pulses or in both components (Fig. 26.38). The many forms of this condition occur in about 1 in 4000 males and three to five times less in females.

Patients with IHH usually are of normal height in early or middle adolescent years, whereas patients with CDP usually have a normal growth rate for bone age but are short for chronologic age. In contrast to CDP patients, those with hypogonadotropic hypogonadism usually do not respond to GnRH stimulation, nor do they have a pulsatile LH profile commensurate with bone age. Although serum concentrations of plasma FSH and LH and urinary gonadotropins are low, the differences are relative rather than absolute and are not diagnostic for an individual.

Hypogonadotropic hypogonadism can involve puberty and reproduction alone or can be a manifestation of a life-threatening condition such as a brain tumor. Hypogonadotropic hypogonadism may result from a genetic or developmental defect present at birth but remain undetected until the age of expected puberty, or it may be caused by a tumor, inflammatory process, vascular lesion, irradiation, or trauma to the hypothalamus. Similarly, hypogonadotropic hypogonadism may arise from lesions or defects that involve the pituitary gland directly. When GH is affected as well as gonadotropins, impaired growth is manifested by decreased growth velocity, especially during the expected pubertal growth spurt, and short stature.

Isolated Hypogonadal Hypogonadism

A defect involving the GnRH pulse generator or gonadotrophs without an anatomic lesion causes selective deficiency of gonadotropins, producing IHH (Tables 26.17 and 26.18).[477] Puberty fails to begin by 14 years in boys and 13 years in girls, or pubertal maturation is incomplete or transient. In boys, micropenis (the penis is normally formed but stretched length is less than 2 cm in length, which is 2.5 SD below the mean length in average newborn males) or undescended testes or both findings are evidence of a fetal testosterone deficiency caused by gonadotropin deficiency. Prepubertal concentration of gonadal sex steroid values (testosterone in boys; estradiol in girls) and low serum gonadotropin levels or values within the normal range (i.e., normal in the basal state but not in the stimulated state) are characteristic. Concentrations of gonadal sex steroids and gonadotropins are low, pulsatile LH secretion is often virtually absent, and the LH response to GnRH or GnRH agonist administration is deficient in the severe form. The testes are small (1–2 mL), and serum inhibin B, an estimate of seminiferous tubule function, is lower than in CDP in initial studies.[478]

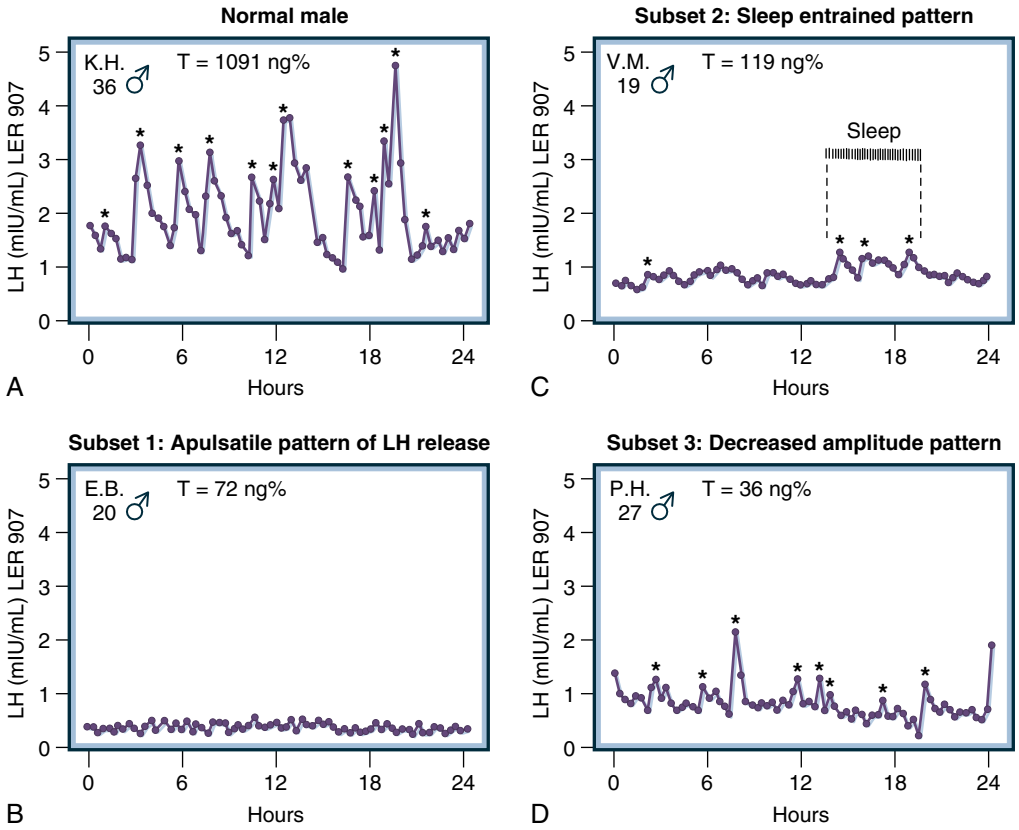
IHH may occur in families (about 20–30% of patients), or it may occur sporadically. There are a growing list of gene defects that cause hypogonadotropic hypogonadism.[479] Some patterns of inheritance are rare and follow the classic mendelian schema; others are oligogenetic, whereby multiple defects of common genes synergize to bring about a condition no single one of them could cause alone.[454,480]

Oligogenicity is more common in IHH than CDP, and IHH genes are more common in IHH than CDP, suggesting a different genetic architecture in IHH compared to CDP.[456]

In contrast to CNS tumors (in which patients usually have GH deficiency and growth failure) and to CDP (in which patients are short for chronologic age), height is appropriate for age in patients with IHH (Fig. 26.39). Because the levels of estradiol are too low to cause epiphyseal fusion at the normal age, increased arm span for height and a decreased ratio of upper to lower body segments (i.e., eunuchoid body proportions) are present. If the condition is left untreated, growth continues, and adult height is tall.[481–483]

Remarkably, about 22% of affected patients with IHH and severe delay of puberty spontaneously increase their testicular size and enter full puberty.[484] Five men who experienced reversal once again became hypogonadal. Thus long-term follow-up is essential.

Kallmann Syndrome. Anosmia or hyposmia resulting from agenesis or hypoplasia of the olfactory lobes or sulci is associated



• **Fig. 26.38** Various patterns of pulsatile luteinizing hormone (LH) secretion that can occur in isolated hypogonadotropic hypogonadism (B–D) are compared with LH secretion in a normal man (A). (A) Discrete LH pulses occur about every 2 hours in a normal 36-year-old man. (B) Typical apulsatile LH pattern is associated with a low testosterone (T) concentration usually found in isolated hypogonadotropic hypogonadism. (C) Pattern of developmental arrest with low-amplitude nocturnal LH pulses is apparent only during sleep. (D) Low-amplitude LH pulse pattern occurs during sleep and wake periods. To convert LH values to international units per liter, multiply by 1.0. (From Spratt DI, Crowley WF. Hypogonadotropic hypogonadism: GnRH therapy. In: Krieger DT, Bardin CW, eds. *Current Therapy in Endocrinology and Metabolism, 1985-1986*. Toronto, Canada: BC Decker; 1985:155–159.)

TABLE 26.17 Isolated Gonadotropin Deficiency

Males more commonly affected
Familial (more common in females) or sporadic (more common in males)
Height normal for age; tall adult height if untreated
Eunuchoid skeletal proportions
Delayed bone age
Small, often cryptorchid testes: diameter <2.5 cm prepubertal size; phallus may be small
Normal adrenarche
Examine for anosmia or hyposmia (Kallmann syndrome)
Look for associated malformations (facial, central nervous system, skeletal, renal)

with GnRH deficiency in Kallmann syndrome, the most common form of IHH[485] (see Table 26.18). The condition was first observed in 1856 during the autopsy of a 40-year-old man with micropenis, small cryptorchid testes, and absence of the olfactory bulbs, but Kallmann described a familial pattern in 1944 (Fig. 26.40). The prevalence is 1 of every 10,000 males and 1 of every 40,000 females. Although the loss of olfaction usually correlates with the degree of GnRH deficiency, even in complete anosmia (found in the classic KAL1 form), the GnRH deficiency may be partial (see "Isolated Luteinizing Hormone Deficiency").[486] Because affected individuals often do not notice impaired olfaction, testing with graded dilutions of pure scents is necessary to determine partial anosmia. Patients with Kallmann syndrome have diminished or absence of nocturnal pulses of gonadotropins found in normal prepubertal boys, although daytime values are equal. Undescended testes are common in this and all types of hypogonadotropic hypogonadism in boys.[483] The magnitude of the GnRH deficiency correlates with the size of the testes. Micropenis occurs in about one-half of males with Kallmann syndrome because of absence of the elevated pituitary gonadotropins characteristic of the normal midgestation fetus (Fig. 26.41).

Associated defects that are inconsistently present include cleft lip, cleft palate, imperfect facial fusion, seizure disorders, short metacarpals, pes cavus, neurosensory hearing loss (rarely found in the X-linked form), cerebellar ataxia and nystagmus, ocular motor abnormalities, unilateral or rarely bilateral renal aplasia or dysplasia, and mirror movements of the upper extremities (i.e., bimanual synkinesia), limited to the X-linked form (see Table 26.18).

KAL1. In classic, X-linked KAL1, fetal GnRH neurosecretory neurons do not migrate from the olfactory placode to the medial basal hypothalamus, where they normally constitute the

TABLE 26.18 Features of Kallmann Syndrome

Clinical

GnRH deficiency: absent or arrested puberty
Anosmia or hyposmia
In infancy: microphallus; cryptorchidism
Normal stature and growth in childhood
Normal adrenarche
Eunuchoid proportions
Associated midline defects (e.g., cleft lip, cleft palate, midline cranial anomalies)
MRI: aplasia or hypoplasia of olfactory bulbs and/or sulci

Prevalence

Approximately 1 in 7500 males, 1 in 50,000 females; 10% prevalence of Klinefelter syndrome

Inheritance

Sporadic and familial cases; genetic heterogeneity
X linked
- X-linked recessive (Kallmann et al)
- X chromosome deletion: Xp22.3 (Meitinger et al)

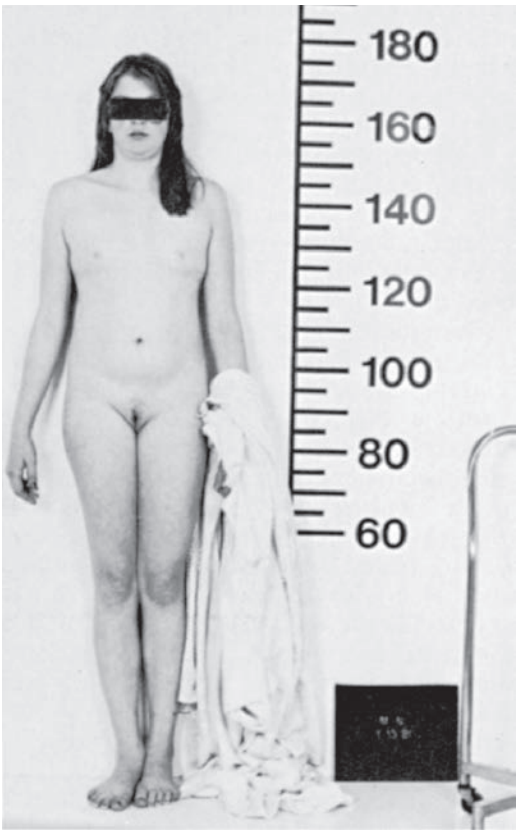
Autosomal
- Dominant (sex limitation) (Santen and Paulsen; Merriam et al)
- Recessive (White et al)

Anatomy

Developmental field defect
Aplasia or hypoplasia of olfactory bulb and sulcus
Arrested migration of GnRH neurosecretory neurons from olfactory placode to medial basal hypothalamus

GnRH, Gonadotropin-releasing hormone; *MRI,* magnetic resonance imaging.

Data from Kallmann F, Schonfeld WA, Barrera SW. Genetic aspects of primary eunuchoidism. *Am J Ment Defic.* 1944;48:203–236; Meitinger T, Heye B, Petit C, et al. Definitive localization of X-linked Kallmann's syndrome (hypogonadotropic hypogonadism and anosmia) to Xp22.3: close linkage to the hypervariable repeat sequence CRI-S232. *Am J Hum Genet.* 1990;47:664–669; Merriam GR, Beitins IZ, Bode HH. Father-to-son transmission of hypogonadism with anosmia: Kallmann's syndrome. *Am J Dis Child.* 1977;131:1216–1219; Santen RJ, Paulsen CA. Hypogonadotropic eunuchoidism. I. Clinical study of the mode of inheritance. *J Clin Endocrinol Metab.* 1973;36:47–54; White BJ, Rogal AD, Brown KS, et al. The syndrome of anosmia with hypogonadotropic hypogonadism: a genetic study of 18 new families and a review. *Am J Med Genet.* 1983;15:417–435.

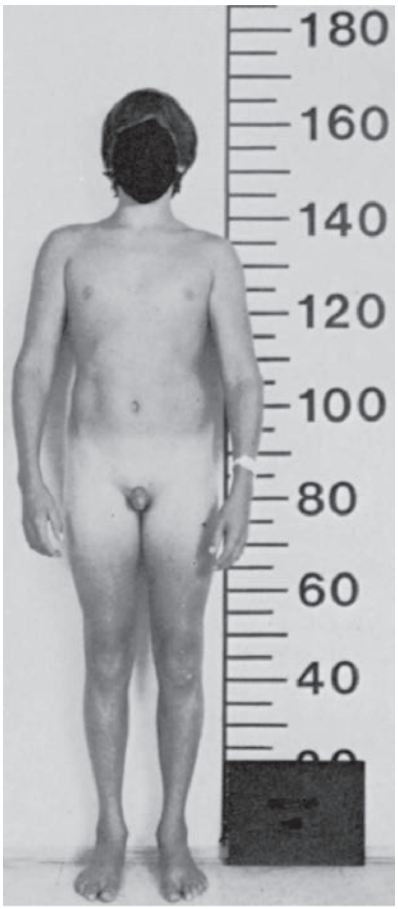


• **Fig. 26.39** A girl aged 18 years and 8 months has isolated gonadotropin deficiency (i.e., sexual infantilism and primary amenorrhea). Her height is 173 cm (+1 SD [standard deviation]), weight was 66.5 kg (+1 SD), and the skeletal age was 13 years. Adrenarche with pubic hair development occurred at age 13.5 years. At the time of the photograph, pubic hair was in stage 3, and there was slight breast and nipple development resulting from a previous short course of estrogen therapy. Immature labia minora and majora were observed, and no estrogen effect was seen on the vaginal mucosa. Olfactory testing results were normal. The plasma luteinizing hormone (LH) (LER-960) level after gonadotropin-releasing hormone (GnRH) administration rose from 0.5 to 1.8 ng/mL (a prepubertal response). Serum estradiol was undetectable. The dehydroepiandrosterone sulfate (DHEAS) level was 92 μg/dL (appropriate for pubic hair stage 2). Notice the discrepancy between adrenarche and gonadarche. For conversion to SI units, see Figs. 26.19 and 26.30. (From Styne DM, Grumbach MM. Puberty in the male and female: its physiology and disorders. In: Yen SCC, Jaffe RB, eds. *Reproductive Endocrinology*. 2nd ed. Philadelphia, PA: WB Saunders; 1986:313–384.)

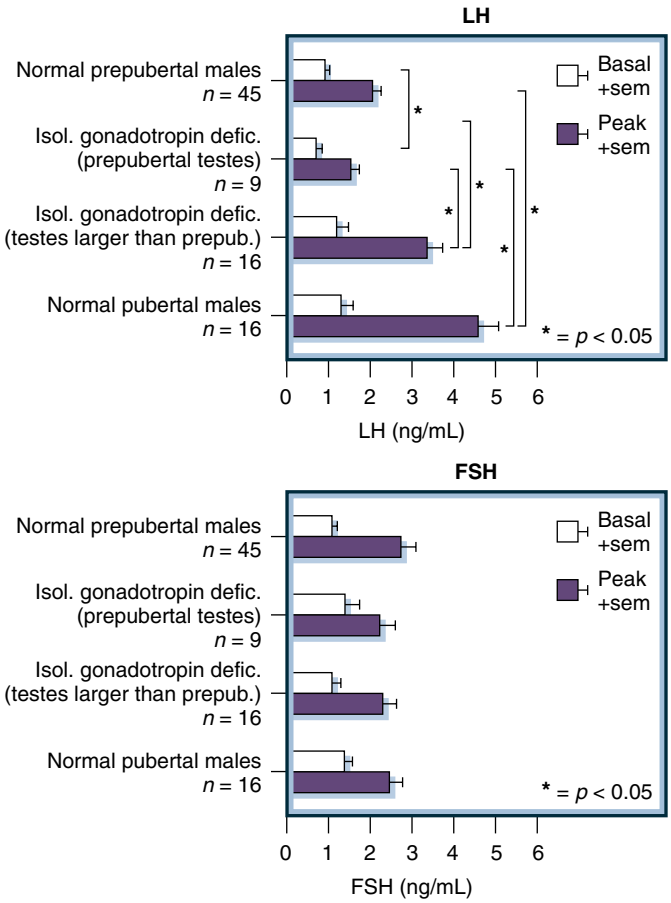
GnRH pulse generator, but instead end in a tangle around the cribriform plate and in the dural layers adjacent to the meninges beneath the forebrain.[372] Abnormal or absent olfactory bulbs or folds are seen on MRI scans with other changes in brain morphometry resulting.[487] Coronal and axial cranial MRI scans of the olfactory bulbs and sulci, unilaterally or bilaterally, reflect this defect in about 90% of cases and can point to the diagnosis, especially in affected infants and prepubertal-age children (Fig. 26.42).

An increasing number of genes are implicated in Kallmann syndrome, starting from the classic *ANOSM1* (previously *KAL1*) gene in the X-linked form first described, but only 30% of patients can be shown to have an identifiable gene defect. Further digenetic and oligogenetic inheritance is now recognized and may explain the genetic heterogeneity of the syndrome and the variation in phenotype encountered within a family—that is, 10% to 20% of IHH are explained with oligogenetic inheritance. Recently, mutations in genes "synexpressed" with fibroblast growth factor 8 were found in IHH and Kallmann syndrome (*FGF17, IL17RD, DUSP6, SPRY4*, and *FLRT3*), supporting this oligogenetic nature of the condition.[488]

A variety of deletions and mutations of the *ANOSM1* gene have been described, including large and small (exon) deletions, point mutations, and a variety of nonsense mutations leading to frameshift and premature stop codons. The ANOSM1 mutations are more prevalent in Japanese than in white patients, and they can be associated with normal olfactory function.[489] The defect in some rare patients with no ANOSM1 mutation but X-linked inheritance may be located in the promoter region of the *ANOSM1* gene. Kallmann syndrome associated with X-linked ichthyosis caused by steroid sulfatase deficiency, mental retardation, and chondrodysplasia punctata occurs in a contiguous gene syndrome. Only 14% of familial cases and 11% of sporadic cases involve mutations in the *ANOSM1* gene on the X chromosome,



• **Fig. 26.40** A boy aged 15 years and 10 months had isolated gonadotropin deficiency and anosmia (Kallmann syndrome). He had undescended testes, but after administration of 10,000 U of human chorionic gonadotropin (hCG), the testes descended and were palpable in the scrotum. His height was 163.9 cm (+1.5 SD [standard deviation]); the upper-to-lower body ratio was 0.86, which is eunuchoid. The phallus measured 6.3 × 1.8 cm, and the testes were 1.2 × 0.8 cm. The concentration of plasma luteinizing hormone (LH) was less than 0.3 ng/mL; the follicle-stimulating hormone (FSH) level was 1.2 ng/mL; and the testosterone level was 16 ng/dL. After 100 μg of gonadotropin-releasing hormone (GnRH), the plasma LH level (LER-960) was 0.7 ng/mL, and the FSH level (LER-869) was 2.4 ng/mL. For conversion to SI units, see Figs. 26.19 and 26.20. (From Styne DM, Grumbach MM. Puberty in the male and female: its physiology and disorders. In: Yen SCC, Jaffe RB, eds. *Reproductive Endocrinology*. 2nd ed. Philadelphia, PA: WB Saunders; 1986:313–384.)

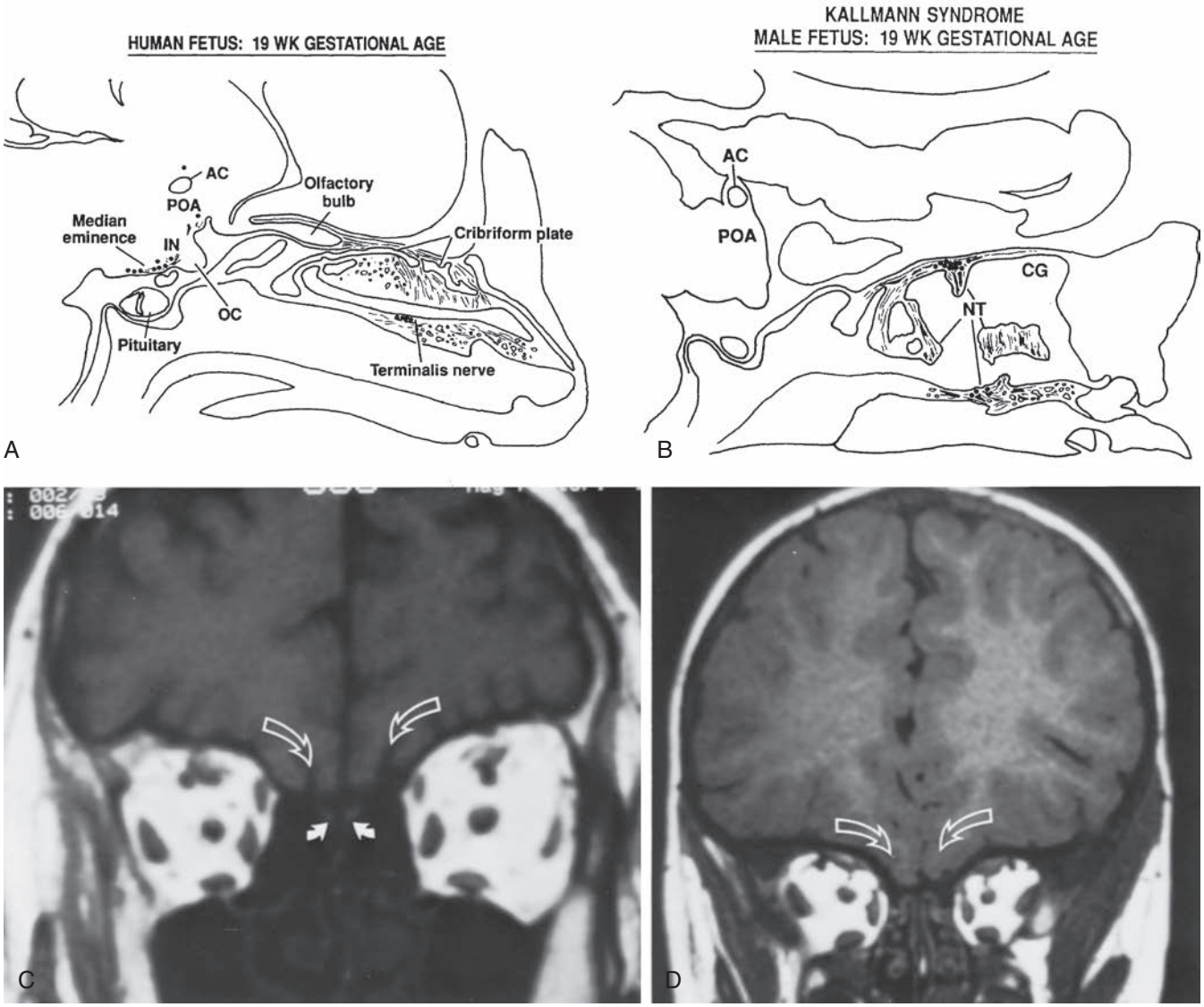


• **Fig. 26.41** Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) responses to the administration of gonadotropin-releasing hormone (GnRH) in 25 males with an isolated gonadotropin deficiency with or without anosmia were segregated according to whether the volume of the testes was prepubertal or greater than 2.5 cm^3. Testicular volume in those with testes larger than 2.5 cm^3 was as large as 4 cm^3. Basal and GnRH-stimulated gonadotropin levels after the intravenous injection of 100 μg GnRH (peak value) are shown (p <0.05). For conversion to SI units, see Fig. 26.16. *sem,* standard error of the mean. (From Van Dop C, Burstein S, Conte FA, et al. Isolated gonadotropin deficiency in boys: clinical characteristics and growth. *J Pediatr.* 1987;111:684–692.)

but these patients are more likely to have complete absence of gonadotropin secretory pulses and absence of migration of GnRH neurons to the hypothalamus.[490] Hypogonadotropic hypogonadism is rarely caused by a mutation in the *ANOSM1* gene in females.

KAL2. The autosomal dominant form is known as Kallmann syndrome type 2 (KAL2), and the associated gene is fibroblast growth factor receptor 1 (*FGFR1*, previously *KAL2*) with a gene map locus of 8p11.2-p11.1 and its ligand FGF8. Mutations result in autosomal dominant Kallmann syndrome, autosomal dominant normosmic hypogonadotropic hypogonadism, or delayed puberty. Evaluation of FGF8 in patients with idiopathic hypogonadotropic hypogonadism revealed inactivating mutations of FGF17, IL17RD, DUSP6, SPR-Y4, and FL-RT3.[488] A Kallmann syndrome patient had a premature termination of FGFR1 resulting in a soluble receptor with dominant negative effect, which produced a truncated FGFR1 without the transmembrane and intracellular domains.[491] Intracerebral administration of anti-FGFR1 antibodies causes weight loss in rodents; FGF21, which is secreted by the liver under the stimulatory effects of beta-klotho (KLB), an obligate coreceptor mediating FEF21 signaling, is also involved in nutritional metabolism. While there is to date no report of mutation FGF21 causing hypogonadism, mutations in KLB impair FGF21 signaling leading to GnRH deficiency of various degrees of severity.[492] Since KLB and FGF21 genes are expressed on GnRH neurons, they may provide a means in which GnRH neurons can detect nutritional status and determine whether it is appropriate to allow reproduction to progress or cease.[493]

KAL2 is associated with developmental delay, choanal atresia, short stature, congenital heart defects, and sensorineural hearing loss, and its presentations are more varied than those of KAL1.



• **Fig. 26.42** Comparison of the brain and nasal cavities of a normal 19-week-old male fetus (A) and those of a male fetus of similar age with Kallmann syndrome caused by an X chromosome deletion at Xp22.3 (B). In the normal fetal brain, the gonadotropin-releasing hormone (GnRH) neurosecretory neurons *(black dots)* are located in the hypothalamic area, including the medial basal hypothalamus, the anterior hypothalamic area, and of interest regarding hypothalamic hamartoma as an ectopic GnRH pulse generator, the premammillary and retromammillary areas. A small cluster of GnRH neurons exists among the fibers of the terminalis nerve on the floor of the nasal septum. In the male fetus with Kallmann syndrome, no GnRH neurons were detected in the hypothalamic region, including the basal hypothalamus, median eminence, and preoptic area. The GnRH cells fail to migrate to and enter the brain from their origin in the nose; these cells end in a tangle beneath the forebrain on the dorsal surface of the cribriform plate and in the nasal cavity. *AC,* anterior commissure; *CG,* crista galli; *IN,* infundibular nucleus; *NT,* terminalis nerve; *OC,* optic chiasm; *POA,* preoptic area. (C and D) show magnetic resonance imaging scans of brain (coronal section, TI-weighted image). (C) Normal olfactory sulci *(open arrows)* and bulbs *(solid arrows)* in a 15-year-old boy. (D) Absent olfactory sulci *(open arrows)* and bulbs in a 17-year-old, anosmic, sexually infantile boy with Kallmann syndrome.

The loss-of-function mutation of the *FGFR1* gene interferes with migration of the olfactory cells to the olfactory bulb. Anosmin-1, a neuronal protein, may act through *FGFR1* to bring about FGF signaling. ANOSM1 partially escapes inactivation in females, and it is postulated that enough ANOSM1 may be produced in affected females, despite *FGFR1* haploinsufficiency, to maintain adequate FGF signaling and allow olfactory function and GnRH neuron migration. One kindred of Kallmann syndrome contained four women with *FGFR1* mutations that were transmitted to affected male offspring, although the mothers had normal reproduction and olfaction.[494]

Although gain-of-function mutations of the *FGFR1* gene are associated with craniosynostosis, a loss-of-function mutation is not associated with lack of fusion of the cranial sutures. One kindred with an *FGFR1* mutation (Arg622X) in the tyrosine kinase domain, in which some of the manifestations were temporary, is

reported; the mother of the proband had delayed puberty, and the maternal grandmother had anosmia, whereas the proband with KAL2 exhibited normal LH levels, testosterone production, and spermatogenesis after prior testosterone therapy.[495]

Studies of mice revealed that the early emergence of GnRH neurons from the embryonic olfactory placode requires FGF8 signaling as a ligand mediated through *FGFR1*.[496] About 30% of FGF8/FGFR1 loss-of-function mutations are associated with cleft palate, whereas *FGFR1* mutations may rarely lead to cartilage abnormalities in the ears, nose, or digits.[496] An unusual kindred with a proband demonstrating severe ear anomalies, mandibular hypoplasia, thoracic dystrophia, and other usual findings was associated with an Arg622 mutation in the *FGFR1* gene, and investigation for hypogonadism is indicated when such facial abnormalities occur.[497] Prevalence of *FGFR1* mutations in the Japanese population is equal to the prevalence among whites. Although renal aplasia is characteristic of ANOSM1 mutations, and cleft palate and dental agenesis are characteristic of *FGFR1* mutations in KAL2, these findings can occur in Kallmann patients without ANOSM1 or *FGFR1* mutations.[489]

KAL3. Apparent autosomal recessive inheritance characterizes other kindreds with Kallmann syndrome type 3 (KAL3), for which the affected gene is *PROKR2*. Unilateral renal agenesis, hypotelorism, cleft lip and palate, and a midline cranial fusion defect occur. Fibrous dysplasia, sleep disorder, severe obesity, synkinesia, and epilepsy are described in patients with *PROK2* or *PROKR2* mutations, and 3% of IHH patients were affected in one study. Knockout mice lacking the *PROK2* gene (formerly called *KAL4*), which encodes prokineticin 2, an 81–amino acid peptide that signals through the G protein–coupled product of the *PROKR2* gene (formerly called *KAL3*), had defective development of the olfactory bulbs and failed migration of GnRH neurons.[498] This model led to demonstration of loss-of-function mutations in *PROKR2* or *PROK2* in 9% of patients with Kallmann syndrome,[499] who were mostly heterozygous, although homozygous and compound heterozygous mutations were also described. Some families appear to have autosomal dominant inheritance.

Other Forms of Kallmann Syndrome. The human equivalent of the mouse nasal embryonic GnRH factor gene *(Nelf)* is *NELF*; a mutation of this gene is found in patients with IHH and in Kallmann syndrome.[500]

A mutation in the binding domain of the membrane coreceptor neuropilin-1 *(SEMA3A)* gene is found in Kallmann syndrome in an autosomal dominant pattern.[501] This mutation was found in some patients who already had mutations in other Kallmann syndrome genes, further suggesting the oligogenic nature of the disorder. Semaphorin 3E (SEMA3E) is a protein involved with axonal growth, and missense mutations were found in two brothers with Kallmann syndrome.[502]

About 30% of Kallmann syndrome patients have hearing impairment, and of those about 38% have heterogeneous mutations of the *SOX10* gene.[503] Deafness may also be found in the CHARGE syndrome (discussed later), but in the absence of other CHARGE findings a search for a mutation in *SOX10* is indicated.

Mutations in heparin sulfate 6-*O*-sulfotransferase 1 *(HS6ST1)* are found in patients and families with IHH and Kallmann syndrome.[504,505] This abnormality is found in complex inheritance patterns as patients may also have mutations in *FGF1* and *NELF*. Thus *HS6ST1* mutations may not be sufficient to cause disease but may add to it.

Recently, Kallmann syndrome has been linked to mutations in *HESX1* (found in septo-optic dysplasia, discussed later)[506] and *FEZF1*.[507]

Congenital hypogonadotrophic hypogonadism can be associated with many other clinical features not involving the reproductive system. The CHARGE syndrome is characterized by coloboma or microphthalmia, coloanal stenosis or atresia, middle and inner ear defects, various cranial nerve defects (including anosmia or hyposmia [including absent olfactory bulbs]), and IHH. In autosomal dominant familial cases, causative mutations were found in *CHD7*, which encodes a chromatin-remodeling factor, which in a small percentage of cases were found in patients with Kallmann syndrome and in some patients with IHH due to apparent loss-of-function mutations. Hypogonadotropic hypogonadism may be more frequent if there is deafness and hypoplasia of the semicircular canals.[508]

Patients with demyelinating sensorimotor polyneuropathy, developmental delay, and severe hypoglycemia initially, with later nonautoimmune insulin-dependent diabetes mellitus, had mutations in DMXL2, which codes for rabconnectin-3α.[509]

Kallmann syndrome has been reported in patients with heterozygous mutations in the WDR11 gene, which is involved in the development of olfactory neurons.[502,510]

The protein CCDC141 is expressed in GnRH neurons and in the back in activating mutations led to normal osmotic hypogonadotropic hypogonadism.[511] Mutations in FEZF1 were found in two families with Kallmann syndrome.[507] This gene promotes the expression of a protease, which allows the olfactory receptor neurons to enter the brain along with GnRH neurons.

Mutations in SMC-HD1 led to the absence of the nose in 41 individuals, most of whom had hypogonadism along with anosmia.[512]

The Gordon Holmes syndrome presents with congenital hypogonadotropic hypogonadism, normosmia, and cerebellar atrophy/ataxia; this syndrome is associated with mutations in the *OTUD4, RNF216,* and *PNPLA6* genes.[513]

The various forms of Kallmann syndrome result from heterogeneous mutations in which the phenotype can vary. For example, a 20-year-old man with the complete picture of Kallmann syndrome had an identical twin brother (proved by genetic fingerprinting) with anosmia but a normal adult phenotype and normal plasma testosterone and gonadotropin concentrations.

Other postulated defects that may interfere with GnRH neuron migration are mutations in the genes for neural cell adhesion molecules (NCAM) and related proteins, such as tenascin, laminin, and phosphacan. Various glycoconjugates may also be involved.

Other Forms of Isolated Hypogonadotropic Hypogonadism. Only about 15% of normosmic hypogonadotropic patients have a definable genetic defect. The combination of human genetic studies and mouse models has led to the discovery of many genes involved in gonadotropin regulation.[514] Inheritance of hypogonadotropic hypogonadism (Table 26.19) with none of the other features of Kallmann syndrome may be found in autosomal dominant (gene map locus 19p13.3, 9q34.3), autosomal recessive (8p21-p11.2), or X-linked recessive (Xp21)[515,516] disorders. Males with cerebellar ataxia and deficient gonadotropin production are reported in kindreds with X-linked inheritance (possibly a variant form of Kallmann syndrome), and hypogonadotropic hypogonadism may be associated with the multiple lentigines and basal cell nevus syndromes.

Gonadotropin-Releasing Hormone Gene Mutations. The GnRH gene *(GNRH1)* would seem a likely candidate for the cause of hypogonadotropic hypogonadism, but although mutations of the GnRH

TABLE 26.19 Molecular Basis for Developmental Disorders Associated With Hypogonadotropic Hypogonadism

Gene	Phenotype	Complex Phenotype
	ISOLATED HYPOGONADOTROPIC HYPOGONADISM	
Kallmann Syndrome or Normosmic IHH (With the Same Mutant Gene)		
KAL1 (Xp22.3)	X-linked Kallmann syndrome	Anosmia/hyposmia, renal agenesis, dyskinesia
FGFR1 (KAL2) (8p11.2)	Autosomal dominant Kallmann syndrome (± recessive)	Anosmia/hyposmia, cleft lip/palate
FGF8 (ligand for FGFR1) (10q25)		
NELF (9p34.3)	Autosomal dominant (?) Kallmann syndrome	
PROK2 (3p21.1)	Autosomal recessive Kallmann syndrome	
PROKR2[a] (20p12.3)		
CHD7 (8p12.1)	Autosomal dominant (some)	CHARGE syndrome includes hyposmia
Normosmic Isolated Hypogonadotropic Hypogonadism		
GNRH1 (8p21-11.2)	Autosomal recessive	
GNRHR[a] (4q13.2-3)	Autosomal recessive (± dominant)	
GPR54[a] (19p13.3)	Autosomal recessive	
SNRPN		Prader-Willi syndrome
Lack of function of paternal 15q11-q13 region or maternal uniparental disomy		Obesity
LEP (7q31.3)	Autosomal recessive	Obesity
LEPR (1p31)	Autosomal recessive	Obesity
NR0B1 (DAX1) (X21.3-21.2)	X-linked recessive	Adrenal hypoplasia
TAC3 (12q13-12)	Autosomal recessive	
TACR3 (4q25)	Autosomal recessive	
Multiple Pituitary Hormone Deficiencies		
PROP1 (POU1F1)	Autosomal recessive GH, PRL, TSH, and LH/FSH (less commonly, later-onset ACTH deficiency)	
HESX1 (RPX)	Autosomal recessive; and heterozygous mutations	Septo-optic dysplasia
	Multiple pituitary deficiencies, including diabetes insipidus, but LH/FSH uncommon	
LHX3	Autosomal recessive GH, PRL, TSH, FSH/LH	Rigid cervical spine
PHF6	X linked; GH, TSH, ACTH, LH/FSH	Börjeson-Forssman-Lehmann syndrome: mental retardation; facies

[a]A G protein–coupled receptor.

ACTH, Corticotropin; *CHD7,* chromatin-remodeling factor; *DAX1,* dosage-sensitive sex reversal-adrenal hyperplasia congenita critical region on the X chromosome, gene 1; *FGF,* fibroblast growth factor; *FSH,* follicle-stimulating hormone; *GH,* growth hormone; *GNRH,* gonadotropin-releasing hormone; *GPR54,* kisspeptin G protein–coupled receptor 54; *HESX1*, homeobox gene expressed in ES cells; *IHH,* idiopathic hypogonadotropic hypogonadism; *LEP,* leptin; *LH,* luteinizing hormone; *LHX3,* lim homeobox gene 3; *NELF,* nasal embryonic luteinizing hormone–releasing factor; *NR0B1,* nuclear receptor family 0, group B, member 1; *PHF6,* plant homeodomain–like finger gene; *PRL,* prolactin; *PROK2,* prokineticin 2; *PROP1,* prophet of Pit-1; *R,* receptor; *SNRPN,* small nuclear ribonucleoprotein polypeptide SmN; *TAC3,* neurokinin 3; *TSH,* thyroid-stimulating hormone.

Modified from Semple RK, Topaloglu AK. The recent genetics of hypogonadotrophic hypogonadism—novel insights and new questions. *Clin Endocrinol.* 2010;72(4):427–435.

See text for details.

receptor gene *(GNRHR)* were identified years ago, mutations in the *GNRH1* gene were not demonstrated until 2009. An autosomal recessive form had been described in the mouse (hyg/hyg), in which there is a deletion of part of the GNRH gene. There appears to be normal migration of GnRH neurons to the medial basal hypothalamus based on information from the mouse model.[517]

GNRH1 mutation is a very rare condition with a prevalence of under 1% in isolated populations and probably far lower in the general population. A homozygous *GNRH1* frameshift mutation in human beings, characterized by the insertion of an adenine at nucleotide position 18 (c.18-19insA) in the sequence encoding the N-terminal region of the signal peptide–containing protein

precursor of GnRH (prepro-GnRH), was found in a teenage brother and sister who had normosmic IHH.[518] When expressed in vitro, the mutant peptide did not demonstrate immunoreactive GnRH. Only 1 of 310 patients with severe, congenital, normosmic IHH (with micropenis, bilateral cryptorchidism, and absent puberty) had a homozygous frameshift mutation that is predicted to disrupt the three C-terminal amino acids of the GnRH decapeptide and to produce a premature stop codon.[517] Of four patients with normosmic IHH, one had a nonsynonymous missense mutation in the eighth amino acid of the GnRH decapeptide; one had a nonsense mutation that causes premature termination within the GnRH-associated peptide (GAP), which lies C-terminal to the GnRH decapeptide within the GnRH precursor; and two had sequence variants that cause nonsynonymous amino acid substitutions in the signal peptide and GnRH-associated peptide.

Gonadotropin-Releasing Hormone Receptor Mutations. Mutations of the gene encoding the type 1 GnRH receptor (*GNRHR*, gene map locus 4q21.2) that affect the G protein–coupled, seven-transmembrane segments lead to various degrees of familial and sporadic hypogonadotropic hypogonadism with normosmia. A *GNRHR* mutation is found in about 40% to 50% of cases of familial, autosomal recessive, normosmic IHH and in about 17% of sporadic cases of normosmic IHH.

Mutations in hypergonadal hypogonadism with amino acid substitutions in the extracellular N-terminal domain (Thr32Ile), the second extracellular loop (Cys200Tyr), the third intracellular loop (Leu266Arg), and the sixth transmembrane helix (Cys279Tyr) were found to affect specific GnRH binding.[519,520] Except for Thr32Ile, there was no significant inositol phosphate accumulation after GnRH stimulation, demonstrating loss of function even if binding was accomplished. However, an increased dose of GnRH allowed stimulation of the gonadotropin subunit and GnRHR promoters and the ability to partially activate extracellular signal-regulated kinase 1 and stimulate cAMP response element (CRE)–luciferase activity. A higher dose of GnRH caused the Cys200Tyr mutant to stimulate gonadotropin subunit and GnRHR promoter activity because this mutant reduces cell surface receptor expression.

Another human GnRH receptor *(GNRHR)* gene mutation of a highly conserved sequence located in the second-transmembrane helix impairs *GNRHR* effector coupling due to loss of surface expression of the receptor and leads to a severe manifestation of IHH.[521]

Certain GnRH receptor defects may be rescued by membrane-permeant pharmacologic agents that can act as a folding template (chaperones) that rescue the structural defects caused by the mutations and allow function to occur (i.e., ligand binding and restoration of receptor coupling to effector).[522] This approach may allow a therapeutic approach to conditions caused by this and other mutations in receptors that result in protein misfolding.

The clinical presentation of patients with mutations in the GnRH receptor is heterogeneous, and impairment of signal transmission is highly variable (e.g., severe features of IHH, sexual infantilism, long-delayed puberty with reversal in adulthood, relatively mild hypogonadism and infertility), even within the same pedigree and especially in patients with compound heterozygous mutations. For example, two women with a normal pubertal history with hypothalamic amenorrhea had mutations in the *GNRHR* gene[523] while the other had mutations in the *FGFR1, PROKR2,* and *ANOSM1* genes. When the mutation is homozygous, the phenotype is related to the number of mutations. However, when it is monoallelic, there is no relationship, which indicates that other genes interact with the single allelic mutation in *GNRH1*.[524] Biallelic mutations in *GNRHR* were found in two girls (compound heterozygous c.317A>G p.[Gln106Arg] and c.924_926delCTT p.[Phe309-Del] and homozygous c.785G>A p.[Arg262Gln]) with incomplete pubertal development or secondary amenorrhea, and twin boys with incomplete pubertal development had compound heterozygous for GNRHR mutations c.317A>G p.(Gln106Arg) and c.785G>A p.(Arg262Gln).[525] With the increasing number of mutations of the GnRH receptor found in partial and reversible normosmic IHH, this gene is suggested to be the first to evaluate in such cases.[526] In all types of congenital gonadotropin deficiencies, male patients are likely to manifest micropenis (penile length <2 cm at birth and in infancy) due to lack of fetal gonadotropin stimulation of fetal testes during the last half of gestation. Boys with congenital GH deficiency have micropenis even if gonadotropin function is normal. Because testosterone therapy is effective in increasing penile size (see later discussion), sex reversal is not indicated in these cases of microphallus.[527]

KISS1/KISS1R Axis Mutations. The KISS1/KISS1R axis plays a role in the increased amplitude of GnRH signaling in puberty. KISS1/KISS1R axis mutations are rare but instructive. Of 30 normosmic subjects with hypogonadotropic hypogonadism who were evaluated, one person had two missense mutations in KISS1R (Cys223Arg in the fifth transmembrane helix and Arg297Leu in the third extracellular loop); the former had no activity, and the latter manifested as mildly decreased signaling ability.[528] Homozygous deletions of 155 nucleotides in the *KISS1R* gene encompassing the splicing acceptor site of the intron 4–exon 5 junction and part of exon 5 were found in all affected family members with IHH,[529] but unaffected family members had no deletion or only one mutant allele. Another kindred had a Leu48Ser mutation in the second intracellular loop (IL2) of KISS1R, and another had two separate mutations in the gene,[352] Arg331Xaa and Xaa399Arg. The latter patient had decreased secretion of GnRH and decreased response to GnRH administration. A line of mice transfected with the affected gene exhibited hypogonadotropic hypogonadism with decreased GnRH in the hypothalamus but were responsive to GnRH or gonadotropin administration. Agonist stimulation may stabilize the switch II region of Gα to promote the opening of Gα switch II to facilitate exchange of guanosine diphosphate (GDP) and guanosine triphosphate (GTP).[530] The Leu148Ser mutation does not affect the expression, ligand-binding properties, or protein interaction network of KISS1R, but diverse KISS1R functional responses are markedly inhibited.

X-Linked Congenital Adrenal Hypoplasia and Hypogonadotropic Hypogonadism. A rare deletion or mutation in the dosage-sensitive sex reversal-A (DSS) adrenal hypoplasia congenita gene on the X chromosome gene 1 (*NR0B1*, formerly called *DAX1*; gene map locus Xp21.3-p21.2) leads to an X-linked recessive disorder of adrenocortical organogenesis.[531] The gene encodes an orphan receptor, a member of the nuclear receptor superfamily, that is a putative transcriptional repressor mapping to the Xp21 locus. A double dose of *NR0B1* is associated with a female phenotype or ambiguous genitalia in 46,XY males. The NR0B1 protein has a novel domain in the N-terminus that contains two putative unique zinc finger motifs, and the C-terminus contains a conserved ligand-binding domain that binds DNA, localizes in the nucleus, and contains a transcriptional silencing domain that antagonizes the steroidogenic factor 1 (SF1, also called NR5A1) transactivation function. NR0B1 has an SF1 response element in the 5′-promoter region that is another orphan member of the nuclear hormone receptor superfamily. Both NR0B1 and SF1 are expressed in the adrenals, gonads,

pituitary, and hypothalamus, raising the possibility of an important interaction between these two genes and their products.

Rare abnormalities of NR0B1 are characterized by severe glucocorticoid, mineralocorticoid, and, at puberty, androgen deficiency. The abnormal structure of the adrenal cortex resembles that of the fetal zone because it consists of disorganized, vacuolated, cytomegalic cells with a normal mature cortex. The severe primary adrenal insufficiency with hyponatremia, hyperkalemia, acidosis, and hypoglycemia is characterized by failure to thrive, vomiting, poor feeding, dehydration, circulatory collapse, and increased pigmentation and is lethal if not treated early in life in affected boys.

Adrenoleukodystrophy may manifest with adrenal failure long before neurologic symptoms develop, and some cases of X-linked Addison disease may represent this diagnosis. This condition is in the differential diagnosis of adrenal hypoplasia. Plasma renin activity is high; plasma cortisol and aldosterone levels are low. Symptomatic adrenal insufficiency may first manifest in later childhood. In male infants, signs of salt wasting are usually the most prominent feature, but cortisol deficiency is detectable, and adrenal insufficiency includes deficient secretion of the zona reticularis steroids, DHEA and DHEAS. An early sign of elevated ACTH is increased skin pigmentation. The testes are undescended in fewer than one-half of patients; micropenis is rare, but urogenital abnormalities and hearing loss occasionally are present. Boys who do not present with clinical evidence of adrenal insufficiency in infancy often have a more insidious onset during childhood or adulthood.

In a pedigree in which two affected boys had a hemizygous *NR0B1* nonsense mutation and neonatal onset of adrenal insufficiency, a maternal aunt who was homozygous for the mutation had sexual infantilism and primary amenorrhea, but even after decades of follow-up, she maintained normal adrenal function. A maternal grandfather who carried the same mutation was asymptomatic.[532] This pedigree highlights the limitations and complexities of genotype and phenotype correlations. Most commonly, due to hypogonadotropic hypogonadism, signs of sexual maturation at the age of puberty (e.g., pubic and axillary hair, testicular enlargement) are lacking, and the concentrations of serum FSH, LH, and testosterone are low. Delayed puberty is a manifestation in some female carriers of a *NR0B1* mutation.

Intragenic mutations in *NR0B1* (i.e., frameshift mutations, nonsense mutations, and missense mutations) indicate that the hypogonadotropic hypogonadism is an intrinsic characteristic of the disorder, a manifestation of the single-gene mutation and not a result of involvement of a contiguous gene. The *NR0B1* gene is expressed in the adrenal cortex, in testes (and weakly in the ovary), and in the hypothalamus and pituitary. There is evidence of GnRH deficiency and an abnormality in the gonadotrophs, yielding a mixed picture of hypothalamic and intrinsic gonadotroph defects with absent or erratic pulsatile secretion of LH. Even if basal immunoreactive LH and FSH levels are normal, gonadotropins seem to lack bioactivity. In some affected boys, the GnRH pulse generator and pituitary gonadotropin apparatus is intact and functional in infancy and early childhood, and the GnRH-gonadotroph defects do not manifest until later in childhood or during the peripubertal period. Azoospermia unresponsive to gonadotropin treatment was detected in a few affected men.[508,515,533,534]

A deletion of the adrenal hypoplasia congenita locus (at Xp21) can include the glycerol kinase *(GK)* and Duchenne muscular dystrophy *(DMD)* genes if it extends centromerically or produce developmental delay if there is extension toward the telomere, leading to contiguous gene syndromes.

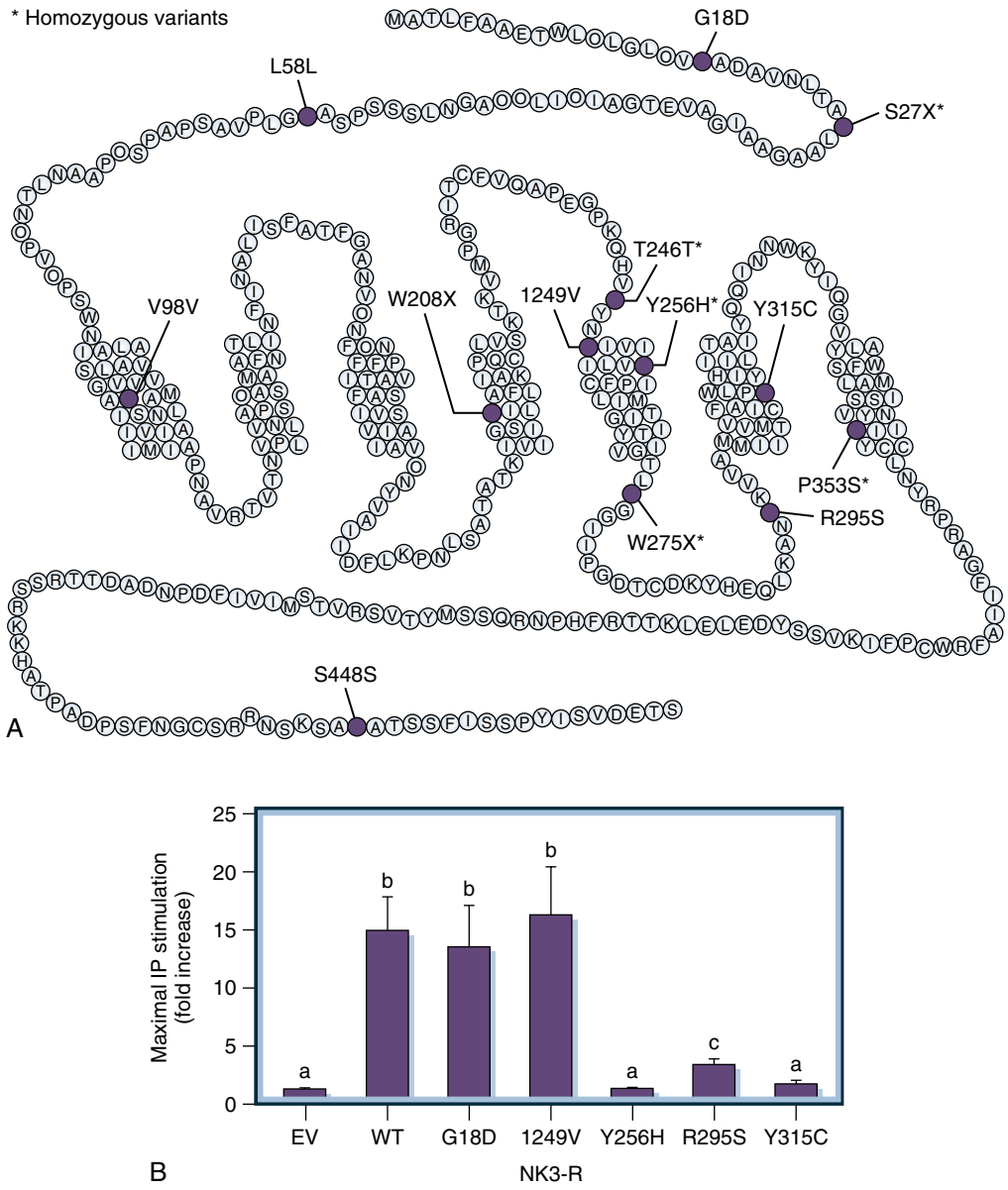
Other mutations of the X chromosome may be associated with IHH. Two brothers were reported who had hypogonadotropic hypogonadism, obesity, and short stature associated with a maternally inherited pericentric inversion (X) (p11.4q11.2). Because the breakpoint is not related to other genes associated with pubertal disorders, it is not clear whether this is a functional relationship or a coincidence.[535]

The *TAC3* gene codes for tachykinin-3, which is cleaved to neurokinin B, a member of the tachykinin superfamily of 127 neuropeptides that includes substance P and neurokinin A. Its cognate G protein–coupled receptor 126 is neuromedin-K receptor 3 (NK3R), which is encoded by TACR3. There are neurons in the arcuate nucleus of the hypothalamus that coexpress kisspeptins and NKB, the kisspeptin/NKB/Dyn (KNDy) neurons that also express dynorphin A (endogenous opioid peptide) and NK3R.[536] Loss-of-function mutations occur in this system in familial, congenital hypogonadotropic hypogonadism; administration of pulsatile GnRH caused resumption of gonadotropin secretion[537–539] (Fig. 26.43). A survey of 345 patients with normosmic hypogonadotropic hypogonadism found 13 rare, distinct nucleotide sequence coding variants (three nonsense, six nonsynonymous, and four synonymous mutations [one predicted to affect splicing]) in *TACR3* and one homozygous single-base-pair deletion, resulting in complete loss of neurokinin B. Of the 16 males for whom phenotype information was available, 15 had microphallus, and none of the females had spontaneous thelarche. When subjects were assessed after discontinuation of therapy, six of seven males and four of five females demonstrated evidence for reversibility of their hypogonadotropism.[540] Continuous infusion of kisspeptin to two patients with loss-of-function mutations in neurokinin B (TAC3) or its receptor (TAC3R) led to pulsatile gonadotropin secretion, indicating that pulsatile administration of kisspeptin is not necessary to institute pulsatile GnRH secretion.[363]

Mutations in the prohormone convertase 1 gene (*PCSK1*, also called *PC1*) lead to extreme childhood obesity, hypocortisolemia, defects in conversion of proinsulin to insulin leading to hypoglycemia, diabetes insipidus, and isolated partial hypogonadotropic hypogonadism, allowing spontaneous pubertal development but primary amenorrhea. The hypogonadal hypogonadism probably resulted from impaired processing of GnRH or neuropeptides involved in its secretion. Findings in another subject extended to gastrointestinal disturbance, small intestinal malabsorption related to monosaccharide and fats, and elevation in progastrin and proglucagon levels, showing that prohormone processing in enteroendocrine cells was abnormal.[541]

Isolated Luteinizing Hormone Deficiency. Isolated LH deficiency (fertile eunuch syndrome) is associated with deficient testosterone production (which responds to hCG administration) and decreased virilization in the presence of mature testicular size and variable spermatogenesis; the disorder may be idiopathic or may result from a hypothalamic pituitary neoplasm. A homozygous Gln106Arg mutation in the first extracellular loop of the GnRH receptor at gene map locus 4q21.2 was associated with normal testicular volume (17 mL) but with apulsatile, low gonadotropin values and low testosterone[542] values in one subject. After hCG stimulation, he developed adequate spermatogenesis to father a child, and after cessation of hCG treatment, he demonstrated adult testosterone values and pulsatile gonadotropin secretion, an example of reversibility of the syndrome.

Isolated Follicle-Stimulating Hormone Deficiency. Homozygous or compound heterozygous mutations in the FSH β-subunit



• **Fig. 26.43** (A) Schematic of mutations in the NK3-R, which is encoded by *TAC3*. (B) Effects of mutations in *TACR3* on neurokinin B (NKB)–mediated activation of signal transduction. COS7 cells transfected with wild-type (WT) G18D, I249V, Y256H, R295S, and Y315C NK3R or empty vector (EV) were treated with NKB (10-7 M) for 1 hour. A significant increase in inositol phosphate (IP) accumulation occurred in cells transfected with WT, G18D, or I249V NK3R. In contrast, there was a marked reduction in NKB-stimulated IP production in cells transfected with Y256H, R295S, or Y315C NK3-R, or with EV. *a*, *b*, and *c* denote significantly different fold increases in IP accumulation. (From Gianetti E, Tusset C, Noel SD, et al. *TAC3/TACR3* mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. *J Clin Endocrinol Metab.* 2010;95:2857–2867.)

have been reported in three females and two males with delayed puberty or poorly developed secondary sex characteristics and with primary amenorrhea but normal adrenarche in the women.[543] The LH concentration was elevated, the serum level of estradiol was low, and immunoactive FSH was absent. Two of the three women had a homozygous nonsense mutation (Val61X) in the FSH β-subunit gene at gene map locus 11p13, and the other was a compound heterozygote (Cys51Gly/Val61X). The women had antral follicles but no progression, demonstrating that FSH action may not be necessary for development to the antral stage. The two men had azoospermia; small, soft testes; and absence of serum FSH. One had normal puberty and normal LH and testosterone values, with a missense mutation (Cys82Arg), and the other had slightly delayed puberty, low testosterone and inhibin B levels, high LH levels, and a nonsense mutation (Val61X). The low testosterone may indicate a necessity for FSH to promote testosterone production.

Follicle-Stimulating Hormone Receptor Mutations. Hypergonadotropic hypogonadism is noted with rare mutations in FSH receptors.[544] Women may have several mutations, but only five men are described as homozygous for the Finnish p.Ala189Val *FSHR* gene mutation. Although the affected women had amenorrhea, some of the men were fertile and all progressed through puberty with normal or slightly small testes. The defect in the

FSH receptor appears to be less severe than the absence of the ligand. Variations in FSHR are associated with age of thelarche in healthy Danish girls, while variance in LIN28B is related to age of menarche and thelarche; this demonstrates a differential impact of specific gene loci on age of thelarche and menarche.[545]

Developmental Defect of the Midline

Septo-optic or optic dysplasia is caused by abnormal development of the prosencephalon, leading to small, dysplastic, pale optic discs with a double outline and pendular (evenly moving side to side) nystagmus; blindness may occur. The prevalence is 1.9 and 2.5 per 100,000 births.[546] A midline hypothalamic defect may cause GH deficiency, diabetes insipidus, and ACTH, TSH, and gonadotropin deficiency. Short stature and delayed puberty may result, although CPP is an alternative.[546] The septum pellucidum is often absent in association with optic hypoplasia or dysplasia, which is readily demonstrable by imaging techniques.[547] In the University of California at San Francisco (UCSF) series, the syndrome was associated with decreased maternal age; recent series found maternal age of 20 to 24 years to carry the highest risk.[545] The pituitary may be hypoplastic because of the lack of hypothalamic stimulatory factors, and the neurohypophysis may have an ectopic location identified by the location of the posterior pituitary hot spot on MRI.

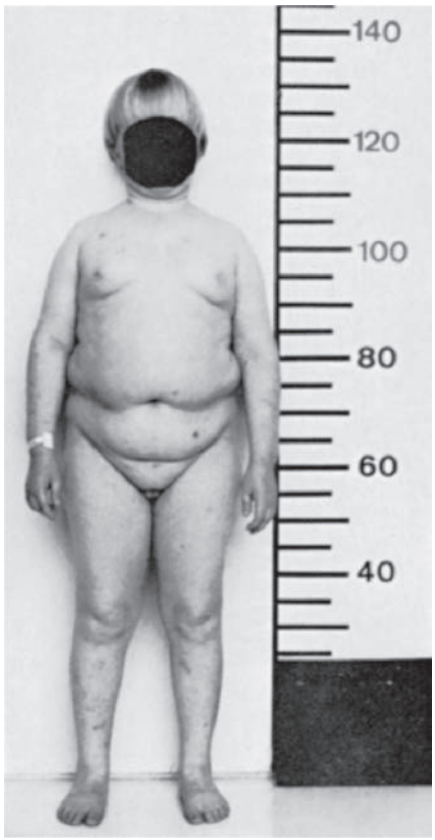
Abnormalities of the corpus callosum and cerebellum are common on MRI. Four groups are described: those with normal MRI results, those with abnormalities of the septum pellucidum and with a normal hypothalamic pituitary area, those with abnormalities of the hypothalamic pituitary area and a normal septum pellucidum, and those with abnormalities in both areas.[548] No endocrine abnormalities were described in the first group, but the others had progressively more endocrine abnormalities, with precocious puberty most common in the second group. Early diagnosis is important because of the risk of sudden death associated with adrenal insufficiency.

The condition is usually sporadic, but inherited cases involving transcription factors HESX1, SOX2, SOX3, and OTX2 are reported.[549]

The solitary median maxillary incisor syndrome is associated with the eponymous midline defect and with a prominent midpalatal ridge (torus palatinus) and hypopituitarism. The defect in this autosomal dominant condition is in the sonic hedgehog gene *(SHH)* at gene map locus 7q3.[550]

Other congenital midline defects ranging from complete dysraphism and holoprosencephaly to cleft palate or lip are associated with hypothalamic-pituitary dysfunction. Delayed puberty is rarely described in duplication of the hypophysis. Myelomeningocele (myelodysplasia) is associated with endocrine abnormalities, including hypothalamic hypothyroidism, hyperprolactinemia, and elevated gonadotropin concentrations, and with CPP.

Long-term follow-up of hypogonadotropic hypogonadism or partial pubertal development revealed that even with defined genetic defects, restoration of normal gonadal function, including pulsatile LH secretion and spermatogenesis in adulthood, may occur in over 20% of cases.[551,552] A recent series of 308 patients demonstrated that 22% underwent reversal.[484] One had undetectable olfactory bulbs demonstrating that this anatomic finding does not necessarily lead to long-term infertility. There was a higher prevalence of neurokinin B signaling (10%) of the subjects undergoing reversal, but none had mutations in KAL1. Five men undergoing reversal reverted back to hypogonadotropic hypogonadism. Long-term follow-up is necessary, even for patients with established anatomic and genetic defects.



• **Fig. 26.44** A 20-year-old man with idiopathic hypopituitary dwarfism and deficiencies of gonadotropins, thyrotropin, corticotropin, and growth hormone had a history of arrested hydrocephalus. His height was 129 cm (–8 SD [standard deviation]), the phallus was 2 cm long, and the testes measured 1.5 × 1 cm. He had received thyroid hormone and glucocorticoid replacement. The basal luteinizing hormone (LH) level was less than 0.2 ng/mL (LER-960), follicle-stimulating hormone (FSH) level was 0.5 ng/mL (LER-869), and testosterone level was less than 0.1 ng/mL. In response to 100 μg of gonadotropin-releasing hormone (GnRH), the plasma LH concentration increased slightly to 0.6 ng/mL, and there was no increase in the plasma testosterone level. The excretion of urinary 17-ketosteroids was 1.1 mg/24 hours. The bone age was 10 years, and the volume of the sella turcica was small on skull radiographs. For conversion to SI units, see Figs. 26.19 and 26.20. (From Styne DM, Grumbach MM. Puberty in the male and female: its physiology and disorders. In: Yen SCC, Jaffe RB, eds. *Reproductive Endocrinology*. 2nd ed. Philadelphia, PA: WB Saunders; 1986:313–384.)

Idiopathic Hypopituitary Dwarfism

In addition to *HESX1* mutations, autosomal recessive mutations in homeobox genes encoding transcription factors involved in the early aspects of pituitary development lead to hypogonadotropic hypogonadism and other pituitary hormone deficiencies.[553,554] *PROP1* mutations at gene map locus 5q cause GH and TSH deficiency and produce delayed puberty or late onset of secondary hypogonadism in adulthood and rarely cause ACTH deficiency (Fig. 26.44).[555] In one study of 73 patients with idiopathic multiple pituitary hormone deficiencies, 35 had a mutation in *PROP1*. Homozygous Arg73Cys mutation of *PROP1* allowed spontaneous puberty in 2 of 10 affected family members. ACTH deficiency may develop later than other deficiencies and is more rarely a feature of *PROP1* deficiency.

Homozygous mutations occur in the *LHX3* gene at gene map locus 9q34.3. It encodes a member of the LIM class of

homeodomain proteins, which are associated with multiple pituitary hormone deficiencies, including LH and FSH, and often with severe restriction of head rotation.[556] *LH4* and *GLI2* mutations may cause isolated GH deficiency or combined pituitary hormone deficiencies, including gonadotropin deficiency.[557,558]

The familial forms of multiple pituitary hormone deficiencies with autosomal recessive or X-linked inheritance are less common. The degree of hormone deficit and the age at onset of pituitary hormone deficiencies may vary within a single kindred having the same genetic defect.

The X-linked form of hypopituitarism can be associated with duplication of the *SOX3* gene.[559] Deficiency of SOX2, a transcription factor involved in early hypothalamic-pituitary embryonic development, leads to anterior pituitary hypoplasia. Patients with *SOX2* mutations have major eye abnormalities, including anophthalmia, microphthalmia, and coloboma. They also have hypogonadotropic hypogonadism as the most common pituitary defect, in contrast to most other types of pituitary hypoplasias, demonstrating GH deficiency most frequently.[560]

There is an association between breech delivery (especially for male infants), perinatal distress, and idiopathic hypopituitarism.[126] Malformations of the pituitary stalk demonstrable by MRI are common in these patients. Other types of birth traumas or complications may lead to hypopituitarism as well.

Common to many patients with congenital hypopituitary dwarfism is early onset of growth failure; late onset of diminished growth is an ominous finding, suggesting the presence of a CNS tumor.

Isolated GH deficiency allows spontaneous pubertal development when the bone age reaches the pubertal stage of 11 to 13 years, usually after the corresponding chronologic age is reached. Associated gonadotropin deficiency does not allow spontaneous puberty, even when the bone age advances to the pubertal stage during GH therapy.

Miscellaneous Conditions

Prader-Willi Syndrome. Prader-Willi syndrome is an autosomal dominant disorder that combines a tendency for intrauterine growth retardation, delayed onset and poor fetal activity, infantile central hypotonia, and lethargy, followed by early-onset childhood hyperphagia, pathologic obesity, and carbohydrate intolerance (leading to type 2 diabetes in 25% of patients at a mean age of 20 years). Features include short stature, small hands and feet, mild to moderate mental retardation, and emotional instability, including perseveration, obsessions, and compulsions. Almond-shaped eyes, a triangular mouth, and narrow bifrontal diameter combined with delayed puberty and hypogonadotropic hypogonadism caused by combined hypothalamic and gonadal dysfunction are characteristic.[561] Despite the late or absent puberty, there is a tendency to early adrenarche (14%) or even precocious puberty rarely (3.6%).

Affected boys usually have a micropenis and cryptorchidism (100% in a large series[562]), and an underdeveloped scrotum (69%) is common. In a study of 37 adults with Prader-Willi syndrome, none achieved full genital development, and primary testicular defects were suggested.[563] Serum AMH levels were near the lower limits of normal, inhibin B levels were consistently low or undetectable, and in the adults, FSH levels were high, although LH levels were normal. Two adults had undetectable levels of LH and FSH, but in contrast to the others, they had high AMH levels. Female subjects exhibit underdevelopment of the labia majora, labia minora, or clitoris (76%). Amenorrhea occurs in about one-half of cases (53%), and irregular menses or spotting is common in others. Weight reduction may lead to menarche in some females, because severe obesity may play a role in the impaired puberty in some patients. Dietary therapy during years 2 through 10 can provide effective treatment of obesity but may decrease growth, although contemporaneous GH therapy may overcome slow growth.[564]

The role of relative GH deficiency in this disorder is uncertain and controversial. The FDA approved Prader-Willi syndrome as an indication for recombinant human GH treatment without a requirement for assessing GH secretion. Genetic testing is used to confirm the clinical diagnosis of the syndrome. GH treatment (in a dose of 0.24 mg/kg/week subcutaneously given six to seven times per week) was shown in long-term, randomized, controlled trials to decrease body fat; increase fat utilization, LBM, linear growth, and energy expenditure; and possibly improve physical strength and motor development.[565] Children with Prader-Willi syndrome are at risk for sudden death due to gastrointestinal, respiratory, or cardiac complications.[566] However, the report of sudden deaths due to respiratory complications during GH treatment led to a recommendation for evaluation for sleep apnea or respiratory difficulties before instituting GH therapy. More recent data cast doubt on the beneficial effects of GH on body composition and BMI, but higher parental educational status was correlated with better clinical outcome.[986]

This distinct genetic disorder, with a frequency of about 1 case in 15,000 to 30,000 individuals, is rarely familial (i.e., the recurrence risk depends on the type of the genetic defect). It is caused by abnormalities involving the long arm of chromosome 15 in the q11-q13 region. Approximately 70% of Prader-Willi cases are caused by a paternal deletion of 15q11-q13 (commonly 3–5 megabase pairs long); 20% to 25% of cases involve maternal uniparental disomy (isodisomy or heterodisomy) in which both chromosomes 15 are derived from the mother, possibly by nondisjunction during maternal meiosis, representing a striking example of genomic imprinting.[561] In 2% to 5% of cases, an imprinting center defect has been detected. Lack of a functional paternal 15q11-q13 region, caused by any of a variety of genetic mechanisms, can result in the syndrome. One imprinted gene, that for small nuclear ribonucleoprotein-associated polypeptide SmN *(SNRPN)*, which is implicated in splicing pre-mRNA, is expressed in the brain, including the hypothalamus, and has been advanced as one explanation of some characteristics of the syndrome.

Elevated serum concentrations of the GH secretagogue and orexigenic gastrointestinal hormone ghrelin are found in the basal state in Prader-Willi syndrome.[567] Increased levels are identified after meals, when values should be suppressed, and are a possible cause of the insatiable appetite. Administration of the somatostatin analogue octreotide leads to a decrease in basal ghrelin values and some decrease in values after meals, but no change in appetite was demonstrated as yet.

Laurence-Moon and Bardet-Biedl Syndromes. The Laurence-Moon syndrome and the Bardet-Biedl syndrome were previously separated as rare autosomal recessive traits, with retinitis pigmentosum and hypogonadism of various types. Currently the conditions are considered as one, and the present term is *Bardet-Biedl syndrome*. The estimated incidence is 1 in 160,000 in northern European populations and 1 in 13,500 in some Arab populations.[568] The findings are developmental delay, spastic paraplegia, postaxial polydactyly, onset of obesity (usually in early infancy), and renal dysplasia. Hypgonadism is characteristic, and males are

infertile as are most females. The genetically and phenotypically heterogenous Bardet-Biedl syndrome is linked to 16 genes, which account for 80% of cases. The basic defect is a ciliopathy. The Biemond syndrome II has similar features, with iris coloboma, hypogenitalism, obesity, polydactyly, and developmental delay, but it is a distinct entity.

Functional Gonadotropin Deficiencies and Other Chronic Conditions. The effects of malnutrition, which can lead to functional hypogonadotropic hypogonadism, should be separated from the primary effects of chronic systemic disease, some of which have direct effects on the function of the hypothalamic-pituitary unit or the gonads. Weight loss from any cause to less than 80% of ideal weight for height can lead to gonadotropin deficiency and low serum leptin levels; weight regain usually restores hypothalamic-pituitary gonadal function over a variable period, although the weight needed to restart menstrual periods varies among individuals and is related to the weight at which menstruation first ceased.[569]

If adequate nutrition and body weight are maintained in patients with regional enteritis or chronic pulmonary disease, gonadotropin secretion is usually adequate. Cystic fibrosis is also associated with delay in puberty and the age of PHV, in large part through malnutrition; improved management and newborn screening have increased PHV and advanced the age of PHV, but neither has reached normal values.[454,570,571] The age of menarche in girls with cystic fibrosis is related to maternal age, as expected, but is delayed by approximately 1 year compared with menarche in the mother, an effect that is mainly related to nutritional status.[570,987] However, even with normal pubertal progression, boys with cystic fibrosis almost universally have oligospermia caused by obstruction of the spermatic ducts, which is unrelated to nutritional status. The greater prevalence of reproductive difficulties in male patients with cystic fibrosis compared with female patients may reflect the greater prevalence of the cystic fibrosis transmembrane regulator (CFTR) in male reproductive tissues (e.g., epididymis, vas deferens), and more viscid luminal contents, which ultimately damage the testes and can lead to absence of the epididymides and the vasa deferentia. Normal ovaries do not express CFTR, and endometrial tissue expresses it only after puberty, with variable levels found in cervical epithelium and the fallopian tubes. Even though the *CFTR* gene and its protein are expressed in the human hypothalamus, mutations in the corresponding gene did not appear to affect LH and FSH secretion in an immortalized mouse hypothalamic GnRH-secreting cell line.

Boys and girls with sickle cell disease have delayed pubertal development by about 2 years and delayed menarche of about 6 months even with modern treatment techniques.[572] This may be due to nutritional status. Boys with sickle cell anemia often exhibit impaired Leydig cell function caused by ischemia of the testes or gonadotropin deficiency or both.

Thalassemia carries the risk of hemochromatosis due to transfusional iron deposition in the pituitary and hypothalamus; as a consequence, 60% to 80% patients may have hypogonadotropic hypogonadism and impairment of growth.[573] The gonads can be stimulated by exogenous gonadotropins, and satisfactory sexual development, including fertility, can be promoted by the use of hCG and human FSH in many patients without gonadal damage, although pituitary and gonadal damage may be severe in children with poorly controlled disease. Primary hypothyroidism is prevalent in this condition, but it is only part of the problem of sexual maturation. Desferrioxamine therapy may cause skeletal dysplasia and compromise pubertal growth, and growth failure due to GH deficiency may also affect pubertal growth.[574] Decreased BMD in thalassemia makes early recognition and treatment of the problem all the more important.

Cytotoxic effects of the alkylating agents used to prepare patients for bone marrow transplantation in this condition add to the problem. Treatment after the onset of puberty is safer for gonadal function in boys but not necessarily in girls. Girls with early bone marrow transplantation and apparently normal pubertal development have elevated serum FSH levels and menstrual abnormalities ranging up to amenorrhea,[575] suggesting that gonadal impairment is universal in girls with thalassemia major after bone marrow transplantation.

Puberty is significantly delayed in children with prenatally acquired human immunodeficiency virus (HIV) infection compared to those exposed but not affected.[988] Modern treatment regimens are predicted to decrease this delay.

Chronic gastrointestinal disease (e.g., Crohn disease) is often accompanied by delayed puberty, and therapy to restore nutrition, if successful, enables puberty to progress. The pubertal growth spurt is compromised by active inflammatory bowel disease, especially if glucocorticoid therapy is necessary. Celiac disease decreases the growth rate in childhood and adolescence, but with appropriate dietary restrictions, adult height appears to be normal.

Chronic renal disease is associated with delayed pubertal development and decreased pulsatile gonadotropin secretion due to a decrease in the mass of bioactive and immunoactive LH secreted rather than an alteration of the frequency. Successful renal transplantation usually restores gonadotropin secretion and improves growth. Immunoreactive gonadotropin concentrations may be elevated, presumably because of impaired renal clearance, but the response to GnRH is blunted in severe renal impairment. TeBG is elevated in chronic renal failure, and the level of free testosterone is low. Survivors of renal transplantation who are undergoing immune suppression and alternate-day steroid treatment often have delayed onset of puberty and decreased pulsatility of GH and gonadotropins at night.

Patients with nephrotic syndrome have poor pubertal growth, poor secondary sexual development, and deficient gonadotropin secretion in a pattern resembling CDP. Glomerulonephritis treated with alternate-day glucocorticoid therapy leads to a late, diminished, but prolonged pubertal growth spurt that can result in a normal final height.

Children with early onset of leukemia and early long-term remission experience puberty at an appropriate age or with only a slight delay, whereas patients with initial symptoms of leukemia in late childhood may have considerable delay of pubertal development. Radiation treatment to the CNS may cause hypogonadotropic hypogonadism or GH deficiency or both, and irradiation of the abdomen or pelvis and certain types of chemotherapies, especially if administered during puberty, may impair gonadal function and cause primary hypogonadism, although ovarian function may return even in the face of elevated serum gonadotropin levels.[568] Total-body irradiation for bone marrow transplantation exerts the most significant effects, such as severe GH deficiency in 50%, hypothyroidism in 56%, and hypogonadism in 83% of males, and 100% of women had ovarian failure; insulin resistance was found in 83% and dyslipidemia in 61%.[576] Children with leukemia treated with CNS irradiation had a diminished pubertal growth spurt and diminished final height. Long-term follow-up studies

demonstrate the rising incidence of the metabolic syndrome survivors and high lifetime risk for cardiovascular disease in childhood cancer.[577]

Other Endocrine Conditions and Puberty. Hypothyroidism may delay the onset of puberty or menarche (except in extreme cases in which puberty starts early); treatment with levothyroxine reverses this pattern, but there is likely to be a permanent loss of height if the diagnosis is delayed.

Diabetes mellitus type 1 is associated with delayed menarche.[578] Remarkably, this may occur no matter the degree of glycemic control. Prepubertal children are most vulnerable to poor glycemic control, and pubertal subjects exhibit normal growth unless severe hyperglycemia occurs. The degree of control necessary to avoid these complications cannot be exactly quantified, but adolescents with even moderately poor control frequently manifest some degree of growth impairment and delayed puberty or irregular menses. Mauriac syndrome is characterized by poorly controlled diabetes, hepatomegaly with fatty infiltration of the liver, and delayed puberty that is likely related to poor nutritional status.[579]

Cushing disease can be associated with delayed onset or arrest of gonadarche, although excessive virilization is an alternative finding.

Anorexia Nervosa and Variants.[580,581,990]

Anorexia Nervosa. Anorexia nervosa,[582] a common cause of gonadotropin deficiency in adolescence, is a functional disorder. Prevalence is increased among girls (it is the third most common chronic disease of adolescent girls), but it is rarer in boys. This condition has the highest mortality rate of all psychiatric disorders[583]; weighted mortality rates (i.e., deaths per 1000 person-years) were 5.1 for anorexia nervosa, and 1.7 standardized mortality ratios were 5.86 for anorexia nervosa. It is characterized by a distorted body image, obsessive fear of obesity, and food avoidance that can cause severe self-induced weight loss (to less than 85% of normal weight for age and height or a BMI <17.5 kg/m^2 after cessation of growth), primary or secondary amenorrhea in affected females, widespread endocrine disorders, and even death. Specific diagnostic details are provided in the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (DSM-IV) criteria of the American Psychiatric Association[581]:

A. Restriction of energy intake relative to requirements, leading to a significantly low body weight in the context of age, sex, developmental trajectory, and physical health. Significantly low weight is defined as a weight that is less than minimally normal or, for children and adolescents, less than that minimally expected.
B. Intense fear of gaining weight or of becoming fat, or persistent behavior that interferes with weight gain, even though at a significantly low weight.
C. Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight.

There are subtypes:

Restricting type and binge-eating/purging type

The onset of amenorrhea may precede the onset of severe weight loss. Other common features include onset in middle adolescence, hyperactivity, defective thermoregulation with hypothermia and sensitivity to cold, constipation, bradycardia and hypotension, decreased BMR, dry skin, fine or downy hypertrichosis, peripheral edema, and parotid enlargement. The pathogenesis is multifactorial and includes a genetic factor and a well-characterized psychologic component. Before the diagnosis of anorexia nervosa is made, organic disease must be excluded; for example, a girl with macroprolactinoma may present with signs consistent with anorexia nervosa. The prevalence of anorexia nervosa is increased among individuals with Turner syndrome.

Anorexia nervosa has considerable endocrine ramifications.[584] The concentrations of plasma FSH, LH, leptin, and estradiol and the excretion of urinary gonadotropins are characteristically low. There may be a reversion to a circadian rhythm of LH secretion and to the sleep-associated increase in episodic LH secretion or LH response to GnRH characteristic of early puberty, or the amplitude of the pulsatile episodes may be diminished, as in the pattern of prepubertal children, if onset occurs during puberty. Pulsatile administration of intravenous GnRH at intervals of 90 to 120 minutes can produce LH pulses that are indistinguishable from the normal pubertal pattern, demonstrating functional GnRH deficiency. Serum leptin levels are low, consistent with the strikingly decreased mass of adipose tissue, and increase with regain of weight. Other hormonal changes include increased mean concentrations of plasma GH and plasma cortisol; low levels of plasma IGF1, DHEAS, and triiodothyronine (T_3) with normal levels of thyroxine (T_4) (unless the low thyroxine syndrome is present) and TSH; a decreased rise in serum prolactin after administration of thyrotropin-releasing hormone (TRH) or insulin-induced hypoglycemia; and a diminished capacity to concentrate urine. This condition must be considered in the differential diagnosis of growth failure in younger subjects.

Lower heart rates are characteristic and a dangerous sign of severe disease and not to be confused with an athletic bradycardia in view of the excessive exercise the patient engages in. A lower systolic blood pressure, lower body temperature, anemia, and leukopenia are found in persons with anorexia nervosa. The ratio of bone age to chronologic age is significantly lower in girls with anorexia nervosa and correlates positively with duration of illness and markers of nutritional status. All measures of BMD are lower, and the most significant predictors of bone density are LBM, BMI, and age at menarche. Treatment of decreased bone density in these individuals is accomplished with improved nutrition, and a degree of catch-up in bone density occurs, although reversion to normal may not occur.

Normal endocrine and metabolic function may follow weight gain, but amenorrhea may persist for months, suggesting persistent hypothalamic dysfunction. In view of the associated mortality rate, parenteral alimentation may be indicated in resistant patients with severe weight loss, especially in those with infection or an electrolyte imbalance. However, refeeding syndrome with attendant hypophosphatemia may result if not handled appropriately. Treatment of this disorder requires skillful management, understanding, patience, and psychiatric consultation in a team approach. Unfortunately, an evidence-based approach to optimal treatment is not plentiful, and the difficulty in obtaining therapy in uninsured individuals remains.[585]

Functional hypothalamic amenorrhea is defined as the absence of menses, low or normal gonadotropin levels, and normal gonadotropin response to GnRH stimulation but lack of or an inadequate midcycle LH surge and a decrease in normal pulsatile secretion (amplitude or frequency, or both) of gonadotropins and hypoestrogenemia without organic abnormality.[523] This condition

can occur in women with normal weight but decreased percentage of body fat. These patients have higher than average cortisol values; decreased levels of free T_4, free T_3, and total T_4 with normal TSH levels; and decreased leptin concentrations that are probably caused by subtle dysfunction of eating patterns and altered energy expenditure. The consequences range from severe estrogen deficiency to anovulation to a short luteal phase. Reduced bone density is a concern. Genes involved in the cause of hypogonadotropic hypogonadism such as *GNRHR*, *KAL1*, and *PROKR2* are found in heterozygous patterns in women with functional hypothalamic amenorrhea, suggesting a genetic susceptibility to the condition that is brought out by various types of stresses[523]; thus functional amenorrhea may not be simply functional.[586,587]

Bulimia Nervosa. Bulimia nervosa is now separated from the diagnosis of anorexia nervosa[582]; DSM-IV diagnostic criteria are as follows:

A. Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following:
   1. Eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than what most individuals would eat in a similar period of time under similar circumstances.
   2. A sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating).
B. Recurrent inappropriate compensatory behaviors to prevent weight gain, such as self-induced vomiting; misuse of laxatives, diuretics, or other medications; fasting; or excessive exercise.
C. The binge eating and inappropriate compensatory behaviors both occur, on average, at least once a week for 3 months.
D. Self-evaluation is unduly influenced by body shape and weight.
E. The disturbance does not occur exclusively during episodes of anorexia nervosa.

Bulimia occurs in about 1.5% of young women. A hand lesion from the induced vomiting (i.e., Russell sign) and an abnormal level of serum electrolytes are useful clinical markers. Abuse of laxatives, diet pills, and diuretics is frequent. Although weight loss is not frequent, amenorrhea is common. Bulimia is especially prevalent in female high school and college students. A history of childhood sexual abuse is more common than in unaffected adolescents. Cessation of growth can occur in infants and young children with psychosocial dwarfism. Stressful social situations can also inhibit growth and physical pubertal development at adolescence.

Exercise, Hypo-ovarianism, and Amenorrhea: The Female Athlete Triad. In 1992, the American College of Sports Medicine defined the female athletic triad as primary or secondary amenorrhea, disordered eating, and osteoporosis.[582] Although there are substantial endocrine effects of excessive athletic training in girls, elite prepubertal and pubertal female athletes suffer relatively few physical injuries. Because there is no demonstrable effect on pubertal development from moderate exercise in subelite female runners, moderate exercise should not be discouraged during adolescence. However, extensive training (10–12 hours/week) may be excessive for prepubertal girls.

Bulimia, anorexia nervosa, or anorexia athletica is most often found in girls engaged in sports that emphasize weight.[587] Teenage ballet dancers are lighter, have less body fat, and have a high incidence of delayed puberty and of primary and secondary amenorrhea than less physically active girls. Factors other than decreased body weight can impair pubertal progression and delay menarche through inhibition of the hypothalamic GnRH pulse generator in healthy ballet dancers and female athletes such as swimmers who are not underweight.

Athletes who began strenuous training before menarche have a delay in menarcheal age.[588] However, genetic influences overlie changes due to weight or activity because there is a positive correlation between the delayed menarche found in athletic girls and the age of menarche of their mothers. Although some studies of artistic and rhythmic gymnasts found delayed menarche when compared with their mothers and sisters, with a more significant delay in artistic gymnasts,[589–591] a recent survey of the literature by a committee of the Scientific Commission of the International Gymnastics Federation found the following: "(1) Adult height or near adult height of female and male artistic gymnasts is not compromised by intensive gymnastics training. (2) Gymnastics training does not appear to attenuate growth of upper (sitting height) or lower (legs) body segment lengths. (3) Gymnastics training does not appear to attenuate pubertal growth and maturation, neither rate of growth nor the timing and tempo of the growth spurt. (4) Available data are inadequate to address the issue of intensive gymnastics training and alterations within the endocrine system."[592] The implication is that these athletes may be shorter and delayed in puberty due to self-selection. There are studies with findings at odds with these conclusions.

Higher bone density is reported in the femurs of gymnasts compared with those of ballet dancers and control subjects, but lower radial bone density in the gymnasts and ballet dancers reflects the effect of the application of force on bone remodeling; a positive relationship between serum leptin and tibial bone density is found.[593] The bone density of female long-distance runners is greater in those with regular menses than in those with menstrual irregularities. Osteopenia in later life may result from amenorrhea in ballet dancers, even with estrogen replacement, and nutrition therapy is considered important to improve outcome.[594]

Thinness and strenuous physical activity appear to act synergistically, but strenuous exercise training by itself may inhibit the GnRH pulse generator, mediated in part by endogenous opioidergic pathways involving β-endorphin. When the strenuous physical activity is interrupted (e.g., by injury), puberty advances, and menarche often occurs within a few months in those with amenorrhea, in some cases before a significant change in body composition or weight. Even though gonadarche is retarded, adrenarche is not delayed.

Female athletes of normal weight who have less fat and more muscle than nonathletic girls (e.g., ice skaters, swimmers) are also at risk for delayed puberty and for primary and secondary amenorrhea. However, the mechanism apparently is different from the hypothalamic amenorrhea in runners and ballet dancers. In swimmers, menstrual cycles frequently are irregular and anovulatory rather than absent, and the plasma concentrations of DHEAS and LH were higher than normal, but plasma estrogen levels were normal.

Prospective study of gymnasts contrasted with swimmers demonstrated decreased growth velocity, stunting in leg length growth, and in some studies decreased height prediction in the gymnasts.[595]

Prolactin levels may be elevated in women athletes and may contribute to the delayed menarche found in this group. Osteopenia can result from the associated chronic hypoestrogenism.[588]

Scoliosis in girls usually develops during the pubertal growth spurt and more often occurs in girls with a more rapid pubertal growth spurt. Ballet dancers have a higher incidence of scoliosis

than the general population and often have delayed puberty and delayed menarche. These girls have decreased leptin levels, but soluble leptin receptor and adiponectin levels were increased throughout puberty; these changes were related to changes in LBM rather than BMI. Idiopathic scoliosis in the general population is associated with a statistically earlier age of menarche and an early adolescent growth spurt. The strongest association with scoliosis is taller stature at the time of the pubertal growth spurt. Adult height in familial constellations of scoliosis does not vary from the family norm.

Although men are less affected than women, men may also be affected by rigorous physical training. They may have decreased LH response to GnRH and decreased spontaneous LH pulse frequency and amplitude; the serum testosterone level is normal or low with extreme activity levels.

Other Causes of Delayed Puberty

Marijuana use has been associated with gynecomastia and is a putative cause of pubertal delay. Untreated Gaucher disease causes delay in pubertal development, but early treatment with enzyme replacement allows puberty to begin on time.[596] Girls with familial dysautonomia have delayed menarche and often have a severe premenstrual syndrome. The condition is ultimately compatible with pregnancy. Chronic infections may delay the onset of puberty.

Central Nervous System Tumors

Extrasellar masses may interfere with GnRH synthesis, secretion, or stimulation of pituitary gonadotrophs. Most patients with hypothalamic-pituitary tumors causing gonadotropin deficiency have one or more additional pituitary hormone deficiencies (or an increased serum prolactin level with prolactinomas). Those with GH deficiency due to a neoplasm have late onset of growth failure compared with those who have idiopathic and familial hypopituitarism, in which growth failure starts early in life. The presence of anterior and posterior pituitary deficiencies in infancy suggests a midline developmental defect, but the development of this combination after infancy ominously suggests an expanding CNS lesion.

Craniopharyngioma. Craniopharyngioma is a rare embryonic malformation of nonglial origin in childhood (0.5–2 new cases per 1 million population annually, or 1.2–4% of pediatric intracranial tumors), a common CNS neoplasm.[597] It is, however, the most common brain tumor associated with hypothalamic-pituitary dysfunction and sexual infantilism and accounts for 80% to 90% of neoplasms found in the pituitary and up to 15% of all intracranial tumors in childhood.[598] Symptoms usually arise before the age of 20 years with a peak incidence between the ages of 6 and 14 years with about 30% to 50% occurring in the pediatric age range. Harvey Cushing introduced the term *craniopharyngioma* and considered them "the most formidable of intracranial tumors."[599]

Various theories of the embryologic origin of this nonglial intracranial tumor are current: One theory favors development from ectodermal remnants of Rathke pouch and another development from residual embryonic epithelium of the anterior pituitary gland and of the anterior infundibulum. Craniopharyngiomas may reside within or above the sella turcica, or more rarely, they may be found in the nasopharynx or the third ventricle.

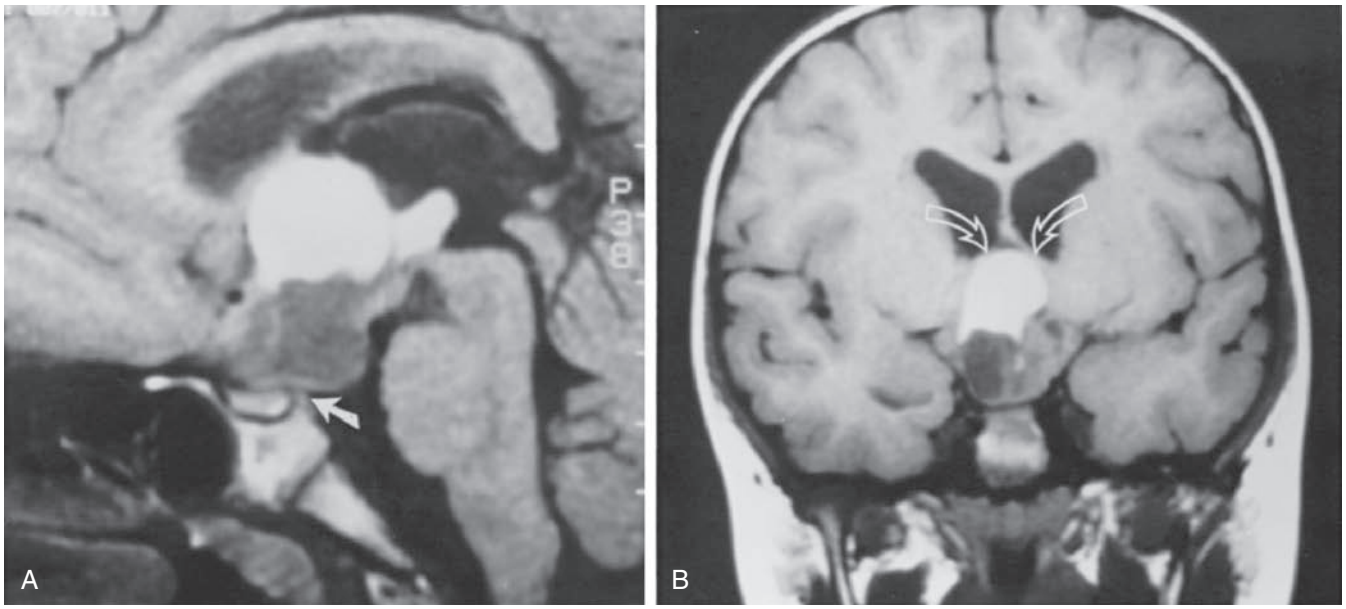
Craniopharyngioma appears to be a monoclonal tumor, and about 50% have cytogenetic abnormalities such as gains in 1q, 12q, and 17q. About 70% of cases of craniopharyngioma in childhood are the adamantinomatous type with cyst formation. These types have dysregulation of the Wnt signaling pathway and a mutation in the β-catenin gene *(CTNNB1)* in contrast to the papillary type of craniopharyngioma that has *BRAF* mutations and is more often found in adult patients.[600,601]

CNS signs of craniopharyngiomas develop as the tumor encroaches on surrounding structures. Symptoms of craniopharyngioma include headache, visual disturbances, short stature, diabetes insipidus, vomiting, and weakness of one or more limbs. Visual defects (including bilateral temporal field deficits), optic atrophy or papilledema, and signs of GH deficiency, delayed puberty, and hypothyroidism are features of craniopharyngiomas. Although most patients are below the mean in height and height velocity at diagnosis, a long, indolent course is possible. Deficiencies of gonadotropins, GH, thyrotropin (TSH, thyroid-stimulating hormone), ACTH, and arginine vasopressin are common. The serum concentration of prolactin is normal or increased. Delayed bone age is common and may point to the onset of tumor growth.

About 70% of patients with a craniopharyngioma have suprasellar or intrasellar calcification (found in fewer than 1% of normal individuals) and an abnormal sella turcica, which are sometimes found on radiographs taken for other indications, including orthodontia. CT (but not MRI) reveals fine calcifications that are not apparent on lateral skull radiographs. MRI scans before and after gadolinium are the diagnostic procedures of choice for suspected craniopharyngioma and can determine whether the tumor is cystic or solid and indicate the presence of hydrocephalus; if necessary a CT scan can be used to search for calcifications, and CT or MRI with contrast can determine whether the tumor is cystic or solid and indicate the presence of hydrocephalus (Fig. 26.45).

Smaller craniopharyngiomas, usually intrasellar, can be treated by transsphenoidal microsurgery, but larger or suprasellar masses usually require craniotomy, and the approach must be individualized. The reported postsurgical 5-year overall survival rate is 88% to 94%, and the reported 10-year overall survival rate is 70% to 92% with a 20-year survival rate of 76%. The combination of limited tumor removal and radiation therapy leads to a satisfactory neurologic prognosis, better cognitive outcome, and better endocrine outcome compared with attempts at complete surgical extirpation. Frequent and early tumor relapse after apparently complete resection and tumor progression after incomplete resection suggest the wisdom of radiation therapy after surgery. Alternative approaches include proton beam therapy; in mainly cystic craniopharyngioma cases, instillation of radioisotopes or sclerosing substances such as bleomycin or interferon-α are being investigated.[602] Nonetheless, the preferred manner of treatment to retain the best quality of life is not yet established, but longitudinal studies such as the randomized multinational trial KRANIOPHARYNGEOM 2007 may answer this question.[602]

Postoperative hyperphagia and obesity (BMI >5 SD above normal) can be striking and correlate with the magnitude of hypothalamic damage on cranial MRI. Injury to the hypothalamic ventromedial nuclei (associated with increased parasympathetic activity and hyperinsulinemia) or to the PVN may cause these findings, and insulin suppression may be helpful. Short-term follow-up studies demonstrate the efficacy of bariatric surgery in the management of obesity in affected patients.[601] Hypothalamic



• **Fig. 26.45** Craniopharyngioma in a short 5-year-old girl with a history of frontal headaches, impaired vision, and poor growth. (A) Midline, sagittal, T1-weighted image shows a hyperintense region superiorly and an inferior hypointense region. The combination of hyperintense and hypointense areas in a non–contrast-enhanced examination is the most characteristic finding for craniopharyngioma. Notice the erosion of dorsum sellae *(solid arrow)* and posterior pituitary bright spot. (B) Coronal, T1-weighted image shows tumor extending upward to the inferior frontal horns, narrowing the foramen of Monro, and causing mild hydrocephalus. The *open arrows* indicate the upper border of the hyperintense area of the tumor.

sparing surgery decreases the risk of postoperative hyperphagia and obesity.[602] Aberrant sleep patterns and even narcolepsy and daytime somnolence may follow surgical treatment of craniopharyngiomas, with melatonin improving sleep patterns in some.[603] Although the endocrine complications are more manageable, the combination of antidiuretic hormone insufficiency (i.e., diabetes insipidus) and impaired sense of thirst remains a complex management problem.

A Rathke cleft cyst is often discovered as an incidental finding on MRI, but it can produce symptoms and signs indistinguishable from those of a craniopharyngioma, such as precocious or delayed puberty.[604] Surgical drainage and excision of the cyst wall are customary approaches.

Other Extrasellar Tumors

Germinomas. Germinomas (i.e., pinealomas, ectopic pinealomas, atypical teratomas, or dysgerminomas) and other germ cell tumors of the CNS are the most common extrasellar tumors that arise in the suprasellar hypothalamic region and in the pineal region that commonly cause sexual infantilism. Germinomas constitute 66% of all intracranial germ cell tumors (GCTs), which make up 3% to 11% of pediatric brain tumors. About 84% are found in the pineal and the neurohypophyseal regions. Peak incidences occur in the second decade and during infancy. They are found more often in males.[605] Polydipsia and polyuria are the most common symptoms, followed by visual difficulties and abnormalities of growth and puberty or movement disorders. Diagnosis may be delayed for months to years because the findings are attributed to psychiatric disorders. Deficiencies of vasopressin and GH are most common, but other anterior pituitary hormone deficiencies (including gonadotropin deficiency) and elevated serum prolactin levels are also frequent. Determination of the concentration of hCG in spinal fluid and in serum and assessment of α-fetoprotein levels provide useful tumor markers in children and adolescents with germ cell tumors. Germ cell tumors in boys cause isosexual GnRH-independent sexual precocity (GISP) by secretion of hCG (see "Sexual Precocity"). Tumors secreting hCG cause precocious puberty in boys, and there has been one case report of an affected girl.

Subependymal spread of germ cell tumors along the lining of the third ventricle is common, and seeding may involve the lower spinal cord and corda equina. MRI with contrast enhancement is useful in the detection of isolated enlargement of the pituitary stalk, an early finding that requires periodic MRI monitoring, especially in patients with diabetes insipidus.[481] The size of the pituitary gland increases by 100% between year 1 and year 15, but the pineal gland does not normally change in size after the first year of life; any later enlargement indicates a mass lesion. Pineal cysts are a rare cause of CPP.

Irradiation is the preferred treatment for pure germ cell tumors such as germinomas; surgery is rarely indicated, except for biopsy to establish a tissue diagnosis.[481] However, attempts to decrease the long-term morbidity of radiation therapy lead to consideration of chemotherapy. Chemotherapy alone is inadequate, but the combination of chemotherapy and radiation therapy can be successful,[485,606] and both treatment methods are recommended for a mixed germ cell tumor. Because testicular germ cell tumors are occasionally found years after successful therapy for CNS germ cell tumors, long-term surveillance is indicated.[557,607]

Hypothalamic and optic gliomas or astrocytomas, occurring as part of neurofibromatosis (von Recklinghausen disease) or arising independently, can also cause sexual infantilism. Gliomas and meningiomas are the most common CNS tumors to develop in childhood cancer survivors treated with CNS radiation, often the young adult or even late teenage years.[608]

Pituitary Adenomas. Only 2% to 6% of all surgically treated pituitary tumors occur in childhood and adolescence, with about 1 in 1 million children affected.[598] Most functional pituitary adenomas are ACTH secreting, with prolactinomas or GH secreting or nonfunctioning adenomas occurring less commonly. Most pituitary tumors are monoclonal lesions caused by mutations of *GNAS*. Adolescent onset of pituitary team tumors may be the first manifestation of multiple endocrine neoplasia type I or familial isolated pituitary adenoma (FIPA).[609] With higher sensitive imaging techniques the presence of a pituitary incidentaloma, a previously unsuspected pituitary lesion that is discovered on an imaging study performed for an unrelated reason, may be discovered.[610] Evaluation of secretory activity of such a lesion, consideration of mass effects, and follow-up to monitor a change in size are important, but some lesions detected will not be related to the pubertal abnormality.

Incidence of prolactinoma is low in childhood but one in five present in the age group 15 to 24 years.[611] A survey of 44 cases reported that 61% of prolactinomas were macroadenomas (more often in boys; hypopituitarism and growth failure were common) and 39% were microadenomas (more often in girls; delayed puberty was common).[612] Only 2 of these 29 patients had delayed onset of puberty, although primary amenorrhea was the presenting symptom in 13 of 20 pubertal females. Presenting symptoms included oligomenorrhea and galactorrhea in the girls and headache in the boys. Galactorrhea may be demonstrable only by manual manipulation of the nipples (blood samples for prolactin should be obtained before examination or many hours later, because manipulation of the nipples raises prolactin levels).

Dopaminergic therapy is often successful in decreasing prolactin values. The dopamine agonist bromocriptine may decrease serum prolactin concentrations and decrease tumor size, which is a useful approach before surgery of large macroprolactinomas is undertaken and when resection of the adenoma is incomplete. Transsphenoidal resection of microprolactinomas in children and adolescents is an effective treatment. Pubertal progression and normal menstrual function in girls usually follow reduction of serum prolactin levels. Pituitary apoplexy followed cabergoline treatment of a macroprolactinoma in a 16-year-old girl[613]; this complication has been seen in adults treated with bromocriptine, and tricuspid regurgitation may be a cumulative effect.[614] High serum levels of macroprolactin, a complex of immunoglobulin G and monomeric prolactin with little biologic activity in vivo, cross-react in commercial prolactin assays, leading to a finding of pseudohyperprolactinemia; high prolactin values should be rechecked with subfractionation after polyethylene glycol precipitation.[615]

Other Central Nervous System Disorders Leading to Delayed Puberty

Langerhans Cell Histiocytosis. Langerhans cell histiocytosis (i.e., Hand-Schüller-Christian disease or histiocytosis X) is a clonal proliferative disorder of Langerhans histiocytes or their precursors. It is characterized by the infiltration of lipid-laden histiocytic cells or foam cells in skin, viscera, and bone.[616] The cause is not clear because there are features of a neoplasm and features of a reactive immunologic disorder. Diabetes insipidus, caused by infiltration of the hypothalamus or the pituitary stalk, is the most common endocrine manifestation, with GH deficiency and delayed puberty possible. The lung, liver, and spleen, cystlike areas in flat and long bones, and the dorsolumbar spine may be involved. "Floating teeth" within rarefied bone of the mandible, absent or loose teeth, and exophthalmos due to infiltration of the orbit are seen. Mastoid or temporal bone involvement may lead to chronic otitis media. Treatment with glucocorticoids, antineoplastic agents, and radiation therapy is promising in terms of survival, but more than 50% of patients have late sequelae or disease progression. The natural waxing and waning course of this rare disease makes evaluation of therapy difficult and highlights the importance of national treatment protocols.[616]

Postinfectious Inflammatory Lesions of the Central Nervous System, Vascular Abnormalities, and Head Trauma. Tuberculous or sarcoid granulomas of the CNS are associated with delayed puberty. Hydrocephalus may cause delayed puberty that can be reversed with decompression, as may pressure from a subarachnoid cyst.

Irradiation of the Central Nervous System. Irradiation of the CNS for treatment of tumors, leukemia, or neoplasms of the head and face may result in the gradual onset of hypothalamic-pituitary failure.[617] Although GH deficiency is the most common hormone disorder resulting from irradiation, gonadotropin deficiency, hypothyroidism, and decreased bone density also occur.[618,619] Exposure of the hypothalamic pituitary axis to 30 Gy carries a recurrence rate of about 6 for premature ovarian failure in women and requirement for testosterone treatment in men.[620]

Self-reported fertility was reported to be lower in women who received CNS radiotherapy for acute lymphoblastic leukemia at about the time of menarche,[621] although the average age of women in this long-term study was in the early 20s, and longer follow-up of fertility may change the results. Irradiation of the CNS in early life predisposes the patient to later onset of secondary CNS tumors sometimes in just a few years after treatment of the first tumor.[608]

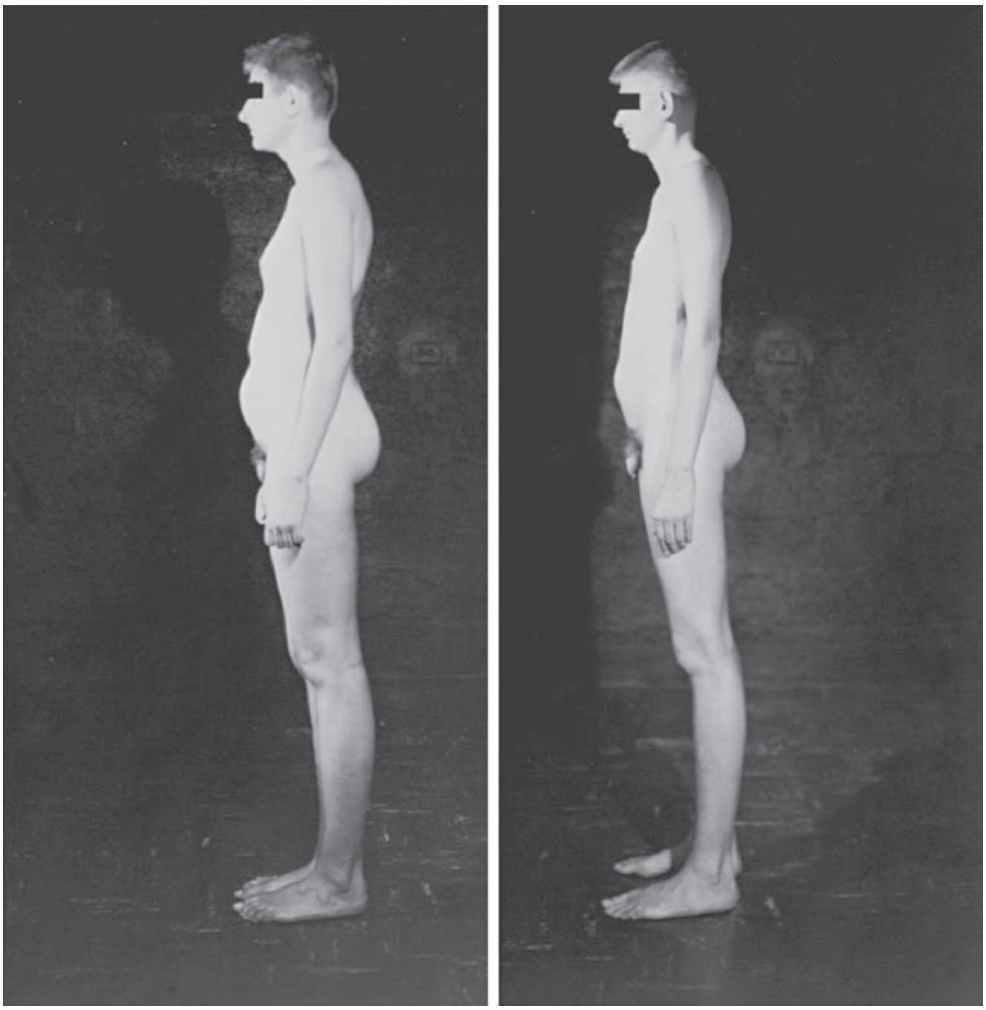
Fröhlich Syndrome. Fröhlich syndrome or adiposodysgenesis is a constellation of endocrine abnormalities, combining findings of obesity and hypogonadism due to a hypothalamic-pituitary disorder. Remarkably, the original description involved a patient with tuberculosis involving the hypothalamic-pituitary axis.

Hypergonadotropic Hypogonadism: Sexual Infantilism Caused by Primary Gonadal Disorders

The most common forms of primary gonadal failure are associated with sex chromosome abnormalities and characteristic physical findings.[590,622] Testicular or ovarian dysfunction as an isolated finding is less commonly a cause of pubertal hypergonadotropic hypogonadism.

Boys

Klinefelter Syndrome and Its Variants (see Chapter 24). Klinefelter syndrome (i.e., syndrome of seminiferous tubular dysgenesis) and its variants occur in approximately 1 in 1000 males, and they are the most common forms of male hypogonadism.[622–624] The invariable clinical features include small, firm testes as an adult (<3.5 cm long); impaired spermatogenesis; and a male phenotype, usually with gynecomastia and long legs but not long arms[622] (Fig. 26.46). Prepubertally, patients can be detected by the disproportionate length of the extremities, decreased U/L body ratio without an increase in arm span rather than eunuchoid proportions, in which arm span and leg length are increased; however, less than 10% are diagnosed prepubertally. With increasing use



• **Fig. 26.46** 47,XXY Klinefelter syndrome in 17-year-old identical twins. At age 15, gynecomastia was observed. The twins had a eunuchoid habitus and poorly developed male secondary sexual characteristics. Both were 187 cm tall; arm spans were 187 cm and 189.5 cm; the voices were high pitched; the testes measured 1.8 × 1.5 cm; and penis length was 7.5 cm. Gynecomastia and signs of androgen deficiency were more evident in the twin on the left. Urinary gonadotropin levels were greater than 50 mU/24 hours. The testes exhibited extensive tubular fibrosis, small dysgenetic tubules, and clumping or pseudoadenomatous formation of Leydig cells; germ cells were rare. The microscopic appearance was typical of seminiferous tubule dysgenesis. (Patient data from Grumbach MM, Barr ML. Cytologic tests of chromosome sex in relation to sexual anomalies in man. *Recent Prog Horm Res.* 1958;14:255–324.)

of karyotype determination in children with behavior problems and in pregnancy for various reasons, diagnosis earlier in life may increase. Tall stature for family size is common in this disorder due to the disproportionate growth of the legs and appears to be related to multiple copies of the *SHOX* gene[624]; however, normal or short stature does not eliminate the diagnosis.

Infants with Klinefelter syndrome have normal INSL3 levels transiently increased at 2 to 3 months of age during the minipuberty of infancy, testosterone levels were below the median of control subjects but within the normal range, and inhibin B and AMH levels were also within normal range.[624] However, FSH levels were above normal in 25% of patients, indicating early evidence of testicular deficiency. Muscle tone is also lower in some.[625]

There is a normal increase in the concentrations of testosterone, INSL3, and inhibin B before puberty. By midpuberty testosterone and INSL3 concentrations remain in the low-normal range, AMH levels were undetectable in XXY adolescents but decreased at an age later than in normal boys, and inhibin B levels decreased from normal to the low levels characteristic of adult Klinefelter syndrome during late puberty after an unequivocal increase in serum testosterone (>2.5 nmol/L) levels and degeneration of Sertoli

cells.[626] Serum LH and FSH usually rise by midpuberty. Rarely, low gonadotropin concentrations occur when hypogonadotropic hypogonadism is associated with 47,XXY Klinefelter syndrome or coexisting constitutional delay.

Prepubertal testes show only subtle histologic changes, although the testes are small, and the germ cell content is reduced, whereas Sertoli cells are normal in abundance and appearance before 2 years of age. Older prepubertal subjects have normal seminiferous tubules. Hyalinization and fibrosis of the seminiferous tubules and pseudoadenomatous changes of the Leydig cells develop after puberty; adult-type spermatogonia are found in peripubertal boys, but older boys had no germ cells; and the testes degenerate in an accelerated manner at the onset of puberty.[627] However, there is a 44% success rate for sperm retrieval from testicular tissues and a 55% success rate for use of microdissection testicular sperm aspiration. Intracytoplasmic sperm injection led to the birth of 101 children, in whom there was no apparent increase in congenital or genetic defects.[628] Cryopreservation of testicular tissue is recommended before degeneration progresses,[629] although coverage for such procedure may be difficult to obtain.

There is variation in Leydig cell function among childhood and pubertal subjects, but the plasma concentration of testosterone fails to rise to normal adult levels. The onset of puberty usually is not delayed, but impaired Leydig cell reserve and low testosterone levels may lead to slow progression or arrest of pubertal changes. Testosterone replacement should be considered when the LH level rises above the normal range of values but is not necessary in all subjects in early puberty.[630] Serum estradiol-to-testosterone ratios and TeBG levels are higher than those in normal males, which indicates an increased estrogen effect and decreased testosterone effect that may account in part for the gynecomastia characteristic of Klinefelter syndrome. Testosterone administration does not appear to reduce the gynecomastia, but dihydrotestosterone may help. Aromatase inhibitors or estrogen receptor antagonists do not seem to be effective treatment for the gynecomastia of Klinefelter syndrome. If the gynecomastia does not regress within 2 years, reduction mammoplasty is required. Monitoring for breast cancer is important in these susceptible individuals.

The *AR* gene is located on the X chromosome and encodes a ligand-dependent transcription factor with highly polymorphic CAGn trinucleotide repeats in the coding sequence of the first exon. The length of the translated polyglutamate tract in the N-terminal transactivation domain of the resulting protein and the length of this polyglutamate tract are inversely proportional to receptor transactivation activity. The shorter the repeat sequence within the range of normal variation, the more active the AR, and small changes in this activity may result in more significant effects in Klinefelter syndrome, in which testosterone secretion may already be impaired. A negative correlation between CAGn repeat length and penile length, but not testicular size, in children has been reported.[581,631] Another study found a positive association between CAGn repeat length and body height, an inverse relationship to bone density and arm span to body height, and the presence of longer CAGn repeats for gynecomastia and smaller testes[632] in adults. A paternal origin of the extra X chromosome is associated with later onset of puberty and longer CAG repeats.[627]

Behavior and Development in Klinefelter Syndrome. Neurobehavioral abnormalities, primarily in language, speech, learning, and frontal executive functions, are common, even universal, in Klinefelter syndrome, but severe retardation is uncommon.[244] These problems may lead to evaluation in childhood and the prepubertal recognition of the syndrome. The prevalence of adjustment problems in adolescence is increased. Adults with Klinefelter syndrome have shorter education, lower income, earlier retirement, and increased unemployment, and more rarely marry than the national average. Fatality among Klinefelter syndrome men was significantly increased (hazard ratio, 1.9).[633] The crime rates for sexual abuse and arson are significantly increased, whereas traffic offenses and drug-related crime are significantly decreased.

The global IQ in unselected populations of Klinefelter syndrome subjects is normal or near normal, but verbal IQ, in contrast to that of patients with Turner syndrome, is usually lower (e.g., 10–20 points) than performance IQ.

Prepubertal Klinefelter syndrome patients have reduced left hemisphere specialization for verbal tasks and enhanced right hemisphere specialization for nonverbal tasks. However, these abnormalities tended to normalize after puberty began, suggesting hemispheric reorganization during puberty. Hypotheses are advanced supporting the effect of prenatal testosterone on cerebral dominance and on language and reading pathology.

There is controversy about the indication for testosterone treatment of infants or adolescents with Klinefelter syndrome. Although there is a growing feeling among parents that testosterone treatment in the infancy or early pubertal period improves language, reading, behavior, and self-image in boys with Klinefelter syndrome, no well-controlled studies supporting this contention are available, and long-term studies are needed.[634,991]

Other Aspects of Klinefelter Syndrome. Conditions associated with Klinefelter syndrome include aortic valvular disease and ruptured berry aneurysms (6 times the normal rate); breast carcinoma (20 times the rate in normal men and one-fifth that of women); other malignancies such as acute leukemia, lymphoma, and germ cell tumors at any midline site; systemic lupus erythematosus; and osteoporosis in about 25% of affected adults. There is an increased risk of diabetes mellitus, thyroid disease, fatigue, varicose veins, and essential tremor.

About 20% of mediastinal germ cell tumors are associated with Klinefelter syndrome, and they occur at a younger age than the mediastinal germ cell tumors that are not associated with the syndrome. With rare exceptions, these germ cell tumors, which may be located in the midline anywhere from the CNS to the pelvis, secrete hCG and induce sexual precocity. Klinefelter syndrome needs to be considered in boys with hCG-secreting germ cell tumors, especially if the tumor is located in the mediastinum or CNS.

Other Forms of Primary Testicular Failure

Cancer Survivors. CHEMOTHERAPY Chemotherapy and direct radiotherapy affect testicular function, and as more children survive with effective therapy for cancer, delayed puberty and adult infertility will result. Cancer therapy, especially irradiation of the gonads or the use of alkylating chemotherapeutic agents, affects testicular function and can lead to adult infertility.[635] Some courses of therapy can cause severe damage to germinal cells without apparent effect on Leydig cells. Chemotherapy for childhood Hodgkin disease, including chlorambucil, vinblastine, Mustargen (mechlorethamine), Oncovin (vincristine), procarbazine, and prednisone (COPP/MOPP), may allow spontaneous progression through puberty, but FSH and LH concentrations may be elevated, and the inhibin B concentrations decrease during puberty. The basal serum FSH level and rise in LH and FSH levels after GnRH correlate with the dose of cyclophosphamide. COPP/MOPP chemotherapy for Hodgkin disease can cause severe damage to Sertoli and germinal cells, but it has less effect

on Leydig cells, even if therapy occurred in the prepubertal period. Lower dosing or limiting therapy to less than three courses is suggested to decrease these complications.[636] Normal basal LH values may raise hope that Leydig cell function is normal, but if there is an exaggerated rise of the LH level after GnRH, compensated Leydig cell damage is present. The combination of Adriamycin (doxorubicin), bleomycin, vinblastine, and dacarbazine (ABVD regimen) can cause germ cell depletion. Although initially it was thought that some degree of prepubertal gonadal maturation was necessary before these drugs could cause gonadal damage, gonadal damage can occur earlier as a result of therapy in the prepubertal period but may not be demonstrable until the age of puberty. Chemotherapeutic agents used in the treatment of nephrotic syndrome or leukemia, such as cyclophosphamide or chlorambucil, have led to Sertoli cell, Leydig cell, and germ cell damage in prepubertal patients; these effects are sometimes reversible, which is indicative of gonadal damage.

Radiation Therapy. Radiation to the gonads can cause primary testicular failure, usually resulting in azoospermia, although normal testosterone secretion may be associated with elevated LH and FSH values (compensated Leydig cell failure); the gonads must be shielded from the treatment, if possible. Direct radiation doses of more than 2 Gy will likely impair spermatogenesis. Exposure to more than 20 Gy carries a recurrence rate of almost 11 of requiring testosterone as an adult.[620]

Preservation of sperm by freezing is being investigated for pediatric cancer patients (in Klinefelter syndrome patients), but the ethical and logistics problems of collecting sperm from adolescents remain substantial, all the more in prepubertal children. Not all sperm may be affected by treatment, and functional sperm may be recovered by microdissection of seminiferous tubules even in individuals who appear to have testicular failure. This may allow fertilization by successful intracytoplasmic sperm injection even if the quantity of sperm is low. The genes responsible for apoptosis are activated when DNA damage from radiation or chemotherapy occurs; manipulation of such genes may allow reestablishment of fertility in the future. Oncofertility is a new field of investigation that aims to preserve fertility in children and teenagers subject to cancer therapy, and new methods will undoubtedly appear.

Survey of adults who were not offered fertility preservation or with whom such an issue was not even discussed at time of diagnosis shows that they feel considerable distress. However, sperm banking in puberty or prepuberty presents ethical concerns as to the procedure, the cost of banking, and the uncertainty of outcome and is not presently standard therapy.[637]

Concern for decreased bone density and risk for fractures is also raised in cancer survivors who have undergone radiation treatment of the hypothalamic-pituitary axis.

Testicular Biosynthetic Defects. The 46,XY disorder of sex development is caused by 17α-hydroxylase/17,20-lyase deficiency resulting from mutations in CYP17A1 at gene map locus 10q24.3; it is associated with sexual infantilism and a female phenotype.[638] The testosterone biosynthetic defect blocks the synthesis of testosterone and adrenal androgens, impairing masculinization at all stages of development. Associated cortisol deficiency and increased mineralocorticoid secretion in this condition lead to hypertension, decreased serum potassium levels, and metabolic alkalosis. Elevated serum progesterone levels and decreased plasma renin activity are helpful diagnostic features.[639] Glucocorticoid replacement suppresses ACTH and mineralocorticoid excess and corrects the electrolyte abnormalities, but no sexual development occurs unless exogenous gonadal steroids are administered. Less severe deficiencies are associated with ambiguous genitalia. *CYP17A1* mutations leading to isolated 17,20-lyase deficiency are rare.

A rare autosomal recessive condition is steroidogenic acute regulatory (StAR) protein deficiency, in which the ability to produce C21, C19, and C18 steroids is lost and a severe impairment of the conversion of cholesterol to pregnenolone results; severely affected patients have lipid-laden adrenal glands.[640] The large adrenal glands may be visualized on ultrasound, CT, or MRI. Death often occurs in infancy if untreated because of unrecognized glucocorticoid and mineralocorticoid deficiencies. Affected individuals physically appear to be sexually infantile females, whether their karyotype is 46,XY or 46,XX; because of the absence of gonadal or adrenal androgen production, the affected XY phenotypic females do not develop secondary sexual characteristics, including pubic hair.[622] However, XX females with a null mutation develop female sex characteristics at puberty, including pubic hair and multicystic ovaries, but they also have primary or secondary amenorrhea. In contrast to the fetal testis, the fetal ovary, which is insensitive to FSH and steroidogenically inactive, is undamaged in fetal life and remains so until the onset of puberty. Under FSH stimulation during puberty and with recruitment of ovarian follicles, the ovaries undergo progressive damage and cyst formation. Ovarian damage and impairment of ovarian StAR-independent steroidogenesis appear to be related to lipid deposition in the ovary.[641]

Luteinizing Hormone Resistance. Presumptive evidence of LH resistance caused by an LH receptor abnormality on the Leydig cell was reported in an 18-year-old boy with a male phenotype, no male secondary sexual development, gynecomastia, elevated plasma LH levels, and early pubertal plasma testosterone concentrations that did not increase after hCG administration; there was no elevation of testosterone precursor levels.[642] The testes were prepubertal in size and had the microscopic appearance of normal prepubertal testes. Plasma membrane receptor preparations from the testes bound only one-half as much radiolabeled hCG as control testes.

In affected males, this autosomal recessive disorder is caused by a mutation in *LHCGR*, the gene encoding the G protein–coupled, seven-transmembrane LH/hCG cell receptor at gene map locus 2p21. Mutations that cause a more severe compromise in *LHCGR* function are associated with XY disorders of sex development. Homozygous deletion of exon 10 or the homozygous missense mutations Ser616Tyr and Ile625Lys of the LH receptor are associated with micropenis (but not hypospadias) due to partial impairment of LH receptor function, leading to a discordance with a poor response to LH but not to hCG.

Nephropathic cystinosis in boys leads to hypergonadotropic hypogonadism.

Anorchia and Cryptorchidism. Cryptorchidism is the condition in which one or both testes have not reached the bottom of the scrotum before birth. Animal and human data demonstrate that testicular descent is influenced by testosterone and INSL3; for example, androgen resistance leads to lack of descent, as also occurs in transgenetic mice without INSL3. When testes are not descended, they may be located in high scrotal, suprascrotal, or inguinal positions or can be nonpalpable, which includes ectopic testes as well.[643] Data collection is variable, but analysis of the literature shows the undescended testis rate in term appropriate weight for gestational age (AGA) babies is 1.0% to 4.6%, whereas in premature or SGA infants it is 1.1% to 4.3%.[644] By 1 year the undescended testis rate in term AGA boys was 1.0%

to 1.5%, at 6 years 0.0% to 2.6, at 11 years 0.0% to 6.6%, and at 15 years 1.6% to 2.2% of boys. Testes may ascend after birth, ascensus testis, which leads to a higher prevalence of undescended testes in prepuberty than at birth in several international reports, and these patients are not always included in cryptorchidism surveys. Study of more than 1 million Danish boys showed the concordance rate of cryptorchidism was 3.4% for paternal half-brothers, 6% for maternal half-brothers, 8.8% for full brothers, 24.1% for dizygotic twin brothers, and 27.3% for monozygotic twin brothers demonstrating effects of genetics and intrauterine environment.[645]

A 46,XY male without palpable testes may have intra-abdominal testes, which carry an increased risk of malignant degeneration; anorchia (i.e., vanishing testes syndrome, caused by perinatal torsion), in which no testes are found at laparotomy; or retractile testes, a variation of normal. About 50% of bilateral, nonpalpable testes are undescended, and the other 50% are testicular remnants from vanishing testes that usually do not contain germ cells, are found in the scrotum, are not at risk for carcinoma, and need not be removed if the history is certain.[646] If there is a male phenotype and male internal ducts, functioning fetal testes capable of secreting testosterone and AMH were present early during fetal life but degenerated thereafter. Serum gonadotropins follow the normal U-shaped curve of high values in infancy and puberty with lower values in midchildhood in anorchia, although the values are above normal.[647] Serum AMH and inhibin B are nondetectable in anorchia.[648] Ultrasound is often used to locate undescended testes, but the accuracy is poor: A meta-analysis demonstrated that a positive ultrasound result increases and negative ultrasound result decreases the probability that a nonpalpable testis is located within the abdomen from 55% to 64% and 49%, respectively.[649] Laparoscopy, however, is recommended in diagnosis of nonpalpable testes.[650]

Discovery of unilateral cryptorchidism may represent the presence of a descended testis on one side and none on the other side, and this presents a diagnostic dilemma. Compensatory hypertrophy occurs if there is no contralateral testis, and this aids the diagnosis. A Japanese study demonstrated that the mean contralateral testicular length and volume in the boys with an absent testis were 22.4 mm and 2.20 mL compared with 16.6 mm and 1.10 mL in boys with a testis present and 16.6 mm and 1.18 mL in control subjects; the optimal cutoff value of 21 mm in length and 1.6 mL in volume led to a predictive accuracy for an absent testis of 87.3%, sensitivity of 81.8%, and specificity of 95.5%, with 85.5%, 84.8%, and 86.4% for the volume, respectively.[651] Because the finding does not universally predict monorchia, laparoscopy can be used for diagnosis of this condition if ultrasound is unsuccessful. If there is a unilateral undescended testis, the increased relative risk of carcinoma in the contralateral descended testis is 1.74 (95% CI, 1.01–2.98), which is lower than in the unilateral undescended testes (6.33; 95% CI, 4.30–9.31).[652]

Hormonal treatment of undescended testes may be successful in causing descent, but evidence is scarce and meta-analysis is difficult owing to variation in dosage and length of treatment. There is no compelling evidence that hormonal therapy in the short term is harmful, but unsuccessful medical therapy should not be allowed to significantly delay surgical therapy. In one schema, a single dose of 1500 IU hCG is administered intramuscularly, and serum levels of LH, FSH, testosterone, inhibin B, and AMH are measured at baseline and at 72 hours afterward. The test was positive in Prader-Willi syndrome when the maximum testosterone level after 72 hours was 2 to 20 times higher than baseline levels in infants aged 3 to 12 months and 5 to 10 times higher, between 2.5 and 9 nmol/L, in infants aged 1 to 4 years.[653] Administration of an hCG dose, 250 IU for infants aged 3 to 12 months and 500 IU intramuscularly for infants aged 1 to 4 years, twice a week for 6 weeks led to a descent of testes in 62% who had a positive stimulation test, and 23% of the testes reached a stable scrotal position. There are studies of longer term administration of hCG or GnRH to either foster a rise in testosterone for diagnosis or for descent of testes for treatment of boys without Prader-Willi syndrome with variable results. The lack of a rise in testosterone concentration, in conjunction with an increased plasma concentration of FSH and LH or an augmented gonadotropin response to GnRH, and decreased AMH and inhibin B are evidence for the diagnosis of bilateral anorchia. Because testicular descent normally occurs by 1 year of age, orchidopexy is recommended between 6 and 12 months in those who have testes that do not descend with medical treatment or upon discovering cryptorchidism after 12 months of age.[654] One study linked testicular descent to an adequate neonatal surge of LH and testosterone by 4 months in AGA infants and by 6 months in premature infants; these study authors recommended treatment be considered earlier than at 1 year.

Cryptorchidism is associated with an increased risk of infertility. Cryptorchid testes may demonstrate congenital abnormalities and may not function normally even if brought into the scrotum early in life. Two critical steps in the maturation of germ cells are described in the normal prepubertal testis that do not occur in the unilaterally undescended testes. First, at 2 to 3 months of age, the gonocytes (primitive spermatogonia) in the fetal stem cell pool transform into the adult dark spermatogonia, which become the adult stem pool (possibly related to the early infancy surge in LH, FSH, and testosterone). Second, at 4 to 5 years of age, meiosis begins, and primary spermatocytes appear. The contralateral descended testis is affected but less so than the undescended testis. Identification of the gonocyte transformation has influenced recommendations on the timing of orchidopexy. Postpubertal orchidopexy is associated with a greater than 85% prevalence of azoospermia or oligospermia. It has been surmised that cryptorchid testes, even if replaced in the scrotum, may never have normal spermatogenic function as a consequence of an early abnormality in germ cell maturation, vascular damage to the testicular circulation during orchidopexy, or an intrinsic testicular defect. Fertility potential varies depending upon preoperative history and laboratory results. Testicular dysgenesis may be indicated by increased gonadotropin levels, and early surgery may be indicated; normal gonadotropin levels and a decreased germ cell number may indicate transient hypothalamus-pituitary-gonadal hypofunction with a poor fertility prognosis; if there are normal gonadotropins, inhibin B, and germ cell number, there is a good fertility prognosis, too.[655] A study of men with cryptorchidism reported a paternity rate of 65% for men who had had bilateral cryptorchidism, compared with 90% for the formerly unilateral cryptorchid men and 93% for control men; the reduction in fertility was supported by semen and hormone analyses.[656] Successful fertilization was reported by the use of intracytoplasmic injection of sperm extracted from the testes of cryptorchid men who had orchiopexy after puberty.[657] Patients undergoing orchiopexy may sustain subtle damage to the vas deferens, leading to the later production of antibodies to sperm that may result in infertility.

Cryptorchidism is associated with an increased risk of cancer of the testes, which is rising. The incidence of testicular carcinoma in England at all ages increased from 2 per 100,000 in 1909 to 4.4 per 100,000 in 1999, with a rise noticed during puberty.[658] Such data are nation specific, and there is an approximately 10-fold range between countries.[646] A recent Swedish study reported the overall relative risk of testicular carcinoma in cryptorchidism is 2.5 to 8, with the highest risk associated with intra-abdominal rather than inguinal testes and higher risks observed for those with abnormal chromosomes, syndromes, or late or no orchiopexy. Undescended testes remain at a higher temperature than descended testes, and undescended testes have a maturation arrest at the conversion of the gonocytes to spermatogonia, which appears to direct the testes toward malignant degeneration. There is a very small risk of carcinoma of the testes in prepuberty, but the absence of carcinoma in situ in prepuberty is not an assurance that carcinoma will not develop in adult life. Periodic sonography of the testis of affected patients is recommended after the onset of puberty.

Adverse environmental factors may be important in the apparent increase in testis cancer, cryptorchidism, hypospadias, and low semen quality, which are all qualities described in the testicular dysgenesis syndrome (TDS).[659] The complexities of clustering such findings without a pathophysiologic explanation has led to criticism of the concept.[654] This remains an important area of research.

Retractile testes can descend into the scrotum but then reascend. They are considered a normal variation, but a requirement for orchiopexy was reported for 22.7% in a series of 150. The finding of one case of testicular carcinoma in a boy with spontaneous descent in this series led to a suggestion of following such cases in the long term.[660] The risk of breast cancer associated with gynecomastia is increased in men with a history of undescended testes, orchiopexy, orchitis, testicular injury, infertility, or any cause of delayed puberty.

Small for Gestational Age. SGA predisposes males to reproductive problems and is also associated with the TDS. Males born SGA tend to have smaller testes and lower testosterone and higher LH[661] levels, suggesting an impairment of fertility, as is found in SGA females. Adult males born SGA have increased aromatase and 5α-reductase activities, leading to elevated levels of estradiol and dihydrotestosterone.[662] Of most concern is the fact that elevated estradiol levels in males increase the risk of testicular cancer and do not provide the protective effect on cardiovascular health as found in females. The level of inhibin B is elevated significantly in SGA boys (i.e., mean birth weights >2 SD below the mean for gestational age), although other studies with higher-birth-weight SGA boys demonstrated no such chages.[647,663]

Girls

Syndrome of Gonadal Dysgenesis and Its Variants.[622]. The most common form of hypergonadotropic hypogonadism in the female is the syndrome of gonadal dysgenesis (Turner syndrome and its variants), a sporadic disorder with an incidence of 1 per 2500 liveborn girls in which all (i.e., X chromosome monosomy with haploinsufficiency) or part of the second sex chromosome (i.e., partial sex chromosome monosomy) is absent.[590,646] About 99% of 45,X conceptuses abort spontaneously, and 1 in 15 spontaneous abortions has a 45,X karyotype. The 45,X karyotype is associated with female phenotype, short stature, sexual infantilism, and various somatic abnormalities.

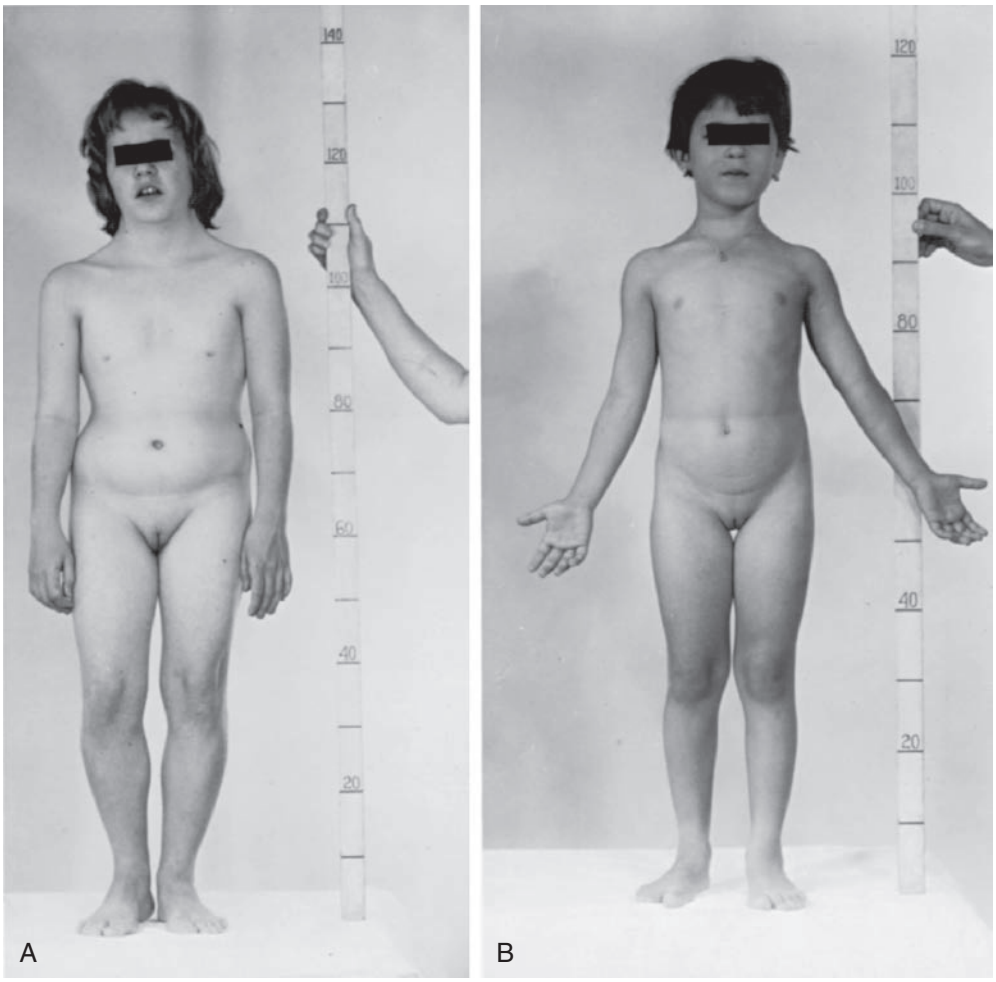
Sex chromosome mosaicism or structural abnormalities of an X or Y chromosome (affects about 40% of individuals with Turner syndrome) may modify the features of this syndrome. The syndrome of gonadal dysgenesis and its variants are found in a continuum ranging from the typical 45,X phenotype to a normal male or female phenotype.[590,622] Comprehensive recommendations for the diagnosis and management of Turner syndrome were presented by an international committee.[649]

45,X Turner Syndrome.[993] Short stature and sexual infantilism are typical features of sex chromatin–negative 45,X gonadal dysgenesis (Turner syndrome), which is the karyotype found in approximately 60% of cases[622] (Fig. 26.47). The short stature is caused by loss of a homeobox-containing gene that is located on the pseudoautosomal region (PAR1) of the short arms of the X (p22) and Y (p11.3) chromosomes and encodes an osteogenic factor. This short stature homeobox-containing *(SHOX)* gene[664] was previously called the pseudoautosomal homeobox osteogenic gene *(PHOG)*. Because it is located on the PAR1 of the short arm of the X and Y chromosomes, it escapes X-chromosome inactivation. *SHOX* haploinsufficiency is responsible for, in addition to abnormal growth, mesomelic growth retardation and Madelung deformity of the wrist (i.e., bilateral bowing of the radius with a dorsal subluxation of the distal ulna as found in Leri-Weill dyschondrosteosis [i.e., *SHOX* haploinsufficiency]). Langer mesomelic dysplasia, which includes severe dwarfism with striking hypoplasia or aplasia of the ulnar and fibula, is caused by *SHOX* nullizygosity. *SHOX* haploinsufficiency appears to be responsible for 2 SD of the approximately 3 SD deficit in stature and the skeletal abnormalities in Turner syndrome. On the other hand, patients with complete gonadal dysgenesis and tall stature had a 45,X/46,X der(X) and three doses of the *SHOX* gene due to the *SHOX* duplication on the der(X) chromosome.

A diagnosis of Turner syndrome may be straightforward in its classic sense, but often there is a delay in diagnosis. For instance, the Danish study found that about 15% are diagnosed below 1 year of age, 33% during the adolescent years, and over 38% during adulthood.[665] The age of diagnosis may be coming down with increased education of providers and the general public, however. The standardized mortality rate for all causes of death for Turner syndrome was 2.86 with the highest prevalence among females with 40 5X and 45 isoXq karyotypes.

Turner syndrome may be recognized in the newborn period or before. More than 90% of Turner fetuses spontaneously abort. The 45,X abortuses have edema and large hygromas of the neck that may be seen on prenatal ultrasound studies. This lymphatic defect is the basis for the loose skinfolds that ultimately form the webbed neck (i.e., pterygium colli). Affected newborn infants may also have lymphedema of the extremities; the term *Bonnevie-Ullrich syndrome* has been applied to newborn infants with these features of Turner syndrome.

Frequent features are distinct facies with micrognathia, a fish-mouth appearance, high-arched palate with dental abnormalities, epicanthal folds, ptosis, low-set or deformed ears, short neck with low hairline, webbing (i.e., pterygium colli), and recurrent otitis media, often leading to impaired hearing (25% of affected adults require hearing aids[622]). A broad, shieldlike chest leads to the appearance of widely spaced nipples, and the areolae are often hypoplastic. Skeletal defects include short fourth metacarpals and cubitus valgus (which may develop after birth), Madelung deformity of the wrist (in about 7%), genu valgum, and



• **Fig. 26.47** (A) A girl aged 14 years and 10 months with the typical form of the syndrome of gonadal dysgenesis (Turner syndrome). The X chromatin pattern was negative, and the karyotype was 45,X. She was short (height, 134.5 cm; height age, 9 years and 5 months) and sexually infantile except for the appearance of sparse pubic hair. She exhibited characteristic stigmata of the syndrome: a short, webbed neck; shield-like chest with widely separated nipples; bilateral metacarpal signs; puffiness over the dorsum of the fingers; cubitus valgus; increased number of pigmented nevi; characteristic facies; and low-set ears. The bone age was 13.5 years; the urinary 17-ketosteroid level was 5.1 mg/day; and the urinary gonadotropin level was greater than 100 mU/day. Vaginal smears and the urocytogram showed an immature pattern in which cornified squamous cells were absent. Female secondary sexual characteristics were induced with estrogen therapy, and the cyclic administration resulted in periodic estrogen withdrawal bleeding. (B) A 45,X girl aged 9 years and 11 months with Turner syndrome. Apart from short stature (height, 118 cm; height age, 6 years and 11 months), increased pigmented nevi, and subtle changes in the fingers and toes, she had few somatic anomalies. In contrast to the patient on the left, the main clinical feature was short stature.

scoliosis. There are extensive pigmented nevi,[622] a tendency to keloid formation, and hypoplastic nails. Lymphatic obstruction leads to the infantile puffiness of extremities and pterygium colli and to a distinctive shape of the ears. Cardiovascular anomalies affect the left side of the heart and include coarctation of the aorta in about 10% (40% of these have associated webbing of the neck), aortic stenosis, and bicuspid aortic valves; the latter individuals are at risk for a dissecting aortic aneurysm. An echocardiogram of the cardiovascular system must be performed, and prophylactic antibiotics are indicated if an anatomic abnormality is demonstrated. Elevated values on the ambulatory arterial stiffness index (AASI) may be another risk factor for later serious cardiac disease.[666] Long-term regular follow-up of adults with Turner syndrome with echocardiography is recommended every 5 years in adulthood.[659] Abnormal pelvicalyceal collecting systems, abnormal position or alignment of the kidneys, and an abnormal vascular supply to the kidney are encountered in 30% to 60% of patients. Recurrent urinary tract infections are common. Defects of the gastrointestinal system include intestinal telangiectasias and hemangiomatoses that rarely can lead to massive gastrointestinal bleeding. The prevalence of inflammatory bowel disease, chronic liver disease, and colon cancer is increased. Autoimmune diseases, such as Hashimoto thyroiditis (16-fold relative risk) and Graves disease, are common, and an association with juvenile rheumatoid arthritis and psoriatic arthritis has been described.

The age of diagnosis of Turner syndrome continues to be delayed, with the exception of newborns with the striking phenotype of the Bonnevie-Ullrich syndrome or those diagnosed on amniocentesis. It is recommended that all prepubertal age girls below −2 SD who have at least two somatic stigmata of the syndrome have a karyotype analysis; early diagnosis is key for optimal management of the growth failure and the detection of occult features of the syndrome.

Pelvic ultrasonography or MRI usually permits the detection of even a small, infantile uterus and reveals streak gonads. Ultrasensitive estrogen bioassays can confirm decreased ovarian function in girls with Turner syndrome because estradiol values are significantly lower than those found in average girls in puberty. Long-term follow-up of affected women previously treated with GH and estrogen demonstrated normal adult uterine length only in those with 45,X/46,XX karyotypes, whereas those with pure 45,X karyotypes had a smaller uterus length and volume.[667] The streak gonads result in sexual infantilism, but in about 10% of cases, puberty, menarche, and (rarely) pregnancy may occur.[667] Women with some of the variants have been able to achieve fertility and deliver normal infants, although cardiovascular risks increase the danger. Affected adults can undergo hormone replacement to prepare the uterus to receive a donated embryo and proceed to delivery. Unfortunately, some patients receiving a donated ovum have died because of dissection or rupture of the aorta, and caution should be used in considering this technique.[622]

Intrauterine growth retardation with a mean deficit in birth length of 2.6 cm (−1.24 SD) and a slow childhood growth rate result in a loss of about 8 to 9 cm (−3 SD) by age 3 years in girls with Turner syndrome.[660,668] A major portion of the height deficit occurs during the first 3 years of life. Decreased growth rate occurs at the time of expected puberty, and the pubertal growth spurt is absent in those without pubertal development. Untreated individuals with Turner syndrome in the United Kingdom and United States have a mean adult height of approximately 142 to 143 cm, which is about 20 cm less than the average height of typical women; the adult stature of these patients correlates with midparental height and with the height of unaffected women of the same ethnic group. Haploinsufficiency of the *SHOX* gene is estimated to contribute two-thirds of the height deficit. It is postulated that a second gene on the short arm of the X chromosome that does not undergo X-chromosome inactivation contributes the other one-third of the deficit. In girls with Turner syndrome with spontaneous puberty, pubertal height velocity was transiently higher than in girls with amenorrhea, but adult height was not different.[669] Specific growth curves are available for plotting the growth of affected children.[622]

GH treatment is approved by the FDA for Turner syndrome to increase growth and adult height.[670] The addition of estrogen therapy at low doses in addition to GH has a modest additive effect on adult height; untreated height was 144.6 ± 5.5 cm, 140.8 ± 5.0 cm for estrogen treatment alone, 147.9 ± 7.2 cm for the GH treatment alone, and 149.3 ± 6.6 cm for GH and estradiol combined over an average 7.2 ± 2.5-year treatment/observation period.[992] The average height gain in various studies has varied from 4 to 16 cm, and a systematic review of the literature shows a 5-cm gain to be the most likely outcome.[671] This variability in gain in height is incompletely understood, but many factors have been implicated, including the age of initiation of therapy, dose duration, age (especially number of years from beginning hGH treatment) at the beginning of estrogen replacement, number of injections per week, compliance, and whether the last measured height represented the adult height. Early initiation of hGH therapy (e.g., 2–8 years of age) leads to greatest effect. GH treatment of Turner patients has been safe, and untoward events are no more common than in other conditions treated with GH. There is some degree of improvement of the abnormal body proportions of Turner syndrome with hGH treatment, but the disproportionate growth of the foot may dissuade some girls from continuing treatment to maximal benefit on height. Five-year follow-up of young adults with Turner syndrome demonstrated continued beneficial effects of GH on blood pressure, lipid levels, and increased adult height.[672]

A nationwide survey of 632 Danish girls with Turner syndrome demonstrated an increased prevalence of fractures, mainly in the forearm, compared with control subjects. The prevalence was higher still in the absence of ovarian function and in girls with family history of fractures and presumed familial disorders of bone density.[673] It appears that estrogen therapy is critical for the prevention and repair of osteoporosis, but for adolescents and adults, the optimal dose preparation and site of delivery for the prevention of osteoporosis are not known. GH treatment of Turner syndrome for at least 1 year showed no difference in volumetric BMD, although LBM was higher and fat mass was lower than in the control subjects.[674]

About 50% of patients with Turner syndrome have a tendency toward impaired glucose tolerance without GH treatment; in some, this may be caused by associated obesity, and risk of type 2 diabetes mellitus is increased. Although glucose values do not change with GH therapy, insulin levels reversibly rise during treatment, indicating an additional degree of insulin resistance caused by the GH.[675] Turner syndrome patients as young as 11 years can already have elevated serum cholesterol concentrations before treatment with GH or estrogen.

The biphasic pattern of gonadotropin secretion in normal infancy and childhood is exaggerated in Turner syndrome[420] (see Fig. 26.27).

The appearance of pubic hair (i.e., pubarche) is often delayed in the syndrome of gonadal dysgenesis, even though adrenarche, as assessed by the increase in concentration of plasma DHEAS, occurs at the normal age.[442] Girls with ovarian failure demonstrate early adrenarche, and therefore higher serum values of DHEAS, but later pubarche, whereas those who demonstrate at least beginning breast development follow a course of adrenarche similar to that of unaffected girls. This suggests that ovarian function is necessary to convert DHEA to the active androgens responsible for the appearance of pubic hair in normal girls. The pubic hair of affected individuals is sparse, but estrogen therapy increases the growth of pubic hair despite a lack of increase in adrenal androgen secretion, and estrogen affects pubic hair appearance.

Behavior and Development of Turner Syndrome. Counseling and a peer support group are exceedingly important components of long-term management. Girls with Turner syndrome younger than 6 years did not perceive that they had a problem with height, but by 7 to 12 years and especially by 13 to 15 years, affected Turner girls have a strong desire for GH therapy and even unrealistic expectations of what GH therapy can accomplish in terms of adult height. GH therapy improved self-esteem even if there remained a significant difference in height between Turner girls and the normal range. Height gained with GH therapy did not affect quality of life, although cardiac defects and otologic complications did.[676] Girls who have discontinued GH therapy after reaching adult height showed no evidence of depression but still had remaining problems with self-perception and bodily attitude

despite significant height gains. Psychologic problems in Turner syndrome are not necessarily diminished with GH treatment and an increase in adult height.

Turner syndrome girls resemble normal girls in verbal and language skills, and IQ is normal when verbal ability, including comprehension and vocabulary, are considered, but visual-constructional or visual-perceptual spatiotemporal processing, visuomotor coordination, and mathematical ability (particularly in geometry) may be impaired, leading to a decrease in the performance of IQ tests due to mistakes on operation and alignment processes.[677] Girls with 45,X mosaicism associated with a 46,XX cell line, 45,X/46,XX, scored closer to normal than those with other types of mosaicisms. Only 3.3% of girls with Turner syndrome have developmental delay in the absence of a variant of Turner syndrome caused by a ring X chromosome. It is useful to monitor the patient's progress in high school mathematics. There are consistent MRI abnormalities in the right parietal lobe and the occipital lobes, and decreased volumes in these areas are implicated in defects in visual-spatial processing. These anatomic data relate to the difficulties in visual-spatial skills found in most studies of girls with Turner syndrome, because these difficulties are most closely linked to the right parietal region.

There is an increased risk of impaired social adjustment in Turner syndrome. Although there was evidence that the origin of the remaining X chromosome in classic Turner syndrome affected behavior due to imprinting, recent evidence could not support the effect of imprinting on social behavior.[678]

Transition of girls with Turner syndrome to adult care is best carried out by an experienced team, which is ideally composed of an endocrinologist, cardiologist, nephrologist, reproductive endocrinologist, audiologic physician, ENT (ear, nose, and throat) surgeon, plastic surgeon, dentist, and psychologist because of the multiplicity of complications that affected individuals may encounter in the areas of growth failure, cardiovascular disease, gonadal failure, and learning disabilities.[677]

Sex Chromatin–Positive Variants of the Syndrome of Gonadal Dysgenesis. Mosaicism of 45,X/46,XX; 45,X/47,XXX; or 45,X/46,XX/47,XXX chromosomes is associated with a chromatin-positive buccal smear and usually with fewer manifestations of the syndrome of gonadal dysgenesis. Likewise, structural abnormalities of the X chromosome can be associated with fewer phenotypic features of the syndrome. Lack of genetic material on the long or the short arm of the second X chromosome can cause decreased gonadal function; loss of all or part of the short arm of the X leads to the physical findings of Turner syndrome.[622] Depending on the location and extent of the deletion on the short arm of the X chromosome, these patients may be more likely to have modest pubertal growth and some spontaneous pubertal development.

Sex Chromatin–Negative Variants of Gonadal Dysgenesis. These variants include 45,X/46,XY mosaicism and structural abnormalities of the Y chromosome. Affected individuals have phenotypes that vary from those of classic gonadal dysgenesis to those of ambiguous genitalia to phenotypic males.[622] Patients may present with short stature, delayed puberty, and a history of hypospadias repair. There is variable testicular differentiation, ranging from a streak gonad to functioning testes. Patients with mosaicism involving a Y cell line or abnormalities of the Y chromosome are at risk for neoplastic transformation of the dysgenetic testes. Gonadoblastomas, which are benign, nonmetastasizing tumors, may arise within the gonad and produce testosterone or estrogens. The neoplasm may become calcified sufficiently to be detected on an abdominal radiograph. The appearance of feminization or virilization in a patient with dysgenetic gonads and a Y cell line may indicate gonadoblastoma formation. Of greater significance is the increased prevalence of malignant germ cell tumors, arising within the dysgenetic gonad or gonadoblastoma. Examples are dysgerminomas, mature teratomas, and testicular intraepithelial neoplasia.[679] These tumors occur more often in postpubertal subjects and rarely in children.[621]

46,XX and 46,XY Gonadal Dysgenesis. The term *pure gonadal dysgenesis* refers to phenotypic females with sexual infantilism and a 46,XX or 46,XY karyotype without chromosomal abnormalities.[621]

Familial and Sporadic 46,XX Gonadal Dysgenesis and Its Variants. The usual phenotype of 46,XX gonadal dysgenesis includes normal stature, sexual infantilism, bilateral streak gonad, normal female internal and external genitalia, and primary amenorrhea. The streak gonad occasionally produces estrogens or androgens, but malignant transformation is rare. Incomplete forms of this condition may result in hypoplastic ovaries that produce enough estrogen to cause some breast development and a few menstrual periods, followed by secondary amenorrhea. This heterogeneous syndrome occurs sporadically or with autosomal recessive inheritance,[621] and in some instances it is associated with other congenital malformations. Some familial cases have been associated with sensorineural deafness (i.e., Perrault syndrome).

Familial and Sporadic 46,XY Gonadal Dysgenesis and Its Variants. A phenotype that includes female genitalia with or without clitoral enlargement, normal or tall stature, bilateral streak gonads, normal müllerian structures, sexual infantilism, and a eunuchoid habitus is typical of 46,XY gonadal dysgenesis. About 15% of the patients have a deletion or mutation in the sex-determining region Y *(SRY)* gene. If the dysgenetic testes produce significant amounts of testosterone, slight clitoral enlargement may occur at birth, and virilization may ensue at puberty. The incomplete form of 46,XY gonadal dysgenesis may involve any degree of ambiguity of the external genitalia and internal ducts. All patients with dysgenetic gonads and a Y chromosome or an *SRY* gene are susceptible to neoplasia or carcinoma formation in the gonad. The evidence on optimal therapy is not supported by high-quality evidence, but gonadectomy is indicated at diagnosis, especially if the gonad cannot be palpated.[673]

The disorder is usually transmitted as an X-linked or sex-limited autosomal dominant trait or less commonly as an autosomal recessive trait.[621]

Other Causes of Primary Ovarian Failure. The prevalence of primary ovarian failure is increasing as a consequence of the long-term effects of cytotoxic chemotherapy and radiation therapy as these agents prolong the lives of children and adolescents with cancer. The same pattern occurs for males with testes that have been treated with these modalities.

Chemotherapy. Successful treatment of childhood acute lymphoblastic leukemia has become commonplace. Chemotherapy and radiation therapy to the CNS or ovaries exert damage in a dose-dependent manner.[680] Chemotherapy known to adversely affect ovaries carries about a recurrence rate of 6 for premature ovarian failure.[620]

Attempts to protect the gonads by suppressing the pituitary-gonadal axis with gonadal steroids or GnRH agonists are probably ineffective.[681] Oncofertility is a new field of investigation that aims to preserve fertility in children and teenagers subject to cancer therapy and is leading to improved methods of preservation.[682,683] A major problem is lack of discussion of possible

method of preservation by oncologists with the families. Careful endocrine follow-up of children and adolescents treated with chemotherapy or radiation therapy is essential.

Radiation Therapy. Ovarian transposition, moving the ovaries out of the radiation field if they are not the target of therapy, before radiation therapy is compatible with normal menses, pubertal development, and pregnancy in the few cases reported.[684] The uterus may be affected by radiation and may not expand normally during pregnancy. Hypothalamic-pituitary exposure to 30 Gy carries a recurrence rate of 6 for ovarian insufficiency.

Autoimmune Oophoritis. Premature menopause may occur at any age before the normal climacteric and has been reported in adolescent girls. Cessation of ovarian function usually manifests as secondary amenorrhea.[685] Autoimmune oophoritis can cause ovarian failure leading to primary amenorrhea, oligomenorrhea, arrest of puberty, and occasionally cystic enlargement of the ovaries. Most often it is associated with other autoimmune endocrinopathies, especially autoimmune Addison disease, in which it may precede the onset of adrenal insufficiency, but it rarely occurs in isolated premature ovarian failure. Autoimmune oophoritis occurs in more than 20% of patients with autoimmune adrenal insufficiency. Various autoantibodies have been detected in autoimmune oophoritis, including autoantibodies to cytochrome P450 steroidogenic enzymes; some are organ specific, whereas others react with antigens in more than one tissue and more than one cell type. Glucocorticoid therapy may improve, at least temporarily, ovarian function.

Type I autoimmune polyglandular insufficiency, also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), is a rare systemic autoimmune disorder with an array of clinical features, including hypoparathyroidism, adrenal insufficiency, gonadal failure, diabetes mellitus, pernicious anemia, hypothyroidism, chronic hepatitis, mucocutaneous candidiasis, dystrophic nail hypoplasia, vitiligo, alopecia, keratinopathy, and intestinal malabsorption. Thirty-six percent of women with APECED exhibited ovarian failure before age 20, whereas only 4% of affected men had testicular failure by that age. This autosomal recessive disorder is caused by more than 42 mutations in the *CDK14 (AIRE1)* gene at gene map locus 21q22.3.

Homozygous Galactosemia. Homozygous galactosemia due to mutation in the galactose-1-phosphate uridylyltransferase *(GALT)* gene is associated with primary ovarian failure, from failure to develop puberty to primary or secondary amenorrhea and premature menopause, but puberty is usually normal in males, and the risk of testicular dysfunction is low; compound heterozygotes have normal onset of puberty. Dietary restriction programs have not prevented the ovarian failure, nor are other means of avoiding ovarian failure effective.[686] The pathogenesis of galactose-induced ovarian toxicity remains unclear but probably involves galactose itself and its metabolites, such as galactitol and uridine diphosphate galactose. Most cases are detected by newborn screening programs.

Haploinsufficiency of the FOXL2 Gene. A rare autosomal dominant disorder involving eyelid dysplasia and premature ovarian failure is caused by haploinsufficiency of the *FOXL2* gene, a member of the winged helix/forkhead family of transcription factors.[687] The eyelid abnormalities include small palpebral fissures, ptosis, and a small skinfold extending inward and upward from the lower lid (i.e., epicanthus inversus). The gene is expressed in the follicular cells, and the mutations that lead to haploinsufficiency are associated with an increased rate of follicular atresia. The degree of ovarian failure varies from primary amenorrhea to irregular menses and premature ovarian failure, with ultrasound findings ranging from normal-appearing ovaries to streak gonads with an inconsistent number of primordial follicles found on ovarian biopsy. The infertility component of the syndrome is limited to females. Animal studies provide insights into other genetic mutations that may cause premature ovarian failure in humans, including mutations of *BMP15*, *FMR1*, *POF1B*, and *FOXO3A*.[688]

Congenital Disorders of Glycosylation-1: Carbohydrate-Deficient Glycoprotein Syndrome Type Ia. The congenital disorders of glycosylation-1 (i.e., carbohydrate-deficient glycoprotein syndrome type Ia) include an autosomal recessive disorder associated with circulating glycoproteins deficient in their terminal carbohydrate moieties, including a wide range of glycoproteins, enzymes, binding proteins, and coagulation factors.[689] A typical isoform pattern of serum transferrin detected by isoelectric focusing is used as a diagnostic test. The dominant clinical feature is the neurologic manifestations of involvement of the central and peripheral nervous system. Among the other organ systems affected is the pituitary-gonadal system.

The hypergonadotropic-hypogonadism is more severe in females because males virilize at puberty. The ovary and the pituitary are affected. Affected girls have sexual infantilism; the ovaries are hypoplastic or atrophic. High serum FSH and LH levels exhibited normal electrophoretic isoform patterns, but they appeared to have decreased but not absent FSH bioactivity in an FSH bioassay.

Follicle-Stimulating Hormone Receptor Resistance: Gene Mutations and Hypergonadotropic Hypogonadism. The FSH receptor is a member of the G protein–linked receptor, seven-transmembrane superfamily. It has a large, extended extracellular ligand-binding domain. An autosomal recessive disorder due to a mutation in the extracellular ligand-binding domain of the FSH receptor in affected females in six Finnish families mainly from the north central region resulted in delayed (40%) or normal puberty but primary amenorrhea, elevated gonadotropin levels, and hypergonadotropic ovarian dysgenesis with arrest of ovarian follicular development at the primary follicle stage and continued atresia.[642] The clinical features are very similar to the findings in FSH-deficient mice generated by targeted disruption of the gene encoding the FSHβ-subunit. This disorder likely is responsible for most cases of the resistant ovary syndrome. The FSH receptor gene contains nine small exons (1–9) that encode the extracellular ligand-binding domain and one large exon 10 that designates the remainder of the receptor, including the seven-transmembrane and intracellular domains. The Finnish mutation, an Ala1989Val substitution, is in the extracellular domain. Expression of the mutation in transfected cells indicated a small FSH effect on cAMP production, a striking reduction of FSH-binding capacity, but apparently normal binding affinity.

The FSH receptor mutation in the Finnish patients is not a null mutation. It remains to be determined whether the loss or complete inactivation of the FSH receptor leads to failure of puberty and sexual infantilism or to estrogen synthesis by the immature ovarian follicles described in the FSHβ-subunit knockout mouse. Affected males in these families are normally masculinized at puberty but tend to have small testes. They have a variable degree of spermatogenic insufficiency, but not azoospermia, increased plasma concentrations of FSH and LH, decreased inhibin B levels, and normal plasma testosterone values.[690]

Luteinizing Hormone and Human Chorionic Gonadotropin Resistance. LH/hCG resistance due to mutations in the gene encoding the seven-transmembrane LH-CGR is discussed in

Chapter 24. In the affected XY individual, this autosomal recessive disorder leads to various degrees of male pseudohermaphroditism; the mildest form is represented by an isolated micropenis.[691] Less severe mutations of the *LHCGR* may be associated with delayed puberty. In affected females, LH/hCG resistance does not affect pubertal maturation but does lead to amenorrhea with high serum LH levels but normal FSH and estradiol concentrations.

Polycystic Ovary Syndrome. PCOS, or functional ovarian hyperandrogenism, does not delay the onset of puberty but often delays menarche or causes menstrual abnormalities.[692] It can have serious long-term metabolic consequences such as dyslipidemia and insulin resistance over and above androgen excess and reproductive difficulties (see upcoming discussion).

Noonan Syndrome. Individuals with Noonan syndrome (i.e., pseudo-Turner syndrome, Ullrich syndrome) have webbed neck, ptosis, down-slanting palpebral fissures, low-set ears, short stature, cubitus valgus, and lymphedema, which explains why this phenotype has been called *pseudo-Turner syndrome*.[622] Features that differentiate these individuals from those with Turner syndrome include triangular facies, pectus excavatum, right-sided heart disease (e.g., pulmonary stenosis, often with valve dysplasia; atrial septal defect) compared with the left-sided heart disease in Turner syndrome, hypertrophic cardiomyopathy, varied blood clotting defects, and an increased incidence of developmental delay. Females with Noonan syndrome have normal ovarian function. Males have normal differentiation of external genitalia but may have undescended testes; germinal aplasia or hypoplasia and impaired Leydig cell function may be present. Puberty is often delayed as 35% of boys enter puberty after age 13.5 years and 44% of girls enter puberty after age 13 years.[693] Stature is decreased after normal birth length and weight, with a mean adult height of 162.5 cm (63.9 inches) for men and 152.7 cm (60.1 inches) for women, usually following the −2 SD curve. The pubertal growth spurt is often delayed or attenuated.

Noonan syndrome is inherited as an autosomal dominant trait.[622] A gene implicated in Noonan syndrome has been localized to the long arm of chromosome 12 (12q24.2-q24.31), and a mutation in *PTPN11* was identified, but at least three other gene mutations have been identified. Noonan syndrome is linked to the chromosomal band 12q24.1, and eight genes in the RAS-MAPK signaling pathway cause Noonan syndrome: *PTPN11, SOS1, KRAS, NRAS, RAF1, BRAF, SHOC2*, and *CBL*.[693] This disorder with a mutation in the (RAS) rat sarcoma-mitogen-activated protein kinase pathway is classified as one of the RASopathies.[694] The incidence is estimated at 1 case in 1000 to 1 in 2500 individuals. One parent may have features of the syndrome in 40% to 60% of cases. About 50% of patients are thought to have new mutations.

Administration of hGH is approved by the FDA for use in Noonan syndrome. Response to GH is decreased with *PTN11* mutations in some but not all[695] reports, and there is an increase in risk for neoplasia in this genotype as well.[696] GH therapy can allow affected children to reach the lower normal range of stature.

Frasier Syndrome. Germline mutations in exon 8 or 9, coding for zinc fingers 2 or 3 of the Wilms tumor suppressor gene, *WT1*, leads to Denys-Drash syndrome, which includes Wilms tumor, male pseudohermaphroditism, and early nephrotic syndrome with diffuse mesangial sclerosis. Frasier syndrome includes male pseudohermaphroditism, nephrotic syndrome with focal and segmental glomerular sclerosis, and the development of gonadoblastoma and is associated with mutations in the second splice donor site in intron 9 leading to a decrease in the KTS+ isoforms. Although most patients with Frasier syndrome present with ambiguous genitalia, this diagnosis should be considered for any phenotypic female with end-stage renal disease (due to focal segmental glomerulosclerosis) and sexual infantilism. The karyotype may be 46,XY or 46,XX.

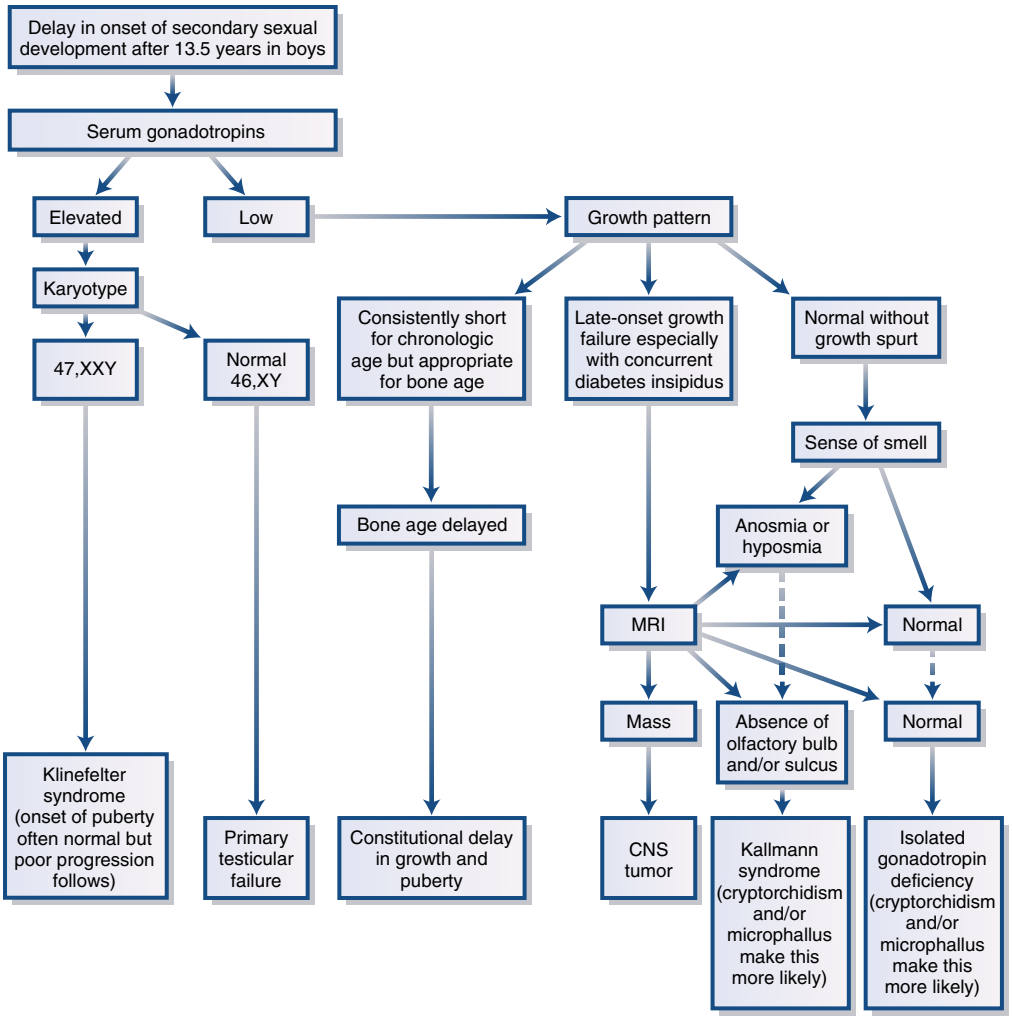
Williams-Beuren Syndrome. Williams-Beuren syndrome is a microdeletion disorder, or contiguous-gene-deletion disorder caused by deletion of the Williams-Beuren syndrome chromosome region; it was previously known as Williams syndrome. The prevalence is about 1 in 10,000 persons. While it is generally known for its characteristic facial appearance and unusual personality, many other features, including endocrine conditions, occur. While the guidelines of the Academy of Pediatrics state that precocious puberty is rare in this condition,[697] CPP is reported in about 18% of affected girls in a recent series of 24 patients, and these cases responded to GnRH agonist therapy.[698]

Diagnosis of Delayed Puberty and Sexual Infantilism

When girls remain prepubertal at 13 years or boys remain prepubertal at 14 years (although some say 13.5 years), the physician must make a clinical judgment about who are variants of the norm and who require extensive evaluation and treatment (Figs. 26.48 and 26.49; Tables 26.20 and 26.21). A boy who has not completed secondary sexual maturation within 4.5 years after onset of puberty or a girl who does not menstruate within 5 years after onset may have a hypothalamic, pituitary, or gonadal disorder. The diagnosis of hypergonadotropic hypogonadism is readily established by elevation of random plasma LH and FSH concentrations. However, differentiating the diagnosis of hypogonadotropic hypogonadism from constitutional delay in growth and adolescence remains difficult in spite of decades of study owing to the overlap in physical and laboratory findings for the two conditions (see Table 26.21).[699] Most boys with pubertal delay have a self-limited variant in the tempo of growth and pubertal onset, CDP.

Medical history must elicit all symptoms of chronic or intermittent illnesses and all details pertaining to growth and development. Questions about the patient's sense of smell are essential. Has puberty failed to occur, or did it begin but failed to progress or even regress? Disorders of pregnancy, abnormalities of labor and delivery, and birth trauma, if part of the patient's history, suggest that a congenital or neonatal event may be related to the delay in puberty. Poor linear growth and poor nutritional status during the neonatal period and childhood may reflect long-standing abnormalities of development. Family history may reveal disorders of puberty or infertility, anosmia, or hyposmia in relatives and delay in the age at onset of puberty in parents or siblings. Recalled age of pubertal onset is relatively reliable in women but less often accurate in men. A history of consanguinity is important in the detection of autosomal recessive disorders.

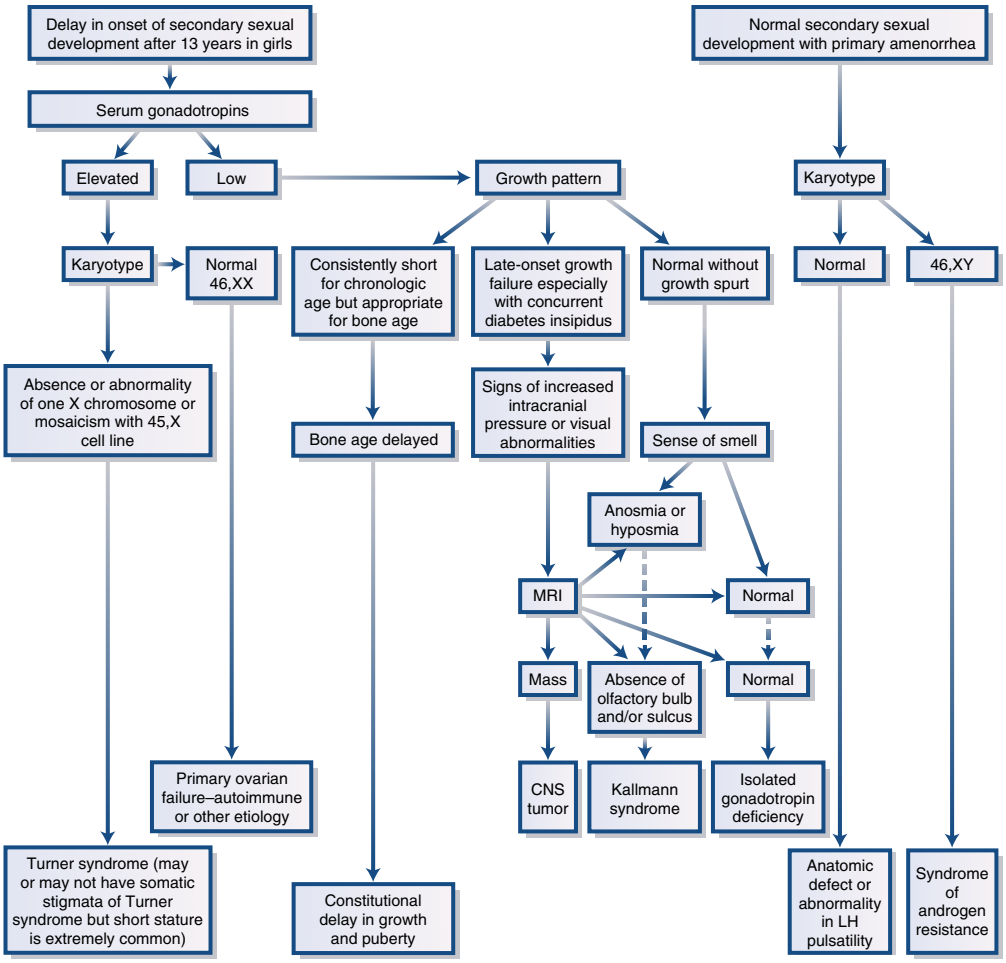
The physical examination starts with accurate determination of height, weight, and BMI. A growth chart is plotted to represent graphically growth velocity from birth (see Chapter 25). Late-onset growth failure usually indicates a serious condition requiring immediate evaluation. Weight is plotted to determine states of malnutrition. BMI should be calculated and plotted by age and gender in this era of epidemic obesity to further determine nutritional status. The height velocity should be documented over a period of at least 6 months, preferably 12 months. The U/L segment ratio and the arm span are measured



• **Fig. 26.48** Flow chart for the evaluation of delayed puberty in boys. *CNS,* central nervous system; *MRI,* magnetic resonance imaging.

and compared with the height. The signs of puberty are assessed, and the stage of secondary sexual development is determined by physical examination according to the standards presented earlier (see Figs. 26.5, 26.6, and 26.10). Questionnaires with pictures are used to allow a child to determine his or her own stage of puberty in some studies but do not replace the physical examination, as there is a tendency to overestimate development early in puberty and underestimate it late in puberty. The length and width of the testes are measured in boys, or the volume is assessed using an orchidometer. The length and diameter of the gently stretched penis are determined in boys, and the diameter of glandular breast tissue and areolar size are determined in girls. The presence or absence of galactorrhea is documented. Obese boys often appear to have a small penis because of excessive adipose tissue surrounding the phallus; only when the fat is retracted can the full extent of phallic development be assessed. This is among the most common causes of inappropriate referral for hypogonadism. The extent of pubic and axillary hair is assessed, as is the degree of acne or comedones. The possibility of cryptorchidism or retractile testes should be determined if no testes are palpated in the scrotum. Neurologic examination, including examination of the optic discs and visual fields by frontal confrontation perimetry, may reveal findings, suggesting the presence of a CNS neoplasm or a developmental defect, and may suggest that more accurate ophthalmologic evaluation is needed. Determination of olfaction is important because many patients with Kallmann syndrome wait years for the correct diagnosis to be made even in the presence of classic findings; physicians must remain alert to the possibility of this diagnosis and to the fact that congenital anosmia may not be noted by the patient or family for years. The stigmata of gonadal dysgenesis (i.e., Turner syndrome) or the small testes and gynecomastia of Klinefelter syndrome may suggest a karyotypic abnormality. Complete physical examination, including the lungs, heart, kidney, and gastrointestinal tract, is important in the search for a chronic disorder that may delay puberty.

Laboratory studies (Table 26.22) include determination of plasma LH and FSH concentrations by sensitive third-generation assays in pediatric endocrine laboratories, measurement of the rise in the LH level after GnRH or GnRH agonist administration, and determination of testosterone concentrations in boys and estradiol levels in girls in pediatric endocrine laboratories using HPLC-MS/MS, all with pediatric standards for that laboratory. One of the national endocrine laboratories should be used for determinations of the hormones of puberty, because most local laboratories are interested only in differentiating the normally higher adult values



• **Fig. 26.49** Flow chart for the evaluation of delayed puberty in girls. *CNS,* central nervous system; *LH,* luteinizing hormone; *MRI,* magnetic resonance imaging.

from inappropriately low levels, and they cannot determine the gradations of the low levels found in puberty.[265] Results of commercial immunochemiluminometric assays for LH and FSH are reported to be more sensitive for use in pediatrics than immunofluorometric assays (IFMA).[700] Several national laboratories began using liquid chromatography with tandem mass spectrometry methods for improved sensitivity and specificity and determinations in children (and women) for increased accuracy.[265] Newer ultrasensitive bioassays are not yet available commercially for the determination of low values of testosterone and estradiol or for total androgen or estrogen. Measurements of T_4 and TSH concentrations in boys and girls are usually necessary, and prolactin is often useful as well.

Genetic testing is available either by targeted evaluation or by panels developed by the commercial laboratories for hypogonadotropic hypogonadism. Targeted evaluation may be carried out by looking for mutations of FGF8 in affected patients with cleft palate or lip or skeletal abnormalities, signs of CHARGE syndrome in evaluating for mutations in CHD7, bimanual synkinesia or renal agenesis in looking for mutations of KAL1, or in those with severe obesity looking for mutations in leptin and leptin receptors or PCSK1.[374] Genetic counseling should be offered to the affected families. This is more easily carried out when there is an autosomal dominant, autosomal recessive, or X-linked condition than for oligogenetic conditions at present.

Radiographic examination may include bone age determination and, if the history or physical examination is consistent with a CNS lesion, an MRI of the brain, with specific attention to the pituitary and hypothalamic area using contrast. MRI (T2-weighted) coronal views of the olfactory tract will be useful in the diagnosis of Kallmann syndrome. CT can detect calcification, in contrast to standard MRI scans, although calcification can be found on plain radiographs, in some cases. Ultrasound evaluation of the uterus and ovaries provides useful information about the state of development of these structures but only if the ultrasonographer has experience with children and young adolescents. DXA evaluation of bone density should be considered in hypogonadotropic hypogonadism.

Assessment of karyotype should be considered for all undiagnosed short girls, even in the absence of somatic signs of Turner syndrome and especially if puberty is delayed or unexplained short stature is involved. Karyotype assessment should be performed for boys with suspected Klinefelter syndrome stigmata or behavior.

A presumptive diagnosis of constitutional delay in growth and adolescence is made if the history and growth chart reveal a history of short stature but a consistent growth rate for skeletal age (and no signs or symptoms of hypothalamic lesions), if the family history includes parents or siblings with delayed puberty, if the physical examination (including assessment of the olfactory threshold) is normal, if optic discs and visual fields are normal,

TABLE 26.20 Differential Diagnostic Features of Delayed Puberty and Sexual Infantilism

Condition	Stature	Plasma Gonadotropins	GnRHa Test LH Response	Plasma Gonadal Steroids	Plasma DHEAS	Karyotype	Olfaction
Constitutional delay in growth and adolescence	Short for chron. age, usually appropriate for bone age	Prepubertal, later pubertal	Prepubertal, later pubertal	Low, later normal	Low for chron. age, appropriate for bone age	Normal	Normal
Hypogonadotropic Hypogonadism							
Isolated gonadotropin deficiency	Normal, absent pubertal growth spurt	Low	Prepubertal or no response	Low	Appropriate for chron. age	Normal	Normal
Kallmann syndrome	Normal, absent	Low	Prepubertal or no response	Low	Appropriate for chron. age	Normal	Anosmia; pubertal growth spurt or hyposmia
Idiopathic multiple pituitary hormone deficiencies	Short stature and poor growth since early childhood	Low	Prepubertal or no response	Low	Usually low	Normal	Normal
Hypothalamic-pituitary tumors	Late-onset decrease in growth velocity	Low	Prepubertal or no response	Low	Normal or low for chron. age	Normal	Normal
Primary Gonadal Failure							
Syndrome of gonadal dysgenesis (Turner syndrome) and variants	Short stature since childhood	High	Hyperresponse for age	Low	Normal for chron. age	45,X or variant	Normal
Klinefelter syndrome and variants	Normal to tall	High	Hyperresponse at puberty	Low or normal	Normal for chron. age	47,XXY or variant	Normal
Familial XX or XY gonadal dysgenesis	Normal for age	High	Hyperresponse	Low	Normal for chron. age	46,XX or 46,XY	Normal

chron., Chronologic; *DHEAS,* dehydroepiandrosterone sulfate; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone.

TABLE 26.21 Endocrine Diagnosis of Constitutional Delayed Adolescence and Hypogonadotropic Hypogonadism

No single test reliably discriminates between the two diagnoses.
Onset of puberty in boys is indicated by
- Testes >2.5 cm in diameter
- Serum testosterone concentration >50 ng/dL
- Pubertal LH response to GnRH bolus
- Pubertal pattern of LH pulsatility

GnRH, Gonadotropin-releasing hormone; *LH,* luteinizing hormone.

TABLE 26.22 Endocrine and Imaging Studies in Delayed Adolescence

Initial assessment
- Plasma testosterone or estradiol
- Plasma FSH and LH
- Plasma thyroxine (and prolactin)
- Bone age and lateral skull roentgenograph
- Test of olfaction

Follow-up studies
- Karyotype (short, phenotypic females)
- MRI with contrast enhancement
- Pelvic ultrasonography (females)
- GnRH test
- hCG test (males)
- Pattern of pulsatile LH secretion
- Visual acuity and visual fields

FSH, Follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *hCG,* human chorionic gonadotropin; *LH,* luteinizing hormone; *MRI,* magnetic resonance imaging.

and if the bone age is significantly delayed. In classic cases, MRI of the hypothalamic-pituitary region may not be necessary. The rate of growth in these patients is usually appropriate for bone age; a decrease in growth velocity occurs in some normal children just before the appearance of secondary sexual characteristics and may awaken concerns if such a pattern occurs in these subjects. The onset at puberty correlates better with bone age than with chronologic age in delayed puberty, although bone age is not any better at estimating the onset of puberty in normal boys than is chronologic age and cannot be considered a highly accurate test.[164] Elevated concentrations of gonadotropins and gonadal steroids to early pubertal levels precede secondary sexual development by several months; measurements of serum LH, FSH, estradiol, or testosterone levels in appropriate assays may help to predict future development. The GnRH stimulation test was the gold standard for the determination of whether a child is in puberty, but GnRH is not available in the United States. Under 3 years of age, children may have peaks of gonadotropins and sex steroids similar to pubertal subjects. In healthy Danish girls below 3 years of age LH increases up to 9.2 IU/L after GnRH test, and the stimulated LH/FSH ratio did not exceed 0.43. The third-generation LH assays are sufficiently sensitive to allow in most boys the determination of the onset of endocrine puberty with a single blood sample, but a dynamic GnRH test is still often performed using GnRH agonists. The measurement of gonadotropins 1 hour (or 4 hours in some reports) after a subcutaneous injection of GnRH agonist is the current method of testing dynamic secretion of gonadotropins in the absence of native GnRH supplies.

Measurement of an 8 AM serum testosterone level provides an accurate indication of impending pubertal development; a value of greater than 0.7 nmol/L (20 ng/dL) predicts enlargement of testes to greater than 4 mL by 12 months in 77% of cases and in 15 months in 100% of cases, whereas of those with a value less than 0.7 nmol/L, only 12% entered puberty in 12 months and only 25% entered puberty in 15 months. This technique may help predict spontaneous pubertal development, but it still requires considerable watching and waiting.[460] A single inhibin B value under 35 pg/mL holds promise in eliminating CDP in boys.[260,488] A method has been proposed to reliably separate constitutional delay in puberty from idiopathic hypogonadotropic hypogonadism in girls; a basal inhibin B value of less than 20 pg/mL or a GnRH agonist stimulated follicle-stimulating hormone less than 11 IU/L at 4 hours favors IHH.[701] For boys a method to reliably separate CDP from IHH is proposed to be a basal LH of less than 0.3 IU/L or a stimulated LH at 4 hours after GnRH agonist of less than 5.3 IU/L or an inhibin B less than 111 pg/mL.[702] A recent series of 174 boys with delayed puberty found that a history of cryptorchidism carried the highest risk of hypogonadism of all types and the combination of small testes (<1.1 mL) and low inhibin B (10–50 ng/L) carried the highest risk of CHH.[703] Low AMH values are found in severe IHH.[703] Functional hypogonadotropic hypogonadism or CDP were more likely if growth velocity was less than 3 cm per year. These methods require continued evaluation before they become standard clinically. Watchful waiting may remain the procedure of choice when a patient does not fulfill the delineated criteria and does not fall into a diagnosable grouping.

The presence of red flags is suggested to be a strong indication of gonadotropin deficiency rather than CDP. Thus lack of prior minipuberty of infancy (such as cryptorchidism or micropenis) or the presence of congenital defects, including anosmia, deafness, mirror movements, renal agenesis, dental/digital anomalies, clefting or coloboma, or findings of CHARGE syndrome, make the diagnosis of gonadotropin deficiency more likely.[704] Genetic testing for many of the genes that cause Kallmann syndrome is available; it is suggested that priorities for such testing may be best focused upon subjects with anosmia or hyposmia, synkinesia, dental agenesis, digital bony abnormalities, and hearing loss.[705] Genetic testing for *PROP* mutations is commercially available for combined hypopituitarism.

A typical patient with isolated gonadotropin deficiency is of average height for age and has eunuchoid proportions; has low plasma concentrations of gonadal steroids, LH, and FSH; and has no increase or a blunted response of LH after GnRH or GnRH agonist administration. The amplitude and usually the frequency of LH pulses are decreased when serial blood samples are studied over a 24-hour period. In some forms of Kallmann syndrome, the sense of smell is absent or impaired. However, as stated, differentiation of isolated gonadotropin deficiency in the absence of hyposmia or anosmia from CDP may be difficult at initial study. Gonadotropin-deficient patients may be as short as those with constitutional delay in growth and adolescence, and concentrations of LH and FSH in hypogonadotropic hypogonadism may be indistinguishable from those of normal prepubertal children or children with constitutional delay. Sometimes, years of observation are necessary to detect the appearance of spontaneous and progressive signs of secondary sexual development or to document rising concentrations of gonadotropins or gonadal steroids before the diagnosis is clear. There is a tendency for hypogonadotropic patients to undergo adrenarche at a normal age and to have higher DHEAS concentrations than those with constitutional delay in growth, and this pattern is helpful in making the differential diagnosis.[442] In most cases, absence of the first signs of sexual maturation or failure of a rise in gonadotropins or gonadal steroid levels by age 18 years in the presence of a normal concentration of serum DHEAS for chronologic age supports the diagnosis of isolated gonadotropin deficiency.

Patients with deficiency of gonadotropins combined with deficiency of other pituitary hormones require careful evaluation for a CNS neoplasm, especially if the defects are acquired. Visual field or optic disc abnormalities support the diagnosis of CNS tumor; even if these tests are normal, cranial MRI should be done to evaluate the pituitary gland and stalk and the hypothalamic region. MRI appears superior to CT for detecting mass lesions and developmental abnormalities of the hypothalamic-pituitary region.

Treatment of Delayed Puberty and Sexual Infantilism

Patients with constitutional delay in growth and adolescence ultimately have spontaneous onset and progression through puberty. Often, reassurance and continued observation to ensure that the expected sexual maturation occurs are sufficient. However, the stigma of appearing less mature than one's peers can cause psychologic stress. These individuals may be unable to participate in the dating activities their friends are starting; smaller size may lead them to avoid participation in athletics; immature appearance may lead to ridicule, especially in the locker room; and schoolwork may suffer because of their poor self-image. Some children feel such intense peer pressure and low self-esteem that only the appearance of signs of puberty can reassure them and enable them to participate in sports and social activities with their peers. Poor

self-image in late-maturing boys may carry into adulthood, even after normal puberty ensues. Growth retardation appears more often responsible for most of the stress rather than the delay in pubertal development itself.

For psychologic reasons, for boys 14 years old or older who show no signs of puberty, a 3-month to 6-month course of testosterone enanthate, cypionate, or cyclopropionate (50-mg dose given intramuscularly every 4 weeks) may be helpful. Because starting with the higher dose of 100 mg can lead to priapism in treatment-naïve boys, care, lower dosage, and short-acting preparations are advisable. Decades of experience confirm no effect on adult height of low dosages in the short term.[706] The low dose of testosterone enanthate is considered to be safe but can raise apolipoprotein B (apoB) and decrease HDL cholesterol and apoAI levels (estradiol increases HDL cholesterol and decreases triglycerides, LDL cholesterol, and apoB). Although the use of exogenous androgens may improve self-image and start the secondary sexual changes of puberty, low-dose androgen use does not increase final height. Low-dose estrogen may be invoked in the treatment of CDP in girls in a similar manner.

If during the 3 to 6 months after discontinuing gonadal steroid therapy spontaneous puberty does not ensue or the concentrations of plasma gonadotropins and plasma testosterone in boys or plasma estradiol in girls do not increase, the treatment may be repeated. Only one or two courses of therapy usually are necessary. When treatment is discontinued after bone age has advanced, for example, to 12 to 13 years in girls or 13 or 14 years in boys, patients with constitutional delay usually continue pubertal development on their own, whereas those with gonadotropin deficiency do not progress and may regress. Oral testosterone undecanoate was successfully used for less than 1 year in a large study of boys with constitutional delayed puberty with apparently no ill effects.[707]

Functional hypogonadotropic hypogonadism associated with chronic disease is treated by alleviating the underlying problem. Delayed puberty in this situation is usually a result of inadequate nutrition and low weight or excessive energy expenditure. When weight returns to normal values, puberty usually occurs spontaneously, although there will likely be months of delay. Treatment with T_4 allows normal pubertal development in hypothyroid patients with delayed puberty.

The transition of care from the range of age normally considered to be adolescence, administered by a pediatric practitioner, to adult life, administered by a practitioner experienced in adult endocrinology, presents important implications for a change in the manner in which medical care is presented. Changes in the individual's autonomy and implications of legal distinctions arise, and some have suggested that this transition period should be considered another stage of the life span.[708]

Congenital or acquired gonadotropin deficiency as a result of a lesion or surgery requires replacement therapy at an age approximating the normal age of onset of puberty (Tables 26.23 and 26.24). Present practice in hypogonadotropic hypogonadism is replacement with gonadal steroid therapy, but there is research into the use of gonadotropins or GnRH to stimulate the adolescent age gonad in a more physiologic manner; at present it is not clear which is the optimal approach in teenagers, but sex steroid replacement is most often used.

An exception may occur when GH deficiency coexists with gonadotropin deficiency; if bone age advancement and epiphyseal fusion are brought about by testosterone or estradiol replacement before therapy with GH causes adequate linear growth, adult height will be compromised. However, if puberty is not initiated early enough, the patient may suffer psychologic damage. It is advisable to initiate puberty in these patients with low-dose gonadal steroids by age 14 in boys and age 13 in girls, regardless of the definitive diagnosis of gonadotropin deficiency. Isolated GH-deficient patients may have a delayed onset of puberty; with GH administration, puberty usually occurs at an appropriate age but may progress faster than in normal individuals. Children with GH deficiency showed a correlation between the age of onset of induced puberty and adult height for those who were also gonadotropin deficient, whereas those who underwent spontaneous puberty, which occurred earlier than the age of hormone-induced puberty in the gonadotropin-deficient children, had a decreased adult height. This supports the advisability of waiting to initiate puberty in GH-deficient and gonadotropin-deficient children. Height at the onset of puberty is also correlated with adult height in GH-deficient children. However, artificially delaying puberty with a GnRH analogue to attempt to achieve a greater final height has been attempted in isolated cases of GH deficiency or with normal-variant short stature, but concern over decreased bone density seen in subjects treated with GnRH analogues led to warnings about the use of this agent in GH-deficient patients. There is inadequate evidence of efficacy to recommend this therapy.[470]

Microphallus due to hypothalamic deficiencies may be treated with one or two 3-month courses of testosterone enanthate, with 25 mg per month given intramuscularly to enlarge the size of the penis.[327] Although concern was raised that early testosterone therapy might not allow the attainment of a normal adult penile size, experience has shown otherwise. The concern that the penis might not respond to androgens later in life if exposed to testosterone in childhood, a pattern observed in the rat, proved incorrect. Positive psychologic outcomes and attainment of normal stretched penile

TABLE 26.23 Management and Treatment of Delayed Puberty

OBJECTIVE
Determine site and cause of abnormality.
Induce and maintain secondary sexual characteristics.
Induce pubertal growth spurt.
Prevent the potential short-term and long-term psychologic, personality, and social handicaps of delayed puberty.
Ensure normal libido and potency.
Attain fertility.
THERAPY
Concerned But Not Anxious or Socially Handicapped Adolescent
Reassurance and follow-up (tincture of time)
Repeat evaluation (including serum testosterone or estradiol) in 6 mo
Psychosocial Handicaps, Anxiety, Highly Concerned
Therapy for 4 mo
Boys: testosterone enanthate 100 mg IM q4wk at 14-14.5 yr of age, or overnight transdermal testosterone patch
Girls: ethinyl estradiol 5-10 mg/day PO or conjugated estrogens 0.3 mg/day PO or overnight ethinyl estradiol patch at 13 yr of age
No therapy for 4-6 mo; reevaluate status including serum testosterone or estradiol; if indicated repeat treatment regimen

IM, Intramuscularly; *PO*, orally; *q*, every.

TABLE 26.24 Hormonal Substitution Therapy in Hypogonadism

Boys

Goal: to approximate normal adolescent development when diagnosis is established

Initial therapy: at 13 yr of age, testosterone enanthate (or other long-acting testosterone ester) 50 mg IM every month for about 9 mo (6-12 mo)

Over the next 3-4 yr: gradually increase dose to adult replacement dose of 200 mg q2-3wk

Testosterone gel is coming into widespread use as discussed in the text

Begin *replacement therapy in boys with suspected hypogonadotropic hypogonadism* by bone age ≤14 yr

To induce fertility at appropriate time in hypogonadotropic hypogonadism: pulsatile GnRH or FSH and hCG therapy

Girls

With a firmly established diagnosis of hypogonadism (e.g., girls with 45,X gonadal dysgenesis), begin hormonal substitution therapy at 12-13 yr of age

Goal: to approximate normal adolescent development

Initial therapy: ethinyl estradiol 5 mg by mouth or conjugated estrogen 0.3 mg (or less) by mouth daily for 4-6 mo or preferably estradiol transdermally

After 6 mo of therapy (or sooner if breakthrough bleeding occurs), begin cyclic therapy:

- Estrogen: first 21 days of month
- Progestogen (e.g., medroxyprogesterone acetate 5 mg PO) 12th to 21st day of month
- Gradually increase dose of estrogen over next 2-3 yr to conjugated estrogen 0.6-1.25 mg or ethinyl estradiol 10-20 mg daily for first 21 days of month or estradiol patch

In hypogonadotropic hypogonadism, to induce ovulation at appropriate time: pulsatile GnRH or FSH and hCG therapy

FSH, Follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *hCG,* human chorionic gonadotropin; *PO,* orally; *q,* every.

length have been reported.[527,709] It is appropriate to treat male infants and children with micropenis due to gonadotropin or GH deficiency with short courses of androgens to enlarge the penis into the normal childhood range. Patients with isolated congenital GH deficiency occasionally have micropenis that may be successfully treated with GH replacement alone. It is not appropriate to sex reverse a male infant because of microphallus caused by fetal testosterone or GH deficiency.

Episodic administration of GnRH elicits pulsatile LH and FSH release and gonadal stimulation in prepubertal children or hypogonadotropic patients. Portable pumps are used to administer episodic GnRH over prolonged periods. Pulsatile GnRH therapy can induce puberty and promote the development of secondary sexual characteristics and spermatogenesis in men and ovulation in women,[710] but it is not practical for the routine induction of puberty in adolescent boys and girls with gonadotropin deficiency. Pregnancy can be achieved with this regimen in women and spermatogenesis in men with hypogonadotropic hypogonadism. A lower frequency of GnRH administration favors FSH secretion, whereas a faster frequency favors LH secretion and ultimately has been associated with a PCOS-like picture.

Human menopausal gonadotropin and hCG can be used as effective substitutes for recombinant human pituitary LH and FSH to produce full gonadal maturation, but this regimen is cumbersome and expensive. At present, long-term gonadal steroid replacement therapy is the treatment of choice for hypothalamic or pituitary gonadotropin deficiency until fertility is desired.

Hypergonadotropic hypogonadism is treated by replacement of testosterone in boys and estradiol in girls. For treatment of gonadal dysgenesis, estrogen therapy should be initiated when the patient is age 13 (bone age >11 years) to allow secondary sexual development at an appropriate chronologic age. Klinefelter syndrome is compatible with various degrees of spontaneous masculinization at puberty; some patients require testosterone replacement. The concentration of plasma testosterone and LH should be monitored every 6 months during puberty and yearly thereafter. If the LH level rises more than 2.5 SD above the mean value or the testosterone level decreases below the normal range for age, testosterone replacement therapy is indicated. FSH is increased due to lack of inhibin from affected testes and may not decrease with testosterone treatment.

Patients receiving gonadal steroid replacement follow the same treatment regimen whether the diagnosis is hypogonadotropic hypogonadism or hypergonadotropic hypogonadism (see Table 26.24). Various testosterone preparations are available with several routes of administration. Alkylated testosterone preparations should be avoided because of the risk of peliosis hepatis (i.e., hemorrhagic liver cysts), which is not related to dose or duration of treatment; although regression is possible with discontinuation of testosterone treatment, progression to liver failure can occur. Males may receive testosterone enanthate, propionate, or cypionate (50 mg every 4 weeks intramuscularly) at the start, although priapism has been reported with the higher starting dose (100 mg) in a testosterone-naïve boy; later, the dosage is gradually increased to 200 to 300 mg every 2 to 3 weeks. Low-dose replacement therapy is appropriate until well into the pubertal growth spurt.

Dermal preparations of testosterone have been used in studies of delayed puberty. Testosterone may be administered by cutaneous patch on nonsexual skin to cause secondary sexual development in hypogonadal adolescents; patches may be given at night to recreate the diurnal variation of testosterone seen in early puberty. Physiologic values of serum testosterone may be reached with these patches, along with secondary sexual development. A teenage boy may be less likely to apply a patch daily, and biweekly or monthly injections may allow better compliance; nonetheless, 2.5-mg and 5-mg dermal testosterone patches may be useful in motivated teenagers. Testosterone gel preparations, usually rubbed onto the forearms or shoulders, are approved for adults but not for adolescents. Contact with the skin, clothing, or towels used by a patient treated with androgen gel can cause virilization in young children or women. Testosterone ointment may be used as therapy for microphallus to enlarge the size of the phallus intentionally, but a normal infant or child contacting the skin of an individual treated with testosterone gel (before it is absorbed) runs the risk of unplanned testosterone effects.[711] A dose of 10 mg of 2% transdermal testosterone given for 6 months achieved similar effects to 50 mg of intramuscular testosterone in terms of height velocity, although the control group in comparison achieved testicular enlargement while the treated groups did not.[712] A 1% testosterone gel given daily to patients age 12 to 17 years with Klinefelter syndrome or anorchia raised their serum testosterone levels to the pubertal range over a 6-month period of treatment: Cough was the major adverse reaction.[713] Transdermal testosterone is not approved for use in teenagers with delayed puberty.

The type and route of estrogen administration for girls are being reconsidered. Many suggest the use of 17β-estradiol rather than ethinyl estradiol owing to more physiologic effects; there is evidence in the treatment of transgender individuals that the use of ethinyl estradiol or conjugated estrogen carries a higher risk for venous and arterial thrombosisis.[704] Transdermal estrogen administration is presently recommended to avoid the first pass effect and hepatic clotting factor production as injected estrogen transits the liver and stimulates the production of proteins such as C-reactive protein, angiotensin precursor, and activated protein C, which are involved in cardiac complications; adult women treated with injectable estrogen had experienced increased risk of deep vein thrombosis and pulmonary thromboembolism, breast cancer, decreased serum insulin-like growth factor-I concentrations, and reduced lean body mass but increased fat mass. This contrasts with dermal estrogen, which is administered in lower dosages and reaches its therapeutic targets relatively unchanged and in lower, more physiologic concentrations; there is no change in these proteins with dermal estrogen administration.[714,715] For girls 13 years of age or older, transdermal estrogen has been used in clinical trials for decades in the treatment of delayed puberty or hypogonadism with beneficial results on physical development and bone density and is entering widespread use.[716]

Initially, girls 12 to 13 years old are given estradiol alone in an attempt to mimic normal estrogen exposure characteristic of puberty. Estrogen patches, fractions of Evorel patches (0.05–0.07 μg/kg in younger girls or 0.08–0.12 μg/kg in older girls every night) where it is desirable to have more rapid breast development, demonstrate achievement of reasonable levels of serum estrogen.[716] Evorel patch is available in Europe; in the United States, one-sixth to one-eighth of a Vivelle-Dot patch is administered once or twice per week.[677] There is individual variation up to 10% in absorption, but in an individual similar plasma levels are achieved using 2 mg daily oral 17β-estradiol, 2 mg daily gel, and 100-mg patch.[704] However, these patches are not FDA approved for such use at this time. From a clinical experience 50 girls were given dosage that increased every 6 months until an adult dose of 100 to 200 μg daily or one to two pumps of 0.06% estrogen gel daily demonstrated efficacy. Estradiol gel in an increasing dose from 0.1 to 1.5 mg given over a 5-year period is reported to be safe and effective as replacement therapy in girls with Turner syndrome.[717] As with testosterone, children must not be in contact with the preparation because untoward estrogen effects may occur. However, it is important to note that to date dermal estrogen preparations are not approved for teenagers. If oral estradiol is used an initial dose might be 0.1 mg increasing toward adulthood to 1 to 2 mg daily.

Progestational agents are added after breakthrough bleeding occurs or when the girl reaches Tanner stage 4 after the start of cyclic estrogen therapy to avoid causing endometrial hyperplasia and to enhance artificially induced menstrual periods along with estradiol. Progestational agents may be initiated as 5 to 10 mg of progesterone first given 5 days per month increasing to 14 days per month toward adulthood. Estradiol might be administered the first 21 days of the cycle with progesterone added the last 14 days of the artificial cycle.

The maintenance dose should be the minimal amount to maintain secondary sexual characteristics, sustain withdrawal bleeding, and prevent osteoporosis. Undesirable effects are uncommon but may include weight gain, headache, nausea, peripheral edema, and mild hypertension.

There is a concern about the increased risk of endometrial and breast carcinoma in patients receiving chronic estrogen replacement therapy, including patients with Turner syndrome.[718] The use of progestational agents to antagonize the effect of estrogens reduces the risk of endometrial cancer, but the best answer about the optimal dose of estrogen, route of administration, and dose of progesterone to enhance development without unduly increasing the risk of cancer must come from future studies. Estrogen replacement is important for its antiosteoporosis action on bone. Bone density is decreased in Turner syndrome in part because of hypogonadism at puberty, and this tendency becomes more severe with age in patients who discontinue or do not receive estrogen replacement therapy. Transdermal estrogen can increase bone density in subjects with Turner syndrome who have finished statural growth.[719] We lack adequate controlled studies on optional sex steroid replacement regimens in young adolescent women.

Biosynthetic hGH therapy in Turner syndrome causes an increase in growth rate, with an increase in adult height approaching or reaching the lower range of the normal growth curves possible, as stated earlier.

Patients with hypopituitarism may complain of sparse pubic hair growth or, in girls, total absence of pubic hair. Pubic hair thickens further in affected males with hCG treatment, which adds the testicular contribution of testosterone to the exogenous testosterone therapy. GH therapy in GH-deficient and gonadotropin-deficient males enhances the steroidogenic response of the testes to hCG administration. Adolescent or young adult women have been given a low dose (25 mg) of long-acting intramuscular testosterone every 4 weeks to stimulate the growth of pubic hair without virilization. Oral DHEAS treatment is suggested to improve pubic hair growth in hypopituitary girls.[720] However, these androgens are not presently standard practice.

Aromatase inhibitors have been used to enhance growth in short children, including those with constitutional delay in puberty, by decreasing estrogen levels that would ordinarily advance bone age and limit future growth.[471] Unfortunately there are limited data on actual adult height achieved as most studies predict adult height at a point before the child actually ceases growth.[721]

As in the male, psychologic counseling is beneficial for girls to deal with the impact of chronic medical visits, infertility after an extensive medical treatment, and perhaps a less than ideal physical development compared to peers. Adults affected by hypogonadotropic hypogonadism comment that while their providers may attend to their medical treatment, they often do not address their emotional needs. Further problems occur when patients are lost to follow-up as they make the transition to adult care.[722]

Sexual Precocity[994]

If iso-sexual precocity results from premature reactivation of the hypothalamic GnRH pulse generator/pituitary gonadotropin-gonadal axis, the condition is GnRH dependent and is termed *CPP* (i.e., *central* [or previously, *complete* or *true*] *precocious puberty*). Pulsatile LH release has a pubertal pattern, and the rise in the concentration of LH after GnRH or GnRH agonist administration is indistinguishable from the normal pubertal pattern of serum LH. If extrapituitary secretion of gonadotropins or secretion of gonadal steroids independent of pulsatile GnRH stimulation leads to virilization in boys or feminization in girls, the condition is termed *incomplete, pseudoprecocious,* or *peripheral precocious puberty*. The production of excessive estrogens in males leads to inappropriate feminization, and the production of increased

androgen levels in females leads to inappropriate virilization; these conditions are termed *contrasexual precocity* or *heterosexual precocity*. Disorders causing sexual precocity are therefore separated into those in which the increased secretion of gonadal steroids depends on GnRH stimulation of pituitary gonadotropins and those in which it is unrelated to activation of the hypothalamic GnRH pulse generator.

In all forms of sexual precocity, increased gonadal steroid secretion increases height velocity, somatic development, and the rate of skeletal maturation; because of premature epiphyseal fusion, sexual precocity can lead to the paradox of tall stature in childhood but short adult height (Table 26.25). Untreated females with idiopathic CPP demonstrated a mean adult height of 151 to 155 cm.[723] In the few reports of adult height in boys with untreated precocious puberty, mean adult stature was 155.4 cm ± 8.3 SD, with all subjects well below midparental height and far below the father's height from the data available.[724,725]

Serum alkaline phosphatase reflects growth, and IGF1 concentrations reflect the degree of sexual development rather than chronologic age, as do most chemistry and hematology values. The serum concentrations of the propeptide of type III procollagen (P-III-NP) in normal puberty and in CPP parallel the normal pubertal growth curve and also parallel the changes in growth rate in children treated with GnRH agonists. Blood pressure matches that of normal subjects of the same height and gender after correcting for bone age rather than chronologic age according to the latest standards for blood pressure.[726]

In boys (Fig. 26.51), the testes usually enlarge under gonadotropin stimulation before any other signs of puberty are seen; in girls, an increase in the rate of growth, the appearance of breast development, enlargement of the labia minora, and maturational changes in the vaginal mucosa are the usual presenting signs, with variable manifestations of pubic hair depending on the age at onset. Progression of secondary sexual maturation may be more rapid than normal, but a waxing and waning course of development may occur. Spermatogenesis in males and ovulation in females often occur, and fertility is possible. The rapid growth is associated with the increased GH secretion and elevation of serum IGF1 levels resulting from stimulation by estradiol. The ratio of bone age to chronologic age and the rise of IGF1 above normal values for chronologic age are predictive of outcome: More mildly affected children progress less rapidly and tend to maintain their target height, and this may represent a benign entity.

Central Precocious Puberty: Complete Isosexual Precocity

As noted earlier, the classic lower age limit beneath which the diagnosis of precocious puberty is applied was 8 years in girls. However, this limit was reconsidered leading into the amended diagnosis of sexual precocity (Table 26.26) as the appearance of any sign of secondary sexual maturation before 7 years for white girls and 6 years for African-American girls. These newer lower cutoffs assume that there are no signs or symptoms of CNS disorders or other concurrent diseases that might cause sexual precocity, as evaluation is indicated in those cases regardless of age. Further, the newer lower limits usually applied to girls with elevated body mass index values and if the subject does not have an increased BMI evaluation may still be indicated. Thus careful evaluation is essential at these lower age ranges in girls who should have only minimal slowly progressive signs of sexual precocity. These newer limits remain controversial, so the cautions described must be heeded.

In the UCSF series of more than 200 patients with CPP, girls had true precocious puberty (i.e., GnRH-dependent CPP) five times more commonly than boys, and idiopathic CPP was eight times more common in girls than in boys (Table 26.27). Others reported a 10-fold increased prevalence of precocious puberty in girls compared with boys. CNS abnormalities occurred at least as often as idiopathic CPP in boys, whereas in girls neurologic lesions were one-fifth as common as idiopathic disorders. Therefore, it is essential to search for a CNS cause for CPP, especially in boys, because sexual precocity may be the only manifestation of a CNS tumor (Tables 26.28 and 26.29). However, most children referred for evaluation have the benign variants leading to premature thelarche or premature adrenarche.

TABLE 26.25 Historical Controls of Untreated Children With True Precocious Puberty

Study	No. Patients (Women/Men)	FINAL HEIGHT (CM)[a]	
		Women	Men
Thamdrup			
Sigurjonsdottir and Hayles	26/8	151.3 ± 8.8	155.4 ± 8.3
	40/11	152.7 ± 8.0	156.0 ± 7.3
Werder et al	4/0	150.9 ± 5.0	
Lee	15/0	155.3 ± 9.6	
University of California, San Francisco	8/4	153.8 ± 6.8	159.6 ± 8.7
Total	93/23	152.7 ± 8.6	155.6 ± 7.7

[a]Mean ± 1 SD (standard deviation).

From Paul D, Conte FA, Grumbach MM, et al. Long-term effect of gonadotropin-releasing hormone agonist therapy on final and near-final height in 26 children with true precocious puberty treated at a median age of less than 5 years. *J Clin Endocrinol Metab.* 1995;80:546–551.

Data from Lee PA. Medroxyprogesterone therapy for sexual precocity in girls. *Am J Dis Child.* 1981;135:443–445; Sigurjonsdottir TJ, Hayles AB. Precocious puberty: a report of 96 cases. *Am J Dis Child.* 1968;115:309–321; Thamdrup E. *Precocious Sexual Development: A Clinical Study of 100 Patients.* Springfield, IL: Charles C Thomas; 1961; Werder EA, Murset G, Zachmann M, et al. Treatment of precocious puberty with cyproterone acetate. *Pediatr Res.* 1974;8:248–256.

TABLE 26.26 Classification of Sexual Precocity

TRUE PRECOCIOUS PUBERTY OR COMPLETE ISOSEXUAL PRECOCITY (GNRH-DEPENDENT SEXUAL PRECOCITY OR PREMATURE ACTIVATION OF THE HYPOTHALAMIC GNRH PULSE GENERATOR)

- Idiopathic true precocious puberty
- CNS tumors
  - Optic glioma associated with neurofibromatosis type 1
  - Hypothalamic astrocytoma
- Other CNS disorders
  - Developmental abnormalities including hypothalamic hamartoma of the tuber cinereum
  - Encephalitis
  - Static encephalopathy
  - Brain abscess
  - Sarcoid or tubercular granuloma
  - Head trauma
  - Hydrocephalus
  - Arachnoid cyst
  - Myelomeningocele
  - Vascular lesion
  - Cranial irradiation
- True precocious puberty after late treatment of congenital virilizing adrenal hyperplasia or other previous chronic exposure to sex steroids
- True precocious puberty due to gain of function mutations:
  - in KISS1R/GRP54 gene
  - in *KISS1* gene

INCOMPLETE ISOSEXUAL PRECOCITY (HYPOTHALAMIC GNRH-INDEPENDENT)

Males

- Gonadotropin-secreting tumors
  - hCG-secreting CNS tumors (e.g., chorioepitheliomas, germinoma, teratoma)
  - hCG-secreting tumors located outside the CNS (hepatoma, teratoma, choriocarcinoma)
- Increased androgen secretion by adrenal or testis
  - Congenital adrenal hyperplasia (CYP21 and CYP11B1 deficiencies)
  - Virilizing adrenal neoplasm
  - Leydig cell adenoma
  - Familial testotoxicosis (sex-limited autosomal dominant pituitary gonadotropin-independent precocious Leydig cell and germ cell maturation)
  - Cortisol resistance syndrome

Females

- Ovarian cyst
- Estrogen-secreting ovarian or adrenal neoplasm
- Peutz-Jeghers syndrome

Both Sexes

- McCune-Albright syndrome
- Hypothyroidism
- Iatrogenic or exogenous sexual precocity (including inadvertent exposure to estrogens in food, drugs, or cosmetics)

VARIATIONS OF PUBERTAL DEVELOPMENT

- Premature thelarche
- Premature isolated menarche
- Premature adrenarche
- Adolescent gynecomastia in boys
- Macroorchidism

CONTRASEXUAL PRECOCITY

Feminization in Males

- Adrenal neoplasm
- Chorioepithelioma
- CYP11B1 deficiency
- Late-onset adrenal hyperplasia
- Testicular neoplasm (Peutz-Jeghers syndrome)
- Increased extraglandular conversion of circulating adrenal androgens to estrogen
- Iatrogenic (exposure to estrogens)

Virilization in Females

- Congenital adrenal hyperplasia
  - CYP21 deficiency
  - CYP11B1 deficiency
  - 3βHSD deficiency
- Virilizing adrenal neoplasm (Cushing syndrome)
- Virilizing ovarian neoplasm (e.g., arrhenoblastoma)
- Iatrogenic (exposure to androgens)
- Cortisol resistance syndrome
- Aromatase deficiency

CNS, Central nervous system; *CYP11B1*, 11-hydroxylase; *CYP21*, 21-hydroxylase; *GnRH*, gonadotropin-releasing hormone; *hCG*, human chorionic gonadotropin; *3βHSD*, 3β-hydroxysteroid dehydrogenase 4,5-isomerase; *KISS1R/GPR54*, kisspeptin/G protein–coupled receptor 54.

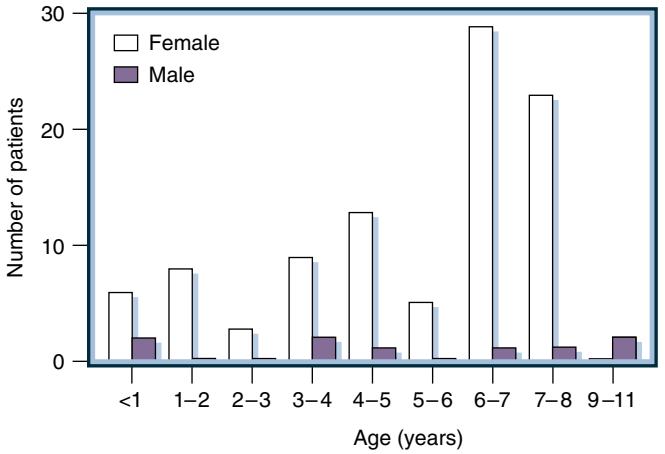
Modified from Grumbach MM. True or central precocious puberty. In: Kreiger DT, Bardin CW, eds. *Current Therapy in Endocrinology and Metabolism, 1985-1986*. Toronto, Canada: BC Decker; 1985:4–8.

Idiopathic True or Central Precocious Puberty[159,160,316,423,726,727]

In otherwise healthy girls, those with onset of puberty at 6 to 8 years of age often represent one end of the normal range of age at puberty onset; those with constitutional delay in growth and adolescence fall at the opposite end of the normal range of variation. These girls with low normal age of onset of puberty are often considered to have early puberty; a meta-analysis demonstrated that treatment with GnRH agonist is not needed to achieve mid-parental height.[728,729]

Children may develop CPP with no familial tendency and no signs of organic disease; these children have idiopathic CPP. This condition, which may manifest in infancy (see Table 26.27), is commonly associated with electroencephalographic abnormalities. The age at onset is 6 to 7 years (the new boundaries for age of onset of normal puberty suggest some of the 7-year-olds may be in the normal range) for about 50% of affected girls, 2 to 6 years for about 25%, and during infancy for 18% (Fig. 26.50). Patients with organic forms of CPP, especially if associated with hypothalamic hamartoma, have an earlier mean age at onset than those with the idiopathic form.

Many girls in this subset have clinical and hormonal features that fall between those of premature thelarche and CPP (e.g., more rapid growth rate) and are typical of neither condition[730]; this is



• **Fig. 26.50** Age at onset of idiopathic true precocious puberty in 106 children. At all ages, the frequency is greater in females than in males. The peak prevalence in girls is between ages 6 and 8 years. (From Kaplan SL, Grumbach MM. The neuroendocrinology of human puberty: an ontogenetic perspective. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:1–68.)

denoted exaggerated thelarche. About 10% of girls with apparently classic premature thelarche progress to definite CPP, but there are no signs at the time of first presentation to differentiate them from girls who continue with the pattern of premature thelarche and even biochemical measurements may not define them.[731] In most patients in this situation, the onset of breast development was first noticed after 2 years of age. Therapy may not be indicated if a pubertal pattern of pulsatile LH secretion during sleep is not present or if the basal LH measured by ultrasensitive assay or the LH response to exogenous GnRH or GnRH agonist is prepubertal or the uterus on sensitive ultrasound is less than 34 mm. (The upper limit of uterine length in the prepubertal state is 35 mm, as stated later.) Regular follow-up is thus important.

The nature of the striking sex difference in the prevalence of idiopathic CPP (females > males), compared with that of constitutional delay in growth and puberty (males > females) is poorly understood, although genomic data are accumulating. There may be a history of early maturation in families of affected subjects. CPP may be transmitted as an autosomal dominant trait with incomplete dominance in boys and girls.[726]

Before beginning treatment, it is essential to establish the progressive nature of the sexual precocity. In a subset of girls, the tempo is relatively slow and the sexual precocity may not be sustained. A small proportion of patients with CPP proven by a pubertal response of LH to GnRH and increased pulsatile LH secretion at night may revert spontaneously to a more immature pubertal state, persist without further progression, or fluctuate between progression and regression. There is a continuum in girls with CPP from premature thelarche, through unsustained or slowly progressive precocious puberty, to the relatively rapid progression of sexual maturation once it has begun.[732] If the growth rate slows to normal for age, skeletal maturation progresses in accordance with chronologic age, and there is little to no risk of impairment of adult height[733]; estrogen and IGF1 concentrations are normal or only slightly elevated. If height prediction is normal at the time of diagnosis rather than reduced, the patient does not require therapy; the efficacy of treatment with GnRH in those with onset after 6 years of age is inconsistent and rarely leads to increased adult stature (some sources say the effects of GnRH treatment are decreased after initiation of treatment after 8 years).[470] In some girls, we have observed within a period of 1 to 2 months the return of a prepubertal pattern of LH pulsatility during sleep, a prepubertal LH response to GnRH, and a concentration of plasma estradiol equivalent to the prepubertal state. Unlike typical patients, such girls do not exhibit the initial hyperresponse of plasma estradiol and LH to the GnRH agonist or the physical changes of the estrogen effect, and they tend to have lower serum IGF1.

The uterus and ovaries increase in size in CPP. The ovaries also may develop a multicystic appearance (but not a polycystic appearance) that may remain even after successful treatment with a GnRH agonist. The pituitary gland undergoes hypertrophy in early infancy, puberty, and pregnancy and also increases in size on MRI imaging in patients with CPP (see later).

CPP in females does not lead to premature menopause. However, there is increased risk in girls for the development of carcinoma of the breast in adulthood as noted earlier.[734] Psychosexual development is advanced modestly in patients with sexual precocity (about 1.5 years in girls with idiopathic CPP).

The gonadotropin and gonadal steroid concentrations in plasma, the LH response to GnRH administration, and the amplitude and frequency of LH pulses are in the normal pubertal range in CPP (Figs. 26.52 and 26.53). The third-generation gonadotropin assays allow diagnosis of CPP by the determination in a single serum sample of LH in the basal state in most girls.[260] Gonadotropin determination 60 or 240 minutes after a single subcutaneous dose of GnRH or GnRH agonist can diagnose CPP with high specificity and sensitivity. Adrenarche usually does not accompany gonadarche in girls with CPP who are younger than 5 or 6 years of age[442]: Pubic hair is sparse or absent initially in such girls. When the onset of CPP occurs after age 6 years, it is usually associated with adrenarche that is early for chronologic age but not for bone age.

In the UCSF experience and that of others, normal pregnancies have occurred in women with idiopathic CPP, CPP triggered by a CNS abnormality, or premature menarche.[735] Pregnancy is reported in the lay press in a patient with CPP at 5 years of age, an unfortunate result of childhood sexual abuse and CPP.

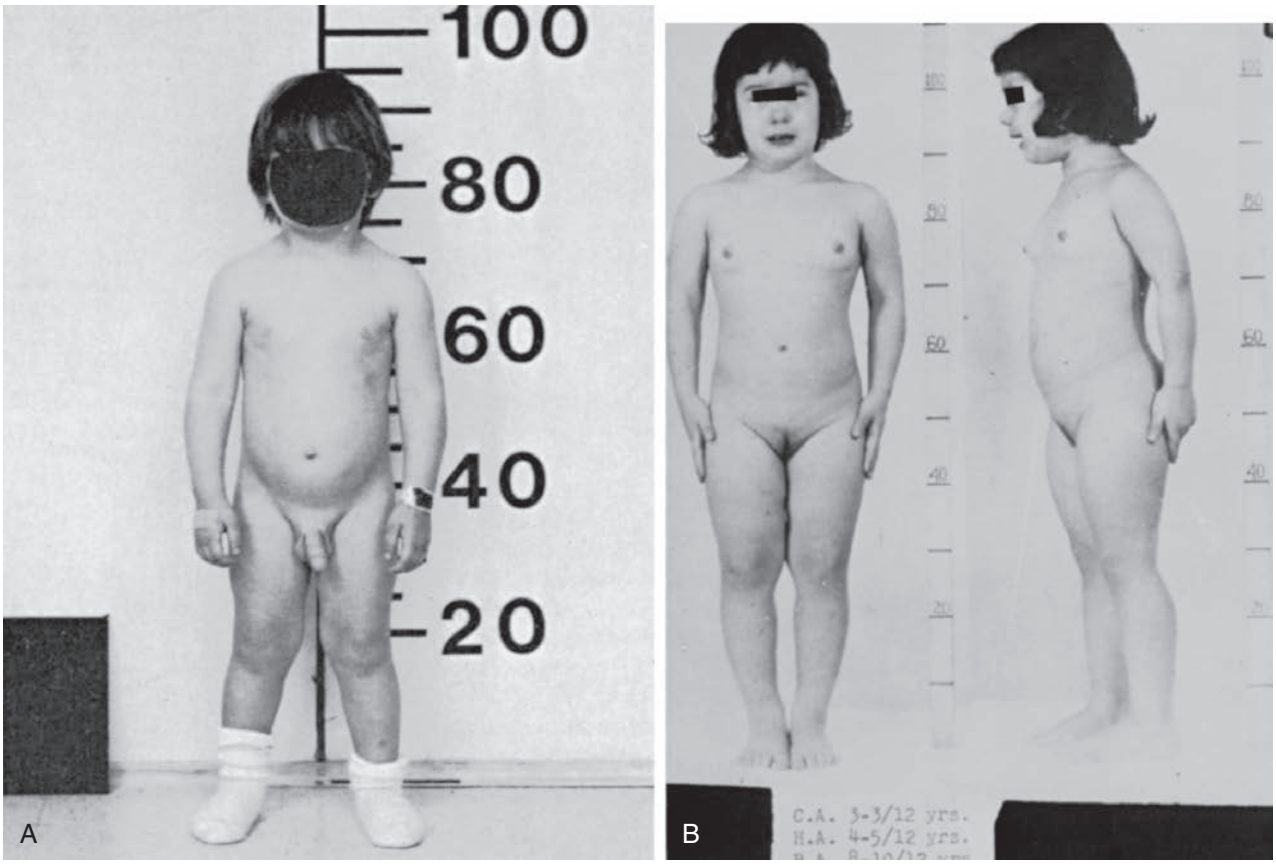
Long-term follow-up of 142 women up to 50 years of age who had central precocious puberty demonstrated no significant health conditions compared to the control population.[736] Treatment with gonadotropin-releasing hormone agonist or lack of treatment did not change this conclusion. In an upcoming discussion, however, a concern over the occurrence of PCOS is raised.

An increase in the prevalence of CPP girls in Korea between 2014 and 2010 is reported.[737] There was a lesser but measurable increase in prevalence in boys as well.

Gain-of-Function Mutations as Cause of Central Precocious Puberty

KISS and KISSIR/GRP54 Mutations

KISS Mutations. Whereas inactivating mutations in the KISS1R receptor cause hypogonadotropic hypogonadism, recently the first detected mutations in the gene encoding KISS and KISSR associated with precocious puberty were reported.[727] Two novel *KISS1* missense mutations were discovered in three children with idiopathic CPP; these mutations were absent in 400 control alleles. The first was an adopted Brazilian girl who had slowly progressing thelarche since birth, but acceleration of growth, bone age maturation, and secondary sexual development occurred at 7 years of age and was reversed with GnRH agonist treatment and had borderline pubertal GnRH-stimulated LH peak levels.[738] She had a



• **Fig. 26.51** (A) A boy aged 2 years and 5 months with idiopathic precocious puberty. He had pubic hair and phallic and testicular enlargement by 10 months of age. At 1 year, his height was 86 cm (+4 SD [standard deviation]); the phallus measured 10 × 3.5 cm, and the testes measured 2.5 × 1.5 cm. The plasma luteinizing hormone (LH) level was 1.9 ng/mL (LER-960); the follicle-stimulating hormone (FSH) level was 1.2 ng/mL (LER-869); and the testosterone level was 416 ng/dL. After 100 μg of gonadotropin-releasing hormone (GnRH), the plasma LH level increased to 8.4 ng/mL, and the FSH level increased to 1.8 ng/mL, a pubertal response. When photographed, the patient had been treated with medroxyprogesterone acetate for 1.5 years. His height was 95.2 cm (+1 SD), the phallus was 6 × 3 cm, and the testes were 2.4 × 1.3 cm. Basal concentrations of LH (LER-960) were 0.9 ng/mL; the FSH (LER-869) level was 0.8 ng/mL; and the testosterone level was 7 ng/dL. After 100 μg of GnRH, LH concentrations rose to 2.3 ng/mL, whereas FSH concentrations did not change when he was on treatment with medroxyprogesterone acetate. For conversion to SI units, see Figs. 26.19 and 26.20. (B) A girl aged 3 years and 3 months with idiopathic true precocious puberty had recurrent vaginal bleeding since she was 9 months old. Her height age was 4 years and 5 months; bone age was 8 years and 10 months. (A, from Styne DM, Grumbach MM. Puberty in the male and female: its physiology and disorders. In: Yen SCC, Jaffe RB, eds. *Reproductive Endocrinology*. 2nd ed. Philadelphia: WB Saunders; 1986:313–384.)

TABLE 26.27 Distribution by Sex of Children With Idiopathic and Neurogenic Precocious Puberty

	IDIOPATHIC		NEUROGENIC	
Series	Male	Female	Male	Female
Thamdrup (1961)	4	34	7	11
Wilkins (1965)	13	67	10	5
Sigurjonsdottir and Hayles (1968)	8	54	16	16
University of California, San Francisco (1981)[a]	13	121	26	45

[a]Unpublished data.

Data from Sigurjonsdottir TJ, Hayles AB. Precocious puberty: a report of 96 cases. *Am J Dis Child.* 1968;115:309–321; Thamdrup E. *Precocious Sexual Development: A Clinical Study of 100 Patients.* Springfield, IL: Charles C Thomas; 1961; Wilkins L. *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence.* Springfield, IL: Charles C Thomas; 1965.

TABLE 26.28 Etiology of True Precocious Puberty[a]

Etiology	No. Females	No. Males
Idiopathic	121	13
CNS: hypothalamic tumors, including hamartomas	11	15
Arachnoid cyst	2	1
Hydrocephalus	6	1
Head trauma (child abuse)	1	
Perinatal asphyxia, cerebral palsy	3	1
Encephalitis or meningitis	3	1
Sex chromosome abnormalities (47,XXY; 48,XXXY)		2
Nonspecific seizure disorder or mental retardation	26	16
Degenerative CNS disease		3
Congenital virilizing adrenal hyperplasia with secondary true precocious puberty		3

[a]Data from University of California, San Francisco, Pediatric Endocrine Clinic.

CNS, Central nervous system.

From Kaplan SL, Grumbach MM. Pathogenesis of sexual precocity. In Grumbach MM, Sizonenko PC, et al, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:620–660. © 1990, The Williams & Wilkins Co., Baltimore.

TABLE 26.29 Classification of CNS Tumors Associated With Isosexual Precocity at UCSF

10% of all true precocious puberty patients: CNS tumors, hypothalamic ($n = 26$)
Males—IPP/organic precocious puberty = 13/15 (0.9:1)
Females—IPP/organic precocious puberty = 121/11 (12:1)
GnRH-dependent true precocious puberty
- Astrocytoma: 3 M, 5 F
- Hamartomas: 3 M, 3 F
- Neurofibromatosis: 5 M, 1 F
- Craniopharyngioma: 2 F

GnRH-independent incomplete sexual precocity
- hCG-secreting tumor,[a] 4 M

[a]CNS and extra-CNS neoplasms.

CNS, Central nervous system; *F,* female; *GnRH,* gonadotropin-releasing hormone; *hCG,* human chorionic gonadotropin; *IPP,* idiopathic true or central precocious puberty; *M,* male; *UCSF,* University of California, San Francisco.

heterozygous activating mutation in the *KISS1R* (p.Arg386Pro), which led to prolonged activation of intracellular signaling pathways in response to kisspeptin.

A 1-year-old boy with CPP had a heterozygous missense mutation in *KISS1* and a c.C369T transition in exon 3 of the *KISS1* gene, resulting in substitution of a proline by a serine at position 74 of kisspeptin-1(1-145) (p.Pro74Ser). His *KISS1* product was thought to be more resistant to degradation, leading to greater kisspeptin bioavailability and leading to a greater capacity to stimulate signal transduction compared to the wild-type kisspeptin; there was no indication of a change of affinity for the receptor. Because his mother and maternal grandmother, who had normal pubertal development, also carried the p.Pro74Ser mutation in the heterozygous state, the mutation was thought to have incomplete sex-dependent penetrance.[727] Two unrelated girls with CPP had homozygous mutations in c.417 C3G in exon 3, leading to the substitution of a histidine by an aspartic acid at position 90 of kisspeptin-1 (p.H90D). It was not clear what biologic changes this mutation brought about to cause the precocious puberty, but heterozygous H90D mutations were previously found in rare patients with IHH. The prevalence of *KISS* mutations causing CPP in children previously thought to have idiopathic CPP remains to be determined but appears to be low. No new mutations of *KISS1* causing CPP have been found at this time.[428]

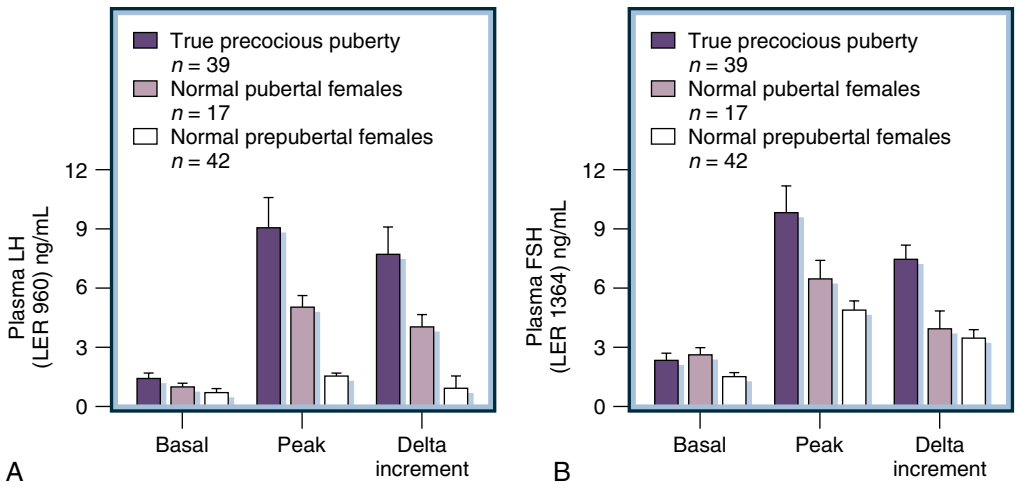
Loss-of-Function Mutations as Cause of Central Precocious Puberty

MKRN3. Ten different loss-of-function mutations of *MKRN3*, an imprinted gene located on the long arm of chromosome 15q11.2 in the Prader-Willi syndrome critical region, which encodes the makorin ring finger protein 3, was found in 22 patients, mostly girls, with CPP, all of whom inherited their mutation from their fathers as the maternal allele of this gene is silenced by methylation, a case of monoallelic expression.[428] No patient had findings of Prader-Willi syndrome. There was no family history reported in most cases in spite of the genetic inheritance of the trait; this is suggested to be an example of the underreporting of the familial nature of CPP in many cases as mothers more often are the parent to bring the child to the doctor. This mutation represents the most common cause of familial CPP. The age range of the onset of secondary sexual development in affected girls was 3 to 6.5 years with a mean of 5.9 years and median 6 years and in boys was 5.9 to 9.7 years with a mean of 8.5 years and median 8.8 years; this genetic dimorphism is reminiscent of the earlier age of normal puberty in girls compared to boys and demonstrates that girls are more affected by the mutation. A single girl diagnosed prepubertally had pubertal development by about 6 years.[739] These ages sometimes overlap with the new guidelines for precocious puberty in girls, which start at 7 years in white girls and 6 years in African-American girls with high BMI values, indicating that some girls starting puberty at these lower limits may have familial CPP. As the age of onset of puberty in affected patients was years after birth it is speculated that *MKRN3* does not play a role in suppression of pubertal development after the minipuberty of infancy but does play a role in the reactivation of the GnRH pulse generator closer to the normal age of pubertal onset. Affected patients are often but not always successfully treated with GnRH agonists. A single girl is described with a genetic alteration in the 4-nt proximal promoter region of *MKRN3*. Her mother had menarche at 10 years but did not have the same 4-nt mutation.[429] A boy and a girl with mutations in the *MKRN3* gene were asymptomatic carriers.[740] A normal-functioning *MKRN3* gene is considered to be a brake for the onset of puberty, and absence of this brake leads to precocious puberty.[425]

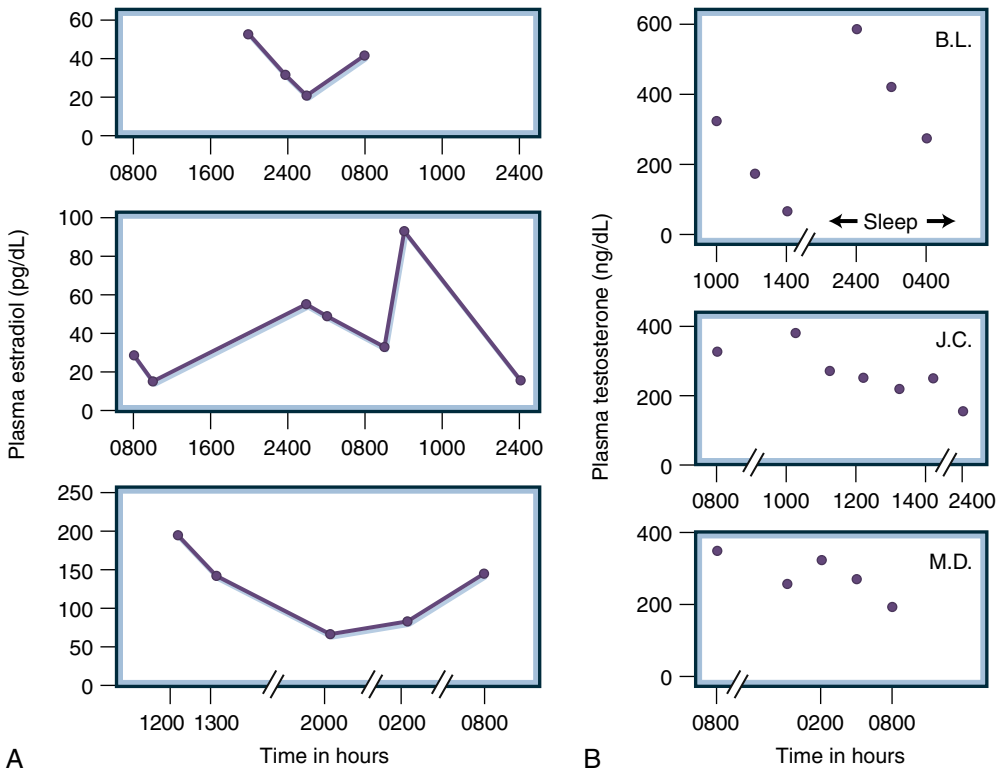
DLK1. Delta-like 1 homolog (DLK1) is another paternally imprinted gene recently reported to be associated with precocious puberty in girls.[741]

Central Nervous System Tumors Causing True Precocious Puberty

Sexual precocity may be the first manifestation of a hypothalamic tumor of any cell type when it arises in or impinges on the posterior hypothalamus. Neurologic symptoms such as headaches and



• **Fig. 26.52** (A) Mean basal plasma luteinizing hormone (LH) level (LER-960) and mean peak and increment after intravenous gonadotropin-releasing hormone (GnRH) (100 μg) in normal prepubertal and pubertal females and in females with idiopathic true precocious puberty. The mean peak and increments of plasma LH are higher in true precocious puberty than in normal puberty. (B) Basal follicle-stimulating hormone (FSH) level (LER-1364) and mean peak and increment after intravenous GnRH (100 μg) in normal prepubertal and pubertal females with true precocious puberty. The concentration of FSH and the response to GnRH were greater in females with true precocious puberty and normal puberty than in prepubertal females. (From Kaplan SL, Grumbach MM. Pathogenesis of sexual precocity. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:620–660.)



• **Fig. 26.53** (A) Serial determinations of plasma estradiol in three girls with idiopathic true precocious puberty. Notice the striking fluctuations in values. (B) Serial determinations of plasma testosterone in three boys with true precocious puberty (B.L. and J.C. have a hypothalamic hamartoma; M.D. has the idiopathic form). For conversion to SI units, see Figs. 26.19 and 26.20. (From Kaplan SL, Grumbach MM. Pathogenesis of sexual precocity. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:620–660.)

visual disturbances may develop, and children may have diabetes insipidus, hydrocephalus, or optic atrophy caused by an enlarging tumor in addition to precocious puberty. CPP resulting from CNS tumors (see Table 26.29) has about the same prevalence in boys as in girls in the population; however, boys have a lower overall prevalence of precocious puberty, and neurologic abnormalities account for two-thirds of those with CPP. It is more likely to find a CNS tumor in a boy with CPP than a girl. In the UCSF experience, a CNS tumor was present in at least half of this group.

A CNS neoplasm must be considered in the differential diagnosis of any patient with CPP. The location of CNS tumors causing CPP makes surgical removal difficult. A conservative approach calls for biopsy of the neoplasm and radiation or chemotherapy or both, depending on the pathologic findings. Optic and hypothalamic glioma (often associated with neurofibromatosis), astrocytoma, ependymoma, and, rarely, craniopharyngioma may cause CPP by impinging on the neural pathways that inhibit the GnRH pulse generator in childhood or as a consequence of cranial irradiation for treatment of a brain tumor. Pineal neoplasms may be associated with loss of upward gaze (Parinaud syndrome) due to brainstem compression.

The prevalence of CPP is increased after cranial irradiation for local tumors or leukemia even if radiotherapy targets the pituitary gland.[300] There is a 29% prevalence of CPP following cranial irradiation of the hypothalamic pituitary area for CNS tumors; there is a risk of decreased adult height, often due to delayed diagnosis and treatment.[742] Further, late onset gonadotropin deficiency is reported in about 33% of the irradiated subjects. The combination of GH deficiency and CPP can occur in children previously subjected to therapeutic irradiation of the CNS in association with a CNS neoplasm and in those with a variety of other CNS abnormalities, including developmental malformations and head trauma.[300] The lack of GH may not be apparent because of increased growth resulting from the elevated gonadal steroid levels; GH-deficient children with CPP grow more slowly than GH-sufficient children with CPP but faster than GH-deficient children without sexual precocity. GH-deficient children with CPP have IGF1 concentrations that are intermediate between the higher levels found in GH-sufficient children with sexual precocity and the lower levels found in prepubertal GH-deficient children.

GH deficiency and CPP can occur with CNS radiation doses of only 18 to 47 Gy, whereas gonadotropin deficiency, TSH deficiency, ACTH deficiency, and hypoprolactinemia usually occur with doses greater than 40 Gy.[743] The advance in the age of onset of puberty is positively correlated with the age of diagnosis of the condition for which the radiation therapy was given (i.e., earlier age at onset of puberty with earlier age at diagnosis) and is positively correlated with BMI at diagnosis. Newer radiation treatment regimens using lower doses of radiation for various malignancies may have less influence on advancing the age of menarche and may lead to less long-term morbidity. Treatment with a combination of GH and GnRH agonist is indicated in these patients and results in better growth and improved height prognosis when compared with the use of GnRH agonist alone. Because GH secretion is related to BMI, it is important to rule out a decrease in GH secretion caused by increased BMI in CPP before interpreting the decrease as evidence of GH deficiency.

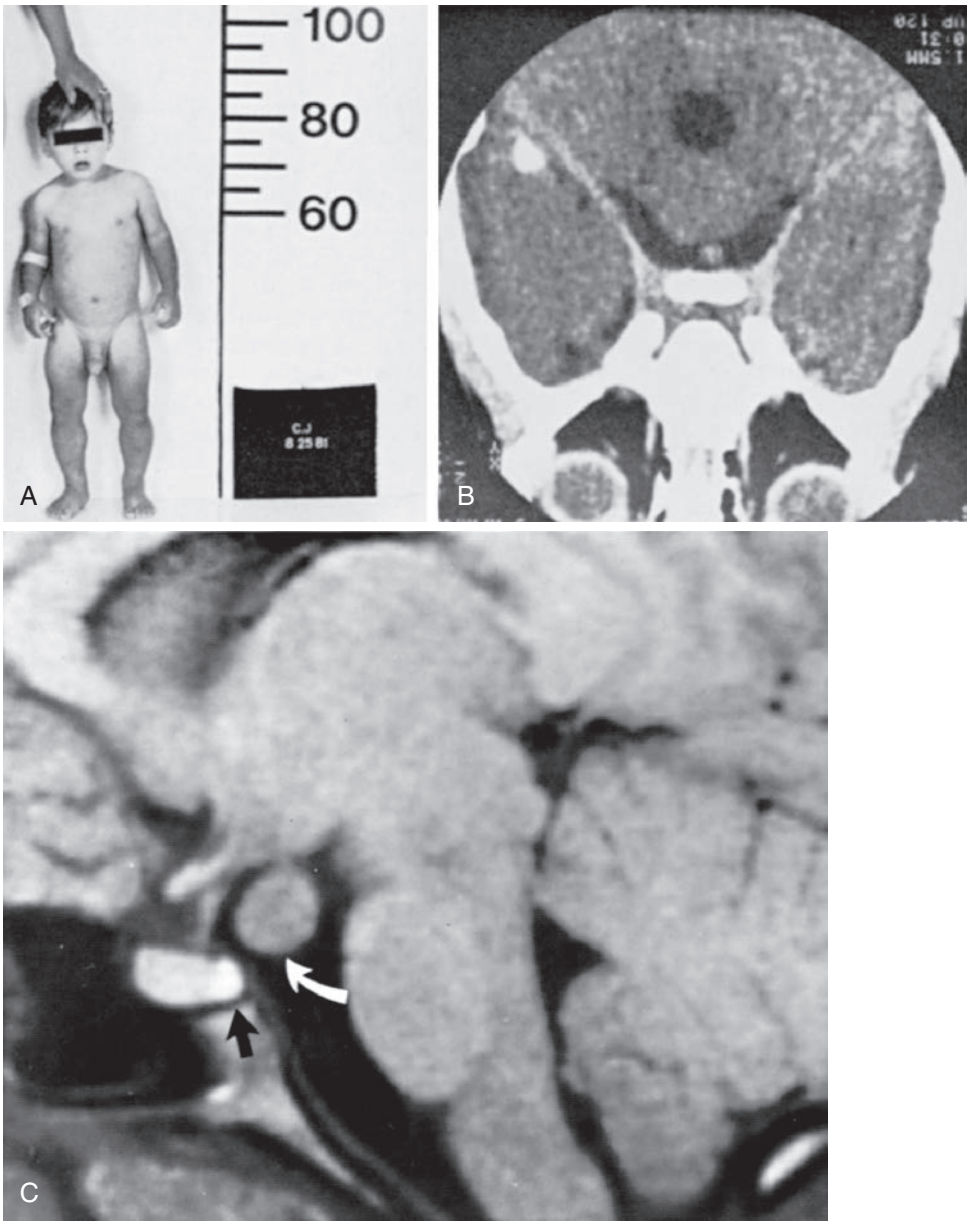
Hamartomas of the Tuber Cinereum. Hamartomas are congenital malformations composed of a heterotopic mass of nervous tissue containing GnRH neurosecretory neurons, fiber bundles, and glial cells; they are frequently associated with CPP (Fig. 26.54), which usually manifests before the patient is 3 years of age (Table 26.30).[423,718,744] Hypothalamic hamartomas may be sessile or pedunculated and are usually attached to the posterior hypothalamus between the tuber cinereum and the mammillary bodies. These masses project into the suprasellar cistern, and the pedunculated hamartoma has a distinct stalk. They present a characteristic appearance that does not change with time. Hamartomas of the tuber cinereum are not true neoplasms,[395,396,744] as long-term follow-up demonstrated lack of growth on monitoring by periodic CT or MRI.[396,745,746] Hamartomas appear on CT or MRI scans as an isodense, abnormal fullness of the interpeduncular, prepontine, and posterior suprasellar cisterns, occasionally with distortion of the anterior third ventricle. Their appearance and location relate to the clinical manifestation, with distortion of the third ventricle more closely associated with the occurrence of seizures. There is no enhancement with contrast material, and T2-weighted MRIs provide the best visualization of the lesion (Fig. 26.55).[396] However, the solid component of hamartomas may be missed when associated with a subarachnoid cyst if lower resolution MRI studies are invoked.[747]

The etiologic development of the hypothalamic hamartoma may be the converse of the lack of migration of GnRH neurons in Kallmann syndrome due to absent production of adhesion molecules coded by the *KAL1* gene. We may postulate that in hypothalamic hamartoma, the KAL1 protein, among other axon-guiding factors, may cause most of the total complement of about 1500 GnRH neurons to migrate to the hamartoma; alternatively, there may be a stimulus to progenitor cells that are capable of synthesizing GnRH to do so while located in the hamartoma.

Hamartomas associated with CPP contain ectopic GnRH neurosecretory cells that are similar to the GnRH-containing neurons in the medial basal hypothalamus. This developmental abnormality exerts its endocrine effects through elaboration and pulsatile release of GnRH. GnRH-containing fibers have been identified passing from the hamartoma toward the median eminence. We have suggested that the GnRH-containing neurosecretory neurons in the tumor are unrestrained by the intrinsic CNS mechanism that inhibits the normal GnRH pulse generator and act as an ectopic GnRH pulse generator,[326] either independently or in synchrony with the GnRH neurosecretory neurons in the medial basal hypothalamus, to produce intermittent secretory bursts of GnRH[326] (see Fig. 26.29). The GnRH is transported to the pituitary by way of the portal circulation and elicits pulsatile release of LH. If the hamartoma were to secrete GnRH in a continuous fashion, CPP would not occur, because the GnRH receptors would be desensitized. About 10% of hypothalamic hamartomas are not associated with CPP. The hamartomas associated with CPP tend to be more likely to contact the infundibulum or tuber cinereum and less likely to distort the third ventricle and were larger than those that are not, but the size of the hamartoma is not related to the age of onset of puberty.[748]

Hypothalamic hamartomas are postulated to elicit their effects via neurons that are able to produce GnRH within the tumor, through the ability to control neurons synaptically connected to GnRH neurons, or through neuronal networks that include GnRH neurons in the hypothalamic hamartoma itself or signaling-competent astrocytic and ependymoglial cells.[749] The precocious sexual development can be controlled by treatment with GnRH agonist therapy.[750]

All hypothalamic hamartomas in a recent series contained GnRH, GnRHR, and TGFα, and some contained *KISS1, GPR54*, and *GRM1A* (which encodes the isoform of the



• **Fig. 26.54** (A) A 17-month-old male infant with hamartoma of the tuber cinereum and true precocious puberty. When the patient was 8 months of age, secondary sexual development was observed, and the patient was misdiagnosed as having congenital virilizing adrenal hyperplasia. He was treated with glucocorticoids, which slowed his growth but did not affect his sexual development and bone age advancement. When he was first seen at 17 months, his height was 84.2 cm, his weight was 14.8 kg, pubic hair was stage 2, penis was 10.4 × 2.2 cm, testes were 1.5 × 2.8 cm, and the scrotum was thinned and rugated. The bone age was 4.25 years. After gonadotropin-releasing hormone (GnRH) administration, the luteinizing hormone (LH) level rose from 0.5 to 3.1 ng/dL (LER-960), the follicle-stimulating hormone (FSH) level rose from 0.5 to 1.2 ng/mL (LER-869), and the testosterone level rose from 409 to 450 ng/dL. The dehydroepiandrosterone sulfate (DHEAS) was 17 μg/dL (preadrenarchal value). The patient was treated with a potent, long-acting LH-releasing hormone (LHRH) agonist, deslorelin (D-Trp^{6}Pro^{9}NEt-GnRH), which resulted in arrest of his pubertal advancement and a striking decrease in the plasma concentration of testosterone, LH pulses, and the response to exogenous GnRH. (B) Computed tomography demonstrated a 1.5-cm mass posterior and rostral to the dorsum sella, which depressed the flow of the third ventricle. For conversion to SI units, see Figs. 26.19, 26.20, and 26.30. (C) Sagittal, T1-weighted magnetic resonance image shows a hypothalamic hamartoma *(white arrow)* in a 4-year-old boy with true precocious puberty. The posterior pituitary hot spot is designated by the *black arrow*. (B, from Styne DM, Grumbach MM. Puberty in the male and female: its physiology and disorders. In: Yen SCC, Jaffe RB, eds. *Reproductive Endocrinology*. 2nd ed. Philadelphia: WB Saunders; 1986:313–384.)

metabotropic glutamate receptor 1A, which rises in the female monkey with puberty) with no relationship as to whether CPP occurred or not.[748] Earlier two young girls with rapidly progressive CPP did not contain immunoreactive GnRH neurons, but the mass showed a network of astroglial cells containing TGFα.[751] This suggests that some hypothalamic hamartomas, by virtue of increased production of TGFα and of neuregulins synthesized by hypothalamic and astroglial cells through paracrine mechanisms, effect the release of bioactive factors, including prostaglandin E_2, that act on GnRH neurons to increase GnRH secretion. However, these hamartomas were much larger than the typical hypothalamic hamartoma associated with CPP; the mass bulged into the third ventricle in one girl, and in the other girl, the pituitary gland was hypertrophic and bulged through the diaphragma sellae. Activation of the GnRH pulse generator through a mass effect and compromise of restraint mechanisms may be the mechanism of CPP rather than TGFα signaling in these cases.

Before 1980, only 37 patients were in the literature with hamartomas of the tuber cinereum, but many more have been reported since the advent of CT and MRI brain scans (see Fig. 26.54 and Table 26.30). Of girls with CPP studied at the National Institutes of Health, 16% had a hypothalamic hamartoma, 40% had other CNS abnormalities, and 60% had idiopathic CPP. Among boys with CPP, 10% had idiopathic CPP, 50% had a hypothalamic hamartoma, and the rest had other CNS abnormalities, including hypothalamic neoplasms.[752]

Hypothalamic hamartomas that cause CPP can be associated with laughing (gelastic), petit mal, or generalized tonic-clonic seizures; developmental delay; behavioral disturbances; and dysmorphic syndromes beginning as early as the neonatal period. Males are more likely to have seizures with these lesions, and seizures can be caused by a hamartoma in the absence of precocious puberty. The occurrence of seizure is uncommon when the mass diameter of the hamartoma is less than 10 mm, whereas a larger mass is associated with a higher risk.

Although some have advocated neurosurgical removal of these hamartomas because of the occurrence of precocious puberty alone, we do not recommend neurosurgical extirpation in the absence of strong evidence of growth of the mass or of an associated complication such as intractable seizures or hydrocephalus.[423,753] Gelastic seizures are less amenable to antiepileptic therapy than other hamartoma-related seizures and may require surgical treatment; endoscopic technique is increasingly used,[754,755] and Gamma Knife procedures are used, especially for small lesions.[756] Refractory seizures have replaced precocious puberty as the main reason to perform surgery in cases of hamartoma of the tuber cinereum. The endocrine result of surgery on these lesions is rarely reported, but in one series of 29 subjects, hypernatremia, low T_4, low GH, and weight gain were encountered.[757] Prior unsuccessful surgery may increase the risk for endocrinopathy in these patients. The postoperative endocrine disturbances appear to be transient and mild or asymptomatic, but increased appetite and weight gain appeared in 25% of the subjects and may present an enduring problem. Although there are cases in which removal of a hypothalamic hamartoma led to reversal of the pubertal process, deaths have been reported after attempted operative removal. Medical therapy with GnRH agonist in lieu of surgery is the optimal treatment of precocious puberty associated with these hamartomas if seizures are absent or under control.

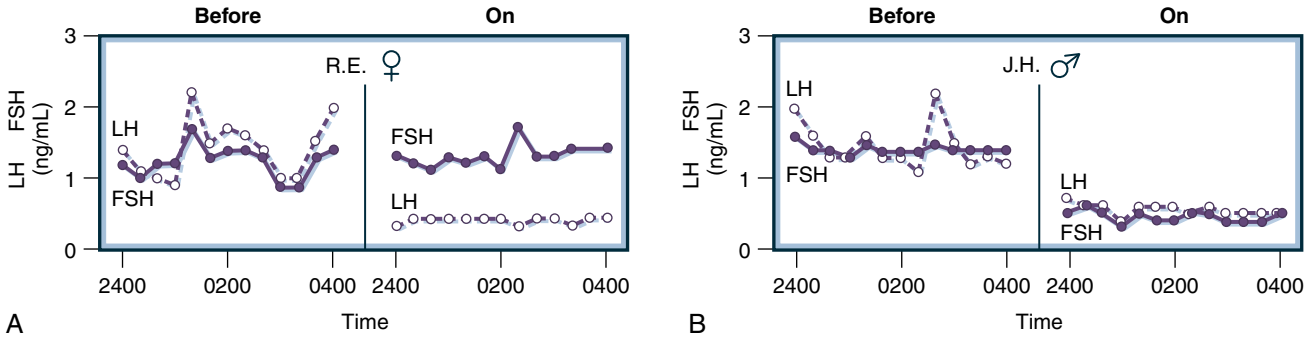
The Pallister-Hall syndrome is associated with hypothalamic hamartomas, precocious puberty (but rarely) with or without seizures, polydactyly, imperforate anus, bifid epiglottis, and hypopituitarism with seizures.[758]

TABLE 26.30 Clinical and Laboratory Characteristics of Children With True Precocious Puberty Caused by Hypothalamic Hamartoma

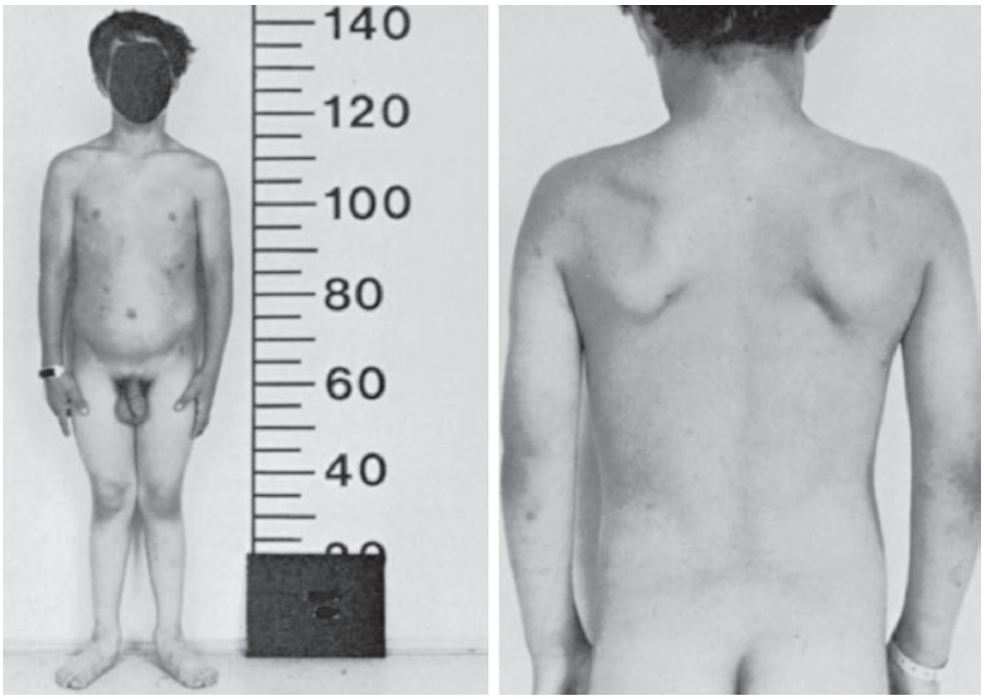
Characteristic	UCSF (6 M, 6 F)	Hochman et al.[a] (18 M, 9 F)
Age at Onset of Pubertal Signs		
Birth to 1 yr	4	6
1-2 yr	4	17
2-4 yr	3	6
7 yr	1	1
Neurologic Signs		
Seizures, including gelastic type	3/12	11/24
Headache and visual symptoms	1/12	5/24
None	7/12	7/24

[a]Data from Hochman HI, Judge DM, Reichlin S. Precocious puberty and hypothalamic hamartoma [literature review]. *Pediatrics.* 1981;67:236–244.

F, Female; *M,* male; *UCSF,* University of California, San Francisco.



• **Fig. 26.55** Pulsatile luteinizing hormone (LH) secretion before and during gonadotropin-releasing hormone (GnRH) agonist therapy in a boy (B) and a girl (A) with true precocious puberty resulting from a hypothalamic hamartoma. For conversion to SI units, see Fig. 26.19. *FSH,* follicle-stimulating hormone.



• **Fig. 26.56** A boy aged 8 years and 8 months with neurofibromatosis and precocious puberty resulting from a hypothalamic glioma. He had tonic-clonic seizures at 2.5 years and rapid growth starting at 4 years; an enlarged penis and testes and the presence of pubic hair were first noticed at 7.5 years. At this time, his height was 139.9 cm (+1.4 SD [standard deviation]); the phallus was 9 × 3 cm, the right testis measured 5.5 × 3.2 cm, and the left measured 5.4 × 2.9 cm. He had stage 3 pubic hair and 24 large café au lait spots. Computed tomography and pneumoencephalography revealed a 1.5 × 2.5–cm, hypothalamic mass, which was treated with irradiation. The plasma concentration of luteinizing hormone (LH) was 0.5 ng/mL (LER-960); the follicle-stimulating hormone (FSH) level was 0.4 ng/mL (LER-869); and the testosterone level was 221 ng/dL. After 100 μg of intravenous LH-releasing hormone (LHRH), the peak concentration of LH was 4.9 ng/mL, and that of FSH was 1.4 ng/mL, a pubertal response. For conversion to SI units, see Figs. 26.19 and 26.20. (From Styne DM, Grumbach MM. Puberty in the male and female: its physiology and disorders. In: Yen SCC, Jaffe RB, eds. *Reproductive Endocrinology*. 2nd ed. Philadelphia: WB Saunders; 1986:313–384.)

Neurofibromatosis Type 1. Neurofibromatosis type 1 (NF1 or von Recklinghausen disease) is associated with a propensity to develop the optic chiasmal tumors that are the most common cause (but not the only cause) of the development of CPP in a child with neurofibromatosis.[759] Most optic gliomas appear during the first decade of life, but only 20% to 30% become symptomatic; these tumors rarely progress in the years after diagnosis. The tumor suppressor *NF1* gene, located on the long arm of chromosome 17 (q11.2), which has a high mutation rate, encodes a 327-kDa protein, neurofibromin, that is widely expressed even though NF1 involves mainly tissues derived from the neural crest. A wide variety of mutations of the *NF1* gene are reported, especially deletions, nonsense mutations, and truncating mutations distributed over the coding region. In sporadic cases, the new mutation originates in the paternally derived *NF1* allele in most instances, suggesting a role for genomic imprinting. Concentrations of midkine (MK) and stem cell factor, but not epidermal growth factor, are substantially increased in the serum of NF1 patients compared with healthy control subjects and serve as a diagnostic feature.[760] Serum MK levels increase dramatically in patients older than 18 years of age, apparently as a feature of pubertal development. Because serum from patients with NF1 enhances proliferation of human neurofibroma-derived primary Schwann cells and endothelial cells, enhanced levels of circulating growth factors contribute to diffuse tumorigenesis in NF1.

NF1 is characterized by multiple pigmented areas and overgrowth of nerve sheaths and fibrous tissue elements (Fig. 26.56). Multiple café au lait spots are frequent and are smoother in outline (coast of California appearance) than those of the McCune-Albright syndrome (coast of Maine appearance). The diagnosis is made if two or more of the following are observed[761]:

1. Six or more café au lait macules, the greatest diameter being more than 5 mm in prepubertal subjects or more than 12.5 mm in postpubertal subjects
2. Two or more neurofibromas of any type or one plexiform neurofibroma
3. Freckling in the axillae or inguinal region
4. Optic glioma
5. Two or more iris Lisch nodules (ophthalmic hamartomas that occur more frequently after the onset of puberty)
6. A distinctive osseous lesion such as sphenoid dysplasia or pseudoarthrosis
7. A first-degree relative with NF1 according to the criteria described previously

Neurofibromas of the skin in neurofibromatosis may be subcutaneous, sessile or deep, plexiform masses in children; pedunculated lesions develop in later childhood. Internal neurofibromas cause most of the complications. Bone abnormalities such as cysts and pseudoarthrosis, hemihypertrophy, bowing, scoliosis, and skull and facial defects are common (20% of patients);

dumbbell-shaped tumors of spinal nerve roots may cause pain, sensory and motor dysfunction, and bone erosions; gliomas or neurofibromas of any part of the CNS, including the optic nerves and the hypothalamus, may calcify. Lisch nodules of the iris are frequent, particularly in adults. Sarcomatous degeneration occurs in 5% to 15% of patients. Other neoplasms include CNS astrocytomas that often involve the visual pathways, ependymomas, meningiomas, neurofibrosarcomas, rhabdomyosarcomas, and nonlymphocytic leukemias. Pheochromocytoma may develop in affected adults.

The clinical manifestations of neurofibromatosis include seizures, visual defects, and either delayed puberty or CPP, although many have growth hormone deficiency.[759] Although some manifestations of NF1 are quite common (e.g., café au lait spots, which were found in 99% of a series of 297 subjects), precocious puberty is rarer in some reports (found in 3.2% of that same series)[762] but found in about 33% in a report of 40 patients.[759] GH deficiency is possible at presentation, but after radiation therapy for associated optic glioma, GH, TSH, ACTH, and gonadotropin deficiency may develop. Developmental delay occurs more often in this population but usually is not severe; there is also an increased incidence of psychiatric disease. Most affected children have some manifestations of the disease by 1 year of age. Screening MRI scans are recommended for early detection of CNS tumors.

Other Central Nervous System Conditions

CPP may occur secondary to encephalitis, static cerebral encephalopathy, brain abscess, or sarcoid or tuberculous granulomas of the hypothalamus, with or without tuberculous meningitis. CPP can occur after severe head trauma (usually in girls), and it has been associated with cerebral atrophy or focal encephalomalacia occurring after cerebral edema (causing cerebral injury), complicating diabetic ketoacidosis. Children with nontumor hydrocephalus, even if shunted, experience earlier pubertal development, and those who have not been adequately treated may develop CPP. Delayed puberty is an alternative outcome in a minority of affected children. The growth pattern of children with severe hydrocephalus often includes poor prepubertal growth and an early pubertal growth spurt leading to decreased final height.

Arachnoid Cysts. Arachnoid cysts arising de novo, after infection or after surgery, can cause premature sexual development, possibly with associated GH deficiency.[326,763] Head nodding, abnormal gait, and abnormalities of visual fields are reported in 30% to 40% of cases. Erosion or enlargement of the sella turcica into a J shape may occur. Decompression and extirpation of a suprasellar arachnoid cyst can reverse the sexual precocity (see Fig. 26.30). Late endocrine effects such as growth hormone deficiency can be found after surgery to approach suprasellar arachnoid cyst.[764]

Other Central Nervous System Abnormalities. Other CNS abnormalities associated with CPP but without demonstrable lesions on imaging study include epilepsy, laughing seizures, developmental delay, cerebral palsy, and the posttraumatic state.[765] Septo-optic dysplasia (described earlier) may be associated not only with multiple pituitary hormone deficiencies and delayed puberty but also, rarely, with CPP.[546] There may be coexisting deficiencies of some pituitary hormones and excessive secretion of others, including prolactin.

Patients with myelomeningocele (myelodysplasia) have an increased prevalence of endocrine abnormalities, including hypothalamic hypothyroidism, hyperprolactinemia, and elevated gonadotropin concentrations, which in some patients is associated with CPP.

Miscellaneous Causes

Central Precocious Puberty in Children Adopted From Developing Countries. There was a 15-fold to 20-fold increased prevalence of CPP reported among children (with established birth dates) from developing countries who were adopted into Denmark, and other countries have reported similar tendencies. In Sweden, adopted Indian children had pubertal growth spurts similar to those of Swedish children, but adult height was decreased, with the loss of height in childhood and the early puberty apparently being responsible. Environmental influences, many related to the present obesity epidemic, are posited to exert effects in the reported decrease in age at menarche among children from the developing world. These children, who suffer poor prenatal nutrition and often are SGA, are adopted into families in developed countries, and in that environment of affluence they experience precocious puberty.[4,766]

It is not clear why adopted children may develop precocious puberty, and some even question this phenomenon itself; underreported age of adopted children fits an explanation of the tendency to ascribe precocious puberty to adoption and more critical analysis of the data is sought.[767] Foreign children who immigrated to Belgium with their biologic families from developing countries had greatly elevated concentrations of p,p′-DDE, a derivative of the organochlorine pesticide dichlorodiphenyltrichloroethane (DDT), raising the possibility of a role for endocrine disrupters in their CPP,[768] although this is contradicted in the experience of other counties. Older age at adoption and immigration are risk factors for precocious puberty. One study showed that children of immigrant groups who were born in their new country may have earlier puberty than children of the predominant ethnic group of that country; these influences might be genetic, or they might be related to cultural and dietary differences but could complicate the analysis.[31] However, a detailed analysis in Spain showed that the relative risk of CPP in domestic and internationally adopted children compared with those born in Spain was 27.82 (19.99 – 38.77), whereas the relative risk among immigrants was 1.55 (0.97 – 2.38); the authors suggest that psychologic factors may be the cause of this difference rather than immigration, nutrition, or pesticide exposure.[744] Remarkably, children adopted from South Korea do not appear to have the onset of puberty as early as those from other countries that were studied.[744]

The use of GnRH agonist in addition to GH treatment to increase adult height is reported in adopted children with CPP, but the combination is not supported by adequate evidence to recommend this combination in adopted children or any children with CPP.[470] The combination of adoption, living in a culture foreign to the background at birth, and precocious puberty makes these children vulnerable to psychic trauma, which must always be considered.

True Precocious Puberty After Virilizing Disorders. Correction of long-standing virilization may be followed by development of CPP with activation of the hypothalamic-pituitary gonadotropin-gonadal system. This secondary CPP occurs in congenital virilizing adrenal hyperplasia with advanced bone age when glucocorticoid replacement therapy starts after 4 to 8 years.[290,727] CPP has also been documented in children who received or were exposed to androgens or estrogens for long periods during early childhood for a variety of medical conditions.

TABLE 26.31 Objectives for the Management and Treatment of True Precocious Puberty

Detection and treatment of an expanding intracranial lesion
Arrest of premature sexual maturation until the normal age at onset of puberty
Regression of secondary sexual characteristics already present
Attainment of normal mature height; suppression of the rapid rate of skeletal maturation
Prevention of emotional disorders and handicaps and alleviation of parental anxiety; promotion of understanding by counseling, early sex education, and acceleration of social age
Reduction of risk of sexual abuse and early sexual debut
Prevention of pregnancy in girls
Preservation of future fertility
Diminishment of the increased risk of breast cancer associated with early menarche

From Grumbach MM. True or central precocious puberty. In: Krieger DT, Bardin CW, eds. *Current Therapy in Endocrinology and Metabolism, 1985-1986.* Toronto, Canada: BC Decker; 1989:4–8.

TABLE 26.32 Action of Gonadotropin-Releasing Hormone Agonists in True Precocious Puberty

A selective, highly specific pharmacologic clamp on the secretion of gonadotropin that produces a medical gonadectomy
- Chronic administration induces desensitization of the pituitary gonadotroph to the action of endogenous GnRH

As a consequence:
- Inhibition of pulsatile secretion of LH and FSH
- Inhibition of gonadotropin secretion results in a striking decrease in gonadal steroid output by testes or ovaries and reduction in gonadal size

FSH, Follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone.

Marfan Syndrome. Marfan syndrome may be associated with tall stature and early PHV and menarche compared with North American averages.[745]

Management of Central Precocious Puberty

Table 26.31 addresses the major psychosocial and clinical goals of therapy for CPP. Three principal agents were used in the medical treatment of idiopathic or neurologic CPP: medroxyprogesterone acetate, cyproterone acetate, and superactive GnRH agonists.

Medroxyprogesterone Acetate and Cyproterone Acetate. Medroxyprogesterone and cyproterone reversed or arrested the progression of secondary sexual characteristics but had no apparent effect or only a small effect on adult height, especially in affected girls.[622,746] Medroxyprogesterone acetate inhibits gonadotropin secretion by its action on the hypothalamic GnRH pulse generator/pituitary gonadotropin unit and has a direct suppressive effect on gonadal steroidogenesis through 3βHSD2. Medroxyprogesterone acetate has glucocorticoid action and can suppress ACTH and cortisol secretion, increase appetite and lead to excessive weight gain, and induce hypertension and a cushingoid facies and appearance.

Cyproterone acetate has been used outside the United States for the treatment of CPP with advantages and disadvantages similar to those of medroxyprogesterone acetate. Cyproterone acetate has antiandrogenic, antigonadotropic, and progestational properties. Cyproterone acetate suppresses the secretion of ACTH and the plasma concentration of cortisol. Fatigue and weakness are common side effects, probably as a consequence of secondary adrenal insufficiency. This agent lacks gluconeogenic activity and does not appear to produce cushingoid features.

The long-term effects of these agents on fertility are not known. For the treatment of CPP, medroxyprogesterone acetate and cyproterone acetate have now been replaced by the much more effective GnRH agonists; however, they may be used as backup agents for the occasional patients who develop untoward effects from GnRH agonist therapy.

Superactive Gonadotropin-Releasing Hormone Agonists. The GnRH agonists, synthetic analogues of the amino acid sequence of the natural GnRH decapeptide, are the treatment of choice for CPP of any cause (Tables 26.32 and 26.33). After initial stimulation, these pharmacologic agents suppress pulsatile LH and FSH release, gonadal steroid output, and gametogenesis, similar to the effects of continuous administration of natural GnRH, which suppresses gonadotropin secretion after an initial, brief stimulation of gonadotropin release.[336,747] The agonist binds to the GnRH receptor on gonadotrophs, and this is followed by desensitization of the gonadotroph to GnRH and downregulation and loss of receptors. Desensitization persists after receptor levels return to normal as a result of uncoupling of the receptors from the intracellular signaling effector pathway. This regimen functions as a selective, highly specific pharmacologic clamp on the secretion of gonadotropins without interfering directly with release of the other pituitary hormones. In essence, the regimen produces a reversible medical gonadectomy (see Table 26.32).

The superactive agonist analogues of GnRH have about 15 to 200 times the potency of the natural GnRH decapeptide, prolonged action, and low toxicity (see Table 26.33). Replacing the glycine-amide terminus of GnRH with alkyl amines (i.e., ethylamine [NEt]), as in (Pro^9-NEt)GnRH; substituting certain D-amino acids at position 6, as in (D-Trp^6)GnRH; or making bulky hydrophobic alterations at position 6, as in (D-Nal[2]^6)GnRH increases the potency and duration of action. These changes make the molecule more resistant to enzymatic degradation, increase the binding affinity of the analogue for the receptor on the pituitary gonadotroph, increase hydrophobicity, and, with some analogues, increase binding to plasma proteins.

The suppressive effects of the GnRH agonists on gonadotropin secretion make them useful in the treatment of CPP, and they are also used in endometriosis, prostatic carcinoma, and in delaying puberty during consideration of definitive treatment in transgender use. Every-4-week and every-12-week formulations of leuprorelin (leuprolide acetate) have been FDA approved for treatment of CPP.[769] Long-term studies have established the efficacy and safety of the every-4-week injection, but only short-term studies are available for the every-12-week version. Triptorelin has been recently approved for the treatment of central precocious puberty in children; it can be given as an injection every 6 months.[770] The bioavailability of agonists given intranasally is much reduced, as reflected in the need to use a high dose at more frequent intervals, and they are rarely used at present. A subcutaneous implant of histrelin was approved for 12-month treatment of CPP, and recent studies show efficacy in gonadotropin suppression for 2 years after implant.[771] Increased predicted adult height is demonstrated with this treatment.[772] The effectiveness of GnRH agonists in the

TABLE 26.33 Gonadotropin-Releasing Hormone Agonists: Pharmacologic Treatment of True Precocious Puberty[a]

Hormone	Potency	Formula	Dosage Form	Dose	References
Structure of Natural GnRH	GnRH (potency 1): <Glu-His-Pro-Ser-Trp-Gly-Leu-Arg-Pro-Gly-NH2> 1 2 3 4 5 6 7 8 9 10				
Substitutions in GnRH Agonist Analogues					
Deslorelin: D-Trp[6], -Net	150	(D-Trp[6]Pro[9]NEt)GnRH	SQ Depot-IM	4-8 mg/kg/day	Grumbach and Kaplan, Kaplan and Grumbach, Styne et al,[750] Pescovitz et al,[752] Boepple et al,[773] Comite et al (1986),[421] Comite et al (1981), Oerter
Nafarelin: D-Nal(2)[6]	150	[D-Nal(2)[6]Pro[9]NEt]GnRH	SQ Intranasal	4 mg/kg/day 800-1600 mg/day	Grumbach and Kaplan, Kaplan and Grumbach, Comite et al (1981)
Leuprolide: D-Leu[6], -Net	20	(D-Leu[6]Pro[9]NEt)GnRH	SQ Depot-IM	20-50 mg/kg/day 140-300 mg/kg/mo	Boepple and Crowley, Eshet et al, Kaplan and Grumbach (1991)[b]
Buserelin: D-Ser(tBu)[6], -Net	20	[D-Ser(tBu)[6]Pro[9]NEt]GnRH	SQ Intranasal	20-40 mg/kg/day 1200-1800 mg/day	Drop et al, Bourguignon et al, Holland et al, Rappaport et al, Suwa et al, Luder et al, Donaldson et al, Rime et al
Triptorelin: D-Trp[6]	35	(D-Trp[6])GnRH	SQ Depot-IM	20-40 mg/kg/day 60 mg/kg/mo	Kauli et al, Roger et al
Histrelin: D-His(Bzt)[6], -Net	150	[D-His(Bzt)[6]NEt]GnRH	SQ implant	12-mo pellet	

[a]Superscripted numbers indicate substitution at that position of the specified amino acid; -NEt indicates replacement of the terminal glycine-amide with ethylamine.

[b]Unpublished data.

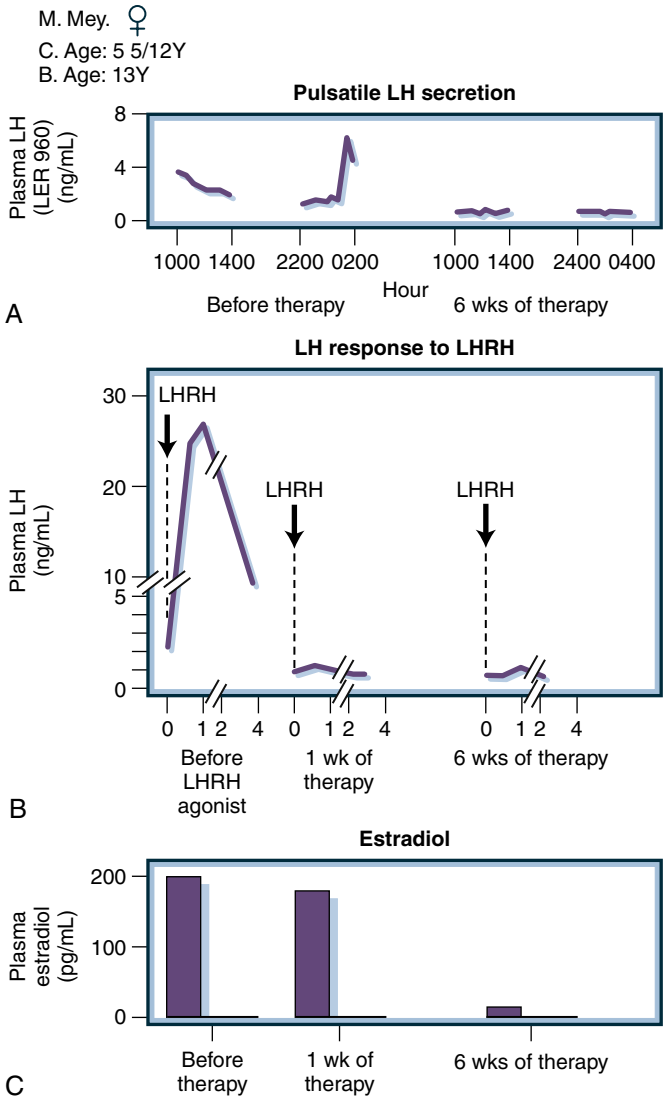
GnRH, Gonadotropin-releasing hormone; *IM,* intramuscular; *SQ,* subcutaneous.

Modified from Grumbach MM, Kaplan SL. Recent advances in the diagnosis and management of sexual precocity. Acta Paediatr Jpn. 1988;30:S155–S175.
Data from Boepple PA, Crowley WFJ. Gonadotrophin-releasing hormone analogues as therapeutic probes in human growth and development: evidence from children with central precocious puberty. Acta Paediatr Scand Suppl. 1991;372–338; Bourguignon JP, Van Vliet G, Vandeweghe M, et al. Treatment of central precocious puberty with an intranasal analogue of GnRH (buserelin). Eur J Pediatr. 1987;146:555–560; Comite F, Cutler GBJ, Rivier J, et al. Short-term treatment of idiopathic precocious puberty with a long-acting analogue of luteinizing hormone-releasing hormone: a preliminary report. N Engl J Med. 1981;305:1546–1550; Donaldson MD, Stanhope R, Lee TJ, et al. Gonadotrophin responses to GnRH in precocious puberty treated with GnRH analogue. Clin Endocrinol (Oxf). 1984;21:499–503; Drop SL, Odink RJ, Rouwe C, et al. The effect of treatment with an LH-RH agonist (buserelin) on gonadal activity growth and bone maturation in children with central precocious puberty. Eur J Pediatr. 1987;146:272–278; Eshet R, Duz Z, Silbergeld A, et al. Erythrocytes from patients with low concentrations of IGF1 have an increase in receptor sites of IGF1. Acta Endocrinol. 1991;125:354–358; Grumbach MM, Kaplan SL. Recent advances in the diagnosis and management of sexual precocity. Acta Paediatr Jpn. 1988;30:S155–S175; Holland FJ, Fishman L, Costigan DC, et al. Pharmacokinetic characteristics of the gonadotropin-releasing hormone analog D-Ser (tBU) 6Pro9NEt luteinizing hormone-releasing hormone (buserelin) after subcutaneous and intranasal administration in children with central precocious puberty. J Clin Endocrinol Metab. 1986;63:1065–1070; Kaplan SL, Grumbach MM. True precocious puberty: treatment with GnRH-agonists. In: Delemarre-Van de Waal H, Plant TM, van Rees GP, et al, eds. Control of the Onset of Puberty. Amsterdam, The Netherlands: Elsevier; 1989:357–373; Kauli R, Pertzelan A, Ben-Zeev A, et al. Treatment of precocious puberty with LHRH analogue in combination with cyproterone acetate: further experience. Clin Edocrinol (Oxf). 1984;20:377–387; Luder AS, Holland FJ, Costigan DC, et al. Intranasal and subcutaneous treatment of central precocious puberty in both sexes with a long-acting analog of luteinizing hormone-releasing hormone. J Clin Endocrinol Metab. 1984;58:966–972; Oerter KE, Manasco P, Barnes KM, et al. Adult height in precocious puberty after long-term treatment with deslorelin. J Clin Endocrinol Metab. 1991;73:11235–11240; Rappaport R, Fontoura M, Brauner R. Treatment of central precocious puberty with an LHRH agonist (buserelin): effect on growth and bone maturation after three years of treatment. Horm Res. 1987;28:149–154; Rime JL, Zumsteg U, Blumberg A, et al. Long-term treatment of central precocious puberty with an intranasal HLRH analogue: control of pituitary function by urinary gonadotrophins. Eur J Pediatr. 1988;147:263–269; Roger M, Chaussain JL, Berlier P, et al. Long term treatment of male and female precocious puberty by periodic administration of a long-acting preparation of D-Trp6-luteinizing hormone-releasing hormone microcapsules. J Clin Endocrinol Metab. 1986;62:670–677; Suwa S, Hibi I, Kato K, et al. LH-RH agonistic analog (buserelin) treatment of precocious puberty: collaborative study in Japan. Acta Paediatr Jpn. 1988;30:S176–S184.

treatment of CPP varies with the potency of the analogue, dose, route of administration, and compliance.

Treatment of CPP with a potent GnRH agonist results in 1 to 3 days of increased FSH and LH release and a rise in circulating gonadal steroid levels, followed after 7 to 14 days of treatment by suppression of pulsatile secretion of LH and FSH and of the pubertal LH response to the administration of native GnRH (Figs. 26.57 and 26.58). The isoforms of gonadotropins tend toward a more basic charge. A plasma estradiol concentration of less than 18 pmol/L (5 pg/mL) in girls or a plasma testosterone level of less than 0.7 nmol/L (20 ng/dL) in pediatric assays using HPLC-MS/MS in boys indicates adequate gonadal suppression; this occurs within about 2 to 4 weeks in girls and 6 weeks in boys. GnRH agonist therapy does not affect the secretion of adrenal androgens or sexual hair growth.[750,773]

Changes in secondary sexual characteristics within the first 6 months of therapy (Fig. 26.59) include reduction in breast size and decrease in pubic hair, cessation of menses if present before treatment, and decreased size of the uterus and ovaries as assessed by pelvic sonography in girls. Some girls have recurrent episodes of hot flushes and moodiness. In boys, pubic hair thins, the testes decrease in size, acne and seborrhea regress, penile erections and masturbation become much less frequent, the high energy level and aggressive behavior diminish, and self-esteem improves.



• **Fig. 26.57** Effect of administration of the gonadotropin-releasing hormone (GnRH) agonist deslorelin (4 μg/kg/day subcutaneously) on pulsatile secretion of luteinizing hormone (LH) (A), LH response to GnRH (B), and plasma concentration of estradiol (C) in a 5-year, 1-month-old girl with idiopathic true precocious puberty. This patient, who had a bone age of 13 years when treatment was begun, has been administered deslorelin for 7 years. During this period, the estimated predicted final height increased by 15 cm. Surprisingly, the bone age advanced by only about 6 months on serial examinations for several years. *LHRH,* LH-releasing hormone. (Modified from Grumbach MM, Kaplan SL. Recent advances in the diagnosis and management of sexual precocity. *Acta Paediatr Jpn.* 1988;30:S155–S175.)

Height velocity decreases by about 60% during the first year of therapy, with greater decreases found in those with the most advanced bone age and taller relative height.[774] Skeletal maturation slows dramatically during the first 3 years, to a rate that often is less than the progression in chronologic age. From the second year on, height velocity for bone age is usually appropriate (Fig. 26.60). Bone age is suggested to represent a surrogate for growth plate senescence due to prior exposure to estrogens. Those with the longest courses before treatment commences, the most advanced physical findings, and the most rapid bone age advancement before therapy have the lowest growth velocities on treatment.

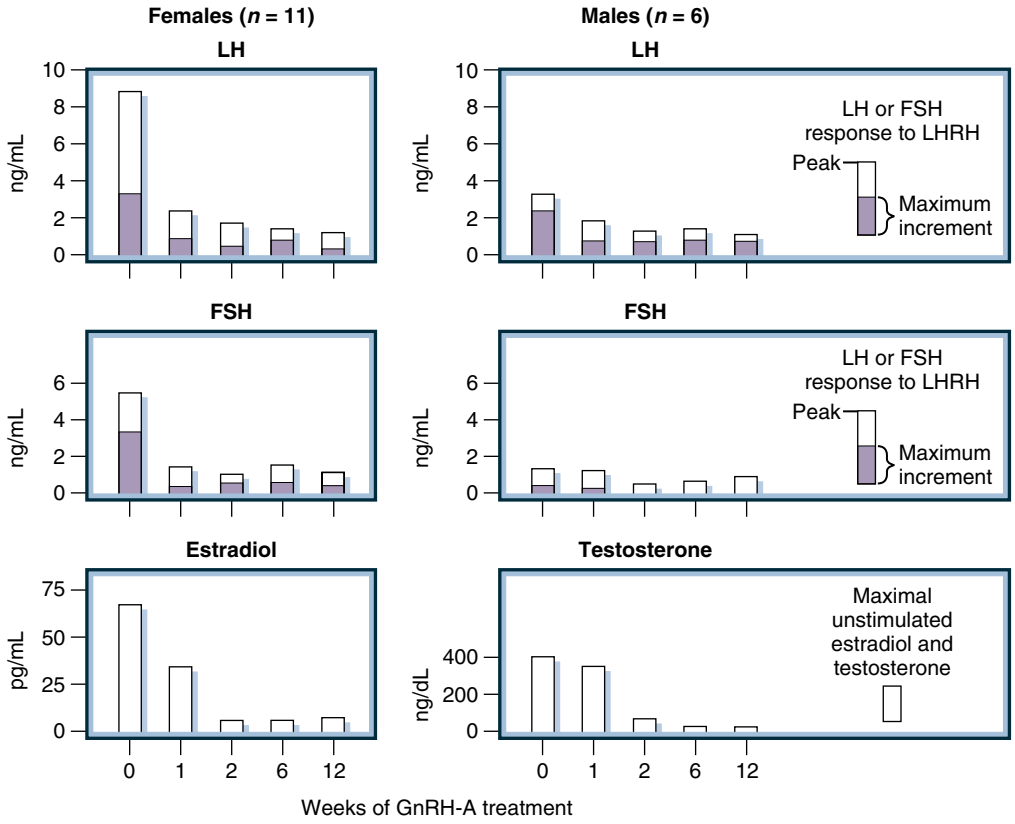
The rate of growth during treatment with GnRH agonists is inversely related to the bone age at the start of therapy[308]; best results occur when treatment is begun soon after the onset of precocity and when the bone age is advanced by only a few years.[775] There is a striking benefit for those children treated before 5 years of age (girls' height, 164.3 ± 7.7 cm) compared with those treated after age 5 years (157.6 ± 6.6 cm) or with untreated patients (152.7 ± 8.6 cm).[722] Adult height in children treated with GnRH agonists is improved, especially when therapy starts before 6 years of age rather than after 8 years of age[470,724,776] (Table 26.34). The literature on treatment in children with onset of puberty after 6 years of age varies but generally shows no proven efficacy in increasing adult height.[777] An adult height within the target height occurs with therapy in about 90% of girls and boys.[778] We recommend treatment of all affected children with onset of puberty before 6 years of age to ensure an optimal prognosis for adult height. The optimal age at which to discontinue therapy is undetermined because the posttreatment growth spurt is important in determining adult height.[470]

The addition of hGH treatment to the GnRH regimen is a consideration when growth velocity is reduced sufficiently over a 6-month period to compromise predicted final height but must be considered experimental.[779] This is in contrast to the concern raised regarding therapy with GnRH agonist and GH in normal variant short stature, which is not proved by evidence-based study.[470]

In a preliminary study, the GnRH antagonist, cetrorelix, appeared to bring about more rapid suppression of gonadotropin secretion and to eliminate the flare-up of gonadotropin secretion after administration of GnRH agonist; it is used in assisted reproductive treatment.[777] There are no reports at present concerning the use of cetrorelix as a sole treatment of precocious puberty, but in one unusual case, a girl with purported gonadotropin-independent precocious puberty responded to this treatment, bringing up a possible direct effect of the agent on ovarian function.[780] This agent suppresses ovarian but not adrenal function and was proposed to be useful as a test to differentiate the origin of severe hyperandrogenism in adolescence.

Girls with CPP have a tendency toward obesity that is unrelated to treatment with GnRH agonist.[470] Serum leptin values in patients with precocious puberty remain in line with the average for children of similar BMI and pubertal development.[781] There is conflicting data on weight gain while on treatment with GnRH agonists. A longitudinal study 101 girls, 23% obese at start of therapy, suggested that, with adequate gonadotropin suppression, BMI for age may improve after at least 2 years of therapy.[782] A study of 117 girls showed the increased BMI during treatment often reverted to normal after cessation of treatment.[783] However, a longitudinal study of 333 girls, some to adult height, demonstrated increasing BMI SDs with treatment continuing to adulthood.[784] These conflicting findings make it difficult to predict the effect of GnRH agonist on BMI in individuals.

The IGF1 concentration in CPP correlates best with the stage of puberty and the plasma concentration of testosterone or estradiol. Treatment with GnRH agonist reduces the level of IGF1 to the normal range for bone age but not for chronologic age.[316] Gonadal steroids increase plasma IGF1 concentrations in CPP, as in normal puberty. Secretion of GH is increased in CPP to a level comparable to that observed in normal puberty. Treatment with GnRH agonist usually results in decreased secretion of GH, most



• **Fig. 26.58** Deslorelin treatment (4 μg/kg/day subcutaneously) of girls and boys with true precocious puberty. Effects were seen during the first 12 weeks of treatment on the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) response to a challenge with gonadotropin-releasing hormone (GnRH) (mean peak response and maximum increment) and on the maximal unstimulated concentration of plasma estradiol in the girls and of plasma testosterone in the boys. Notice the relatively rapid change from pubertal values to prepubertal values. For conversion to SI units, see Figs. 26.19 and 26.20. *LHRH,* LH-releasing hormone. (From Styne DM, Harris DA, Egli CA, et al. Treatment of true precocious puberty with a potent luteinizing hormone releasing factor agonist: effect on growth, sexual maturation, pelvic sonography, and the hypothalamic pituitary gonadal axis. *J Clin Endocrinol Metab.* 1985;61:142–181. Copyright by The Endocrine Society.)

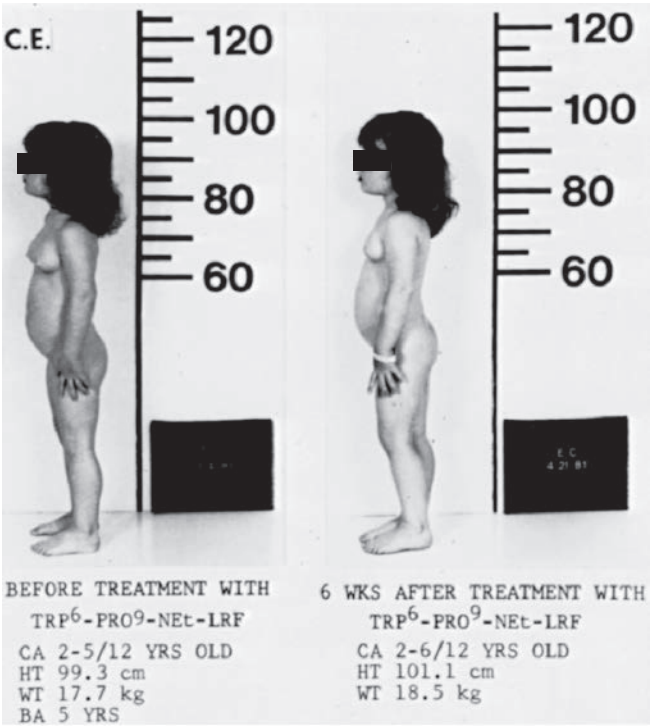
strikingly during sleep, and in a decreased GH response to provocative stimuli.

The depot formulations of GnRH agonists provide continuous exposure to the drug with a single intramuscular injection every 4 to 26 weeks (as described earlier) and minimize the problem of compliance. However, irregular or inadequate treatment or poor compliance results in persistent or intermittent increase in the concentration of plasma gonadal steroids, leading to decreased growth but advancing bone age.

Regular assessment is essential, initially at intervals of 3 to 6 months with the use of the injectable formulations, and should include periodic determinations of plasma testosterone levels in boys and estradiol levels in girls by HPLC-MS/MS; change in basal concentrations of LH and FSH as measured by third-generation assays with pediatric standards or in the LH and FSH response to exogenous GnRH or GnRH agonists; growth, bone age, and secondary sexual characteristics; and, in girls, serial evaluations of ovarian morphologic appearance and uterine size by pelvic sonography. A decrease in the size of the ovaries and uterus on pelvic sonography occurs with successful GnRH agonist treatment.[750] Because native GnRH is not available, the rise in serum LH and FSH is evaluated 60 or 240 minutes after administration of the GnRH agonist.[257–259] The LH and FSH responses to GnRH agonist are suppressed with effective therapy, but because standards differ among laboratories, actual cutoff values may differ in clinical practice, depending on location. A 1-year Silastic implant of the histrelin GnRH agonist, although it involves a surgical procedure, eliminates the need to titrate the dose and repeat testing for maximal effect; a second treatment year is reported to be effective.[771] There may be low pubertal values of LH even with successful suppression of advancement of puberty.[785]

Urinary excretion of LH correlates with the stage of pubertal development in normal subjects and is elevated in CPP; these methods are proposed as a noninvasive manner to monitor or diagnose CPP.[786]

When treatment is discontinued, even after 8 years, gonadal suppression is reversed within a few weeks to months, as manifested by a rise in the concentration of plasma gonadal steroids, progression of sexual maturation, and return of menses. Menarche occurred at an average of 1.2 to 1.5 years after discontinuation of therapy (range, 0–60 months). Ovulation had occurred in 50% of girls by 1 year after menarche and in 90% of those studied 2 or more years after menarche, and pregnancies are reported in GnRHA-treated girls.[735,787] Mean ovarian volume was found to remain greater than in normal subjects, and the LH response to GnRH was less than the normal response.

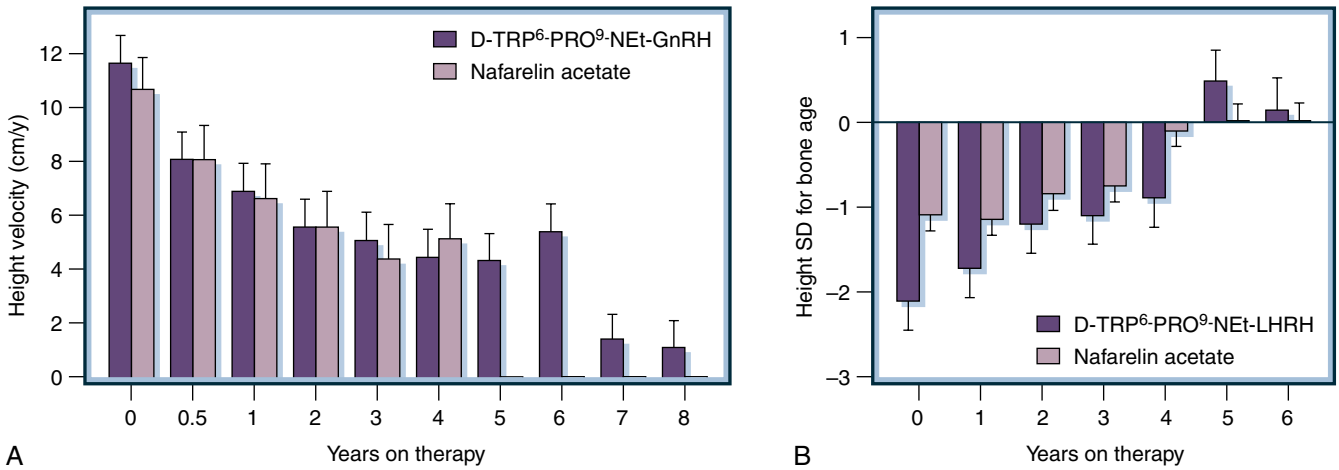


• **Fig. 26.59** A girl aged 2 years and 5 months with true precocious puberty after 6 weeks of deslorelin therapy (4 μg/day subcutaneously). Notice the regression in the size of the breasts; however, the rapid rate of growth had not decreased. At the end of 1 year of therapy, the growth rate was suppressed to 4 cm/year, and bone age advanced only 1 year. *BA,* bone age; *CA,* chronologic age; *HT,* height; *WT,* weight. (From Styne DM, Grumbach MM. Puberty in the male and female: its physiology and disorders. In: Yen SCC, Jaffe RB, eds. *Reproductive Endocrinology*. 2nd ed. Philadelphia: WB Saunders; 1986:313–384.)

The reversible nature of the therapy has also been confirmed in boys with CPP, because gonadotropins in the basal or GnRH-stimulated state return to normal pubertal values by 1 year after cessation of therapy.[788] Testicular size may take longer to reach normal values.

A report of 46 women studied 12.5 years after cessation of treatment with GnRH agonist revealed an adult height 1.6 cm, or 0.3 SD, below target height, with no evidence of reproductive impairment or apparent PCOS or hirsutism.[789] However, an increased prevalence of PCOS was reported in young women (mean age, 18.1 years) with a history of CPP, with onset at a mean age of 7.65 years.[790] Of the patients, 32% had PCOS according to the Rotterdam definition, and 30% had PCOS according to the Androgen Excess Society; the most common presentation was clinical or biochemical hyperandrogenism, or both, and polycystic ovarian morphologic appearance. The study authors did not find any other predictive factors for the development of PCOS when the diagnosis of CPP was made. These girls would not have qualified for the diagnosis of CPP based on the newest criteria, so it is of concern that these girls with early rather than precocious puberty had such a high prevalence of PCOS. GnRH agonist treatment may actually decrease the incidence of PCOS compared to the incidence in untreated patients.[735] Thus the prevalence of PCOS after Lupron for CPP is unclear.[777]

An international consensus conference convened by the Lawson Wilkins Pediatric Endocrine Society and the European Society for Pediatric Endocrinology reviewed the world literature on the use of GnRH to determine, in an evidence-based approach, the appropriate use of the agent in precocious puberty. However, few controlled prospective studies have been performed with GnRH analogues in children, so many of the conclusions relied



• **Fig. 26.60** Effect of gonadotropin-releasing hormone (GnRH) agonist therapy in true precocious puberty on growth. (A) Changes in mean height velocity (cm/year ± 1 SE [standard error]) after the initiation of GnRH agonist therapy with D-Trp^{6}Pro^{9}Net (deslorelin) *(darker bars)* or with nafarelin *(lighter bars)*. A sharp decrease in height velocity occurred within 1 year. (B) Mean (±1 SE) height for bone age before and during GnRH agonist treatment. The discrepancy between height and the more advanced bone age decreases (reverts to normal) with chronic GnRH agonist treatment. *SD,* standard deviation. (From Kaplan SL, Grumbach MM. True precocious puberty: treatment with GnRH agonists. In: Delemarre-Van de Waal H, Plant TM, van Rees GP, et al, eds. *Control of the Onset of Puberty*. 3rd ed. Amsterdam, The Netherlands: Elsevier; 1989:357–373.)

TABLE 26.34 Effect of GnRH Agonist Treatment on Adult or Near-Adult Height and Height Gain

Chronologic Age at Start of Therapy	No. Patients	MEAN CURRENT HEIGHT (CM) Female	Male	Mean Height Gain (cm)[a]
Untreated[b]				
Total	116	152.7 ± 8.6	155.6 ± 7.7	
<5 yr	41	150.2 ± 7.6	153.3 ± 7.1	
>5 yr	75	153.4 ± 8.4	161.3 ± 6.0	
GnRH-Treated[c]				
UCSF	26	160.5 ± 6.6	166.3 ± 12.2	
<5 yr	11	164.3 ± 7.7	172.1	10.0 F; 11.1 M
>5 yr	15	157.6 ± 6.6	163.3 ± 13.0	4.0 F; 6.0 M
Reference Untreated Study				
Oerter et al	40	157.8 ± 5.9	168.8 ± 8.3	5.2 F; 6.7 M
Kauli et al	8	151.2 ± 5.9		5.8 F
Boepple and Crowley	26	154.4		4.1 F

[a]Final predicted height minus initial predicted height (Bayley-Pinneau method).

[b]Final height.

[c]Final or near-final height.

F, Female; *GnRH,* gonadotropin-releasing hormone; *M,* male; *UCSF,* University of California, San Francisco.

From Paul D, Conte FA, Grumbach MM, et al. Long-term effect of gonadotropin-releasing hormone agonist therapy on final and near-final height in 26 children with true precocious puberty treated at a median age of less than 5 years. *J Clin Endocrinol Metab.* 1995;80:546–551.

Data from Boepple PA, Crowley WFJ. Gonadotrophin-releasing hormone analogues as therapeutic probes in human growth and development: evidence from children with central precocious puberty. *Acta Paediatr Scand Suppl.* 1991;372–338; Kauli R, Pertzelan A, Ben-Zeev A, et al. Treatment of precocious puberty with LHRH analogue in combination with cyproterone acetate: further experience. *Clin Endocrinol (Oxf).* 1984;20:377–387; Oerter KE, Manasco PK, Barnes KM, et al. Effects of luteinizing hormone-releasing hormone agonists on final height in luteinizing hormone-releasing hormone-dependent precocious puberty. *Acta Paediatr Suppl.* 1993;388:62–68; discussion 69.

in part on collective expert opinion. The major conclusions were as follows[451,470]:

- GnRH analogues exert benefit in increasing adult height in children with early-onset CPP (<6 years in girls) and are not routinely recommended after that age.
- The psychosocial effects of CPP and their alteration by GnRH analogues require additional study.
- The use of GnRH analogues does not appear to cause weight gain or long-term diminution of BMD.
- The use of GnRH analogues for conditions other than CPP, such as to increase adult height in children with idiopathic short stature or SGA, or with GH treatment in children, is not recommended.

Affected girls do not appear to manifest increased stress with this condition, although mothers do in preliminary studies.[791] Although psychosocial factors and parental anxiety that adversely affect the well-being of the child need to be assessed in the decision to initiate GnRH agonist treatment, this therapy cannot be routinely recommended for such concerns (Table 26.35).

Adverse Effects. Rare reactions to GnRH agonists include local and systemic allergic reactions, including asthmatic episodes when the agent is given intranasally. Rare cases of anaphylaxis occur in response to gonadotropin-releasing hormone agonist.[792] The prevalence of a sterile abscess at the site of intramuscular injection of long-acting repository preparations, including

TABLE 26.35 Indications for Therapy With Gonadotropin-Releasing Hormone Agonists in True or Central Precocious Puberty

In children with clinical and unequivocal endocrine features of idiopathic true precocious puberty:

- Rapid advancement over a period of 6-12 mo of secondary sex characteristics, height, height velocity, and bone age (increased >2.5 SD for chronologic age) in affected boys and girls
- A plasma testosterone concentration sustained >2.5 nmol/L (>75 ng/dL) in boys <8 yr of age determined by sensitive, specific immunoassay
- A plasma estradiol concentration, recurrently ≥36 pmol/L (≥10 pg/mL) determined by a sensitive, specific assay capable of quantifying low concentrations of estradiol
- Onset of menarche (and recurrent menses) in girls <9 yr of age
- Psychosocial factors and parental anxiety, including evidence that the child's psychosocial well-being is adversely affected

In children with neurogenic or organic true precocious puberty, especially those with associated GH deficiency, the course is almost invariably progressive and LHRH treatment should not be delayed.

GH, Growth hormone; *LHRH,* luteinizing hormone–releasing hormone; *SD,* standard deviation.

leuprorelin and triptorelin, is clearly increased (5–10%); these reactions are unpredictable and intermittent, and in most instances are related to the polylactic and polyglycolic polymer and not to the GnRH agonist itself. Switching to daily subcutaneous injections of nondepot preparations or to intranasal preparations is rarely associated with a recurrence. A small increase in serum prolactin above normal limits was described in girls after treatment with GnRH agonist, but galactorrhea was not observed. Volumetric BMD and peak bone mass are normal during and after discontinuation of GnRH therapy. Calcium and vitamin D intake must be ensured during treatment to achieve optimal skeletal health. However, high fruit and vegetable intake (defined as >3 servings per day, lower than the US government recommended intake of 3 vegetables and 2 fruit in younger children) is desirable in all children and may serve in early puberty as a factor to increase bone density, possibly because of decreasing calcium excretion in the urine.[793]

Four patients were reported to have developed slipped capital femoral epiphyses during or just after treatment of CPP with GnRH agonist.[794] Slipped capital epiphyses occur mostly during the earliest phase of puberty, when growth is beginning to increase, and not after fusion of the triradiate cartilage, so these cases may have a different etiologic course than that found in average pubertal children.

There is a concern over maintenance of bone density when sex steroids are reduced with the use of GnRH agonists. There is evidence, however, that adolescents previously treated with gonadotropin-releasing hormone agonist for precocious puberty have normal bone density.[795]

Recently, reports of young women complaining of various maladies who were previously treated for precocious puberty with GNRH agonist appeared. They speak of pain in the jaw, fibromyalgia, degenerative disc symptoms, thinning bones and depression, or other psychologic symptoms. These have generally appeared on lay websites but the FDA is gathering such reports for ongoing surveillance to determine whether these are long-term effects related to Lupron well after treatment.[796]

Other Treatment for Precocious Puberty. The GnRH agonists are useful in conjunction with GH in the management of organic or neurogenic CPP with associated GH deficiency (usually as a result of irradiation of the brain). However, the use of GnRh agonists has been advocated even in the absence of precocious puberty to allow a longer period of GH treatment before epiphyseal fusion. A few, usually short-term studies have evaluated the combination, with variable results. This regimen is experimental, and its cost effectiveness needs to be considered (see earlier discussion).[470]

Aromatase inhibitors (e.g., letrozole) decrease or eliminate the effect of estrogen on bone age advancement. They have been useful to increase height prognosis in familial sexual precocity in boys (see upcoming discussion).[787] A study of Greek girls with decreased predicted adult height due to early puberty demonstrated that the combination of an aromatase inhibitor with a gonadotropin-releasing hormone agonist will increase predicted adult height.[797] Long-term controlled studies are necessary to establish the safety and efficacy and, especially, the effects on adult bone density, which remains a worrisome subject in the use of aromatase inhibitors in children[475] (Table 26.36).

Psychosocial Aspects. Psychologic management is a critical aspect of the care of children with CPP. With the advanced physical maturation for chronologic age, these children tend to seek friends closer to their size, strength, and physical development. Difficulties may arise because they lack the social skills of older children. Sex education of the child and the family is essential and must be given in a skillful, sensitive, and explicit manner; the risks of sexual abuse (in both sexes) and of pregnancy need to be discussed.[798]

The parents need to be informed about the management of menses. The onset of sexual activity may be earlier than average but usually remains within the normal range. Menarche before or at 11 years increases the risk of higher delinquency rates and stronger negative peer influences.[799]

It is imperative to provide support in handling the increased height, the advanced sexual maturation, and the effects of gonadal steroids on behavior, activity, and emotional stability. The unrealistic demands and expectations that arise from the discrepancy between the child's physique and his or her chronologic, mental, and psychosexual age require wise counseling, as do the reactions to ridicule by peers and the concern about being different from age-mates. Some of these problems have been mitigated by school acceleration, in which the child is advanced by one or two grades, if this is consistent with the mental and emotional development. These comments are applicable to children with all forms of sexual precocity; the effectiveness of GnRH agonists has reduced but not eliminated many of these issues in CPP.

The psychological evaluation of the effects of precocious puberty children must take into account their family situation and other social stressors lest these factors create effects on the child and are incorrectly attributed to precocious puberty.[800] A series of 10 girls suggested an increased level of stress before treatment for central precocious puberty with a diminution of the stress with appropriate treatment.[801] However, one detailed study of 15 girls found remarkable similarity between girls with precocious puberty and controls, except that those with precocious puberty had some tendency to increased emotional lability.[802]

TABLE 26.36 Potential Use of Aromatase Inhibitors or Estrogen Receptor Antagonists in Disorders of Growth and Sexual Maturation

Growth Disorders or Variants of Normal Growth

Isolated growth hormone deficiency
- To restrain epiphyseal maturation

Genetic short stature/constitutional delay in growth
- To restrain epiphyseal maturation

Sexual Precocity

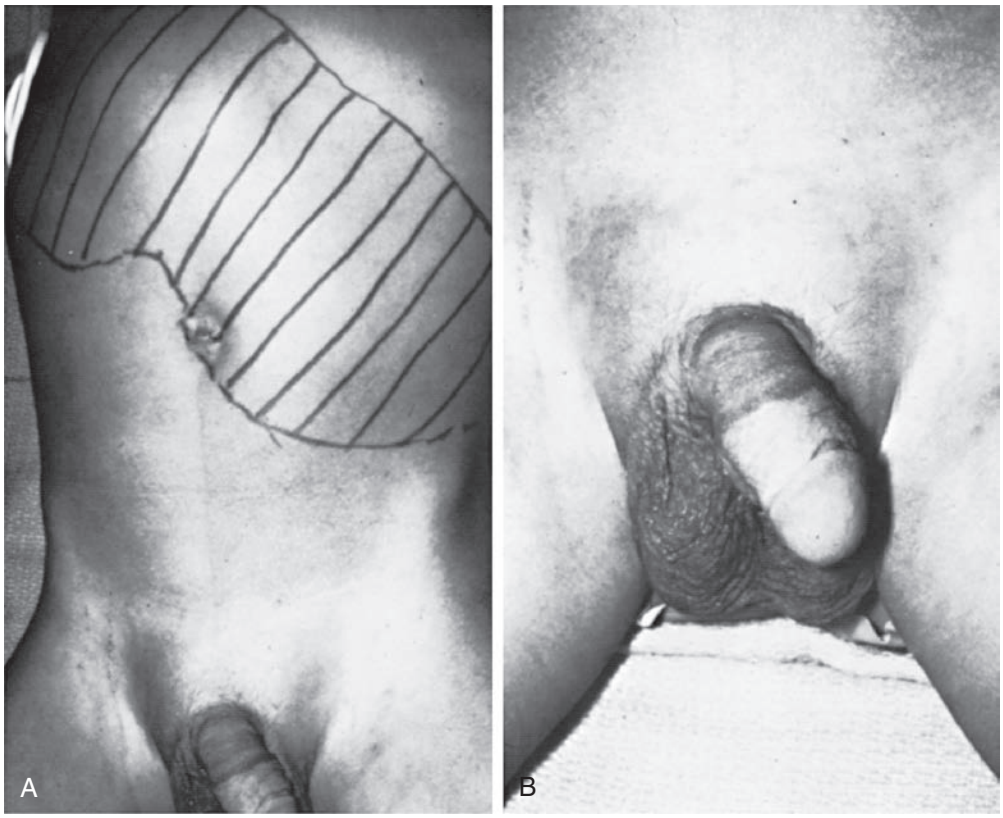
Congenital virilizing adrenal hyperplasia in male and female
- To reduce dose of glucocorticoid
- To inhibit conversion of C19 steroids to estrogens (or estrogen action)
- With or without use of 17,20-lyase inhibitor of antiandrogen

Testotoxicosis
- To inhibit conversion of C19 steroids to estrogens

McCune-Albright syndrome
- To inhibit conversion of C19 steroids to estrogens (or estrogen action)

Adolescent Gynecomastia
- To inhibit estrogen synthesis (or estrogen action)

From Grumbach MM. Estrogen, bone, growth, and sex: a sea change in conventional wisdom. *J Pediatr Endocrinol Metab.* 2000;13:S1439–S1455.



• **Fig. 26.61** A boy aged 1 year and 5 months with a human chorionic gonadotropin (hCG)–secreting hepatoblastoma. Notice the outline of the large liver (A) and the penile enlargement (B). The testes were 2 × 1 cm, and pubic hair was stage 2. The plasma hCG level was 50 mIU/mL; the plasma testosterone level was 168 ng/dL; and the plasma α-fetoprotein level was 160,000 ng/mL. Metastatic lesions in both lungs were seen on the chest radiograph. To convert testosterone values to SI units, see Fig. 26.19. To convert hCG values to international units per liter, multiply by 1.0. To convert α-fetoprotein values to micrograms per liter, multiply by 1.0. (From Kaplan SL, Grumbach MM. Pathogenesis of sexual precocity. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:620–660.)

Peripheral Precocious Puberty or Incomplete Isosexual Precocity: Gonadotropin-Releasing Hormone–Independent Sexual Precocity[995]

In incomplete or peripheral forms of precocious puberty (IPP), such as GISP, the secretion of testosterone in boys and estrogen in girls is independent of the hypothalamic GnRH pulse generator (see Table 26.26). There is no pubertal LH response to GnRH or GnRHA, and there is no pubertal pattern of pulsatile LH secretion. Patients do not respond to chronic GnRH agonist therapy with suppression of gonadal steroid output. ISP is a consequence of gonadal or adrenal steroid secretion that is independent of GnRH, iatrogenic exposure to gonadal steroids, or, in boys, rare hCG-secreting or LH-secreting tumors. It can be considered a paraneoplastic syndrome caused by tumor production of hormones or a pathologic alteration of gonadotropin secretion.[803] Measures of tumor markers such as hCG or AFP may be helpful in the evaluation of peripheral precocious puberty but not of CPP.

Boys

Chorionic Gonadotropin–Secreting Tumors. Several types of germ cell tumors secrete hCG, which may cross-react in some polyclonal LH assays (although they are rarely used currently) and will lead to a positive pregnancy screen. Boys with hCG-secreting neoplasms have slightly enlarged testes (although not to a size consistent with the size of the phallus and other male secondary sex characteristics, because the seminiferous tubules are not affected), and it may be difficult to differentiate these patients from boys in the early stages of CPP on the basis of physical examination alone. However, plasma hCG levels are elevated without an increase in the concentration of FSH or LH measured in specific assays and will lead to a positive pregnancy screen.

Boys with hepatoma or hepatoblastoma present with hepatomegaly or firm, irregular liver nodules, anemia, and precocious puberty[804]; these are among the most serious of hCG-secreting tumors (Fig. 26.61). hCG is localized to multinucleated tumor giant cells; α-fetoprotein was found in the embryonal-type tumor cells of the hepatoblastoma in one case. The mean age at onset is 2 years, 8 months, but average survival time was only 10.7 months after diagnosis in previous series, but newer chemotherapy can lead to longer survival.[805]

Infantile choriocarcinoma is also associated with elevated hCG and is thought to originate in the placenta; infants may be diagnosed at 1 month of age, with a survival time of only 3 months.[806] About 20% of mediastinal germ cell tumors occur in boys with 47,XXY or mosaic Klinefelter syndrome, a prevalence 30 to 50 times more common than in unaffected boys. Plasma α-fetoprotein is a useful additional marker for yolk sac (endodermal sinus) or mixed germ cell tumors; the cells in the tumor that secrete α-fetoprotein appear to differ from those that secrete hCG. Rarely, the germ cells contain enough aromatase activity to convert

circulating C19 precursors (of adrenal origin after adrenarche) to estradiol, which in some instances is sufficient to induce breast development.[807]

Some teratomas, chorioepitheliomas, and mixed germ cell tumors in the hypothalamic region (or in the mediastinum, lungs, gonads, or retroperitoneum); certain pineal tumors (usually a germ cell tumor or mixed germ cell tumor); and, less commonly, a chorioepithelioma or its variants cause sexual precocity in boys by secreting hCG rather than by activating the pituitary gonadotropin-gonadal axis via the hypothalamic GnRH pulse generator.

Intracranial germ cell tumors account for 3% to 11% of malignant CNS tumors in children and adolescents, with a predominance in the Far East. Germ cell tumors of the hypothalamus or pineal region constitute fewer than 1% of primary CNS tumors in Western countries but account for 4.5% of such tumors in Japan. The prevalence of intracranial germ cell tumors is 2.6 times greater in males than in females, but germ cell tumors in the suprasellar-hypothalamic region do not exhibit a sex predominance and are generally associated with pituitary hormone deficiencies, including diabetes insipidus and delayed puberty.[481] Germ cell tumors do not cause gonadotropin-induced ISP in females because of the paucity of effects of hCG in prepubertal females. However, CPP may occur through disinhibition of the hypothalamic GnRH pulse generator by local mass effects.[808] Calcification of the pineal is found in 8% to 11% of children age 8 to 11 years, and by itself is not indicative of a germ cell tumor.

Germ cell tumors that secrete hCG are rarely located in the thalamus and basal ganglia. In "true" pure CNS germ cell tumors (germinomas), hCG cannot be readily detected in the circulation but may be detected in the cerebrospinal fluid.[481] In mixed germ cell tumors, hCG is commonly found in the blood as well as in cerebrospinal fluid. Extremely elevated levels of hCG in a CNS tumor suggest a primary intracranial choriocarcinoma or germ cell tumor with a high risk of tumor hemorrhage during biopsy, and surgical removal or debulking rather than diagnostic biopsy may be the initial operative approach.[809]

Mixed germ cell tumors and especially pure germinomas are radiosensitive, and if the bone age is less than 11 years, sexual precocity may regress, only to progress later into normal puberty. Long-term survival is reported in 88% of patients with CNS germ cell tumors after appropriate therapy. However, testicular germ cell tumors are occasionally found years after successful therapy for CNS germ cell tumors, so long-term surveillance is always indicated.[606]

Pineal cysts are a rare cause of CPP.[810] All pituitary adenomas, including gonadotropin-secreting pituitary adenomas, are exceedingly rare in children. An LH-secreting pituitary adenoma (basal serum LH of 900 IU/L with no rise after GnRH) and a prolactin-secreting pituitary adenoma (215 μg/L) caused sexual precocity in two boys with serum testosterone levels of 7 nmol/L (200 ng/dL).[811] Prepubertal values returned after removal of these chromophobe adenomas with suprasellar extension.

Precocious Androgen Secretion Caused by the Adrenal Gland

Virilizing Congenital Adrenal Hyperplasia. Virilizing CAH caused by a defect in 21-hydroxylation (CYP21 deficiency) leads to elevated androgen concentrations and masculinization and is a common cause of GISP in boys.[812] Approximately 75% of patients with CYP21 deficiency have salt loss resulting from impaired aldosterone secretion as well as low serum sodium and high serum potassium concentrations. Increased plasma concentrations of 17-hydroxyprogesterone, increased levels of urinary 17-ketosteroids and pregnanetriol, and advanced bone age and rapid growth are characteristic. Recent discovery of alternative steroidogenic pathways toward the production of virilizing androgens in this condition, the backdoor pathway, holds promise for new methods of evaluation of optimal treatment regimens.[813,814]

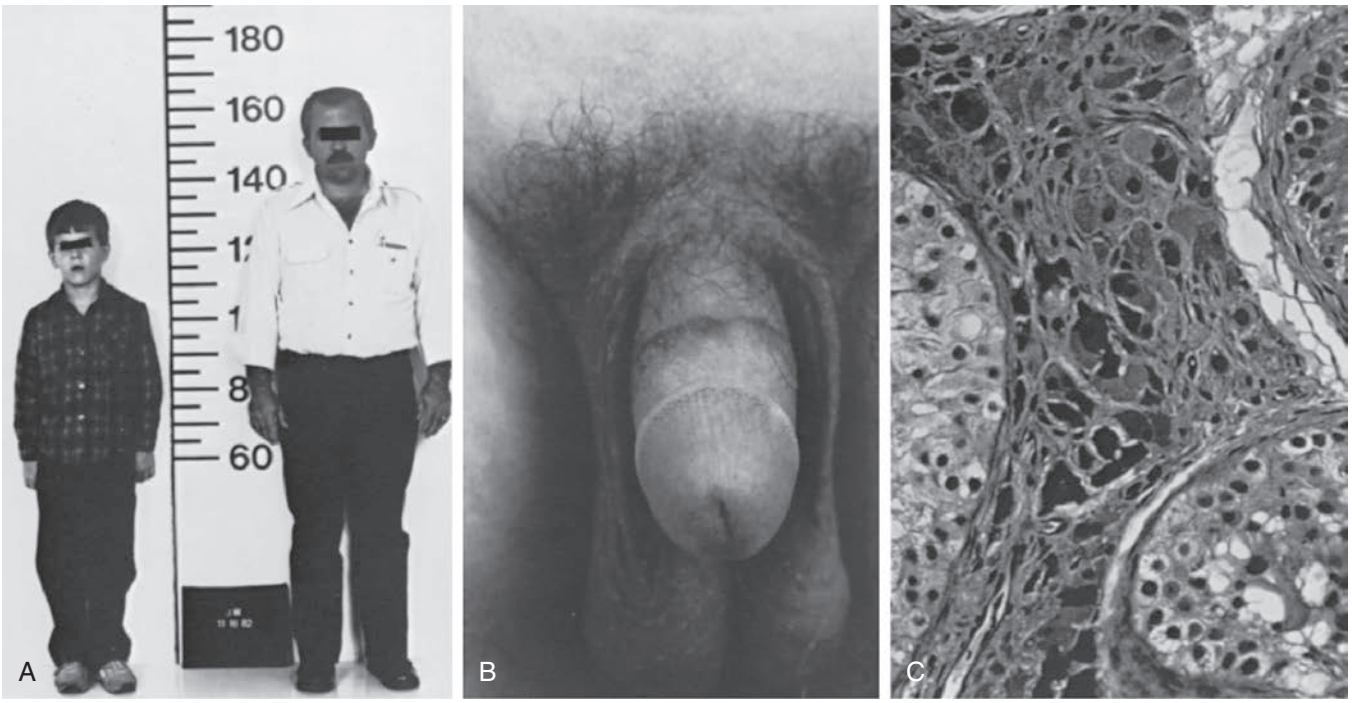
Treatment with glucocorticoids suppresses the abnormal androgen secretion and arrests virilization; treatment with mineralocorticoids, when necessary, corrects the electrolyte imbalance. Virilizing CAH accompanied by hypertension occurs in 11β-hydroxylase deficiency (CYP11B1 deficiency); the progressive virilization ceases and the blood pressure falls to normal with glucocorticoid therapy. All forms of CAH are inherited as autosomal recessive traits. Untreated virilizing CAH causes anovulatory amenorrhea in females and oligospermia in males; these conditions are reversible with treatment. Delayed treatment of virilizing CAH may reveal GnRH-dependent CPP (secondary CPP) as a consequence of the advanced somatic and hypothalamic maturation resulting from long exposure to adrenal androgen. Further difficulty is presented in the treatment of CAH during the pubertal years, when androgen secretion normally increases, and increased clearance of glucocorticoids at puberty in girls may alter dosing requirements.

Adrenal rests, or heterotopic adrenal tissue in the testes, are found in over 90% of boys with CAH. They may enlarge (sometimes to massive size) and may mimic bilateral or unilateral interstitial cell tumors. The size of these testicular adrenal rest tissues (TART) was thought to be related to poor control of CAH during childhood and adolescence, but recent evidence shows no relationship between biochemical control or plasma ACTH concentration and growth patterns, raising the likelihood that other control mechanisms perhaps originating in fetal life determine the size of these tumors.[815] MRI sonography, including Doppler flow studies of the testes, is useful to define the extent and nature of the testicular masses. These tumors can significantly decrease fertility, but surgical management, including enucleation of the tumor(s), has been useful to prevent further damage to the testes and improve the potential for fertility in some but not all studies.

As with most chronic diseases originating in childhood, transition of care to adult providers during late teenage years is essential. Comprehensive care clinics are suggested to offer the best method to provide care to children and adults with CAH.[816]

Virilizing Adrenal Tumor. Virilizing adrenal carcinomas or adenomas secrete large amounts of DHEA and DHEAS and, on occasion, testosterone. Glucocorticoids do not suppress the increased secretion of adrenal androgens to the normal range for age in carcinoma, as they do in CAH. Cushing syndrome resulting from adrenal carcinoma may cause ISP and growth failure in boys. Rarely, an adrenal adenoma produces both testosterone and aldosterone, leading to sexual precocity and hypertension with hypokalemia.

NR0B1 (DAX1) Gene Mutations. Two cases of *NR0B1* frameshift mutations demonstrated adrenal failure and GISP that were suppressible by glucocorticoid therapy but not by GnRH agonist. The exceedingly high ACTH levels, possibly acting through the human melanocortin 1 receptor present in human Leydig cells, may have been the underlying cause of the increased steroidogenesis and testosterone secretion that were reversed by glucocorticoid treatment. Because *NR0B1* inhibits transactivation of SF1, a regulator of steroidogenic genes, loss of *NR0B1* inhibition of SF1 transcriptional activity also may have had a role.[817,818]



• **Fig. 26.62** (A) A boy aged 5.5 years and his 28-year-old father with familial testotoxicosis. The boy exhibited signs of sexual precocity by 3 years of age. His height was 130.6 cm (+4.8 SD [standard deviation]), and his bone age was 12.5 years. The plasma testosterone level was 267 ng/dL; the dihydrotestosterone level was 46 ng/dL; and the dehydroepiandrosterone sulfate (DHEAS) level was 23 μg/dL. The plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were low, and neither rose after treatment. Pulsatile LH secretion was not demonstrable. Treatment with deslorelin, a gonadotropin-releasing hormone (GnRH) agonist, had no effect. The father had begun sexual maturation by 3 years of age and had reached a final height of 162.6 cm in his early teens. The plasma testosterone level was 294 ng/dL; the LH level was 0.5 ng/mL (LER-960); and the FSH level was 0.5 ng/mL (LER-869). The father had an adult-type LH and FSH response to GnRH; the LH level increased to 7.5 ng/mL, and the FSH level increased to 2 ng/mL. At least 28 male family members over nine generations are affected. To convert dihydrotestosterone values to nanomoles per liter, multiply by 0.03467. For other conversions to SI units, see Figs. 26.19 and 26.20. (B) External genitalia of the 5.5-year-old boy. The penis measured 12 × 2.8 cm, the right testis was 4 × 2 cm, and the left testis was 3.5 × 2.5 cm. (C) Testis of the boy showed Leydig cell maturation without Reinke crystalloids and spermatogenesis (Mallory trichrome stain).

Leydig Cell Tumor. Testicular tumors are rare in childhood, representing 1% to 2% of all pediatric solid tumors, and Leydig cell tumors make up only 1.5% of those.[819] Androgen-producing Leydig cell tumors rarely are malignant and are slow growing but must be treated or epiphyseal fusion will limit height and precocious puberty will affect social development. They derive from primordial mesenchyme, are classified as interstitial cell tumors, and occur most frequently around the age of 4 to 5 years. Unilateral enlargement (often nodular) of the testis usually occurs in boys with this neoplasm (although 5–10% of cases are bilateral); in contrast, both testes are usually of normal size (small) for chronologic age in boys with CAH or a virilizing adrenal tumor.[820] Although LH receptor–activating mutations were detected in several boys with sporadic Leydig cell adenomas,[779] no known mutations can be found in others. There is a trend toward testis sparing surgery or radiographic observation rather than orchidectomy in benign testicular lesions.[821]

Pituitary Gonadotropin–Independent Familial Premature Leydig Cell and Germ Cell Maturation: Familial or Sporadic Testotoxicosis.[331,779,822,823,997]. Pituitary gonadotropin–independent familial premature Leydig cell and germ cell maturation, or testotoxicosis,[822,824,825] causes boys to develop secondary sexual characteristics with penile enlargement, which may be present at birth, and bilateral enlargement of testes to the early or midpubertal range, although the testes often are smaller than expected in relation to penile growth and pubertal maturation (Fig. 26.62). Premature maturation of Leydig and Sertoli cells and spermatogenesis occur. Leydig cell hyperplasia may occur; the Leydig cells in affected boys produce dimeric inhibin B as well as testosterone, and Leydig cells and spermatogonia stain positively for the α and β_B segments of inhibin. The rate of linear growth is rapid, skeletal maturation is advanced, and muscular development is prominent. The presence of prepubertal basal and GnRH-stimulated gonadotropin concentrations, lack of a pubertal pattern of LH pulsatility (as measured by immunologic or bioassay techniques), and normal pubertal or adult testosterone levels and clearance of testosterone are characteristic (Table 26.37). The onset of adrenarche and its biochemical marker, serum DHEAS, correlate with bone age rather than chronologic age.

Treatment with an GnRH agonist does not suppress testicular function or maturation in the initial phase.[822] In late childhood or early adolescence, fertility is achieved and an adult pattern of LH secretion and response to GnRH is demonstrable[825]; secondary GnRH-dependent CPP may be superimposed on

TABLE 26.37 Testotoxicosis: Clinical and Laboratory Characteristics

Sex-limited autosomal dominant inheritance; activating mutation in the gene encoding the LH receptor
Early onset of sexual precocity in boys, with bilateral testicular enlargement
Prepubertal immunologic and biologic LH response to GnRH; prepubertal LH pulse secretory pattern
Concentration of plasma testosterone in pubertal range
Premature Leydig cell and seminiferous tubule maturation
No CNS, adrenal, or testicular abnormalities demonstrable by radiologic or hormonal studies
Lack of suppression of plasma testosterone or physical signs of puberty by GnRH agonist

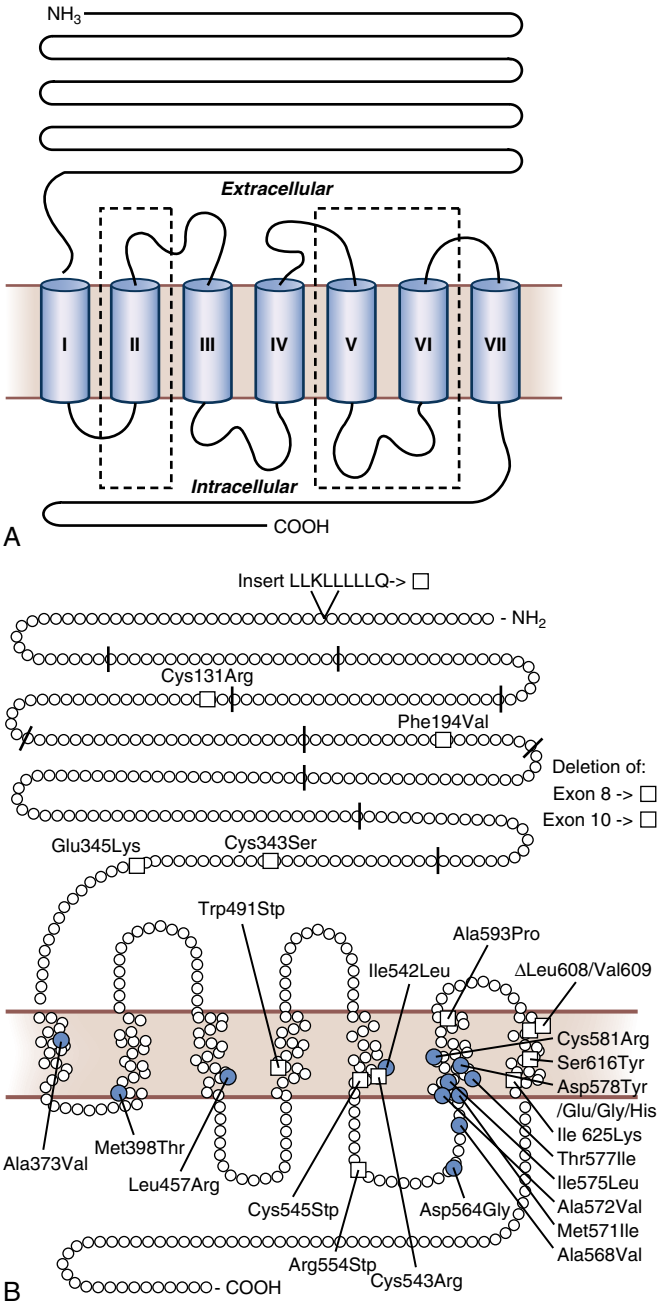
CNS, Central nervous system; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone.

the substrate of testotoxicosis. In some adults, impaired spermatogenic function is associated with elevated concentrations of plasma FSH. Testotoxicosis may occur sporadically, probably as a consequence of a germline mutation or even a postzygotic one, but it is usually inherited as a sex-limited autosomal dominant trait. A kindred with nine generations of affected males has been reported; obligatory female carriers of the trait were unaffected, because constitutional activation of the LH receptor on the ovary causes no ill effects.[826]

Heterozygous activating mutations of the heterotrimeric G_s protein–coupled LH-CGR that in concert transduce the LH/hCG signal to the main effector, adenyl cyclase, are the cause of testotoxicosis[827] (Fig. 26.63). The LH receptor cloned from the human is a glycoprotein of 80 to 90 kDa that belongs to a subfamily of the seven transmembrane–spanning, G protein–coupled receptors. The gene is localized to chromosome 2p21 (the same as for the FSH receptor); it spans at least 70 kilobases and contains 11 exons separated by 10 introns. The large glycosylated N-terminal extracellular hormone binding domain of the 701–amino acid LH-CGR is encoded by exons 1 through 10. A single exon, the large exon 11, encodes the entire G protein–linked transmembrane domain with its seven α-helic segments connected by alternating extracellular and intracellular loops, the intracellular domain, and the three untranslated regions—almost two-thirds of the receptor.[828]

Fourteen constitutive activating heterozygous missense mutations are reported in more than 60 patients[827] (see Fig. 26.63). Nine mutations were located between amino acid residues 542 and 581, suggesting a mutation hot spot. There appears to be a limited repertoire of mutations in American boys, consistent with a founder effect. European pedigrees are more diverse. A model of the transmembrane domain of the receptor provides novel suggestions for the structural and functional effects of these activating mutations.[829] Transfected cultured cells with these mutations exhibited increased basal cAMP production in the absence of agonist, observations consistent with a constitutive activating mutation.[823] Various possibilities for the conformational changes in the LH receptor that lead to its constitutive activation have been considered. Inactivating mutations of the LH-CGR and their clinical consequences are discussed earlier in this chapter.

Although affected boys do not respond to chronic administration of a GnRH agonist with suppression of testosterone



• **Fig. 26.63** (A) The serpentine, seven-transmembrane, G_s protein–coupled human luteinizing hormone/human chorionic gonadotropin (hLH/hCG) receptor with its large extracellular domain and the intracellular domain. The seven-helic transmembrane domains are indicated by roman numerals. (B) Mutations in the LH receptor protein are shown in the schematic structure of the LH receptor protein and localization of the inactivating *(open squares)* and activating *(blue-filled circles)* mutations in the human LH receptor. Notice the cluster of mutations in the VI transmembrane helix and third cytoplasmic loop. The Asx578Gly mutation is the most common activating mutation. The *short lines* across the amino acid chain separate the 11 exons. (A, redrawn from Yano K, Kohn LD, Saji M, et al. A case of male limited precocious puberty caused by a point mutation in the second transmembrane domain of the luteinizing hormone choriogonadotropin receptor gene. *Biochem Biophys Res Commun.* 1996;220:1036–1042; B, from Themmen APN, Huhtaniemi IT. Mutations of gonadotropins and gonadotropin receptors. *Endocr Rev.* 2000;21:551–583.)

TABLE 26.38 Pharmacologic Therapy for Sexual Precocity

Disorder	Treatment	Action and Rationale
GnRH Dependent		
True or central precocious puberty	GnRH agonists	Desensitization of gonadotrophs; blocks action of endogenous GnRH
GnRH Independent		
Incomplete sexual precocity		
Girls		
Autonomous ovarian cysts	Medroxyprogesterone acetate	Inhibition of ovarian steroidogenesis; regression of cyst (inhibition of FSH release)
McCune-Albright syndrome	Medroxyprogesterone acetate[a]	Inhibition of ovarian steroidogenesis; regression of cyst (inhibition of FSH release)
	Third-generation aromatase inhibitor (e.g., letrozole)	Inhibition of P450 aromatase; blocks estrogen synthesis
Boys		
Familial testotoxicosis	Ketoconazole[a]	Inhibition of CYP17 (mainly 17,20-lyase activity)
	Flutamide or bicalutamide and letrozole or anastrozole	Antiandrogen Inhibition of aromatase; blocks estrogen synthesis
	Medroxyprogesterone acetate[a]	Inhibition of testicular steroidogenesis

[a]If true precocious puberty develops, an LHRH agonist can be added.

CYP17, Cytochrome P450 17α-hydroxylase/17,20-lyase; *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *LHRH,* luteinizing hormone–releasing hormone.

Modified from Grumbach MM, Kaplan SL. Recent advances in the diagnosis and management of sexual precocity. *Acta Paediatr Jpn.* 1988:30:S155–S175.

secretion, testosterone secretion, height velocity and rate of bone maturation, and aggressive and hyperactive behavior have reportedly been decreased by treatment with oral medroxyprogesterone acetate[822] (Table 26.38).

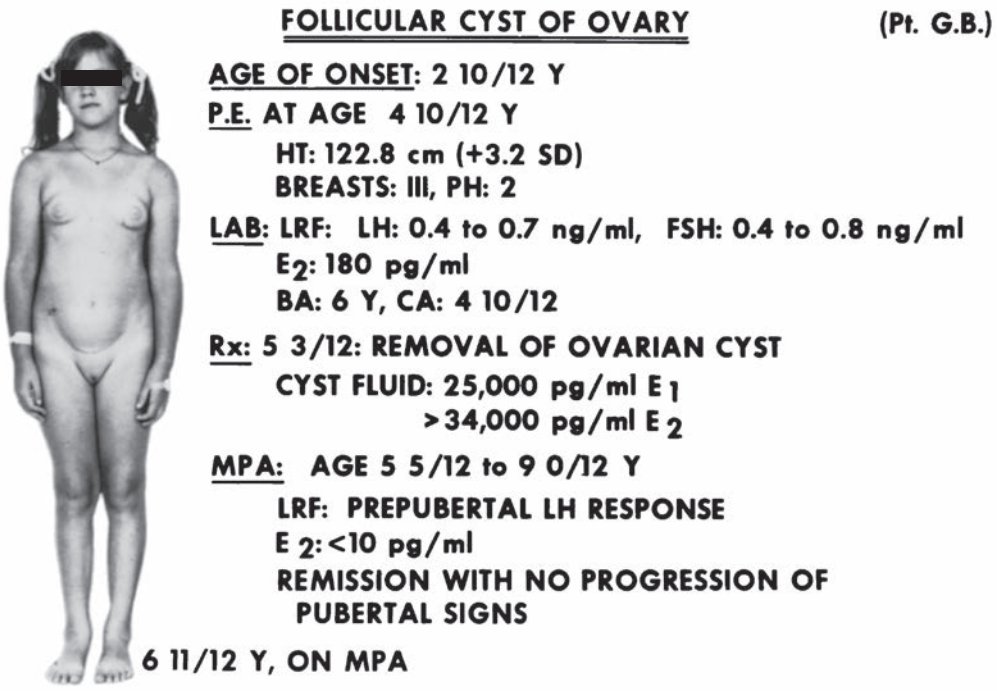
Ketoconazole, an orally active substituted imidazole derivative, suppresses gonadal and adrenal biosynthesis by inhibiting the enzyme CYP17, which regulates both 17-hydroxylation and the scission (17,20-lyase) of 17α-hydroxypregnenolone (Δ^5-17P) to DHEA (see "Nature and Regulation of Adrenal Androgens"). In the dosage used for treatment of testotoxicosis (median dose 16.2 mg/kg/day to a maximal dose of 700 mg/day[830]), the agent produces a mild transient decrease in cortisol secretion and interferes with binding of testosterone to TeBG. Secondary CPP often occurs when the bone age advances to or has already reached the pubertal range (usually >11.5 years), at which time the addition of a GnRH agonist is appropriate.[827] Ketoconazole can cause hepatic injury, which is usually mild and reversible, but in rare cases hepatotoxicity is severe. Reversible renal injury, rash, and interstitial pneumonia were reported in a patient who tolerated lower doses, suggesting a dose-response effect. Nonetheless, five patients treated with ketoconazole experienced no side effects other than one mild and transient elevation of liver enzymes; they had appropriate age of onset of true puberty and reached an adult height almost identical to the target height (a mean increase of 8 cm over the initially predicted height), suggesting great benefit of this therapy in this condition.[830,831]

The antiandrogen (and antimineralocorticoid) spironolactone, in combination with testolactone, an inhibitor of aromatase (CYP19), the key enzyme in the conversion of androgens to estrogens, is also used to treat testotoxicosis.[832] The addition of a GnRH agonist is a useful step to suppress pituitary gonadotropin secretion and secondary CPP that may later develop.[829] More potent nonsteroidal antiandrogens (e.g., flutamide, nilutamide) and aromatase inhibitors (e.g., letrozole) inhibit the rate of skeletal maturation and linear growth by suppressing estradiol synthesis, potentially with greater therapeutic efficacy.[159,160] The combination of bicalutamide and anastrozole is proposed as another approach.[833] Report of 20-year follow-up of these therapies demonstrated efficacy with few adverse effects. Table 26.38 lists the various agents used in the treatment of testotoxicosis.

A study of an untreated boy with testotoxicosis (GISP) had the expected pattern of rapid growth and early cessation, and the adult height of 174 cm was within target height range (171.5–188.5 cm), indicating the critical importance of individual approaches to affected boys when considering treatment to maximize height.[832]

Follow-up of boys with testotoxicosis indicates an increased risk of seminoma in adult life and Leydig cell adenoma in later childhood.[779,834] One boy with testotoxicosis developed nodular Leydig cell hyperplasia at 10 years of age.[835] These cases support a relationship between an activating mutation of the gene encoding the LH receptor and of Leydig cell tumors.[836]

Gonadotropin-Independent Sexual Precocity and Pseudohypoparathyroidism Type Ia. A mutation in $G_s\alpha$ can constitutively activate or inactivate adenyl cyclase.[837] Two boys who presented in infancy with classic pseudohypoparathyroidism type Ia (PHPIa), a disorder characterized by resistance to hormones whose action is mediated by cAMP, developed signs of sexual precocity with the hormonal characteristics of testotoxicosis (i.e., gonadotropin-independent sexual precocity) at about 24 months of age. Whereas the alanine residue is usually absolutely conserved in all heterotrimeric G pro-



• **Fig. 26.64** A girl aged 4 years and 10 months with recurrent, "autonomous" follicular cysts of the ovary. For conversion to SI units, see Fig. 26.19. *BA,* bone age; *CA,* chronologic age; E_2, estradiol; *FSH,* follicle-stimulating hormone; *LH,* luteinizing hormone; *LRF,* luteinizing hormone-releasing factor; *MPA,* medroxyprogesterone acetate (oral); *P.E.,* physical examination; Rx, treatment. (From Kaplan SL, Grumbach MM. Pathogenesis of sexual precocity. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:620–660.)

teins, both of these patients had a unique Ala366Ser mutation in one allele of the $G_s\alpha$ gene. PHPIa is caused by a wide variety of inactivating mutations in $G_s\alpha$ that lead to an approximately 50% reduction in $G_s\alpha$ activity in functional assays.

The paradox of a $G_s\alpha$ mutation causing both inactivation with PHP and constitutive activation with testotoxicosis was resolved by the in vitro demonstration that, unlike other activating mutations of $G_s\alpha$, which involve mutations inhibiting its intrinsic GTPase activity and decreasing the rate of hydrolysis of GTP to GDP, the mutation in these two boys caused accelerated dissociation of GDP at 33°C in transfected Leydig cells but was rapidly degraded at 37°C in a lymphoma cell line[838] and at 33°C and 37°C in skin fibroblasts transfected with the mutation. These observations explain the clinical consequences of increased $G_s\alpha$ activity in the testis, which are 3°C to 5°C cooler than the body, and the tissue specificity and temperature dependency of the mutation. The mother of one patient appeared to be a mosaic for the $G_s\alpha$ mutation; in the other boy, a germline mutation was likely.

Girls

GISP in girls (see Table 26.26) is caused by autonomous estrogen secretion by an ovarian cyst or tumor or an adrenal neoplasm or by inadvertent exposure to estrogen. A series of 129 patients demonstrated that the prevalence of non-gonadotropin–dependent precocious puberty was 14 in 1000 children if congenital adrenal hyperplasia was excluded. Girls were affected four times more than boys due to the prevalence of ovarian cysts followed by McCune-Albright syndrome in girls.[837] Girls harboring a teratoma or teratocarcinoma (or a CNS germ cell tumor) that secretes hCG have experienced sexual precocity caused by concurrent estrogen secretion by the tumor but no effect from the hCG alone; these girls also may have galactorrhea, especially if chorionic somatomammotropin (hCS or hPL) is also secreted.

Autonomous Ovarian Follicular Cysts. The most common childhood estrogen-secreting ovarian mass and ovarian cause of sexual precocity is the follicular cyst.[838] Antral follicles up to about 8 mm in diameter are common in the ovaries of normal prepubertal girls and may be seen in third trimester fetuses and newborn infants.[839] They may appear and regress spontaneously. Larger follicular cysts may be discovered because of the presence of an abdominal mass or abdominal pain, especially after torsion or as an unexpected finding on pelvic sonography performed for other reasons. Occasionally, the antral follicles secrete estrogen and may form large masses, or the follicular cysts may recur and cause recurrent signs of sexual precocity and acyclic vaginal bleeding. Enlarged antral follicles or cysts occur in premature thelarche, CPP, and transient or incomplete sexual precocity. In some patients with ovarian follicular cysts, the transient or recurrent sexual precocity is GnRH independent (Fig. 26.64). The concentration of estradiol fluctuates, usually correlating with changes in the size of the follicular cyst(s) when monitored by pelvic sonography, and may increase to levels found in granulosa cell tumors, although values may also be in the pubertal range.

These patients do not have increased plasma granulosa cell tumor markers such as AMH or inhibin.[840] The concentration of LH is suppressed, a pubertal pattern of pulsatile LH secretion is absent, and the LH rise induced by GnRH is prepubertal. A constitutive activating mutation of the FSH receptor has not been described in a female, but a heterozygous mutation, Asp567Gly, was detected in the third intracellular loop of the FSH receptor in a hypophysectomized man who, despite the gonadotropin

deficiency, was fertile and had normal-size testes.[827] Accordingly, the possibility that some girls with recurrent ovarian cysts harbor an activating mutation of the FSH receptor seems worthy of study. The McCune-Albright syndrome may lead to recurrent ovarian cysts even in the apparent initial absence of other features of this disorder due to somatic activating mutations in the gene encoding the α-subunit of the heterotrimeric G_s protein. The luteinization of follicular cysts may be related to subtle elevations and increased pulses of plasma FSH. Ovarian cysts and sexual precocity have been associated with the fragile X syndrome in girls.[841]

Estradiol-secreting ovarian cysts occur in preterm infants born before 30 weeks of gestation; they are associated with edema of the labia majora and, in some instances, of the lower abdominal wall.[842] The LH and FSH response to GnRH in these patients suggests GnRH dependence, and treatment with medroxyprogesterone acetate is associated with regression of the cysts. A case of massive ovarian edema associated with ovarian cysts found in a 6-month-old with breast and pubic hair development has been reported.[843]

GnRH agonists are useful in the treatment of ovarian follicular cysts associated with CPP (GISP) but not of so-called autonomous cysts. However, autonomously functioning ovarian follicular cysts, whether recurrent or manifesting in an isolated episode, often respond to treatment with oral medroxyprogesterone acetate (or cyproterone acetate outside of the United States), which seems to prevent recurrence, to accelerate involution of the follicular cysts,[843] and to reduce the risk of torsion. The use of a potent aromatase inhibitor such as letrozole to reduce estradiol secretion is another potential approach to treatment and has been successfully used in girls with no stigmata of McCune-Albright syndrome other than autonomous ovarian cysts.[844,845] Surgical intervention is rarely indicated; a large or persistent cyst can be reduced by puncture at laparoscopy, and the size of the cyst can be monitored readily by pelvic sonography.

While it is a rare cause of ovarian tumors, an FSH-secreting and TSH-secreting adenoma of the pituitary gland led to extremely large cysts in a 9-year-old girl's ovaries but was only discovered after menopausal FSH levels led to the diagnosis of the tumor at 19 years of age.[846]

Ovarian Tumors. Ovarian tumors are the most common genitourinary tumors of girls,[847,848] accounting for about 1% of all tumors in girls younger than 17 years of age, but they are rare in the prepubertal period. Most are benign according to most reports,[849] but a referral center might find a majority of tumors are malignant.[850] Most ovarian tumors arise from germ cells or sex cord–stromal cells in childhood with fewer than 20% being of epithelial origin, whereas in adults, most are of epithelial origin.[851] Early diagnosis of most childhood tumors of the ovary allows successful cure, unlike ovarian cancer in adult women. Most of these girls present with pain or an abdominal mass. Tumors smaller than 5 cm at diagnosis are more likely to be nonneoplastic, and those larger than 10 cm are more likely to be neoplastic.[838,852] Malignant ovarian tumors evaluated by ultrasonography, computed tomography, or magnetic resonance imaging are mostly solid or heterogeneous and are larger than benign tumors.[853] The successful use of tumor markers for diagnosis varies by cause. For example, in one series, cystic teratomas resulted in lactate dehydrogenase (LDH) elevation and an increased erythrocyte sedimentation rate; immature teratomas produced elevated levels of LDH, α-fetoprotein, and cancer antigen 125 (CA125), and granulosa cell tumors had elevated sex steroids (estradiol or testosterone[850] or both).[854]

Granulosa cell tumor of the ovary is rare in childhood, although theca cell tumors are even less common.[855] Because most granulosa cell tumors produce estrogen, incomplete isosexual precocity occurs in the youngest girls affected. Characteristic histologic features of juvenile granulosa cell tumors include nodular architecture, follicle formation, abundant interstitial and intrafollicular acid mucopolysaccharide-rich fluid, irregular microcysts, individual cell necrosis, and high mitotic activity (mean activity, 11 mitotic figures per 10 high-power fields). Size can vary from 2.5 to 25 cm, with a mean diameter of 12 cm. The interstitial mucinous fluid consists predominantly of hyaluronic acid. Prognosis is good, with the mortality rate being about 3%, but delay in treatment leads to substantial complications. In one series, girls who presented with ISP and were correctly diagnosed had no intra-abdominal spread and had Federation of Gynecology and Obstetrics (FIGO) stage IA disease, whereas those who presented with acute abdominal symptoms had 50% prevalence of intra-abdominal spread and two recurrences after surgery. When the diagnosis was made after normal puberty had begun, some girls experienced virilization or abdominal symptoms; 80% had intra-abdominal spread, and 30% had recurrence with FIGO stage IIC.

Approximately 80% of granulosa cell tumors can be palpated on bimanual examination, whereas fewer than 5% are bilateral or clinically malignant. The concentration of plasma estradiol may increase to high levels, and serum FSH and LH concentrations are usually suppressed. AMH and inhibin are sensitive tumor markers and are used to screen for metastases especially if elevated at diagnosis[856]; an elevated estradiol concentration in an affected patient younger than 9 years of age or an abnormal rise in concentration of plasma AMH or inhibin at any age suggests recurrence or metastasis. Girls affected with stage I disease may be treated by surgery alone, but a more advanced grade will require chemotherapy.[857]

Occasionally, gonadoblastomas in streak gonads, rare lipoid tumors, cystadenomas, and ovarian carcinomas secrete estrogens, androgens, or both. Even with successful resection of a gonadal sex steroid–secreting neoplasm, the child is at risk for secondary CPP in the future. Gonadal tumors composed of a mixture of germ cells and sex cord–stromal cells are distinct from gonadoblastoma. They may be benign or malignant, with the Sertoli-Leydig cell tumors reported to be most aggressive.[858] α-Fetoprotein and other tumor markers aid in diagnosis. However, a 558-g ovarian dysgerminoma was found in a 7-year-old girl with precocious puberty and no elevation of serum tumor markers.[859]

Peutz-Jeghers Syndrome. Peutz-Jeghers syndrome, an autosomal dominant syndrome, is usually caused by mutations in the gene located on 9p13.3 that encodes a serine/threonine protein kinase, STK11, leading to haploinsufficiency of this novel tumor-suppressing gene.[88,860] This condition is characterized by mucocutaneous pigmentation of the lips, buccal mucosa, fingers, and toes; gastrointestinal hamartomatous polyposis; and a predisposition to malignancy.

It is associated with a rare, distinctive sex cord tumor with annular tubules in both boys and girls. Estrogen secretion by the tumor may lead to feminization in girls and incomplete sexual precocity in boys. Less frequently, a feminizing Sertoli-Leydig cell tumor has been found in patients with Peutz-Jeghers syndrome. The rare feminizing large-cell calcifying Sertoli cell tumors of the testes present with gynecomastia in boys and are found in Peutz-Jeghers syndrome and Carney complex, the latter of which is most often caused by *PRKAR1A* mutations, the gene encoding regulatory subunit type 1 of protein kinase A.

Sex cord–stromal cell tumors derive from the celomic epithelium or mesenchymal cells of the embryonic gonads and are composed of granulosa, theca, Leydig, and Sertoli cells. Estrogen secretion from these tumors can cause ISP in girls, and androgen secretion can cause virilization. Inhibin A and B activin are produced, as is AMH; all serve as useful tumor markers. Sex cord–stromal cell tumors not associated with Peutz-Jeghers syndrome are malignant in 25% of cases; these tumors can grow quite large, but those associated with Peutz-Jeghers syndrome are often small and multiple, and they contain calcifications.[861] Estrogen excess in Peutz-Jeghers syndrome[1008] has been treated with aromatase inhibitors.[474]

Girls with this disorder should be examined at regular intervals for the presence of gonadal tumors by pelvic sonography. Peutz-Jeghers syndrome should be considered in boys with unexplained gynecomastia, especially if in the prepubertal years.

Adrenal Tumors. Adrenocortical tumors are rare in childhood (0.6% of all childhood tumors and 0.3% of all malignant childhood tumors), but most produce steroid hormones, whereas those in adults usually do not. The median age at diagnosis is 4 years, but 41% of these tumors manifest before 2 years and 71% before 5 years of age. Most cause virilization or Cushing syndrome, but adrenal tumors may produce estrogen as well as androgens and can cause sexual precocity in a girl or gynecomastia in a boy.[862] One adrenal adenoma found in a 7-year-old girl expressed the gene for aromatase, demonstrating that the tumor could directly produce estrogen[863] to a level of 145 pg/mL, within the range found in adrenal carcinomas.

Boys and Girls

McCune-Albright Syndrome. McCune-Albright syndrome[846,1003] occurs about twice as often in girls than in boys; it is sporadic and is caused by somatic activating mutations (Cys or His to Arg201) in exon 8 of the gene *(GNAS1)* encoding the α-subunit of the trimeric GTP-binding protein ($G_s\alpha$) that stimulates adenyl cyclase.[864,865] This leads to a constitutive ligand-free activation of cellular function in a mosaic distribution, leading to a high variability of organ involvement and degree of severity.[474] It is characterized by the triad of irregularly edged hyperpigmented macules (café au lait spots of the coast of Maine type); a slowly progressive bone disorder, polyostotic fibrous dysplasia, that can involve any bone and is frequently associated with facial asymmetry and hyperostosis of the base of the skull; and, more commonly in girls, GISP (Fig. 26.65 and Table 26.39). At least two of the features must be present to consider the diagnosis.

Autonomous hyperfunction most commonly involves the ovary, but other endocrine involvement includes the thyroid gland (nodular hyperplasia with thyrotoxicosis or euthyroid status), adrenal gland (multiple hyperplastic nodules with Cushing syndrome that may occur in the neonatal period and may be followed by adrenal insufficiency),[866] pituitary gland (adenoma or mammosomatotroph hyperplasia with gigantism, acromegaly, and hyperprolactinemia), and parathyroid glands (adenoma or hyperplasia with hyperparathyroidism).[867,868] Hypophosphatemic vitamin D–resistant rickets or osteomalacia occurs either because of overproduction of a phosphaturic factor, phosphatonin, that is secreted by the bone lesions or because of an intrinsic renal abnormality leading to the excess generation of nephrogenous cAMP in the proximal tubule and, as a result, decreased reabsorption of phosphate. Hepatocellular dysfunction may occur due to expression of the mutant activating gene in liver cells, which leads to jaundice associated with hepatobiliary disease, and pancreatitis.

• **Fig. 26.65** A girl aged 7 years and 4 months with gonadotropin-releasing hormone (GnRH)–independent sexual precocity associated with McCune-Albright syndrome. She had breast development since infancy, and it increased noticeably at about 3 years of age; 6 months later, episodes of recurrent vaginal bleeding began. Growth of pubic hair was noticed at about 4 to 5 years. At age 5.5 years, the bone age was 6 years and 11 months; height was +1 SD (standard deviation) above the mean value for age. By 6.5 years, when she was seen at the University of California, San Francisco, the bone age had advanced to 9 years, and height was at +1 SD. Breasts were at Tanner stage 4, and pubic hair at stage 3. Extensive, irregular café au lait macules cover the right side of the face, left lower abdomen and thigh, and both buttocks. A bone survey showed widespread involvement of the long bones with typical polyostotic fibrous dysplasia, the floor of the anterior fossa of the skull was sclerotic, and the diploic space had widened. She has had two pathologic fractures through bone cysts in the right upper femur. Notice the osseous deformities. Plasma estradiol concentrations were consistently in the pubertal range; the luteinizing hormone (LH) response to GnRH was prepubertal. Results of thyroid function studies were normal, including the thyrotropin response to thyrotropin-releasing hormone administration, and antithyroid antibodies were not detected. Treatment with oral medroxyprogesterone acetate suppressed menses and arrested pubertal development but did not slow skeletal maturation. Her final height is 142 cm (–2.5 SD). Menstrual cycles are regular.

Another nonendocrine manifestation is cardiac disease, and patients carry the risk of cardiac arrhythmia and sudden death. This is a sporadic condition that can be concordant or discordant in monozygotic twins.

Considering children with at least one of the signs of McCune-Albright syndrome, 24% had the classic triad, 33%

TABLE 26.39 Clinical Manifestations of McCune-Albright Syndrome in 158 Reported Patients[a]

Manifestation	% OF PATIENTS			Mean Age at Diagnosis (yr) and Range	Comments
	Total (*N* = 158)	Male (*n* = 53)	Female (*n* = 105)		
Fibrous dysplasia	97	51	103	7.7 (0-52)	Polyostotic more common than monostotic
Café au lait lesion	85	49	86	7.7 (0-52)	Variable size and number of lesions, irregular border (coast of Maine)
Sexual precocity	52	8	74	4.9 (0.3-9)	Common initial manifestation
Acromegaly/gigantism	27	20	22	14.8 (0.2-42)	17/26 of patients with adenoma on MRI/CT
Hyperprolactinemia	15	9	14	16 (0.2-42)	23/42 of acromegalic patients with PRL
Hyperthyroidism	19	7	23	14.4 (0.5-37)	Euthyroid goiter is common
Hypercortisolism	5	4	5	4.4 (0.2-17)	All primary adrenal
Myxomas	5	3	5	34 (17-50)	Extremity myxomas
Osteosarcoma	2	1	2	36 (34-37)	At site of fibrous dysplasia, not related to prior radiation therapy
Rickets/osteomalacia	3	1	3	27.3 (8-52)	Responsive to phosphorus plus calcitriol
Cardiac abnormalities	11	8	9	(0.1-66)	Arrhythmias and CHF reported
Hepatic abnormalities	10	6	10	1.9 (0.3-4)	Neonatal icterus is most common

[a]Evaluations include clinical and biochemical data; other rarely described manifestations include metabolic acidosis, nephrocalcinosis, developmental delay, thymic and splenic hyperplasia, and colonic polyps.

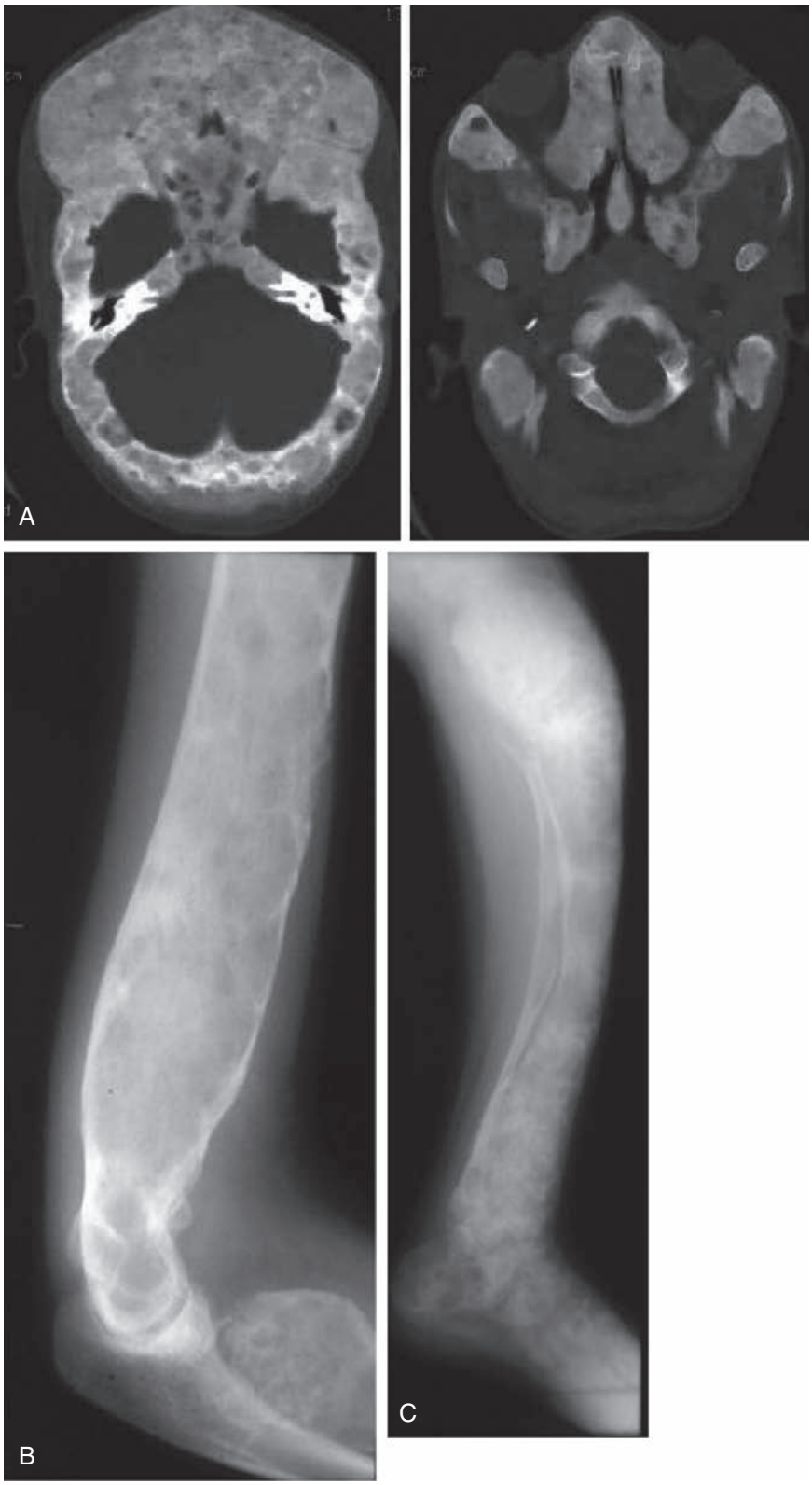
CHF, Congestive heart failure; *CT,* computed tomography; *MRI,* magnetic resonance imaging; *PRL,* prolactin.

Modified from Ringel MD, Schwindinger WF, Levine MA. Clinical implication of genetic defects in G proteins: the molecular basis of McCune-Albright syndrome and Albright hereditary osteodystrophy. *Medicine.* 1996;75:171–184.

had two signs, and 40% had only one classic sign. The mutation was detected in 46% of blood samples from patients presenting the classic triad but in only 21% and 8% of samples from patients with two signs or one sign, respectively. If an affected tissue was available, the mutation was found in more than 90% of the patients no matter what the number of signs. The mutation was found in 33% of the 39 cases of isolated peripheral precocious puberty. Patients with monostotic fibrous dysplasia, isolated peripheral precocious puberty, neonatal liver cholestasis, or the classic McCune-Albright syndrome all had the same molecular defect.[818] Whereas most endocrine organs involved in McCune-Albright syndrome were not associated with parent specificity,[869] pituitary adenomas secreting GH expressed NESP55 transcripts, which are monoallelically expressed from the maternal alleles rather than from the exon 1A paternal allele. Mutation of *GNAS1* involving Arg201His replacement is associated with apparent premature or exaggerated thelarche and early menarche.[870,871]

Most patients have pigmented skin lesions in infancy that usually increase in size along with body growth.[872] The irregularly bordered café au lait macules usually do not cross the midline, but they may; they often are located on the same side as the main bone lesions and have a segmented distribution.

The skeletal lesions in the cortex are dysplastic and are filled with spindle cells with poorly organized collagen support; they take the form of scattered cystic areas of rarefaction on radiography and often result in pathologic fractures and progressive deformities (Fig. 26.66). Technetium bone scintigraphy has been the most sensitive approach to the detection of bone lesions before they are visible radiographically. Fractures are most common between the sixth and the tenth years but decline thereafter, and they are more frequent if phosphaturia is present.[873] Patients referred for fibrous dysplasia of the bone in one or more locations are often found to have endocrine or dermatologic manifestations of McCune-Albright syndrome as well as *GNAS1* mutations, so suspicion should be kept high when evaluating fibrous lesions.[874] If the skull is involved, there may be entrapment and compression of optic or auditory nerve foramina, which can lead to blindness, deafness, facial asymmetry, and ptosis. Asymmetry of the jaw is another manifestation of McCune-Albright syndrome. Fifty percent of affected children in one series manifested bone abnormalities by 8 years of age.[872] Increased serum GH



• **Fig. 26.66** Bone lesions in McCune-Albright syndrome. (A) The skull has severe thickening primarily at the base due to fibrous dysplasia. The auditory and optic nerves can be caught in narrowed foramina, but that is not the case in these patients. (B, C) Distortions of the long bones can develop into a "shepherd's crook" appearance. Notice the multiple bone cysts. (D) Bone scan shows the areas of remodeling that "light up," depending on the area affected in individual patients. There are examples of patients primarily affected in the craniofacial area or in the appendicular area, or both areas, and the axial skeleton. (Courtesy Michael T. Collins, M.D., National Institutes of Health, Bethesda, MD, and Sandra Gorges, M.D., University of California, Davis, CA.)

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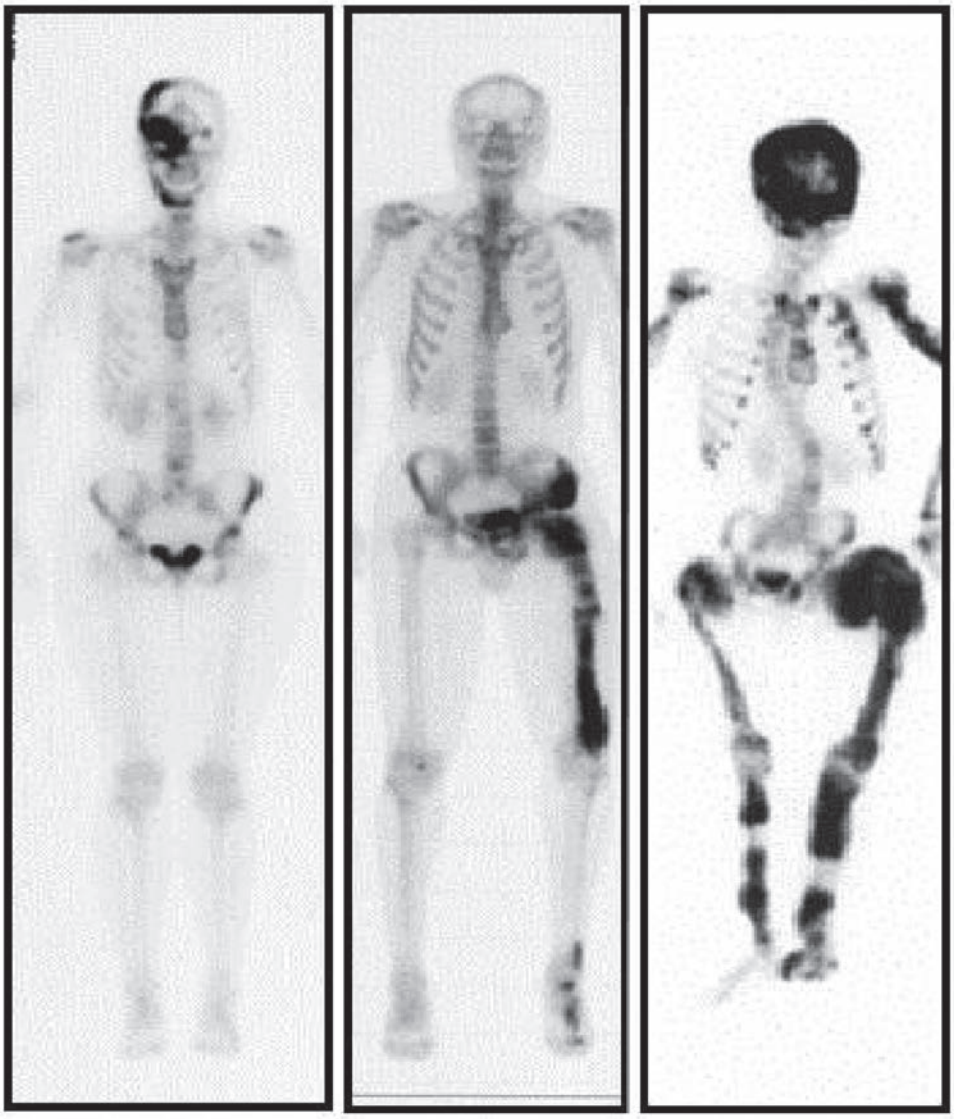

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• **Fig. 26.66 cont'd** See page 1147 for legend.

levels occur in up to 30% of cases and have an adverse effect on the skull deformities, depending on the age at onset; somatostatin analogues are usually effective in suppressing GH.[875,876] Irradiation of the hypothalamic-pituitary area may be invoked, but it carries a risk of later occurrence of sarcoma. Rapid control of elevated GH can be achieved by the use of long-acting somatostatin agonists such as pegvisomant, which is often but not always successful.[877]

The sexual precocity often begins during the first 2 years of life and is frequently heralded by menstrual bleeding; the cause is autonomously functioning luteinized follicular cysts of the ovary in girls (Table 26.40).[865] The ovaries contain no corpora lutea and commonly exhibit asymmetric enlargement as a result of a large solitary follicular cyst that characteristically enlarges and then spontaneously regresses, only to recur (Fig. 26.67).[865] Serum estradiol is elevated (at times to extraordinary levels); in contrast, the LH response to GnRH is prepubertal, and the pubertal pattern of nighttime LH pulses is absent at the onset and during the initial years. Later in the course of the sexual precocity, when the bone age approaches 12 years, the GnRH pulse generator becomes operative and ovulatory cycles ensue. However, adults carry the risk of acyclic hyperestrogenemia, infertility, and a potential risk of estrogen-dependent cancer, including breast cancer.[878]

An affected girl may progress from GnRH-independent puberty to GnRH-dependent puberty (see Table 26.40). GnRH agonists are not effective for treatment in the GnRH-independent stage. Testolactone, fadrozole, and anastrozole have had equivocal or no success in controlling manifestations in initial studies.[475] After a single case report of treatment with tamoxifen, an antiestrogen, showed decreases in bone age advancement, growth rate, menses, and pubertal development, a multicenter trial demonstrated the utility of this agent in decreasing vaginal bleeding and decreasing the rate of bone age advancement and growth rate in

TABLE 26.40 A Patient With McCune-Albright Syndrome and Recurrent Ovarian Cysts

Chronologic Age (yr)	Bone Age (yr)	Height (cm)	Physical Signs	Basal and Post-LHRH (ng/mL)[a,b]	Plasma Estradiol (pmol/L [pg/mL])	Radiography (Long Bones)
$1^{4}/_{12}$	$1^{3}/_{12}$	81.1	Café au lait pigmentation, B2, PH1	LH 0.6-1.3 FSH 1.9-3.2	40 (11)	Normal
			Vaginal bleeding (×2 mo)	DHEAS <50 ng/mL (<0.14 mmol/L)		
$1^{8}/_{12}$			B1, PH1			
$2^{6}/_{12}$	$2^{6}/_{12}$	92.4	B2, PH2	LH 0.6-1.1	55-66 (15-18)	Normal
			Vaginal bleeding	FSH 1.9-3.2		
				DHEAS <50 ng/mL (<0.14 mmol/L)		
$3^{1}/_{12}$		98.3	B1, PH1			
$3^{10}/_{12}$	$3^{10}/_{12}$		B2, PH1	LH 1.1-2.0	51-95 (14-26)	Normal
				FSH 1-1.7		
$4^{3}/_{12}$			B1, PH1		7.3-7.3 (20-20)	Polyostotic fibrous dysplasia of femurs
$5^{11}/_{12}$	6	123.4	B3, PH2	LH 1.1-4.3		
			Vaginal bleeding (×2 mo)	FSH 1.0-2.0		
$6^{6}/_{12}$	$7^{10}/_{12}$	128.5	B3, PH2			
			Oral medroxyprogesterone acetate, 10 mg bid stated		<5	
$7^{10}/_{12}$	$8^{10}/_{12}$	136.8				
$8^{7}/_{12}$		142.2				

[a]Matched standard reagents were LER-960 for LH and LER-869 for FSH. To convert ng/mL to IU/L, multiply LH value by 3.8 and FSH value by 8.4.

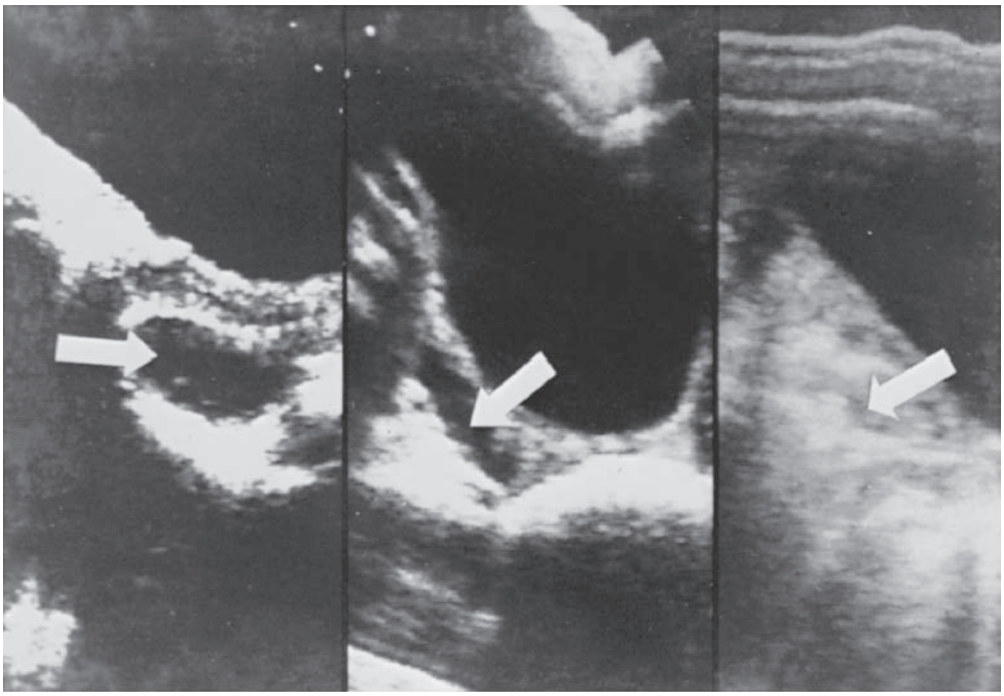
[b]Note the prepubertal LH response to GnRH consistent with GnRH-independent sexual precocity until age 5 11/12 yr and the pubertal LH response at 5 11/12 yr, consistent with the development of secondary true precocious puberty (GnRH-dependent). Note discrepancy between gonadarche and adrenarche as evidenced by preadrenarchal concentration of DHEAS.

B, Breast stage; *bid*, twice a day; *DHEAS*, dehydroepiandrosterone sulfate; *FSH*, follicle-stimulating hormone; *GnRH*, gonadotropin-releasing hormone; *LER*, matched standard reagent; *LH*, luteinizing hormone; *LHRH*, luteinizing hormone–releasing hormone; *PH*, pubic hair stage.

affected girls.[879] However, ovarian and uterine volumes remained elevated. The longest term study demonstrated that letrozole can suppress bone age advancement and preserve adult height far over controls.[880]

Sexual precocity is rare in boys with McCune-Albright syndrome[865]; however, testicular disease occurs as frequently in affected boys as ovarian disease in girls.[881] Affected boys may have asymmetric enlargement of the testes in addition to signs of sexual precocity. The histologic changes and hormonal findings are reminiscent of those observed in testotoxicosis: The seminiferous tubules are enlarged and exhibit spermatogenesis, and Leydig cells may be hyperplastic, the most common histologic finding.[865,881] A 3.8-year-old boy with McCune-Albright syndrome (several café au lait lesions on the back and polyostotic fibrous dysplasia) had an Arg201His mutation detected in bone and testis tissue and the unusual feature of macroorchidism (right testis, 9 mL; left testis, 7 mL), but sexual precocity was absent. Basal and GnRH-stimulated gonadotropins and sex steroid levels were prepubertal, but serum inhibin B and AMH concentrations were strikingly elevated. On histologic examination of the testes, most of the seminiferous tubules were slightly increased in diameter and filled with Sertoli cells but lacked a lumen. The tubules stained intensively for inhibin β_B-subunit; mature Leydig cells were absent.[882] An increased incidence of the rare condition testicular microlithiasis was described in boys with McCune-Albright syndrome evaluated by ultrasonography.[883,884]

McCune-Albright syndrome may occur concordantly or discordantly in monozygotic twins; familial cases have not been convincingly described. In 1986, Happle[885] posited that the disorder

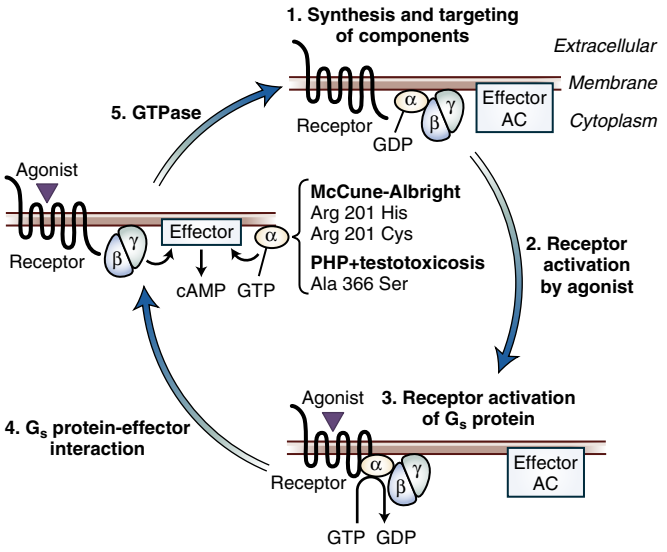


• **Fig. 26.67** Serial pelvic ultrasonograms at 2-week intervals of a 6-year-old girl with McCune-Albright syndrome. Breast development and vaginal bleeding coincided with the enlargement of the ovarian cyst; *white arrows* denote the decreasing size of the cyst. With the spontaneous regression of the large ovarian cyst, the breasts regressed in size, and vaginal bleeding ceased. (From Kaplan SL, Grumbach MM. Pathogenesis of sexual precocity. In: Grumbach MM, Sizonenko PC, Aubert ML, eds. *Control of the Onset of Puberty.* Baltimore, MD: Williams & Wilkins; 1990:620–660.)

is caused by an autosomal dominant lethal gene that results in loss of the zygote in utero and that cells bearing this mutation survive only in embryos mosaic for the lethal gene. Early somatic mutation would lead to a mosaic cell pattern of the distribution of cells containing the mutation. The severity of the disorder would depend on the proportion of mutant cells in various embryonic tissues. The description of somatic mutations in human endocrine tumors that convert the peptide chain of the G_s protein into a putative oncogene (referred to as a *gsp* mutation) raised the possibility of a similar defect in McCune-Albright syndrome that both affects a differentiated function such as a signaling pathway and mediates the regulation of proliferation. These hypotheses are now established, because mutations in the gene encoding the α-subunit of the stimulatory G protein for adenyl cyclase was identified in the tissues of children with the McCune-Albright syndrome.

The heterotrimeric guanine nucleotide–binding proteins (G proteins) are a subfamily within the large superfamily of GTP-binding proteins and serve to transduce signals from a large number of cell surface receptors with a common structural motif of seven-transmembrane–spanning domains to their intracellular effector molecules, including enzymes and ion channels; in essence, they couple serpentine cell surface receptors to effectors (Fig. 26.68). For G_s, the stimulatory G protein, the effector is adenyl cyclase, which is controlled by G_s and an inhibitory G protein,[836] G_i. The heterotrimer is composed of an α-subunit (39–45 kDa) that binds GTP, has intrinsic GTPase activity, and converts GTP to GDP; a β-subunit (35–36 kDa); and a smaller β-subunit (7–8 kDa). The latter two subunits are tightly but noncovalently associated with each other. Each of the subunits is encoded by a distinct gene. The G proteins function as conformational switches. The GDP-liganded α-subunit is bound to the βγ-subunits and is in an inactivated state. When the cell surface receptor is activated by its ligand or agonists, the GDP is catalytically released from the α-subunit, enabling GTP to bind. This leads to dissociation of the GTP-activated α-subunit from the bound βγ-subunits and activation of the effector, adenyl cyclase. When GTP is hydrolyzed by the intrinsic GTPase activity of $G_s\alpha$, the α-subunit and βγ-subunit reassociate, and the α-subunit is in the off or inactive conformation. The three-dimensional structure of the heterotrimeric G proteins has been determined.

Activating heterozygous mutations in the $G_s\alpha$-subunit that occurred as an early postzygotic event are described in the McCune-Albright syndrome. The somatic constitutive activating mutation, which leads to excess cAMP production and, in some tissues, cAMP-induced hyperplasia, has a mosaic pattern; the proportion of the hyperactive mutant compared with normal cells varies in different tissues, contributing, at least in part, to the varied clinical findings, the severity, the sporadic nature of the syndrome, and the discordant occurrence in monozygotic twins. A germline mutation is presumed to be lethal to the embryo. Two gain-of-function somatic missense mutations have been described in this disorder, both of which involve the arginine 201 residue of the α-subunit.[836] This is the site of covalent modification by cholera toxin: Either a cysteine or a histidine is substituted for arginine 201 (see Fig. 26.68). The arginine 201 residue is critical for α-subunit GTPase activity, and each of these two mutations decreases the GTPase activity of the $G_s\alpha$-subunit, leading to constitutive activation. These activating mutations have been found in all tissues affected by the syndrome, including bone lesions.



• **Fig. 26.68** The G protein guanosine triphosphatase (GTPase) cycle. The heterotrimeric guanine nucleotide-binding proteins (G proteins), which are composed of three subunits (α, β, γ), couple cell surface receptors consisting of a single serpentine polypeptide having seven-helic membrane-spanning domains with an effector. In this instance, adenylate cyclase (AC) catalyzes the transformation of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). The G protein stimulation subunit α (G_s) mediates the stimulation of cAMP generation. In the inactive, unstimulated state, the G protein is a heterotrimer, and guanosine diphosphate (GDP) is tightly bound to the α-subunit. When the cell surface receptor is activated by its cognate agonist, the receptor catalyzes the release of the tightly bound GDP, which enables GTP to bind to the α-subunit. The GTP-bound α-subunit (αGTP) dissociates from the tightly bound βγ dimer, and both play a role in the G protein activation of the effector, adenylate cyclase. The intrinsic GTPase activity of the α-subunit ends the stimulation of the effector by converting the bound αGTP to αGDP; as a consequence, the α-subunit again returns to its inactive state and reassociates with high affinity with the βγ subunit, yielding the α, β, γ heterotrimer. Disorders of signal transduction can arise from germ cell or somatic mutations at any of the five stages of the cycle. The gain-of-function, activating somatic mutations in the *GNAS1* gene that encodes the G G_sα-subunit and leads to McCune-Albright syndrome *(shown in the bracket),* involves the highly conserved arginine 201 residue. These mutations inhibit the intrinsic GTPase activity of the α-subunit and therefore the conversion of the bound GTP to GDP. The Ala366Ser mutation *(shown in the bracket)* was detected in two boys, both of whom had pseudohypothyroidism 1a (PHP1a) and testotoxicosis. The mutant protein was constitutively activated in the Leydig cells at the scrotal temperature (32–33°C), leading to testotoxicosis, but it was rapidly degraded at body temperature, 37°C, which led to PHP1a. (Modified from Spiegel AM. Mutations in G proteins and G protein-coupled receptors in endocrine disease. *J Clin Endocrinol Metab.* 1996;81:2434–2442.)

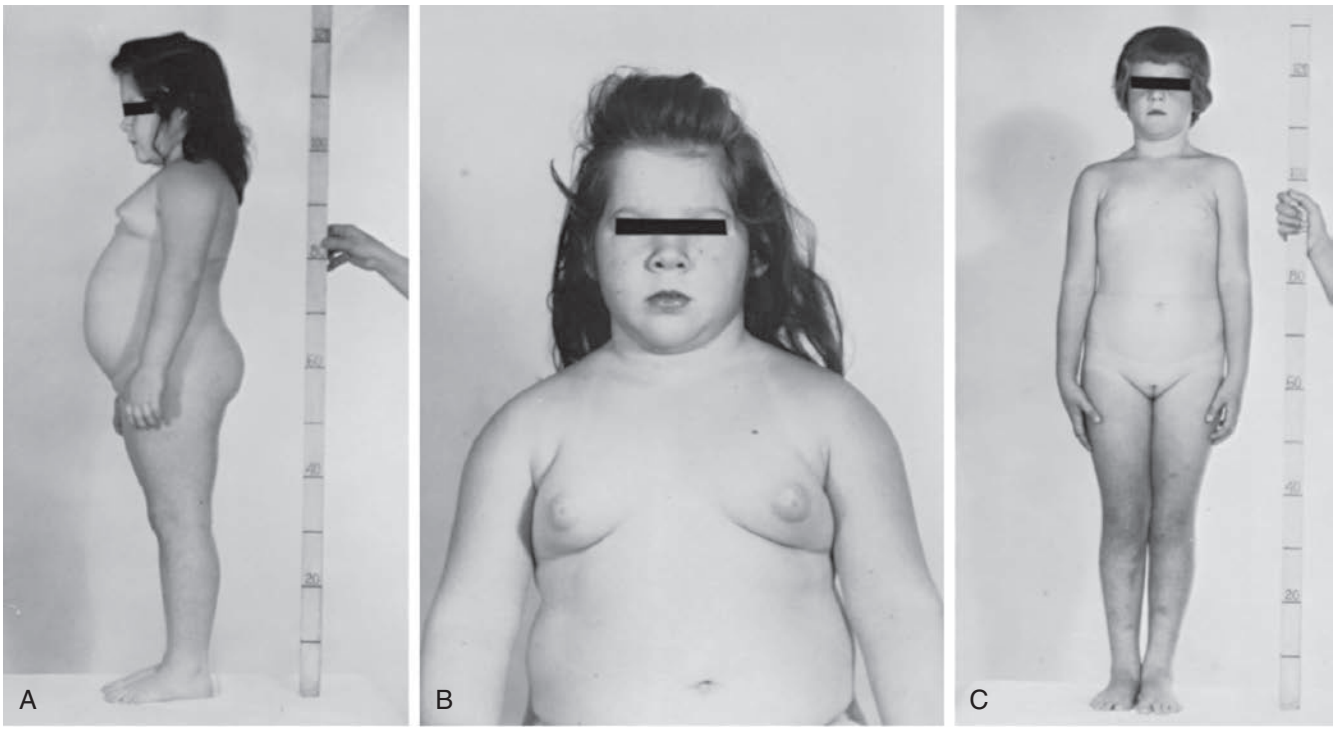
Adults with this syndrome have continued hyperfunction of the endocrine organs, but other comorbidities are reported. For example, a GNAS-positive breast cancer is reported in a woman, two GNAS-positive muscle myomas, platelet dysfunction in four, and various types of respiratory and cardiovascular complications were found in a series of 16 affected adults.[886] There are reports of fertility in adults with McCune-Albright syndrome.

Juvenile Hypothyroidism. Long-standing untreated primary hypothyroidism, usually a consequence of Hashimoto thyroiditis, is an uncommon cause of incomplete ISP in both girls and boys and occurs in association with impaired growth and delayed skeletal maturation.[323] If the concentration of plasma prolactin is elevated, galactorrhea may be demonstrable, more commonly in affected girls than boys (Figs. 26.69 and 26.70). Girls have breast development, enlarged labia minora, and estrogenic changes in the vaginal smear, usually without the appearance of pubic hair; some girls have irregular vaginal bleeding that could proceed to metrorrhagia, and solitary or multiple ovarian cysts may be demonstrable by pelvic sonography or on physical examination.[887] It is important to recognize the condition to avoid unnecessary surgery for the ovarian cysts or the accompanying pituitary enlargement, which would be a tragic mistake in view of the success of medical management. In about 80% of boys with juvenile hypothyroidism, the testes are enlarged because of an increase in the size of the seminiferous tubules, but signs of virilization and Leydig cell maturation are absent, and the plasma concentration of testosterone is prepubertal. Enlargement of the sella turcica and the pituitary gland in the face of hypersecretion of TSH (see Fig. 26.70) as well as associated galactorrhea has led to the misdiagnosis of a pituitary neoplasm. The hypothyroidism, incomplete sexual maturation, galactorrhea, and pituitary enlargement are reversed or corrected by levothyroxine therapy within a few months.

In 1960, Van Wyk and Grumbach[323] suggested that the syndrome resulted from hormonal overlap in negative feedback regulation with increased secretion of gonadotropins, prolactin, and TSH as a consequence of the chronic hypothyroidism. With the advent of radioimmunoassays for pituitary hormones, increased prolactin secretion was documented in children and adults with primary hypothyroidism and in affected girls with the syndrome. GH release is usually decreased, as in uncomplicated primary hypothyroidism.

However, the explanation for the sexual maturation remains uncertain. Pubertal development in primary hypothyroidism is usually delayed and is only rarely advanced for chronologic age. By the use of radioimmunoassays or other methods for FSH and LH in which the cross reaction with TSH is negligible, an increased (pubertal) concentration of plasma immunoreactive and bioactive FSH, but not LH, has been detected. Bioactive LH activity is also low. Increased FSH pulsatility, mainly at night, but not LH release was demonstrated in patients with the syndrome and in some children with primary hypothyroidism who did not exhibit premature sexual maturation. The increased FSH release[888] and the high FSH/LH ratio (in contrast to that observed in normal puberty) seem to account for the increased ovarian estrogen secretion in girls and for the enlarged testes without signs of virilization in affected boys; the suggestion is that FSH-induced Sertoli cell proliferation is an important determinant of mature testis size. Prolactin is postulated to suppress LH in this condition.

Pulsatile TSH release is increased at night, and administration of TRH appears to increase FSH release in normal children (but not in adults). Moreover, the FSH response to TRH, but not GnRH, is augmented in primary hypothyroidism, and this response can occur in gonadotropin-secreting pituitary adenomas. It is likely that the incomplete sexual precocity and the increased prolactin secretion and galactorrhea are a consequence of the increased release of TRH, the increased sensitivity of the mammotrophs and gonadotroph to TRH, or both.[323] This mechanism, which has gained support, would explain the relatively rapid and complete reversal of the syndrome by levothyroxine treatment. Human recombinant TSH at a dose about 1000-fold greater than that of human FSH evoked a dose-dependent cAMP response in COS7 cells transfected with the



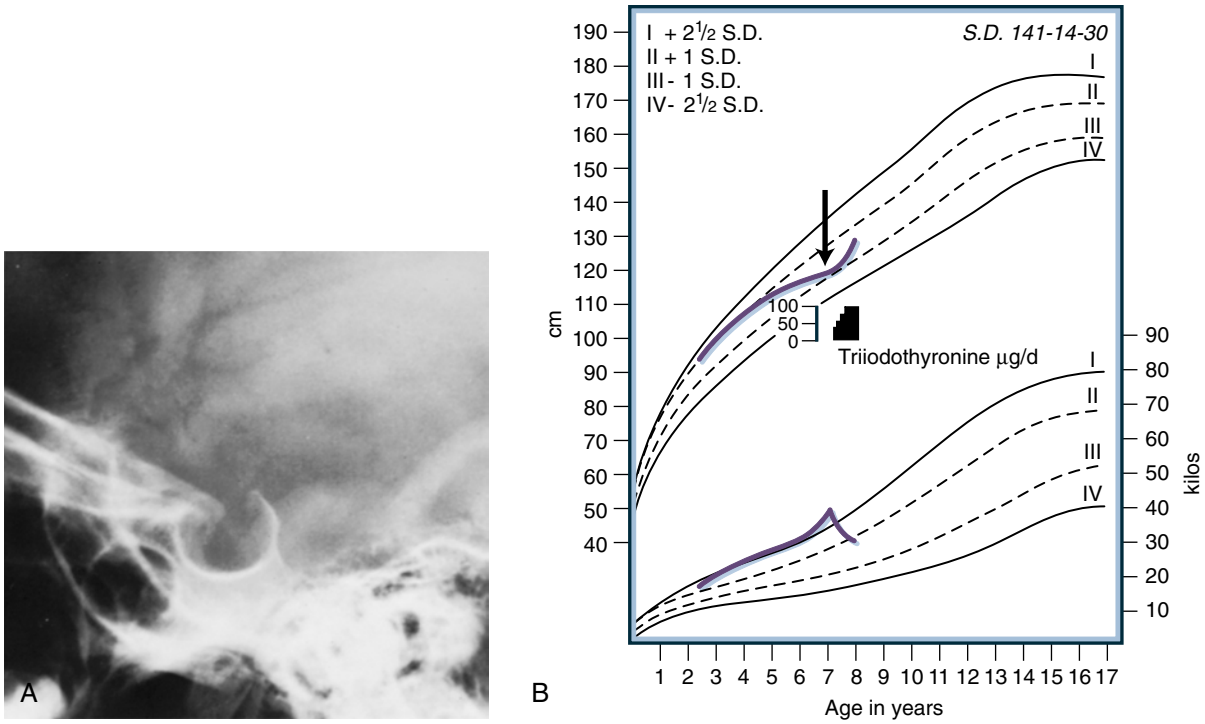
• **Fig. 26.69** (A, B) Severe, chronic hypothyroidism of Hashimoto thyroiditis in a girl aged 7 years and 1 month with sexual precocity (without pubic or axillary hair), episodic vaginal bleeding, and galactorrhea. She had symptoms of hypothyroidism and a sharply decreased rate of growth over the previous 2 years (height, −1 SD [standard deviation]; bone age, 5 years and 3 months). Breast development was Tanner stage 3, the labia minora were enlarged, and the vaginal mucosa was dull pink, thickened, and rugated, with evidence of an estrogenic effect. No acne, seborrhea, or hirsutism was present. The uterus was of adolescent size, and the endometrial mucosa was in a proliferative phase. Urinary gonadotropins were barely detectable by bioassay. (C) Striking change in appearance after 8 months of thyroid hormone treatment. She had grown 7 cm in height and lost 8.1 kg in weight. The breasts had decreased in size, galactorrhea was no longer demonstrable, the labia minora had regressed, and the vaginal mucosa was pink and glistening (no estrogen effect). Ten weeks after the initiation of thyroid hormone replacement therapy, she developed a right slipped capital femoral epiphysis that was repaired surgically; recovery was uneventful.

human FSH receptor, which suggests another possible but less likely mechanism for the FSH-dependent (or FSH-like–dependent) but GnRH-independent sexual precocity.[889] A direct effect of severe hypothyroidism on the prepubertal testis that leads to overproliferation of Sertoli cells also has been advanced as an explication of the macroorchidism.

The syndrome is rare but a few cases combine the Van Wyk-Grumbach syndrome with the Kocher-Debre-Semelaigne syndrome, which manifests as lower extremity muscle pseudohypertrophy, elevation of serum creatinine and creatinine kinase concentrations, delayed contraction and relaxation of reflexes, and myxedema; both manifestations of hypothyroidism can be treated with appropriate doses of thyroxine.[890,891]

Iatrogenic Sexual Precocity and Endocrine Disruptors. Prepubertal children are remarkably sensitive to exogenous gonadal steroids and may show signs of sexual maturation resulting from overlooked sources of androgens or estrogens, such as ingested or absorbed tonics, lotions, or hair creams or hair straighteners that contain or are inadvertently contaminated with an estrogen.[892] Dermal exposure to estrogen may add up to more than 300 μg, far in excess of a therapeutic dose and possibly greater in infants and children exposed to estrogen dermal gel. Compounds containing tea tree and lavender oils caused gynecomastia in three prepuberal boys and demonstrated estrogenic activity in vitro.[893,894] A short course of application of estrogen cream is used to treat labial adhesions, but long courses may lead to breast development or even withdrawal bleeding. In addition to breast development, pigmentation of the areolae and the linea alba and the appearance of pubic hair may be seen in children exposed to dermal estrogen. Children who touch the skin or the towels of men using androgen gel therapy may themselves develop virilization.[895] The administration of hCG to boys with undescended testes may induce secretion of testosterone sufficient to cause incomplete sexual precocity.

FDA guidelines define a limit of not more than 1% of normal daily estrogen production in prepubertal children as a safe intake of estrogen[835]; this is equivalent to 0.43 ng/day for boys and 3.24 ng/day for girls, based on data from extremely sensitive estrogen assays, but food is still a suspected source of endocrine disruptors.[835,892] Epidemics of gynecomastia in boys and thelarche in girls among schoolchildren in Italy were suspected to be caused by contaminated meat. During a 10-year period, more than 600 cases of gynecomastia in boys and premature thelarche or incomplete sexual precocity in girls were discovered in Puerto Rico; this is the highest prevalence reported in the world, about 10 to 15 times higher than that measured in a survey in Olmsted, Minnesota.[896,897] Maternal ovarian cysts were demonstrated in two-thirds of affected Puerto Rican girls. The clandestine use of



• **Fig. 26.70** (A) Radiograph of the skull of a patient with hypothyroidism shows an enlarged pituitary fossa in the lateral view. The dorsum sellae was thin and demineralized, and the floor had a double contour line. The area of the sella turcica was 150 mm^2. Pneumoencephalography showed a suprasellar mass impinging on the cisterna chiasmatica. After thyroid hormone treatment for 8 months, the volume of the sella had decreased 30% to 100 mm^2, the dorsum sellae had remineralized, and the double floor was no longer evident. (B) Growth curve illustrates the decrease in growth rate despite sexual precocity and the catch-up growth induced by thyroid hormone therapy. (From Van Wyk JJ, Grumbach MM. Syndrome of precocious menstruation and galactorrhea in juvenile hypothyroidism: an example of hormonal overlap in pituitary feedback. *J Pediatr.* 1960;57:416–435.)

estrogen preparations in animals to stimulate weight gain, leading to ingestion of estrogen-contaminated meat from these animals, was raised as a possible cause, but this was neither confirmed nor excluded by selected analyses of meat, poultry, and milk in Puerto Rico by the US Department of Agriculture.

There are rising concerns that endocrine-disrupting chemicals (EDCs), defined as "an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function" exert many effects on growth and development of children, including pubertal development.[852,898] Far more conclusive evidence of an adverse effect of EDCs comes from animal, rather than human, studies, and some of the data on human beings derive from industrial accidents and very high-level exposure rather than the lower level exposures most individuals experience, so EDCs must be studied in more detail in human beings. Method of ascertainment, laboratory methods, activity and half-life of various molecules of interest, and the frequent difficulties in drawing conclusions from associations rather than controlled studies present complexities in interpreting data.

Girls who were breastfed or exposed during intrauterine life to polybrominated biphenyls (PBBs) after an accidental exposure of their mothers in Michigan experienced early menarche (by about 1 year of age) and early appearance of pubic hair but not breast development, compared with girls who were not exposed or breastfed.

The NHANES 2003-2004 data demonstrate a slightly lower age at menarche with higher serum polybrominated diphenyl ether (PBDE, a flame retardant) concentrations.[899,900] On the contrary, a recent longitudinal study in Hispanic American children demonstrated a later age of menarche while boys had an earlier onset of pubic hair development with in utero exposure to PBDE.[901]

Exposure to polychlorinated biphenyls (PCBs) in inner-city girls with reduced BMI values reportedly delayed thelarche.[63] There was a 9.56 increase in relative risk for precocious puberty treated by GnRH agonist in a localized area in Italy, compared with surrounding areas, suggesting the presence of an endocrine disruptor in the area.[902] Recent longitudinal studies demonstrate no effect on secondary sexual development in girls with a slight decrease in the age of puberty in boys by elevated levels of phthalate excretion in urine.[900] A Chinese study found elevated levels of LH which corresponded to decreased levels of testosterone in children with constitutional delay in puberty who had elevated phthalates.[903]

Widespread exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), an extremely potent antiestrogenic xenobiotic, in Italy revealed that girls younger than 8 years of age at the time of exposure, who presumably had the highest dose per BMI compared with older girls, showed a tendency for a decrease in age at menarche, suggesting that age of exposure to environmental endocrine disruptors modulates their effects.[904] Blood concentrations of the phenols, 2,5-dichlorophenol and 2,4-dichlorophenol, were inversely proportional to the age of menarche in 440 girls included in the NHANES for 2003 to 2008.[904] Follow-up of adolescents

who were exposed to di-(2-ethylhexyl)-phthalate (DEHP), a component of polyvinyl chloride (PVC) that is used in plastic tubing and medical devices, when they underwent extracorporeal membrane oxygenation (ECMO) as neonates, demonstrated no effects on pubertal development despite findings of disruption of development in animals exposed to this substance, demonstrating difficulties in translating animal data to human beings.[905]

Increased lead levels in Mohawk girls living near the border of New York and Canada delayed the age of menarche, whereas increased levels of PCB promoted it[906]; surprisingly, variations in BMI exerted no effect of these toxic substances, which are concentrated in adipose tissue. In the NHANES III survey, a serum lead level of 0.7 to 2.0 μg/dL delayed menarche and pubic hair development, and African Americans exposed to 3 μg/dL also experienced delay in breast development.[908] A long-term study of puberty in Chapaevsk, Russia, an area highly contaminated with industrial waste, demonstrated 43% reduced odds of entering stage 2 genitalia among boys age 8 to 9 years with serum lead levels equal to or greater than 5 μg/dL.[909]

There is a high frequency of reproductive problems among adult Danish men, including impaired semen quality, testicular cancer, and increased rate of infantile testicular cancer; these disorders occur in a pattern that is attributed to environmental disruptors, described in the testicular dysgenesis syndrome.[669] A higher rate of hypospadias in Danish newborns and smaller testes with lower serum inhibin B, compared with Finnish infants, suggested that environmental agents played a role. Phthalates were found in the breast milk of mothers from both countries, and although there was no relationship to the finding of hypospadias, there was an indication of altered reproductive hormones in the boys in a pattern suggesting effects on Leydig cells. There may be a genetic component to susceptibility to the TDS based upon GWAS analysis, indicating a gene X environment basis for the disorder.[848]

Insulin-like peptide 3 (INSL3) is a testicular product associated with testicular descent in a chronologic pattern; amnionic values of INSL3 during the development of normal genitalia are affected by exposure to phthalates, which is postulated to offer a potential mechanism of the etiology of TDS, although this is not proven to date.[910,911]

Longitudinal observation of girls in Copenhagen, Denmark, using physical examination of breast tissue, showed a significant decline in the age of Tanner stage 2 breast development (estimated mean age, 9.86 years in 2006–2008 vs 10.88 years in 1991) and the age at menarche (13.13 vs 13.42 years, respectively); there was no change in serum gonadotropins, but the serum estradiol concentration was decreased in 2006.[54] Changes in BMI did not occur in this study between the cohorts, leaving open the possibility of endocrine-disrupting chemicals as an explanation.

Boys exposed to PCBs and polychlorinated dibenzofurans (PCDFs) in utero from contamination of rice ingested by their mothers had decreased testosterone and defects in postpubertal sperm production in a preliminary study, as well as increased estrogen compared with control subjects, although there was no difference on physical examination or in age at onset of puberty.[912] Boys exposed to DDT in utero did not demonstrate any abnormalities in puberty in a study from Philadelphia, Pennsylvania.[854]

Proximity to traffic in a longitudinal cohort study of 437 girls found an earlier onset of signs of puberty of 2 to 9 months compared to unexposed girls. This suggests exposure to air pollutants affects the onset of puberty, although no pollutants were measured in this study.[913] The complexities of the study of endocrine disrupters on pubertal timing are complicated by type, amount, developmental age at exposure, and other factors that are difficult to tease apart.[914] In the United States, chemicals can enter the environment before their safety is proved, leading to an ever-expanding list of potential EDCs without data to prove their effects. Environmental disrupters have a wide range of deleterious effects upon neurodevelopment as well as the endocrine system.[915]

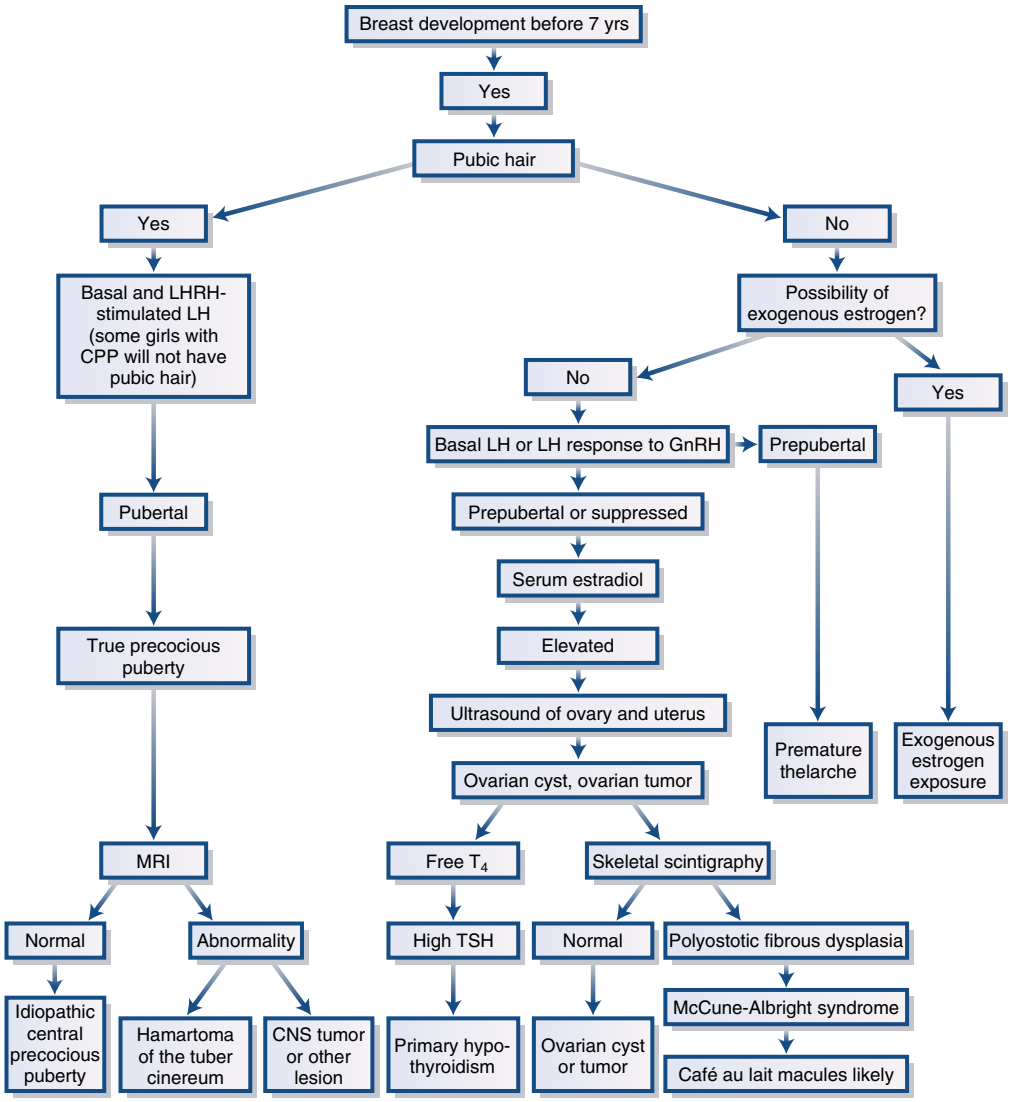
Well-designed longitudinal studies are needed in this area.[910]

Diagnosis of Sexual Precocity

As with delayed puberty the majority of patients referred for precocious puberty will prove to have variations of normal development or to be inappropriately referred due to a misinterpretation of the normal ages of puberty by the referring physicians. However, precocious puberty can certainly be the outward sign of a serious condition. Thus the separation of patients with self-limited benign disorders, such as premature adrenarche, premature thelarche, or normal but early puberty, from those with serious or even potentially fatal disorders is the first step in evaluation (Figs. 26.71, 26.72, and 26.73; Table 26.41). The history may reveal symptoms suggesting perinatal CNS abnormalities or injuries, previous infections, adventitious ingestion of or exposure to gonadal steroids, or the presence of similar conditions in family members. Previous measurements should be plotted on a growth chart to determine height velocity and the age at onset of any increase in the rate of growth.

Important aspects of the physical examination include description of the secondary sexual development according to Tanner (sexual maturation) stages; measurement of the penis (length gently stretched and width) and the testes in boys (greatest diameter without the epididymis or volume by comparison with an orchidometer) and of the breast tissue in girls (areolar and glandular diameters); and examination for comedones and acne, oily skin, facial and body hair, pubic and axillary hair development, axillary apocrine gland odor, muscular development, and galactorrhea. A careful examination of the external genitalia should be done with a nonrelated chaperone present. A thorough neurologic examination is indicated, with emphasis on assessment of the visual fields and optic discs in a search for signs of increased intracranial pressure; evaluation for skin lesions associated with the McCune-Albright syndrome or neurofibromatosis; and examination for abdominal, gonadal, or adnexal masses and for coexisting endocrine disease. Bone age is determined in all cases, although it is an imperfect measure and the reader must be experienced. Dental development can be determined as a rough index of pubertal development[916] but is rarely invoked clinically except in forensic cases.[917]

Ultrasonography of the ovary and uterus is exceedingly useful in the evaluation of affected girls, because standards are available for shape and volume of the uterus and the ovaries.[918] The largest measurements of uterine size by sonography in infants and children are found at puberty and in the neonatal period. The upper limit of uterine length in the prepubertal state is 3.5 cm. A uterine volume of greater than 1.8 mL is specific for the onset of normal puberty, but increased ovarian volume is less specific. Patients with premature thelarche were indistinguishable from age-matched control subjects when this sonographic standard was used.[128] The presence of microcysts and macrocysts of the ovary also can be detected on ultrasound examination. Cysts may be found in the ovaries in patients with CPP or

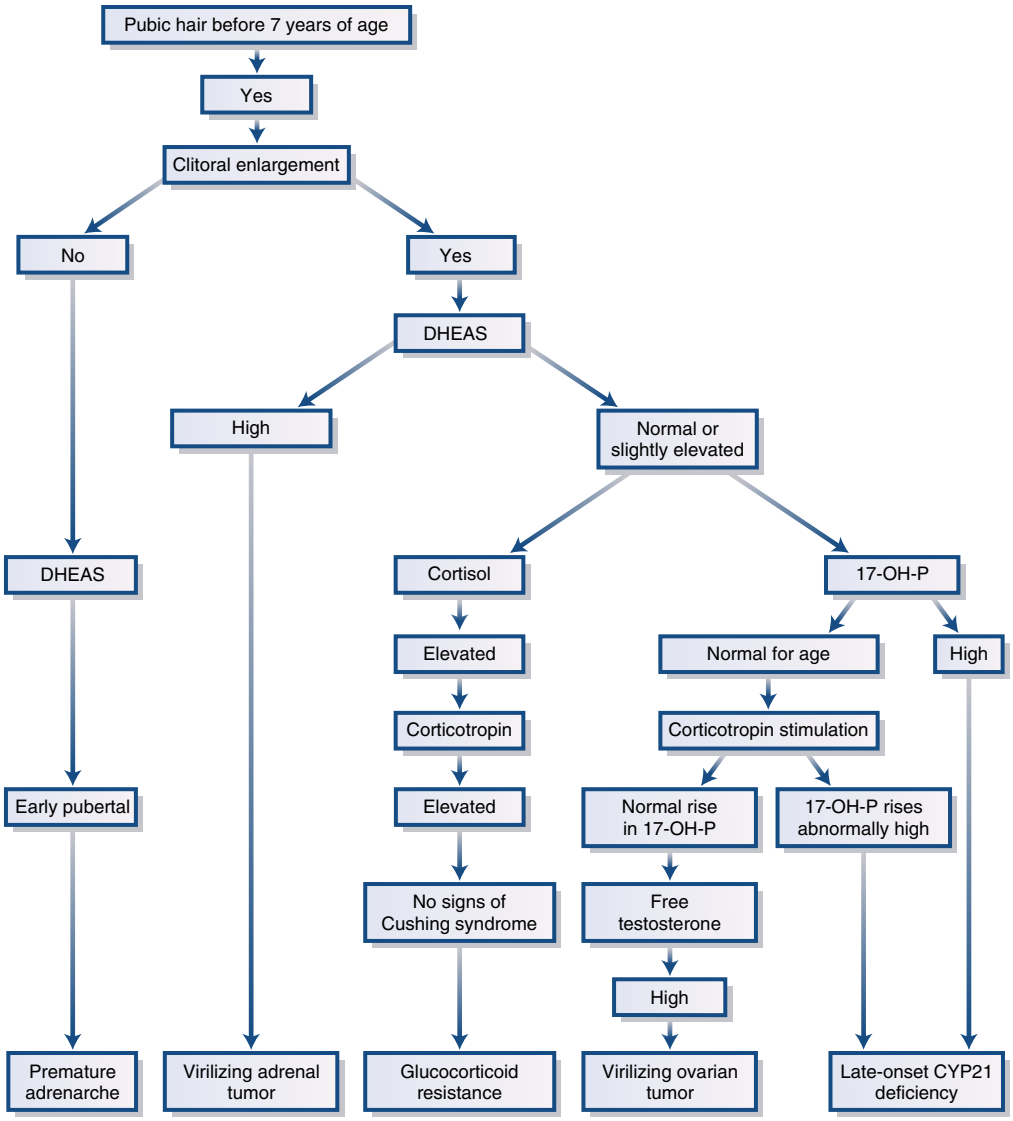


• **Fig. 26.71** Flow chart for diagnosing sexual precocity in girls. *CNS,* central nervous system; *CPP,* central precocious puberty; *FSH,* follicle-stimulating hormone; *GnRH,* gonadotropin-releasing hormone; *LH,* luteinizing hormone; *LHRH,* LH-releasing hormone; *MRI,* magnetic resonance imaging; T_4, thyroxine; *TSH,* thyroid-stimulating hormone.

GnRH-independent ISP; they usually are smaller than 9 mm in the former and larger than 9 mm in the latter[919] condition. Ovarian volume is reportedly the best indicator of precocious puberty, and uterine length was best for the differentiation of premature thelarche from premature puberty.[920] However, in the earliest stages of pubertal development ultrasonography of the pelvis may not differentiate the patient from the prepubertal state, although gonadotropin values will do so.[921] The presence of an endometrial stripe was indicative of precocious puberty.[128] Ultrasonography of the breast is reported as a method of determining rapidly progressive versus slowly progressive or transient precocious puberty; accuracy is increased when breast ultrasound findings are added to those of uterine ultrasound and the other factors discussed previously.[922]

CPP in males usually begins with enlargement of the testes, followed by other signs of secondary sexual maturation. A Leydig cell tumor usually causes asymmetric enlargement of the testes, whereas an extragonadal hCG-secreting tumor is associated with less marked testicular enlargement than occurs at the same stage of masculinization in CPP. Testicular adrenal rest tissues may enlarge in boys with CAH and may be bilateral, although they are unlikely to closely mimic normal pubertal testicular development. An elevated hCG level with prepubertal gonadotropin values or GnRH test indicates an ectopic, autonomous, gonadotropin-secreting tumor. If this tumor is in the CNS, abnormalities will likely be present on MRI or CT brain scans. Enlargement of the liver or a mediastinal, retroperitoneal mass in boys with sexual precocity suggests an hCG-producing hepatic or germ cell tumor; the possibility of Klinefelter syndrome needs to be considered in the latter case.

It is essential that laboratory tests for sex steroids and gonadotropin be carried out by appropriate methods in the experienced laboratories. Simply ordering LH or FSH levels in a standard laboratory will usually lead to a determination of whether there are high levels with gonadal failure such as menopause or normal levels for adults, but rarely can such an assay determine subtle changes that are characteristic of childhood and adolescence. A highly sensitive third-generation assay with pediatric standards

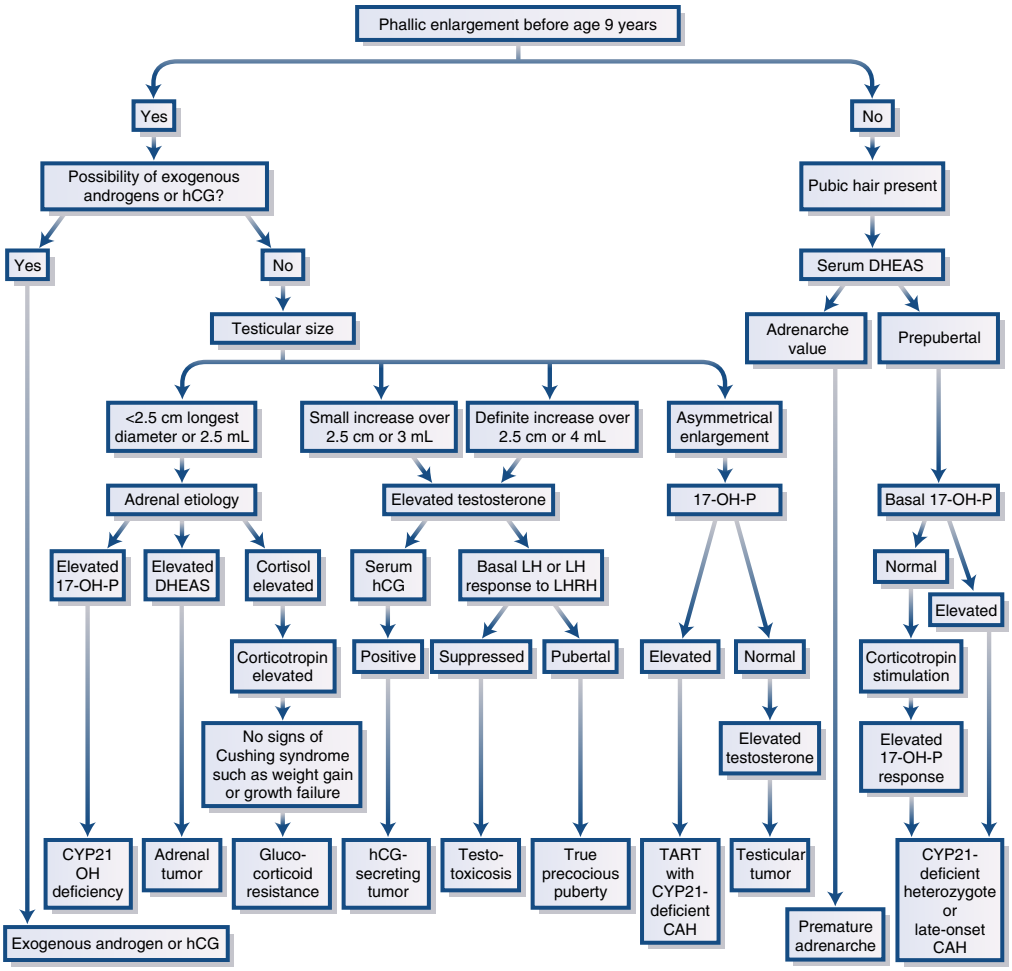


• **Fig. 26.72** Flow chart for the evaluation of pubic hair in normal phenotypic girls before 7 years. *DHEAS,* dehydroepiandrosterone sulfate; *17-OH-P,* 17-hydroxyprogesterone.

must be ordered. Further, standard testosterone assays can differentiate between a normal male and one with gonadal failure but cannot determine the low levels characteristic of the early stages of puberty. For children and adolescents (and indeed for women) an HPLC-MS/MS method with pediatric standards is necessary; the same type of assay must be used for the determination of estradiol levels in girls.[266]

Measurements of basal plasma gonadotropin concentrations and the LH response to administration of GnRH (presently not available) or GnRH agonist or the amplitude and frequency of LH pulses, especially at night, using third-generation assays with pediatric standards, as well as measurements of the plasma concentration of testosterone in boys or of estradiol in girls using LC/MS-MS assays, are of primary importance in diagnosis. Patients with a basal LH level 0.3 IU/L or higher had subsequent pubertal progression, whereas 39 of 41 patients with a basal LH 0.2 IU/L or lower did not progress, resulting in 100% specificity (95% CI 92–100%) and 90.5% sensitivity (69.6–98.8%) in one report.[261] Girls early in the course of CPP may have elevation of estradiol (this is not always noted, however) associated with increasing LH levels but not necessarily an increase in the concentration of FSH. Boys will have rising values of testosterone as puberty commences. Pubertal concentrations of LH and FSH, a pubertal mode of pulsatile LH secretion (initially during sleep), or pubertal LH response to GnRH or GnRH agonist confirms the diagnosis of CPP (and in boys differentiates CPP from familial testotoxicosis). Determination of T_4 concentration (usually free T_4) is indicated when hypothyroidism as a cause of sexual precocity is suspected.

A CNS tumor must be considered as a potential cause of premature activation of the hypothalamic GnRH pulse generator, especially in boys. The evaluation for a CNS tumor as a cause of CPP is similar to the investigation of an hCG-secreting tumor of the CNS.[923] Although CT scanning is a well-established procedure for determining the presence of a CNS abnormality, MRI with contrast is more sensitive for the detection of small tumors in the hypothalamus, such as a hamartoma of the tuber cinereum (see Fig. 26.55). The use of contrast adds to diagnostic certainty and is recommended for MRI of the CNS. All boys with CPP



• **Fig. 26.73** Flow chart for diagnosing sexual precocity in a phenotypic male. *CAH,* congenital adrenal hyperplasia; *DHEAS,* dehydroepiandrosterone sulfate; *hCG,* human chorionic gonadotropin; *LH,* lutein-izing hormone; *LHRH,* LH-releasing hormone; *17-OH-P,* 17-hydroxyprogesterone; *TART,* testicular adrenal rest tissue.

should have CNS MRI evaluation, but girls do not always receive the same recommendation because a CNS tumor is less likely in girls than in boys to be the cause of CPP. However, studies using MRI or CT brain scans indicate that the hypothalamic hamartoma is more prevalent in both boys and girls with so-called idiopathic CPP than was previously suspected. An unselected group of girls with precocious puberty and no other symptoms underwent CNS MRI; 15% were found to have intracranial trauma, and the investigators found no clinical difference between those girls and the other 85% of girls studied, supporting the use of CNS MRI for girls with precocious puberty.[924]

The height of the pituitary gland on MRI correlates with advancing age and with pubertal development[925]; patients with CPP had pituitary heights exceeding 6 mm on average, whereas those with precocious thelarche had lower heights. The shape of the pituitary gland is also of importance: A convex appearance rather than a flat top is associated with CPP of any cause.[749] Physiologic enlargement of the pituitary gland characteristic of puberty is the source of many needless neurosurgery referrals.[926] The size and shape of the pituitary gland do not decrease with successful GnRH therapy.

T1-weighted images indicate a convex upper border of the pituitary gland in both normal patients and those with CPP, indicating the similarity in the physiologic changes in both pubertal conditions. Pituitary gland hyperplasia (height >1 cm) is a rare finding reported in CPP. The empty sella syndrome is less frequently observed in patients with CPP than in patients with pituitary hypofunction. Empty sella is found in 10% of children who are imaged for suspected hypothalamic-pituitary disorders, including hypogonadotropic hypogonadism.[927]

The premature appearance of pubic hair, phallic enlargement, and other signs of virilization in a male without enlargement of the testes or the liver suggests the diagnosis of virilizing CAH, virilizing adrenal tumor, or, more rarely, Cushing syndrome. Measurement of plasma 17-hydroxyprogesterone and DHEAS concentrations and their suppressibility with glucocorticoids will distinguish 21OH-deficient CAH from a virilizing adrenal tumor. If growth rate is suppressed, the possibility of primary hypothyroidism or Cushing syndrome is raised; elevated plasma concentrations of cortisol, urinary free cortisol, 17-hydroxycorticosteroid, or salivary cortisol after suppression with dexamethasone confirm the latter diagnosis. The appearance in a girl of pubic hair and other signs of virilization, such as clitoral enlargement, acne, deepening voice, muscular development, or growth spurt, is caused by virilizing CAH, virilizing adrenal tumor, or virilizing ovarian tumor. Cushing syndrome caused by an adrenocortical

TABLE 26.41 Differential Diagnosis of Sexual Precocity

Disorder	Plasma Gonadotropins	LH Response to GnRHa	Serum Sex Steroid Concentration	Gonadal Size	Miscellaneous
Gonadotropin Dependent					
True precocious puberty	Prominent LH pulses (premature reactivation of GnRH pulse generator)	Pubertal LH response initially during sleep	Pubertal values of testosterone or estradiol	Normal pubertal testicular enlargement or ovarian and uterine enlargement	MRI of brain to rule out CNS tumor or other abnormality; skeletal survey for McCune-Albright syndrome (by US)
Incomplete Sexual Precocity (Pituitary Gonadotropin-Independent)					
Males					
Chorionic gonadotropin-secreting tumor in males	High hCG, low LH	Prepubertal LH response	Pubertal value of testosterone	Slight to moderate uniform enlargement of testes	Hepatomegaly suggests hepatoblastoma; CT scan of brain if chorionic gonadotropin-secreting CNS tumor suspected
Leydig cell tumor in males	Suppressed	No LH response	Very high testosterone	Irregular, asymmetric enlargement of testes	
Familial testotoxicosis	Suppressed	No LH response	Pubertal values of testosterone	Testes symmetric and >2.5 cm but smaller than expected for pubertal development; spermatogenesis occurs	Familial; probably sex-limited, autosomal dominant trait
Virilizing congenital adrenal hyperplasia	Prepubertal	Prepubertal LH response	Elevated 17-OHP in CYP21 deficiency or elevated 11-deoxycortisol in CYP11B1 deficiency	Testes prepubertal	Autosomal recessive, may be congenital or late-onset form, may have salt loss in CYP21 deficiency or hypertension in CYP11B1 deficiency
Virilizing adrenal tumor	Prepubertal	Prepubertal LH response	High DHEAS and androstenedione values	Testes prepubertal	CT, MRI, or US of abdomen
Premature adrenarche	Prepubertal	Prepubertal LH response	Prepubertal testosterone, DHEAS, or urinary 17-ketosteroid values appropriate for pubic hair stage 2	Testes prepubertal	Onset usually after 6 yr of age; more frequent in CNS-injured children
Females					
Granulosa cell tumor (follicular cysts may present similarly)	Suppressed	Prepubertal LH response	Very high estradiol	Ovarian enlargement on physical examination, CT, or US	Tumor often palpable on physical examination
Follicular cyst	Suppressed	Prepubertal LH response	Prepubertal to very high estradiol	Ovarian enlargement on physical examination, CT, or US	Single or recurrent episodes of menses and/or breast development; exclude McCune-Albright syndrome
Feminizing adrenal tumor	Suppressed	Prepubertal LH response	High estradiol and DHEAS values	Ovaries prepubertal	Unilateral adrenal mass
Premature thelarche	Prepubertal	Prepubertal LH, pubertal	Prepubertal or early estradiol response	Ovaries prepubertal	Onset usually before 3 yr of age

TABLE 26.41 Differential Diagnosis of Sexual Precocity—cont'd

Disorder	Plasma Gonadotropins	LH Response to GnRHa	Serum Sex Steroid Concentration	Gonadal Size	Miscellaneous
Premature adrenarche	Prepubertal	Prepubertal LH response	Prepubertal estradiol; DHEAS or urinary 17-ketosteroid values appropriate for pubic hair stage 2	Ovaries prepubertal	Onset usually after 6 yr of age; more frequent in brain-injured children
Late-onset virilizing congenital adrenal hyperplasia	Prepubertal	Prepubertal LH response	Elevated 17-OHP in basal or corticotropin-stimulated state	Ovaries prepubertal	Autosomal recessive
In Both Sexes					
McCune-Albright syndrome	Suppressed	Suppressed	Sex steroid pubertal or higher	Ovarian (on US); slight testicular enlargement	Skeletal survey for polyostotic fibrous dysplasia and skin examination for café au lait spots
Primary hypothyroidism	LH prepubertal; FSH may be slightly elevated	Prepubertal FSH may be increased	Estradiol may be pubertal	Testicular enlargement; ovaries cystic	TSH and prolactin elevated; T_4 low

CNS, Central nervous system; *CT,* computed tomography; *CYP,* P450 cytochrome isoenzyme; *DHEAS,* dehydroepiandrosterone sulfate; *GnRH,* gonadotropin-releasing hormone; *hCG,* human chorionic gonadotropin; *LH,* luteinizing hormone; *MRI,* magnetic resonance imaging; *17-OHP,* 17-hydroxyprogesterone; T_4, thyroxine; *TSH,* thyrotropin; *US,* ultrasonography.

carcinoma can result in virilization associated with growth failure, and a virilizing adrenocortical carcinoma can manifest with so much androgen effect that the signs of Cushing syndrome are not apparent, as rapid growth and virilization are first noted; estradiol may be secreted by these tumors as well as androgens. Virilizing ovarian tumors may be detected by pelvic ultrasonography.

The appearance of pubic hair without other signs of puberty in boys or girls is usually a result of premature adrenarche but may alternatively be the first sign of sexual precocity or of adrenal virilism from other causes.

In a girl, breast development associated with dulling and thickening of the vaginal mucosa leading to a more pink than red appearance and enlargement of the labia minora indicate significant estrogen secretion or iatrogenic exposure to estrogen. The differential diagnosis includes CPP, an estrogen-secreting neoplasm, aromatase excess, and a cyst of the ovary. If the plasma concentrations of gonadotropins are in the pubertal range, if LH pulses of pubertal amplitude are detected, or if a pubertal LH response to GnRH or GnRH agonist is elicited, CPP is present. In one report, a child had pubertal-level serum LH due to heterophile antibodies that interfered with the LH assay and fallaciously elevated the values in the basal and stimulated state; after addition of antimouse antibody, LH values decreased.[928] As always, clinical observation should be congruent with laboratory findings, and the assays should be highly sensitive and associated with valid pediatric standards. Estradiol concentrations in girls early in normal puberty or CPP are in the prepubertal range for much of the day, and a single determination may be inadequate to reflect ovarian function.[160]

If the concentration of plasma estradiol is elevated but gonadotropin levels are low, an estrogen-secreting cyst or neoplasm is present or exogenous estrogens are the cause. Ovarian tumors of moderate size can be palpated by bimanual examination. Advances in pelvic sonography allow the delineation of ovarian cysts or tumors and the determination of uterine size and the size of the endometrial stripe.[128] An estrogen-secreting neoplasm of the ovary is usually accompanied by high estradiol concentrations, but some ovarian cysts are associated with concentrations of estradiol as high as those in granulosa cell tumors; the differential diagnosis between these cysts and ovarian neoplasms rarely requires exploratory laparotomy or laparoscopy and usually can be resolved by pelvic sonography and the use of tumor markers. Breast development in the absence of other estrogen effects is almost always a result of premature thelarche.

Contrasexual Precocity: Feminization in Boys and Virilization in Girls

Boys

Feminization in a boy before the age of puberty is rare. Rarely, an estrogen-secreting adrenal adenoma or a chorionepithelioma causes gynecomastia. Gynecomastia has been reported in a 1-year-old boy with 11β-hydroxylase deficiency and in boys with late-onset CAH.[929]

Aromatase Excess Syndrome. Gynecomastia in prepubertal boys can be caused by increased extraglandular aromatization of C19 steroids of adrenal origin, such as androstenedione, and subsequent increased extraglandular estrogen production in sporadic or familial cases. The autosomal dominant form leads to excess estrogen synthesis from C19 precursors due to aromatase overexpression, especially in fat and skin; it is a consequence of gain-of-function mutations of *CYP19*, the gene that encodes aromatase, resulting from a chromosome arrangement that gives rise to a cryptic promoter.[930] An autosomal dominant pattern of prepubertal gynecomastia and adult hypogonadism but not short stature in the presence of elevated serum estrone (with little or no elevation of estradiol) has been reported; the mutations in the *CYP19* gene in these patients appeared to be different from those in the families described earlier with gain-of-function mutations resulting from gene inversions.[931] In a Turkish kindred, there was a potential rearrangement between *CYP19* and *TRPM7* genes on chromosome 15q21.2 as a cause of aromatase excess syndrome. Aromatase excess syndrome is caused by duplications involving *CYP19A1* and simple and complex rearrangements generated by

both recombination-mediated and replication-mediated mechanisms, independent of the known rearrangement-inducing DNA features or late-replication timing.[932]

Feminizing Testicular Tumors. Feminizing testicular tumors may cause gynecomastia in boys younger than 6 years of age who have the Peutz-Jeghers syndrome.[860,933] Aromatase is absent or is present in barely detectable amounts in prepubertal testes, but maximal amounts appear in late puberty. In normal testes, aromatase is predominantly present in the Leydig cells, but in testicular tumors of Sertoli cells or Leydig cells (e.g., associated with the Peutz-Jeghers syndrome), the Sertoli cells of the tumor express aromatase. Both testes may be enlarged, and the histologic examination indicates sex cord or Sertoli cell tumors that form annular tubules and often have areas of calcification; increased estradiol secretion is noted in the basal state, and a further rise occurs after hCG administration. Otherwise, feminizing Sertoli cell tumors are very rare in boys.[934] Sonography or MRI of the testes may be useful in making the diagnosis.

In one series, 5% of 581 boys referred for evaluation of gynecomastia were prepubertal at diagnosis (mean age, 9 years), and in 93% no underlying cause was identified.[935] Spontaneous resolution was recorded in 6 boys, no change was found in 15, and further breast enlargement was found in 6. Prepubertal gynecomastia can also be caused by neurofibromatosis.

Girls

Adrenal Causes of Virilization. CAH resulting from 21-hydroxylase or 11β-hydroxylase deficiency or from androgen-producing tumors of the adrenal can cause virilization (see earlier discussion of their occurrence in males). Nonclassic or late-onset forms of CAH do not demonstrate ambiguous genitalia, but there is evidence of androgen effect in prepuberty or the teenage years. The etiology is usually 21OH deficiency. 3βHSD/$\Delta^{4,5}$-isomerase deficiency is a rare type of CAH characterized by elevated levels of Δ^5-17P, DHEA, and DHEAS, as well as decreased secretion of aldosterone and cortisol in the severe form. Severely affected patients have mineralocorticoid and glucocorticoid deficiencies and may die in infancy. Excess adrenal androgens lead to virilization in utero and to ambiguous external genitalia, including clitoral enlargement in females with continued virilization after birth. Milder forms of this disorder can cause hirsutism in women. Women with a 46,XY phenotype and incomplete forms of androgen resistance syndrome or 17βHSD3 deficiency may have virilization as well as breast development at the time of expected puberty. Aromatase deficiency due to mutations in the *CYP19* gene, which encodes aromatase, is associated with intrauterine masculinization of the external genitalia in affected 46,XX individuals and with progressive virilization, lack of female secondary sex characteristics, multicystic ovaries at the age of puberty, tall stature, and osteopenia.[272,274,301,303]

Cushing syndrome resulting from adrenal carcinoma usually manifests as growth failure with or without virilization, obesity, and moon facies; striae may not appear until months to years later.

Syndrome of Glucocorticoid Resistance. The syndrome of glucocorticoid resistance has variable manifestations. Some patients demonstrate hyperandrogenic signs such as acne, hirsutism, male-type baldness, menstrual irregularities, and oligoanovulation and infertility.[936] Dexamethasone decreases the excessive adrenal androgen secretion, virilization, and advancing bone age found in general glucocorticoid resistance.

Virilizing Ovarian Tumors. Arrhenoblastoma, also called Sertoli tumor of the ovary, is the most common virilizing ovarian tumor, but it is rare in children.[871] Recently somatic missense mutations affecting the RNase IIIb domain of DICER1 that alter DICER1 function in a manner that perturbs microRNA processing to become oncogenic are common in these nonepithelial ovarian tumors.[937] Lipoid-cell tumors of the ovary and gonadoblastomas of the ovary are even more unusual sources of androgens.

Variations of Pubertal Development

Premature Thelarche

Unilateral or bilateral breast enlargement without other signs of sexual maturation (e.g., sexual hair, growth of the labia minora, growth of the uterus) is not uncommon in infancy and childhood and is termed *premature thelarche*. The disorder usually occurs by age 2 (>80% of cases) and rarely after age 4.[938] In a retrospective study in Minnesota, the incidence of premature thelarche was 21.2 per 100,000 patient-years, 60% of cases were identified in patients between 6 months and 2 years of age, and most cases regressed within 6 months to 6 years after diagnosis, although a few persisted until puberty. The prevalence of premature thelarche appears to be increasing in Denmark.[940] When 10-year to 35-year follow-up of premature thelarche was available, no untoward effects on later health, growth, or fertility were evident.[897] Breast enlargement usually regresses after a few months but occasionally persists for years or until the onset of normal puberty; in about half of affected girls, the breast development, which is characteristically cyclic, lasts 3 to 5 years, and this is found with age of onset over 2 years.[939] Usually significant nipple and areola development is absent, and estrogen-induced thickening and dulling of the vaginal mucosa is uncommon. Enlargement of the uterus on ultrasonography (volume >1.8 mL, length >36 mm) is rare. Measurement of the ellipsoid volume of the uterus (V = longitudinal diameter × anteroposterior diameter × transverse diameter × 0.523) is the most sensitive and specific discriminator between premature thelarche and early[128] CPP and provides better early discrimination than the LH response to GnRH or GnRH agonist. Growth in stature is normal.

This is a benign, self-limited disorder that is compatible with normal pubertal development at an appropriate age; usually, only reassurance and follow-up are necessary. However, the appearance of premature thelarche can be the harbinger of further sexual maturation in a minority of cases. Although onset occurring soon after birth and before 2 years of age carries a higher prognosis for regression, 14% of two large series did progress no matter the age of onset of premature thelarche.[940] Because the breast development may be unilateral, it is important to consider the condition in girls who have unilateral breast development so that needless worry about a breast neoplasm is not stimulated in the parents and no unnecessary surgical procedure is carried out. Removal of tissue in premature thelarche may leave the child with no possibility of future breast development. In selected instances, sonography of the breast is useful to distinguish unilateral premature thelarche from less benign conditions but is of limited value in differentiating premature thelarche from precocious puberty.[941] The most common cause of a breast mass in a pubertal girl is fibroadenoma, and although metastatic disease may locate in the pubertal breast, breast carcinoma is exceedingly rare in young patients. Plasma estradiol levels are prepubertal in most standard assays but were slightly higher for age in patients with premature thelarche determined by a highly sensitive estrogen bioassay.[942,943] However, there is usually no significant increase in plasma levels of TeBG or in

thyroxine-binding globulin, which are indicators of estrogen action on circulating plasma proteins, and TeBG is suggested as one method of differentiating premature thelarche from PP.[940]

The urocytogram often reveals an estrogen effect on squamous epithelial cells in the urine in premature thelarche.[840,944]

Some endocrine measurements are proposed to differentiate PT from CPP. The concentration of serum FSH may be in the pubertal range, nocturnal FSH pulsatility has been detected, and the rise in FSH elicited by administration of GnRH may be augmented for chronologic age, with an FSH/LH ratio higher in precocious thelarche than in normal individuals or in girls with CPP. Girls under 48 months with mild premature thelarche had $17\beta E_2$ levels below 32 pmol/L using extraction RIA, while those with CPP have values over 70.[935] However, these methods are not infallible in differentiating the two conditions.

As postulated for some recurrent ovarian cysts, premature thelarche appears to result from the ovarian response to transient increases in FSH levels and possibly from variations in ovarian sensitivity to FSH. The LH response to GnRH is prepubertal in all cases.[437,945] Plasma inhibin B and FSH levels are higher in girls with precocious thelarche than in control subjects, in a range similar to that observed in patients with precocious puberty. Activin concentrations have not been reported. The possible role of a paracrine-acting pituitary factor in stimulating FSH independent of GnRH is not known.

Sonograms of the ovary may show one or several cysts larger than 0.5 cm that disappear and reappear, usually correlating with changes in the size of the breasts, but the volume of the ovary and uterus is prepubertal.[128] In clinical practice, it is rare to find a cyst at the time of presentation and on ultrasonic study.

Exaggerated thelarche is described as premature thelarche with the added findings of advanced bone age and increased growth rate, which are estrogen effects. The endocrine measurements in the basal state are in the normal prepubertal range, but after GnRH agonist stimulation, the level of FSH (but not of LH) rose higher than in control subjects or in patients with CPP. Mutation of *GNAS1* involving Arg201His is associated with apparent premature or exaggerated thelarche and early menarche.[730]

Unfortunately, there are no guidelines that can determine which girl will undergo progression from premature thelarche to precocious puberty. Clinical follow-up is essential to determine which course will occur.

Premature Isolated Menarche

Rarely, girls begin periodic vaginal bleeding at between 1 and 9 years of age without any other signs of secondary sexual development. The bleeding can recur for 1 to 6 years and then cease. At the normal age of puberty (3–11 years later), secondary sexual development and menses ensue and follow a normal pattern, as does stature.[946]

Fertility was later demonstrated after a normal onset of puberty in women with this variant of pubertal development. The cause is uncertain, but it may be a counterpart of premature thelarche. There is a predominance of FSH secretion, but the gonadotropin secretion pattern is not characteristic of CPP.[947] Isolated menarche may appear before other manifestations of sexual precocity in patients with the McCune-Albright syndrome and in those with the premature sexual maturation that can occur in juvenile hypothyroidism.

Before the diagnosis of premature menarche is accepted, all other causes of vaginal bleeding and precocious estrogen secretion and of exposure to exogenous estrogens should be excluded, including neoplasms, granulomas, infection of the vagina or cervix, and presence of a foreign body. A careful examination for trauma, such as that caused by sexual abuse, is indicated. Urethral prolapse may be misdiagnosed as vaginal bleeding.

Premature Adrenarche

Premature adrenarche causes the precocious appearance of pubic hair (i.e., pubarche), axillary hair and apocrine axillary odor, comedones, and acne, without other signs of puberty or virilization. It is characterized by premature and mild adrenal hyperandrogenism.[444,948] The term *premature adrenarche* refers to the rise in serum concentrations of adrenal androgens that cause the appearance of the pubic hair, which is termed *premature pubarche*. In the past, this designation was assigned when these clinical features appeared before age 8 years in girls or 9 years in boys. Although in boys the 9 years still seems appropriate as a cutoff point, the age of 8 years may no longer be appropriate for girls, according to the results of previously quoted studies showing earlier onset of puberty in recent years (mean ages are shown in Table 26.1). We recommend that the diagnosis of premature pubarche should be limited to African-American girls younger than 6 years of age and Caucasian American girls younger than 7 years, which should affect the age at which laboratory studies are initiated unless there are other signs of virilization, such as clitoromegaly or rapid growth.

Premature adrenarche is about 10 times more common in girls than in boys.[289,949,950] The prevalence is increased in children with CNS abnormalities without a clear sex difference; the electroencephalogram may be abnormal in the absence of other neurologic findings. Familial transmission is uncommon. Premature adrenarche is commonly slowly progressive and does not have an untoward effect on either the onset or the normal progression of gonadarche or final adult height.[444] Nonetheless, there is a relationship between reduced fetal growth leading to intrauterine growth retardation and subsequent SGA and the increased prevalence of premature adrenarche, and probably ovarian hyperandrogenism in life, although the relationship may vary among different ethnic groups.[951]

Plasma concentrations of DHEA, DHEAS, androstenedione, testosterone, 17-hydroxyprogesterone, and Δ^5-17P are usually comparable to values normally found in pubic hair stage 2. However, some patients have low concentrations of adrenal androgens, indicating that conversion of these molecule to more active androgens or a rise in androgen receptor activity is as important in the development of premature pubarche as the adrenal androgen secretion itself.[444] An ACTH stimulation increases serum DHEA and DHEAS concentrations and the excretion of urinary 17-ketosteroids, but the concentrations of plasma 17-hydroxyprogesterone and Δ^5-17P do not increase to the levels found in individuals with virilizing forms of CAH.[884] Shorter *AR* gene CAG number, indicative of increased androgen sensitivity, is reported in some girls with precocious adrenarche, suggesting that increased sensitivity to low androgen levels may be the basis rather than high androgen values per se. As in CAH, dexamethasone suppresses adrenal androgen and androgen precursor secretion.[952]

Serum gonadotropin levels in the basal state and after GnRH are in the prepubertal range in patients with premature adrenarche.[437] Premature adrenarche occurs independently of gonadarche and results from some unknown factor other than increased secretion of GnRH or ACTH. Bone age, height, and weight gain are slightly advanced for chronologic age, but normal adult height is commonly achieved, except, rarely, in some individuals with unusually high levels of adrenal androgens, hirsutism, acne, and a

bone age more than 2 years advanced or 2.5 SD above the mean value for chronologic age.[953] In a follow-up study of 20 girls, the functional adrenal hyperandrogenism in premature adrenarche was limited to childhood.

Premature adrenarche may be considered to be a developmentally regulated, normal variation in the differentiation, growth, and function of the zona reticularis of the adrenal cortex, marked biochemically by the precocious increase in the concentration of plasma DHEAS to more than 40 μg/dL.[290] The latter is probably related to the independent increase of 17,20-lyase activity in the developing zona reticularis, which is mediated by increased phosphorylation of serine and threonine residues on the CYP17 enzyme, and the increased abundance of cytochrome b_5 and of electron-donating redox partners such as cytochrome P450 oxidoreductase and cytochrome b_5, which are essential for the 17,20-lyase activity of this functional microsomal enzyme (see Fig. 26.36).[884] A rise in intra-adrenal cortisol may inhibit 3βHSD activity, thereby increasing DHEA according to in vitro evidence.[948] Nonetheless, the factors stimulating development and function of the zona reticularis, independent of ACTH, remain elusive.

The appearance of premature pubarche can be a manifestation of nonclassic 21-hydroxylase deficiency CAH caused by homozygous or compound heterozygous missense mutations in the *CYP21* gene.[954] This condition can readily be detected by a plasma 17-hydroxyprogesterone above 200 ng/dL (6 nmol/L) in the basal state or above 2000 ng/dL or 72 nmol/L in response to ACTH, which is at least 10 SD above the mean value for age. The prevalence of nonclassic 21-hydroxylase deficiency in children apparently presenting with premature adrenarche is low except in some ethnic groups (e.g., Hispanics, Italians, Ashkenazi Jews), in which the prevalence may be as high as 20% to 30%.

The cause of the observed mild deficiency in 3βHSD activity is unknown, but it may be multifactorial and may lead to a wide range in secretory capacity of the zona reticularis. A family constellation was described with a dominant pattern of inheritance of elevated adrenal androgens and androgen precursors that manifested as premature pubarche[949]; later-affected individuals developed hirsutism and anovulation. Several investigators joined to propose hormonal standards for the diagnosis of 3βHSD deficiency in cases of apparent premature pubarche and stated that ACTH-stimulated Δ^5-17P values must exceed 294 nmol/L for Tanner stage 2 (17 ± 5 nmol/L) or the ratio of Δ^5-17P to cortisol (F) must be at least 363. Studies relating genotype and hormonal analyses in basal and ACTH-stimulated conditions have confirmed that significant elevations of the Δ^5-17P-to-F ratio are necessary to prove true 3βHSD deficiency in genetically proven disease and that this is a rare disorder in patients presenting with putative premature adrenarche.[955] However, patients with a constellation of findings indicating PCOS may have subtler elevations of these values and present a picture of adrenal impairment of 3βHSD activity in the absence of mutations in the gene coding for the enzyme; these children presenting with premature pubarche are postulated to develop clinical PCOS at a later age.

The phenotype of premature pubarche is also associated with the rarer nonclassic 11β-hydroxylase deficiency. Mutation of *HSD3B2* or *HSD3B1* is an uncommon cause of premature pubarche, exaggerated adrenarche, and hirsutism in adolescent girls and women.

DHEA stimulates sebaceous gland activity,[956] and prepubertal acne or comedones may appear in association with elevated serum DHEAS concentrations in some children without the appearance of pubic hair, suggesting that a variant of premature adrenarche may manifest in this manner.[145] More significant androgen effects (e.g., clitoral or penile enlargement, rapid growth, hirsutism, deepening of the voice) exclude premature adrenarche and indicate a more severe form of hyperandrogenism.

Although premature adrenarche was usually considered to be a benign condition with no substantial long-term risk, accumulating observations indicate that girls with premature adrenarche are at increased risk of developing functional ovarian hyperandrogenism and PCOS, hyperinsulinism, acanthosis nigricans, and dyslipidemia in adolescence and adult life, especially if fetal growth was reduced and the birth weight was low.[950] Affected girls have BMI values similar to those of control subjects but differing distribution of fat; they are more likely to have increased waist circumference along with measures of insulin resistance.[957]

The concept of exaggerated adrenarche was first advanced in relation to a postulated childhood antecedent of PCOS. It has been extended to include rare instances of premature adrenarche associated with excessive responses of Δ^5-17P, DHEAS, and androstenedione to ACTH found in women with functional adrenal hyperandrogenism.

A report of a patient with premature adrenarche, advanced bone age, excessive acne, hyperandrogenic anovulation, very low DHEAS levels, and increased androgen levels demonstrated a mutation in PAPSS2, an enzyme that generates the sulfate donor 3′-phosphoadenosine-5′-phosphosulfate (PAPS), which is required for conversion of DHEA to DHEAS by the enzyme SULT2A1. Although the child was described as having premature pubarche, the androgen effects were greater than those usually encountered in this condition. The DHEA level was not elevated for age, but androstenedione was high owing to inadequate formation of DHEAS that decreases the conversion of DHEA to androstenedione. This presentation would ordinarily suggest an ovarian cause of the virilization. This monogenetic defect must be added to the differential diagnosis of premature adrenarche.[292]

Polycystic Ovary Disease[958,959]

PCOS is the most common endocrine disease of reproductive age women; it is estimated to affect 10% of women.[960] The hallmarks of this condition are hyperandrogenism, hirsutism,[961] anovulation, amenorrhea or oligomenorrhea, and insulin resistance; there is compensatory hyperinsulinemia, with its attendant risk of major metabolic sequelae, including type 2 diabetes mellitus, dyslipidemia, an increased propensity to coronary heart disease, and, in about 50% of affected women, obesity. PCOS is considered to be equivalent to the metabolic syndrome in men with its many manifestations in females. A 2013 review of diagnostic criteria supports the use of the Rotterdam criteria for diagnosis, noting the weakness of the method as well. Thus diagnosis rests upon the presence of two of the following criteria: androgen excess, ovulatory dysfunction, or polycystic ovaries.[691] Some prefer *hyperinsulinemic androgen excess* as a better indicator of the basis of the disorder in view of the fact that polycystic ovaries are not required for diagnosis.[950] Indeed criteria for the diagnosis of polycystic ovary morphologic appearance may require a greater number of follicles (>25 on ultrasound) than recommended by the Rotterdam criteria, and standards may yet change.[933] The use of AMH in the diagnosis of PCOS is under consideration but remains controversial owing to differences in study criteria and in the assays used to report AMH values.[962] At least it can be said

that AMH appears to correlate with polycystic appearance and androgen manifestations in many studies and in the future may become a diagnostic criterion.

Premature adrenarche in some populations is a risk factor for the later development of the PCOS and functional ovarian hyperandrogenism in adolescent and adult women; the magnitude of this risk is unknown, but it appears to be rare, except in girls with a history of SGA.[963] However, catch-up growth after SGA may be as important a factor in the development of PCOS, and even prematurity may be a risk. In an 880-member cohort of 8-year-olds, serum androstenedione and DHEAS levels were directly related to weight gain between 1 and 3 years and current weight and inversely related to birth weight.[878] However, a Dutch study could not confirm such a relationship between increase in premature adrenarche and SGA birth weight in 181 subjects born SGA compared with 170 subjects born with AGA.[964]

In certain ethnic groups, and especially in African-American and Hispanic girls, there is a greater association of premature adrenarche with the metabolic syndrome (obesity, hyperinsulinism, dyslipidemia, and other factors that increase the risk of later coronary heart disease) and the development of PCOS in late adolescence and early adulthood, especially if decreased insulin sensitivity and acanthosis nigricans accompany the premature adrenarche.[965]

As discussed earlier, hyperinsulinism is associated with many metabolic and endocrine conditions and functional ovarian hyperandrogenism that, in some cases, is heralded by premature adrenarche. Lifestyle modification is the primary approach to the treatment of PCOS and although success is achievable,[966] the failure rate is substantial. Oral contraceptives are often invoked but may be considered to treat the effects rather than the cause based on insulin resistance and excessive adiposity; nonetheless, the use of oral contraceptives with progestational and antiandrogen effects can regulate menstrual periods,[951] although long-term studies of progestation-only contraceptives in teenagers with PCOS are lacking.[967] Therapeutic approaches to reduce insulin resistance, especially the use of insulin sensitizers, have been introduced into the therapy for PCOS. The most widely used drug is metformin because of its low prevalence of adverse effects and therapeutic efficacy. Substantial data support the safety and efficacy of its use in adolescents with insulin resistance,[951] although metformin is not FDA approved for such use. Although flutamide in higher doses has caused hepatotoxicity, low-dose flutamide (1 mg/kg) is reportedly safe and effective in hirsute young women (but has not been proved to have such a safety profile in obesity or steatohepatitis)[968]; abstinence or contraception is essential when using this teratogenic agent. Thiazolidinediones are not recommended because of safety concerns.[691]

In a trial involving girls just past menarche who had a history of low birth weight and premature adrenarche and were therefore at risk for development of PCOS, metformin prevented this predicted course.[963] Treatment of 8-year-old girls who had similar risk factors appeared to diminish the risk during short-term studies. The beneficial effects on body composition, dyslipidemia, insulin resistance, and other parameters were present only during therapy; they reverted to increased risk factors after discontinuation of metformin. Weight loss is documented with the combination of metformin and oral contraceptives.[969]

The Endocrine Society recommends the oral contraceptives pill (OCP) as the first-line treatment for PCOS.

Adolescent Gynecomastia[970]

Normal boys, usually in the early stages of puberty, may have either unilateral breast enlargement (~25% of boys)[971] or bilateral breast enlargement (~50–65% of boys to varying degrees); this commonly occurs between chronologic ages 14 and 14.5 years or with pubic hair stages 3 and 4.[972] The physical exam aims to detect glandular tissue, but due to the epidemic of childhood and adolescence obesity differentiation of glandular tissue from adipose tissue has become more difficult. "Pinching" the tissue under the areole between the thumb and forefinger and comparing this to other areas of the chest helps in the determination of glandular tissue.[973] After such palpation, determination of serum prolactin might be misleading high, so blood samples must be taken before or well after.

In affected boys, the plasma concentrations of testosterone and estrogen are normal for their stage of puberty. Some have suggested that the ratio of estrogen to androgen or an increase in the ratio of testosterone to dihydrotestosterone is a cause. In a prospective study, adolescent boys with gynecomastia had a lower mean free testosterone concentration, lower weight, higher plasma TeBG levels, and a tendency toward earlier onset of puberty and more rapid progression through puberty.[971] In one study, a significant decrease in the concentration ratio of plasma androstenedione to estrone and estradiol and a similarly low ratio of DHEAS to estrone and estradiol were described in boys with pubertal gynecomastia who had normal ratios of plasma estrone and estradiol to testosterone. It was postulated that decreased adrenal production of androgens or (more likely) increased peripheral conversion of adrenal androgens to estrogens was a factor in the development of pubertal gynecomastia.[974]

Trials of estrogen receptor antagonists (e.g., tamoxifen, raloxifene) show promise, but more study is required.[975,976] Aromatase inhibitor administration had mixed results in gynecomastia. One study of boys (average age, 13 years) with gynecomastia involving a mean of 7 months of treatment with anastrozole demonstrated a substantial decrease in breast area (63%) and volume (53%), as measured manually and by ultrasonography, compared with watchful waiting[977]; however, larger studies are recommended to prove the utility of this approach because another study found no such result.

Pubertal gynecomastia is due to ductular proliferation in fibrous connective tissue.[978] The condition usually resolves spontaneously within 1 to 2 years after onset,[972] and reassurance and continued observation are often adequate treatment. Nevertheless, some boys have conspicuous gynecomastia, and if it lasts longer than 2 years (5–20% in various studies), it is likely to become permanent. These children may have sufficient psychologic distress to warrant a reduction mammoplasty. Indeed the psychologic stress appears unrelated to the duration or severity, and counseling should be considered in appropriate boys.[979] Liposuction is an alternative approach, but its efficacy in adolescent gynecomastia remains to be established. Untreated persistent gynecomastia persists into adulthood. The histologic examination of physiologic gynecomastia tissue rarely reveals carcinoma, so routine disease examination may be unnecessary.[980]

Gynecomastia is a component of Klinefelter syndrome, anorchia, primary and secondary hypogonadism, biosynthetic defects in testosterone synthesis, increased aromatase activity in adipose and other tissues (aromatase excess syndrome),[1008] Sertoli cell tumors, adventitious exposure to estrogens in meat or cosmetics, and variants of the androgen resistance syndromes, including Rosewater syndrome (familial hypogonadism and gynecomastia)

and Reifenstein syndrome (hypospadias, hypogonadism, and gynecomastia). These disorders usually have characteristic findings or environmental circumstances that allow ready differentiation from the normal gynecomastia of puberty.[622] Gynecomastia has been described in association with the administration of drugs such as cimetidine, spironolactone, digitalis, atypical antipsychotics, and phenothiazines; with GH therapy; and with the use of marijuana. The aromatase excess syndrome is described earlier.

Macroorchidism

Macroorchidism is defined as testes twice the normal size for age without androgenization. It is a rare manifestation of the McCune-Albright syndrome[884] and an occasional finding in prepubertal boys with long-standing primary hypothyroidism (see Van Wyk-Grumbach syndrome). This form of testicular enlargement appears to result from increased FSH secretion, independent of a pubertal increase in LH secretion or a pubertal LH response to GnRH. Testicular adrenal rests in CAH can cause bilateral macroorchidism, usually with nodules, as can lymphoma. In the McCune-Albright syndrome, an activating mutation in the $G_s\alpha$ gene primarily expressed in the Sertoli cells can cause macroorchidism due to Sertoli cell proliferation and hyperfunction with increased concentration of serum inhibin B and AMH but without increased testosterone levels due to Leydig cell hyperplasia, elevated gonadotropins, or signs of puberty.[327] Macroorchidism is a feature of severe aromatase deficiency in young male adults[303] and in men with an FSH-secreting pituitary macroadenoma. Bilateral megalotestis (testicular volume, 26 mL) in adults can occur as a normal variant.[981] One may speculate that some instances of bilateral macroorchidism are the result of a heterozygous constitutive activating mutation of the FSH receptor. As noted earlier, prepubertal enlargement of the testes was reported with a single-base-pair deletion at codon 434 (1301delT) of the *NR0B1/DAX1* gene and led to prepubertal testosterone and gonadotropin values.[818]

Immunoglobulin superfamily 1 *(IGSF1)* gene stimulates transcription of the thyrotropin-releasing hormone receptor by decreasing activity of the TGFβ1-Smad signaling pathway while increasing synthesis and biopotency of TSH; it also downregulates the activin-Smad pathway so that FSHB decreases. Thus with mutation in the *IGSF1* gene, central hypothyroidism and macroorchidism develop.[982]

Testes may enlarge during treatment with aromatase inhibitors.

The fragile X syndrome is associated with developmental delay, a long face and large prominent ears, and macroorchidism in 80% of affected pubertal boys. Macroorchidism may be evident only after careful measurements. The enlarged testes are caused by increased interstitial volume and excessive connective tissue, including increased peritubular collagen fibers, rather than by an increase in the seminiferous tubules. Enlargement of the testes is demonstrable in the prepubertal period in most patients with fragile X syndrome, but the onset of true macroorchidism (>4 cm) occurs only in the later prepubertal period.[983]

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The complete list of references is available online at ExpertConsult.com.

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27 Hormones and Athletic Performance

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CHAPTER OUTLINE

KEY POINTS

- Physical activity exerts an important influence on the endocrine system, modulating synthesis and secretion of several hormones. Almost every organ and system in the body is affected by physical activity and exercise, mainly mediated through the endocrine and neuroendocrine system.
- Exercise mode, intensity, and duration, age, gender, and individual fitness level, and environmental and psychological factors may affect endocrine responses to physical activity.
- Several hormones influence physical performance and body composition, with a bidirectional interrelationship between exercise and hormones.
- Hormone abuse has become a widespread habit among professional and recreational athletes. Anabolic androgenic steroids, growth hormone and growth hormone secretagogues, insulin-like growth factor 1, insulin, erythropoietin, and glucocorticosteroid properties are used and misused in sports. Specific methods to detect hormone abuse are described.
- Professionals involved in sports medicine and in endocrinology are presented with an elucidation of the complex interactions between physical activity and the endocrine system. Hormone abuse in competitive and recreational sports exerts negative consequences for long-term health.

Effect of Athletic Performance on Hormonal Systems

Catecholamines

Norepinephrine and epinephrine are closely coupled in their actions and respond rapidly to exercise to redistribute blood flow to meet metabolic demands. Norepinephrine increases from a resting level of 1.2 to 3.0 nmol/L to levels as high as 12.0 nmol/L at maximal exercise. Resting concentrations of epinephrine are 380 to 655 pmol/L. With maximal exercise, epinephrine concentrations can increase up to 3300 pmol/L. Both of these hormones progressively increase as workload increases.[1] Following exercise, plasma levels of catecholamines return to resting levels in a matter of minutes.

Mild exercise produces little or no response in catecholamines, whereas at moderate exercise levels norepinephrine significantly increases with minimal change in circulating epinephrine. At intense or prolonged exercise levels, both hormones increase significantly. Acute, short-duration maximal exercise can significantly increase norepinephrine and epinephrine levels. This rapid response suggests that the levels are primarily regulated via neural release mediated by activation of the sympathetic nervous system. Spillover from active muscle during exercise appears to be the primary contributor, but the kidneys are a possible other source.[1] Moreover, alteration in the ratio of norepinephrine to epinephrine, with a greater increase in the release of epinephrine from the adrenal medulla during exercise, suggests a possible hypothalamic mediation in the response to exercise.

Graded exercise produces a lower catecholamine response than continuous prolonged exercise. The responses are directly related to workload and oxygen uptake, and they are greater with small muscle groups than with large muscle groups.

Many studies report a higher adrenaline response to exercise in endurance-trained subjects compared with untrained subjects in response to intense exercise at the same relative intensity as all-out exercise. This higher capacity to secrete adrenaline was observed both in response to physical exercise and to other stimuli such as hypoglycemia and hypoxia.[2] This phenomenon can partly explain the higher physical performance observed in trained subjects compared with untrained subjects. More recently, these findings have also been reported in anaerobic-trained subjects in response to supramaximal exercise.

The combination of mental and physical challenges can elicit exacerbated catecholamine responses above that of a single challenge alone. Webb and colleagues[3] demonstrated that acute mental challenges and

physical stress together elicit a greater catecholamine and cardiorespiratory response than a single challenge, and this response is affected by fitness level (being higher in individuals with higher fitness levels). These findings suggest that fitness may be of benefit to those engaged in situations where dual-stressors are common (emergency responders, military personnel, etc.), as greater activation of the sympathoadrenal axis may be essential to safety and survival.

Interestingly, studies in women remain scarce; the results are more conflicting than in men, and the physical training type (aerobic or anaerobic) effects on catecholamine response remain to be specified.

Epinephrine and norepinephrine are responsible for many adaptations both at rest and during exercise. These include cardiovascular and respiratory adjustments and substrate mobilization and utilization.[2] Redistribution of circulation to working muscles and to the skin for heat loss and sweating is mediated through changes in catecholamines directly or indirectly via other intermediate hormones. Moreover, catecholamines may mediate mental performance improvement that occurs through exercise.[2]

Fluid Homeostasis–Vasopressin–Renin-Angiotensin-Aldosterone System

During physical exercise, there is considerable loss of water and electrolytes in sweat, which is necessary to maintain body temperature by dissipating heat generated from muscle use. The rate of fluid loss owing to sweating may be as high as 1500 mL/hour. The loss of fluids is replaced by the subsequent ingestion of liquids, which is modulated by thirst. The replacement of electrolyte is the result of the normal intake of food. Renal function is the major mechanism by which electrolytes are conserved following exercise.

The maintenance of fluid and electrolyte homeostasis during physical exercise depends on the action of arginine vasopressin (AVP), natriuretic peptides, the renin-angiotensin-aldosterone (RAA) axis, and catecholamines. These hormonal systems are modified in response to exercise, with different patterns depending on the amount of relative work performed, the duration of exercise, and the training status. Other factors influencing the response of hormones to exercise include the mode of exercise, environmental factors, age and gender of the subjects, and several medical/physiologic conditions.[1,4] Hormones involved in the regulation of fluid and electrolyte homeostasis show a relatively consistent response among individuals.

AVP concentrations increase during exercise up to 24 pg/mL, and elevated levels persist for more than 60 minutes following maximal exercise. The stimulus for the increase in AVP during exercise is the increase in plasma osmolality and reduction in blood volume. Animal experiments have demonstrated an increase in activation of hypothalamic neurons indicating an increased vasopressin content and performance above the anaerobic threshold.[5] Thus, the response of vasopressin appears to be associated with the onset of anaerobic metabolism, which is also related to increases in stress hormones such as cortisol and adrenocorticotropic hormone (ACTH).

A study in runners using pharmacologic manipulation with a receptor-specific agonist and antagonist to activate and inhibit the vasopressin type 2 receptor (V2R) has provided indirect evidence that AVP response during exercise regulates water losses from body fluids only in the kidney with no effects on sweat and salivary glands.[6]

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), which may be altered by exercise,[1] also elicit a natriuretic effect. ANP is increased with exercise in a linear response. In case of prolonged exercise, ANP undergoes an initial increase, a subsequent fall, and then a re-elevation of levels, persisting until completion of the exercise.[1] The increase in ANP with exercise appears to be related to stretch of the atrium due to volume changes, neurologic inputs, and sodium intake.[1] BNP response to exercise is modulated by sodium intake, hydration status, and hypoxia.[1,7] BNP is not consistently altered in normal subjects in response to acute exercise except under conditions of high altitude/hypoxia, whereas it increases with long-duration exercise such as a 100-km ultramarathon.[7]

The RAA system responds to acute exercise with a significant increase of activity. In fact, increased values of plasma renin activity (PRA) are reported following maximal exercise.[1] The increase in PRA occurs at submaximal workloads of more than 60% to 70%. With the increase in PRA during exercise, there is a concomitant increase in angiotensin II (A-II), which partially mediates the increase in circulating aldosterone concentrations up to 250 to 3300 pmol/L. Elevated levels of aldosterone may persist for days after the end of exercise, depending on water and sodium intake. The primary activator of the RAA system during exercise is the sympathetic nervous system. Stimulation of the release of renin is modulated by changes in renal sympathetic nerve activity resulting in an increase in local norepinephrine. The increase in aldosterone with exercise is assumed to be mediated by the increase in A-II in response to activation of the RAA system. However, inhibition of angiotensin-converting enzyme does not attenuate the increase in aldosterone with maximal exercise in healthy subjects.[8] Other factors involved in the activation of aldosterone production include sodium intake, potassium balance, and levels of ACTH. Elevated levels of aldosterone may persist for days after the end of exercise depending on water and sodium intake.[1]

The persistent increase of aldosterone long after the end of exercise may be associated with reductions in plasma osmolality and sodium concentrations due to ingestion of water to replace total body water losses.[1] Thus, the interaction of several regulating factors is involved in mediating the response of aldosterone.

In contrast with acute exercise, PRA is reduced after exercise training, whereas no effect is observed on serum aldosterone or A-II. Significant reductions in systolic and diastolic blood pressure following exercise training have been observed with no relation between PRA changes and changes in blood pressure.[4]

Hypothalamus-Pituitary-Adrenal Axis

Glucocorticoids

Since the pioneering studies of Davies and Few,[9] it has been known that exercise of an appropriate intensity is a potent stimulus for cortisol secretion. Glucocorticoids exert many beneficial effects in exercising humans, increasing the availability of metabolic substrates for the need of energy of muscles, maintaining normal vascular integrity and responsiveness, and protecting the organism from an overreaction of the immune system in the face of exercise-induced muscle damage.[10]

During acute exercise, the hypothalamus-pituitary-adrenal (HPA) axis responds to numerous stimuli demonstrating the regulatory and integrative functions of the HPA axis: neuronal homeostatic signals (chemoreceptor, baroreceptor, and osmoreceptor stimulation), circulating homeostatic signals (glucose, leptin, and ANP), and inflammatory signals (interleukin 1, interleukin 6, and tumor necrosis factor α).[11,12]

In humans, repeated brachial blood samplings have shown that the dynamics of HPA axis activation during exercise associate

stimulation of hypothalamic corticotropin-releasing hormone and AVP secretion (with a prominent role of corticotropin-releasing hormone), synthesis, and release of ACTH from pituitary corticotroph cells preceding the increase of cortisol.[13]

Response to Endurance Exercise

Two major factors modulate the HPA axis response to endurance exercise: the intensity and the duration of exercise.[10,13] The minimum intensity of exercise necessary to produce a cortisol response from the HPA axis is 60% of maximal oxygen uptake ($\dot{V}O_2max$), with a linear increase between the intensity of exercise and the increase in plasma cortisol concentrations for exercises above 60% $\dot{V}O_2max$.[13,14] Below this intensity threshold, such as during light and prolonged exercise (<60% $\dot{V}O_2max$), ACTH and cortisol concentration may increase, with a duration threshold around 90 minutes of exercise at 40% $\dot{V}O_2max$.[14] These thresholds are independent of training.

Several other factors modify the response of the HPA axis to physical activity. The cortisol response to exercise is modulated by hypohydration, meals, and time of day. Independently of external thermal stress, hypohydration (up to 4.8% body mass loss) greatly amplifies the exercise-induced cortisol response to exercise. This enhancement probably results from an increased core temperature and cardiovascular demand consecutive to decreased plasma volume.[15] Meals also stimulate cortisol release in humans. Exercise performed immediately after food ingestion results in a blunted cortisol response to the exercise stimulus. Finally, the cortisol response to exercise is significantly modulated by time of day, which influences the incremental response to exercise (greater increase in the evening than in the morning) but not the peak response to exercise.[13]

Endurance training has been compared with chronic stress in humans. When the HPA axis is repeatedly challenged by exercise, humans demonstrate modifications in the activity of the HPA axis suggesting an adapting process to endurance training. Several studies have shown that in endurance-trained subjects, the HPA axis activity in resting conditions is similar to that of healthy sedentary subjects.[14,16] Accordingly, plasma cortisol at 0800 hours, nycthemeral cortisol rhythm, overnight and 24-hour urinary free cortisol, seasonal rhythmicity of cortisol excretion, and cortisol response to the dexamethasone suppression test in resting endurance-trained subjects are similar to those of age-matched untrained subjects.[13,14,16]

However, when the HPA axis is challenged, endurance-trained subjects demonstrate a decreased pituitary sensitivity to the negative feedback of glucocorticoids, which explains their capacity to successfully achieve a second bout of exercise separated by a short rest period.[14] Different mechanisms can be involved in this adaptation. At the central nervous system level, neuropeptides and corticosteroid receptors (glucocorticoid receptors, mineralocorticoid receptors) in the brain and anterior pituitary play a major role in the regulation of circulating cortisol levels. At the peripheral level, tissue sensitivity to glucocorticoids may also be different between endurance-trained and sedentary subjects.[17] Altogether, these adaptation processes are finalized to protect the body from the severe metabolic and immune consequences of increased cortisol levels.

Response to Resistance Exercise

Resistance (strength) exercise is also a potent stimulus to the HPA axis. In humans, a bout of resistance exercise acutely increases cortisol secretion and then plasma cortisol concentrations.[18]

Two major factors modulate HPA axis response to resistance exercise: the intensity and the volume (sets × repetitions × intensity) of the exercise performed. Resistance protocols of moderate to high intensity, high in volume, stressing large muscle mass, and using short rest intervals produce the greatest acute hormonal elevations (ACTH and cortisol).[18]

Cortisol has multiple roles during resistance exercise: meeting the greater metabolic demand of resistance training; remodeling of muscle in response to a bout of exercise, as cortisol regulates muscle protein content by inhibiting muscle protein synthesis; and stimulating protein degradation likely via activation of the ubiquitin pathway.[13] Moreover, cortisol can also influence neuromuscular function (i.e., neuronal activity, intracellular signaling, muscle force) through various rapid or short-term mechanisms. Cortisol may contribute to the muscular properties of the peripheral nervous system by rapidly modulating calcium influx in skeletal muscle cells.[19]

Mineralocorticoids

The RAA system is closely coupled and responds to acute exercise. PRA values increase following maximal exercise.[1] Progressively higher increases in aldosterone levels have been observed with increasing degrees of exertion. Elevated levels of aldosterone may persist for days after the end of exercise depending on water and sodium intake.[1] The interaction of several regulating factors is involved in mediating the response of aldosterone. These include the sympathetic nervous system, renin activity, A-II, sodium intake, potassium balance, blood volume reductions, and levels of ACTH.[1] In contrast with acute exercise, PRA is reduced after endurance training, whereas no effect is observed on serum aldosterone or A-II.[4]

Endorphins

Exercise influences the release of β-endorphins depending on intensity and duration of the physical activity. If a threshold intensity is exceeded, endogenous opiate levels start to increase. Incremental graded exercise tests elevate β-endorphin levels 1.5- to 7-fold.[20] Several studies suggest that circulating β-endorphins can increase with an appropriate minimal intensity of exercise (>60% $\dot{V}O_2max$) but this is not always the case.[20] Instead of a critical intensity related to aerobic capacity, other studies related the increase in β-endorphins to the lactate threshold.[20] Moreover, other factors, such as diet and immune function, can influence the β-endorphin response. Very little is known about the influence of training status on the release of β-endorphins, and study results are often inconsistent.

Interestingly, the psychological and physiologic stress related to competitive practice has been proposed to stimulate the secretion of endorphins to counter the negative effects of competitive stress.[21] A physiologic purpose of endogenous opiate increase in athletes can be the modulation of pain and the improvement of mood.[22] Altogether, the action of endogenous opiates can be described as a rewarding system that makes the athlete continue physical activity.

Hypothalamic-Pituitary-Gonadal Axis

Male Gonadal Axis

The effects of physical activity on the hypothalamic-pituitary-gonadal (HPG) axis in males vary with the intensity and duration of the activity, the fitness level of the individual, and his nutritional-metabolic status. Relatively short, intense exercise usually

increases serum testosterone levels, whereas more prolonged exercise usually decreases them.[23-25] Increased serum testosterone levels have been reported during relatively strenuous free and treadmill running, weight training, rock climbing, and ergometer cycling.[26]

The testosterone response increases with increased exercise load.[27] Similar workloads produce similar responses regardless of whether the load is aerobic or anaerobic.[28] Increased and decreased ambient temperature, altitude, and dehydration have no effect on testosterone response to intense exercise.[29] Acute exercise-induced testosterone increments are also seen in older men despite their different hormonal milieu.[30]

Exercise-associated increase in circulating testosterone is considered not to be mediated by luteinizing hormone (LH) due to the inconsistent LH response and to the evidence that testosterone levels increase more quickly than LH in response to exercise. Possible mechanisms such as hemoconcentration, reduced clearance, and/or increased testosterone synthesis may be involved.[26] However, the timing of testosterone response differs from that of other circulating steroids (e.g., androstenedione and dehydroepiandrosterone increase simultaneously with cortisol), thus suggesting that specific testicular mechanisms are involved.[31] These mechanisms may include the activation of the sympathetic system, which stimulates testicular testosterone production during exercise via a direct neural pathway in some species.

In contrast to the short-term testosterone increment, a suppression of serum testosterone levels occurs during and subsequent to more prolonged exercise, and to some extent in the hours following intense short-term exercise.[32,33] A variety of systems could influence the decrease of testosterone synthesis during and subsequent to prolonged exercise. A lack of significant elevation in basal LH, coupled with a reduced testosterone concentration, has been reported, reflecting hypogonadotrophic-hypogonadism characteristics.[34,35] Moreover, altered basal prolactin has been described in response to prolonged exercise.[34,36]

Leptin and ghrelin levels, which may be influenced by physical exercise, have been suggested to be altered in exercise-hypogonadal men. Leptin is an adipocyte-released hormone associated in part with communicating satiety and energy reserve status to the hypothalamus.[37] It is also linked to reproductive function in both women and men. Acute and chronic exercise can reduce leptin concentrations independent of changes in body adiposity.[38,39]

Ghrelin is another hormone associated with appetite regulation. Evidence from several experimental studies in animals and in humans suggests that ghrelin may function as a metabolic modulator of the HPG axis, with predominant inhibitory effects in line with its role as a signal of energy deficit.[40] Acute and chronic exercise has been shown to increase ghrelin concentration levels.[38,41] However, no research has yet examined whether ghrelin levels in exercise-hypogonadal men are normal.

Another potential disruptive hormone to the HPG axis is cortisol. Cumming and associates[42] have demonstrated that the direct infusion of cortisol in men results in concurrent declines in testosterone levels. However, in the hormonal profile studies reporting the existence of low testosterone in trained men, none have reported elevated resting cortisol levels.[26] Thus, at this time, the role of cortisol in the changes found in the gonadal axis of trained men is in need of further study.

Reductions in testosterone levels, if extensive and prolonged, can compromise a person's health status. Relative to athletes, these reductions can negatively impact on the adaptation process associated with skeletal muscle (i.e., myoplasticity), which is fundamental to the exercise training progression and improvement in performance.[43,44]

It is important to note that hormonal changes involved in overtraining and/or decreased energy availability can decrease the production of testosterone. Participation in sports where leanness is considered a competitive advantage, such as running, cycling, wrestling, lightweight rowing, and gymnastics, has been linked to lower body mass index, eating disorders, and low energy availability.[33,45] Low energy availability in the context of anorexia nervosa has been associated with low testosterone levels in males.[46] Hagmar and associates[47] evaluated athletes from 26 different sports and divided them into those who participated in leanness sports and those who did not. The leanness sport athletes had lower body fat, higher spinal bone mineral density (BMD), lower serum free testosterone and leptin, and higher insulin-like growth factor 1 (IGF1) binding protein.

Research in exercising men demonstrates the existence of a select group who, through chronic exposure to endurance exercise training, developed alterations in their reproductive hormonal profile (i.e., persistently low basal resting testosterone concentrations).[34] In particular, the majority of these men exhibit clinically "normal" testosterone concentrations, but these concentrations are at the low end of the normal range or even reach subclinical status. The health consequences of such hormonal changes are increased risk of abnormal spermatogenesis, male infertility problems, and compromised bone mineralization.[34] The prevalence of such health problems seems low, but investigative studies examining this condition and its consequences are few in number.[34] The specific terminology used to refer to this condition has not been universally agreed on. In 2005, Hackney and associates[48] proposed use of the phrase "the exercise-hypogonadal male" as a label for this condition.

The effects of intense training on male fertility are controversial. Some studies have found changes in semen from exercise, noting decreases in sperm motility, quality, and number,[49-51] whereas others have not found such alterations.[52,53] Several studies, however, did not control for energy availability. Thus, like much of the work in female athletes, research in male athletes will be more easily interpretable in the future if energy availability is accurately quantified and factored into the analysis.[33]

Female Gonadal Axis

The endocrine equilibrium that regulates the reproductive function in women can be affected by physical and psychological factors. In females, short-term consequences of physical exercise on the HPG axis have not been as widely studied as in males. Differences in hormonal responses between the sexes are believed to be dependent on basal hormone concentrations (i.e., sex differences in basal estradiol and testosterone).[33] A few studies have demonstrated increases in total testosterone after acute bouts of resistance and endurance training in women.[54-57] Studies evaluating acute changes in estradiol are challenging given that estradiol levels change widely during a typical menstrual cycle. Nevertheless, multiple investigations have found an acute increase in estradiol immediately after exercise.[54,58] This increase in estradiol seems to be responsible for greater lipid and less carbohydrate oxidation in women compared with men during exercise and seems to be protective for muscle during the stress of exercise.[33]

The long-term impact of exercise and training on hormone levels has been more widely studied in females, particularly in the setting of decreased energy availability. Women who engage in regular high-intensity exercise may be at risk of developing menstrual disturbances such as delayed menarche, oligomenorrhoea, amenorrhea, and luteal phase defects.[59]

Although factors such as physical and/or psychological stress of competition have been postulated to underlie exercise-induced reproductive disorders, evidence accumulated to date indicates that negative energy balance is the primary cause of the impairment of normal reproductive function commonly observed in female athletes.[60,61] In 1939, Selye[62] reported that "the ovaries undergo atrophy and more or less permanent anestrus ensues" when young female rats were forced to exercise for prolonged periods. Earlier, Selye[63] observed a *general adaptation syndrome* also involving hypertrophy of the adrenal glands, cessation of growth and lactation, shrinkage of the liver, loss of muscular tone, a fall in body temperature, and the disappearance of adipose tissue. In 1980, Warren[60] was the first to suggest that menstrual disorders in dancers are disrupted by an "energy drain." In 1984, Winterer and associates[64] hypothesized that lack of sufficient metabolic fuels to meet the energy requirements of the brain causes an alteration in brain function that disrupts the gonadotropin-releasing hormone (GnRH) pulse generator, although the mechanism of this alteration was unknown. The energy availability hypothesis is supported by endocrine observations of athletes. Amenorrheic athletes display low 24-hour blood glucose, low 24-hour insulin, and high 24-hour insulin-like growth factor binding protein 1 (IGFBP1),[65] loss of the leptin diurnal rhythm,[66] and low T3 levels in the morning.[67] Loucks and associates[68] found that low energy availability reduced LH pulse frequency and increased LH pulse amplitude, and that exercise stress had no suppressive effect on LH pulsatility beyond the impact of the energy cost of exercise on energy availability. LH pulsatility was disrupted regardless of whether energy availability was reduced by extreme energy restriction alone, by extreme exercise energy expenditure alone, or by a combination of moderate dietary energy restriction and moderate exercise energy expenditure. Dietary supplementation prevented the suppression of LH pulsatility by exercise energy expenditure.

To investigate the dose-response relationship between energy availability and LH pulsatility in exercising women, Loucks and Thuma[69] manipulated the energy availability of 29 regularly menstruating, habitually sedentary, young women, administering a clinical dietary product to set energy availability at 45 and 10, 20, or 30 kcal/kg fat-free mass (FFM) for 5 days. LH pulsatility was disrupted only below 30 kcal/kg FFM per day. This was consistent with many studies of amenorrheic runners, all of which indicated energy availabilities less than 30 kcal/kg FFM per day,[70] and with the only prospective study of the refeeding of amenorrheic athletes, in which menstrual cycles were restored in runners by increasing energy availability from 25 to 31 kcal/kg FFM per day.[71]

In female athletes, low energy availability is a component of the *female athlete triad*, a term used to describe the interrelationship of decreased energy availability, subsequent HPG axis inhibition leading to menstrual irregularity, and decreased BMD.[45] The triad was first described by the American College of Sports Medicine in the 1990s. In 2007, the American College of Sports Medicine published a revised position stand on the female athlete triad,[72] correcting the former misunderstanding of the triad as a narrow syndrome consisting of disordered eating, amenorrhea, and osteoporosis by describing the triad more broadly as the harmful effects of low energy availability on menstrual function and BMD.[61] The International Olympic Committee (IOC) proposed an expansion of the concept of the female athlete triad to include males and coined the term *relative energy deficiency in sport*.[73] The development of that term had three main purposes: (1) to draw awareness to the fact that energy restriction can have negative consequences in men in addition to women; (2) to highlight other potential negative health and performance consequences of low energy availability in athletes besides bone problems; and (3) to encourage expansive research into the potential myriad effects of low energy availability in various populations, including Paralympic athletes.

The discovery of leptin in 1994 was fundamental in clarifying the relationship between negative energy balance and reproductive dysfunction. Various sets of data suggest that leptin may serve as a signal to the CNS with information on the critical amount of adipose tissue stores necessary for GnRH secretion and pubertal activation of the HPG axis. Several unfavorable metabolic situations are associated with low plasma leptin, increased secretion of hypothalamic neuropeptide Y, and hypogonadism, and a causal relationship has been evoked. Severe dietary restriction in juvenile female rats is associated with low plasma leptin and sexual immaturity. Cessation of food restriction leads to immediate increase in plasma leptin followed by sexual maturation.[74] Leptin administration for the relative leptin deficiency in women with hypothalamic amenorrhea improves reproductive, thyroid, and growth hormone (GH) axes and markers of bone formation, confirming that leptin is required for normal reproductive and neuroendocrine function[75] and can restore ovulatory cycles.[76]

Impaired production of gonadotropins, which leads to luteal phase deficiency and anovulation, is a common hormonal finding with exercise-induced menstrual disturbances, but several other hormones may show significant alterations.

The HPA axis activation may be involved in the gonadal axis functional disruption during physical exercise. The so-called stress hypothesis holds that exercise activates the HPA axis, which disrupts the GnRH pulse generator by another unknown mechanism. Amenorrheic athletes may display mildly elevated cortisol levels, and this observation is the basis for attributing their amenorrhea to stress. However, since cortisol is a glucoregulatory hormone activated by low blood glucose levels, the mild hypercortisolism observed in amenorrheic athletes may reflect a chronic energy deficiency rather than exercise stress.[61]

The involvement of endogenous opioid peptides and catecholestrogens in provoking menstrual irregularities in women athletes has been also suggested.[77] In basal circumstances, β-endorphins may decrease LH levels by suppressing hypothalamic GnRH; some catecholestrogens may suppress LH levels, whereas others seem to potentiate and induce LH surge. The activities of both β-endorphins and catecholestrogens depend on the essential presence of a sufficiently estrogenic environment. In addition, both endogenous opioid peptides and some of the catecholestrogens appear to be able to suppress prolactin release, probably by interfering with its inhibiting factor, dopamine. The increased plasma concentrations of β-endorphin, which are found after physical exercise, give rise to speculations as to their involvement in the frequently appearing menstrual irregularities in female athletes.[77]

Hyperandrogenism has been suggested as a possible alternative mechanism underlying oligomenorrhea or amenorrhea in some female athletes with menstrual disturbances.[78-80] Sports that emphasize strength over leanness, such as swimming and rowing, are not associated with low weight and restrictive eating patterns, yet athletes engaged in these sports are vulnerable to menstrual irregularities as well. The endocrine profile of athletes engaged in these sports is characterized by mildly elevated LH levels, elevated LH/follicle-stimulating hormone ratios, and mild hyperandrogenism rather than hypoestrogenism. Interestingly, hyperandrogenic female athletes have a more anabolic body composition and higher $\dot{V}O_2$max and performance values than female athletes with menstrual disturbances but normal androgen concentrations.[78]

Prolactin

Blood prolactin levels increase during exercise, and this response appears proportional to the exercise intensity.[36] Provided that the intensity is adequate, the increase in prolactin is quite rapid. Nonetheless, short-term, graded exercise may result in a peak hormonal response after the exercise ends. In a prolonged exercise session, the prolactin response is proportional to the intensity at which it is performed. However, extending the duration of exercise can augment the magnitude of the prolactin response.[36]

The mechanisms by which prolactin increases with exercise are unclear. Prolactin levels may increase when the anaerobic threshold is reached, perhaps concomitantly with a GH increase.[81] In some situations, such as competitive sports, excessive emotional stress can cause an anticipatory increase in prolactin even before the initiation of exercise.[82] Moreover, the prolactin increase may be related to changes in body temperature and dehydration, is reduced with habituation and hypoxia, and is unresponsive to metabolic events.[83]

Vigorous, high-intensity anaerobic exercise results in greater prolactin response than that typically seen in submaximal steady-state aerobic exercise.[28] The effect of resistance exercise, which is intermittent in nature, on prolactin has not been studied extensively.

The chronic effects of exercise training on basal, resting prolactin levels are yet unclear and in need of further research. Some studies have found increases in resting levels, whereas others have found decreased levels.[36] These contradictions seem to be related to differences in training protocols (intensity, frequency, and duration of training sessions).

GH/IGF1 Axis

In 1963, Roth and associates[84] demonstrated that plasma levels of GH increase during exercise, and it was later shown that exercise is the most potent physiologic stimulus to GH release.[85] Most of the current knowledge regarding the GH response to exercise is based on studies of the effect of aerobic-type exercise.

The GH response to exercise is dependent on the duration and intensity of the exercise bout, the fitness level of the exercising subject, the refractoriness of pituitary somatotroph cells to the exercise stimuli, and other environmental factors.[86] Lactate and nitric oxide are suggested to be afferent stimulation for the exercise-induced GH response.[87] A linear dose-response relationship between exercise intensity and the GH secretory response was demonstrated, with escalating GH release across the range of exercise intensities (25–175% of lactate threshold).

The exercise duration should be at least 10 minutes, because exercise of shorter duration both below and above the lactate threshold was not accompanied by increases in circulating GH levels.[86] Exercise-induced GH peak occurs 25 to 30 minutes after the start of exercise, irrespective of the exercise duration.[86] Thus, when the task is brief, a peak may be reached after its cessation, but when the task is long (e.g., 45 minutes), the GH peak occurs while the individual is still exercising.

The nature of the exercise may also influence the GH response. Although the continuous exercise protocols may be comparable to competition events, the endurance-type training undertaken by many athletes involves intermittent or interval exertion. Comparing exercise at equivalent total workloads, GH levels are lower with continuous protocols (40–45% $\dot{V}O_2$max) as opposed to interval protocols, with twice the work rate for half the time, reflecting the greater metabolic stress and lactate levels in the latter.[88] With resistance exercise, incremental GH responses have also been described.[89] The important determinants appear to be the relationship between load and frequency of individual repetitions. Greater GH increments have been reported following "hypertrophy" protocols (moderate loads, high number of repetitions) than "strength" protocols (heavy loads, low repetitions) in both men and women.[90]

There is conflicting evidence regarding the neuroendocrine pathways that regulate GH secretion during exercise. Mechanisms involving cholinergic, serotoninergic, α-adrenergic, dopaminergic, and opioidergic pathways have been proposed.[91] There may be interactions among the pathways, and they may operate at different exercise intensities. In young males, regular but not acute exercise is associated with higher GH production and also augments GH stimulation of GH release by GH-releasing hormone.[92] It has been hypothesized that this is due to decreased hypothalamic somatostatinergic activity and higher GH pulsatility.

The GH response to exercise is greater in women than in men—as is the unstimulated GH output over time—and declines with aging.[91] In fact, even in early middle age (mean age, 42 years), the GH response to exhaustive exercise is greatly attenuated compared with younger subjects (mean age, 21 years).[93] However, it is difficult to separate the effects of aging from changes in body composition, because body fat increases with aging and GH secretory rates are reduced in overweight subjects.[94]

Environmental and nutritional factors, as well as some pathologic states, may interfere with the GH response to exercise. Cappon and associates[95] showed that a high-fat meal could inhibit the magnitude of GH response to exercise, the inhibition of the exercise-induced GH response being correlated with circulating levels of somatostatin.

High ambient temperature may increase circulating GH levels,[96] whereas low temperature attenuates GH release.[97] Obesity and/or polycystic ovarian syndrome are characterized by attenuated GH response to exercise.[98]

Exercise exerts acute effects on other components of the GH/IGF1 axis. The effect of exercise on circulating IGF1 has been examined by several investigators with differing results depending on the intensity, duration, and type of exercise performed. Schwarz and associates[99] demonstrated IGF1 increases following 10 minutes of exercise of both below and above the lactate/anaerobic threshold. This study suggests that the increase in IGF1 accompanying exercise is not related to GH.

The transient nature of IGF increases suggests that hemodynamic or metabolic effects of exercise per se might play a role. Exercise in humans is accompanied by the rapid "hemotransfusion" of hemoconcentrated blood from the spleen into the circulation by increased blood flow to the exercising muscle and by loss of plasma water. These phenomena might explain, at least in part, an increased IGF concentration by changes in IGF flux and/or volume of distribution.

Longer periods of exercise training, however, stimulate *IGF1* gene expression both in the central neuroendocrine and local tissue components of the GH-IGF1 system. Eliakim and associates[100] showed that muscle IGF1 protein concentrations in rats can increase with endurance training despite the lack of change in muscle IGF1 mRNA or serum IGF1.

Few studies have investigated the response of IGFBPs to exercise. IGFBP1 levels have been shown not to change during 30 minutes of moderate exercise[90] but to increase transiently after acute exercise.[101] The physiologic role of the postexercise increase in IGFBP1, given IGFBP1 inhibition of IGF1 metabolic actions, may be to prevent late hypoglycemia.

Schwarz and associates[99] have demonstrated that IGFBP3 levels increased with both low- and high-intensity exercise and that high-intensity exercise increased IGFBP3 proteolysis. A transient increase in IGFBP3 levels in response to an acute exercise has also been confirmed by Wallace and associates,[101] who described an acute increase of all components of the ternary complex, IGF1, IGFBP3, and the acid-labile subunit.

Eliakim and associates[102] have described that functional and structural indices of fitness were correlated with mean overnight GH levels, growth hormone–binding protein (GHBP), and serum IGF1 levels in late pubertal adolescent girls. Moreover, thigh muscle volume was inversely correlated with IGFBP2 and IGFBP4.

The acute increase in serum GHBP in response to acute exercise has also been described by Wallace and associates[101]; considering that GHBP at rest acts as a damper on GH oscillation, these authors speculate that the postexercise GHBP increase may prolong the GH signal, increasing the GH-mediated signal for postexercise protein synthesis, tissue repair, and muscle glycogen replenishment. The increment in the serum GHBP concentration may represent either increased synthesis from the liver or reduced clearance.[101]

Hypothalamus-Pituitary-Thyroid Axis

Exercise has effects on thyroid function, which can be viewed as an adaptive mechanism associated with enhanced performance possibly serving to provide a better balance between energy consumption and expenditure.[103] Short-term incremental exercise (≤20 minutes) increases blood TSH (thyroid-stimulating hormone, or thyrotropin) levels, with a critical intensity threshold of approximately 50% or more of $\dot{V}O_2max$ necessary to induce significant changes.[36] Even though TSH is elevated, most research involving short-term exercise indicates that total and free thyroxine (T_4) and T_3 are not immediately affected.[36] However, total T_4 and T_3 levels can increase following such exercise, although these findings appear primarily due to exercise-induced hemoconcentration.[104]

The influence of more prolonged submaximal exercise (approaching 60 minutes) on thyroid hormones is controversial. Some investigations report no effect on blood TSH levels, whereas others have found TSH and/or free T_3 to increase progressively with high-intensity steady-state workloads.[36] These divergent findings are difficult to interpret due to the highly variable exercise sessions (i.e., different durations and intensities of exercise) and the varying blood sampling protocols employed.

Energy balance plays an important role in the body's thyroid hormone response to exercise. Loucks and Heath[105] found a decrease in T_3 and free T_3 along with an increase in reverse T_3 in healthy women undergoing aerobic exercise testing with low caloric intake. This "low T_3 syndrome" was not seen in individuals receiving a higher caloric diet. An increase in reverse T_3 concentrations has been described as one of the more consistent findings, particularly when a caloric energy deficiency is associated with exercise.[103]

Insulin and Glucose Metabolism

Physical activity affects the metabolism of glucose and other intermediate substrates in normal subjects and in subjects with diabetes. The effects of exercise on carbohydrate metabolism are complex and involve type, intensity, and duration of exercise; changes in body composition; alterations in other behaviors, such as food intake; degree of insulin deficiency; and a complex time course of the glucose-insulin response.[106]

In healthy individuals, no major alterations in blood glucose levels are usually seen during exercise, despite the increase in glucose utilization by skeletal muscle. With the onset of activity, activation of the α-adrenergic system results in inhibition of insulin release from the pancreas. This results in an increased rate of lipolysis in the periphery, as well as a stimulation of hepatic glucose output. As glucose levels begin to fall, glucagon levels rise, further stimulating hepatic glucose output. Finally, as plasma glucose drops toward hypoglycemic levels, epinephrine is released, further stimulating hepatic glucose production and increasing lipolysis in the periphery. The increased availability of free fatty acid for muscle metabolism helps to restrain the rate of glucose utilization. It has been shown that when one of these mechanisms fail, the others can largely compensate, avoiding the development of hypoglycemia.[106]

Training induces a reduction in basal insulin levels and in exercise-associated changes in glucagon and insulin, increases insulin sensitivity at rest and in response to a glucose load, and reduces insulin decline during acute exercise.[107] Regular exercise has become an integral part of the treatment recommendations for patients with type 2 diabetes, because it improves insulin sensitivity and reduces average blood glucose concentrations.[108,109] Physical training results in an increase in insulin-stimulated glucose disposal and improves glucose control in type 2 diabetes. However, the increase in insulin sensitivity is rapidly lost if exercise is not performed on a regular basis. Exercise may also be effective in delaying or preventing the development of type 2 diabetes.[109,110]

Erythropoietin

Plasma erythropoietin (EPO) levels are not usually affected by single bouts of strenuous exercise at sea level. The lack of major effects of normoxic exercise on EPO production is plausible, because the oxygen sensor in control of EPO synthesis is not located in the skeletal muscle or the heart but in the kidneys.[111] Generally, there are no major differences in basal red blood cell (RBC) count, hematocrit, blood hemoglobin concentration, and mean corpuscular hemoglobin concentration values in athletes compared with nonathletes. Hemoglobin mass has been described to be increased in elite endurance athletes.[112] However, sportsmen, particularly those in endurance sports, may present with relatively low hemoglobin concentrations and hematocrit values. This "sports anemia" is usually a pseudoanemia due to an enlarged plasma volume. If there is an increased rate of intravascular hemolysis caused by compression, the RBC lifespan will be reduced.[111]

Performance-Enhancing (Ab)use of Hormones

Anabolic Androgenic Steroids

The use of ergogenic (performance-enhancing) substances by athletes continues to be a growing problem, and androgens are among the most frequently abused drugs.[113] The global lifetime prevalence rate of using anabolic-androgenic steroids (AASs) is 6.4% for males and 1.6% for females.[114] Classical sport disciplines are not the only ones involved; the phenomenon is similarly widespread among bodybuilders, and thus the drugs used have been collectively classified as appearance- and performance-enhancing drugs (APEDs).

Although doping has been practiced since antiquity, often with placebo or toxic effects, truly effective APEDs only became available with the rise of modern pharmacology, and particularly following the isolation and synthesis of testosterone and AASs. Testosterone came into clinical use shortly after its synthesis in 1935,[115] and its first documented use for doping was by German rowers in 1952 (to maintain their marital duties during exhausting training) and by Russian weight lifters in 1954 to enhance their power.[116]

The IOC introduced antidoping regulations for the first time in 1967 and performed the first antidoping testing in the 1972 Munich Olympics. In 1976, androgens were placed on the IOC doping list.[113]

Interestingly, an early and comprehensive review of previous results concluded that there was little evidence for supraphysiologic doses of testosterone or synthetic AASs having any appreciable effect on muscle size or strength in healthy men.[117] However, many of the studies reviewed had a lack of adequate control and standardization. Conclusions from more recent reviews suggested that the administration of AASs could consistently result in significant increases in strength if male athletes satisfied certain criteria, including the timing of doses and dietary factors.[118,119] In 1996, Bhasin and associates[120] demonstrated that the administration of supraphysiologic doses of testosterone in combination with exercise in male weight lifters induces a greater increase in muscle size and strength than exercise alone or testosterone treatment alone (i.e., the effects of combining supraphysiologic doses of testosterone with exercise are additive). Subsequent work showed that increases in FFM, muscle size, strength, and power are highly dose dependent and correlated with serum testosterone concentrations.[121,122] In fact, significant increases in muscle size and strength occur only with doses of 300 mg per week and higher.[121] The increase in muscle size is due to a hypertrophy that results from an increase in cross-sectional areas of both type I and type II muscle fibers and an increase in myonuclear number.[123]

The anabolic effects of androgens are primarily mediated through androgen receptor signaling. Androgen receptors are expressed in the satellite cells and other stem-like cells in the interstitium of the skeletal muscle fibers.[124] A growing body of evidence supports the hypothesis that testosterone and dihydrotestosterone promote myogenic differentiation of mesenchymal, multipotent stem cells and inhibit their differentiation into the adipogenic lineage.[125,126]

The AASs used for nontherapeutic purposes are endogenous androgens (e.g., androstenedione, dehydroepiandrosterone [DHEA]), 17β-esters of testosterone (e.g., cypionate, enanthate, heptylate, propionate, undecanoate, buciclate), 17α-alkyl derivatives of testosterone (e.g., methyltestosterone, fluoxymesterone, oxandrolone, stanozolol), 19-nortestosterone (nandrolone), 17β-esters of 19-nortestosterone (e.g., decanoate, phenpropionate), 19-norandrostenedione and 19-norandrostenediol, and tetrahydrogestrinone. More than 100 different AASs have been developed, with most of them being used illegally, synthesized in clandestine laboratories, commercialized without medical prescription or safety controls, and sometimes unknown to the scientific world[127] (Table 27.1).

The updated list of AASs is available on the following website: https://www.wada-ama.org/en/prohibited-list.[128]

Intramuscular injections are used far more frequently than oral formulations. The dose of AASs used by athletes varies considerably and is often thought to exceed 10 to 40 times the recommended therapeutic dose. In addition, combinations of androgens are used more frequently than single agents. Multiple androgens may be combined in a practice known as stacking, in which two or more androgens are added in progressively increasing doses over a period of several weeks. Athletes also often use a practice called *cycling*, in which weeks of androgen use are followed by periods of drug holiday; this routine is based on the unproven premise that cycling prevents desensitization to massive doses of androgen. The phrase "building a pyramid" refers to the progressive increase in the doses of androgens during a cycle. Toward the end of a cycle, athletes may reduce the doses of androgens or switch to other drugs (e.g., human chorionic gonadotropin or aromatase inhibitors or estrogen antagonists) that they believe will reduce the likelihood of testicular suppression. In most surveys, the duration of steroid administration or steroid cycle lasts between 4 and 12 weeks.[129]

TABLE 27.1 Performance-Enhancing Hormones

Anabolic Androgenic Steroids

17β-Esters of testosterone (cypionate, enanthate, heptylate, propionate, undecanoate, buciclate)
17α-Alkyl derivatives of testosterone (methyltestosterone, fluoxymesterone, oxandrolone, stanozolol)
19-Nortestosterone (nandrolone)
17β-Esters of 19-nortestosterone (decanoate, phenpropionate)
19-Norandrostenedione
19-Norandrostenediol
Tetrahydrogestrinone

Peptide Hormones

Growth hormone
Insulin-like growth factor 1
Insulin
Erythropoietin

Adverse Effects

The side effects associated with AAS use are numerous and involve multiple organ systems[113,116,130] (Table 27.2). Systematic investigations of the adverse effects of androgens in athletes and recreational body builders have been difficult for many reasons. With the exception of the association between hepatic dysfunction and the use of some oral AASs, many of the reports of serious side effects in otherwise healthy individuals have come from anecdotal case studies or small retrospective studies based largely on self-reported data.[130] Confounding factors, such as undiagnosed preexisting conditions, family history, and concurrent use of other drugs, further dampen the credibility of case reports. Moreover, because most anabolic steroids are obtained on the black market and are of dubious quality, there is potential for adverse medical events independent of steroid use. However, data from larger observational studies suggest that the majority (88–96%) of AAS users experience at least one minor subjective side effect, including acne (40–54%), testicular atrophy (40–51%), gynecomastia (10–34%), cutaneous striae (34%), and injection site pain (36%).[131]

AAS-induced hypogonadism (ASIH) is common among former AAS abusers and usually presents as hypogonadotropic hypogonadism due to abrupt decreases in plasma androgen levels following AAS cessation.[132] Scientific data on ASIH are limited, but the condition is characterized by symptoms and signs of hypogonadism (e.g., testicular atrophy, low plasma testosterone levels, impaired spermatogenesis, erectile dysfunction, fatigue, decreased libido, and depressive symptoms) and is considered to resolve spontaneously within 6 to 12 months.[116,133] However, studies investigating the recovery phases of young men with ASIH are scanty. A growing number of studies reporting cases in which ASIH manifestations persisted years after AAS cessation suggest that ASIH is a more permanent condition in a substantial proportion of former AAS abusers.[132] This emerging group of young men may become a considerable public health concern in the coming years.

TABLE 27.2 Side Effects of Anabolic Androgenic Steroids

Cardiovascular
Cardiomyopathy
Lipid disorders (decreased HDL, increased LDL)
Increased platelet aggregation
Increased hematocrit
Elevated blood pressure

Cosmetic
Gynecomastia
Acne
Hair loss
Cutaneous striae

Reproductive-Endocrine
Libido changes
Subfertility
In Males
Testicular atrophy
Impaired spermatogenesis
Erectile dysfunction
Prostate diseases
In Females
Hirsutism
Breast atrophy
Voice deepening
Virilization (clitoromegaly)
Menstrual disturbances

Hepatic
Cholestasis
Steatosis
Tumors
Hepatocellular adenoma and carcinoma
Hepatic angiosarcoma and cholangiocarcinoma

Psychological
Aggression
Mood swings
Anxiety
Psychosis
Irritability
Dependence
Withdrawal
Depression

Injection Related
Infection
Bruising
Fibrosis
Injection site pain

HDL, High-density lipoprotein; *LDL,* low-density lipoprotein.

In a recent cross-sectional case-control study involving 37 current AAS abusers, 33 former AAS abusers, and 30 healthy control participants, Rasmussen and associates[132] demonstrated that the group of former AAS abusers exhibited significantly lower plasma total and free testosterone, had smaller testicular sizes, and featured a higher proportion of participants with depressive symptoms, fatigue, erectile dysfunction, and decreased libido than the control group more than 2 years after AAS cessation.

AASs may also induce dyslipidemia, cardiovascular disease, insulin resistance, glucose intolerance, diabetes mellitus, and liver disease.[113,130] In general, the ingestion of oral C17α-alkylated anabolic steroids causes an average 30% decrease in high-density lipoprotein and an average 30% increase in low-density lipoprotein.[134] The mechanisms for this effect are unknown but apparently include an increase in the activity of hepatic triglyceride lipase that catabolizes high-density lipoprotein particles. Most studies indicate that injectable non-C17α-alkylated anabolic steroids, such as testosterone and nandrolone esters, exert minimal adverse effects on blood lipids.[129,134] It is unclear if the adverse changes in blood lipids as a function of testosterone use actually lead to an increase in the incidence of coronary artery disease.

AASs may also influence platelet aggregation and the myocardium, although the relationship between these effects and cardiovascular disease is unclear. Occasional reports of cardiomyopathies and arrhythmias associated with steroid use have been published, and several mechanisms have been proposed.[130]

Liver disease is a well-documented side effect of most, but not all, C17α-alkylated AASs, the exception being oxandrolone. In contrast, most non-C17α-alkylated steroids exert minimal hepatoxicity. Liver pathologies associated with anabolic steroids include cholestasis, peliosis hepatis, hepatocellular adenoma and carcinoma, and hepatic angiosarcoma and cholangiocarcinoma.[113,130]

Potential effects on the reproductive system include infertility and testicular atrophy in men, and menstrual and genital tract alterations and infertility in women.[116] Although all AASs suppress the hypothalamic-pituitary axis to some extent, the resulting infertility in males is generally reversible. The effects of AAS use on fertility in women is unknown.[116] Menstruation is either diminished or absent in steroid users, but ovulation may occur.[135]

Balding is common in those who use AASs that undergo 5α reduction to potent androgens. In some male steroid users, gynecomastia may be caused by increased circulating estrogen associated with the use of aromatizable androgens and/or human chorionic gonadotropin, with decreased clearance of circulating estrogens as a result of impaired hepatic function, and/or with a temporary state of hypotestosteronemia following AAS withdrawal.[130]

Several studies have suggested that AAS use may lead to significant psychological morbidity. Anxiety, psychosis, irritability, increased aggression, and antisocial and violent behavior have been described to be associated with AASs. In addition, dependence, withdrawal symptoms, and depression may accompany or follow the nonmedical use of AASs.[130]

Finally, there are concerns about potential effects of androgens on the risk of prostate disease, although the long-term effects of supraphysiologic doses of androgens on the risk of prostate cancer, benign prostatic hypertrophy, and lower urinary tract symptoms remain unknown.[116]

Detection

All known AASs can be detected via urinalysis for a period of time following the last dose. Detection of the misuse of exogenous substances is preferably done using gas chromatography/mass spectrometry (GC-MS) complemented by liquid chromatography/tandem mass spectrometry.[136] To improve the selectivity and sensitivity, traditional GC-MS methods are accompanied by high-resolution mass spectrometry and/or tandem mass spectrometry techniques.[137]

Endogenous androgens and their metabolites occur naturally in the human body, and thus specific indicators for the detection

of the exogenous administration of these steroids are required.[137] For screening purposes, a set of urinary concentrations of several endogenous steroids or metabolites is determined by the GC-MS method used for the detection of steroid abuse. The method of steroid profiling was first introduced into routine doping control by Donike and associates[138] in 1983 (testosterone to epitestosterone ratio). The most important steroid profile parameters in doping control are the ratios of testosterone/epitestosterone, androsterone/etiocholanolone, androstane/testosterone, and 5α/5β forms of androstane-3,17-diol. The administration of steroids like testosterone or its precursors (e.g., androstenediol, androstenedione, or DHEA) or metabolites (e.g., dihydrotestosterone or epitestosterone) has been proved to alter one or more of the parameters of the urinary steroid profile.[138] Consequently, monitoring the steroid profile parameters allows to screen for potential misuse.

Growth Hormone

GH has been used as a drug of abuse in sport since the early 1980s, although the first scientific studies demonstrating a clear-cut physiologic role for GH in adults were only published in the peer-reviewed medical literature in 1989.[139,140]

Human GH (hGH) misuse in sports predates its use in adult endocrinology by 10 years, although cadaveric GH had been used in children since the 1950s. This practice was stopped by clinicians (but not athletes) when cases of Creutzfeldt-Jakob disease were discovered in patients who had previously received hGH. The clinical use of GH resumed following US Food and Drug Administration approval of the first synthetic recombinant hGH (rhGH) in 1985. Other formulations subsequently followed.[141]

GH has several properties that are attractive to athletes wishing to improve their performance, but it is mainly misused because of its anabolic and lipolytic effects. GH administration leads to increased muscle mass and a decrease and redistribution of body fat from central to peripheral depots, thereby potentially improving the power to weight ratio of an athlete.[141]

In 2008, Liu and associates[142] completed a systematic review of 44 randomized controlled trials that compared GH treatment with no GH treatment in healthy adults. Their review suggests that although GH administration may increase FFM, strength and exercise performance did not improve with GH, and edema and fatigue were more common in the GH-treated subjects. The authors concluded that claims that GH improves athletic performance are not supported by the available scientific literature.

Graham and associates[143] performed a well controlled study that showed an effect on strength and peak power output in a group of well-defined abstinent steroid abusers using rhGH. A study in recreational athletes demonstrated that in the short term, GH significantly increased lean body mass, reduced fat mass, and improved sprint capacity but not strength, power, or endurance.[144]

Because GH is a banned substance, the doses that athletes use are difficult to evaluate. It has been suggested that athletes abusing GH take rhGH three to four times per week at a dose of 10 to 25 IU per day to increase their lean body mass.[145]

GH abuse extends beyond professional sports and is also present among adolescents engaged in sports in schools. This widespread use presents a public health problem because GH use is accompanied by adverse effects, and long-term use can lead to serious morbidity.[146]

Adverse Effects

The side effects of GH treatment in GH-deficient adults are well documented and include edema, arthralgias, myalgias, sweating, fatigue, and dizziness.[141,147] Athletes typically take pharmacologic doses that are likely 10 or more times greater than typical replacement doses. Although the long-term adverse effects of these megadoses are not known at present, some insight may be obtained from patients with acromegaly. Acromegalic patients have an increased risk of insulin resistance and diabetes, hypertension, cardiomyopathy, and certain forms of cancer (colorectal, thyroid, breast, and prostate).[148]

Finally, the risk of infections such as human immunodeficiency virus/acquired immunodeficiency syndrome or hepatitis due to the use of nonsterile or contaminated syringes[145] and the availability on the black market of GH that comes from extracts of human pituitary glands and represents a source for Creutzfeldt-Jakob disease must be taken into account.

Detection

Two different strategies have been proposed to detect GH doping in sports.[149] For the marker method "pharmacodynamic endpoints of GH use," the GH-2000 and GH-2004 consortiums identified biochemical parameters of the IGF system, such as IGF1, IGFBP3, and the acid-labile subunit, as suitable markers of GH use in combination with procollagen cleavage products that also show a clear-cut increase following GH use. The combination of IGF1 and the procollagen III N-terminal extension peptide is proposed to provide a set of markers that allow the detection of GH abuse in athletes for up to 2 weeks after the last application.

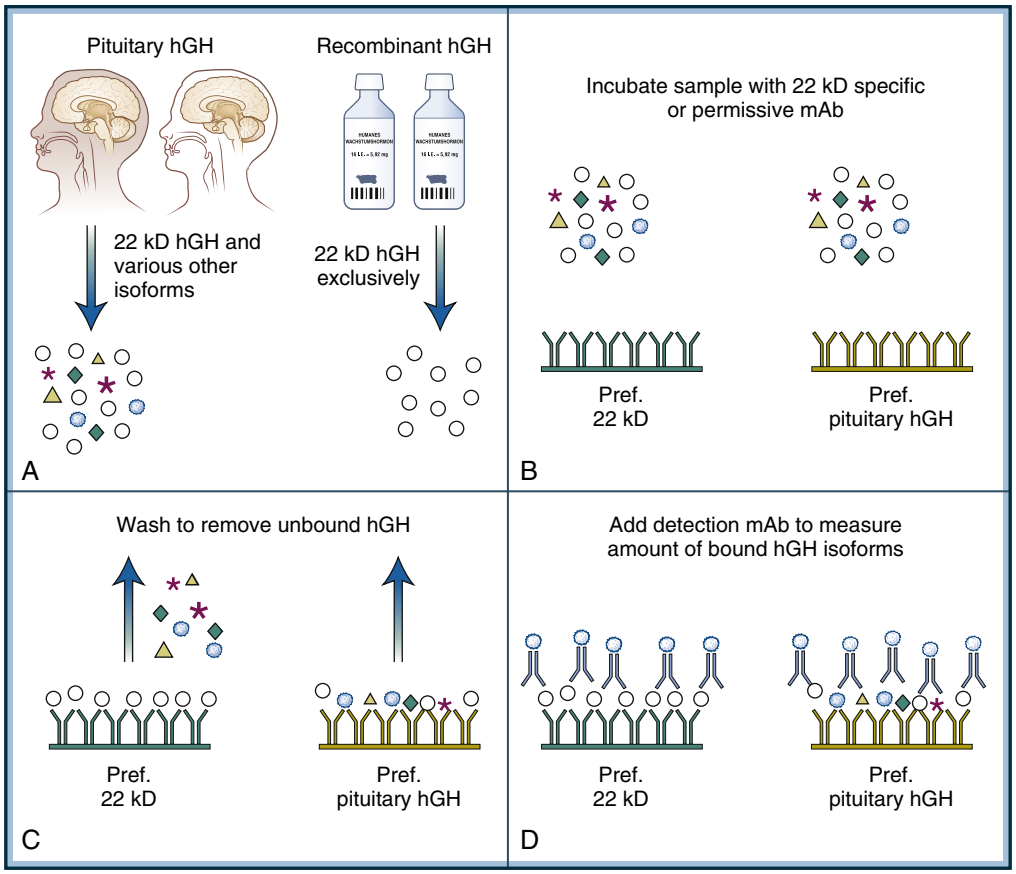
The "GH-isoform method" exploits the difference in isoform composition between recombinant GH consisting mostly of monomeric 22-kilodalton (kDa) hGH while the pituitary secretes a variety of GH isoforms including a 20-kDa form lacking 14 amino acids, as well as amidated and acylated isoforms, and dimers, oligomers, and fragments of GH. After peripheral injection of recombinant 22-kDa hGH, the pituitary's production of GH isoforms is reduced by negative feedback via IGF1. Subjecting serum samples to two immunoassay analyses—one of which is specific for 22-kDa monomeric hGH, whereas the other recognizes the majority of isoforms released from the pituitary—allows the calculation of an isoform ratio (Fig. 27.1).

The isoform test is an excellent strategy to detect GH doping, provided that it is administered shortly after the last GH dose (within 24–36 hours, depending on the dose),[150] realistically probably within 12 to 24 hours.[146] Due to this short window of opportunity, the test is not well suited for in-competition testing. Its use in unannounced out-of-competition testing, however, should be more successful in catching GH abusers, and recently it has been used mostly in that setting.[146]

GH Secretagogues

GH secretagogues are peptides or nonpeptidic agents that act to release GH from the pituitary. There is evidence that they are being used by athletes as an indirect method for GH doping. Secretagogues include GH-releasing hormone and its analogues, ghrelin analogues (known as GH-releasing peptides) or GHSs (GH secretagogues in a narrower sense), and amino acids (e.g., arginine or ornithine). GHSs have short-lived effects and provide a relatively weak boost in GH exposure compared with what can be achieved by direct GH administration.[146] Nevertheless, GHSs may be attractive to athletes who want to avoid detection because the GH released is endogenous and therefore not detectable by the GH isoform test.[146]

Currently, there are no published studies of the effects of GHSs on performance, but investigations have shown that they increase GH secretion and potentially could improve athleticism through this mechanism.[141]



• **Fig. 27.1** (A) While the pituitary gland secretes a variety of molecular forms of growth hormone (GH), recombinant GH consists predominantly of monomeric 22 kD GH. (B) Serum samples are incubated with immobilized monoclonal antibodies (mAbs) against GH preferentially binding to either 22 kD GH *(left)* or the other pituitary-derived molecular forms *(right)*. (C) Unbound GH forms and other sample constituents are washed away before (D) incubation with an independently labeled anti-GH antibody binding to all GH molecular forms. The result of the differential immunoassay is then expressed as the ratio between the two different isoform measurements. *hGH,* human growth hormone.

Insulin-like Growth Factor 1

Recombinant human IGF1 (rhIGF1) is used in clinical practice, but a variety of IGF1 compounds and IGF1 analogues are also advertised on the internet, and many have been available on the black market for several years.[151] The rationale for using rhIGF1 as an ergogenic aid differs little from that of rhGH. The potential benefits include increased muscle protein synthesis, increased glycogen synthesis, and free fatty acid availability,[152] but in contrast to GH, IGF1 is not lipolytic.

Abuse of IGF1 is more recent than that of rhGH. In fact, until recently, IGF1 was only available in limited supply, partly because there is no natural source for this hormone and partly because there were no pharmaceutical preparations. This situation changed with the development of two compounds, mecasermin (Increlex) by Tercica (Brisbane, CA) and mecasermin rinfabate (iPLEX) by Insmed (Richmond, VA). Both preparations received US Food and Drug Administration approval for clinical use in the treatment of growth failure in children with severe primary IGF1 deficiency or with GH gene deletion who have developed neutralizing GH antibodies.[153] Although there are no confirmed cases of IGF1 misuse by athletes, the drug seems to be already popular among amateur bodybuilders. IGF1 is extensively discussed on internet bodybuilding forums. The list of purported benefits include increases in muscle size and strength, improvements in energy and endurance, benefits to the immune system, and increased bone density.[151]

Very few studies have been undertaken to examine the effects of IGF1 on performance. Guha and associates[154] demonstrated a 9% increase in $\dot{V}O_2$max in recreational athletes following 28 days of administration of rhIGF1/rhIGFBP3 despite no change in body composition. The mechanism underlying this change is unknown, but previous studies have shown that the intravenous administration of rhIGF1 caused an increase in cardiac output, heart rate, and stroke volume in healthy adults.[155] IGF1 may have a role in the regulation of vascular tone through alterations in nitric oxide synthesis, allowing improved intramuscular blood flow. Serum IGF1 correlates positively with hemoglobin concentration, which may enhance oxygen delivery to exercising muscle. Finally, an anabolic effect on respiratory muscle strength may improve inspiratory effort.[141]

Adverse Effects

The adverse effects that have been reported with Increlex include hypoglycemia, jaw pain, headache, myalgias, and fluid retention.[156] The most common adverse effects observed so far with the use of mecasermin rinfabate include local injection site erythema and lipohypertrophy, although headaches, increased liver and kidney size, and altered liver function tests have also been reported.[153]

It also seems reasonable to hypothesize that IGF1 could result in adverse effects similar to acromegaly.[152]

Detection

At present, there is no specific test to detect IGF1 abuse, and detection of this form of doping poses many challenges. IGF1 is excreted in urine at low concentrations, and methods for measuring urinary

IGF1 are complex and time consuming. Several factors contribute to renal clearance of IGF1: a significant increase in urinary IGF1 concentration is observed as part of the proteinuria that occurs in response to exercise. Blood sample collection, rather than urine, will thus be required for IGF1 doping tests.[151]

The GH-2004 research group has been investigating methods for detection of IGF1 misuse, and a test is being developed based on principles of the successful GH-2000 marker method. Commercial immunoassays for these markers have been validated for antidoping purposes, but new methods, including IGF1 measurement by use of mass spectrometry, are hoped to improve the performance of the tests and to help in the detection of athletes who are doping with these peptide hormones.[151,157]

Insulin

Insulin stimulates the uptake of glucose into muscle and fat by making available an increased number of glucose transporters (Glut-4) at the cell membrane. However, its main effect is inhibitory to lipolysis, glycolysis, gluconeogenesis, ketogenesis, and proteolysis.[158]

Insulin regulates hepatic glucose output by inhibiting gluconeogenesis and promoting glycogen storage. Similarly, in muscle cells, insulin-mediated glucose uptake enables glycogen to be synthesized and stored, and for carbohydrates, rather than fatty acids or amino acids, to be utilized as the immediately available energy source for muscle contraction. Although insulin stimulates the uptake of amino acid into cells and promotes protein synthesis in a range of tissues at high insulin concentrations, the major action of insulin is to inhibit proteolysis, which occurs at lower insulin concentrations.[152]

The theoretic performance benefits of insulin are mediated by an increase in muscle glycogen storage and the inhibition of proteolysis but have not been demonstrated in clinical or scientific trials.[152] The first suggestions of insulin as an anabolic agent were published in two bodybuilding magazines in 1996, which were commented on in the *British Journal of Sports Medicine* in 1997.[159] At the Winter Olympic Games in Nagano in 1998, a Russian medical officer inquired as to whether the use of insulin was restricted to type 1 diabetes.[160] This drew attention to its role as a potential performance-enhancing drug and led to its ban in 1999 by the IOC.[153]

Various reports since then have described the misuse of insulin to improve performance and muscle strength, and synthetic analogues were the subject of several studies describing the beneficial effects of biotechnologically modified insulins.[161]

Adverse Effects

The most common adverse effect of insulin use is hypoglycemia. Most athletes who abuse insulin usually balance the ingestion of carbohydrate when injecting rapidly acting insulin analogues. Another problem associated with insulin is weight gain, although most competitive athletes are accustomed to diet and follow training regimens that allow them to have strict control over weight gain.[152,153]

Detection

So far, no screening tool is available providing fast and reliable information on possible insulin misuse. Only sophisticated procedures including immunoaffinity purification followed by liquid chromatography and tandem mass spectrometry have enabled the detection of synthetic insulins in doping control blood or urine samples.[162] Insulin analogues have been designed to alter their pharmacokinetics by genetic substitutions of one or two amino acids from human insulin. These small differences can be utilized to differentiate between native and exogenous insulin.[153]

Erythropoietin and the Erythropoietin System

EPO, a glycoprotein hormone naturally produced in the kidney and the liver, is an essential growth factor for the erythrocytic progenitors in the bone marrow. Tissue hypoxia is the physiologic stimulus for EPO expression and erythropoiesis. Once released, it serves to stimulate an increase in hemoglobin. In this way, it increases the oxygen-carrying capacity of the blood.[111]

Successful cloning of the human *EPO* gene allowed for the production of recombinant human EPO (rhEPO) and later the approval to treat patients with anemia. More recently, several newer generations of EPO analogues have been produced.[163]

Because hemoglobin mass correlates with the aerobic capacity, some athletes seek to increase erythropoiesis by pharmacologic means. Treatment with rhEPO may enhance performance by raising hemoglobin concentration. Studies show that when rhEPO is applied to healthy volunteers in low dosages, $\dot{V}O_2$max is increased by 6% to 12% when the hematocrit is raised to approximately 0.50. In addition, the time to exhaustion (in the laboratory) is increased by up to 50% at a given level of $\dot{V}O_2$max.[164] When rhEPO administration is discontinued, $\dot{V}O_2$max remains elevated for at least 3 weeks.[111]

Apart from rhEPO, several other erythropoiesis-stimulating agents have been developed. Darbepoetin alfa is an rhEPO glycosylation analogue approved for use in 2002 in the European Union and United States. It has the same mechanism of action as rhEPO—binding and activation of the EPO receptor—but it has a longer serum half-life and an increased in vivo potency. Another long-acting erythropoiesis-stimulating agent is methoxy polyethylene glycol-epoetin beta (methoxy PEG-epoetin beta). The half-life of methoxy PEG-epoetin beta amounts to 130 to 140 hours on intravenous injection. The prolonged in vivo survival of darbepoetin alfa and methoxy PEG-epoetin beta is in part due to a reduced EPO-R binding affinity.[111]

EPO mimetic peptides are synthetic cyclic peptides of about 20 amino acids that signal through the EPO-R but show no sequence homology to EPO. The clinically most advanced product was peginesatide, a pegylated homodimer of two EPO mimetic peptides, which was initially approved in the United States for treatment of anemic patients with chronic renal failure but subsequently recalled after lethal acute hypersensitivity reactions occurred in several patients.[165]

Sotatercept (ACE-011) is a novel recombinant erythropoietic protein. It is composed of the extracellular part of the activin type II receptor and the Fc domain of human immunoglobulin G1. In a clinical phase I study, single intravenous injections of sotatercept proved to increase blood hemoglobin levels, RBC numbers, and the hematocrit.[166] However, the mechanisms underlying the effect of sotatercept or of analogue compounds on erythropoiesis are still poorly understood.[111]

The expression of the *EPO* gene (chromosome 7q22) is under the control of several transcription factors, including GATA-2, which inhibits EPO expression. GATA antagonists are organic small molecule compounds that prevent GATA-2 from suppressing the EPO promoter. GATA antagonists may be misused in sports, because they have been shown to increase EPO concentrations, hemoglobin levels, and endurance performance in mice, although human studies have not yet been reported.[111,167]

Hypoxia-inducible factor (HIF) stabilizers, such as α-ketoglutarate competitors, stimulate erythropoiesis by increasing EPO expression in the kidneys and at extrarenal sites. Advantages of HIF stabilizers—compared with recombinant proteins—include the oral

route of administration. However, the HIFs can activate more than 1000 genes apart from EPO, including genes for proteins that are involved in tumor growth, such as vascular endothelial growth factor. However, some of the HIF-activated genes encode proteins that may increase physical performance independent from the stimulation of erythropoiesis.[111]

EPO gene transfer is theoretically possible but medically little advanced. In vivo allogeneic EPO transfer can cause immune reactions. Furthermore, antidoping research has provided techniques to specifically amplify intronless DNA sequences allowing the detection of small amounts of transgenic DNA in blood.[168] Ex vivo EPO transfer is performed by use of transfected autologous or allogeneic cells that are transferred from in vitro cultures into the individual. The autologous ex vivo approach has been investigated in clinical trials.[169] However, the method has made little progress and has not stepped beyond the clinical trial phase, even after many years of study. Taken together, for technical difficulties, gene doping with EPO is probably not applied in sports, at least not yet.[111]

Adverse Effects

Artificially raising hemoglobin levels can have dangerous consequences. In contrast to the effect of endurance training, which results in an increased plasma volume, the administration of rhEPO produces a selective increase in red cell mass. If the hematocrit exceeds 0.50, blood viscosity and cardiac afterload increase significantly. The main risk of erythrocytosis with a hematocrit greater than 0.55 include heart failure, myocardial infarction, seizures, peripheral thromboembolic events, and pulmonary embolism.[170] Moreover, EPO withdrawal may be implicated in neocytolysis—that is, the hemolysis of young red blood cells in the presence of an increased hematocrit.[171]

Detection

The authorities of sports forbade the use of EPO in 1990, and now any analogue or mimetic is also included in the list of prohibited substances of the World Anti-Doping Agency (WADA). The challenge of detecting the misuse of EPO has given strength to the proposal of several strategies.[172] The marker methods rely on the measurement of some hematologic and serum parameters, and their comparison with populational or individual limit values.[173] An indirect test based on the measurement of five blood parameters, including reticulocyte hematocrit, serum EPO, hematocrit, soluble transferrin receptor, and the percentage macrocytes, was developed by Parisotto and associates.[174] Two models were developed. The "on" model used all parameters to detect recent use of EPO. The "off" model used three parameters to detect EPO use with more retrospectivity.[173] An advantage of indirect methods to detect rhEPO is its universal coverage of different types of analogues and mimetics, a field in clear expansion.

The most powerful way to directly discriminate between endogenous and rhEPO is probably based on the glycosylation differences existing between both types of molecules.[175] In 1995, Wide and colleagues[176] proposed, for the first time, a method able to separate both types of molecules in blood and urine. This technique was reliable, as it allowed to clearly identify the presence of rhEPO in urine and blood. However, although the proposed method was powerful as long as the biologic samples were collected within 24 hours after the last rhEPO injection, it appeared to be far less sensitive on samples collected later after injection.

In June 2000—a few weeks before the Sydney Olympic Games—Lasne and de Ceaurriz[177] presented in *Nature* an innovative test based on the isoelectric separation of urinary EPO isoforms on a polyacrylamide gel followed by a double blotting process.[178] In the past 10 years, this test has been adapted to several recombinant EPO forms, such as darbepoetin-α, whose isoforms are located in the most acidic part of the gel, epoetin-δ, or more recently, generic (biosimilar) or "copy" EPOs.

Glucocorticosteroids

Glucocorticosteroids (GCs) were first purified and manufactured in the 1930s and 1940s following the discovery of their potent anti-inflammatory actions.[179] GCs are widely used in medicine and have shown unchallenged therapeutic potential in several chronic inflammatory and other diseases.

GCs are also widely used in sports medicine for the treatment of conditions such as asthma and acute injuries. Nevertheless, their beneficial effect in certain conditions in sports, where inflammation is only a secondary reaction, remains to be validated.[180]

According to the World Anti-Doping Code, all orally, rectally, intravenously, and intramuscularly administered GCs are prohibited, and their medical use requires a standard therapeutic use exemption. Administration of GCs by all other routes requires an abbreviated therapeutic use exemption, except dermatologic preparations, which are not prohibited.[180] Because of the complexity of GCs, determining the boundaries between their medical use and abuse is a constant challenge for antidoping organizations.

The first use of GCs as performance-enhancing drugs was described in the 1960s.[179] With regard to athletes, the most interesting systemic effect of GCs is energy production by stimulation of gluconeogenesis and mobilization of amino acids and fatty acids. A possible increase in cardiovascular performance is a matter of debate, as there is no evidence for such effects. As a consequence, systemic GCs have been misused for decades to enhance performance, and they once belonged to the group of most commonly used doping substances in sports.[180] Indeed, the expected effects of the use and abuse of GCs are numerous: neurostimulatory effects at cerebral GC receptors could attenuate central impressions of fatigue, and anti-inflammatory and analgesic effects could inhibit sensations of muscle pain on effort and raise the fatigue threshold.[181]

Few studies have examined the effects of GCs on exercise performance. An extensive review of the scientific literature performed by Duclos[182] showed two types of results: studies supporting the hypothesis that there is no relationship between performance and corticosteroid use in humans (negative studies) and studies supporting the hypothesis that there are relationships between performance and corticosteroid use in humans (positive studies).

Inconsistencies found regarding the ergogenic effect of GC administration in humans may be attributed to (1) the GC administration dosage, route, and mode (acute or short term); (2) the type, duration, and intensity (submaximal, maximal) of exercise tested; (3) the participants (highly trained or professional vs. recreational trained); (4) the differences in diet, such as whether or not experiments are food controlled and whether or not subjects fasted; and (5) GC intake coupled or not with intensive training.[182]

It is noteworthy that data reported by the "negative studies" mainly was derived from acute administration of GCs. Animal and human studies performed using higher doses of GCs and/or longer periods of administration (positive studies) clarified the effects of GC based on scientific evidence and clearly demonstrated that GCs have ergogenic effects in both animals and humans.[182]

Adverse Effects

GCs have pleiotropic effects, causing several adverse effects, especially at higher doses and for long periods, such as osteoporosis, insulin resistance, and cardiovascular conditions (e.g., hypertension and atherosclerosis).[183] Moreover, a major (possibly life-threatening) complication can arise on the withdrawal of GCs: acute adrenal insufficiency.

Detection

The detection of the administration of GCs is complicated by the fact that the human body produces these steroids naturally. Several groups have described protocols and screening methods for the analysis of endogenous GC urinary metabolites using liquid chromatography/mass spectrometry. Some methods allow for the analysis of a large number of analytes from most of the classes in the WADA Prohibited List, including anabolic agents, β2-agonists, hormone antagonists and modulators, diuretics, stimulants, narcotics, glucocorticoids, and β-blockers, and do so while meeting the WADA sensitivity requirements.[184]

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28

Endocrinology and Aging

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CHAPTER OUTLINE

KEY POINTS

- Treatment of subclinical hypothyroidism in older individuals is not associated with clinical benefits. In younger individuals, it is associated with fewer ischemic heart disease events, but this is not evident in older people (>70 years).
- Subclinical hyperthyroidism is associated with an increased risk of total and ischemic heart disease mortality, as well as incident atrial fibrillation. Currently, treating subclinical hyperthyroidism is recommended when the thyroid-stimulating hormone level is <0.1 mU/L, and confirmed after a 3- to 6-month period, in all individuals 65 years of age or older; postmenopausal women not on bisphosphonates or estrogen; and patients with cardiac risk factors, heart disease, or osteoporosis. At a thyroid-stimulating hormone level between 0.1 and 0.5 mU/L, treatment should be considered only for individuals 65 years of age or older and in patients with cardiac disease or hyperthyroid symptoms.
- Aging is associated with late-day and evening increases in cortisol levels; earlier morning cortisol maximum (phase advance)—for example, 6:30 AM (older) versus 9:00 AM (younger); lower circadian amplitude; and more irregular cortisol secretion patterns.
- Estrogen hormone therapy is currently only recommended in the perimenopausal period in those women suffering from menopausal symptoms. Hormone therapy is not indicated for cardioprotection for women in their 70s.
- The current recommendation is not to treat asymptomatic older men with age-related decline in testosterone levels. Clinicians can consider offering testosterone therapy on an individualized basis to older men with low testosterone levels and clinically significant symptoms of androgen deficiency. Control of prostate size, prostate-specific antigen levels, and hematocrit is mandatory.
- During the aging process, growth hormone–insulin-like growth factor 1 axis activity declines. At present, there is no evidence to recommend medical intervention in the growth hormone–insulin-like growth factor 1 axis as an antiaging effort to prolong life or rejuvenate healthy elderly people.

Global life expectancy at birth is currently 69.1 years for men and 73.8 years for women.[1] Twenty-nine countries have an average life expectancy of 80 years or higher. Life expectancy increases accelerated in most regions from 2000 onward, and overall there was a global increase of 5 years in life expectancy between 2000 and 2015. Average global life expectancy at birth is predicted to further increase by 4 years by 2030.[1] Between 1950 and 2050, the number of people in the world older than 80 years is expected to increase from 14.5 million to 394.7 million.[2]

It is not clear, however, whether these additional years will be satisfactory. Healthy life expectancy in high-income countries at birth in 2015 for males and females combined was 69.8 years, 8.9 years lower than total life expectancy at birth. In general, healthy life expectancy is on average 11.7% shorter than life expectancy (ranging from 9.3–14.7% between countries).[1] Most data indicate a modest gain in the number of healthy years lived but a far greater increase in years of compromised physical, mental, and social function.[3] The number of days with restricted activity and admissions to hospitals and nursing homes increases sharply after 70 years of age.[4] The compression-of-morbidity hypothesis[5] suggests that it may be possible to reduce cumulative lifetime morbidity. Since chronic illness and disability usually occur late in life, cumulative lifetime disability could be reduced if primary prevention measures postpone the onset of chronic illness. Indeed, smoking, body mass index, and exercise patterns in midlife and late-adulthood are important predictors of subsequent disability.[6] Not only do persons with better health habits live longer, but in such persons, disability is postponed and compressed into fewer years at the end of life.

Aging and Physical Frailty

Throughout adult life, all physiologic functions start to decline gradually.[7] There is a diminished capacity for cellular protein synthesis, a decline in immune function, an increase in fat mass, a loss of muscle mass and strength, and a decrease in bone mineral density.[7] Most older adults die of atherosclerosis, cancer, or dementia, but in an increasing number of the "healthy" oldest old, loss of muscle strength is the limiting factor that determines their chances of an independent life until death.

Age-related disability is characterized by generalized weakness, impaired mobility and balance, and poor endurance. In the oldest old, this state is termed *physical frailty*, defined as "a state of reduced physiological reserves associated with increased susceptibility to disability."[8] Clinical correlates of physical frailty include falls, fractures, impairment in activities of daily living, and loss of independence. Falls contribute to 40% of admissions to nursing homes.[9]

Loss of muscle strength is an important factor in the development of frailty. Muscle weakness can be caused by aging of muscle fibers and their innervation, osteoarthritis, and chronic debilitating diseases.[10] A sedentary lifestyle, decreased physical activity, and disuse, however, are also important determinants of the decline in muscle strength.

In a study of 100 frail nursing home residents (average age 87 years), lower extremity muscle mass and strength were closely related.[11] Supervised resistance exercise training (45 minutes three times a week for 10 weeks) doubled muscle strength and significantly increased gait velocity and stair-climbing power. This finding demonstrates that frailty in the elderly population is not an irreversible effect of aging and disease but can be influenced and perhaps even prevented.[11] Further, in nondisabled elderly persons living in the community, objective measures of lower extremity function are highly predictive of subsequent disability.[12] Prevention of frailty may be achieved by caloric and protein support, vitamin D supplementation, reduction in polypharmacy, and working (training).[13] However, exercise is difficult to implement in the daily routine of the aging population, and the number of dropouts from exercise programs is very high.

Part of the aging process involving body composition (i.e., loss of muscle [strength] and bone, increase in fat mass) might also be related to changes in the endocrine system.[14] Current knowledge has shed light on the effects of long-term hormone replacement therapy on body composition, as well as on atherosclerosis, cancer formation, and cognitive function.

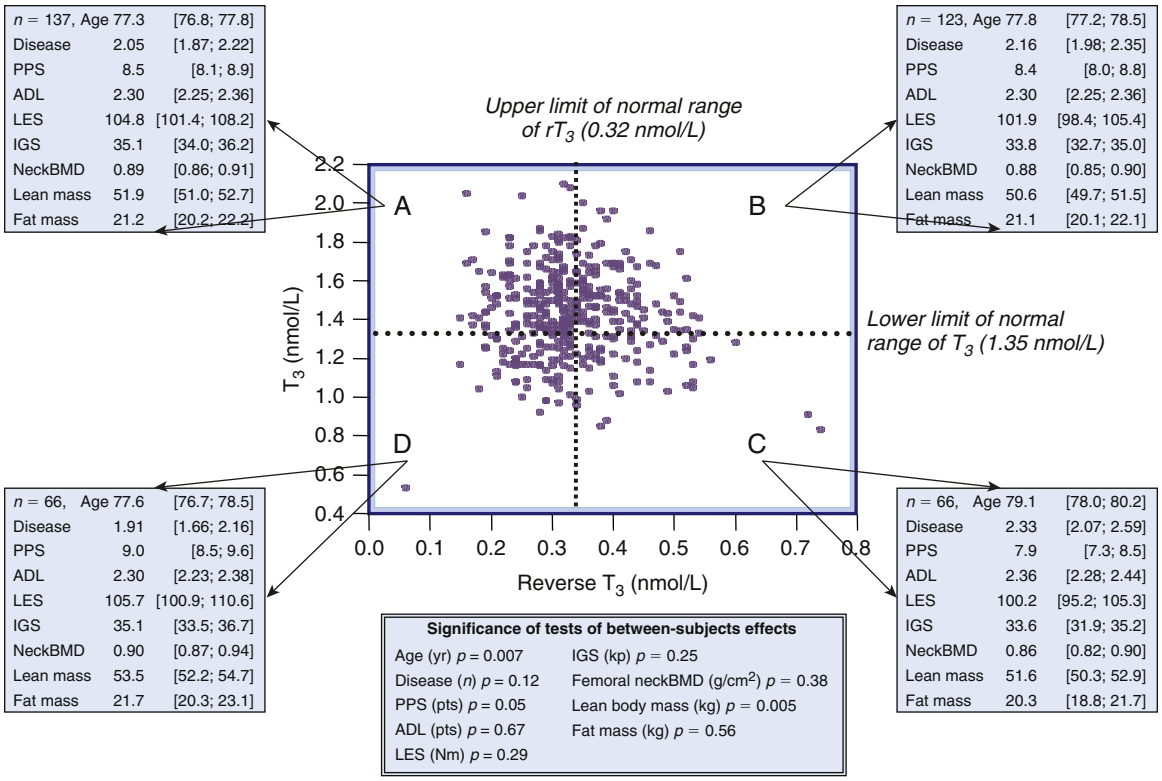
The Endocrinology of Aging

The two most important clinical changes in endocrine activity during aging involve the pancreas and the thyroid gland. Approximately 40% of individuals age 65 to 74 years and 50% of those older than 80 years have impaired glucose tolerance or diabetes mellitus, and in nearly 50% of elderly adults with diabetes, the disease is undiagnosed.[15] These adults are at risk for development of secondary, mainly macrovascular, complications at an accelerated rate. Pancreatic, insulin receptor, and postreceptor changes associated with aging are critical components of the endocrinology of aging. Apart from decreased (relative) insulin secretion by the beta cells, peripheral insulin resistance related to poor diet, physical inactivity, increased abdominal fat mass, and decreased lean body mass contributes to the deterioration of glucose metabolism.[16] Dietary management, exercise, hypoglycemic agents, and insulin are the four components of treatment for these patients, whose medical care is costly and intensive (see Chapter 27).

Age-related thyroid dysfunction is also common.[17] Lowered plasma thyroxine (T_4) and increased thyrotropin concentrations occur in 5% to 10% of elderly women.[17] These abnormalities are primarily caused by autoimmunity and are thus an expression of age-associated disease rather than a consequence of the aging process. Normal aging is accompanied by an increase in serum thyrotropin levels and seems to be dependent on the regional iodine intake.[18-21] Serum free T_4 levels remain largely unaffected during aging, but decreased peripheral degradation of T_4 results in a gradual age-dependent decline in serum triiodothyronine (T_3) concentrations.[17,22] The magnitude and pattern of changes in thyroid function during aging, however, are highly variable among individuals.[21,23] This slight decrease in plasma T_3 concentrations occurs largely within the broad normal range of the healthy elderly population and has not been convincingly related to functional changes during the aging process. It remains to be clarified why serum thyroid-stimulating hormone (TSH) levels increase with advancing age. Several mechanisms, like a changed pituitary sensitivity or affected TSH glycosylation and thus TSH bioactivity, have been proposed.[19] The detrimental effects of overt thyroid dysfunction in elderly individuals are clearly recognized. However, the clinical relevance of mild forms of hypo- and hyperthyroidism are a matter of debate.

Subclinical hypothyroidism (SCH) is present in about 4% to 8.5% of adults in the United States without known thyroid disease.[24] Subtle thyroid dysfunction in the oldest-old fraction of the elderly population (i.e., those >85 years) is often present. Although SCH in younger individuals is associated with an increased risk of atherosclerosis,[25] such an association is not present in elderly subjects older than 65 years.[26,27] In addition, an observational study of real-life practice performed from data obtained from the United Kingdom General Practitioners Research Database showed that treatment of SCH with levothyroxine was associated with fewer ischemic heart disease events in younger individuals, but this was not evident in older people (>70 years).[26] Treatment with levothyroxine in persons with SCH older than 65 years for 1 year did not provide improvement of hypothyroid symptoms or tiredness.[28] In fact, in 85-year-old "healthy" individuals, hypothyroidism was, in the subsequent 4 years, accompanied by lower all-cause and cardiovascular mortality when compared with euthyroid individuals.[29] In a group of 400 males with a mean age of 78 years, low serum free T_4 and T_3 (with normal reverse T_3 [rT_3]) concentrations were associated with better physical performance and 4-year survival, whereas subjects with low serum T_3 and high rT_3 concentrations (i.e., fulfilling the criteria for "low T_3 syndrome") did not show a survival advantage and had lower physical activity[23] (Fig. 28.1). These findings were confirmed in other studies that link lowered thyroid hormone levels to less frailty.[30,31] These studies support the concept that some degree of physiologically decreased thyroid activity at the tissue level might even have favorable effects in the oldest-old subjects, but caution should be exercised when interpreting the predictive value of thyroid dysfunction in old subjects, which may give a double-faced "Janus response" if not considered in the appropriate context.[32,33]

Subclinical hyperthyroidism seems to be associated with an increased risk of total and ischemic heart disease mortality, and incident atrial fibrillation, as was concluded by recently pooled individual data from 10 prospective cohort studies.[34] In addition, subclinical hyperthyroidism is associated with an increased



• **Fig. 28.1** Overview of the values of triiodothyronine (T_3) and reverse T_3 (rT_3) within a population of 403 elderly men. The *spotted lines* indicate the normal values of T_3 and reverse T_3. *PPS*, physical performance score; *ADL*, number of problems in activities of daily living; *LES*, maximum leg extensor strength; *IGS*, isometric grip strength; *BMD*, bone mineral density. (From van den Beld AW, Visser TJ, Feelders RA, et al. Thyroid hormone concentrations, disease, physical function, and mortality in elderly men. *J Clin Endocrinol Metab.* 2005;90[12]:6403–6409.)

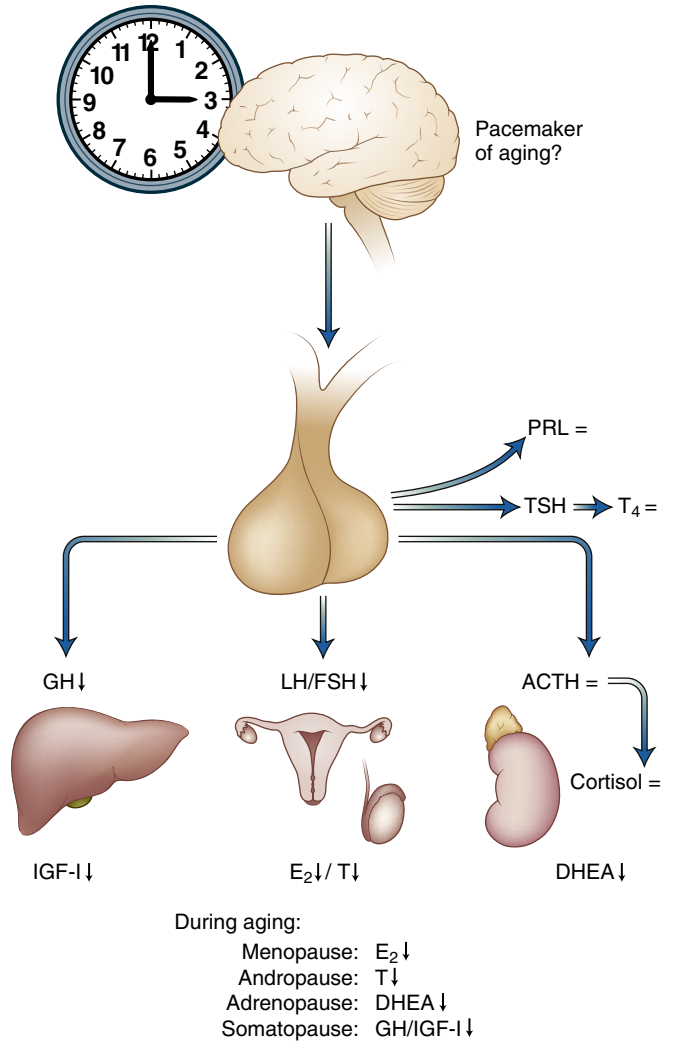
fracture risk.[35] Controversy persists about whether treatment is warranted. The latest consensus guidelines recommend treating subclinical hyperthyroidism after diagnosing the underlying disorder, based on the level of TSH suppression. When the TSH is less than 0.1 mU/L, and confirmed after a 3- to 6-month period, treatment is advised in individuals with hyperthyroid symptoms; all individuals 65 years of age or older; postmenopausal women not on bisphosphonates or estrogen; and patients with cardiac risk factors, heart disease, or osteoporosis. At a TSH level between 0.1 and 0.5 mU/L, treatment should be considered only for individuals 65 years of age or older and in patients with cardiac disease, osteoporosis, or hyperthyroid symptoms.[36] Furthermore, a recent systematic review concluded that there is a substantial body of evidence to support the association between subclinical hyperthyroidism and cognitive impairment. There is, however, lack of evidence to suggest that antithyroid treatment might delay the development of dementia.[37]

In addition, cortisol homeostasis is influenced by age, mostly via unknown mechanisms. Most clinical studies show circadian cortisol changes with age (reviewed by Veldhuis and colleagues[38]). Aging is associated with late-day and evening increases in cortisol levels; earlier morning cortisol maximum (phase advance)—for example, 6:30 AM (older) versus 9:00 AM (younger); lower circadian amplitude (24-hour decrement for peak minus nadir or attenuated wake-evening slopes); and more irregular (less orderly) cortisol secretion patterns.

The question remains to which degree these alterations reflect or cause aging-associated changes in functional ability, cognition, and possibly depression. A few studies have investigated associations between parameters of the hypothalamic-pituitary-adrenal axis and functional parameters. More dynamic activity of the hypothalamic-pituitary-adrenal axis (i.e., a greater diurnal decline) seems to be associated with better physical performance in later life,[39] as well as better cognitive function.[40] In a longitudinal setting, higher urinary cortisol levels are associated with an increased risk in Alzheimer disease pathology.[41] Further, data from a prospective cohort study of more than 400 men and women, with a mean age of 61 years, demonstrated that a flatter slope in cortisol levels across the day was associated with increased risk of all-cause 6-year mortality.[42] Results from the Longitudinal Amsterdam Study of Aging showed that higher morning salivary cortisol levels were associated increased mortality in men, and that higher nighttime salivary cortisol levels were associated with increased mortality in women.[43] Future clinical research is necessary to determine whether measuring diurnal cortisol measures is relevant in identifying those who might benefit from intervention therapies.

Three other hormonal systems exhibit lowered circulating hormone concentrations during normal aging, and these changes have thus far been considered mainly physiologic (Figs. 28.2 and 28.3). Hormone replacement strategies have been developed, but many aspects remain controversial, and replenishing hormone blood levels to those found in 30- to 50-year-old patients has not yet uniformly proved to be beneficial and safe.

The most dramatic and rapidly occurring change in women around age 50 years is *menopause*.[44] Cycling estradiol production during the reproductive years is replaced by very low, constant



• **Fig. 28.2** During aging, declines in the activities of several hormonal systems occur. *PRL*, prolactin; T_4, thyroxine; *TSH*, thyroid-stimulating hormone. *Left*. A decrease in growth hormone (GH) release by the pituitary gland causes a decrease in the production of insulin-like growth factor 1 (IGF1) by the liver and other organs (somatopause). *Middle*. A decrease in release of gonadotropin luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and decreased secretion at the gonadal level (from the ovaries, decreased estradiol [E_2] from the testicle, decreased testosterone [T]) cause menopause and andropause, respectively. (Immediately after the initiation of menopause, serum LH and FSH levels increase sharply.) *Right*. The adrenocortical cells responsible for the production of dehydroepiandrosterone (DHEA) decrease in activity (adrenopause) without clinically evident changes in corticotropin (adrenocorticotropic hormone [ACTH]) and cortisol secretion. A central pacemaker in the hypothalamus or higher brain areas (or both) is hypothesized, which together with changes in the peripheral organs (the ovaries, testicles, and adrenal cortex) regulates the aging process of these endocrine axes.

estradiol levels. For many years, the prevailing view was that menopause resulted from exhaustion of ovarian follicles. An alternative perspective is that age-related changes in the central nervous system and the hypothalamic-pituitary unit initiate the menopausal transition. The evidence that both the ovary and the brain are key pacemakers in menopause is compelling.[44]

Changes in the activity of the hypothalamic-pituitary-gonadal axis in men are slower and more subtle. During aging, a gradual decline in serum total and free testosterone levels occurs.[45] *Andropause* is characterized by a decrease in testicular Leydig cell numbers and their secretory capacity, as well as by an age-related decrease in episodic and stimulated gonadotropin secretion.[46,47] The primary site of the aging effect appears to be the Leydig cell's ability to respond to luteinizing hormone (LH) with increased testosterone production.

The second hormonal system demonstrating age-related changes is *adrenopause*, a term that describes the gradual decline in circulating levels of dehydroepiandrosterone (DHEA) and its sulfate (DHEAS).[48,49] Adrenal secretion of DHEA gradually decreases over time, whereas corticotropin secretion, which is physiologically linked to plasma cortisol levels, remains largely unchanged. The decline in DHEA and DHEAS levels in both sexes therefore contrasts with the maintenance of plasma cortisol levels and seems to be caused by a selective decrease in the number of functional zona reticularis cells in the adrenal cortex instead of being regulated by a central (hypothalamic) pacemaker of aging.[50]

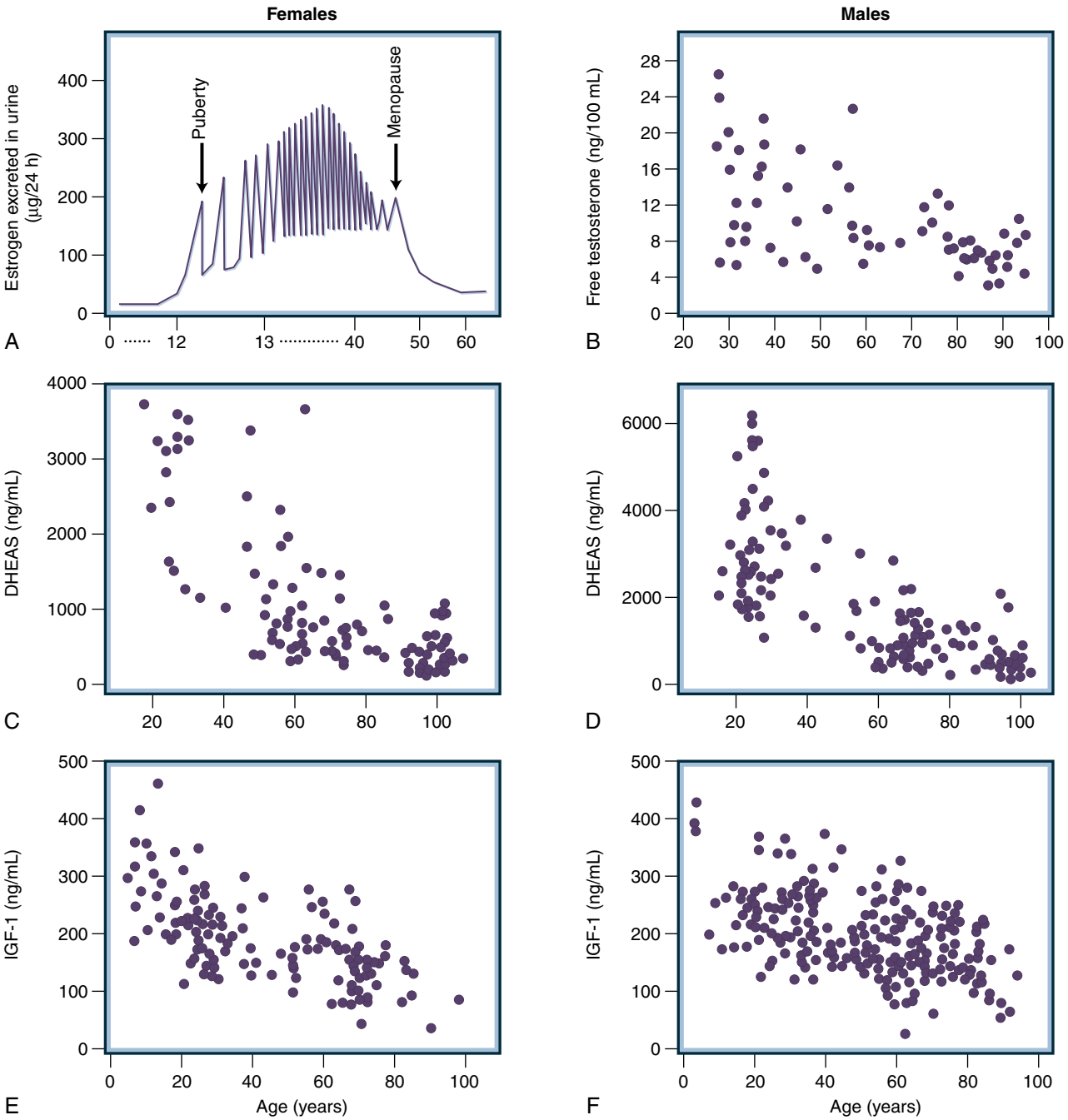
The third endocrine system that gradually declines in activity during aging is the growth hormone (GH)–insulin-like growth factor 1 (IGF1) axis[51] (see Fig. 28.3[219]). Mean pulse amplitude and duration and fraction of GH secreted, but not pulse frequency, gradually decrease during aging. In parallel, a progressive drop in circulating IGF1 levels occurs in both sexes.[51,52] There is no evidence for a peripheral factor in this process of *somatopause*, and its triggering pacemaker seems mainly localized in the hypothalamus because pituitary somatotropes, even in the oldest old, can be restored to their youthful secretory capacity by treatment with GH-releasing peptides (see later discussion).

It is unclear whether changes in gonadal function (menopause, andropause) are interrelated with the processes of adrenopause and somatopause, which occur in both men and women. In addition, functional correlates (decrease in muscle size and function and in fat and bone mass, progression of atherosclerosis, and decline of cognitive function) have not been demonstrated to be directly causally related to these changes in endocrine activity. However, several effects of normal aging closely resemble features of (isolated) hormonal deficiency (hypogonadism, GH deficiency), which in subjects in middle adulthood are successfully reversed by replacement of the appropriate hormone.[53,54] Although aging does not simply result from a variety of hormone deficiency states, medical intervention in the processes of menopause, andropause, adrenopause, or somatopause might prevent or delay some aspects of the aging process.

Menopause

Menopause is the permanent cessation of menstruation resulting from the loss of ovarian follicular function and is diagnosed retrospectively after 12 months of amenorrhea. In most women, vasomotor reactions, depressed mood, and urogenital complaints accompany this period of estrogen decline. In the subsequent years, the loss of estrogens is followed by a high incidence of cardiovascular disease, loss of bone mass, and cognitive impairment. The average age of menopause (51.4 years) has not changed over time and seems to be largely determined by genetic factors.

In the past decade, antimüllerian hormone (AMH) has been proposed as a marker to predict age at natural menopause. In women, AMH is exclusively produced by granulosa cells of ovarian follicles during the early stages of follicle development. After an initial increase until early adulthood, AMH concentrations slowly decrease with increasing age until becoming



• **Fig. 28.3** Changes in the hormone levels of normal women (*left*) and men (*right*) during the aging process. A and B, Estrogen secretion throughout an individual normal woman's life (expressed as urinary estrogen excretion) (A) and mean free testosterone (T) index (the ratio of serum total testosterone to sex hormone–binding globulin levels) during the life span of healthy men (B). C and D, Serum dehydroepiandrosterone sulfate (DHEAS) concentrations in 114 healthy women (C) and 163 healthy men (D). E and F, The course of serum insulin-like growth factor 1 (IGF1) concentrations in 131 healthy women (E) and 223 healthy men (F) during aging. Note the difference in the distribution of ages in the different panels. (A and B from Guyton A. *Textbook of Medical Physiology,* 8th ed. Philadelphia: Saunders; 1991:899. C and D modified from Ravaglia G, Forti P, Maioli F, et al. The relationship of dehydroepiandrosterone sulfate [DHEAS] to endocrine-metabolic parameters and functional status in the oldest-old. Results from an Italian study on healthy free-living over-ninety-year-olds. *J Clin Endocrinol Metab*. 1996;81[3]:1173–1178. E and F modified from Corpas E, Harman SM, Blackman MR. Human growth hormone and human aging. *Endocr Rev.* 1993;14[1]:20–39.)

undetectable about 5 years before menopause when the stock of primordial follicles is exhausted.[55] In a long-term follow-up study, 257 women were followed for 11 years. It was demonstrated that using age and AMH, the age range in which menopause will subsequently occur can be individually calculated.[56,57] A study of 401 women participating in the Penn Ovarian Aging Study demonstrated that in women with a baseline AMH level below 0.20 ng/mL, the median time to menopause was 5.99 years (95% confidence interval [CI], 4.20–6.33) in the 45- to 48-year age group and 9.94 years (95% CI, 3.31–12.73) in the 35- to 39-year age group. With higher baseline AMH levels above 1.50 ng/mL, the median time to menopause was 6.23 years in the oldest age group and more than 13.01 years in the youngest age group. Smoking significantly reduced the time to menopause.[58]

Perimenopausal Use of Hormone Therapy

Typical symptoms that result from the sudden decrease in estrogen production around menopause are menstrual cycle disorders, vasomotor changes (hot flushes, night sweats), and urogenital complications (atrophic vaginal irritation and dryness, dyspareunia, atrophic urethral epithelium leading to micturition disorders). Additional symptoms are irritability, mood swings, joint pain, and sleep disturbances. Frequency, severity, onset, and duration of symptoms vary widely between individuals and between ethnic groups. About 75% of women in Western societies experience so few troublesome symptoms during the menopausal transition that hormone therapy (HT) is not needed or requested.[59] HT rapidly alleviates the symptoms of menopause. Hot flushes and vasomotor instability, as well as symptoms of urogenital atrophy, rapidly disappear on the start of HT.

Long-Term Hormone Replacement Therapy

Because life expectancy is increasing, the time a woman spends after menopause constitutes more than one third of her life. A decade ago, long-term use of HT (5–10 years) was considered to offer advantages with regard to the prevention of the three chronic disorders most common in the elderly: cardiovascular diseases, osteoporosis, and dementia. In the early 1900s, several cross-sectional and prospective studies demonstrated a statistically significant reduction in coronary heart disease in menopausal women taking HT. Grady and colleagues[60] presented a meta-analysis of published observational studies and reported that HT was associated with a reduction in fatal coronary heart disease by one third. A meta-analysis of 25 observational studies conducted between 1976 and 1996 showed that the relative risk for coronary heart disease in women who ever used HT compared with never users was 0.70.[61]

The Nurse's Health Study was a comprehensive investigation conducted in 121,700 female nurses age 30 to 55 years. In the latest report, compiled with data from 70,533 postmenopausal nurses followed for 20 years, the overall risk of coronary heart disease in current users of HT was reduced, with a relative risk of 0.61.[62]

Over the past 15 years, however, findings from several prospective randomized controlled trials (RCTs) have fully changed the attitudes concerning the benefits and harms of HT. The Women's Health Initiative (WHI) trial comprised two large, randomized, placebo-controlled clinical trials, including estrogen-only and combined estrogen-progestin studies in more than 161,000 "healthy" postmenopausal women, age 50 to 79 years.[63] It was expected that the WHI would definitively answer whether or not estrogen is cardioprotective. However, the estrogen-progestin versus placebo trial, which involved more than 16,000 women, was discontinued early because of an increase in cardiovascular complications (coronary heart disease, stroke, and venous thromboembolism) and increased incidence of breast cancer in the treatment group.[63] Although important benefits were also seen (risk reduction for fractures and colon cancer), there was concern that the risks of combined estrogen-progestin outweighed the benefits. The estrogen-only versus placebo trial included nearly 11,000 women who had undergone hysterectomy and therefore did not require a progestin. This trial was also stopped early because a small increase in breast cancer and coronary heart disease risk was seen, although hip fracture risk was reduced.[64]

Three other RCTs supported the absence of benefit of HT for prevention of coronary heart disease and ischemic stroke.[65,66] These studies were carried out in postmenopausal women with documented ischemic stroke or transient ischemic attack,[67] in women after documented myocardial infarction,[65] or in women with documented coronary heart disease.[66]

Taken together, the WHI, which studied women presumed healthy at recruitment, and the 3 other trials carried out in women with documented cardiovascular disorders argue strongly against the earlier assumptions made on the basis of observational studies that estrogen users had a 30% to 40% reduced risk of coronary heart disease mortality and morbidity relative to nonusers.

A series of commentaries has addressed the differences in outcome between the observational studies and the randomized trials.[68,69] Healthy user bias, the age at which study participants started HT, the different estrogen and progestin preparations, and dose all have been mentioned as possible confounders.

Subsequently, reassessment of the data from the WHI and other studies has led to a different interpretation of the data, in that groups of peri- and early postmenopausal women may in fact derive cardiovascular benefits from hormone replacement therapy. In this regard, much interest has been focused on the "timing hypothesis," which states that estrogens are atheroprotective if used in an early phase of atherosclerosis development. In an arm of the WHI in which 50- to 59-year-old women were treated with conjugated estrogens, coronary artery calcium scores were slightly but significantly lower.[70] The timing hypothesis is also supported by a recent Danish study in which it was shown that participants who were assigned to HT and were younger than 50 years at baseline had a 65% lower risk of a combined outcome of all-cause mortality, heart failure, or myocardial infarction. Unfortunately, there was no placebo, and the trial was not blinded.[71] Further, an independent subanalysis from the WHI also supported the timing hypothesis. This study also demonstrated that the duration of treatment is of importance. Comparison of the rate ratios for years 1 through 6 versus years 7 through 8 showed a statistically significant (p = 0.003) reduction in cardiovascular disease risk after more than 6 years of use of conjugated equine estrogens (CEE) versus placebo.[72] In summary, the majority of long-term large observational studies and several small RCTs strongly suggest that menopausal HT should be protective against atherosclerosis if initiated early but is potentially harmful if administered to women who already have mature at-risk plaque.[73]

Subsequently, several studies have confirmed the risk of breast cancer, which increases with longer duration of HT.[74-76] In the Million Women Study, current users of estrogen had an increased

TABLE 28.1 Absolute Risks and Benefits of Clinical Events With Estrogen-Progestin and Estrogen-Only Therapy Compared With Placebo in the Women's Health Initiative Trial.[a]

	ESTROGEN-PROGESTIN THERAPY		ESTROGEN THERAPY	
Health Event	Absolute Risk (per 10,000 women/yr)	Absolute Benefit (per 10,000 women/yr)	Absolute Risk (per 10,000 women/yr)	Absolute Benefit (per 10,000 women/yr)
Coronary heart disease	8	—	—	3
Stroke	8	—	11	—
Breast cancer	8	—	—	8
Venous thromboembolism	18	—	8	—
Colorectal cancer	—	7	1	—
Hip fracture	—	5	—	6
Any fracture	—	47	—	56
New-onset diabetes	—	15	—	14

[a]For overall hazard ratio, 95% confidence interval, and adjustments, see the original article.

Modified from Hodis HN. Assessing benefits and risks of hormone therapy in 2008: new evidence, especially with regard to the heart. *Cleve Clin J Med.* 2008;75 Suppl 4:S3–S12.

risk of incident invasive breast cancer of 30%, whereas in women using estrogen plus progestin, this risk had doubled. Breast cancer risk was unchanged in both the older and younger women with a prior hysterectomy treated with estrogen only. Past users of HT also had no increased risk.[77]

In addition, the earlier expectations from observational studies that estrogen use might prevent cognitive decline were not confirmed by randomized, placebo-controlled trials. Estrogen therapy alone did not reduce dementia or mild cognitive impairment in women 65 years of age or older, but the estrogen-progestin combination resulted in slightly increased risks for both end points.[78]

The efficacy of HT in the prevention of osteoporotic fractures remains undisputed with regard to both hip and other fractures[79] (Table 28.1).

The findings of the WHI trial are so important and have been so broadly publicized that they have created the perception that HT, in general, always carries risks that exceed its benefits. Interestingly, recently, data have been published presenting the analysis of two combined studies of the WHI with extended duration of follow-up of 18 years. Compared with placebo, no differences were found in all-cause mortality (cardiovascular and cancer related) in the CEE plus medroxyprogesterone acetate treated group (treated for a mean of 5.6 years) nor in the CEE trial only (treated for a mean of 7.2 years). Mortality risk is similar compared with the placebo group despite an increased risk of breast cancer in the CEE plus medroxyprogesterone acetate treated group.[80] However, it remains unclear whether longer duration of treatment would be of benefit and outweigh the risks.

Given the noted uncertainties, HT can be considered in the perimenopausal period in those women suffering from menopausal symptoms.[81] For menopausal women older than 60 years or more than 10 years past menopause with bothersome vasomotor symptoms who do not have contraindications or excess cardiovascular or breast cancer risk, it is suggested to initiate estrogen therapy for those without a uterus and estrogen plus progesterone for those with a uterus. An association between endometrial cancer and estrogen use was observed many years ago. Ten years of unopposed estrogen use increases the risk for endometrial cancer 10-fold.[60] For this reason, the HT regimens were supplemented with progestogens, which almost completely prevented this excess risk for endometrial cancer.

Presently advised doses of estrogen were originally designed to prevent bone loss, and progestogen regimens were opposed to prevent endometrial cancer. Several estrogen and progestogen preparations are available for HT.[59] Components of available preparations vary in their effects on different target tissues. Commercial preparations differ in their clinical effect by design, and individual women differ in their responses. HT can be administered orally, transdermally, topically, intranasally, or as subcutaneous implants.

Although HT in the perimenopausal state can cause some symptoms (e.g., vaginal discharge, uterine bleeding, and breast tenderness), it relieves many other symptoms, including hot flushes and the severity of night sweats.[82] Grady has estimated that about one serious adverse event will occur among every thousand 50-year-old women using HT for 1 year. The HT regimen used in the WHI trial combined 0.625 mg/day of CEE and 2.5 mg/day of medroxyprogesterone acetate. The dose of estrogen necessary to diminish perimenopausal symptoms, however, can be lower in many women. A dose of 0.3 or 0.45 mg/day of CEE is effective at diminishing the number and the intensity of hot flushes as well.[83]

Selective Estrogen Receptor Modulators

In the search for optimal hormone replacement therapy during menopause, it was observed that tamoxifen has variable antiestrogenic and estrogenic actions in different tissues.[84,85] Tamoxifen suppresses the growth of estrogen receptor–positive breast cancer cells. Long-term treatment of menopausal patients with breast cancer with tamoxifen also lowered the incidence of new (contralateral) breast cancer by 40%. In addition, the number of cardiovascular incidents decreased by 70%, and the age-related decrease in bone mineral density was partially prevented.[86]

These initially puzzling observations were explained by the fact that tamoxifen and other compounds such as raloxifene have selective estrogen receptor–modulating effects, exerting antiestrogenic actions on normal and cancerous breast tissue but agonistic actions on bone, lipids, and blood vessel walls.[87] These effects of tamoxifen and raloxifene may be explained by differential stabilization of the conformation of the estrogen receptor, which facilitate interactions with coactivator or corepressor proteins, and subsequently initiate or suppress transcription of target genes. These specific interactions in the target cell lead to tissue selective actions.[88] Several other selective estrogen receptor modulators (SERMs) have been evaluated for the treatment of breast cancer, osteoporosis, and menopausal symptoms.[89]

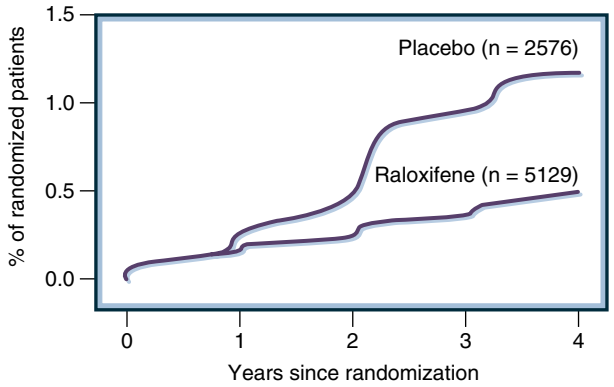
The efficacy and safety of raloxifene for the prevention of osteoporosis in postmenopausal women were demonstrated in a study that found a 2.5% increase in bone mineral density in the lumbar spine and hip in a group of postmenopausal nonosteoporotic women treated with raloxifene for 2 years.[90] A significant reduction of vertebral fracture risk by raloxifene was subsequently demonstrated.[91] A meta-analysis confirmed the effects of raloxifene treatment on reduction of vertebral fracture risk in postmenopausal women without an effect on nonvertebral fracture risk.[92]

In a placebo-controlled clinical trial of 10,000 postmenopausal women with an increased risk of coronary heart disease followed for 5 or 6 years, raloxifene had an overall neutral effect on the incidence of cardiovascular events.[93] The incidence of all strokes did not differ between the raloxifene participants and placebo-treated participants. However, in the raloxifene-treated individuals, there was a higher incidence of fatal stroke, especially in smokers, as well as venous thromboembolic events (incidence rates per 100 women-years 0.22 vs. 0.15 [$p < 0.05$] and 0.39 vs. 0.27 [$p = 0.02$], respectively).[94] Raloxifene, in contrast to tamoxifen and estrogen, does not stimulate endometrial thickness or vaginal bleeding.[90] With regard to side effects, raloxifene causes an increased incidence of leg cramps and hot flushes.[95]

Endocrine approaches to breast cancer prevention have been increasingly successful in recent years. Four trials of tamoxifen administration for 5 years or longer in women at increased risk of breast cancer showed an about 50% reduction in breast cancer, but only for estrogen receptor–positive disease (Fig. 28.4[220]) (refer to Howell[96] for further discussion). Follow-up indicates that there was a carryover effect of tamoxifen after the completion of treatment at 5 years, so the preventive effect at 10 years is significantly greater than at 5 years.

Raloxifene has been compared with placebo in three trials: one in women with osteoporosis,[91,97] one in women with or at risk of cardiac disease,[93] and one in which raloxifene was compared with tamoxifen in women at high risk of breast cancer[98]: a 66%, a 44%, and a 50% risk reduction of breast cancer was noted after 4 to 5 years of raloxifene therapy, respectively. The last trial showed that raloxifene was as effective as tamoxifen.[98] As with the previous tamoxifen studies, raloxifene was only reducing the risk of estrogen receptor α–positive tumors.

In the United States, a 60-mg dose of raloxifene is now indicated for the treatment and prevention of osteoporosis in postmenopausal women, for reduction in risk of invasive breast cancer in postmenopausal women with osteoporosis, and in postmenopausal women at high risk for invasive breast cancer.[99,100] Although approved for use in the European Union for the prevention of vertebral fractures, lasofoxifene was never marketed. In addition, bazedoxifene is approved for use in the European Union. Four RCTs show that bazedoxifene decreases the incidence of new vertebral fractures (relative risk, 0.69: 95% CI, 0.52–0.93). Bazedozifene treatment was not associated with an increased risk of breast cancer or with increases in endometrial thickness or carcinoma.[101-103] Tibolone is a compound that also regulates estrogen activity selectively at different tissues. It has a high efficacy in the treatment of climacteric symptoms and the prevention of bone loss. The compound is approved in many countries but not in the United States. In a 5-year trial, tibolone (2.5 mg/day) was associated with a 45% decrease in vertebral fractures, a 26% decrease in nonvertebral fractures, a 68% decrease in invasive breast cancer, and a 69% decreased risk of colon cancer.[104] However, the tibolone-treated group had an increased risk of stroke (relative hazard, 2.19 [$p = 0.02$]).



• **Fig. 28.4** Effect of raloxifene administration (60–120 mg/day) on the cumulative incidence of breast cancer in 7705 postmenopausal women (mean age, 66.5 years) with osteoporosis. Statistical significance of the difference between the groups was $p < 0.001$. (Modified from Cummings SR, Eckert S, Krueger KA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. *JAMA*. 1999;281[23]:2189–2197.)

Androgen Replacement

In premenopausal women, androgen production originates equally from the adrenal glands and the ovaries. Androgen production in women declines with aging. After menopause, circulating androgen levels decrease by more than 50%.

At present, there is increasing awareness of the impact of low androgen levels on emotional and sexual well-being in perimenopausal women. No single androgen level is predictive of low female sexual function, and the majority of 1423 women age 18 to 75 years with low DHEAS levels had normal sexual function.[105]

The efficacy and safety of testosterone treatment for hypoactive sexual desire disorder in postmenopausal women was studied in a double-blind, placebo-controlled, 52-week trial in which more than 800 women participated.[106] Treatment with a patch delivering 300 μg of testosterone per day resulted in a modest but meaningful improvement in sexual function. Similar results were found in a smaller study among 272 women treated with a testosterone patch for 6 months.[107] The long-term effects of testosterone, including effect on the breast, remain uncertain.

HT, SERMs, or No Treatment?

The issue of hormonal therapy in postmenopausal women is controversial, and many aspects remain unresolved. The idea that HT is a global risk reduction strategy has been abandoned.

Although the general benefits of HT in the short term during and after the menopausal transition are evident in women suffering from estrogen withdrawal symptoms, the balance of the effects of long-term HT after menopause points in general to a negative outcome with more harm than benefits. The evolution of the association of HT and cardiovascular risk from protection to harm and now to possible protection again has resulted in controversy and confusion.[108,109]

HT is usually prescribed for women 45 to 60 years of age who are experiencing vasomotor symptoms. HT is not indicated for cardioprotection for women in their 70s or for women who do not suffer from vasomotor symptoms or urogenital atrophy. Data clearly demonstrate, however, that clinicians can prescribe and women can use (low-dose) HT confidently during the time when therapy is most needed. The debate regarding the potential benefits of HT on cardiovascular risk and/or mortality for women who start therapy in proximity to the menopausal transition is not resolved. The benefit and risk of any therapy, including HT, should be reassessed periodically in each individual based on future scientific evidence.[109]

Currently, a vast armamentarium of other pharmacologic treatments to reduce cardiovascular and bone risks is available; these include cholesterol-lowering statins, β-blockers, SERMs, and bisphosphonates. An optimal choice of these different lifestyle drugs for menopausal women requires individualization of the treatment decision. Coronary artery disease, for example, is a complex disorder resulting from an interaction of genetic predisposition and environmental factors. Risk factor modification (diet, smoking, physical activity) should be advised. Secondary prevention of coronary artery disease and atherosclerosis includes lipid-lowering drugs, aspirin, nitrates, and β-blockers.[110] For women with existing osteoporosis, HT is effective. However, SERMs and especially bisphosphonates come close or are better in their fracture-reducing effects. Recognition of an increased risk for breast cancer in menopausal women is an important consideration in the choice for SERMs. Chemoprevention of breast cancer with raloxifene has become a major consideration in the pharmacologic choice for risk reduction in the long-term preventive treatment of postmenopausal women.

Andropause Versus Late-Onset Hypogonadism

Role of Testosterone During Aging

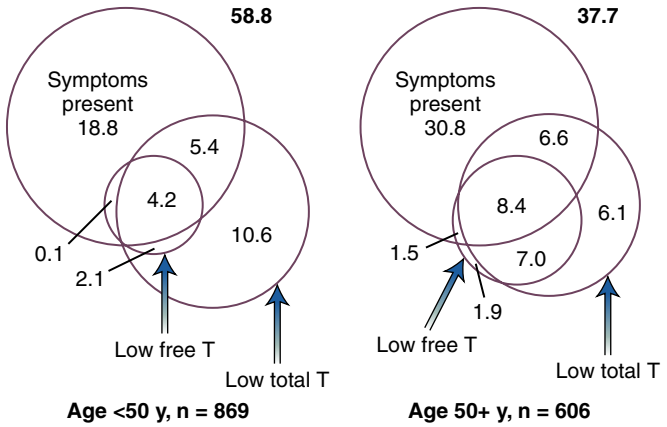
Age-associated hypogonadism does not develop as clearly in men at andropause as in women at menopause. The key difference is the gradual, often subtle change in androgen levels in men versus the precipitate fall of estrogen production in women. It is generally agreed that as men age, there is a decline in serum total testosterone concentration that begins after the age of 40 years. In cross-sectional studies, the annual decline in total and free testosterone is 1.0% and 1.2%, respectively. The higher decline in free testosterone levels is related to the increase in sex hormone–binding globulin (SHBG) levels with age.[45,111] Part of these changes attributed to aging may be confounded by health-related factors such as obesity. It was also reported that serum testosterone was stable across age strata among men self-reporting very good health.[112] It remains unclear whether the well-known biologic changes occurring during aging in men (e.g., reduced sexual activity, muscle mass and strength, and skeletal mineralization) are causally related to these changes in testosterone bioactivity. The gradual decline in testosterone levels is called *late-onset hypogonadism* (LOH). In recent years, there has been major disagreement on how to define androgen deficiency in the elderly man. According to practice guidelines,[113-115] the diagnosis of androgen deficiency in the elderly or LOH, for which testosterone treatment might be considered, should be based on the concurrent presence of consistent symptoms of hypogonadism and unequivocally low serum (free) testosterone.

The biochemical part consists of measuring morning serum total testosterone levels (two times) or determining a "free" testosterone concentration by additionally measuring SHBG levels. Most elderly men have testosterone levels within the normal range, with prevalence estimates of "low" (e.g., <10.4 nmol/L = <300 ng/dL) serum testosterone concentrations generally between 10% and 25%.[116,117] Most of these men with low testosterone levels will not come to clinical attention because testosterone levels are not routinely measured in clinical practice.[118] An important problem with a biochemical approach of the diagnosis of LOH in elderly men is that men with low testosterone may not exhibit clinically significant symptomatology, raising the possibility that large numbers of men, simply by virtue of falling below an arbitrary threshold, are diagnosed as needing testosterone replacement therapy.

In addition, the clinical part to the diagnosis of androgen deficiency of the aging male has important drawbacks.[119] All symptoms and signs of androgen deficiency are nonspecific and readily accounted for or modified by comorbidities and medications. Lethargy, reduced concentration, sleep disturbance, irritability, and depressed mood may relate to physical illness (and side effects of treatment), obesity, and/or lack of physical exercise and other lifestyle issues (e.g., alcohol or drug use), relationship difficulties, and occupational or financial stresses. Indeed, existing screening tools for androgen deficiency lack adequate specificity and sensitivity to be reliably employed in directing clinical diagnosis and treatment.

Araujo and colleagues[118] defined the prevalence of symptomatic androgen deficiency in men by studying the association between symptoms of androgen deficiency (low libido, erectile dysfunction, osteoporosis or fracture, or two of the following symptoms: sleep disturbance, depressed mood, lethargy, or diminished physical performance) and low serum total (<10.4 nmol/L = <300 ng/dL) and free (<0.17 nmol/L = <5 ng/dL) testosterone concentrations. In nearly 1500 men (age 30–79 years), they found 24% with total testosterone less than 10.4 nmol/L and 11% with free testosterone levels less than 0.17 nnol/L. The prevalence of symptoms was as follows: low libido (12%), erectile dysfunction (16%), osteoporosis/fracture (1%), and two or more of the nonspecific symptoms (20%). Although low testosterone levels were associated with symptoms, many men with low testosterone levels were asymptomatic (e.g., in men older than 50 years: 47.6%). In Fig. 28.5, the interrelationships are shown: symptomatic androgen deficiency with low serum testosterone levels (<10.4 nmol/L) was observed in 4.2% of men younger than 50 years and in 8.4% of men older than 50 years. This prevalence rapidly increases with age, amounting to 18.4% among 70-year-old men.

In addition, Wu and colleagues[120] tried to better identify elderly men with LOH. They surveyed a random population sample of 3369 men between the ages of 40 and 79 years (the European Male Aging Study). They proposed minimal diagnostic criteria, consisting of the "syndromic" simultaneous presence of three sexual symptoms (i.e., poor morning erections, decreased sexual interest, and erectile dysfunction), together with a serum



• **Fig. 28.5** Venn diagrams showing the interrelationships among symptoms, low total testosterone (<10.4 nmol/L [<300 ng/dL]), and low free testosterone (<0.17 nmol/L [<5 ng/dL]) among men younger than 50 years (*left*) and those 50 years of age or older (*right*). Numbers displayed are percentages within each area. Positive symptom reports and low total and free testosterone were more common in older men. The presence of symptoms was related more strongly to testosterone levels in older as compared with younger men as indicated by a greater degree of overlap between symptom presence and low total and free testosterone among older (52.4% of men with low total or free testosterone had symptoms) compared with younger (43.1% of men with low total or free testosterone had symptoms) men. The intersection of symptoms and low total and free testosterone levels was more common in older men (prevalence of symptomatic androgen deficiency was 4.2% among men <50 years of age and 8.4% among men 50 years of age or older). Note: Circles for the Venn diagrams are proportional within age strata. *T*, testosterone. (From Araujo AB, Esche GR, Kupelian V, et al. Prevalence of symptomatic androgen deficiency in men. *J Clin Endocrinol Metab*. 2007;92[11]:4241–4247.)

total testosterone levels less than 11 nmol/L (320 ng/dL) and free testosterone levels less than 0.22 nmol/L (6.4 ng/dL); men fulfilling these criteria with serum total testosterone levels less than 8 nmol/L (230 ng/dL) are considered to have severe LOH. Prevalence of such defined LOH in the European Male Aging Study population increased with age, body mass index, and number of coexisting illnesses and was 5.1% in men 70 to 79 years of age.[120] Men with LOH had lower hemoglobin and muscle and bone mass and poorer physical performance and general health compared with their peers; men with low testosterone only, irrespective of sexual symptoms, showed lesser magnitude of associations with the same end points.[121]

In recent years, it has become clear that an association exists between LOH and adverse metabolic conditions, such as obesity, metabolic syndrome, and type 2 diabetes mellitus.[122-124] An obesity-induced estrogen increase seems to play a major role in determining the negative feedback at the pituitary level, therefore inducing hypogonadotropic hypogonadism with low, or inappropriately normal, gonadotropin levels. In addition, insulin resistance may contribute to the low testosterone levels seen in obese men.[122] Comorbidity was also associated with a lower total testosterone level. Three independent meta-analyses published in 2011, as well as a recent large observational study of nearly 2600 men participating in the European Male Aging Study, demonstrated an association between hypogonadism and overall and cardiovascular mortality, but they failed to find any statistical association with incident cardiovascular events.[124-127] These findings support the concept that cardiovascular disease is associated with male hypogonadism.

Testosterone Replacement Therapy

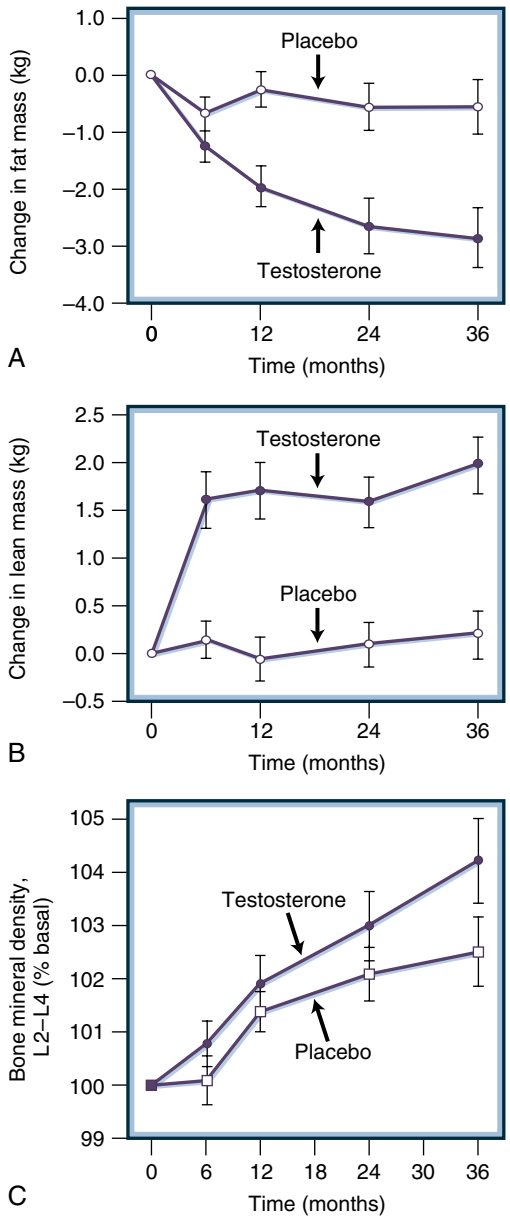
Many persuasive reports in the literature demonstrate that testosterone replacement in men of all ages (young, adult, and old) with clear clinical and severe biochemical hypogonadism instantly reverses vasomotor activity (flushes and sweats); improves libido, sexual activity, and mood; increases muscle mass, strength, and bone mineralization; prevents fractures; decreases fat mass; and decreases fatigue and poor concentration.[54,111,128] In addition, the treatment of normal adult men with supraphysiologic doses of testosterone, especially when combined with resistance exercise training, increased fat-free mass and muscle size and strength.[129]

Most studies reporting the results of androgen therapy in older men were small, short term, noncontrolled, and without uniform end points. The results of one of the first large randomized studies in healthy elderly men seem representative for effects expected of androgen therapy.[130,131] Ninety-six men (mean age 73 years) wore a testosterone patch on their scrotum (6 mg of testosterone per 24 hours) or a placebo patch for 36 months. Mean serum testosterone concentrations in the men treated with testosterone increased from 12.7 ± 2.9 nmol/L (367 ± 7.9 ng/dL) before treatment to 21.7 ± 8.6 nmol/L (625 ± 249 ng/dL; $p < 0.001$) at 6 months of treatment and remained at that level for the duration of the study. The decrease in fat mass (–3.0 ± 0.5 kg) in the testosterone-treated men during the 36 months of treatment was significantly different from the decrease (–0.7 ± 0.5 kg) in the placebo-treated men ($p < 0.001$) (Fig. 28.6A). The increase in lean mass (1.9 ± 0.3 kg) in the testosterone-treated men was significantly different from that in the placebo-treated men (0.2 ± 0.2 kg; $p < 0.001$).

Changes in knee extension and flexion strength, hand grip, walking speed, and other parameters of muscle strength and function were not significantly different in the two groups. Bone mineral density in the lumbar spine increased in both the testosterone-treated (4.2% ± 0.8%) and placebo-treated (2.5% ± 0.6%) groups, but mean changes did not differ between groups (see Fig. 28.6C). However, the lower the pretreatment serum testosterone concentration, the greater the effects of testosterone treatment on lumbar spine bone density after 36 months ($p = 0.02$). A minimal effect (0.9 ± 1.0%) of testosterone treatment on bone mineral density was observed in men with a pretreatment serum testosterone concentration of 13.9 nmol/L (400 ng/dL), but an increase of 5.9% ± 2.2% was found in men with a pretreatment testosterone concentration of 6.9 nmol/L (200 ng/dL).

The subjective perception of physical functioning decreased significantly during the 36 months of treatment in the placebo-treated group ($p < 0.001$) but not in the testosterone-treated group. Interestingly, the effect of testosterone treatment on the perception of physical functioning varied inversely with the pretreatment serum testosterone concentration ($p < 0.01$). There was no significant difference between the two treatment groups with regard to the subjective perception of energy or sexual functions.

To determine definitively whether testosterone treatment of elderly men with low testosterone is efficacious in improving symptoms and objective measures of age-associated conditions, a coordinated set of seven clinical trials were designed. The seven trials included 790 men with measurements of physical function, sexual function, vitality, cognitive function, anemia,



• **Fig. 28.6** A to C, Mean (± standard error) change from baseline in fat mass, lean mass, and bone mineral density of the lumbar spine (L2–L4) as determined by dual-energy x-ray absorptiometry in 108 men older than 65 years who were treated with either testosterone or placebo (54 men each). The decrease in fat mass ($p < 0.005$) and the increase in lean mass ($p < 0.01$) in the testosterone-treated subjects were significantly different from those in placebo-treated subjects at 36 months. Bone mineral density increased significantly in both groups. (A and B from Snyder PJ, Peachey H, Hannoush P, et al. Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. *J Clin Endocrinol Metab.* 1999;84[8]:2647-2653; C from Snyder PJ, Peachey H, Hannoush P, et al. Effect of testosterone treatment on bone mineral density in men over 65 years of age. *J Clin Endocrinol Metab*. 1999;84[6]:1966–1972.)

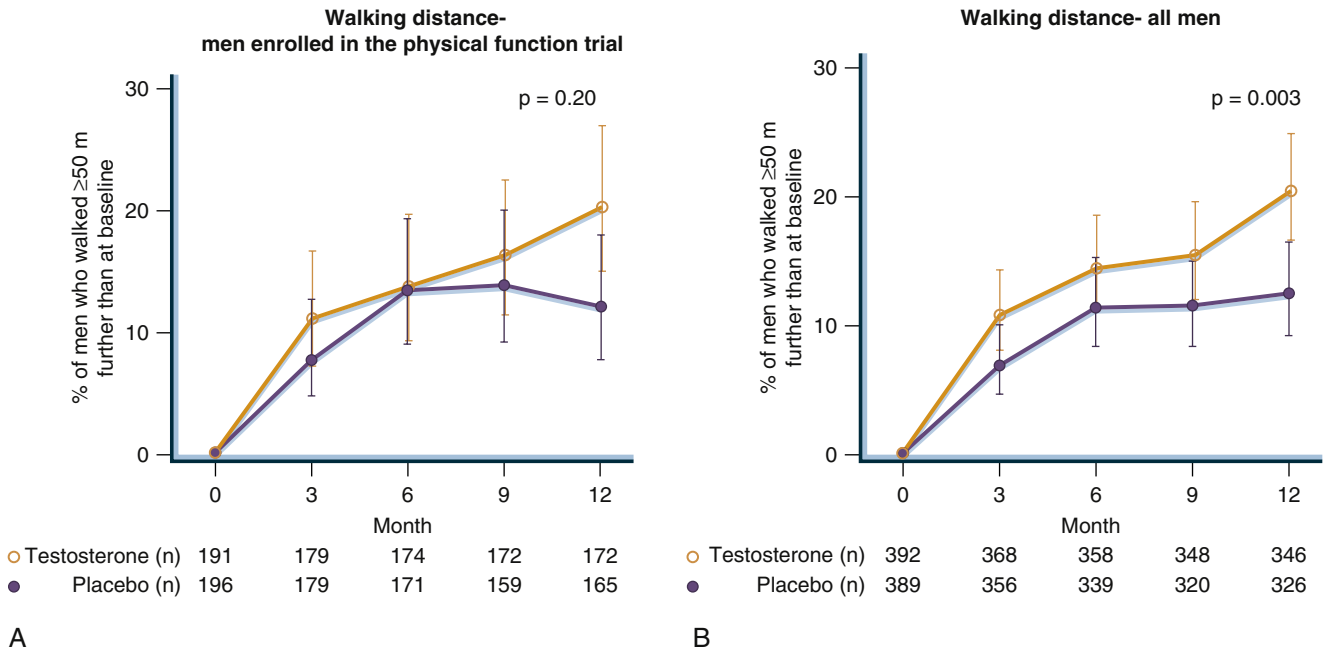
cardiovascular, and bone parameters.[132] These trials show that in treatment of men younger than 65 years for 12 months with testosterone levels below 9.5 nmol/L (275 ng/dL) with a testosterone gel in an adjusted dose to maintain testosterone levels in the normal range of young men, self-reported sexual function increased more with testosterone treatment than with placebo. There was a small difference between the testosterone group and the placebo group in the percentage of men whose 6-minute walking distance increased by at least 50 m[131] (Fig. 28.7). Further, testosterone treatment showed no significant benefit over placebo with respect to vitality, as determined by an increase of at least 4 points in the FACIT-Fatigue score[131] (Fig. 28.8).

With regard to the potential adverse effects of testosterone treatment in healthy elderly men, the studies by Snyder and colleagues[131,133] seem representative. The mean serum prostate-specific antigen concentration did not change during treatment in the placebo-treated group but increased by a small but statistically significant ($p < 0.001$) amount in the testosterone-treated group. Three cases of prostate cancer were observed in the testosterone-treated group versus one in the placebo group. The urine flow rate and volume of urine in the bladder after voiding were similar in the two groups. Hemoglobin and hematocrit did not change in the placebo-treated group, but both increased significantly ($p < 0.001$) in the testosterone-treated group. Seven men treated with testosterone developed persistent erythrocytosis (hemoglobin >17.5 g/dL; hematocrit >52%) during treatment.

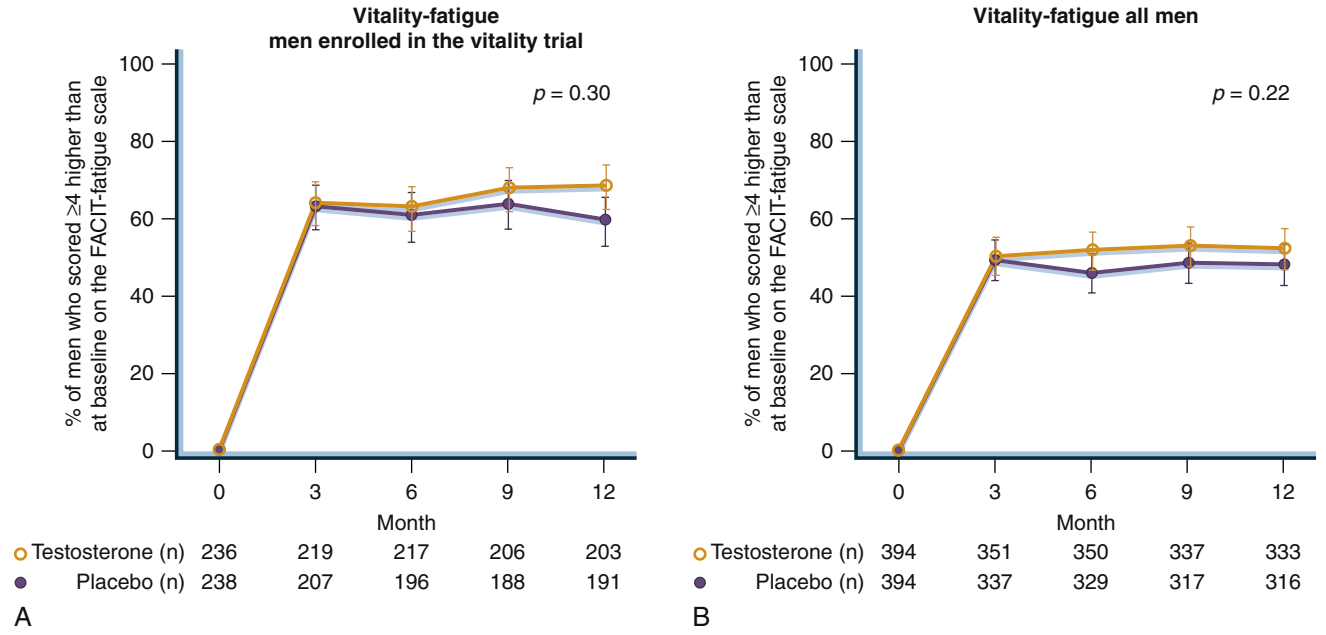
Numerous studies of large populations of healthy men have shown a marked rise in the incidence of impotence to more than 50% in men 60 to 70 years old.[134] Although this increased rate occurs in the same age group who show a clear decline in serum (free) testosterone levels, no causal relationships have been demonstrated. A systematic review and meta-analysis of randomized, placebo-controlled trials concluded that testosterone use in normal men is associated with a small improvement in satisfaction with erectile function and moderate improvements in libido.[135] Other factors, such as atherosclerosis, alcohol consumption, smoking, and the quality of personal relationships, seem to be more important.[136,137] Only in the case of clear hypogonadism is the decrease in libido and potency restored by testosterone replacement therapy.[114,128,133]

No RCTs have been large enough or long enough to determine the effects of testosterone replacement therapy on major adverse cardiovascular events. A meta-analysis suggested that testosterone therapy increases cardiovascular-related events among men. The risk of testosterone therapy was particularly marked in trials not funded by the pharmaceutical industry.[138] The Testosterone Trials,[132] which were published after this meta-analysis, have investigated the effects of testosterone therapy in older men on cardiovascular-related parameters. Treatment with testosterone gel for 1 year in 394 men with symptomatic hypogonadism and testosterone levels less than 275 ng/dL was associated with small reductions in cholesterol and insulin but not with glucose markers or markers of inflammation or fibrinolysis or with troponin compared with placebo.[139] However, testosterone treatment in a subset of 73 men was associated with a significantly greater increase in coronary artery noncalcified plaque volume compared with placebo.[140] In addition, in the Testosterone Effects on Atherosclerosis in Aging Men Trial, a placebo-controlled, randomized, double-blind trial among 308 community-dwelling men older than 60 years, insulin sensitivity did not improve in a subset of 134 nondiabetic men treated with testosterone for 36 months.[141]

The Endocrine Society Clinical Practice Guideline on testosterone therapy in adult men with androgen deficiency syndromes summarized the observed effects and the adverse outcomes of randomized, placebo-controlled trials of testosterone administration over 1 to 3 years in older men with low-normal to low testosterone concentrations.[115]



• **Fig. 28.7** Effect of testosterone on walking distance. Graphs showing percentage of (A) men taking testosterone or placebo and enrolled in the Physical Function Trial and (B) all men enrolled in testosterone trials whose distance walked in 6 minutes increased by 50 or more meters greater than baseline. Data presented as means and 95% confidence intervals. (Redrawn from Snyder PJ, Bhasin S, Cunningham GR, et al. Lessons from the Testosterone Trials. *Endocr Rev.* 2018;39[3]:369–386.)



• **Fig. 28.8** Effect of testosterone on vitality and fatigue. Graphs showing percentage of (A) men taking testosterone or placebo and enrolled in the Vitality Trial and (B) all men enrolled in testosterone trials whose score on the FACIT-Fatigue scale increased by 4 or more points greater than baseline. Data presented as means and 95% confidence intervals. (Redrawn from Snyder PJ, Bhasin S, Cunningham GR, et al. Lessons from the Testosterone Trials. *Endocr Rev.* 2018;39[3]:369–386.)

Which Elderly Men Should Be Treated?

A key lesson from the Heart and Estrogen/Progestin Replacement and WHI studies is that conventional medical practice should not precede substantiation with reliable clinical evidence of safety and efficacy.[142] Androgen replacement in older men is the male counterpart of hormonal therapy in postmenopausal women but differs crucially in that a clear syndrome of androgen deficiency is lacking. On the basis of several suggestive clinical features collected from the history, symptoms, or signs of an elderly man, the biochemical confirmation of androgen deficiency

is sought. In previous discussions of testosterone replacement in older men,[136,143] it was suggested that the biochemical diagnosis of "true" hypogonadism seems certain if the serum total testosterone concentration is less than 6.9 nmol/L (200 ng/dL). This cutoff remains arbitrary and does not answer the question whether healthy elderly men with testosterone levels between 6.9 and 10.4 nmol/L are hypogonadal, or whether such men would benefit from replacement therapy with testosterone. There are not enough data to determine whether low testosterone levels in the elderly are just a marker of poor health and should be treated with testosterone replacement. It has been demonstrated that intercurrent diseases frequently result in a transient, sharp drop in serum testosterone concentrations,[144] whereas frail, elderly men generally tend to have testosterone levels 10% to 15% lower than those of healthy, age-matched control subjects.[145] In addition, functional hypogonadism in the elderly is potentially reversible.[146] Lifestyle modifications, particularly weight loss, as obesity is the strongest risk factor for low testosterone levels in older adults, can lead to higher testosterone levels.[147]

The current recommendation[115] is not to treat asymptomatic older men with age-related decline in testosterone levels. The guideline suggests that clinicians consider offering testosterone therapy on an individualized basis to older men with low testosterone levels measured on more than one occasion and clinically significant symptoms of androgen deficiency, after explicit discussion of uncertainty about the risks and benefits of testosterone therapy. Among experts, there is disagreement on serum levels below which testosterone therapy should be offered to older men with symptoms. When a serum testosterone concentration is found to be low, an additional evaluation with measurements of serum gonadotropins and prolactin is mandatory to exclude pituitary pathology.

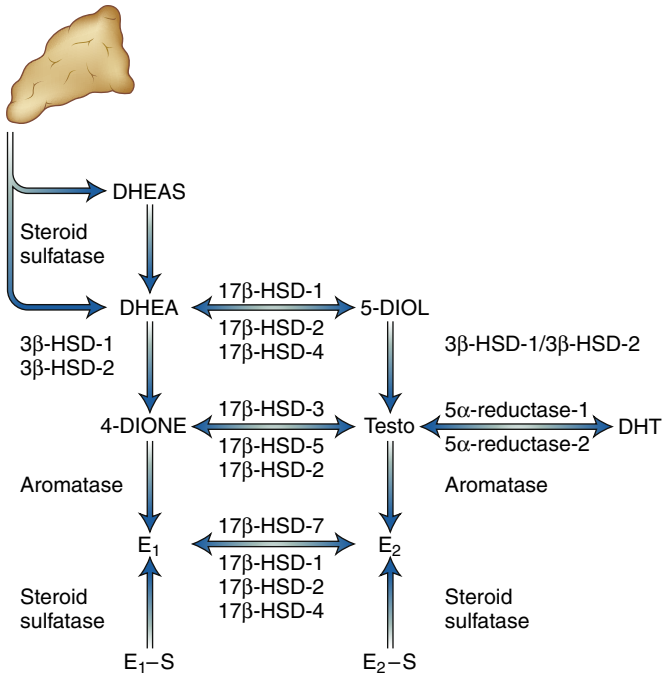
If one decides to start testosterone replacement, the guideline suggests that clinicians aim at achieving total testosterone levels in the lower part of the normal range of young men (400–500 ng/dL (14.0–17.5 nmol/L). The dose should thus be titrated according to serum levels. Considerations concerning the choice of testosterone preparation, as well as the route of administration (oral, injectable, implantable, or transdermal), are discussed in Chapter 8.

At present, the duration of testosterone administration is uncertain. Control of prostate size, prostate-specific antigen levels, and hematocrit is mandatory. The identification of elderly men who might benefit most from testosterone treatment remains uncertain, and the risks to the prostate and increased blood viscosity require further study.

Adrenopause

Role of DHEA During Aging

Humans are unique among primates and rodents because the human adrenal cortex secretes large amounts of the steroid precursor DHEA and its sulfate derivative DHEAS.[148] Serum DHEAS concentrations in adult men and women are 100 to 500 times higher than those of testosterone and 1000 to 10,000 times higher than those of estradiol. In normal subjects, serum concentrations of DHEA and its sulfate are highest in the third decade of life, after which the concentrations of both gradually decrease; therefore, by the age of 70 to 80 years, the values are about 20% of peak values in men and 30% of peak values in women[49] (see Fig. 28.3).



• **Fig. 28.9** Human steroidogenic enzymes in peripheral intracrine tissues. *DHEA*, dehydroepiandrosterone; *DHEAS*, DHEA sulfate; *DHT*, dihydrotestosterone; *5-DIOL*, androsterone-5-ene-3β,17β-diol; *4-Dione*, androstenedione; E_1, estrone; E_2, estradiol; *3β-HSD*, 3β-hydroxysteroid dehydrogenase; *17β-HSD*, 17β-hydroxysteroid dehydrogenase; *Testo*, testosterone. (Modified from Labrie F, Luu-The V, Lin SX, et al. Intracrinology: role of the family of 17 beta-hydroxysteroid dehydrogenases in human physiology and disease. *J Mol Endocrinol.* 2000;25[1]:1–16.)

DHEA and DHEAS seem to be inactive precursors that are transformed within human tissues by a complicated network of enzymes into androgens or estrogens, or both (Fig. 28.9). The key enzymes are aromatase, steroid sulfatase, 3β-hydroxysteroid dehydrogenases (3β-HSD-1 and 3β-HSD-2), and at least seven organ-specific 17β-hydroxysteroid dehydrogenases (17β-HSD-1 to 3β-HSD-7). Labrie and colleagues[148] introduced the term *intracrinology* to describe this synthesis of active steroids in peripheral target tissues in which the action is exerted in the same cells in which synthesis takes place, without release into the extracellular space and general circulation.

In postmenopausal women, nearly 100% of sex steroids are synthesized in peripheral tissues from precursors of adrenal origin, except for a small contribution from ovarian or adrenal testosterone and androstenedione. Thus, in postmenopausal women, virtually all active sex steroids are made in target tissues by an intracrine mechanism. In elderly men, the intracrine production of androgens is also important; less than 50% of the androgen supply is derived from testicular production.

The high secretion rate of adrenal precursor sex steroids in men and women differs from that in laboratory animal models, in which the secretion of sex steroids occurs exclusively in the gonads. In rats and mice, long-term administration of DHEA prevented obesity, diabetes mellitus, cancer, and heart disease, and enhanced immune function as well.[48,50,149]

These experimental animal data have been used to argue that DHEA administration in adult or elderly individuals prolongs the life span and might be an "elixir of youth." Supportive data in humans are few, however, and highly

controversial (see the following). Epidemiologic studies indeed point to a mild cardioprotective effect of higher DHEAS levels in both men and women.[150] In the Rotterdam study, DHEA and DHEAS concentrations are inversely associated with the risk of diabetes.[151] Functional parameters of activities of daily living in men older than 90 years were lowest in those with the lowest serum DHEAS concentrations[49]; in healthy elderly individuals, there was an association between the ratio of cortisol to DHEAS levels and cognitive impairment.[152]

CYP3A7, expressed in the human fetal liver and normally silenced after birth, plays a major role in the 16α-hydroxylation of DHEA, DHEAS, and estrone. A common polymorphism in the *CYP3A7* gene (CYP3A7*1C) causes persistence of the enzymatic activity of the gene during adult life. Between 6% and 8% of the population are heterozygous carriers of this polymorphism, resulting in almost 50% lower DHEAS levels compared with homozygous carriers of the reference allele.[153] Interestingly, no evidence was found that such lowered levels are associated with an acceleration of the aging process.

DHEA Replacement Therapy

A physiologic functional role of DHEA in women has been ascertained in a careful double-blind study. In women with adrenal insufficiency,[154] DHEA administration (50 mg/day) normalized serum concentrations of DHEA, DHEAS, androstenedione, and testosterone. DHEA significantly improved overall well-being, as well as scores for depression and anxiety, the frequency of sexual thoughts, sexual interest, and satisfaction with both mental and physical aspects of sexuality.

Several short controlled trials with DHEA in small groups of elderly individuals provided ambiguous results.[155,156] A 2-year placebo-controlled trial showed no effect of the oral administration of DHEA (at a dose of 75 mg/day in men and 50 mg/day in women) on body composition, muscle strength, or insulin sensitivity, as compared with placebo.[157] In this study involving 87 elderly men with low levels of DHEAS and bioavailable testosterone and 57 elderly women with low levels of DHEAS, DHEA levels increased in both sexes during DHEA administration by about 9.5 μmol/L.

These results are in line with a previous study[158] in 280 healthy subjects 60 to 79 years of age, in which 50 mg of DHEA daily for 1 year did not improve body composition or muscle strength, although in women an increase in libido was noted. In addition, a double-blind RCT with 50 mg of DHEA for 36 weeks in 50 elderly men, 70 years of age or older, with low scores on muscle strength tests did not improve isometric grip strength, leg extensor power, and physical performance.[159]

With regard to one beneficial effect of DHEA administration, the results seem rather consistently positive: increases in bone mineral density in women were repeatedly reported.[157,158,160,161] These positive effects, however, were very small and not more than approximately half those observed with current osteoporosis therapies, such as estrogens and bisphosphonates, and therefore they are unlikely to have a significant effect on the risk of fracture.[157] Review of placebo-controlled RCTs does not show benefits of oral DHEA in postmenopausal women for impaired sexual function, well-being, and cognitive performance, nor does it show favorable effects on lipids and carbohydrate metabolism.[162,163] A study from Weiss and colleagues,[164] however, of both elderly men (n = 92) and women (n = 51) showed that DHEA supplementation in a dose of 50 mg/day with a follow-up of 12 months reduces arterial stiffness.

Results from a meta-analysis showed that among the 644 elderly men enrolled in 8 RCTs measuring bone parameters, DHEA supplementation with a mean follow-up of 52 weeks did not improve lumbar or femoral bone mineral density nor formation or resorption of bone turnover markers.[165] The same meta-analysis including a total of 25 RCTs with 1353 elderly men with a mean follow-up of 36 weeks showed that DHEA supplementation of 50 to 100 mg/day was associated with a reduction of fat mass. No effect was observed for lipid and glycemic metabolism, sexual function, or quality of life.

Local delivery of DHEA, however, may be beneficial, because daily intravaginal administration of 0.50% (6.5 mg) DHEA has shown to improve moderate to severe vaginal dryness at 12 weeks and decrease pain at sexual activity.[166]

Conclusions

DHEAS is a universal precursor for the peripheral local production and action of estrogens and androgens in target tissues such as brain, bone, skin, and adipose tissue. However, the importance of these pathways remains undefined, particularly in men, who have a relatively much higher production of testosterone from testicular origin. DHEA administration in the elderly, compared with placebo, increases serum DHEAS, testosterone, free testosterone, estrone, estradiol, and IGF1 concentrations, and lowers SHBG levels.[157,159,160] It is not known whether this increase in sex steroid levels induced by DHEA administration in the long term is safe with regard to the development or growth of ovarian, prostate, or other types of steroid-dependent cancers. The addition of DHEA (50 mg/day) to the existing large pool of DHEA and DHEAS in elderly individuals, even if they have been selected on the basis of low(ered) circulating levels of these steroids, has very limited clinically meaningful effects, if any.

DHEA, which is currently available as a dietary supplement, is widely used within the United States as an unapproved preventive treatment against aging. There are no convincing arguments at present to recommend the (routine) use of DHEA for delaying or preventing the physiologic consequences of aging, and its safety is unknown.[167,168]

Somatopause

Role of GH and IGF1 During Aging

Elderly men and women secrete GH less frequently and at lower amplitude than do young people.[20] In fact, GH secretion declines approximately 14% per decade in normal individuals.[169,170] In parallel, serum levels of IGF1 (see Fig. 28.3) are 20% to 80% lower in healthy elderly individuals than in healthy young adults.[171] However, whether this decline has adverse or beneficial effects is controversial. In invertebrates and rodents, an attenuation of the insulin/IGF signaling pathway has led to an extension of the life span.[172] In addition, in humans, genetic defects in the somatotropic axis resulting in lower or a decreased IGF1 signaling may be associated with increased survival.[173] Paradoxically, low IGF1 concentrations in humans have been associated with increased risk for cardiovascular disease, stroke, type 2 diabetes mellitus, and osteoporosis. Many studies over the past decade have looked for associations between IGF1 levels and mortality risk. Some studies have found no association[174-176]; however,

other studies have found that lower levels of serum IGF1[177-179] or serum IGF1 bioactivity[180] were associated with greater all-cause mortality, whereas still others found that low IGF1 levels were predictive of extended survival.[181] One study found that higher IGF1 levels were associated with a higher mortality risk,[182] and two studies found a higher risk in subjects with both high and low IGF1 levels.[183,184] A meta-analysis indicated that both high and low IGF1 concentrations are associated with increased cancer and cardiovascular-related mortality in the general population.[185]

IGF1 and IGF2 actions can be modulated by IGF binding proteins (IGFBPs). IGFBP2 is the second most abundant IGFBP in the circulation. This protein has received a lot of attention over the past decade due to its potential role in metabolic syndrome and several forms of cancer. IGFBP2 levels increase with increasing age and decrease with increasing body mass index (BMI).[186,187] A strong association between low IGFBP2 levels and metabolic syndrome and low insulin sensitivity has been identified.[187-190] Surprisingly, however, although it was found that higher IGFBP2 was associated with favorable risk factors, including lower fasting glucose and lower fasting insulin, two studies found a strong association between higher IGFBP2 and mortality in older adults.[187,189] In both studies, the association appeared to be independent of acute-phase reactants (C-reactive protein and interleukin 6). Earlier, it was published that high serum IGFBP2 concentrations were associated with worse physical function, low muscle mass, and low bone density in a population of elderly men.[191] The association of high IGFBP2 concentrations and increased 8-year mortality was independent of physical function. The clinical features of subjects with high IGFBP2 levels are similar to subjects with protein energy malnutrition. Interestingly, these associations were, however, present in a population with a normal BMI and in subjects who lived independently.

IGFBP2 concentrations are overexpressed in several forms of malignancies.[192] Thus far, it is unclear what role IGFBP2 plays in the development and progression of human cancers.[192] In the studies mentioned previously, the associations between high IGFBP2 levels and increased mortality were independent of the presence of malignancy. The role of IGFBP2 in the aging process remains to be elucidated. The concept that this decline in GH and IGF1 secretion contributes to the decline of functional capacity in elderly people (somatopause) is mainly derived from studies in which GH replacement therapy in GH-deficient adults was shown to increase muscle mass, muscle strength, bone mass, and quality of life. A beneficial effect on the lipid profile and an important decrease in fat mass were also observed in these patients.[53,193,194] As in hypogonadal individuals, adult GH deficiency can thus be considered a model of normal aging because several catabolic processes that are central in the biology of aging can be reversed by GH replacement.

GH Therapy

Rudman and colleagues,[195] after a ground-breaking RCT of healthy men, 61 to 81 years old, with serum IGF1 concentrations in the lower third for their age, reported in 1990 that GH treatment (30 mg/kg three times weekly for 6 months) restored the men's IGF1 levels to "normal." In the treatment group, lean body mass rose by 8.8% and lumbar vertebral density increased by 1.6%. The magnitudes of these initial changes were equivalent to a reversal of the age-related changes by 10 to 20 years. However, during continuation of this study to 12 months, the significant positive effect on bone mineral density at any site was lost.[196]

In the subsequent years, it became clear that GH administration in healthy elderly individuals frequently caused acute adverse effects, such as carpal tunnel syndrome, gynecomastia, fluid retention, and hyperglycemia, which were severe enough for an appreciable number of individuals to drop out of these studies. The most disappointing aspect, however, was that no positive effects of GH administration were observed on muscle strength, maximal oxygen consumption, or functional capacity. In contrast, when GH was administered in combination with resistance exercise training, a significant positive effect on muscle mass and muscle strength was recorded that did not differ from that seen with placebo treatment, which suggests that GH does not add to the beneficial effects of exercise.[197,198] A representative example of a well-controlled study[199] of GH administration in unselected elderly men is given in Table 28.2.

In a systematic study of 31 articles describing 18 unique well-defined study populations, the safety and efficacy of GH in the healthy elderly were reviewed.[200] A total of 220 participants who received GH for 107 person-years completed the study. Participants' mean age was 69 years, and they were overweight (mean BMI, 28 kg/m^2). Initial daily GH dose (mean, 14 μg/kg body weight) and treatment duration (mean, 27 weeks) varied. Overall fat mass decreased by 2.1 kg and lean body mass increased by 2.1 kg in those treated with GH, whereas total cholesterol levels decreased by 0.29 mmol/L. Disappointingly, no consistent changes in muscle strength, physical activity, or psychosocial outcomes were observed.

GH is associated with substantial adverse effects.[200] To obtain insights into what can be expected, the details of one particularly well carried out placebo-controlled study in healthy women (n =

TABLE 28.2 Effects of Growth Hormone Administration in Healthy Older Men.

	MEAN CHANGE IN VARIABLE		
Parameter	GH (n = 26)	Placebo (n = 26)	*p* Value
IGF1 (ng/mL)	119.2	7.6	<0.0001
Body Weight and Composition			
Weight, kg	0.5	1.0	>0.2
Lean mass, %	4.3	–0.1	<0.001
Fat mass, %	–13.1	–0.3	<0.001
Bone mineral content, %	0.9	–0.1	0.05
Skin thickness, %	13.4	1.1	0.09
Muscle Strength, %			
Knee extension	3.8	1.3	>0.2
Knee flexion	10.0	8.2	>0.2
Hand grip	–1.5	3.8	0.11
Maximum Oxygen Consumption, %	2.5	–2.0	>0.2

GH, 30 μg/kg three times a week, was administered for 6 months to 52 healthy 69-year-old men with well-preserved functional ability but low levels of IGF1.

GH, Growth hormone; *IGF1*, insulin-like growth factor 1.

From Papadakis MA, Grady D, Black D, et al. Growth hormone replacement in healthy older men improves body composition but not functional ability. *Ann Intern Med.* 1996;124:708–716.

57) and men (n = 74) 65 to 88 years of age is presented[201]: GH administered subcutaneously at an initial dose of 30 μg/kg, three times per week, then reduced to 20 μg/kg for 26 weeks, was associated with carpal tunnel syndrome in 38% of women versus 7% of those taking placebo, and in 24% of men versus 0% taking placebo; edema in 39% of women (0% for placebo) and 30% of men (12% for placebo); and arthralgias in 46% of women (7% for placebo) and 41% of men (0% for placebo). Troublesome was the report that 18 men treated with GH developed glucose intolerance or diabetes compared with 7 men in the nontreatment group.[201]

In an RCT, Blackman and colleagues[201] studied the synergetic effects of a combination of GH and sex steroids in healthy elderly women and men. A slight increase in lean body mass and muscle strength was observed in elderly men treated with GH and testosterone but not in women who received GH and estrogen. In a study of healthy elderly men, coadministration of low-dose GH with testosterone resulted also in slightly beneficial changes (muscle strength, quality of life) compared with GH or testosterone alone.[202] In a partially placebo controlled trial of 112 older men, testosterone and recombinant human GH administration appeared not to worsen cardiometabolic risk in healthy older men after 4 months.[203] Earlier studies demonstrated that pharmacologic doses of GH prevent the *autocannibalistic* effects of acute diseases on muscle mass.[204] Confirmation is needed, however, before GH can gain a place in the treatment of acute catabolic states in frail elderly people.

Other components in the regulation of the GH–IGF1 axis are effective in activating GH and IGF1 secretion. Long-acting derivatives of the hypothalamic peptide growth hormone–releasing hormone, given twice daily subcutaneously for 14 days to healthy 70-year-old men, increased GH and IGF1 levels to those encountered in 35 year olds.[51] These studies suggest that somatopause is driven primarily by the hypothalamus and that pituitary somatotropes retain their capacity to synthesize and secrete high levels of GH.

Two ghrelin-mimetic GH secretagogues (GHSs) have also been demonstrated to be able to restore levels of GH and IGF1 in elderly individuals to those of young adults.[205] Ghrelin, an octanoylated 28–amino acid peptide, stimulates GH secretion via a distinct, endogenous GHS receptor but also has appetite-stimulating activity.[206] These GHSs slightly increased fat-free mass without changing fat mass, muscle strength, and function or quality of life,[207] and changed body composition and increased appetite that was accompanied by small but significant improvements in some measures of physical function[208] in older adults.

The long-term safety of activating GH and IGF1 levels in older people has become a concern because of reports of an association between serum IGF1 concentrations and cancer risk. Individuals with high IGF1 levels (or low IGFBP3 levels) within the broad normal range have an increased risk of prostate, colon, and breast cancer.[209-211] These epidemiologic studies, together with experimental data, suggest that the IGF1 system is involved in tumor development and progression. However, no causal relationship between IGF1 levels and cancer risk has yet been established, and possible medical intervention directed at increasing IGF1 bioactivity in elderly people will in most instances be given toward the end of life, presumably not allowing enough time to affect tumor development or progression.

Conclusions

During the aging process, GH–IGF1 axis activity declines. It is unclear whether changes in body composition and functional capacity are directly related. GH administration in older adults causes an increase in lean body mass and an appreciable loss of fat mass. However, GH treatment does not improve muscle strength and functional capacity in elderly people, despite restoration of circulating IGF1 concentrations to young adult levels. Furthermore, most dose regimens of GH cause appreciable adverse effects, and long-term safety with regard to tumor development and progression remains uncertain. Oral ghrelin mimetics are also capable of restoring GH and IGF1 levels in the elderly population, together with an increase in appetite. Modest functional improvement was observed in one study after 2 years administration.

In the near future, clinical trials with such orally active molecules in frail elderly people or in elderly individuals with clearly lowered IGF1 levels, or both, should be able to delineate the precise role of the GH–IGF1 axis in the aging process. In such trials, much emphasis must be given to safety aspects. At present, there is no evidence to recommend medical or other intervention in the GH–IGF1 axis as an antiaging effort to prolong life or rejuvenate healthy elderly people.[212,213] Only elderly patients with GH deficiency caused by organic diseases, such as pituitary adenomas, clearly benefit from GH replacement therapy.[214]

The Concept of Successful Aging

There is considerable variation in the effects of aging on healthy individuals, with some people exhibiting greater and others exhibiting few or no age-related alterations in physiologic functions. It has been suggested that it might be useful to distinguish between usual and successful patterns of aging.[215] Genetic factors, lifestyle, and societal investments in a safe and healthful environment are important aspects of successful aging.[216] Traditionally, the aging process, including the development of physical frailty toward the end of life, has been considered physiologic and unavoidable.

It has recently become evident, however, that it might not be necessary to accept the grim stereotype of aging as an unalterable process of decline and loss.[215] As life expectancy rises further in the coming decades, the overarching goal should be "an increase in years of healthy life with a full range of functional capacity at each stage of life."[217] Such a compression of morbidity can be achieved by adapting lifestyle measures, but several aspects of the aging process of the endocrine system invite the development of routine medical intervention programs offering long-term replacement therapy with one or more hormones to delay the aging process and to allow humans to live for a longer period in a relatively intact state.[218] However, unfortunately, it should be concluded at present that hormonal interventions with sex steroids, DHEA, GH, and/or oral ghrelin agonists have very limited effects on physical capacity in elderly individuals, whereas adverse effects are often considerable.

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The complete list of references is available online at ExpertConsult.com.

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29

Hormones and Disorders of Mineral Metabolism

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CHAPTER OUTLINE

KEY POINTS

- Parathyroid hormone (PTH), vitamin D, and fibroblast growth factor 23 (FGF23) together regulate the levels of calcium and phosphorus in the bloodstream to keep them relatively constant and slightly above the inherent solubility product of calcium and phosphate. These hormones act largely on cells in intestine, kidney, bone, and parathyroid gland.
- Primary hyperparathyroidism and malignant hypercalcemia are the commonest causes of hypercalcemia. All causes of hypercalcemia can be grouped as either PTH dependent or PTH independent.
- Hypocalcemic disorders can be usefully organized as PTH-related, vitamin D–related, or miscellaneous disorders.
- Disorders associated with abnormal levels of phosphate in the blood are usually caused by diseases of the kidney.
- Magnesium disorders usually reflect abnormalities of influx of magnesium across the intestine, excretion of magnesium by the kidney, or shifts among the intracellular and extracellular compartments.

Basic Biology of Mineral Metabolism: Roles of the Mineral Ions

Calcium (Ca) and phosphorus (P) are the principal constituents of bone, and together they compose 65% of its weight. Bone, in turn, contains nearly all of the calcium and phosphorus and over half of the magnesium in the human body. The quantitatively minor amounts of each of these ions in the extracellular fluid and within cells play crucial roles in normal physiology (Fig. 29.1).

Ninety-nine percent of total body calcium resides in bone, of which 99% is located within the crystal structure of the mineral phase. The remaining 1% of bone calcium is rapidly exchangeable with extracellular calcium; this calcium is equally distributed between the intracellular and extracellular fluids. Extracellular calcium is the principal substrate for the mineralization of cartilage and bone, but it also serves as a cofactor for many extracellular enzymes, most notably the enzymes of the coagulation cascade, and as a source of calcium ions that serve as signaling molecules for a great diversity of intracellular processes. These processes include automaticity of nerve and muscle; contraction of cardiac, skeletal, and smooth muscle; neurotransmitter release; and various forms of endocrine and exocrine secretion.

In blood, approximately 50% of total calcium is bound to proteins, mainly albumin and globulins. The ionized calcium concentration in serum is approximately 1.2 mmol/L (5 mg/dL), and it is this ionized fraction that is biologically active and that is tightly controlled by hormonal mechanisms. Because intracellular cytosolic free calcium concentrations typically are in the range of only 100 nM, a very large chemical gradient (i.e., 10,000:1), augmented by the large negative electrical potential, favors calcium entry into cells through calcium channels. This gradient is maintained by the limited conductance of resting calcium channels and

	Calcium ions	Phosphate ions
Extracellular		
Concentration		
total, in serum	2.5×10^{-3} M	1.00×10^{-3} M
free	1.2×10^{-3} M	0.85×10^{-3} M
Functions	Bone mineral Blood coagulation Membrane excitability	Bone mineral
Intracellular		
Concentration	10^{-7} M	$1-2 \times 10^{-3}$ M
Functions	**Signal for:** • Neuron activation • Hormone secretion • Muscle contraction	• Structural role • High energy bonds • Regulation of proteins by phosphorylation

• **Fig. 29.1** Distribution and function of calcium and phosphate. Note the dramatic differences between intracellular and extracellular concentrations of calcium ion and the dramatically different functions of calcium and phosphate inside cells.

by the energy-dependent extrusion of calcium into the extracellular fluid via high-affinity Ca^{2+}-adenosine triphosphatases and H^+-adenosine triphosphatases (ATPases) and low-affinity sodium-calcium (Na^+-Ca^{2+}) exchangers.

More than 99% of intracellular calcium exists in the form of complexes within the mitochondrial compartment, bound to the inner plasma membrane, or associated with the inner membranes of the endoplasmic reticulum and other compartments. Release of calcium from membrane-bound compartments transduces cellular signals and is tightly regulated. The mechanisms responsible for translocations of intracellular calcium between the cytosol and these sequestered regions have become better understood with the identification of specific receptors for calciotropic signaling molecules such as the inositol triphosphate (IP_3) receptor and ryanodine receptors.

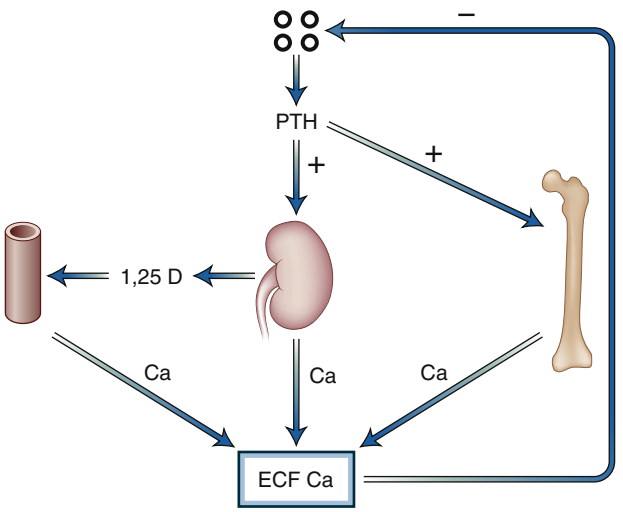
Phosphate is more widely distributed to nonosseous tissues than is calcium. Eighty-five percent of body phosphate is in the mineral phase of bone, and the remainder is located in inorganic or organic form throughout the extracellular and intracellular compartments. In human serum, inorganic phosphate (P_i) is present at a concentration of approximately 1 mmol/L and exists almost entirely in ionized form as either $H_2PO_4^-$ or HPO_4^{2-}. Only 12% of serum phosphate is protein bound, and an additional small fraction is loosely complexed with calcium, magnesium, and other cations. Intracellular free phosphate concentrations are generally comparable to those in the extracellular fluid (i.e., 1–2 mmol/L), although the inside-negative electrical potential of the cell creates a significant energy requirement for translocation of phosphate into cells. This process generally is accomplished through sodium-phosphate cotransport driven by the transmembrane sodium gradient. A number of sodium-phosphate cotransporters have been cloned; various cells and tissues use different species of such transporters with distinctive regulatory characteristics.

Organic phosphate is a key component of virtually all classes of structural, informational, and effector molecules that are essential for normal genetic, developmental, and physiologic processes. Phosphate is an integral constituent of nucleic acids; phospholipids; complex carbohydrates; glycolytic intermediates; structural, signaling, and enzymatic phosphoproteins; and nucleotide cofactors for enzymes and G proteins. The need for the large amounts of phosphate incorporated into cellular constituents during cell proliferation explains why phosphate levels in the blood are regulated by insulin-like growth factor 1 (IGF1), in addition to regulation by hormones of bone mineralization. Of particular importance are the high-energy phosphate ester bonds present in molecules such as adenosine triphosphate (ATP), diphosphoglycerate, and creatine phosphate that store chemical energy. Phosphate plays a particularly prominent role as the key substrate or recognition site in numerous kinase and phosphatase regulatory cascades. Cytosolic phosphate per se also directly regulates a number of crucial intracellular reactions, including those involved in glucose transport, lactate production, and synthesis of ATP. In light of these diverse roles, it is not surprising that disorders of phosphate homeostasis associated with severe depletion of intracellular phosphate lead to profound and global impairment of organ function. Note that none of these roles for intracellular phosphate involves actions of intracellular calcium; the reason we are discussing these together results solely from their intimate relationship in regulating bone mineralization outside cells.

Magnesium is the fourth most abundant cation in the body. Roughly half is found in bone and half in muscle and other soft tissues. As much as half of the magnesium in bone is not sequestered in the mineral phase but is freely exchangeable with the extracellular fluid and therefore may serve as a buffer against changes in extracellular magnesium concentration. Less than 1% of all magnesium in the body is present in the extracellular fluid, where the magnesium concentration is approximately 0.5 mmol/L. The concentration of magnesium in serum normally is 0.7 to 1 mmol/L, of which roughly one-third is protein bound, 15% is loosely complexed with phosphate or other anions, and 55% is present as the free ion. Over 95% of intracellular magnesium is bound to other molecules, most notably ATP, the concentration of which is approximately 5 mmol/L. The intracellular cytosolic free magnesium concentration is approximately 0.5 mmol/L (i.e., 1000-fold higher than that of calcium) and is maintained by an active sodium-magnesium antiporter. The mechanism(s) whereby magnesium enters cells, presumably down a favorable electrochemical gradient, is unknown, although some evidence for regulated channels has been obtained.[1]

Intracellular magnesium, like phosphate, is necessary for a wide range of cellular functions. It is an essential cofactor in enzymatic reactions, including most of the same glycolytic, kinase, and phosphatase pathways that also involve phosphate. Magnesium serves to directly stabilize the structures of a variety of macromolecules and complexes, including deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and ribosomes; is a key activator of the many ATPase-coupled ion transporters; and plays a direct role in mitochondrial oxidative metabolism. As a result, magnesium is critical for energy metabolism and the maintenance of a normal intracellular environment. Extracellular magnesium is crucial for normal neuromuscular excitability and nerve conduction, and many of the clinical consequences of magnesium deficiency or excess reflect abnormalities in this sphere.

The levels of extracellular calcium and phosphate are regulated in a coordinated way that reflects the roles of calcium and phosphate in mineralization of bone. The concentrations of these ions in body fluids are together close to the concentrations that could lead to spontaneous precipitation in soft tissues. In fact, elaborate mechanisms, most poorly understood, have evolved to prevent calcium phosphate precipitation in tissues and yet to allow the controlled deposition of calcium and phosphate in



• **Fig. 29.2** Parathyroid hormone (PTH)–calcium feedback loop that controls calcium homeostasis. Four organs—the parathyroid glands, intestine, kidney, and bone—together determine the parameters of calcium homeostasis. *ECF,* extracellular fluid; *1,25 D,* 1,25-hydroxyvitamin D; –, negative effect; +, positive effect.

bone.[2] The importance of the mineral ions for normal cellular physiology as well as skeletal integrity is reflected in the powerful endocrine control mechanisms that have evolved to maintain their extracellular concentrations within relatively narrow limits. The following sections describe the structures, secretory controls, actions, and interactions of parathyroid hormone, calcitonin, 1,25-dihydroxyvitamin D ($1,25[OH]_2D_3$ or calcitriol), and fibroblast growth factor 23 (FGF23)—the major hormones involved in mineral ion homeostasis. Subsequent sections cover the wide variety of clinical disorders that accompany abnormalities in this hormonal network.

Parathyroid Hormone

Parathyroid hormone (PTH) is the peptide hormone that controls the minute-to-minute level of ionized calcium in the blood and extracellular fluids. PTH binds to cell surface receptors in bone and kidney, thereby triggering responses that increase blood calcium (Fig. 29.2). PTH also increases renal synthesis of $1,25(OH)_2D_3$, the hormonally active form of vitamin D, which then acts on the intestine to augment absorption of dietary calcium, in addition to promoting calcium fluxes into blood from bone and kidney. The resulting increase in blood calcium (and in $1,25[OH]_2D_3$) feeds back on the parathyroid glands to decrease the secretion of PTH. The parathyroid glands, bones, kidney, and gut are thus the crucial organs that participate in PTH-mediated calcium homeostasis.

Parathyroid Gland Biology

Parathyroid glands first appeared in evolutionary history with the exit of amphibians from the sea and a switch from dependence on gills to sole dependence on bone, intestine, and kidney to maintain extracellular calcium homeostasis. Reptiles, birds, and mammals all have parathyroid glands that develop as epithelial specializations from the endoderm of the pharyngeal pouches. Though fish have no discrete parathyroid glands, they do have several PTH-related genes, including two or more that can influence calcium homeostasis.[3]

Parathyroid chief cells have three properties vital to their homeostatic function: First, they rapidly secrete PTH in response to changes in blood calcium. Second, they can synthesize, process, and store large amounts of PTH in a regulated manner. Third, parathyroid cells replicate when chronically stimulated. These functional attributes allow for short-term, intermediate-term, and long-term adaptation, respectively, to changes in calcium availability.

Parathyroid Hormone Biosynthesis

PTH, a protein of 84 amino acids in mammals, is synthesized as a larger precursor, pre-proparathyroid hormone (pre-pro-PTH); with the explosion of genome sequencing in the past decade, the gene has been sequenced in a large number of species (see sequences in Genbank: http://www.ncbi.nlm.nih.gov/gene/5741) from fish to humans. Fig. 29.3 illustrates representative pre-pro-PTH sequences. These pre-pro-PTH sequences share a 25-residue "pre" or signal sequence and a 6-residue "pro" sequence. The signal sequence, along with the short pro sequence, functions to direct the protein into the secretory pathway (Fig. 29.4). During transit across the membrane of the endoplasmic reticulum, the signal sequence is cleaved off and rapidly degraded. The importance of the signal sequence for normal secretion of PTH is illustrated by the hypoparathyroidism inherited in families carrying mutations in the signal sequence of pre-pro-PTH.[4–6]

The role of the short pro sequence is not completely understood; it may help the signal sequence work efficiently and ensure accurate cleavage of the precursor.[7] After cleavage of the pro sequence, the mature PTH(1-84) is concentrated in secretory vesicles and granules. One morphologically distinct subtype of granule contains both PTH and the proteases cathepsin B and cathepsin H. This co-localization of proteases and PTH in secretory granules probably explains the observation that a portion of the PTH secreted from parathyroid glands consists of carboxy-terminal PTH fragments. No amino-terminal fragments of PTH are secreted. Although the possible functions of carboxy-terminal fragments of PTH are still poorly characterized, these fragments do not activate the PTH/PTHrP (PTH-related protein) receptor and may even block bone resorption[8] (see later discussion). The intracellular degradation of newly synthesized PTH thus may provide an important regulatory mechanism. Under conditions of hypercalcemia, the secretion of PTH is substantially decreased, and most of what is secreted consists of carboxy-terminal fragments.[9]

Parathyroid Hormone Secretion

Although catecholamines, magnesium, and other stimuli can affect PTH secretion, the major regulator of PTH secretion is the concentration of ionized calcium in blood. Increased serum ionized calcium leads to a decrease in PTH secretion (Fig. 29.5A). The shape of the dose-response curve is sigmoid. Properties of the parathyroid cell determine the conformation of the sigmoid curve but do not alone determine the point on the curve that represents a physiologic steady state for an individual. This point, usually between the midpoint and the bottom of the curve, is determined by how vigorously target organs respond to PTH.[10] Fig. 29.5C *(solid line)* shows how an individual's calcium level rises in response to increases in PTH; the parathyroid gland's sigmoid curve is the *dotted line*. In the steady state, an individual's blood levels of PTH and calcium represent the intersection of the two lines.

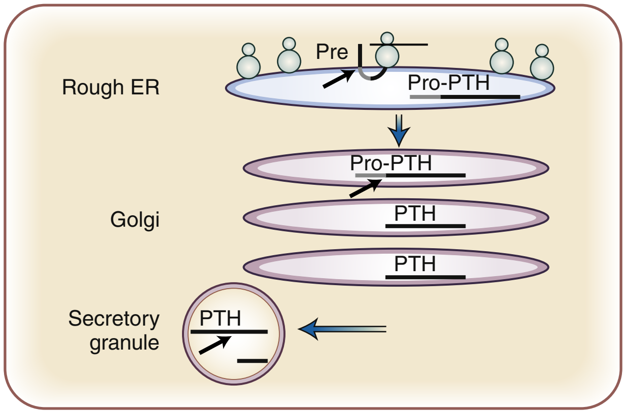
The sigmoid curve reveals several important physiologic properties of the parathyroid gland. The minimal secretory rate is low,

```
                      PRE                ↓ PRO ↓      PTH
             -31                        -6        +1          +10
Human       MIPAKDMAKVMIVMLAICFLTKSDG KSVKKR SVSEIQLMHN
Bovine      MMSAKDMVKVMIVMLAICFLARSDG KSVKKR AVSEIQFMHN
Porcine     MMSAKDTVKVMVVMLAICFLARSDG KPIKKR SVSEIQFMHN
Rat         MMSASTMAKVMILMLAVCFLTQADG KPVKKR AVSEIQLMHN
Canine      MMSAKDMVKVMIVMFAICFLAKSDG KPVKKR SVSEIQFMHN
Chicken     MTSTKNLAKAIVILYAICFFTNSDG RPMMKR SVSEMQLMHN

                      +20          +30          +40            +50
Human       LGKHLNSMERVEWLRKKLQDVHNFVALGAPLAPRDAGSQRPRK
Bovine      LGKHLSSMERVEWLRKKLQDVHNFVALGASIAYRDGSSQRPRK
Porcine     LGKHLSSLERVEWLRKKLQDVHNFVALGASIVHRDGGSQRPRK
Rat         LGKHLASVERMQWLRKKLQDVHNFVSLGVQMAAREGSYQRPTK
Canine      LGKHLSSMERVEWLRKKLQDVHNFVALGAPIAHRDGSSQRPLK
Chicken     LGEHRHTVERQDWLQMKLQDVH..SALE......DARTQRPRN

                  +60               +70                        +80
Human       KEDNVLVE...SHEKSLGEA..........DKADVNVLTKAKSQ
Bovine      KEDNVLVE...SHQKSLGEA..........DKADVDVLIKAKPQ
Porcine     KEDNVLVE...SHQKSLGEA..........DKAAVDVLIKAKPQ
Rat         KEENVLVD...GNSKSLGEG..........DKADVDVLVKAKSQ
Canine      KEDNVLVE...SYQKSLGEA..........DKADVDVLTKAKSQ
Chicken     KEDIVLGEIRNRRLLPEHLRAAVQKKSIDLDKAYMNVLFKTKP.
```

• **Fig. 29.3** Sequences of pre-proparathyroid hormone from six species. Completely conserved residues are in **boldface**. *Arrows* indicate the sites of signal sequence ("pre") and "pro" sequence cleavage. Numbers start at residue +1 of mature parathyroid hormone (PTH); because of gaps, the numbers correspond only to the mammalian and not to the chicken sequence. Amino acids are indicated by the single-letter code: *A,* Ala; *R,* Arg; *N,* Asn; *D,* Asp; *C,* Cys; *Q,* Gln; *E,* Glu; *G,* Gly; *H,* His; *I,* Ile, *L,* Leu; *K,* Lys; *M,* Met; *F,* Phe; *P,* Pro; *S,* Ser; *T,* Thr; *W,* Trp; *Y,* Tyr; *V,* Val.



• **Fig. 29.4** Intracellular processing of pre-proparathyroid hormone (pre-pro-PTH). *Diagonal arrows* indicate sites of cleavage by enzymes that generate pro-PTH in the rough endoplasmic reticulum (ER), PTH in the Golgi, and carboxy-terminal fragments of PTH in the secretory granule.

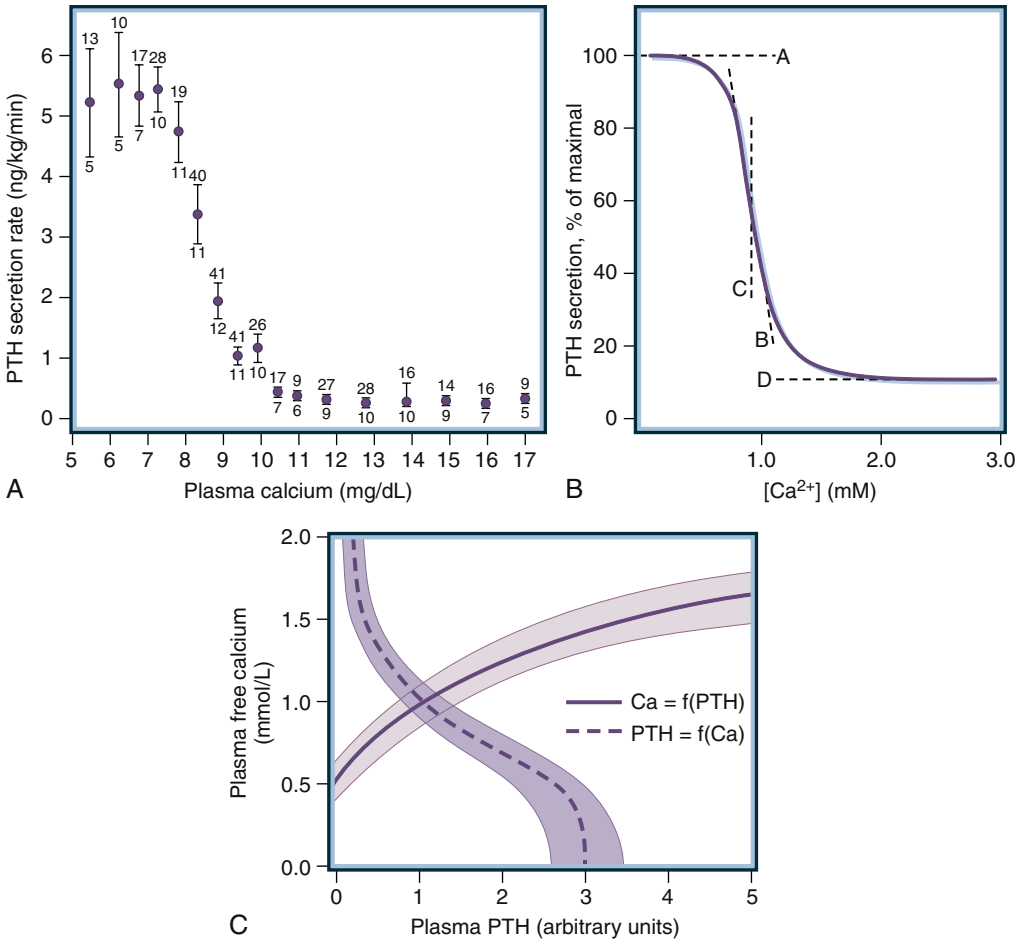
but not zero. The maximal secretory rate represents the reserve of the parathyroid's capacity to respond to hypocalcemia. Because values from normal persons in the steady state are located in the lower portion of the sigmoid curve, the system seems designed to respond more dramatically to hypocalcemia than to hypercalcemia.

Physiologic studies in humans confirmed this sigmoid relationship and have revealed that the parathyroid cell responds both to the absolute level of blood calcium and to the rate of fall of calcium level. Thus PTH levels briefly rise higher during a sudden drop in blood calcium than they do during a more gradual fall in calcium. This property of the parathyroid cell offers an additional protection against sudden hypocalcemia.

The biochemical and cellular determinants of the parathyroid gland's sigmoid response curve are beginning to be defined. A parathyroid calcium-sensing receptor (CaSR) on the parathyroid cell surface is a member of the G protein–coupled family of receptors.[11] The sequence of the receptor suggests that it spans the plasma membrane seven times, like other receptors in the G protein–linked receptor family (Fig. 29.6). A large extracellular domain resembles similar domains in brain metabotropic glutamate receptors as well as bacterial periplasmic proteins designed to bind small ligands, including cations. This domain has been crystalized, and binding sites for calcium, phosphate, and other ligands have been identified.[12] The receptor has been expressed in a number of cell types and has been shown to activate phospholipase C (PLC) and to block stimulation of cyclic adenosine monophosphate (cAMP) production, just as it does in normal parathyroid cells.

The most convincing proof of the identity of the parathyroid CaSR has been the observation that mutations in the receptor gene cause characteristic human diseases. Inactivating mutations cause familial hypocalciuric hypercalcemia (FHH), a disease of defective calcium sensing (see later discussion),[13] whereas activating mutations cause familial hypoparathyroidism with hypercalciuria.[14] Furthermore, mice genetically engineered to have only one functioning copy of the *CASR* gene also have the expected defects in parathyroid calcium sensing.[15] Of importance, calcimimetic compounds that activate the cloned CaSR have been shown to inhibit PTH secretion in humans and are useful in the treatment of secondary hyperparathyroidism.[16,17] Despite the enormous increase in understanding how extracellular calcium activates the parathyroid CaSR, the mechanism whereby this activation leads to a decrease in PTH secretion is poorly understood.

The CaSR is expressed widely. Expression in the renal tubules and calcitonin-producing cells of the thyroid contributes to calcium homeostasis, whereas expression in organs such as the brain points to multiple roles for calcium signaling. Knockout of the CaSR in osteoblasts of mice shows that this receptor regulates osteoblast differentiation and mineralization.[18] The observation that the CaSR also responds to physiologic levels of certain amino



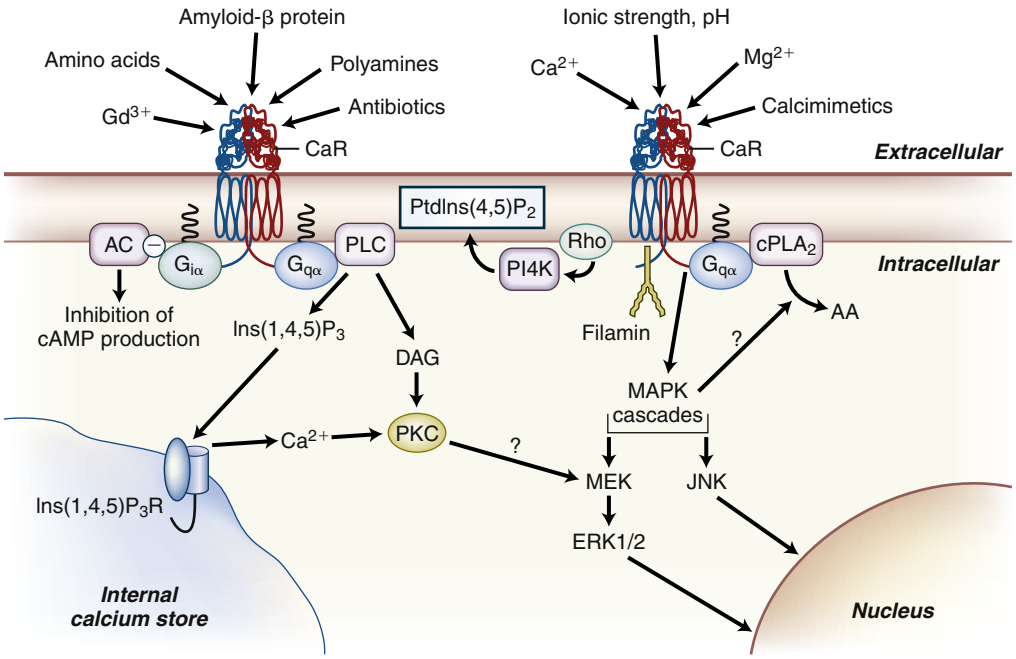
• **Fig. 29.5** Parathyroid hormone *(PTH)* secretion. (A) Secretory response of bovine parathyroid glands to induced alterations of plasma calcium concentration. Calves were infused with calcium chloride or ethylenediaminetetraacetic acid (EDTA), and PTH secretion was assessed by measuring PTH levels in the parathyroid venous effluent. The symbols and *vertical bars* indicate the secretory rate (mean ± SE) in calcium concentration ranges of 1 or 0.5 mg/100 mL. The number of calves and samples are indicated, respectively, by numbers below and above the bars. (B) Sigmoidal curve generated by the equation $Y = ([A - D]/[1 + (X/C)^B]) + D$. Such a curve can be defined by four parameters: the maximal secretory rate *(A)*, the slope of the curve at its midpoint *(B)*, the level of calcium at the midpoint (often called the set-point) *(C)*, and the minimal secretory rate *(D)*; the significance of *A*, *B*, *C*, and *D* is described in the text. (C) Relationships between calcium and PTH levels when each in turn is treated as an independent variable. The *dashed line* represents the sigmoidal relationship between calcium and PTH, when calcium is the independent variable. This curve is the same as that in (A) and (B), but it is turned on its side because the axes are reversed. The *solid line* represents the relationship between calcium and PTH when PTH is considered the independent variable; values for this curve result from measurements made during PTH infusion into parathyroidectomized animals. Actual data are limited, thus the curves should be viewed as illustrative. (A, from Mayer GP, Hurst JG. Sigmoidal relationship between parathyroid hormone secretion rate and plasma calcium concentration in calves. *Endocrinology*. 1978;10:1037–1042; B, modified from Brown EM. Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. *J Clin Endocrinol Metab*. 1983;56:572–581; C, from Parfitt AM. Calcium homeostasis. In Mundy GR, Martin TJ, eds. *Physiology and Pharmacology of Bone*. Berlin: Springer-Verlag; 1993.)

acids[19] suggests that the expression of the CaSR in the gut, parathyroid, and other sites may facilitate the assimilation of multiple nutrients.

Regulation of the Parathyroid Hormone Gene

The minute-to-minute regulation of PTH blood levels can be explained by the two mechanisms already discussed—regulation of PTH secretion by the CaSR and amplification of this regulation by intracellular degradation of stored hormone. Over a longer time frame, the parathyroid cell regulates the expression of the PTH gene as well.

Although $1,25(OH)_2D_3$—the active form of vitamin D—has no direct effect on PTH secretion, it dramatically suppresses PTH gene transcription.[20] This suppression of transcription does not occur when $1,25(OH)_2D_3$ is administered to chronically hypocalcemic animals, however, perhaps because hypocalcemia leads to a fall in parathyroid cell vitamin D receptors (VDRs) or because hypocalcemia increases the expression of calreticulin



• **Fig. 29.6** Signaling by the calcium-sensing receptor. Numerous agonists activate the calcium-sensing receptor *(CaR)* and trigger intracellular pathways. *AA,* arachidonic acid; *AC,* adenylate cyclase; *cAMP,* cyclic adenosine monophosphate; *cPLA$_2$,* cytosolic phospholipase A$_2$; *DAG,* diacylglycerol; *ERK,* extracellular signal–regulated kinase; *$G_{i\alpha}$* and *$G_{q\alpha}$* α-subunits of the i-type and q-type heterotrimeric G proteins, respectively; *Ins(1,4,5)P$_3$,* inositol-1,4,5-trisphosphate; *Ins(1,4,5)P$_3$R,* inositol-1,4,5-trisphosphate receptor; *JNK,* Jun amino-terminal kinase; *MAPK,* mitogen-activated protein kinase; *MEK,* MAPK kinase; *PI4K,* phosphatidylinositol 4-kinase; *PKC,* protein kinase C; *PLC,* phospholipase C; *PtdIns(4,5)P$_2$,* phosphatidylinositol-4, 5-bisphosphate. (From Hofer AM, Brown EM. Extracellular calcium sensing and signaling. *Nat Rev Mol Cell Biol*. 2003;4:530–538.)

in the parathyroid.[21] The capability of hypocalcemia to override the effects of high levels of 1,25(OH)$_2$D$_3$ represents an important defense because it provides a way for the parathyroid cell to synthesize large amounts of PTH and 1,25(OH)$_2$D$_3$ at the same time, when both are needed.

Calcium also regulates the biosynthesis of PTH. In vivo studies show that acute hypocalcemia in rats leads, within an hour, to an increase in PTH messenger RNA (mRNA). In contrast, hypercalcemia leads to little or no change in PTH mRNA. Thus under normal conditions the inhibition by calcium of PTH biosynthesis already is nearly maximal, just as it is for PTH secretion. The parathyroid gland is poised to respond to a fall in calcium much more readily than to a rise. The mechanism for the increase in PTH mRNA in response to hypocalcemia is uncertain; differing experimental paradigms suggest regulation at the levels of gene transcription, mRNA translation, and mRNA stability. The latter mechanism is the one best understood at the molecular level.[22] In the parathyroid cell, peptidylprolyl isomerase Pin1 binds to and leads to the activation of K-homology splicing regulator protein (KSRP), an RNA-binding protein that destabilizes PTH mRNA. Mice without Pin1 have high PTH and PTH mRNA levels, and hypocalcemic rats and rats with chronic kidney disease have low Pin1 levels. Thus Pin1 and KSRP regulate PTH mRNA levels in normal physiology and disease and mediate the effects of calcium and phosphate on PTH mRNA stability.[23]

For decades it has been known that phosphate elevation stimulates PTH secretion largely by lowering blood calcium and 1,25(OH)$_2$D$_3$ levels. Furthermore, a series of studies in vitro[24,25] and in vivo[26] have demonstrated that phosphate can increase PTH secretion directly, independent of effects on blood calcium and 1,25(OH)$_2$D$_3$. Phosphate increases PTH secretion acutely only after a delay and probably works largely through regulation of PTH mRNA levels. The mechanisms that parathyroid cells use to sense phosphate are unknown, but the effect of hypophosphatemia and hyperphosphatemia on PTH mRNA occurs through regulation of PTH mRNA stability through changes in protein binding to the 3′ noncoding region of the mRNA by mechanisms that closely parallel those used by calcium.[27] FGF23, an important phosphate-regulating hormone (see later), activates FGF receptor 1 and its coreceptor, Klotho, on parathyroid cells and thereby suppresses PTH synthesis,[28] though Klotho-independent signaling by FGF23 may be important as well.[29] In renal failure, both FGF23 levels and PTH levels are elevated. This paradoxic observation (given the role of FGF23 to suppress PTH secretion) may be explained by the downregulation of parathyroid cell FGF receptor 1 and Klotho in renal failure.[30,31]

Regulation of Parathyroid Cell Number

Parathyroid cells divide during the growth of young animals but replicate little in adulthood.[32] Parathyroid cell number can dramatically increase, however, in the setting of hypocalcemia, low levels of 1,25(OH)$_2$D$_3$, hyperphosphatemia, or uremia, or during neoplastic growth.

Calcium, acting through the parathyroid CaSR, restrains parathyroid proliferation. The behavior of the parathyroids in patients who lack both copies of the *CASR* gene is consistent with this hypothesis. These neonates exhibit severe primary

hyperparathyroidism with large, diffusely hyperplastic glands that presumably have developed because of insufficient activation of the parathyroid CaSR by extracellular calcium. Furthermore, administration of the calcimimetic compound NPS R-568, which activates the CaSR directly, prevents parathyroid cell proliferation in experimental uremia.

The role of $1,25(OH)_2D_3$, independent of blood calcium, in regulating parathyroid cell proliferation is less well established than that of calcium. That $1,25(OH)_2D_3$ can dramatically affect parathyroid cell number has been shown in vivo in many settings, but such studies cannot rigorously eliminate effects of transient changes in blood calcium. The suppression of proliferation of cultured parathyroid cells by $1,25(OH)_2D_3$[33] suggests that $1,25(OH)_2D_3$ can directly inhibit parathyroid cell replication. In experimental renal failure, the action of $1,25(OH)_2D_3$ to suppress the increase in parathyroid cell transforming growth factor-α (TGFα) and epidermal growth factor (EGF) receptor expression that otherwise would occur may partly explain the dampening of parathyroid cell proliferation.[34] Nevertheless, vitamin D action through the VDR is not essential for control of parathyroid cell number, because calcium alone can prevent parathyroid cell hyperplasia in mice engineered to lack VDRs.[35] Hypocalcemia and experimental renal failure activate the mTORC1 complex, and this increased signaling by mTOR is required for the parathyroid cell proliferation observed.[36]

Although the ability to increase parathyroid cell number in response to physiologic challenge represents an important defense against hypocalcemia, it is a slow response that is not easily reversible. When the need for an increased number of parathyroid cells disappears (e.g., after renal transplantation for uremia), persistent hyperparathyroidism can cause vexing clinical problems for months and years thereafter. The mechanisms for decreasing parathyroid cell number are poorly understood.

Parathyroid Gland Development

Genes involved in making parathyroid cells during development may also regulate PTH synthesis and parathyroid cell number throughout life and may be mutated in human inherited hypoparathyroidism[37]; thus an understanding of parathyroid cell development may have broad clinical implications. Although the genetic mechanisms used to generate parathyroid chief cells during development are largely unknown, the importance of several specific genes has become clear. Studies of gene knockout mice have shown that the hoxa3,[38] pax1,[39] pax9,[40] and Eya1[41] transcription factors are needed to form parathyroid glands as well as many other pharyngeal pouch derivatives, such as the thymus, as reviewed in Liu and associates.[42] Another transcription factor, Tbx1, regulated by the developmental paracrine factor, sonic hedgehog, is expressed early in parathyroid development and is essential for parathyroid cell development. In humans and mice, haploinsufficiency for the transcription factor Tbx1 is likely to be responsible for many of the abnormalities found in DiGeorge syndrome, including hypoparathyroidism.[43] Even though these transcription factors together are essential for the early generation of parathyroid cells, another transcription factor, gcm2 in mice and GCMB in humans, is needed for the continued survival of parathyroid cells.[42] Furthermore, mice or humans[44] missing the *gcm2* and *GCMB* genes, respectively, have no parathyroid glands. In both species, the deletion of *gcm2* or *GCMB* (the human equivalent) is very specific for controlling parathyroid development because no abnormalities in other tissues have been noted. Studies of human hypoparathyroidism have led to the discovery of the likely roles of other transcription factors in parathyroid development. Another transcription factor, MafB, is dependent on GcmB expression and is also important for parathyroid function.[45] Deletion of one copy of MafB in the mouse leads to ectopic parathyroid glands and, in adulthood, suppression of secondary hyperparathyroidism in a renal failure model.[46] Sox3 is a transcription factor expressed in the pharyngeal pouches that give rise to parathyroid cells. Humans with X-linked hypoparathyroidism manifest a deletion-insertion near the end of the *SOX3* gene, a finding that suggests an important role for Sox3 in parathyroid development.[47] People with mutations in the gene encoding the transcription factor GATA3 exhibit a syndrome of hypoparathyroidism, sensorineural deafness, and renal anomalies when only one copy of the gene is mutated.[48] GATA3 synergizes with GcmB and MafB in stimulating PTH gene expression.[49]

Metabolism of Parathyroid Hormone

The earliest radioimmunoassays for PTH demonstrated that the molecular forms of PTH in the circulation differ from those in the parathyroid gland. Characterization of the metabolism of PTH and its fragments has clarified the origins and significance of immunoreactive PTH (iPTH) molecules in the bloodstream.[50] As noted previously, both PTH(1-84) and carboxy-terminal fragments of PTH are secreted from the parathyroid gland; the ratio of inactive PTH to active PTH secretion increases with increasing blood calcium. Secreted intact PTH(1-84) is extensively metabolized by liver (70%) and kidney (20%) and disappears from the circulation with a half-life of 2 minutes. This rapid peripheral metabolism of PTH is unaffected by widely varying levels of blood calcium or $1,25(OH)_2D_3$. Less than 1% of the secreted hormone finds its way to PTH receptors on physiologic target organs. These features of PTH metabolism ensure that the blood level of PTH is determined principally by the activity of the parathyroid glands and that the PTH level can respond rapidly to small changes in the rate of secretion of the hormone.

In the liver, a small amount of PTH binds to physiologically relevant PTH receptors, but most of the intact PTH is cleaved, initially after residues 33 and 36, probably by cathepsins. In the kidney, a small amount of intact PTH binds to physiologic PTH receptors, but most of the intact PTH is filtered at the glomerulus and subsequently bound by a large, membrane-bound luminal protein, megalin[51]; this binding leads to internalization and degradation of PTH by the tubules.[52] Carboxy-terminal fragments are also cleared efficiently by glomerular filtration. In fact, the kidney is the only known site of clearance of carboxy-terminal PTH fragments; these fragments thus accumulate dramatically when the glomerular filtration rate (GFR) falls. Even in the presence of normal renal function, the half-life of carboxy-terminal fragments of PTH exceeds that of PTH(1-84) by several-fold. Consequently, the concentration of carboxy-terminal fragments in the circulation exceeds that of intact PTH, even though intact PTH usually is the major form of PTH secreted from the parathyroid gland.

Careful analysis of PTH fragments using high-performance liquid chromatography (HPLC) and immunologic methods has revealed almost full-length PTH fragments missing the first several amino acids of the hormone, but containing most or all of the remaining hormone sequence.[53] These still incompletely characterized fragments are both secreted from the parathyroid gland and generated by peripheral metabolism of the hormone. Because

they are missing the amino-terminal portion of PTH, they cannot stimulate cAMP production by the PTH/PTHrP receptor, and except in renal failure, they circulate in small amounts. Nevertheless, the possible biologic activity of these and other PTH fragments, possibly through novel receptors, remains an area of active investigation. Experiments with PTH(7-84) suggest that such extended carboxy-terminal fragments may exert potent effects in vivo, opposing those of intact PTH (see later discussion).[8,54,55]

Actions of Parathyroid Hormone

Actions of Parathyroid Hormone on the Kidney

Stimulation of Calcium Reabsorption

Almost all of the calcium in the initial glomerular filtrate is reabsorbed by the renal tubules. Sixty-five percent or more is reabsorbed by the proximal convoluted and straight tubules via a passive, paracellular route.[56] Changes in the transepithelial voltage gradient, determined largely by the rate of sodium reabsorption, control the rate of calcium transport in the proximal tubule, and PTH does little to affect calcium flux in this region. The remaining calcium is largely reabsorbed more distally—20% of the initial filtrate in the cortical thick ascending limb (cTAL) of Henle loop and 10% in the distal convoluted and connecting tubules. In the cTAL, calcium reabsorption also is mainly passive and paracellular, although some transcellular, active calcium transport may occur as well. Efficient paracellular calcium and magnesium movement requires expression of a unique tight-junction protein, paracellin-1, also called claudin-16; mutant paracellin-1 genes underlie a rare renal calcium-wasting and magnesium-wasting disorder.[57] Because paracellular cation transport in the cTAL is driven by the lumen-positive transepithelial voltage gradient that is established by active Na-K-Cl_2 reabsorption, calcium reabsorption there is strongly inhibited by loop diuretics such as furosemide. The CaSR, initially characterized in the parathyroid, also is expressed in the cTAL. When activated by high blood calcium or magnesium, this receptor inhibits Na-K-Cl_2 reabsorption in the cTAL and, thereby, paracellular calcium reabsorption as well. This provides a parathyroid-independent mechanism for controlling renal calcium handling in direct response to changes in blood calcium concentration.

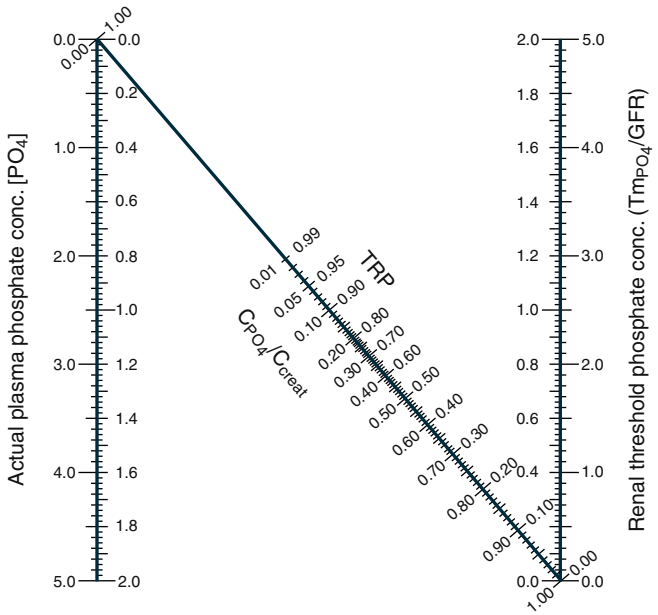
Although PTH modestly stimulates paracellular calcium reabsorption in the cTAL, the primary site for hormonal regulation of renal calcium reabsorption is the distal nephron, which normally reabsorbs nearly all of the remaining 10% of filtered calcium by a unique transcellular active transport mechanism. As depicted in Fig. 29.1, the intracellular level of calcium is extremely low, about 150 nM, compared with the millimolar levels in the glomerular filtrate and the blood. Calcium enters distal tubular cells from the tubular lumen down a highly favorable electrochemical gradient via selective channels (TRPV5 and TRPV6) present on the apical membrane of cells in the distal convoluted tubule (DCT) and connecting tubule (CNT). Intracellular calcium inhibits the activity of these channels, but this is minimized by the avid binding of calcium to calbindin-D28K, which effectively buffers cytosolic calcium and transports it to the basolateral membrane. There, calcium is ejected via active processes involving mainly the sodium-calcium exchanger NCX1 and an ATP-driven calcium pump (PMCA).[58] PTH stimulates DCT and CNT active calcium transport by upregulating several of these components, including TRPV5, calbindin-D28K, and NCX1, both directly and indirectly, via increased synthesis of $1,25(OH)_2D$.[58] PTH acutely increases the flux of calcium through TRPV5 by leading to protein kinase A (PKA) phosphorylation of a residue of TRPV5 that otherwise would bind calmodulin and thereby close the channel.[59]

The amount of calcium in the final urine reflects all of the tubular reabsorption processes just enumerated but also depends crucially on the initial filtered load of calcium. All of PTH's actions serve to raise the blood calcium level so that the filtered load of calcium is high in states of PTH excess. In that setting, even though the rate of distal tubular calcium reabsorption is increased by PTH, the total amount of calcium in the final urine is likely to be high, because of the high initial filtered load.

Inhibition of Phosphate Transport

Phosphate reabsorption occurs mainly in the proximal renal tubules, which reclaim roughly 80% of the filtered load. Some additional phosphate (8–10%) is reabsorbed in the distal tubule (but not in Henle loop), leaving about 10% to 12% for excretion in the urine. The normal overall fractional tubular reabsorption of phosphate (TRP), therefore, is about 90%, although a more reliable measure of renal phosphate handling is the *phosphate threshold* (TmP/GFR), which can be derived from the TRP through the use of a nomogram (Fig. 29.7) based on studies of experimental phosphate infusions in healthy persons and in patients with a variety of diseases that affect phosphate excretion.[60]

Phosphate reabsorption in both proximal and distal tubules is strongly inhibited by PTH, although the proximal effect is quantitatively most important. Phosphate is reabsorbed by a transepithelial route. Transport from the glomerular filtrate into the cell is mediated by specific sodium phosphate (NaPi) cotransporters, several types of which have been cloned and extensively



• **Fig. 29.7** Nomogram for determining renal threshold phosphate concentration *(Tm_{PO4}/GFR)* from the plasma phosphate concentration and the fractional reabsorption of filtered phosphate *(TRP)* or fractional excretion of filtered phosphate (1 – TRP, or C_{PO4}/C_{creat}). Because the blood level of phosphate influences the renal handling of phosphate, the renal threshold phosphate concentration best separates normal from abnormal renal phosphate handling. *C,* clearance; *creat,* creatinine; *GFR,* glomerular filtration rate; *TRP,* tubular resorption of phosphate. (From Walton RJ, Bijvoet OLM. Nomogram of derivation of renal threshold phosphate concentration. *Lancet*. 1975;2:309–310.)

characterized.[61] The low level of sodium within the cell drives the cotransport of sodium and phosphate, even though the phosphate travels up an electrochemical gradient. In response to PTH, the maximum velocity (V_{max}) for sodium phosphate cotransport decreases because NaPi cotransporters (both NaPi-IIa and NaPi-IIc) are rapidly (in 15 minutes) sequestered within subapical endocytic vesicles, after which they are delivered to lysosomes and undergo proteolysis.[62] cAMP and PKA mediate the rapid decrease in phosphate transport in response to PTH, though PLC activation by the PTH receptor is also required for prolonged suppression of phosphate transport by PTH.[63] This response to PTH is dependent upon Na^+/H^+ exchange regulatory factors (NHERFs), which physically interact with both the PTH/PTHrP receptor and the NaPi-II transporters and control the pattern of PTH receptor signaling.[64,65] Conversely, in hypoparathyroidism expression of NaPi protein and mRNA is strongly upregulated.

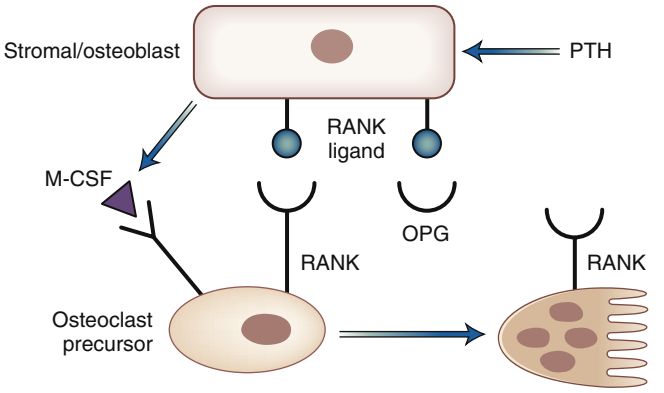
Dietary intake of phosphate also reciprocally regulates the expression and activity of NaPi cotransporters and thus the proximal tubular absorption of phosphate by a mechanism that is independent of PTH. Dietary deprivation of phosphate, for example, leads to a stimulation of phosphate reabsorption that can override the effects of PTH on the proximal tubule. It is likely that this dietary regulation of NaPi expression is mediated by FGF23[66] (see later discussion).

Other Renal Effects of Parathyroid Hormone

PTH stimulates the synthesis of $1,25(OH)_2D$ in the proximal tubule by rapidly inducing transcription of the 25-hydroxyvitamin D (25[OH]D) 1α-hydroxylase gene, an effect that can be overridden by hypercalcemia or by $1,25(OH)_2D$. The interactions of $1,25(OH)_2D$ and PTH in regulating the 25(OH)D 1α-hydroxylase gene involve both PKA-mediated phosphorylation of an activating transcription factor and protein kinase C–mediated demethylation of DNA upstream of the 25(OH)D 1α-hydroxylase gene.[67] PTH inhibits proximal tubular transcription of the 25(OH)D 24-hydroxylase gene and antagonizes the upregulation of 24-hydroxylase activity by $1,25(OH)_2D$. (See discussion under "Metabolism of Vitamin D.") PTH inhibits proximal tubular sodium, water, and bicarbonate reabsorption, mainly via inhibition of the apical Na^+/H^+ exchanger (NHE3) and the basolateral Na^+/K^+-ATPase. PTH also stimulates proximal tubular gluconeogenesis and acts directly on glomerular podocytes to decrease both single nephron and whole kidney GFR.

Actions of Parathyroid Hormone on Bone

The actions of PTH on bone are complicated because PTH acts on a number of cell types both directly and indirectly. For years, the release of calcium from bone through stimulation of bone resorption has been considered to be the major action of PTH on bone. This is only part of the story, however. In fact, PTH administration by any route increases both bone resorption by increasing osteoclast number and bone formation by increasing osteoblast number. Which action dominates depends on the dose of PTH and the route of administration. When PTH is administered continuously, the effect of PTH on bone resorption dominates, and the net result is release of calcium from bone and a decrease in bone mass. This action of PTH thus contributes to the increase in blood calcium seen in the disease, primary hyperparathyroidism. In similar fashion, administration of a soluble form of receptor activator for nuclear factor κB (RANK) ligand (RANKL), a major mediator of PTH's increase in osteoclastic bone resorption (Fig. 29.8), also increases osteoclast number. But RANKL



• **Fig. 29.8** Osteoblast lineage cell control of osteoclastogenesis and osteoclast activity. Parathyroid hormone *(PTH)* acts on PTH/PTH-related protein (PTHrP) receptors on precursors of osteoblasts to increase the production of macrophage colony-stimulating factor (M-CSF) and receptor activator for nuclear factor κB (RANK) ligand and to decrease the production of osteoprotegerin (OPG). M-CSF and RANK ligand stimulate the production of osteoclasts and increase the activity of mature osteoclasts by binding to the receptor RANK. OPG blocks the interaction of RANK ligand and RANK.

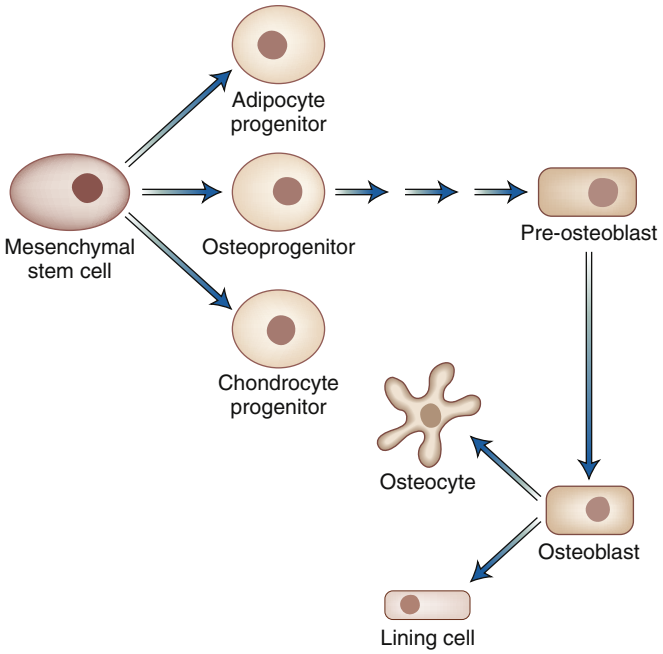
administration causes even lower bone mass than PTH because continuously administered PTH increases osteoblast number more vigorously than RANKL.[68] This finding suggests that one teleologic reason for PTH to increase bone formation is that this separate action of PTH might help preserve bone mass during prolonged requirements for PTH action.

PTH Increases Bone Formation

Administration of low doses of PTH or active amino-terminal fragments of PTH by once-daily subcutaneous injection leads to a net increase in bone mass, with only transient effects on blood calcium. Mechanisms for these divergent effects of PTH are incompletely understood but certainly reflect the variety of cell types in bone that respond directly to PTH, the varying time courses of these responses, and the indirect effects of PTH caused by autocrine and paracrine responses to PTH.[69]

Fig. 29.9 illustrates the cells of the osteoblast lineage (see also Chapter 30). Osteoblasts are probably derived from pluripotent mesenchymal stem cells that can differentiate into chondrocytes, adipocytes, osteoblasts, and other cell types.[70,71] Perivascular cells within human bone can reconstitute bone that supports hematopoiesis after subcutaneous transplantation in mice[72]; these cells may well represent one group of osteoblast stem cells in vivo. Within the osteoblast lineage, committed osteoprogenitor cells divide, become preosteoblastic stromal cells (which can divide further), and then become osteoblasts. Osteoblasts no longer divide and are cuboidal cells found on the bone surface actively laying down new bone. These cells can surround themselves with newly formed bone matrix and extend large numbers of dendritic processes, becoming osteocytes. Alternatively, osteoblasts can stop synthesizing matrix and remain on the bone surface as bone lining cells. Not all preosteoblasts and osteoblasts mature; a variable number die by apoptotic, programmed cell death.[73]

PTH administration can influence movement of cells through the osteoblast lineage.[69] PTH administration in vivo, whether administered continuously or intermittently, increases osteoblast surface and number and bone formation rate. PTH administration increases the number and rate of differentiation of early osteoblast precursors in a murine lineage-tracing model.[74] With



• **Fig. 29.9** Osteoblast lineage. All precursors of osteoblasts can proliferate; osteoblasts are transformed to osteocytes and lining cells without further proliferation. Lining cells can revert to osteoblast function after parathyroid hormone stimulation. At each stage in the lineage, apoptotic cell death is probably an alternative fate.

intermittent PTH administration to rats, the number of bone lining cells decreases as the number of active osteoblasts increases[75]: Subsequent studies using genetically marked mice showed that PTH converts bone lining cells to active osteoblasts.[76] Further, the decreased rate of osteoblast apoptosis after intermittent PTH administration leads to an increase in the number of osteoblasts.[73]

When PTH is administered continuously to rats, mimicking the findings in primary hyperparathyroidism, large numbers of proliferating, alkaline phosphatase–positive fibroblastic cells accumulate in the marrow, perhaps mimicking the findings of osteitis fibrosa in primary hyperparathyroidism. When the infusion of PTH was stopped, the fibroblastic cells disappeared and recently proliferated osteoblasts appeared, suggesting that many of the fibroblastic cells were osteoblast precursors.[77]

In addition to changing osteoblast numbers, PTH changes the activity of mature osteoblasts by a variety of mechanisms. When PTH is added to calvariae in vitro, the osteoblasts decrease their synthesis of collagen I and other matrix proteins. This action may reflect, in part, the action of PTH to steer the essential osteoblast transcription factor Runx2 toward proteosomal destruction.[78] In vivo, however, the most obvious effects of PTH are to increase bone formation by osteoblasts, probably by indirect actions of PTH on autocrine and paracrine pathways. PTH stimulation of osteoblastic cells leads to release of growth factors such as IGF1, FGF2, and amphiregulin from these cells.[79] PTH also decreases the synthesis of dickkopf-1[80] and sclerostin,[81] inhibitors of Wnt signaling[82]; these actions are expected to increase the anabolic actions of Wnt proteins on osteoblasts. Sclerostin is particularly synthesized by osteocytes, thus these cells respond to PTH to regulate the activity of nearby osteoblasts. Further, because bone matrix is a rich source of osteoblast growth factors, the release of these growth factors from this matrix following PTH-induced bone resorption may increase bone formation and bring osteoblastic cells to sites of bone formation.[83] Thus a variety of both direct and indirect actions of PTH can lead to the increased production of bone.

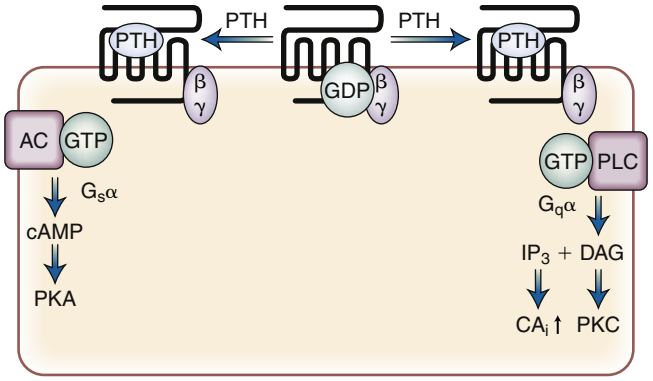
PTH Increases Bone Resorption

Surprisingly, osteoclasts, the bone-resorbing cells derived from hematopoietic precursors, have no PTH receptors on their surfaces. Instead, cells of the osteoblast lineage, including preosteoblasts, osteoblasts, and osteocytes, signal to osteoclast precursors to cause them to fuse and form mature osteoclasts. This signaling also serves to stimulate mature osteoclasts to resorb bone and to avoid apoptosis (see Fig. 29.8). Two cell surface proteins, macrophage colony-stimulating factor (M-CSF) and RANKL, are essential for stimulation of osteoclastogenesis,[84] and RANKL is essential for the activation of mature osteoclasts. The growth factor M-CSF (or CSF1) is expressed both as a secreted protein and as a cell surface protein; the production of both forms is stimulated by PTH.[85] RANKL—also named osteoprotegerin ligand (OPGL), osteoclast-differentiating factor (ODF), and TRANCE—is a membrane-bound member of the tumor necrosis factor (TNF) family; its synthesis is also increased by PTH. RANKL binds to its receptor, RANK, a member of the TNF receptor family. RANK is found both on osteoclast precursors and on mature osteoclasts. The binding of RANKL to RANK can be blocked by osteoprotegerin (OPG), another member of the TNF receptor family. OPG (also called OCIF and TR1) circulates and is also secreted by cells of the osteoblastic lineage. PTH decreases the synthesis and secretion of OPG from these cells. Thus PTH, by increasing RANK and decreasing OPG locally in bone, serves to increase bone resorption.

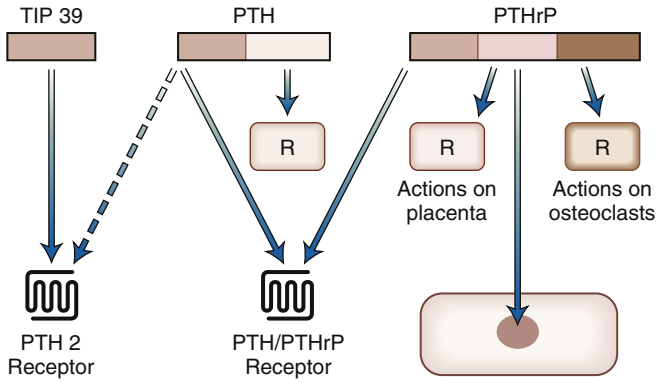
Activation of PTH receptors also causes release of calcium from bone by a less understood mechanism called osteocytic osteolysis. Osteocytes can directly release mineral from the matrix immediately surrounding them. These cells produce proteins associated with bone resorption by osteoclasts, including carbonic anhydrase 2, cathepsin K, and tartrate-resistant acid phosphatase.[86] For example, during lactation in mice, lacunae surrounding osteocytes release calcium; this release depends upon those osteocytes expressing PTH/PTHrP receptors.[87] Little is known about the quantitative importance of osteocytic osteolysis versus osteoclastic bone resorption in various settings.

Molecular Basis of Parathyroid Hormone Action

Ever since the discovery that PTH stimulates the secretion of cAMP into the urine,[88] PTH has been thought to act by triggering a cascade of intracellular second messengers. This guiding hypothesis, in its current form, postulates that all of the actions of PTH result from the binding of the hormone to a receptor on the plasma membrane of target tissues. This receptor is a member of a large family of G protein–linked receptors that span the plasma membrane seven times (Fig. 29.10). The binding of hormone on the outside of the membrane causes conformational changes in the disposition of the seven transmembrane helices that activate the receptor's ability to release guanosine diphosphate (GDP) from the α-subunit of a G protein bound to the receptor. The G protein then binds guanosine triphosphate (GTP) in place of GDP. The GTP-binding α-subunit of the G protein then separates from the βγ-subunits, and the separate subunits of the G protein then modulate the activity of enzymes and channels. The activity of these enzymes and channels then affects proteins farther downstream, eventually leading to the physiologic responses of bone and kidney cells.



• **Fig. 29.10** Parathyroid hormone (PTH)/PTH-related protein (PTHrP) receptors act as nucleotide exchangers. PTH binding to the receptor leads to exchange of guanosine triphosphate (GTP) for guanosine diphosphate *(GDP)* bound to G protein (G_s) α-subunits. G_s α-subunits bound to GTP are released from the receptor and from the βγ-subunits and then activate effectors. $G_s\alpha$ activates adenylate cyclase (AC), leading to the formation of cyclic adenosine monophosphate (cAMP), which then activates protein kinase A (PKA). $G_q\alpha$ and related α-subunits activate phospholipase C (PLC). PLC hydrolyzes phosphatidylinositol 1,4,5-trisphosphate to generate diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). The DAG then activates protein kinase C (PKC), and the IP_3 activates a receptor on microsomal vesicles that directs the movement of calcium (CA_i) from microsomal vesicles into the cytosol.



• **Fig. 29.11** Network of parathyroid hormone (PTH) ligands and receptors (R). PTH and PTH-related protein (PTHrP) closely resemble each other at the amino-terminal region; TIP 39 (tubero-infundibular peptide of 39 residues) is more distantly related. Although only the PTH/PTHrP receptor and the PTH2 receptors have been cloned, biologic actions suggest that there are receptors specific for the carboxy-terminal portion of PTH, as well as distinct receptors for the midregion of PTHrP and for a more distal region of PTHrP. PTHrP is also found in nuclei and may act directly there.

Parathyroid Hormone and Parathyroid Hormone–Related Protein Receptors

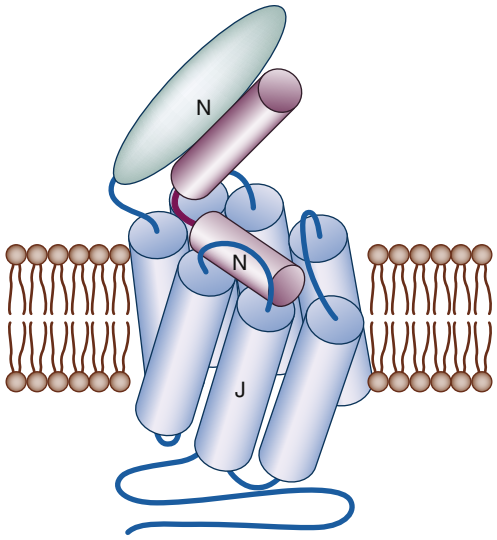
DNA encoding a PTH/PTHrP receptor has been isolated from tissues of many species, including rat, opossum, human, pig, *Xenopus* (toad), and zebrafish,[89] and even some insects.[90] The predicted amino acid sequence of the receptor and direct mapping of inserted epitopes suggest that the receptor spans the plasma membrane seven times, but the sequence does not closely resemble the sequences of most known G protein–linked receptors. Instead it is a member of a distinct subfamily of closely related receptors called family B. Most of these receptors bind peptides of 30 to 40 amino acids in length. Known members include receptors for the secretin family of peptides (secretin, vasoactive intestinal peptide [VIP], glucagon, glucagon-like peptide, growth hormone–releasing hormone [GHRH], pituitary adenylate cyclase–activating peptide, gastric inhibitory peptide), corticotropin-releasing hormone (CRH), calcitonin, and insect diuretic hormones related to CRH. The PTH/PTHrP receptor most closely resembles receptors of the secretin group. The gene encoding the PTH/PTHrP receptor has a complicated structure, with 13 introns interrupting the coding sequence.

The PTH/PTHrP receptor mediates many of the actions of both PTH and PTHrP. The cloned PTH/PTHrP receptor binds amino-terminal fragments of PTH and PTHrP with equal affinity. The receptor is expressed at high levels in kidney and in osteoblasts of bone but is also expressed in a wide variety of tissues, such as smooth muscle, brain, and a variety of fetal tissues, which are thought to be target tissues more for PTHrP than for PTH. When the PTH/PTHrP receptor is ablated in mice, the mutant mice show defects both in calcium homeostasis and in local actions of PTHrP on the growth plates of bone, for example.[91] Mutations in the receptor found in humans are also consistent with this idea (see upcoming discussion). Nevertheless, the scheme of PTH action illustrated in Fig. 29.10 should be considered a simplified outline. It is unlikely that all of the actions of PTH can be explained by interactions with the cloned PTH/PTHrP receptor: Fragments of PTH that seem not to bind the receptor may be biologically active.[92] Furthermore, the carboxy-terminal portion of PTH(1-84) binds a cell surface protein distinct from the PTH/PTHrP receptor.

A second PTH receptor, which can be activated by PTH but not by PTHrP, called the PTH2 receptor (PTH2R), has been cloned. This receptor is expressed in multiple tissues, including brain, vascular endothelium and smooth muscle, endocrine cells of the gastrointestinal tract, and sperm. Expression is not seen in osteoblasts or renal tubules, however. Although PTH activates the human PTH2R well, PTH only poorly activates the PTH2R in rats and other species. Furthermore, a novel ligand called TIP39 (tubero-infundibular peptide of 39 residues) has been characterized and shown to be a potent activator of PTH2R. TIP39 bears only a weak resemblance to PTH or PTHrP and is likely to be a physiologically relevant activator of the PTH2. The functional role of the PTH2R is unknown, but it appears to mediate many actions of TIP39 in the brain and testis; studies of knockout mice implicate TIP39 in regulation of responses to stress[93] and germ cell development.[94] The two cloned PTHRs, as well as distinct receptors for fragments of PTHrP (see later), probably are part of a complex network of ligands and receptors (Fig. 29.11).

Functional Implications of Parathyroid Hormone Structure

Two important advances have helped organize thinking about how PTH activates the PTH/PTHrP receptor. First, it is now clear that all G protein–coupled receptors have structural flexibility that allows them to interact with ligands through the use of multiple conformations.[95] These multiple conformations can bind closely related ligands differently and can lead to differing signaling consequences. Second, high-resolution structures of several family B receptors are now available, in the presence of agonists, antagonists, and G proteins. These structures are consistent with much prior functional work and serve to rationalize many results.[96] They all exhibit a large amino-terminal extracellular domain that binds the carboxy-terminal ends of their respective ligands. The crystal structure of this extracellular domain of the PTH/PTHrP receptor binds the carboxy-terminal portions of either PTH (1-34) or PTHrP (1-36) in similar fashion.[97,98]



• **Fig. 29.12** Binding of PTH(1-34) peptide of the parathyroid hormone (PTH) to the PTH/PTH-related protein (PTHrP) receptor. The amino-terminal *(N)* extracellular domain of the receptor (in *green*) binds rapidly to the carboxy-terminal portion of the ligand. The J domain of the receptor, containing the transmembrane domains and associated loops, binds to the amino-terminal domain of the ligand *(red cylinder marked N).* This binding is slower and may require conformational changes in both the ligand and receptor. These conformational changes then trigger G protein activation, receptor internalization, and other actions. (Courtesy Tom Gardella.)

The structures of the characterized family B receptors show how this binding of the carboxy-terminal portion of the ligands directs the amino-terminal portions of the ligands to interact with the region of the receptor that consists of seven transmembrane domains and associated extracellular loops (Fig. 29.12). The amino-terminal portion of the ligand changes the conformation of the sixth transmembrane domain to allow interaction of the receptor with G proteins to be activated. Amino-terminal fragments of PTH as short as PTH(1-34) have potency at least as great as that of the full-length PTH(1-84).[89] The first several residues of PTH are particularly important for triggering the conformational change in the receptor that results in activation of G_s and adenylate cyclase. Sequences responsible for transmembrane activation of G_s make up most of the first 13 residues of PTH; these are the residues that are highly conserved between PTH and PTHrP. At high concentrations, PTH(1-14) by itself can activate the PTH/PTHrP receptor. This activation domain interacts with the receptor's transmembrane domains and extracellular loops. When the first nine residues of PTH are covalently linked to the receptor's transmembrane domains and extracellular loops, they can activate the receptor. An analogue of PTH(1-14) can also trigger G_q activation, thus activating PLC.[99] These data, plus the observation that a PTH analogue modified at position 1 selectively loses its ability to activate PLC,[100] demonstrate that the amino-terminal portion of PTH is essential for activation of both G_s and G_q. More distal regions of PTH(1-34) can activate protein kinase C and can raise intracellular calcium levels by mechanisms that have not been fully clarified.

Activation of Second Messengers

Precisely how binding of PTH to the extracellular domains of the PTH/PTHrP receptor leads to activation of G proteins is not understood. The crystal structures of the transmembrane portions of other family B receptors resemble those of the more extensively studied rhodopsin family A receptors, but differ in that the potential pocket expected to bind the peptide ligands is larger, forming a V when seen from the side, with transmembrane domains (TMDs) 1, 6, and 7 forming one arm of the V and TMDs 2 to 5 forming the other. This proposed structure, with TMD 1 adjacent to TMDs 6 and 7, is consistent with the behavior of certain mutant PTH/PTHrP receptors.[101,102] Presumably, binding of PTH to several different regions of the receptor changes the relationships of the transmembrane domains[103] such that the receptor's three intracellular loops and carboxy-terminal tail interact with G proteins in an altered way.

Receptors with certain point mutations in the second, sixth, and seventh transmembrane domains can activate G_s even without stimulation by hormone. These mutant receptors were discovered by analyzing the PTH/PTHrP receptors in patients with Jansen metaphyseal chondrodystrophy.[104] Patients with this disorder have signs of parathyroid overactivity (hypercalcemia, hypophosphatemia, and high levels of $1,25[OH]_2D_3$ and urinary cAMP) but low PTH and PTHrP levels. These patients have growth abnormalities, presumably reflecting inappropriately vigorous PTHrP-like actions on growth cartilage. The mutations, all near the bottom of the predicted V-shaped structure of the transmembrane domains,[105] must change the conformation of the intracellular portion of the receptor in a way that resembles the effect of binding of PTH to the normal receptor. The observation that inappropriate activation of the PTH/PTHrP receptor in Jansen chondrodystrophy leads to all of the metabolic abnormalities found in primary hyperparathyroidism is one of the most persuasive pieces of evidence that the cloned PTH/PTHrP receptor does, in fact, mediate the actions of PTH in bone and kidney in humans in vivo.

PTH and PTHrP can both bind to and activate the PTH/PTHrP receptor on the surface of target cells. The fate of the ligand-receptor complexes differs dramatically subsequently, however.[106–108] PTH, but not PTHrP, is internalized in vesicles, along with the PTH/PTHrP receptor, β-arrestin, $G\alpha_s$, and adenylate cyclase. Remarkably, within these vesicles, PTH can signal for longer than PTHrP, which loses its signaling properties when it is internalized. Certain PTH analogs can signal for hours because of their intracellular stabilization and signaling. The tight and acid-resistant binding of PTH to its receptor probably explains the differing properties of PTH and PTHrP, but this distinction and its full functional significance are current topics of active investigation.

Second Messengers and Distal Effects of Parathyroid Hormone

The activation of multiple G proteins by PTH raises questions about the individual roles of each second messenger and their possible interactions. The importance of cAMP as a mediator of the physiologic actions of PTH has been demonstrated by studies in vivo[88] and in vitro.[109] Furthermore, patients with pseudohypoparathyroidism type I, who cannot increase urinary cAMP levels in response to PTH, show clear renal resistance to PTH (see later).

Activation of PLC, with concomitant activation of protein kinase C and synthesis of IP_3, may contribute to physiologic actions of PTH as well, such as inhibition of sodium-phosphate cotransport[110] and stimulation of the renal 25(OH)D 1α-hydroxylase.[111] Mice with mutant PTH/PTHrP receptors that cannot activate PLC have a mild delay in bone development[112]; growth plate abnormalities manifest when G_s is also defective in the growth plates,[113] and abnormalities of phosphate handling by the kidney

occur when challenged with a low-calcium diet.[114] When these mutant mice are challenged with infusions of PTH peptides, their phosphate response is normal at first, but a defect in phosphate handling occurs during prolonged infusion.[63] When these same mutant mice are infused with high levels of PTH, they also exhibit a defect in generation of the expected accumulation of stromal cells in the marrow (osteitis fibrosa) that normal mice exhibit after prolonged PTH infusion.[115] Thus the actions of PTH on bone and kidney that require PLC activation are most clearly seen with high levels of PTH sustained for some time.

The stimulation of one G protein or another by the PTH/PTHrP receptor can vary in different types of cells and even in differing regions of the same cell.[110] In some settings, this choice may be influenced by the interactions of the PTH/PTHrP receptor with intracellular scaffolding proteins, such as NHERF1 and NHERF2 (Na^+/H^+ exchanger regulatory factor). Binding of the PTH/PTHrP receptor to NHERFs is directed by the last four amino acids in the receptor sequence. This binding, particularly prominent at the apical surface of the proximal tubular cells of the kidney, for example, may change the G protein activated by the PTH/PTHrP receptor from predominantly G_s to predominantly G_i.[116]

Target Cell Responsiveness to Parathyroid Hormone

Physiologic responses to PTH depend not only on the concentration of PTH in blood but also on the responsiveness of target cells to PTH. This responsiveness can be modified by previous exposure to PTH or by exposure to a variety of other hormones and paracrine factors. Responsiveness can be changed by alterations at virtually every step in the cellular response to PTH.

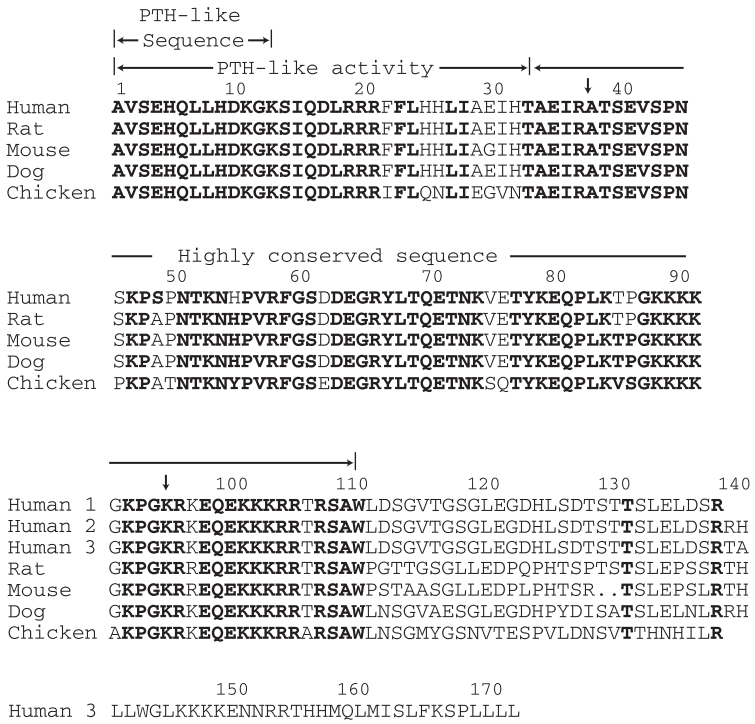
Major regulators of PTH/PTHrP receptor gene expression include, not surprisingly, PTH and $1,25(OH)_2D_3$, both of which can decrease PTH/PTHrP receptor mRNA in certain target cells. In some settings, PTH decreases the amount of immunoreactive and functional receptor on the cell surface without changing the levels of PTH/PTHrP mRNA. This decrease reflects ligand-induced internalization and degradation of receptors. Internalization of receptor is stimulated by PTH binding, which leads to phosphorylation of specific serines found in the receptor's cytoplasmic tail and subsequent internalization directed by binding of β-arrestin to the receptor.[117,118]

Parathyroid Hormone–Related Protein

PTHrP was discovered because the secretion of PTHrP by a wide variety of tumors contributes to the humoral hypercalcemia of malignancy. For this reason, the initial studies of PTHrP in humans and animals stressed the PTH-like structure and properties of the molecule. Subsequent studies soon showed, however, that PTHrP, unlike PTH, is made by a wide variety of tissues, in which it acts locally in ways that may have little relevance to the control of blood calcium.

Gene and Protein Structure

PTHrP sequences in several species, ranging from fish to humans, have been identified[119,120] (Fig. 29.13). In humans, alternative RNA splicing yields transcripts that encode three distinct proteins of 139, 141, and 173 residues that differ only after residue 139.



• **Fig. 29.13** Sequences of parathyroid hormone–related protein (PTHrP) from five species. Completely conserved residues are in *boldface*; note the high level of conservation through residue 111. *Arrows* indicate sites of internal cleavage after residues 37 and 95, which lead to generation of PTHrP(38-94) amide and PTHrP(38-95). Another site of cleavage, generating PTHrP(38-101) and, perhaps, PTHrP(107-139), is not shown.[120] The three human sequences represent proteins synthesized from alternatively spliced messenger RNAs and differ only after residue 139. Amino acids are indicated by the single-letter code (see Fig. 29.3).

Inspection of these sequences suggests that PTHrP has several functionally distinct domains. Eight or nine of the first 13 residues of PTHrP are identical to those in known mammalian PTH sequences. These sequences encompass the known "activation" domain of PTH that interacts with the receptor's transmembrane domain region (see earlier) and are instrumental in the ability of PTHrP to activate PTH/PTHrP receptors.

The sequences in PTHrP(14-34) are also highly conserved. Although these sequences little resemble the corresponding region of PTH, they can displace PTH from the PTH/PTHrP receptor and, in the crystal structure of the amino-terminal domain of the PTH/PTHrP receptor, bind to overlapping but distinct contact sites.[98]

The remaining portion of the PTHrP molecule bears no resemblance to corresponding sequences in PTH. Nevertheless, residues 35 to 111 of PTHrP are strikingly well conserved, with only nine residues differing between mammalian and chicken PTHrP sequences. This sequence conservation is considerably greater than that found in the carboxy-terminal portion of PTH, suggesting that this region of PTHrP has unique and important functions. This region also includes a nuclear localization signal that has been demonstrated to be functional in cultured cells.[121] After residue 111, the PTHrP sequences vary considerably from species to species.

Interspersed within the PTHrP sequences are multiple sites containing one or several basic residues that might serve as post-translational cleavage sites (see Fig. 29.13). Extensive analysis of PTHrP fragments in tumors, cell lines, and transfected cells has shown that several of these sites are, in fact, functional cleavage signals. PTHrP is cleaved after the arginine at residue 37; this cleavage, followed by carboxypeptidase cleavage, generates a PTH-like PTHrP(1-36) fragment as well as the fragments PTHrP(38-94) amide, PTHrP(38-95), and PTHrP(38-101).[122] More carboxy-terminal fragments of PTHrP have been detected in cells as well. Fragments such as PTHrP(107-139) and PTHrP(107-111) can trigger intracellular kinase cascades and affect bone mass in vivo,[123] though the physiologic roles of any of the carboxy-terminal fragments of PTHrP have not yet been established.

In the blood of patients with humoral hypercalcemia of malignancy, multiple immunoreactive species of PTHrP have been found that may well correspond to the fragments of PTHrP in cells and tissue culture media, although precise characterization of these various immunoreactive species is incomplete (see later). Full-length PTHrP may well not circulate, because an amino-terminal–specific immunoaffinity column was unable to extract carboxy-terminal immunoreactivity from the serum of patients with malignant hypercalcemia.[124]

Functions of Parathyroid Hormone–Related Protein

The first actions of PTHrP to be defined were the PTH-like actions associated with the humoral hypercalcemia of malignancy. In this pathologic entity, PTHrP acts as a hormone; it is secreted from the tumor into the bloodstream and then acts on bone and kidney to raise calcium level (see discussion under "Hypercalcemia of Malignancy" later).[125] Whether or not PTHrP circulates at high enough levels in normal adults to contribute to normal calcium homeostasis is an unanswered question. With metastases of breast cancer to bone, locally produced PTHrP can raise serum calcium without necessarily raising blood levels of PTHrP. PTHrP expression by tumors also contributes to the cachexia seen in cancer patients and stimulates the "browning" of adipocytes in tumor models.[126]

PTHrP acts as a calciotropic hormone during fetal life and in lactation. Fetal mice missing the PTHrP gene transport calcium-45 (^{45}Ca) across the placenta inefficiently. This action of PTHrP requires only the midregion of PTHrP and probably involves a receptor distinct from the PTH/PTHrP receptor. Amino-terminal portions of PTHrP and PTH may also be able to increase placental calcium transport, because PTH(1-84) can also increase placental calcium transport in mice missing the PTH gene.[127]

The second setting for humoral actions of PTHrP is lactation. In mice, secretion of PTHrP from the breast into the bloodstream leads to an increase in bone resorption.[128] Calcium then activates the CaSR in breast tissue, increases the movement of calcium into milk, and downregulates expression of PTHrP in the breast.[129] PTHrP therefore probably contributes to the dramatic but largely reversible bone loss during lactation in humans, which is only minimally affected by calcium supplementation.[130] The bone loss is caused both by an increase in osteoclastic bone resorption and by activities of osteocytes to resorb the matrix that surrounds them that require activation of the PTH/PTHrP receptor (osteocytic osteolysis).[87] An exaggeration of this lactational role of PTHrP may explain the rare presentation of hypercalcemia and high PTHrP levels in pregnant and lactating women.[131] Large amounts of PTHrP are also secreted into breast milk, although the role of PTHrP in milk is unknown.

Most of the actions of PTHrP are likely to be paracrine or autocrine.[132] PTHrP is synthesized at one time or another during fetal life in virtually every tissue. Its role in the development of fetal bone has been demonstrated through the striking abnormalities found in genetically engineered mice missing the PTHrP gene. These abnormalities suggest that PTHrP normally keeps chondrocytes proliferating in orderly columns, thereby delaying chondrocyte differentiation.[133] PTHrP blocks the actions of Mef2 and Runx2 transcription factors to activate chondrocyte differentiation by driving class IIa histone deacetylases into the nucleus.[134] The role of PTHrP in many other fetal tissues may analogously involve regulation of proliferation and differentiation. The widespread expression of the PTHrP in fetal life probably underlies the expression of PTHrP in a wide variety of malignancies. As is often the case in malignancy, the expression of PTHrP represents the reinitiation of a fetal pattern of gene expression.

PTHrP is synthesized by many adult tissues. In tissues such as skin, hair, and breast, it is likely that PTHrP regulates cell proliferation and differentiation. PTHrP is also synthesized in response to stretch in the smooth muscle of blood vessels and of the gastrointestinal tract, uterus, and bladder and acts in an autocrine fashion to relax the smooth muscle.[135] PTHrP is also widely expressed in neurons of the central nervous system. Its function in the brain is unknown, but it may protect neurons from excitotoxicity by decreasing flux through voltage-gated calcium channels. An analogous mechanism may explain the role of PTHrP in smooth muscle relaxation.

Many of the actions of PTHrP are mediated by the PTH/PTHrP receptor. Others, such as the activation of placental calcium transport, are probably mediated in part by a distinct receptor, and other actions on bone cells probably involve yet another receptor responsive to more distal portions of PTHrP. Increasing evidence suggests, furthermore, that some actions of PTHrP involve direct nuclear actions of PTHrP.[136] Thus both PTH and PTHrP are likely to use multiple mechanisms to stimulate cells (see Fig. 29.11).

Peptide	Species	Sequence	
CT	Human	**C**GNLST**C**MLGTYTQDFNKFHTFPQTAIGVGAP	-NH2
	Salmon-1	**C**S----**C**V--KLS-ELH-LQTY-R-NT-SGT-	-NH2
	Salmon-2	**C**S----**C**V--KLS-DLH-LQTF-R-NT-AGV-	-NH2
	Salmon-3	**C**S----**C**M--KLS-DLH-LQTF-R-NT-AGV-	-NH2
CGRP	Human α	A**C**DTAT**C**VTHRLAGLLSRSGGVVKNNFVPTNVGSKAF	-NH2
	Human β	-**C**N---**C**----------S----------	-NH2
	Salmon	-**C**N---**C**------DF-N-----GNS----------	-NH2
Amylin	Human	A**C**DTAT**C**VTHRLAGLLSRSGGVVKNNFVPTNVGSKAF	-NH2
ADM	Human	YRQSMNNFQGLRSFG**C**RFGT**C**TVQKLAHQIYQFTDKDKDNVAPRSKISPQGY	-NH2
IMD	Human	TQAQLLRVG**C**VLGT**C**QVQNLSHRLWQLMGPAGRQDSAPVDPSSPHSYG	-NH2
CRSP-1	Porcine	S**C**NTAT**C**MTHRLVGLLSRSGSMVRSNLLPTKMGFKVFG	-NH2
CRSP-2	Porcine	-**C**---S**C**V--KMT-W------VAKN-FM--NVDS-IL	-NH2
CRSP-3	Porcine	-**C**---I**C**V--KMA-W------V-KN-FM-IN--S-VL	-NH2

• **Fig. 29.14** The amino acid sequences of calcitonin (CT), calcitonin gene–related peptide (CGRP), amylin, adrenomedullin (ADM), intermedin (IMD), and calcitonin receptor–stimulating proteins (CSRP) from selected species. The *bold Cs* represent the cysteine residues that form the disulfide linkages critical for the secondary structure of these peptides. The other conserved residues are indicated by a *dashed line* (see Fig. 29.3 for the single-letter amino acid codes).

Calcitonin

Calcitonin has an important role in regulating blood calcium in fish and a demonstrable role in rodents; however, the importance of calcitonin in human calcium homeostasis remains uncertain.

The existence of a second calcium-regulating hormone, in addition to PTH, was first demonstrated during perfusion studies of the thyroid/parathyroid glands of dogs.[137] High calcium perfusion resulted in a rapid decrease in plasma calcium, even more rapid than after parathyroidectomy. This suggested that calcium had stimulated the secretion of a hormone that lowered blood calcium. It was subsequently demonstrated that this missing hormone, named *calcitonin* for its role in regulating the "tone" or level of calcium, was elaborated by the thyroid gland, not the parathyroids. Calcitonin is found in the nonfollicular cells of the thyroid, called C cells, which originate from the neural crest.[138] In fish, the location of the C cells in discrete organs led to the rapid isolation of calcitonin from these ultimobranchial bodies in dogfish, salmon, and several other species. The identification of the glandular origin of calcitonin enabled the isolation of sufficient quantities of calcitonin for sequence analysis[139] and studies of its structure and biologic function.

Synthesis and Secretion

Calcitonin consists of a 32–amino acid polypeptide with an intrachain disulfide bond provided by the cysteines at positions 1 and 7 (Fig. 29.14). These two cysteine residues, along with the carboxy-terminal proline amide and six additional residues, are the only amino acids conserved among the calcitonins isolated from various species. The disulfide linkage and proline amide residues are important for the function of the molecule, although biologically active analogues lacking disulfide bonds have been developed. Interestingly, fish calcitonin is more potent in mammals than is the mammalian hormone. The mature peptide is derived from the middle of a 136–amino acid precursor. The human calcitonin gene, located on the short arm of chromosome 11, contains 6 exons, which are alternatively spliced in a tissue-specific manner to yield the mRNAs encoding calcitonin or calcitonin gene-related peptide (CGRP) (see Fig. 29.14). The mRNA encoding calcitonin is derived by splicing together the first four exons[140] and represents over 95% of mature transcripts in the thyroid C cells. The splicing of the first three exons to exons 5 and 6 results in an mRNA that encodes the 37–amino acid αCGRP peptide. The mRNA encoding αCGRP is expressed in multiple tissues and is the only mature transcript of the calcitonin gene detected in neural tissue. A second CGRP gene encodes the closely related βCGRP. In humans, the predicted sequence of the mature peptide differs from that of αCGRP by only three amino acids (see Fig. 29.14). The βCGRP gene is also found on chromosome 11; its tissue distribution is the same as that of αCGRP.

The synthesis and secretion of calcitonin are tightly regulated. Studies in a porcine model reveal a linear relationship between the secretion of calcitonin and ambient calcium levels.[141] Cell culture studies with calcium ionophores and calcium channel blockers demonstrate that the calcium ion concentration within the C cell determines this secretion rate.[142] The CaSR cloned from parathyroid cells is also expressed in C cells and contributes to the regulation of calcitonin secretion.[143] Other calcitonin secretagogues include glucocorticoids, CGRP, glucagon, enteroglucagon, gastrin, pentagastrin, pancreozymin, and β-adrenergic agents.[144] The physiologic role of the gastrointestinal hormones in regulating calcitonin remains unclear; however, they have been postulated to play a role in the regulation of postprandial hypercalcemia. The secretion of calcitonin is inhibited by somatostatin, which is also secreted by the thyroidal C cells. In vivo[145] and in vitro[146] studies have demonstrated that $1,25(OH)_2D_3$ decreases calcitonin mRNA levels by a transcriptional mechanism.

Calcitonin, when administered acutely, decreases tubular resorption of calcium[147] and impairs osteoclast-mediated bone resorption by a direct action on osteoclasts.[148] In rodents, calcitonin has been shown to play a role in the regulation of postprandial hypercalcemia[149] and the skeletal response to lactation.[150] Studies in calcitonin knockout mice reveal a doubling of bone formation rate in the absence of hormone, accompanied by resistance to ovariectomy-induced bone loss.[151] An increase in bone formation is also found in mice heterozygous[152] or homozygous for calcitonin receptor ablation.[153] This increase in bone formation is

thought to be due to absence of calcitonin-mediated inhibition osteoclast secretion of sphingosine-1-phosphate, which induces bone formation.[153]

The physiologic role of calcitonin in humans, however, remains elusive. The effect of calcitonin on bone density was examined in patients with long-term hypercalcitoninemia secondary to medullary carcinoma of the thyroid (MCT) and in patients with subtotal thyroidectomy resulting in lack of calcitonin secretory reserve.[154] The abnormal calcitonin levels had no influence on the bone density at the lumbar spine and distal radius. Furthermore, long-term, high-dose administration of exogenous calcitonin caused no physiologic abnormalities.[155]

Many of the effects of calcitonin are mediated by a G protein–coupled cell surface receptor in the PTH/secretin receptor family.[156,157] The mRNA encoding this receptor has been found in multiple tissues, including kidney, brain, and osteoclasts. The coupling of this receptor to different G proteins results in the activation of either adenylate cyclase or PLC; in some settings, this is cell cycle dependent.[158]

Calcitonin Family: Calcitonin Gene-Related Peptide, Amylin, Adrenomedullin, Calcitonin Receptor–Stimulating Peptides, and Intermedin

CGRP, amylin, adrenomedullin, calcitonin receptor–stimulating peptide 1 (CRSP1), and intermedin have all been shown to have high-affinity binding sites on cell membranes, and displacement studies suggest that several receptor subtypes for these related ligands are present. However, cloning of specific receptors for these ligands proved difficult, because the functional receptors consist of heterodimers between G protein–coupled receptors and single transmembrane proteins of the RAMP (receptor activity modifying proteins) family.[159,160] Interaction of the calcitonin receptor-like receptor, a relative of the calcitonin receptor, with RAMP1 results in a CGRP receptor, whereas RAMP2 and RAMP3 interactions with the same calcitonin receptor-like receptor generate adrenomedullin receptors. Interaction of RAMP1 with the calcitonin receptor generates an amylin receptor.[161] CGRP receptors have been shown to exhibit ligand-dependent and RAMP-dependent signaling bias among the $G\alpha_s$, $G\alpha_i$, and $G\alpha_{q/11}$ pathways.[162]

CGRP is thought to act as a neurotransmitter and vasodilator rather than as a hormone. In support of this hypothesis, inhibition of CGRP receptor signaling using monoclonal antibodies decreases the frequency of migraines in predisposed individuals,[163,164] and mice lacking αCGRP have been shown to have an increase in mean arterial pressure.[165] Immunohistochemical studies of CGRP in the brain and peripheral nervous system suggest that this neuropeptide also plays an important role in sensory and integrative motor functions.

Three structurally related peptides have been isolated from porcine brain (see Fig. 29.14). These calcitonin receptor-stimulating peptides (CRSPs) are also expressed in the thyroid gland. CRSP1, which is 60% homologous to αCGRP at the amino acid level, binds to the calcitonin receptor, dose-dependently stimulates cAMP production, and inhibits osteoclastogenesis.[166] Consistent with this observation, administration of CRSP1, like that of calcitonin, results in a decrease in serum calcium. The receptors for CRSP2 and CRSP3 have not been identified.[167]

Amylin is highly homologous to CGRP and calcitonin (see Fig. 29.14). Although amylin has been shown to have skeletal actions, the presence of amylin in the pancreas of patients with type 2 diabetes mellitus suggests an etiologic role for this peptide in this disorder.[168] Because amylin slows gastric emptying, promoting satiety and attenuating the postprandial rise in glucagon, analogues of this peptide are currently being used as therapeutic agents for type 2 diabetes and are being investigated for the treatment of obesity. Amylin administration inhibits bone loss associated with ovariectomy and streptozotocin-induced diabetes mellitus in rats.[169,170] Targeted ablation of amylin in mice results in low bone mass due to an increase in bone resorption.[152] Amylin has also been shown to decrease food intake and inhibit gastric acid secretion, protecting against ulcer development in numerous models.[171]

Adrenomedullin (see Fig. 29.14) has vasodilatory effects similar to those of CGRP. In addition to activating CGRP receptors, adrenomedullin binds to specific receptors in the vascular system.[172] Mice lacking adrenomedullin die in midgestation.[173] Haploinsufficiency of RAMP2, which regulates binding of adrenomedullin to the calcitonin receptor, results in hyperprolactinemia, delayed bone development, and decreased bone mineral density.[174] Unlike other family members, adrenomedullin does not inhibit osteoclast activity or formation.[166] The physiologic role of adrenomedullin in the skeleton remains to be clarified.

Intermedin (adrenomedullin-2), the newest member of this family (see Fig. 29.14), was identified by homology screening of expressed sequence tags. It is expressed primarily in the pituitary and the gastrointestinal tract. Intermedin is able to signal through CGRP receptors and competes with CGRP for receptor binding.[175] However, unlike CGRP and adrenomedullin, intermedin is a nonselective agonist for the RAMP coreceptors.

Calcitonin in Human Disease

Calcitonin is secreted by several endocrine malignancies and therefore can serve as a tumor marker. Basal and pentagastrin-stimulated calcitonin levels have been used to identify and follow those at risk for, or affected by, medullary carcinoma of the thyroid (see Chapter 42), although abnormal basal and stimulated levels may be observed in patients on chronic hemodialysis.[176] Calcitonin may also be ectopically secreted by other tumors, including insulinomas, VIPomas, and lung cancers. Severely ill patients, including those with burn inhalation injury,[177] toxic shock syndrome,[178] and pancreatitis,[179] may also have elevated calcitonin levels.

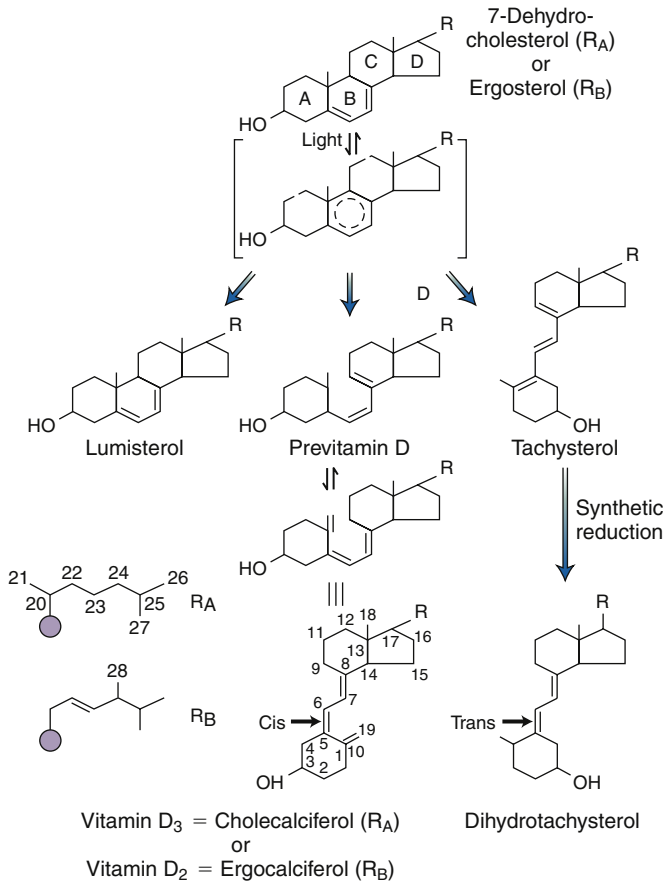
Therapeutic Uses

The observation that calcitonin inhibits osteoclastic bone resorption has led to its therapeutic use for the treatment of several disorders associated with excess bone resorption, including osteoporosis and Paget disease. Calcitonin has also been used for its analgesic effect, in the treatment of patients with vertebral crush fractures, osteolytic metastases, or phantom limb.[180,181] Based on the more beneficial effects of other agents and potential increased risk of malignancy in those being treated with salmon calcitonin, as well as the marginal efficacy of the drug, it is no longer recommended for treatment of osteoporosis.[182]

Vitamin D

Metabolism of Vitamin D

Vitamin D is not a true vitamin, because nutritional supplementation is not required in humans who have adequate sun exposure. When exposed to ultraviolet irradiation, the cutaneous precursor



• **Fig. 29.15** Vitamin D precursors and alternative reaction products. The numbering system for vitamin D carbons and the distinct structures of vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol) are noted, as is the structure of dihydrotachysterol, a synthetic product not produced in vivo. Note that the 3-hydroxyl group of dihydrotachysterol is in a pseudo-1-hydroxyl configuration. This may explain the relatively high potency of dihydrotachysterol in conditions associated with low 1α-hydroxylase activity.

of vitamin D, 7-dehydrocholesterol, undergoes photochemical cleavage of the carbon bond between carbons 9 and 10 of the steroid ring (Fig. 29.15). The resultant product, previtamin D, is thermally labile and over a period of 48 hours undergoes a temperature-dependent molecular rearrangement that results in the production of vitamin D. Alternatively, this thermally labile product can isomerize to two biologically inert products, luminosterol and tachysterol. This alternative photoisomerization prevents production of excessive amounts of vitamin D with prolonged sun exposure. The degree of skin pigmentation, which increases in response to solar exposure, also regulates the conversion of 7-dehydrocholesterol to vitamin D by blocking the penetration of ultraviolet rays.

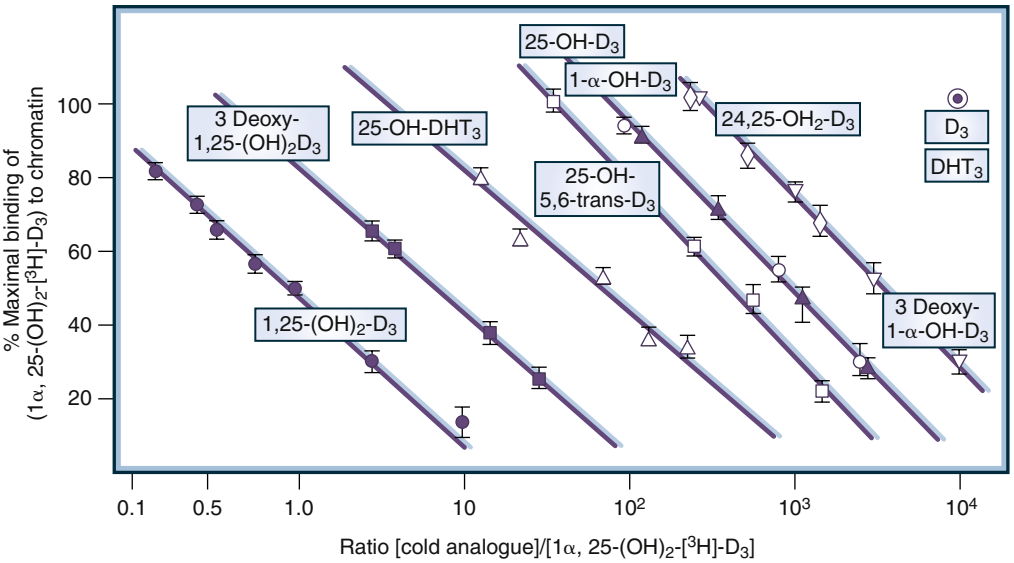
The alternative source of vitamin D is dietary. The elderly, the institutionalized, and those living in northern climates likely obtain most of their vitamin D from dietary sources. However, with increasing avoidance of sun exposure by the general population, ensuring adequate dietary intake of vitamin D has become important for the population at large. Vitamin D deficiency is prevalent and has been shown to contribute significantly to osteopenia and fracture risk. The major dietary sources of vitamin D are fortified dairy products, although the lack of monitoring of this supplementation results in marked variation in the amount of vitamin D provided.[183] Other dietary sources include egg yolks, fish oils, and fortified cereal products. Vitamin D provided by plant sources is in the form of vitamin D_2, whereas that provided by animal sources is in the form of vitamin D_3 (see Fig. 29.15). These two forms have equivalent biologic potencies and are activated equally efficiently by the hydroxylases in humans. While vitamin D_3 has been shown to be more effective at increasing 25-hydroxyvitamin D levels,[184] this effect is dependent upon vitamin D–binding protein (VDBP) genotype and concentration.[185]

Vitamin D is absorbed into the lymphatics and enters the circulation bound primarily to VDBP, although a fraction of vitamin D circulates bound to albumin. The human VDBP is a 52-KDa α-globulin synthesized in the liver. The protein has a high affinity for 25(OH)D but also binds vitamin D and $1,25(OH)_2D$. Approximately 88% of 25(OH)D circulates bound to the VDBP, 0.03% is free, and the rest circulates bound to albumin. In contrast, 85% of the circulating $1,25(OH)_2D_3$ binds to the VDBP, 0.4% is free, and the rest binds to albumin. Mice lacking VDBP have increased susceptibility to $1,25(OH)_2D_3$ toxicity as well as to dietary vitamin D deficiency.[186] Vitamin D–binding protein polymorphisms have been postulated to be the basis for difference in vitamin D levels in several groups, including African Americans and Finns.[187] Measurements of free 25(OH) vitamin D across multiple populations, however, suggest that the monoclonal antibody used in the former study can bring misleading results.[188]

Studies in megalin-deficient patients and megalin null mice demonstrate that VDBP is filtered by the glomerulus and reabsorbed by a megalin-dependent pathway in the proximal renal tubule. Further investigations will be required to determine the importance of this pathway in vitamin D metabolism and the tissues in which megalin-dependent endocytosis plays an important role.

In the liver, vitamin D undergoes 25-hydroxylation by a cytochrome P450–like enzyme present in the mitochondria and microsomes. The half-life of 25(OH)D is approximately 2 to 3 weeks. The 25-hydroxylation of vitamin D is not tightly regulated, therefore the blood levels of 25(OH)D reflect the amount of vitamin D entering the circulation. When levels of VDBP are low, such as in nephrotic syndrome, circulating levels of 25(OH)D are also reduced. The half-life of 25(OH)D is shortened by increases in levels of its active metabolite, $1,25(OH)_2D_3$.

The final step in the production of the active hormone is the renal 1α-hydroxylation of 25(OH)D to $1,25(OH)_2D_3$. The half-life of this hormone is approximately 6 to 8 hours. Like the 25-hydroxylase, the 1α-hydroxylase in the proximal convoluted tubule is a cytochrome P450–like mixed function oxidase, but unlike the 25-hydroxylase, the 1α-hydroxylase is tightly regulated. PTH and hypophosphatemia are the major inducers of this microsomal enzyme, whereas calcium, $1,25(OH)_2D$, and FGF23 repress it.[189,190] Analogous to mice lacking FGF23, mice with inactivating mutations of the FGF23 coreceptor, α-Klotho, a type I membrane protein with homology to β-glycosidases, develop hypercalcemia as a result of increased levels of $1,25(OH)_2D_3$. Like the FGF23 null mice, Klotho null mice have increased levels of the 1α-hydroxylase,[191] and their phenotype is ameliorated by impairment of $1,25(OH)_2D_3$ action. In animal models and in vitro studies, other hormones such as estrogen, calcitonin, growth hormone, and prolactin have been shown to increase 1α-hydroxylase activity; however, the clinical importance of these observations has not been established. Ketoconazole has been shown to decrease levels of $1,25(OH)_2D_3$ in a dose-dependent manner, presumably by interfering with 1α-hydroxylase activity.



• **Fig. 29.16** Relative potency of analogues of $1,25(OH)_2D_3$ (1,25-dihydroxyvitamin D_3) in competitive binding to vitamin D receptors of chick intestinal mucosa. Slopes are plotted for *(left to right)* $1,25\text{-}(OH)_2\text{-}D_3$ (1,25-dihydroxyvitamin D_3); 3 deoxy-$1,25(OH)_2D_3$ (3 deoxy-1,25-dihydroxyvitamin D_3); 25-OH-DHT_3 (25-hydroxydihydrotachysterol); 25-OH-5,6-trans-D_3 (25-hydroxy-5,6-transvitamin D_3); 25-OH-D_3 (25-hydroxyvitamin D_3); 1-α-OH-D_3 (1α-hydroxyvitamin D_3); 24,25-OH_2-D_3 (24,25-dihydroxyvitamin D_3); 3-deoxy-1-α-OH-D_3 (3-deoxy-1α-hydroxyvitamin D_3); D_3 (vitamin D_3); DHT_3 (dihydrotachysterol). (From Proscal DA, Okamura WH, Norman AW. Structural requirements for the interaction of 1α,25-(OH)2-vitamin D3 with its chick intestinal system. *J Biol Chem.* 1975;250:8382–8388.)

The 1α-hydroxylase enzyme is also expressed in keratinocytes, the trophoblastic layer of the placenta, and in granulomas, including sarcoid granulomas, among many other tissues. In granulomatous tissue, the 1α-hydroxylase gene that is expressed is identical to that expressed in the kidney but is not regulated by PTH, phosphate, calcium, or vitamin D metabolites in these cells. Activation of macrophages with interferon-γ[192] or with ligands that activate the heterodimer of toll-like receptors 1 and 2,[193] however, increases the expression of the 1α-hydroxylase in macrophages, whereas treatment of sarcoidosis-associated hypercalcemia with glucocorticoids, ketoconazole,[194] or chloroquine[195] has been shown to lower serum $1,25(OH)_2D_3$ levels. Activation of the VDR in human macrophages induces the antimicrobial peptide cathelicidin and increases killing of intracellular *Mycobacterium tuberculosis*.[193] Thus the pathologic excess of $1,25(OH)_2D_3$ in sarcoidosis may represent an exaggeration of a healthy paracrine response of tissue macrophages. This action can be viewed as a paradigm for many other actions of vitamin D that are mediated more by the local production of $1,25(OH)_2D_3$ than by circulating $1,25(OH)_2D_3$.

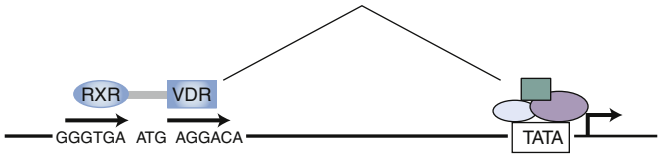
$25(OH)D$ and $1,25(OH)_2D_3$ can also be hydroxylated by vitamin D 24-hydroxylase, which is present in most tissues, including kidney, cartilage, and intestine. $1,25(OH)_2D_3$ increases the activity of the 24-hydroxylase, thereby inducing its own metabolism. The 24-hydroxylated vitamin D metabolites, $24,25(OH)_2D_3$ and $1,24,25(OH)_3D_3$, are not thought to play major biologic roles other than inactivation of $1,25(OH)_2D_3$. Mice and humans with nonfunctional 24-hydroxylase genes demonstrate hypercalcemia, hypercalciuria, and nephrocalcinosis due to vitamin D toxicity.[196,197]

$1,25(OH)_2D_3$ is also metabolized to several inactive products by 23-hydroxylation or 26-hydroxylation and side-chain oxidation and cleavage. This latter side-chain cleavage, resulting in the formation of calcitroic acid, occurs in the liver and intestine, whereas inactivation of $1,25(OH)_2D_3$ in a wide variety of target tissues occurs by 24-hydroxylation. In addition, polar metabolites of $1,25(OH)_2D_3$ are excreted in the bile. Some of these metabolites are deconjugated in the intestine and reabsorbed into the enterohepatic circulation.

Actions of Vitamin D

Vitamin D Receptors

$1,25(OH)_2D_3$ exerts its biologic functions by binding to a nuclear receptor, which then regulates transcription of DNA into RNA. Among the other nuclear receptors, the VDR most closely resembles the retinoic acid, triiodothyronine, and retinoid X receptors (RXRs). The affinity of the receptor for $1,25(OH)_2D_3$ is approximately three orders of magnitude higher than that for other vitamin D metabolites (Fig. 29.16). Although $25(OH)D_3$ is less potent on a molar basis, its concentration in the serum is approximately three orders of magnitude higher than that of $1,25(OH)_2D$. However, its free concentration is only two orders of magnitude greater than that of $1,25(OH)_2D_3$. Therefore, under normal circumstances, it is unlikely that $25(OH)D_3$ contributes importantly to calcium homeostasis. Because the affinity of the vitamin D–binding protein for $25(OH)D_3$ is greater than for $1,25(OH)_2D_3$, in states of vitamin D intoxication (with its associated high levels of $25[OH]D_3$), the free levels of $1,25(OH)_2D_3$ increase because $25(OH)D$ displaces it from the vitamin D–binding protein. $25(OH)D_3$ may therefore play a role in the clinical syndrome of vitamin D intoxication both by its direct biologic effects, when present at toxic levels, and by increasing free levels of $1,25(OH)_2D_3$. Under normal circumstances $25(OH)D$ is not thought to play a role in mineral ion homeostasis; however,



• **Fig. 29.17** Transcriptional activation by 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). A heterodimer of retinoid X receptor (RXR) and vitamin D receptor (VDR) binds to a pair of hexameric sequences separated by three intervening bases (ATG). *Arrows* indicate that the hexamers found in the upregulated rat osteocalcin gene are variants of a consensus sequence, repeated here with identical orientations (direct repeats). Upon binding to DNA, the RXR-VDR heterodimer facilitates formation of a transcription initiation complex, which binds to DNA at and near the TATA sequence.

local activation of this prohormone may contribute to host immune response and barrier function.

The vitamin D receptor acts by forming a heterodimer with the retinoid-X receptor, binding to DNA elements, and recruiting coactivators in a ligand-dependent fashion. These coactivators link the receptor complex to the basal transcription apparatus, thereby regulating transcription of target genes. In most cases, the upregulatory response elements for vitamin D contain hexameric repeats separated by three bases (Fig. 29.17).[198] The mechanism of transcriptional repression by vitamin D varies. For example, VDR-RXR heterodimers repress the 1α-hydroxylase and renin genes by blocking the function of other transcription factors,[199,200] and interaction of the VDR with the Ku antigen, acting as a transcription factor, is required for transcriptional repression of the hPTHrP gene.[201]

Glucocorticoids have been shown to decrease the expression of the VDR gene in osteosarcoma cell lines, whereas $1,25(OH)_2D_3$ increases its expression in many cell types. In the renal proximal convoluted tubule, however, $1,25(OH)_2D_3$ decreases the levels of vitamin D receptors. This decrease has been postulated to lead to decreased activation of the renal 24-hydroxylase by $1,25(OH)_2D_3$ and thereby protect the newly synthesized $1,25(OH)_2D$ from local inactivation.[202]

$1,25(OH)_2D_3$ also has some biologic effects that occur too rapidly for transcriptional mechanisms to be implicated. These so-called nongenomic actions, including a rapid increase in intracellular calcium, activation of phospholipase C, and opening of calcium channels, are observed in several cell types within minutes of exposure to $1,25(OH)_2D_3$. Additional data supporting the hypothesis that nongenomic actions are not dependent on the classic receptor include the identification of specific binding sites for $1,25(OH)_2D_3$ on the antiluminal surface of intestinal cells[203] and a disparity between the affinity of the various vitamin D analogues for the nuclear receptor and their potency in these nongenomic actions. However, both the rapid intracellular accumulation of cGMP in association with the vitamin D receptor and the rapid increase in intracellular calcium in response to $1,25(OH)_2D$ are dependent upon the presence of an intact nuclear receptor, because these effects are not observed in cells derived from patients and mice with VDR mutations.[204] However, 1,25-dihydroxyvitamin D binding to the cell surface receptor PDIA3/Erp57 causes a rapid increase in calcium uptake in enterocytes, which is abrogated by deletion of this receptor.[205] The physiologic importance of the nongenomic actions of vitamin D metabolites[206] has not yet been established.

The VDR is expressed in most tissues and has been shown to regulate cellular differentiation and function in many cell types. Nevertheless, the most dramatic physiologic effects of vitamin D, acting through the VDR, involve regulation of intestinal calcium transport. This is most clearly demonstrated by the phenotype of patients and mice with mutant VDRs (hereditary vitamin D–resistant rickets)[35,207]: Dramatic abnormalities in bone mineralization can be reversed by bypassing the defect in intestinal calcium absorption.[208–210]

Intestinal Calcium Absorption

Under normal dietary conditions, calcium intake is in the range of 700 to 900 mg daily. Approximately 30% to 35% of this calcium is absorbed; however, losses from intestinal secretion of calcium lead to a net daily uptake of approximately 200 mg. Though vitamin D is the major hormonal determinant of intestinal calcium absorption, the bioavailability of mineral ions in the intestinal lumen may be affected by a number of local factors and dietary constituents. Absorption of calcium and magnesium is impaired by bile salt deficiency, unabsorbed free fatty acids in steatorrheic states, and high dietary content of fiber or phytate. Gastric acid is needed to promote dissociation of calcium from anionic components of food or therapeutic preparations of calcium salts. Administration of calcium salts with meals, especially in achlorhydrics, and use of divided doses or more soluble salts such as calcium citrate are commonly used strategies to increase calcium bioavailability.

Calcium is thought to be absorbed by two pathways: a saturable transcellular pathway and a nonsaturable paracellular route. The transcellular pathway is dependent on $1,25(OH)_2D_3$. Although the necessity of vitamin D for paracellular calcium absorption remains controversial, substantial evidence exists that the hormone enhances this pathway as well.[211] Notably, $1,25(OH)_2D_3$ induces the expression of claudin-2 and claudin-12, which contribute to intestinal calcium absorption and are thought to form paracellular channels between neighboring cells.[212]

The most extensively studied mechanism of intestinal calcium absorption involves the transcellular route. This pathway is thought to involve three steps: entry of calcium into the enterocyte (which is the rate-limiting step), transport across the cell, and extrusion across the basolateral membrane.[213]

Entry Into the Enterocyte

$1,25(OH)_2D_3$ induces synthesis of a number of brush border proteins, including the intestinal membrane calcium-binding protein, brush border alkaline phosphatase, and low-affinity Ca^{2+}/Mg^{2+}-ATPase. The activity of these proteins correlates with active calcium transport; however, a causal relationship remains to be established. Two calcium channels, TRPV5 and TRPV6, members of the transient receptor potential vanilloid receptor subfamily containing six membrane-spanning domains, are expressed in the duodenum, the jejunum, and the kidney as well as in other tissues. TRPV6 is thought to play a critical role in intestinal calcium absorption, and $1,25(OH)_2D_3$ increases its expression, as well as that of TRP5.[214] Studies in mice lacking TRPV5 demonstrate that this channel is primarily responsible for renal calcium reabsorption, because mice lacking TRPV5 have enhanced, rather than impaired, intestinal calcium absorption due to their high circulating levels of $1,25(OH)_2D$.[215] Upon entering the enterocyte, calcium binds to components of the brush border complex subjacent to the plasma membrane. Calmodulin is redistributed to the brush border in response to $1,25(OH)_2D_3$ and may play a role in this process, as may the $1,25(OH)_2D_3$-inducible calcium-binding protein, calbindin-9K.

Transcellular Transport

The best studied effect of vitamin D on the enterocyte is the induction of synthesis of the intestinal calcium-binding protein, calbindin-9K. This protein has an EF hand structure that permits the binding of two calcium ions per molecule. The affinity of calbindin for calcium is approximately four times that of the brush border calcium-binding components, so calcium is preferentially transferred to calbindin. Calbindin serves to buffer the intracellular free calcium concentration during calcium absorption. It associates with microtubules and may play a role in the transport of calcium across the enterocyte. However, studies in mice lacking both TRPV6 and calbindin-D9k maintain 40% of normal $1,25(OH)_2D_3$-mediated calcium transport, demonstrating that other hormone-responsive genes may play a significant or compensatory role.[216] Organelles such as the mitochondria, Golgi apparatus, and endoplasmic reticulum also serve as repositories for intracellular calcium.

Exit From the Enterocyte

The transport of calcium across the antiluminal surface of the enterocyte, the final process involved in intestinal calcium absorption, is dependent on $1,25(OH)_2D_3$. The main mechanism of calcium extrusion is the $1,25(OH)_2D_3$-inducible ATP-dependent Ca^{2+} pump (PMCA1b). The affinity of the pump for calcium is approximately 2.5 times that of calbindin. With high calcium intake, a $1,25(OH)_2D_3$-independent Na^+/Ca^{2+} exchanger may play a role in the transfer of calcium across the basolateral membrane as well.

Actions on the Parathyroid Gland

$1,25(OH)_2D_3$ has been shown to regulate gene transcription and cell proliferation in the parathyroids. The hormone also inhibits the proliferation of dispersed parathyroid cells in culture, although the relative contribution of calcium and $1,25(OH)_2D_3$ in the regulation of parathyroid cell proliferation in vivo has not been established. Normocalcemic mice lacking functional vitamin D receptors have normal serum PTH levels and normal-size parathyroid glands, demonstrating that the genomic actions of $1,25(OH)_2D_3$ are not essential for parathyroid cellular homeostasis.[210] $1,25(OH)_2D_3$ has, however, been shown to decrease the transcription of the PTH gene both in vivo and in vitro. This action has been exploited in the use of $1,25(OH)_2D_3$ for the treatment of the secondary hyperparathyroidism associated with chronic renal failure (see discussions under "Parathyroid Hormone Biosynthesis" and "Vitamin D Deficiency").

Actions on Bone

The effects of $1,25(OH)_2D_3$ on bone are numerous. $1,25(OH)_2D_3$ is a major transcriptional regulator of the two most abundant bone matrix proteins: It represses the synthesis of type I collagen and induces the synthesis of osteocalcin. $1,25(OH)_2D_3$ promotes the differentiation of osteoclasts from monocyte-macrophage stem cell precursors in vitro and increases osteoclastic bone resorption in high doses in vivo by stimulating production of RANKL (also called osteoclast differentiating factor) by osteoblasts.[217] Notably, osteoblast-specific knockout of the vitamin D receptor leads to increased bone mass associated with impaired bone resorption due to decreased RANKL expression.[218] Consistent with this, and despite the multiple effects of $1,25(OH)_2D_3$ on the biology of bone in vitro, in vivo studies in $1,25(OH)_2D_3$-deficient rats and in mice lacking functional vitamin D receptors[35,208] suggest that the major osseous consequences of hormone and receptor deficiency can be reversed when mineral ion homeostasis is normalized. In addition, parenteral calcium infusions have been shown to heal the osteomalacic lesions in children with mutant VDRs.[209] These observations suggest that the major role of $1,25(OH)_2D_3$ in bone is to provide the proper microenvironment for bone mineralization through stimulation of the intestinal absorption of calcium and phosphate.

Other Actions of Vitamin D

The effects of $1,25(OH)_2D_3$ on phosphate transport are less well studied than those on calcium transport. $1,25(OH)_2D_3$ has been shown to promote the already efficient intestinal phosphate absorption and to induce the expression of the phosphaturic hormone FGF23.[66]

One of the striking clinical features of profound vitamin D deficiency that is poorly understood is the severe proximal myopathy. While some studies demonstrate VDR expression in skeletal myofibers,[219] both the expression and the role of this receptor in muscle remain controversial. Regardless, in vitro $1,25(OH)_2D_3$ treatment increases 25(OH)D[219] and amino acid uptake into muscle cells and alters phospholipid metabolism. Vitamin D administration has been shown to increase the concentration of troponin C, a calcium-binding protein in muscle that plays a role in excitation coupling and increases the rate of uptake of calcium by the sarcoplasmic reticulum. Vitamin D repletion of severely deficient adults has been shown to improve muscle strength, associated with an increase in mitochondrial function assessed by 31-phosphate nuclear magnetic resonance (NMR) spectroscopy.[220] Vitamin D receptor knockout mice demonstrate a delay in myoblast differentiation[221]; however, little is known regarding the direct role of vitamin D in normal muscle physiology. The myopathy that accompanies vitamin D deficiency is characterized by normal creatinine phosphokinase (CPK) levels, a myopathic electromyogram (EMG), and biopsy findings of loss of myofibrils, fatty infiltration, and interstitial fibrosis. The myopathy resolves within days to weeks of vitamin D replacement and is not related to normalization of mineral ion homeostasis; however, studies in vitamin D–deficient rats demonstrate that maintenance of normal mineral ion levels precludes the development of myopathy.[222] Vitamin D deficiency has also been implicated in statin-induced myopathy.[223] In contrast to these studies, which suggest a beneficial effect of vitamin D on muscle function, monthly administration of 60,000 IU vitamin D has been shown to be associated with an increased incidence of falls in those over the age of 70.[224]

While the vitamin D receptor is widely expressed, the clinical implications of these findings are not well understood. However, recent studies demonstrate that women with vitamin D levels above 30 ng/mL in early and late pregnancy have a lower risk of preeclampsia.[225] Prenatal vitamin D supplementation has also been shown to reduce the risk of early childhood asthma.[226]

Vitamin D Analogs

The recognition that $1,25(OH)_2D_3$ promotes cellular differentiation and inhibits cellular proliferation has led to efforts directed at producing new analogues that retain these effects but do not cause hypercalcemia. Several analogues have been shown to have antiproliferative effects on normal cells as well as on malignant cells in vitro and in xenografts in immunosuppressed mice.[227] In addition, analogues of vitamin D have been shown to synergize with cyclosporine in preventing rejection of transplanted islet cells in a murine model.[228] Nonhypercalcemic analogs have been shown to

suppress PTH synthesis and secretion in rats at doses that stimulate intestinal calcium absorption less than $1,25(OH)_2D_3$. Such analogs are currently used for the prevention and treatment of hyperparathyroidism associated with chronic kidney disease.[229] The antiproliferative effects of vitamin D have been exploited clinically in the treatment of psoriasis.[230] Although analogues with reduced calcemic activity are predominantly used, hypercalcemic crisis after excessive topical use of such compounds can occur.

The physiology underlying the differential biologic effects of these analogues is not completely understood. Altered affinity for the vitamin D–binding protein, metabolism by target tissues,[231] and effects on recruitment of coactivators by the vitamin D receptor may contribute to the unique properties of vitamin D analogs.[232]

Fibroblast Growth Factor-23

FGF23 in Human Disease

The search for the hormonal factor responsible for the hypophosphatemia in patients with tumor-induced osteomalacia (TIO, see Chapter 31) was brought to a close with the identification of the molecular basis for the human disorder, autosomal dominant hypophosphatemic rickets (ADHR).[233] Linkage analyses of affected kindreds identified mutation of the gene encoding FGF23 as the basis for ADHR. The mutation in affected individuals abolishes an RXXR protease recognition motif that is thought to be responsible for the cleavage and inactivation of FGF23.[234,235] The cDNA encoding FGF23 predicts a peptide of 251 amino acids, the first 24 of which comprise a signal peptide. Studies using recombinant FGF23 demonstrate that the full-length mature peptide is required for its biologic activity and that the cleavage site mutated in the ADHR patients is responsible for its inactivation. Cleavage of FGF23 is inhibited by furin inhibition, suggesting that the enzyme responsible is a subtilisin-like proprotein convertase.

Analyses of tumors isolated from patients with TIO reveal a dramatic increase in mRNA expression of FGF23.[236] A significant number of these tumors exhibit a fibronectin:FGFR1 or fibronectin:FGF1 fusion thought to underlie the increase in FGF23 production.[237] The elevated serum levels of FGF23 in patients with TIO have been shown to normalize after removal of the tumor, correlating with resolution of the hypophosphatemia that characterizes this disorder.[238,239] FGF23 is also responsible for the renal phosphate wasting observed in X-linked hypophosphatemia. Increased levels of FGF23 have been reported in disorders characterized by somatic moisaicism, including fibrous dysplasia and epidermal and large congenital melanocytic nevus syndrome. Exome sequencing in the latter individuals demonstrated somatic activating mutations in *HRAS* and *NRAS*, though how this leads to increased serum FGF23 levels is not clear.[240] Conversely, patients with the rare syndrome tumoral calcinosis present with hyperphosphatemia and soft tissue calcium-phosphate deposits. Some of these patients have point mutations in the FGF23 gene that cause abnormal processing of the protein, with low levels of the active hormone in the blood and high levels of inactive fragments.[241–243] Other affected individuals have been shown to have mutations in the FGF23 coreceptor α-Klotho or in GALNT 3, which normally O-glycosylates FGF23.[244,245] Thus human diseases of both increased and decreased FGF23 activity suggest that this factor is an important regulator of phosphate metabolism.

Actions of FGF23

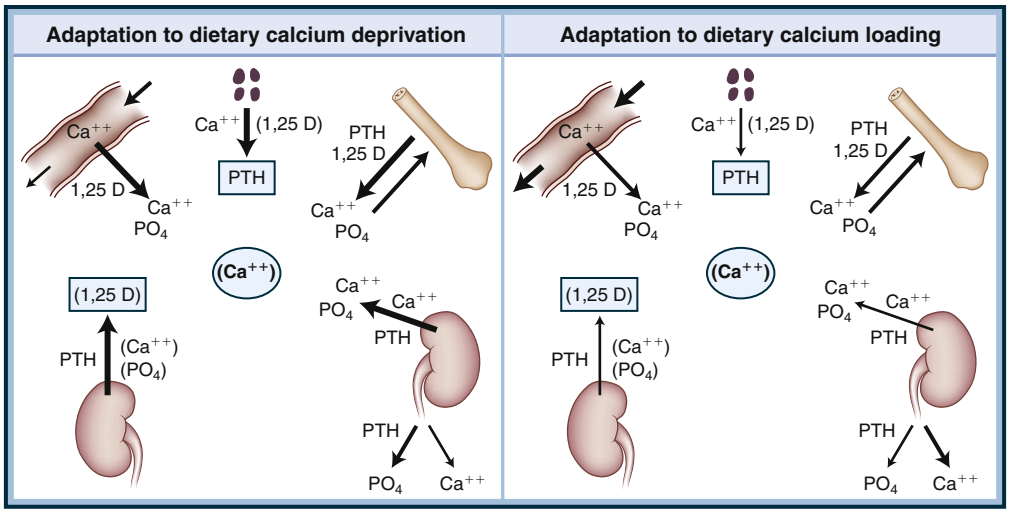
Evidence that FGF23 is a novel hormone that plays a key role in normal phosphate homeostasis has been obtained in murine models of overexpression and ablation. Overexpression of FGF23 or administration of FGF23 to animals results in the development of hypophosphatemia[235] and impaired 1α-hydroxylation of 25(OH)D,[190,246] thus recapitulating the findings observed in patients affected by TIO. Investigations in mice with targeted ablation of FGF23 have proven that endogenous production of this hormone is critical for normal phosphate homeostasis and the regulation of vitamin D metabolism.[247,248] Absence of FGF23 results in impaired renal phosphate excretion, leading to the development of hyperphosphatemia within the first 2 weeks of life. Affected mice also develop hypercalcemia due to high levels of 1,25-dihydroxyvitamin D, a result of the lack of the normal suppressive effect of FGF23 on the renal 25(OH)D 1α-hydroxylase.[190] Ablation of FGF23 results in premature death associated with ectopic mineralization of soft tissues, including the kidney. Impairing 1,25-dihydroxyvitamin D action in these animals prevents the development of hypercalcemia and improves survival, suggesting that the premature death is a direct consequence of impaired mineral ion homeostasis rather than a specific developmental or maturational effect of FGF23.[249]

FGF23 impairs Na-dependent phosphate transport in both intestinal and renal brush border membrane vesicles.[250] It has been shown to decrease the levels of the types IIa, IIb, and IIc Na-dependent phosphate transporters, thereby regulating both intestinal and renal phosphate transport.[251–253] FGF23 decreases circulating levels of 1,25-dihydroxyvitamin D, both by decreasing mRNA levels for the renal 25(OH)D 1α-hydroxylase as well as by increasing expression of the 24-hydroxylase, the key enzyme involved in inactivation of 1,25-dihydroxyvitamin D.[190] FGF23 activates FGF receptor 1 in the presence of α-Klotho, a single-pass transmembrane protein, which acts as a coreceptor.[254] Klotho knockout mice exhibit the same hyperphosphatemia and high $1,25(OH)_2D_3$ levels seen in the FGF23 knockout mice,[191] demonstrating that it plays a critical role in mediating the actions of FGF23.

In chronic kidney disease, FGF23 levels correlate with increased left ventricular mass. While FGF23 has been shown to have direct effects on cardiomyocytes,[255] it also enhances distal renal tubule sodium absorption contributing to hypertension.[256] High FGF23 levels in the setting of hypophosphatemia, such as is seen in humans[257] and mice with X-linked hypophosphatemia and in mice with mutations of the DMP1 gene,[257] do not lead to cardiac hypertrophy, suggesting that cooperative interactions between phosphate and FGF23 may be required.

Regulation of FGF23

Circulating FGF23 levels are increased by dietary phosphorus, serum phosphorus, and 1,25-dihydroxyvitamin D.[66,258,259] Parathyroid hormone induces FGF23 mRNA levels, but also increases furin cleavage, thus limiting target organ effects.[260] Iron deficiency has also been shown to increase expression of FGF23 in bone via an erythropoietin/hypoxia-inducible factor (HIF)-1α–dependent mechanism.[261,262] Interestingly, studies in mice with chondrocyte-specific ablation of the vitamin D receptor have increased circulating FGF23, suggesting that chondrocytes express a vitamin D–regulated repressor of FGF23 production.[263] In addition, studies in mice with mutations in the *PHEX* or *DMP1* genes



• **Fig. 29.18** Homeostatic responses to variations in dietary calcium content. Major homeostatic responses to dietary calcium deprivation or loading are depicted. *Arrow thickness* indicates relative activity of transport or secretory mechanisms, whereas amounts of hormones or transported ions are related to the size of their notations. *Parentheses* indicate an inhibitory regulation. Note that the extracellular calcium concentration is well maintained, although different underlying mechanisms are involved in the two circumstances (see text for details). *1,25 D,* 1,25-dihydroxyvitamin D_3; *PTH,* parathyroid hormone.

demonstrate that these genes suppress FGF23 expression.[248,264] It has been suggested that mutations in both *PHEX* and *DMP1* alter the set-point for extracellular phosphate induction of FGF23.[265] Circulating FGF23 levels are dramatically increased in acute renal injury[266] and in response to lipopolysaccharide (LPS)–induced inflammation. In the latter case, the spleen is a major source of circulating FGF23, demonstrating nonosseous contributions to circulating hormone levels.[266]

In patients with chronic renal failure, an increase in FGF23 levels has been shown to antedate the development of secondary hyperparathyroidism, and thus evaluation of FGF23 levels may be beneficial in predicting which individuals will develop this disorder.[267] Treatment of dialysis patients with sevelamer hydrochloride and calcium carbonate decreases phosphate and FGF23 levels in parallel, implicating increases in serum phosphate or intestinal absorption as the pathophysiologic basis for the increased FGF23 levels in this population.[268] Based on the increase in serum levels of FGF23 seen with iron deficiency and erythropoietin, careful attention to the iron stores of CKD patients and limited use of erythropoietin may also serve to attenuate increases in serum FGF23. Thus FGF23 has emerged as an essential regulator of normal phosphate and $1,25(OH)_2D_3$ homeostasis. Phosphate and $1,25(OH)_2D_3$ increase FGF23 levels; this FGF23 then acts on the renal proximal tubule to suppress synthesis of $1,25(OH)_2D_3$ and to decrease the reabsorption of phosphate.

Calcium and Phosphate Homeostasis

The cytosolic concentrations of intracellular calcium, phosphorus, and magnesium differ markedly, as reviewed previously, and their physiologic roles within cells are diverse and largely unrelated (see Fig. 29.1). In contrast, the concentrations of these mineral ions in extracellular fluid are quite comparable (i.e., 1–2 mmol/L), and it is here that they exert important interactions, both with cells and with one another, that are critical for bone mineralization, neuromuscular function, and normal mineral ion homeostasis. Extracellular calcium and phosphate, in particular, exist so close to the limits of their mutual solubility that stringent regulation of their concentrations is required to avoid diffuse precipitation of calcium phosphate crystals in tissues.

Serum concentrations and total body balances of the mineral ions are maintained within narrow limits by powerful, interactive homeostatic mechanisms. PTH, $1,25(OH)_2D$, and FGF23 regulate mineral ion levels; mineral ion levels, in turn, regulate PTH, $1,25(OH)_2D$, and FGF23 secretion; and these hormones may regulate the production of one another. Calcium sensors in the parathyroid glands control PTH secretion by monitoring the blood concentration of ionized calcium, and those in the kidney act to adjust tubular calcium reabsorption, independently of PTH or $1,25(OH)_2D$. In contrast, the mechanisms of the phosphate sensing needed for normal homeostasis are not understood. The operation of these homeostatic mechanisms can be appreciated by considering the following examples of how the organism adapts to changes in calcium loads (Fig. 29.18).

Dietary calcium restriction, for example, is followed by an increase in the efficiency of intestinal calcium absorption. This increased efficiency results from a sequence of homeostatic responses in which lowered blood ionized calcium activates secretion of PTH, PTH augments synthesis of $1,25(OH)_2D_3$ by the proximal tubules of the kidney, and $1,25(OH)_2D_3$ then acts directly upon enterocytes to increase active transcellular transport of calcium. Enhanced intestinal calcium absorption is quantitatively the most important response to calcium deprivation, but a series of other homeostatic events also occur that limit the impact of this stress. Renal tubular calcium reabsorption is increased by PTH, an effect that is enhanced by increased $1,25(OH)_2D_3$-stimulated expression of calbindin-D28K in the distal tubules. Calcium reabsorption is also enhanced directly by any tendency to hypocalcemia, which is detected by CaSRs in Henle loop (and possibly also in the distal nephron) that control transepithelial calcium movements independent of PTH or $1,25(OH)_2D_3$.

The impact of dietary calcium deprivation is reduced by approximately 15% through release of calcium from bone in response to PTH and $1,25(OH)_2D_3$. The concomitant increase

in net bone resorption causes release of phosphate as well as calcium into the extracellular fluid. Intestinal phosphate absorption also is increased by $1,25(OH)_2D_3$. These phosphate loads are problematic, in that phosphate directly lowers ionized calcium in extracellular fluid, suppresses renal synthesis of $1,25(OH)_2D_3$, and directly inhibits bone resorption. These potentially negative effects of phosphate are obviated by the powerful phosphaturic action of PTH and of FGF23, the secretion of which is promoted by phosphate, calcium, and $1,25(OH)_2D$.

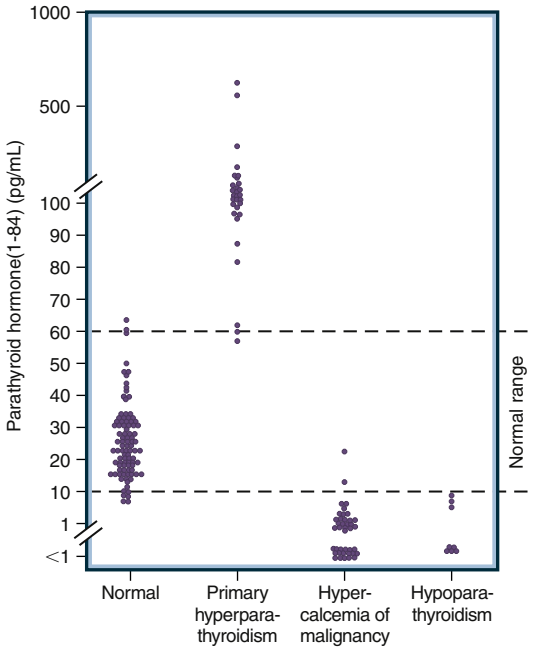
Finally, the possibility of unrestrained secretion of PTH, leading to excessive bone resorption and severe hypophosphatemia, is prevented by the effects of calcium on PTH secretion and by the direct suppressive effect of $1,25(OH)_2D_3$ on the synthesis of PTH and of PTH receptors. As a result of these homeostatic responses, calcium-deprived people maintain near-normal serum calcium and phosphate concentrations but display increased intestinal calcium absorption, increased bone resorption and progressive osteopenia, increased renal tubular calcium reabsorption, decreased renal tubular phosphate reabsorption, low urinary calcium excretion, elevated urinary phosphate excretion, and high serum concentrations of PTH and $1,25(OH)_2D_3$.

Calcium loads induce an opposite series of adaptations: parathyroid suppression, inhibition of renal $1,25(OH)_2D_3$ synthesis, decreased intestinal active transport of calcium, increased renal excretion of calcium and decreased renal excretion of phosphate (secondary to functional hypoparathyroidism), and a decrease in bone resorption sufficient to allow positive skeletal calcium balance. The decline in intestinal calcium absorption is the major safeguard against calcium overload, although this mechanism may be overridden with extraordinarily high intakes of calcium because of the persistence of the passive, non-vitamin D–dependent mode of calcium absorption. Moreover, nonenteral sources of calcium, such as intravenous calcium infusion or excessive net bone resorption (as from immobilization or malignancy), may readily overwhelm the limited homeostatic adaptations that remain once suppressed intestinal calcium absorption is bypassed. In such situations, the kidney rather than the intestine becomes the principal defense against hypercalcemia, and calcium homeostasis becomes critically dependent on adequate renal function. If renal function is impaired in these settings, as frequently occurs clinically, severe hypercalcemia and pathologic calcium deposition in extraskeletal sites may ensue.

Laboratory Assessment of Mineral Metabolism

Parathyroid Hormone

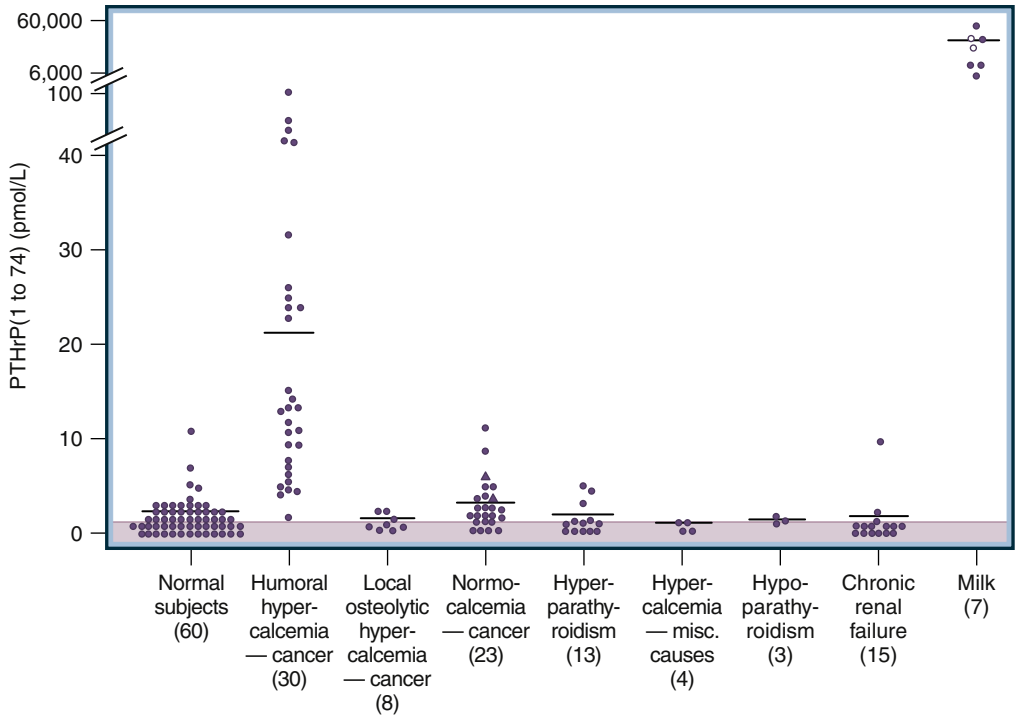
The major challenges in the measurement of blood PTH have been the low levels of circulating PTH and the presence of inactive PTH fragments in far greater abundance than for the intact, biologically active PTH molecule. The measurement of inactive fragments would not be a concern if the ratio of inactive to active PTH molecules remained constant. However, this ratio does change in response to changes in GFR and in parathyroid gland secretory activity (see earlier discussions under "Parathyroid Hormone Secretion" and "Metabolism of Parathyroid Hormone"). Consequently, radioimmunoassays of PTH have suffered from lack of sensitivity and from the inability to measure the biologically active hormone directly.



• **Fig. 29.19** Intact immunoreactive parathyroid hormone (PTH) determined using a two-site immunoradiometric assay in normal subjects and in three different patient groups. Note some overlap between normal people and patients with primary hyperparathyroidism, but no overlap between hypercalcemic patients with primary hyperparathyroidism and those with hypercalcemia of malignancy. (From Segre GV. Advances in techniques for measurement of parathyroid hormone: current applications in clinical medicine and directions for future research. *Trends Endocrinol Metab.* 1990;1:243–247.)

For these reasons, two-site assays that require the presence of amino-terminal and carboxy-terminal sequences of full-length PTH(1-84) on the same molecule have replaced older radioimmunoassays.[269] The assays are sensitive enough to detect PTH in all normal persons. The assays have demonstrated modest circadian variation in PTH levels and some pulsatility in PTH secretion, but these variations have not interfered with the diagnostic usefulness of randomly drawn PTH measurements. Some studies have reported modest increases of PTH levels with age, although others have not. Unlike older radioimmunoassays, the two-site assays demonstrate virtually no overlap in PTH levels between patients with primary hyperparathyroidism and those with nonparathyroid hypercalcemia (Fig. 29.19). Because this distinction represents the most important challenge in the clinical setting, the use of the two-site assay has dramatically facilitated the clinician's task.

This straightforward picture has been complicated by the realization that most two-site assays detect small amounts of PTH fragments that are large but do not extend to the hormone's amino-terminus.[270] These fragments accumulate in significant amounts in patients with renal failure. These observations have prompted the development of two-site PTH assays that use antibodies specific for the first four amino acids of PTH and thus do not detect large fragments of PTH. Although it seems plausible that such assays might prove particularly useful in some clinical situations, their role is presently unclear. They offer no advantage over older two-site assays, for example, in diagnosing primary hyperparathyroidism.[271] These assays do detect a minor peak of PTH immunoreactivity that is also recognized by antibodies directed to the carboxy-terminus of PTH and yet is not recognized by the usual



• **Fig. 29.20** Plasma PTHrP(1-74) determined by two-site immunoradiometric assay in selected patient groups and normal subjects. Also shown are concentrations of PTHrP in human milk *(filled circles)* and in bovine milk *(open circles)*. Two normocalcemic patients with cancer *(filled triangles)* subsequently became hypercalcemic. *Shaded area* denotes levels too low to detect with this assay. *PTHrP,* parathyroid hormone–related protein. (Adapted from Burtis WJ, Brady TG, Orloff JJ, et al. Immunochemical characterization of circulating parathyroid hormone–related protein in patients with humoral hypercalcemia of cancer. *N Engl J Med.* 1990;322:1106–1112.)

"intact" PTH assays, perhaps because of a post-translational modification of PTH in the PTH(15-20) region.[272] Perhaps because of detection of this unusual form of PTH, the PTH immunoreactivity recognized using the antibody to PTH(1-4) is particularly high in patients with parathyroid cancer when compared to patients with benign primary hyperparathyroidism.[273]

Parathyroid Hormone–Related Protein

The measurement of PTHrP in serum presents a series of challenges. The concentration of PTHrP in the bloodstream, even in some patients with PTHrP-mediated malignant hypercalcemia, is not high, and the molecular definition of circulating, biologically active fragments is incomplete. Despite these problems, several groups of investigators have developed assays for PTHrP that can be helpful in the evaluation of a subset of hypercalcemic patients. Radioimmunoassays for amino-terminal portions of PTHrP and two-site assays for amino-terminal and midregion PTHrP[124] separate healthy persons and patients with nonmalignant hypercalcemia from most patients with the humoral hypercalcemia of malignancy (Fig. 29.20). When measured with the most recently developed assays, PTHrP levels are elevated in almost all patients with malignant hypercalcemia without bone metastases and in most patients with hypercalcemia and bone metastases.

In occasional patients, the PTHrP assay has helped distinguish an occult malignancy from other causes of non–PTH-dependent hypercalcemia. Nevertheless, because the diagnosis of malignancy as the cause of hypercalcemia is usually clinically obvious, and the PTH assay can be used to diagnose primary hyperparathyroidism, the role of PTHrP assays in clinical practice is limited.[274]

Calcitonin

Several assays for measuring serum calcitonin are commercially available. The measurements are based on single or double antibody radioimmunoassays or enzyme immunoassays, several of which are sufficiently sensitive to detect calcitonin deficiency.[275] Interference with calcitonin assays has been reported in individuals with a heterophile antibody.[276] The calcitonin monomer is thought to be the biologically active molecule, therefore some investigators believe extraction of the multimeric forms prior to radioimmunoassay provides a more sensitive and specific measurement of serum calcitonin levels. However, the double antibody assays are thought to provide the same information with less sample manipulation. The only clinical use of the calcitonin assay is as a tumor marker, primarily in medullary carcinoma of the thyroid.

Vitamin D Metabolites

Several non-radioligand assays are available for determining the levels of vitamin D metabolites. These assays have shown considerable inter-assay variability, leading to misclassification of the vitamin D status of individuals.[277,278] Therefore mass spectrometry is being increasingly used for measuring 25(OH)D levels. Regardless of the method used, it has been recognized that a central repository for uniform standards is required for validation of these assays and of the laboratories performing them. The National Institute

of Standards and Technology in the United States has developed standard reference materials for this purpose.[279]

The levels of 25(OH)D correlate better with the clinical signs and symptoms of vitamin D deficiency than do the levels of $1,25(OH)_2D_3$. Because the 25-hydroxylation of vitamin D is not tightly regulated, measurements of 25(OH)D more accurately reflect body stores of vitamin D. Measurement of this metabolite should therefore be performed when vitamin D deficiency is suspected.

Measurements of $1,25(OH)_2D_3$ should be reserved for cases in which excessive or impaired 1α-hydroxylation is suspected. High $1,25(OH)_2D_3$ levels can be seen in sarcoidosis, lymphomas, Williams syndrome, and intoxication with 1α-hydroxylated metabolites (see "Parathyroid-Independent Hypercalcemia"). Impaired 1α-hydroxylation can contribute to the hypocalcemia of patients with renal dysfunction, oncogenic osteomalacia, and hereditary defects of vitamin D metabolism (see "Hypocalcemic Disorders").

Fibroblast Growth Factor-23

Currently two types of immunoassays are available for the measurement of serum FGF23 in humans. An assay using two polyclonal antibodies directed against C-terminal epitopes[280] detects most, if not all, circulating forms of FGF23 but does not discriminate between the intact active hormone and the cleaved fragment, which is not thought to have biologic activity. Assays for the intact hormone are classic sandwich assays with antibodies directed against both the N-terminus and C-terminus of the hormone.[235] These latter assays have been shown to be more useful for studying the effects of dietary phosphate on FGF23 levels in humans and thus are thought to provide a more precise determination of the biologically active levels of the hormone in the circulation.[281]

Hypercalcemic Disorders

Parathyroid-Dependent Hypercalcemia

It is useful to delineate two categories of hypercalcemia: (1) that associated with dysfunction of the parathyroid cell and (2) that in which hypercalcemia occurs despite appropriate parathyroid suppression. This distinction is particularly useful clinically because it emphasizes the centrality of the PTH assay in the diagnostic approach to the hypercalcemic patient. Abnormal parathyroid glands are associated with hypercalcemia in three settings: (1) primary hyperparathyroidism, (2) familial hypocalciuric hypercalcemia, and (3) lithium-induced hypercalcemia.

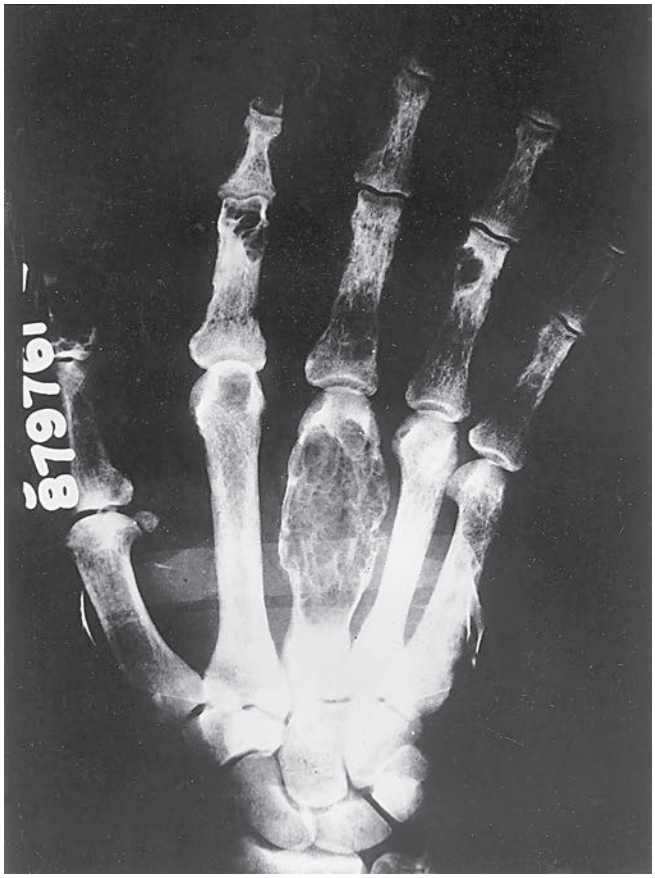
Primary Hyperparathyroidism

In primary hyperparathyroidism, a primary abnormality of parathyroid tissue leads to inappropriate secretion of PTH. In contrast, increased secretion of PTH that is an appropriate response to hypocalcemia is called secondary hyperparathyroidism. The inappropriately high serum concentration of PTH in primary hyperparathyroidism, in turn, sustains excessive renal calcium reabsorption, phosphaturia, and $1,25(OH)_2D$ synthesis, as well as increased bone resorption. These actions of PTH produce the characteristic biochemical phenotype of hypercalcemia and hypophosphatemia, loss of cortical bone, hypercalciuria, and the various clinical sequelae of chronic hypercalcemia. Primary hyperparathyroidism results most often (75–80%) from the occurrence of one or more adenomas in previously normal parathyroid glands, although in 20% of cases diffuse hyperplasia of all parathyroid glands may be present or, rarely, parathyroid carcinoma may be found (<1–2%).[282–285]

Classic Primary Hyperparathyroidism

The bone disease "osteitis fibrosa cystica" first was described by von Recklinghausen in 1891, but the etiologic link between this disease and parathyroid neoplasms was not established until 1925, when Mandl observed clinical improvement following removal of a parathyroid adenoma from a young male with severe bone disease. In early clinical descriptions of primary hyperparathyroidism, the disease emerged as a distinctly uncommon disorder with significant morbidity and mortality rates, in which nearly all affected patients manifested radiographically significant or symptomatic skeletal or renal involvement, or both.

The skeletal involvement in classic primary hyperparathyroidism reflects a striking and generalized increase in osteoclastic bone resorption, which is accompanied by fibrovascular marrow replacement and increased osteoblastic activity. The radiographic appearance (Fig. 29.21) features *generalized demineralization* of bone, with coarsening of the trabecular pattern (due to osteoclastic resorption of the smaller trabeculae); characteristic *subperiosteal resorption*, often most evident in the phalanges of the hands, which gives an irregular, serrated appearance to the outer, subperiosteal cortex and may progress to extensive cortical resorption; *bone cysts*, usually multiple, which contain a brownish serous or



• **Fig. 29.21** Radiograph of the hand of a patient with severe primary hyperparathyroidism. Note the dramatic remodeling associated with the intense region of high bone turnover in the third metacarpal in addition to widespread evidence of subperiosteal, endosteal, and trabecular resorption. (Courtesy Fuller Albright Collection, Massachusetts General Hospital, Boston, MA.)

mucoid fluid, and tend to occur in the central medullary portions of the shafts of the metacarpals, ribs, or pelvis and may expand into and disrupt the overlying cortex; *osteoclastomas*, or *brown tumors*, composed of numerous multinucleated osteoclasts ("giant cells") admixed with stromal cells and matrix, which are found most often in trabecular portions of the jaw, long bones, and ribs; and pathologic *fractures*.

The skull may exhibit a finely mottled, "salt-and-pepper" radiographic appearance, with loss of definition of the inner and outer cortices. Dental radiographs typically show erosion or disappearance of the lamina dura due to subperiosteal resorption, often with extension into the adjacent mandibular bone. The erosion and demineralization of cortical bone may lead to radiographic disappearance of some bones, most notably the tufts of the distal phalanges of the hands, the inferolateral cortex of the distal third of the clavicles, the distal ulna, the inferior margin of the femoral neck and pubis, and the medial aspect of the proximal tibia. The clinical correlates of these changes may include aching bone pain and tenderness, "bowing" of the shoulders, kyphosis and loss of height, and collapse of lateral ribs and pelvis with "pigeon breast" and triradiate deformities, respectively.

The renal manifestations of classic severe primary hyperparathyroidism include recurrent calcium nephrolithiasis, nephrocalcinosis, and renal functional abnormalities that range from impaired concentrating ability to end-stage renal failure. Associated signs and symptoms include recurrent flank pain, polyuria, and polydipsia. No unique features of the stone disease in primary hyperparathyroidism serve to distinguish it from that associated with other, more common causes of calcium kidney stones. The stone disease more often may be recurrent and severe, and in some patients the stones may be composed entirely of calcium phosphate instead of the pure oxalate or mixtures of oxalate and phosphate more commonly encountered in other disorders. In patients diagnosed before 1965, the frequency with which nephrolithiasis complicated primary hyperparathyroidism was as high as 60% to 80% (the frequency is currently <25%), yet in studies of unselected patients conducted throughout the past 50 years, primary hyperparathyroidism has accounted for fewer than 5% of all calcium kidney stones.

Other clinical features that have been reported in association with classic severe primary hyperparathyroidism are conjunctival calcifications, band keratopathy, hypertension (50%), gastrointestinal signs and symptoms (anorexia, nausea, vomiting, constipation, or abdominal pain), peptic ulcer disease, and acute or chronic pancreatitis. The issue of whether primary hyperparathyroidism increases the risk for peptic ulcer disease and pancreatitis remains controversial. Although hyperparathyroidism is associated with a higher risk of hypertension, successful parathyroidectomy has not been shown to correct the hypertension.

Signs and symptoms in primary hyperparathyroidism may result from the involvement of bone (fracture, bone pain) or kidneys (renal colic, renal failure), peptic ulcer disease, pancreatitis, or hypercalcemia per se (weakness, apathy, depression, polyuria, constipation, coma). The presence and severity of neuropsychiatric symptoms, in particular, correlate poorly with the serum calcium concentration, although few patients with severe hypercalcemia are entirely asymptomatic. Elderly persons are most likely to exhibit such symptoms. A peculiar neuromuscular syndrome, first described in 1949 but rarely encountered now, includes symmetric proximal weakness and gait disturbance, with muscle atrophy, characteristic electromyographic abnormalities, generalized hyperreflexia, and tongue fasciculations.[286]

Contemporary Primary Hyperparathyroidism

The clinical spectrum of primary hyperparathyroidism was changed dramatically in the early 1970s by the introduction of routine multichannel serum chemistry screening, which unearthed a large population of patients with previously unsuspected, asymptomatic disease. In Rochester, Minnesota, for example, the annual incidence of the disease increased abruptly from 0.15 to 1.12 per 1000 persons between the prescreening era (1965–1974) and 1975, the year after routine screening was introduced.[287] The peak incidence occurs in the sixth decade of life, and the disease rarely is encountered in patients below age 15. It is two to three times more common in women, who are slightly older at diagnosis than are men. Subsequently, the incidence of primary hyperparathyroidism has fallen; this decreased incidence may not simply be a residual effect of "sweeping the population," because it remained low when serially checked in Rochester—most recently from 1992 to 2001, when the incidence was 0.21 per 1000.[288]

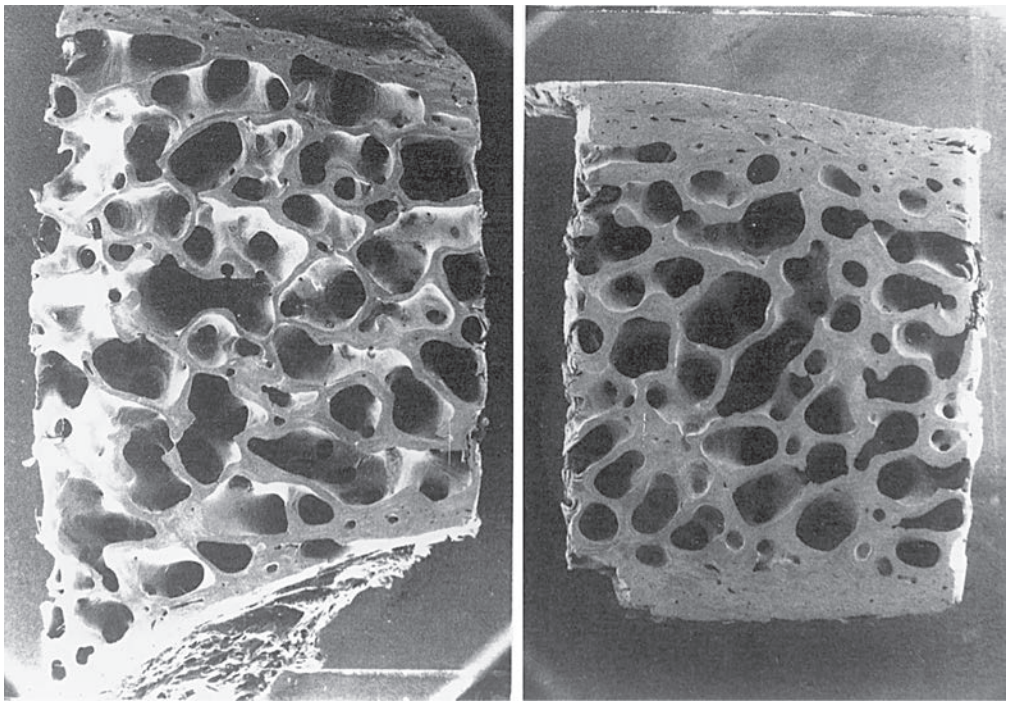
Ascertainment of mild or asymptomatic disease may decline even further in the future because of prevalent economic disincentives to routine serum chemistry screening in the primary care setting. On the other hand, insistence upon overt hypercalcemia as a diagnostic criterion may underestimate the true incidence of the disease. For example, when serum calcium and iPTH were measured in a large population of Swedish women undergoing routine mammographic screening, the prevalence of unsuspected primary hyperparathyroidism, defined by criteria that included the combination of high-normal serum calcium plus elevated or high-normal iPTH, was 2.1%.[289] Two-thirds of these women (72/109) were normocalcemic (10–10.4 mg/dL), yet bone density was reduced in the group as a whole, and the disease was confirmed histologically in 98% of the 61 who had surgery. Further, the widespread practice of evaluating PTH levels in patients with osteoporosis has led to the identification of patients with high PTH levels and normocalcemia.[290] Many of these patients become hypercalcemic during follow-up.

Not surprisingly, given that primary hyperparathyroidism now usually is diagnosed incidentally, few patients are found to have overt signs or symptoms of the classic disease and thus are considered to be asymptomatic. For example, only 2% of patients with primary hyperparathyroidism residing in Olmsted County, Minnesota, and only 17% of 121 patients studied at an academic referral center in New York City had classic disease symptoms.[287,291] In most of them, the relevant symptom was urolithiasis. Many clinicians argue, however, that most patients regarded as having asymptomatic primary hyperparathyroidism and only minimally elevated serum calcium actually suffer from various neuropsychiatric or other symptoms that may improve following curative surgery.[292] These symptoms, however, which include fatigability, weakness, forgetfulness, depression, somatization, polydipsia, polyuria, and bone and joint pain, are common in otherwise normal persons. In the small randomized studies of surgery for primary hyperparathyroidism (see later), the effects of surgery on measures of quality of life have been conflicting.[290,293–295] This remains a critical issue, as the advent of less invasive operative approaches and concerns regarding fracture, cancer, and mortality risk have lowered the threshold for consideration of surgery in many patients with the disease (see later). Throughout this chapter, *asymptomatic primary hyperparathyroidism* refers to patients who lack signs or symptoms of the classic disease, whether or not they experience any of the subtle symptoms mentioned earlier.

The natural history of untreated asymptomatic primary hyperparathyroidism, as currently detected, remains incompletely

understood. Few patients seem to experience progression of disease, as measured by extreme elevations of serum or urinary calcium, appearance of renal dysfunction or nephrocalcinosis, or worsening osteopenia, over many years of observation.[291] On the other hand, late cortical bone loss observed at the femoral neck and distal radius in a small number of patients followed without surgery for 15 years points to the potential importance of continued monitoring in such patients.[296] Also, an excess risk of mortality, mainly from cardiovascular disease, has been noted during extended follow-up of large cohorts of patients with chronic hypercalcemia (and presumed primary hyperparathyroidism) identified by population health screening in Sweden,[297] and similar observations have been made during extended follow-up of postsurgical patients with hyperparathyroidism.[292] Associations of hypertension, hyperuricemia, and glucose intolerance with primary hyperparathyroidism have been implicated, together with hypercalcemia per se, as contributors to this elevated risk.[291] Abnormal cardiac calcification and left ventricular hypertrophy (reversible by successful parathyroidectomy) have been reported in primary hyperparathyroidism as well.[298] Increased cardiovascular mortality risk may be a feature only of severe hyperparathyroidism, as it was restricted to those in the highest quartile of serum calcium in the Olmsted County study, which otherwise showed an overall decreased risk of death.[299] A 40% excess risk of malignancy also was reported among 4163 Swedish patients who had undergone surgery more than a year earlier for (presumably symptomatic) primary hyperparathyroidism.[300] It has been argued that these increased risks for mortality and malignancy, even if confirmed, may apply only to those with primary hyperparathyroidism that is more severe than the asymptomatic version typically encountered today.[291]

Abnormalities of bone in modern, mild primary hyperparathyroidism are far subtler than those associated with the classic disease. Histologically the rate at which new bone remodeling cycles are activated is increased. Because the phase of restorative bone formation at each remodeling site takes much more time than does the initial resorptive phase, such an increase in remodeling rate inevitably increases the ambient volume of the remodeling space and thus the porosity of bone. Depending upon the rate and extent of the accompanying increase in osteoblastic activity and the resulting local balance between net bone formation and resorption, mineralized bone volume may decrease further, remain stable, or even increase (despite an increased remodeling space). For reasons not yet understood, the balance achieved between increased resorption and formation of bone in primary hyperparathyroidism depends not only upon the severity of the hyperparathyroidism but also upon skeletal location. Thus net resorption of endosteal bone may predominate in cortical sites, whereas net apposition of mineral may occur in trabecular bone, when measured through biopsy of the iliac crest[301,302] (Fig. 29.22). Thus bone mineral density may be reduced, particularly at sites of predominantly cortical bone such as the midradius, by as much as 10% to 20%.[303] In contrast, studies using dual-energy x-ray absorptiometry (DXA) demonstrate relative preservation of vertebral bone mass in primary hyperparathyroidism.[304] Despite this evidence of preserved trabecular bone mass in primary hyperparathyroidism, the incidence of vertebral fracture is increased.[305,306] The explanation for this paradox appears to relate to abnormalities in trabecular (and cortical) microarchitecture that can be detected using high-resolution peripheral quantitative computed tomography (CT) or by evaluating the trabecular bone score during DXA measurements.[307,308] These abnormalities reflect shifts from



• **Fig. 29.22** Iliac crest biopsy specimens from a patient with primary hyperparathyroidism *(left)* and a normal control subject *(right)*, viewed by scanning electron microscopy. Note the thin cortices and contrasting maintenance of trabecular bone in the patient. (From Parisien M, Silverberg SJ, Shane E, et al. The histomorphometry of bone in primary hyperparathyroidism: preservation of cancellous bone structure. *J Clin Endocrinol Metab.* 1990;70:930–938.)

plate-like to rod-like microstructure that weaken the bone and presumably account for the elevated risk of vertebral fracture. These defects are improved following successful parathyroidectomy.[309]

Kidney stones now are reported in only 10% to 25% of patients with primary hyperparathyroidism, although some degree of renal dysfunction, either a significant reduction in creatinine clearance or impaired concentrating or acidifying ability, may be found in up to one-third of those with asymptomatic disease. These renal abnormalities are not progressive in the majority of affected patients.[291,310] No parameters of disease severity predict a lower estimated GFR.[311] The association of kidney stones with primary hyperparathyroidism generally is viewed as an indication for parathyroidectomy, however, because successful surgery usually prevents further symptomatic stone disease.[291,292] On the other hand, it is not possible at present to confidently predict, from biochemical measurements in blood or urine, which asymptomatic patients with hyperparathyroidism will go on to develop new stone disease. Stone-formers are more likely to be hypercalciuric than not, but less than one-third of hypercalciuric patients with hyperparathyroidism actually develop stones.

Etiology and Pathogenesis

Parathyroid adenomas are caused by mutations in the DNA of parathyroid cells; these mutations confer a proliferative or survival advantage for affected cells over their normal neighbors.[312,313] As a consequence of this advantage, the descendants of one particular parathyroid cell, a clone of cells, undergo clonal expansion to produce an adenoma.

Multiple chromosomal regions are missing in the parathyroid cells of individual parathyroid adenomas. These genetic deletions probably reflect the deletion of tumor suppressor genes. These chromosomal loci include portions of chromosome 1p–pter (in 40% of adenomas), 6q (in 32% of adenomas), 15q (in 30% of adenomas), and 11q (in 25–30% of adenomas). Many of the 11q deletions are associated, in the undeleted chromosome 11, with mutations in the gene encoding the transcription factor menin, the gene mutated in multiple endocrine neoplasia type 1 (MEN1). Thus this gene is also involved commonly in somatic mutations in patients with sporadic parathyroid adenomas. Somatic mutations have also been found in the mitochondrial genomes of a fraction of chief cell adenomas and have been found even more frequently in so-called oxyphil adenomas, known to exhibit mitochondria with abnormal morphologic appearance.[314] The widespread presence of somatic mutations in sporadic parathyroid adenomas, which are detectable only because large numbers of cells in any one tumor contain the same deletion, constitutes the strongest evidence that parathyroid adenomas are clonal expansions of mutant cells.

Cyclin D1, called initially PRAD1, was the first noted recurrent parathyroid proto-oncogene.[315] This gene was discovered at the breakpoint of an inversion on chromosome 11 in a parathyroid adenoma. This inversion led to the juxtaposition of the PTH gene's regulatory region and the DNA encoding cyclin D1. As a consequence, the cyclin D1 gene was overexpressed. Cyclin D1 is an important regulator of the transition from the G_1 phase of the cell cycle (which follows mitosis) to the S phase (associated with DNA synthesis) and is mutated or amplified in a wide variety of malignancies. Cyclin D1 is overexpressed in about 20% of parathyroid adenomas, though cyclin D1 gene rearrangements have been documented in only 5% of adenomas. Overexpression of cyclin D1 in the parathyroids of transgenic mice leads to formation of parathyroid adenomas and hypercalcemia over many months.[316] The phenotype of these mice demonstrates that cyclin D1 overexpression can cause primary hyperparathyroidism. Cyclin D1 enables the functions of cyclin-dependent protein kinases. More recently, inhibitors of cyclin-dependent protein kinases (encoding the proteins p21, p15, and p18) have been found mutated in the germline and somatically in sporadic parathyroid adenomas, in a pattern that suggests these genes act as tumor suppressors.[317] The gene mutated in multiple endocrine neoplasia, type 1 (encoding menin), is also mutated only in the tumors of a substantial fraction of sporadic parathyroid tumors (reviewed in Costa-Guda and Arnold[318]).

Improvements in DNA sequencing technology have allowed the sequencing of virtually all the exons in the human genome, and this sort of whole-exome sequencing has been performed by three groups.[319–321] The most striking finding of these studies (sequencing 43 tumors between them) was that these tumors averaged roughly 5 exome mutations per tumor (with the exception of one tumor with 110 mutations in association with a mutation in the protection of telomeres 1 gene *[POT1]* known to increase genetic instability). This number is considerably lower than the number of mutations typically found in cancers or even benign tumors that have been sequenced. In both studies 35% of the tumors harbored mutations in the *MEN1* gene, usually in association with deletion of the second *MEN1* gene. No other gene was mutated frequently in these series, though mutation in a known oncogene, an activating mutation in *EZH2,* was found a second time when a larger series of tumors was tested for that mutation.[319] A recent whole-exome sequencing study of 24 parathyroid cancers demonstrated that these tumors harbor a much higher frequency of mutations than do parathyroid adenomas; the genes mutated in parathyroid cancer, so far, appear distinct from the genes mutated in parathyroid adenomas.[322]

As expected for a disease caused by mutations in DNA, parathyroid adenomas occur more frequently in patients who underwent neck irradiation decades earlier, with greater radiation exposure leading to higher risk. Most patients have no definite history of exposure to specific mutagens, however. An intriguing clue that abnormalities of vitamin D physiology may predispose to primary hyperparathyroidism has come from the observation that patients with parathyroid adenomas are more likely than others to inherit a particular allele of the VDR gene.[323] These patients have tumors with particularly low levels of mRNA encoding the VDR. Nevertheless, no mutations in the coding regions of the gene encoding the VDR have been found in parathyroid adenomas.[324]

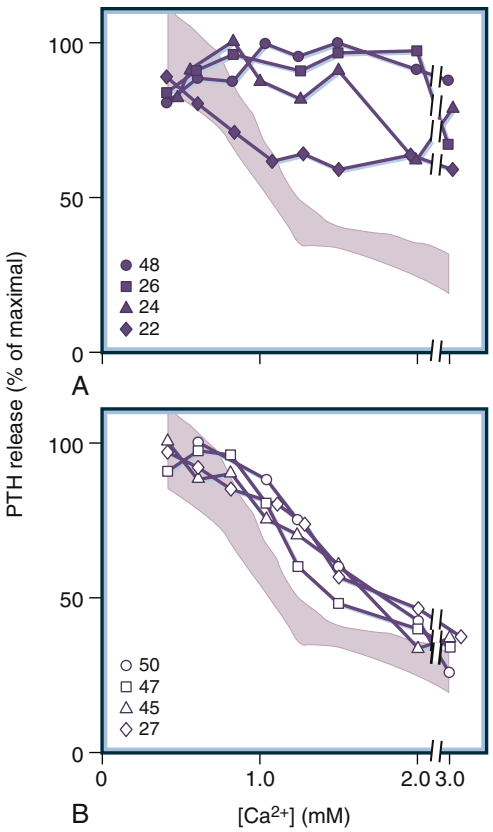
The cause of sporadic primary parathyroid hyperplasia is unknown. The known stimulus for parathyroid cell proliferation—low levels of blood calcium or $1,25(OH)_2D_3$—is not present in this disease. Presumably, some other stimulus outside the parathyroid glands or a genetic abnormality present in all four parathyroid glands leads to inappropriate cell proliferation. Such abnormalities have been found in several inherited forms of parathyroid hyperplasia (see later), but most cases of parathyroid hyperplasia are not found in familial clusters.

The theoretic distinction between adenoma as a clonal proliferation and hyperplasia as a polyclonal growth is clear cut. In some settings, however, clonal expansion can occur in the context of preexisting nonclonal proliferation. The clearest example of this complication has been found in the large glands associated with severe renal failure. In many such glands removed surgically because of hypercalcemia or severe parathyroid-dependent bone disease, evidence for clonal proliferation complicating secondary hyperplasia has been found. Interestingly, the pattern of chromosomal abnormalities in these clonal tumors differs from that

found in parathyroid adenomas in the absence of renal failure.[325] Analogous mechanisms may be operative in a number of settings associated with stimuli to parathyroid cell proliferation, such as X-linked hypophosphatemia and long-term lithium therapy. Furthermore, just as clonal tumors can arise in the setting of *secondary* parathyroid hyperplasia, they can also arise in the setting of sporadic *primary* parathyroid hyperplasia[326] and in MEN1.[327]

The distinction between adenoma and hyperplasia is clinically important, because removal of the one abnormal gland can be expected to cure a parathyroid adenoma, whereas removal of multiple glands is required to successfully treat parathyroid hyperplasia. Unfortunately, differentiating adenoma from hyperplasia from normal parathyroid tissue at pathologic examination is not straightforward. Pathologists distinguish normal from abnormal parathyroid glands by the increase in size and the paucity of fat in abnormal glands. Attempts have been made to distinguish an adenoma from an individual hyperplastic gland on the basis of morphologic features, but no criteria have proved completely reliable.[328] The formation of clonal neoplasms in originally hyperplastic tumors may explain some of the difficulty in pathologic diagnosis.

An increase in cell number is not the only abnormality in primary hyperparathyroidism. The ability of the normal parathyroid cell to suppress PTH secretion in response to hypercalcemia might be expected to protect the individual from sustained hypercalcemia, even if the number of parathyroid cells increased moderately. Unfortunately, parathyroid cells in parathyroid adenomas usually demonstrate abnormalities in their responsiveness to calcium, with a shift in set-point to the right (Fig. 29.23). This set-point shift, combined with the nonsuppressible component of PTH secretion, leads to a new steady state in which both the PTH level and the blood calcium level are higher than normal. The molecular underpinning of the abnormal parathyroid cell responsiveness is beginning to be understood. Parathyroid cells from adenomas respond to changes in extracellular calcium with smaller than normal increases in intracellular calcium, and the amount of CaSR protein on the cell surface is reduced.[329] Perhaps surprisingly, no mutations in the gene encoding the CaSR have been found in parathyroid adenomas. In the experimental model in which overexpression of cyclin D1 results in primary hyperparathyroidism,[316] reduced expression of the CaSR occurs only after cell proliferation has been increased for some time. Thus the decreased expression of the CaSR in parathyroid adenomas is likely to be a secondary response that occurs during tumor formation. One demonstrated regulator of expression of the *CASR* gene in parathyroid cells is the developmental regulator, *gcm2*.[330]



• **Fig. 29.23** Abnormal patterns of parathyroid hormone (PTH) secretion from cells prepared from adenomatous glands and stimulated with varying levels of calcium in tissue culture. The *shaded area* shows the pattern of PTH release (±1 SD) from normal human parathyroid cells. Panel (A) illustrates the pattern from four patients with little suppression of PTH secretion by calcium. Panel (B) illustrates the pattern from four patients with relatively intact mechanism of suppression of PTH secretion by calcium. Even in this group the set-point for calcium suppression is shifted to the right. (From Brown, EM. Calcium-regulated parathyroid hormone release in primary hyperparathyroidism: studies in vitro with dispersed parathyroid cells. *Am J Med*. 1979;66:923–931.)

Inherited Primary Hyperparathyroidism

Although uncommon, inherited forms of primary hyperparathyroidism are clinically important for several reasons. The management of the parathyroid tumors found in familial parathyroid syndromes often differs from that of sporadic primary hyperparathyroidism. Furthermore, extraparathyroidal manifestations of inherited syndromes may need treatment, and awareness of familial clustering should prompt systematic family screening.

Multiple Endocrine Neoplasia Type 1 (see also Chapter 42). MEN1 is caused by inactivating mutations in the tumor suppressor gene encoding menin.[331] Menin is a ubiquitously expressed transcription factor that is part of a complex that targets histone H_3 for methylation,[332] and thereby leads to expression of cell cycle inhibitors in pancreatic islets and other tissues.[333] Menin interacts with many nuclear proteins, and it is not known which interactions are central to the pathogenesis of MEN1.[334] Rarely, mutations in genes encoding cyclin-dependent kinase inhibitors, such as p27, are found in MEN1 patients without menin mutations[335]; some have termed this variant, which presents primarily with parathyroid and pituitary tumors, *MEN4*.[336] These patients can also harbor pancreatic neuroendocrine tumors, gonadal, adrenal, renal, and thyroid tumors.[337,338] Although MEN1 includes tumors of the parathyroid, anterior pituitary, and pancreatic islets, the parathyroid tumors are far more prevalent than the others; 95% of affected patients eventually develop hyperparathyroidism. Most of the parathyroid tumors harbor mutations in both copies of the menin gene; one mutation is inherited and the second occurs in the parathyroid cell whose progeny form the tumor.

The onset of hypercalcemia occurs in the second and third decades of life, though occasional patients present in the first decade. Hypercalcemia never presents at birth or in infancy. The disease involves all four parathyroid glands, although the involvement can be asymmetric and apparently asynchronous. Apart from the earlier age at diagnosis, the presenting clinical picture generally resembles that of sporadic primary hyperparathyroidism, perhaps with somewhat greater loss of bone density.[339] One common complicating feature is that hypercalcemia can

dramatically increase the gastrin levels and symptomatology of patients who also have gastrinomas. Treatment of the parathyroid disease in this setting can greatly simplify the management of the gastric hyperacidity. After parathyroid surgery, hypoparathyroidism and recurrent hyperparathyroidism are more common than in other forms of hyperparathyroidism.[340] The timing and type of surgery are therefore more complicated issues than in sporadic primary hyperparathyroidism. Most authorities agree that parathyroid disease recurs eventually, particularly if fewer than three glands are removed. Some surgeons prefer subtotal parathyroidectomy, whereas others prefer total parathyroidectomy with forearm implantation of a small amount of parathyroid tissue.

Multiple Endocrine Neoplasia Type 2a (see also Chapter 42). Parathyroid disease is a usually late and infrequent (5–20%) occurrence in MEN2a, a disease defined by the clustering of medullary carcinoma of the thyroid, pheochromocytoma, and hyperparathyroidism. The parathyroid disease is typically mild and can be asymptomatic.[338] In some families, hyperparathyroidism is more common; however, these families have the same mutations in the *RET* gene as are found in families without frequent hyperparathyroidism. Both parathyroid hyperplasia and adenoma have been noted at surgery. Because asymptomatic parathyroid hyperplasia has been noted at the time of thyroid surgery, a progression from hyperplasia to adenoma in MEN2a has been suggested. The approach to diagnosis and treatment of hyperparathyroidism is similar to that in sporadic primary hyperparathyroidism, but hyperplasia is more frequently the underlying disorder. The pathogenesis of the hyperparathyroidism is uncertain, but the *RET* gene, mutated in virtually all cases of MEN2a, is expressed in parathyroid cells,[341] so abnormal *RET* expression in parathyroid cells may directly cause parathyroid tumor formation. Hyperparathyroidism does not occur in MEN2b, the variant associated with mucosal neuromas.

Hyperparathyroidism–Jaw Tumor Syndrome. Patients with hereditary hyperparathyroidism–jaw tumor syndrome[342] present with parathyroid adenomas that can be multiple and that are usually cystic. These tumors are often but not invariably associated with fibrous jaw tumors that are unrelated to the hyperparathyroidism. Importantly, the parathyroid tumors are frequently malignant, in contrast to the findings in MEN1 and MEN2. Wilms tumor and polycystic renal disease also have occurred in affected families. The gene mutated in this syndrome, called *CDC73* or *HRPT2*, encodes the nuclear protein, parafibromin.[343] Parafibromin is part of the evolutionarily highly conserved PAF complex that binds RNA polymerase II, regulates chromatin structure, and regulates gene expression.[344–346] Parafibromin binds β-catenin and can mediate Wnt pathway signaling and notch signaling, though whether these properties of parafibromin are related to its tumor suppressor function is unknown.[347] Inactivating mutations in parafibromin are found in a high number of patients with apparently sporadic parathyroid cancer.[348] In a whole-exome sequencing study of sporadic parathyroid cancer, 8 of 17 patients harbored mutations in *CDC73*; half of the patients with *CDC73* mutations also manifest germline mutations, even though they presented as patients with apparently sporadic disease.[322] These findings suggest that all patients with parathyroid cancer should be screened for germline mutations in *CDC73*.

Management of Primary Hyperparathyroidism

The strategy for management of primary hyperparathyroidism has evolved in parallel with the changing presentation of the disease. The only opportunity for permanent cure is surgical removal of the abnormal gland(s), an approach that clearly was appropriate for virtually all patients in whom the classic, severe form of the disease was diagnosed many decades ago and which still is the treatment of choice for those patients who do present with recurrent kidney stones, nephrocalcinosis, clinically overt bone disease, or severe hypercalcemia.

In contrast, the choice of surgical versus medical management for patients with asymptomatic primary hyperparathyroidism remains an open and hotly debated question. Those who favor surgery point to the expected improvement in bone mineral density (at the hip and spine) and left ventricular hypertrophy following successful surgical intervention; evidence of increased risk for fracture, cardiovascular mortality, malignancy, and neuropsychiatric symptoms associated with primary hyperparathyroidism; and the recent successful development of effective minimally invasive surgical procedures (see later). Those who favor an observational approach emphasize the evidence for lack of disease progression in most asymptomatic patients; the small but finite risk of surgical failure and postoperative complications; the probability that excess mortality and cancer risks documented in patients with relatively severe disease may not apply to those with mild, asymptomatic primary hyperparathyroidism; the difficulty in assigning vague neuropsychiatric symptoms to the parathyroid disorder; the lack of evidence (or negative evidence) that hypertension and increased risk of cancer, fracture, or cardiovascular mortality, even if present, are improved by successful parathyroidectomy; and the availability of sensitive techniques for monitoring disease status in nonoperated patients.[291]

Unfortunately no large prospective studies powered to compare clinical outcomes in patients with asymptomatic primary hyperparathyroidism randomly assigned to surgery versus medical management have been conducted. Nevertheless, three valuable smaller, randomized controlled trials of surgery versus observation have been conducted that allow some conclusions about surrogate markers of disease.[293–295] All three trials demonstrated increases in bone density in the spine and hip in the surgical group; these increases were similar to those produced with bisphosphonate therapy in primary hyperparathyroidism (see later). Two of the three studies showed modest improvements in some quality-of-life measures, though the unblinded nature of the studies limits interpretation of these findings. All of the findings reported so far from these studies have been after 2 years or less. As useful as these studies have been, their limitations have forced the field to tap observational studies to draw tentative recommendations based on limited data.

Such provisional recommendations have emanated from a series of conferences: an NIH-sponsored Consensus Conference held in 1990, followed by more informal updates in 2002, 2008, and 2014.[349] The major conclusion of that group was that, although surgery is indicated for symptomatic hyperparathyroidism and always should be considered an appropriate option in asymptomatic hyperparathyroidism, many patients with asymptomatic disease can be safely monitored without surgery for many years. Those suitable for medical observation should have no evidence of significant compromise of skeletal integrity or renal function, should have no history of urolithiasis or gastrointestinal or neuropsychiatric symptoms, and should meet the criteria listed in Table 29.1.[350] Such patients account for at least 50% of those who currently present with primary hyperparathyroidism.

On the other hand, surgery could be preferable if the patient desired surgery even when asymptomatic, if the probability of consistent monitoring seemed low, if concomitant illness seemed

TABLE 29.1 Indications for Surgery in Primary Hyperparathyroidism

- Overt clinical manifestations of disease
  - Kidney stones or nephrocalcinosis
  - Fractures or classic radiographic findings of osteitis fibrosa
  - Classic neuromuscular disease
  - Symptomatic or life-threatening hypercalcemia
- Serum calcium >1 mg/dL above upper limit of normal
- Creatinine clearance <60 mL/min, presence of stone(s) by radiograph, CT, or ultrasound
- Urinary calcium >400 mg/day plus other urinary biochemical indices of stone risk
- Bone mineral density low (T score ≤–2.5) at any site[a]
- Presence of vertebral fracture by radiograph or by vertebral fracture analysis on DXA
- History of fragility fracture
- Young age (<50 years)
- Uncertain prospects for adequate medical monitoring

[a]Z-score ≤–2.5 in premenopausal women and in men <50 years old.

CT, Computed tomography; *DXA,* dual-energy x-ray absorptiometry.

Modified from Bilezikian JP, Khan AA, Potts Jr JT, Third International Workshop on the Management of Asymptomatic Primary Hyperthyroidism. Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the Third International Workshop. *J Clin Endocrinol Metab.* 2009;94:335–339. Based upon recommendations of the 2014 NIH-sponsored Workshop on the Management of Asymptomatic Primary Hyperparathyroidism.

likely to complicate management or obscure significant disease progression, or if the patient was relatively young (<50 years). The latter recommendation reflects the absence of reliable information about the natural history of the disease over many decades of follow-up; the cumulative cost of medical monitoring, which begins to exceed that of surgery by 5 to 10 years; and some data suggesting that young people are more likely than others to have progressive disease.[351] On the other hand, age alone was not viewed as a contraindication to parathyroidectomy, as the procedure has been accomplished with excellent results, with a perioperative mortality rate of 1% to 3%, in large numbers of appropriately selected patients over the age of 75 years. Because hypertension is not thought to be a feature of mild primary hyperparathyroidism, and because hypertension generally is not improved by parathyroidectomy, hypertension was not viewed as an indication for surgery.

Although the Consensus Conference recommendations and subsequent modifications provide a useful framework for decision making, supporting data from large clinical trials are lacking. In a series of 52 asymptomatic patients selected for nonoperative management mainly on the basis of the 1990 Consensus Conference criteria and whose course was followed for 10 years, approximately 25% developed one or more new indications for surgery.[291] Patients who do not meet the Consensus Conference criteria for surgery may nevertheless experience the same postsurgical increase in bone density as those who do.[352] Some have emphasized that evidence of baseline vertebral osteopenia, an unusual finding in primary hyperparathyroidism, should be considered among the criteria for surgery[353] and that surgery also should be considered for postmenopausal women who exhibit vertebral bone loss in the setting of primary hyperparathyroidism.[291]

A common dilemma is the inability to ascertain whether vague but troublesome symptoms such as fatigue, lethargy, weakness (without objective muscle weakness), and depression are due to hyperparathyroidism and thus qualify as "significant" in the context of considering the decision for surgery. Most clinicians do not routinely recommend parathyroidectomy on the basis of such symptoms alone, although dramatic responses to surgery are occasionally seen. With the availability of improved, minimally invasive surgical approaches, the threshold for considering surgery in patients who are significantly disabled by such symptoms clearly is lower now than in the past. Some have advocated, in selected cases, a limited trial of medical therapy to reduce serum calcium (i.e., calcimimetics—see later) and thereby attempt to predict the symptomatic response to surgical cure.

Medical Monitoring of Primary Hyperparathyroidism. The updated NIH Consensus Conference recommendations suggest that patients not treated surgically should be followed carefully, with annual measurement of serum calcium and calculated creatinine clearance and serial determination of bone mineral density at intervals of 1 to 2 years. The most appropriate bone densitometric site is considered to be one that reflects mainly changes in cortical bone (i.e., distal forearm), although the importance of following vertebral bone density as well has been emphasized,[291] and current criteria acknowledge the importance of significant bone loss at any site.[350]

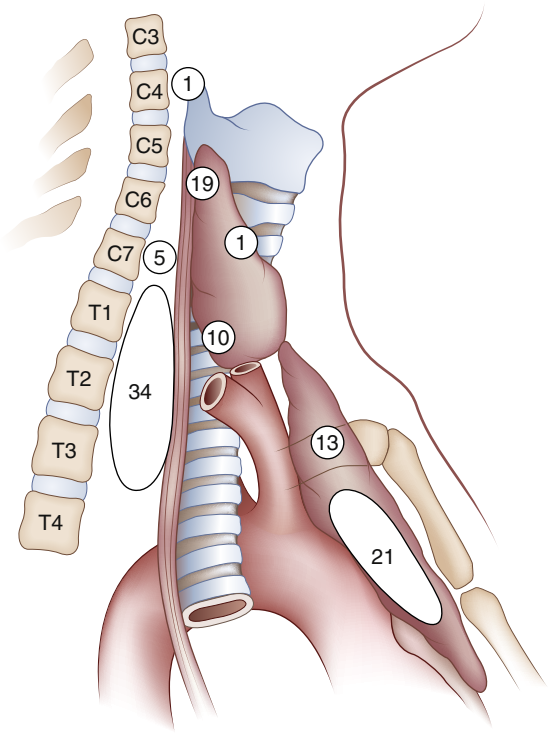
Patients undergoing nonoperative medical management must be cautioned to maintain adequate hydration, to avoid diuretics and prolonged immobilization, and to seek prompt medical attention in the event of illnesses accompanied by significant vomiting or diarrhea. Dietary calcium should not be restricted.

The goal of an effective pharmacologic therapy for primary hyperparathyroidism remains elusive, though studies of sex hormones and selective estrogen receptor modulators, bisphosphonates, and calcimimetics continue. Estrogens and progestins may reduce serum calcium and phosphorus, urinary calcium and hydroxyproline, and histologic evidence of active bone resorption in women with primary hyperparathyroidism, although safety concerns have limited these therapeutic options in postmenopausal women.

Intravenous bisphosphonates have been used successfully in the urgent therapy of hypercalcemia due to primary hyperparathyroidism, and several trials have shown that treatment with oral alendronate for 1 year or more improves bone density at the spine and hip, with only transient effects on serum calcium and PTH.[354,355] The calcimimetics represent a new class of agents that, by sensitizing the CaSR to extracellular calcium, can reduce PTH secretion. Cinacalcet, the first calcimimetic approved for control of secondary hyperparathyroidism in renal disease, was shown to lower serum calcium and PTH in primary hyperparathyroidism (and in some patients with parathyroid carcinoma), although improvement on bone density has not been documented in this population.[356]

Thus in patients for whom surgery for asymptomatic primary hyperparathyroidism is not an option, therapy with oral bisphosphonates can improve bone density without worsening other features of the disease, at least over 2 years of follow-up, and cinacalcet can control blood calcium. Whether these agents or any other medical therapy offers a beneficial long-term alternative to surgery is unknown.

Surgical Treatment of Primary Hyperparathyroidism. Parathyroidectomy is a safe and highly effective approach to definitive treatment of primary hyperparathyroidism. The most serious potential complications of parathyroid surgery—vocal cord paralysis and permanent hypoparathyroidism—occur after fewer than 1% and 4%, respectively, of procedures performed by highly skilled surgeons, although these rates can be much higher in less experienced



• **Fig. 29.24** Sites of ectopic location of 104 parathyroid glands found at reoperation for primary hyperparathyroidism. (From Wang C-A. A clinical and pathological study of 112 cases. *Ann Surg*. 1977;186:140–145.)

hands. Such complications occur most often in patients who require subtotal parathyroid resections for hyperplasia or resection of carcinoma. The surgical cure rate for primary hyperparathyroidism in the best hands is at least 95%.[357,358] Apart from operator inexperience, the usual cause of initial surgical failure ("persistent disease") is the presence of either unrecognized (often very asymmetric) parathyroid hyperplasia or ectopic parathyroid tissue (i.e., intrathyroidal, undescended, retroesophageal, or mediastinal glands)[359] (Fig. 29.24). Up to one in five parathyroid glands may be located ectopically, and this is especially true of supernumerary glands. Recurrent disease, defined as that occurring after an interval of at least 6 to 12 months of normocalcemia, varies in incidence from 2% to 16%. Recurrent hyperparathyroidism usually arises in unresected hyperplastic glands, but rarely it may be due to parathyroid carcinoma, to a second adenoma, or to a multicentric or miliary "parathyromatosis" engendered by inadvertent local seeding of parathyroid tissue (usually hyperplastic) into the neck during previous parathyroid surgery.[282,360]

In the past, there was broad agreement that the best approach is a bilateral neck exploration in which all four parathyroids are identified and all enlarged glands removed. With this procedure, preoperative parathyroid localization studies prior to initial cervical exploration are superfluous, as the positive predictive value of even the best technique (^{99m}Tc-sestamibi scanning) falls well short of the success rate of experienced surgeons unaided by prior imaging.[282,361]

With the advent of preoperative ^{99m}Tc-sestamibi scanning, which can accurately localize 80% to 90% of the single adenomas that account for 75% to 85% of cases, there has been renewed interest in performance of directed unilateral explorations, which reduce operative and recovery-room time, minimize the number of frozen sections required, are associated with significantly fewer postoperative complications, and can more readily be performed using minimally invasive techniques (including local anesthesia and intravenous sedation) that enable same-day discharge.[362] Sestamibi scanning also can identify the occasional mediastinal adenoma and thereby allow the avoidance of an unnecessary neck exploration. On the other hand, the sensitivity and positive predictive value of sestamibi scanning is poor (<50%) in the presence of multiglandular disease (hyperplasia or double adenomas), and thus the test may frequently miss the presence of bilateral disease.[358] To reduce this failure rate, which is unacceptably high in comparison to bilateral exploration, supplemental preoperative ultrasonic imaging or high-resolution CT with dynamic contrast administration (four-dimensional CT [4D-CT])[363] is often used (with or without needle biopsy), and rapid intraoperative PTH assays have been developed to verify successful excision.[364] Because the half-life of intact PTH in blood is very short (<2 minutes), a decline of 50% or more from baseline within 10 minutes or so can signal successful removal of all hyperfunctioning parathyroid tissue. This approach has functioned well in patients with single adenomas, but can be misleading in those with multiglandular disease unless more stringent criteria for cure are applied (i.e., >90% decline, or even normalization, of iPTH).[365]

At present, preoperative imaging enables consideration of a minimally invasive unilateral parathyroidectomy in approximately 70% of those patients thought preoperatively to have sporadic primary hyperparathyroidism due to a solitary adenoma. Surgical cure rates in appropriately selected patients are comparable to those after bilateral neck exploration (i.e., 95–97%),[358] although a recent study in which all patients selected for minimally invasive surgery were also subjected to immediate bilateral neck dissection demonstrated a failure to recognize multiglandular disease in 16% of these subjects.[366] Patients known or suspected to have multiglandular disease, such as those with MEN1 and those younger than 30 years of age, should undergo bilateral neck exploration.[367] Options for patients with hyperplasia include resection limited to visibly abnormal glands, subtotal parathyroidectomy with cryopreservation of tissue, and total parathyroidectomy with immediate autotransplantation (i.e., in the forearm) of some excised tissue. In patients with MEN1, considerations of recurrence rates (30–50% or higher with long-term follow-up) and the timing thereof versus the potential morbidity of surgical hypoparathyroidism tend to favor subtotal parathyroidectomy as the preferred approach at present.

The incidence of parathyroid carcinoma in primary hyperparathyroidism is less than 1%,[285] but this possibility should be strongly considered in patients with unusually severe hyperparathyroidism, a palpable neck mass, hoarseness, evidence of local invasion at surgery, or recurrent hypercalcemia.[368] Even so, parathyroid carcinoma rarely is suspected preoperatively and often eludes diagnosis at the time of initial surgery. When the disease is recognized, vigorous attempts should be made to remove the tumor en bloc. The incidence of local recurrence approaches 50%, however, and distant metastases, particularly to lung, may be heralded by recurrent, severe hyperparathyroidism.[369] Because apparently sporadic and isolated parathyroid cancer can occur in families with parafibromin mutations, a search for such mutations in all patients with parathyroid cancer can facilitate family counseling.[348]

The immediate postoperative management of parathyroidectomy focuses on establishing the success of the surgery and monitoring the patient closely for symptomatic hypocalcemia and for uncommon but potentially serious acute complications such as

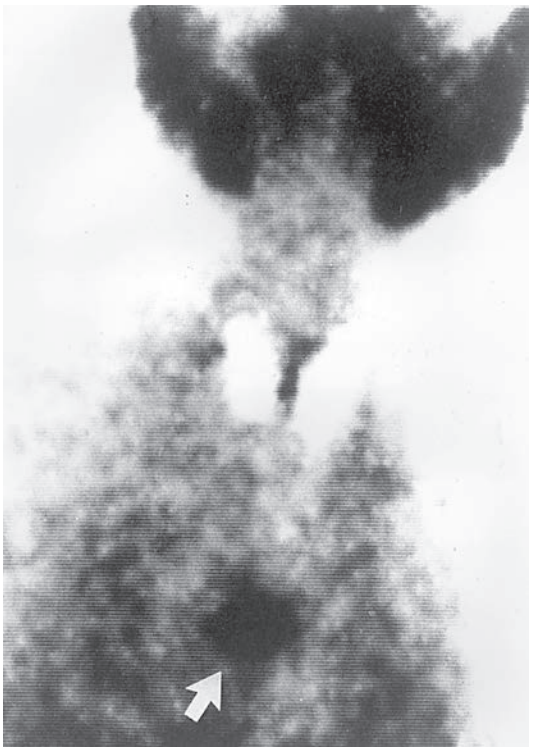
bleeding, vocal cord paralysis, or laryngospasm. After successful resection of a parathyroid adenoma, serum intact PTH levels decline rapidly, often to undetectable concentrations, with a disappearance half-time of about 2 minutes, whereas serum calcium typically reaches a nadir between 24 and 36 hours. Serum PTH returns to the normal range within 30 hours, although measurements of the parathyroid secretory response to hypocalcemia suggest that it does not fully normalize for at least several weeks.[370]

In the past, patients generally were maintained on a low-calcium diet until normalization of serum calcium was clearly documented, ampoules of injectable calcium and other seizure precautions were maintained at the bedside, serum calcium was measured at least every 12 hours until stable, and symptomatic hypocalcemia was promptly treated with calcium, either intravenously (90-mg bolus, 50–100 mg/hour) or orally (1.5–3 g/day). This approach is no longer appropriate for most patients, who are discharged within a few hours after limited surgery. Instead, oral calcium supplements routinely are provided as soon as oral intake is reestablished, and moderate doses of $1,25(OH)_2D$ (0.5–1 μg daily) are added for those with large adenomas and severe hyperparathyroidism or for those in whom alkaline phosphatase had been elevated properatively—that is, patients in whom an impressive calcium requirement can be anticipated, often for many weeks postoperatively, as they remineralize their skeletons. This "hungry bone" syndrome is associated with hypocalcemia, hypophosphatemia, and low urinary calcium excretion.

Serum calcium should be checked at intervals of several days initially to guide adjustment of calcium and vitamin D therapy as needed to achieve a stable result. In those in whom hypocalcemia persists for more than several days, serum PTH should be measured to exclude the possibility of postoperative hypoparathyroidism. Given evidence that bone mineral density continues to increase for at least 1 year after successful parathyroidectomy,[291] it is prudent to continue calcium supplementation for at least that long.

The approach to patients with persistent or recurrent hyperparathyroidism is informed by the recognition that parathyroid hyperplasia or carcinoma, ectopic or supernumerary parathyroid tissue, and postoperative hypoparathyroidism and other complications of further surgery all are more common in this population.[359,361] The first issue to address is whether surgery is indicated. When a presumed adenoma had not been identified initially, the original indications for surgery generally still exist, although some patients may not be suitable candidates for more extensive surgery, such as a median sternotomy, because of concurrent medical illness. Patients with parathyroid hyperplasia may have experienced significant clinical improvement, even after incomplete parathyroidectomy, although those with MEN1 are very likely to experience further progression of their disease.

Preoperative localization studies are recommended for patients with persistent or recurrent disease after a first operation. Scanning with ^{99m}Tc-sestamibi offers the highest sensitivity and accuracy, although other studies (ultrasonography, CT, magnetic resonance imaging [MRI]) may provide additional or confirmatory information.[371] Sestamibi does localize to thyroid nodules, which may accompany parathyroid disease in 20% to 40% of patients, although it tends to wash out of thyroid tissue much more rapidly than from parathyroids. ^{99m}Tc-sestamibi can be combined with ^{123}I scanning to improve distinction of parathyroids from thyroid nodules or with single-photon emission CT (SPECT) imaging to achieve accuracy in localization not possible with planar imaging (Fig. 29.25). On the other hand, sestamibi scanning may fail to reveal small glands (uptake is related to gland size and PTH levels[372]) or to demonstrate multiple abnormal glands in cases of parathyroid hyperplasia, the most common cause of persistent postoperative hyperparathyroidism.[358,373] Use of 4D-CT with synchronous contrast-enhanced multiplanar anatomic reconstruction has been shown to provide sensitivity superior to sestimibi scanning alone for localizing functioning parathyroid tissue in candidates for reoperation.[374]



• **Fig. 29.25** Technetium-99m (^{99m}Tc) sestamibi, iodine-123 (^{123}I) subtraction scanning of a patient with persistent hyperparathyroidism after two previous unsuccessful operations. *Arrow* points to parathyroid adenoma, shown as increased tracer uptake in the aortopulmonary window. (From Thule P, Thakore K, Vansant J, et al. Preoperative localization of parathyroid tissue with technetium-99m sestamibi123I subtraction scanning. *J Clin Endocrinol Metab*. 1994;78:77–82.)

More invasive techniques have been used as well, including angiography and selective venous sampling for measurement of PTH.[375,376] Ultrasound-guided or CT-guided fine-needle aspiration of suspected parathyroid tissue may be used to obtain cytologic or immunochemical confirmation prior to surgery, and intraoperative ultrasonography has been useful in some cases to locate cervical or intrathyroidal glands.[361] Success with video-assisted thoracoscopic resection of documented mediastinal lesions[348,377] offers a less invasive alternative to median sternotomy for this relatively common cause of persistent hyperparathyroidism.

The need for these procedures depends on the experience of the original surgeon and the confidence that the neck was adequately explored initially. For example, among reoperations at one center, over half of the "missed" hyperplastic parathyroid glands in those cases previously explored by a highly experienced parathyroid surgeon were found in the mediastinum or another ectopic location, whereas over 90% of those referred by less experienced surgeons were discovered in a normal anatomic location in the neck.[282]

Following successful surgery for primary hyperparathyroidism, bone mass generally improves by as much as 5% to 10% in the

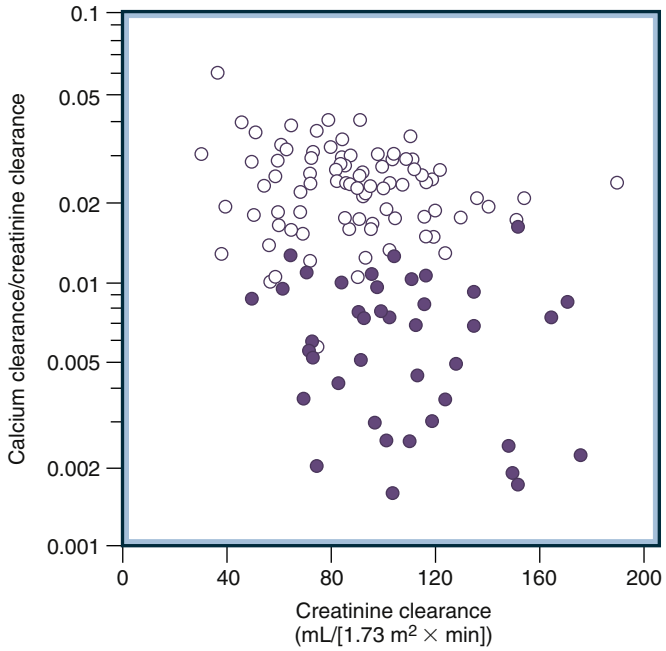
first year at sites rich in trabecular bone (spine, femoral neck), whereas improvement at cortical sites (distal radius) is less predictable.[378,379] Increases at trabecular sites may continue for several years, to as much as 12% to 15% after 10 years, although normal bone mineral density may not be achieved. This improvement, which is most apparent in those with the greatest preoperative reductions in bone mass, may be related in part to rapid remineralization of the previously enlarged bone remodeling volume,[352] but the continued improvement over years suggests a more sustained increase in net bone formation and total bone volume as well.[291]

Familial Hypocalciuric Hypercalcemia

FHH, also appropriately called *familial benign hypercalcemia*, is, in most families, a disorder of autosomal dominant inheritance caused most often by mutations of the *CASR* gene found in parathyroid glands, kidney, and other organs (see earlier discussion of calcium sensing). The mutations, which cause complete or partial loss of function of the CaSR, lead to a shift in the parathyroid cell's set-point for calcium.[380] As a consequence, higher than normal levels of blood calcium are needed to suppress PTH secretion. Furthermore, abnormal function of the CaSR in the renal thick ascending limb leads to increased PTH-independent calcium reabsorption and consequent hypocalciuria. In a minority of patients, mutations in two other genes in the signaling pathway activated by the CaSR have been found. One encodes the α-subunit of the G_{11} heterotrimeric G protein[381] known to participate in responding to the activation of the CaSR, and the other is in the *AP2S1* gene that encodes the α-subunit of adapter protein complex 2, a scaffolding protein needed for formation of clathrin-coated pits needed to internalize the CaSR.[382] Mutations in AP2S1 are considerably more common than mutations in $G\alpha_{11}$ in FHH[383,384]; patients with AP2S1 mutations have particularly severe hypercalcemia and can present with developmental delay and cognitive dysfunction.[383]

The presence of one normal calcium-sensing receptor gene with the abnormal one usually leads to a very mild clinical disorder, although the receptor functions as a dimer, and certain mutations can worsen the function of the normal allele. Rare patients who inherit mutant *CASR* genes from both parents present at birth with severe, life-threatening, primary hyperparathyroidism and almost always require immediate parathyroid surgery. In another genetic variation, a familial form of CaSR-dependent hypercalcemia has been described in association with other autoimmune disorders such as Hashimoto hypothyroidism and celiac sprue, in which autoantibodies directed against the sensor apparently antagonize calcium recognition by the parathyroids and renal tubules.[385,386]

FHH is manifested at birth by hypercalcemia. Although some controversy exists, most observers note that the condition associated with calcium-sensing receptor mutations is usually asymptomatic and that apparent symptoms represent ascertainment bias. Possible exceptions include the occurrence of chondrocalcinosis and perhaps pancreatitis. The blood calcium level is usually less than 12 mg/dL but can be higher. Phosphate measurements are low, as in primary hyperparathyroidism. Blood magnesium levels are high-normal or slightly elevated. PTH levels are inappropriately normal for the degree of hypercalcemia and are occasionally modestly elevated. Urine calcium is usually low, though one novel mutation in the receptor's intracellular tail has been associated with hypercalciuria, possibly because of only mild dysfunction in the kidney.[387]



• **Fig. 29.26** Index of urinary excretion rate for calcium as a function of creatinine clearance. Each point represents the mean of multiple determinations for a hypercalcemic patient with familial hypocalciuric hypercalcemia *(filled circles)* or with typical primary hyperparathyroidism *(open circles)*. The data are based on average 24-hour urinary excretion values and average fasting serum samples. (From Marx SJ, Attie MF, Levine M, et al. The hypocalciuric or benign variant of familial hypercalcemia: clinical and biochemical features in fifteen kindreds. *Medicine*. 1981;60:397–412.)

When patients present as adults, the distinction from mild primary hyperparathyroidism can be difficult. The distinction between FHH and primary hyperparathyroidism is a crucial one, however. Young patients with primary hyperparathyroidism are usually treated surgically and cured. In contrast, hypercalcemia always recurs after surgery for FHH, unless the patient is rendered hypoparathyroid by the removal of all parathyroid tissue. Therefore surgery is contraindicated as therapy for FHH, except in the very rare patient with severe, symptomatic hypercalcemia. No blood or urine measurements are completely reliable for distinguishing between the two conditions, though the ratio of calcium clearance to creatinine clearance distinguishes most patients with FHH from those with primary hyperparathyroidism.[388] Fig. 29.26 shows, however, that this ratio separates the two groups, with modest overlap between the groups. However, because primary hyperparathyroidism is much more common than FHH, most patients with values near the cutoff value of 0.01 for the ratio of calcium clearance to creatinine clearance will have primary hyperparathyroidism and not FHH.[389] Consequently, a case can be made[390] that many of such patients, particularly before parathyroid surgery, should undergo sequencing of their *CASR* gene, now available commercially. The most helpful diagnostic information is the presence of hypercalcemia in an infant relative; such early hypercalcemia does not occur in MEN1. Furthermore, a past history of clearly normal blood calcium, considerably lower than current measurements, makes FHH unlikely if no other reason, such as severe vitamin D deficiency, for a change in blood calcium exists. In the occasionally symptomatic patient, cinacalcet, a calcimimetic drug, can usually lower the blood level of calcium.[391]

Lithium Toxicity

Treatment of bipolar affective disorders with lithium commonly leads to mild, persistent increases in blood calcium.[392,393] Measurement of ionized calcium has shown that ionized calcium is a more sensitive index of lithium effect and was elevated in 24% of consecutive patients in a cross-sectional study.[394] After several years of therapy, clear elevations of PTH levels and modest increases in parathyroid gland size, detected by ultrasonography, often occur. Usually, when lithium therapy is stopped, the blood calcium and PTH normalize within several months. Uncommonly, substantial hypercalcemia and clear hyperparathyroidism ensue. At surgery, both single-gland and multigland disease are found, with a higher fraction of multigland disease than found in primary hyperparathyroidism not associated with lithium therapy.[395,396]

The management of patients with mild lithium-induced hypercalcemia is somewhat complicated. Like patients with mild primary hyperparathyroidism, patients taking lithium usually tolerate mild hypercalcemia without obvious symptoms. These patients can be monitored with protocols similar to those for patients with asymptomatic primary hyperparathyroidism. Close attention must be paid to urine-concentrating ability in these patients, however, because the nephrogenic diabetes insipidus associated with lithium therapy can lead to dehydration and sudden worsening of hypercalcemia. Substantial hypercalcemia should lead to withdrawal of lithium therapy, if possible, with substitution of newer psychopharmacologic agents. If hypercalcemia persists after withdrawal of lithium, decisions about surgery follow the same guidelines as those for patients with primary hyperparathyroidism.

Lithium increases the set-point for PTH secretion when it is added to isolated parathyroid cells in vitro. The set-point for PTH secretion in vivo is shifted to the right in patients who have received lithium for several years as well. A corresponding shift in the concentration of extracellular calcium needed to raise intracellular calcium levels[397] suggests that lithium interferes with the action of the parathyroid CaSR.

Parathyroid-Independent Hypercalcemia

In parathyroid-independent hypercalcemia, PTH secretion is appropriately suppressed. PTH levels, measured using two-site assays, are invariably lower than 25 pg/mL and are usually lower than normal or undetectable. Most affected patients have malignant hypercalcemia, although parathyroid-independent hypercalcemia occurs in a number of other settings as well.[398]

Hypercalcemia of Malignancy

The diagnosis of malignant hypercalcemia is seldom a subtle one.[125] Most malignancies produce hypercalcemia only when they are far advanced; the diagnosis becomes evident after routine studies, guided by the history and physical examination. Patients with malignant hypercalcemia usually die 1 to 2 months after hypercalcemia is discovered. Patients present with the classic signs and symptoms of hypercalcemia: confusion, polydipsia, polyuria, constipation, nausea, and vomiting. Perhaps because of the acuteness of the hypercalcemia and the elderly patient population involved, dramatic changes in mental status, culminating in coma, are relatively common. The diagnosis can be missed because the manifestations often overlap those of the underlying malignancy and because low blood albumin may lead to an apparently normal total blood calcium, despite an elevated blood ionized calcium. Even though the overall prognosis is grim, the diagnosis of malignant hypercalcemia is important to make.

Treatment is usually simple and effective in the short term; such treatment can importantly reverse the patient's symptoms for several weeks and even provide time for a fundamental attack on the underlying tumor, if it is treatable. Treatment consists of restoration of volume, followed by intravenous bisphosphonate or denosumab (see "Management of Severe Hypercalcemia"). Only effective treatment of the underlying neoplasm can significantly influence the long-term prognosis for patients with malignant hypercalcemia.

Although mechanisms in a given patient may be multiple, it is still useful to distinguish hypercalcemia associated with local involvement of bone from that caused by humoral mechanisms. In all cases, resorption of bone plays a pivotal role in the pathogenesis.

Local Osteolytic Hypercalcemia

Hypercalcemia resulting from tumors invading bone occurs most clearly in multiple myeloma and some patients with breast cancer. There is little evidence that the tumor cells themselves resorb bone. Instead, active osteoclasts found near the tumor cells are thought to be the proximate mediators of bone resorption.[399] Myeloma cells and marrow cells associated with myeloma cells secrete numerous cytokines and chemokines capable of stimulating bone resorption, including macrophage inflammatory protein 1 (MIP1), lymphotoxin (tumor necrosis factor-β), and interleukins 1β, 3, and 6. These factors lead to increased expression of RANKL (see Fig. 29.8) on the surface of marrow stromal cells and stimulation of osteoclast formation and activity. RANKL is also found on the surface of myeloma cells, and therefore these cells may directly stimulate the production and activity of osteoclasts. The increased bone resorption not only releases calcium into the circulation but also weakens the bone structurally. Bone is further weakened by the suppression of bone formation by the secretion of dickkopf-1, an inhibitor of Wnt signaling, by myeloma cells.[400–402] In patients with myeloma, treatment with intermittent intravenous bisphosphonates or denosumab inhibits bone resorption and reduces the incidence of bone pain, fracture, and hypercalcemia.[403]

The pathogenesis of hypercalcemia in breast cancer is not completely understood. Extensive metastases to bone are detected in most patients with hypercalcemia and breast cancer; this finding suggests that factors produced in bone by the metastatic tumor cells may be important. Breast cancer cells make a host of cytokines capable of stimulating bone resorption by osteoclasts.[404] The role of tumor-produced PTHrP may be particularly important.[401] A majority of breast cancer patients with hypercalcemia have elevated blood levels of PTHrP. This circulating PTHrP, as well as PTHrP produced in bone by metastatic tumor cells, may generate the hypercalcemia. Primary breast tumors that stain for PTHrP are more likely to result in bone metastases than are those that do not stain for PTHrP; this PTHrP may be instrumental in the establishment of lytic metastases. Animal models indicate that transforming growth factor-β (TGFβ), released from bone matrix by PTHrP-stimulated osteoclastic resorption, may further augment PTHrP secretion by the tumor cells. The latter may be further promoted by estrogen, which may explain the occasional occurrence of hypercalcemia following institution of estrogen or tamoxifen therapy in this disease.[405]

Humoral Hypercalcemia of Malignancy

Albright, in 1941, was the first to propose that a PTH-like humoral factor caused the hypercalcemia in patients with malignancy but few or no bone metastases.[406] Four decades later

biochemical analysis demonstrated that such patients have high blood calcium levels, low blood phosphate levels, and high urinary cAMP levels like those found in primary hyperparathyroidism, but no elevation in iPTH levels.[407] The stimulation of cAMP production was used as an assay to eventually purify PTHrP from human tumors associated with the humoral hypercalcemia of malignancy.[408]

The evidence that PTHrP mediates the humoral hypercalcemia of malignancy in most patients is substantial. As noted previously, PTHrP binds to the PTH/PTHrP receptor and mimics all of the actions of amino-terminal fragments of PTH. Blood levels of PTHrP are elevated in most patients with solid tumors and hypercalcemia. In animal models of the humoral hypercalcemia of malignancy, antibodies against PTHrP can reverse the hypercalcemia.[409]

The acute actions of PTHrP cannot explain all of the findings in patients with the hypercalcemia of malignancy, however. Acutely administered PTHrP increases blood levels of $1,25(OH)_2D_3$ by stimulating the renal 1α-hydroxylase, though the stimulation is less than that induced by PTH.[410] Nevertheless, patients with the humoral hypercalcemia of malignancy usually have low levels of $1,25(OH)_2D_3$.[407] This finding is particularly puzzling, because human tumors from patients with elevated calcium and PTHrP but with low $1,25(OH)_2D_3$ levels stimulate $1,25(OH)_2D_3$ synthesis after they are transplanted into nude mice.[411] Possible explanations for the low $1,25(OH)_2D_3$ levels in patients include the weak activation of the renal 1α-hydroxylase in humans by PTHrP, combined with the inhibition of the 1α-hydroxylase by hypercalcemia[412] or by tumor products.

A second disparity between the acute actions of PTHrP and the findings in patients with malignant hypercalcemia involves the rate of bone formation. Acutely, PTHrP infusions in rats, like PTH, leads to increased bone formation.[413] Nevertheless, in patients with malignant hypercalcemia, bone formation is markedly lower than normal. The explanation for this effect may well lie in the action of other cytokines, immobilization, or particular fragments of PTHrP with novel properties.

The tumors most commonly associated with humoral hypercalcemia include squamous cell cancers of the lung, head and neck, esophagus, cervix, vulva, and skin; breast cancer; renal cell cancer; and bladder cancer. Benign or malignant pheochromocytomas, islet cell tumors, and carcinoids can also overproduce PTHrP, causing hypercalcemia. The aggressive T-cell lymphoma associated with human T-cell lymphotropic virus type 1 (HTLV1) infection is the only hematologic malignancy commonly associated with PTHrP overproduction and hypercalcemia.

Cachexia often accompanies the hypercalcemia of malignancy. In experimental models, administration of a monoclonal antibody to PTHrP ameliorates the cachexia, not simply by lowering blood calcium but also by blocking the ability of PTHrP to convert white adipose tissue to brown, heat-generating adipose tissue.[126]

It is unlikely that PTHrP is the sole cause of the humoral hypercalcemia of malignancy. As noted previously, many cytokines produced by tumors can stimulate bone resorption. The actions of these cytokines have been shown to synergize with those of PTHrP in a number of experimental models. Furthermore, in hypercalcemic patients with non-Hodgkin lymphoma, blood levels of $1,25(OH)_2D_3$ were found to be higher than otherwise expected,[414] and such patients show abnormal sensitivity to 25-hydroxyvitamin D administration.[415] In these hypercalcemic patients, the relative importance of $1,25(OH)_2D_3$ cytokines, PTHrP, and immobilization needs to be clarified. Only a minority of patients with non-Hodgkin lymphoma have clear elevations of either PTHrP or $1,25(OH)_2D_3$.[416]

In a few reported cases, malignant tumors secrete PTH and not PTHrP.[125,417] Although this phenomenon has now been well documented, it should be stressed that in almost all patients with cancer and high PTH levels, concurrent primary hyperparathyroidism, not ectopic PTH production, is the cause of the hyperparathyroidism.

Vitamin D Intoxication

Because the synthesis of $1,25(OH)_2D_3$ is so tightly regulated, extremely large doses of vitamin D, on the order of 100,000 units per day, are required to cause hypercalcemia. Such doses are available in the United States only by prescription, therefore most cases of vitamin D intoxication are iatrogenic. Occasionally, inadvertent ingestion occurs. All over the world, including in the United States, particularly in the era of the internet and widespread enthusiasm about the potential properties of vitamin D, increasing reports of vitamin D intoxication caused by preparations of vitamin D containing much larger amounts of vitamin D than claimed have been published.[418] Patients present with nausea, vomiting, weakness, and altered level of consciousness. Hypercalcemia can be severe and prolonged, because of the storage of vitamin D in fat. As expected, PTH levels are suppressed, and levels of 25(OH)D, which are poorly regulated and reflect levels of ingested vitamin D, are dramatically elevated. In contrast, the levels of $1,25(OH)_2D_3$ are only modestly elevated or can be normal or even low. The modest changes in $1,25(OH)_2D_3$ levels result from the downregulation of the renal 1α-hydroxylase by low levels of PTH and high levels of phosphate, calcium, FGF23, and $1,25(OH)_2D_3$ itself. The cause of the hypercalcemia, when it occurs in the face of normal levels of $1,25(OH)_2D_3$, is uncertain but may reflect the direct action of 25(OH)D and possibly other vitamin D metabolites, which are capable of binding the $1,25(OH)_2D_3$ receptor weakly or which may be locally 1α-hydroxylated by nonrenal 1α-hydroxylases. Of note, vitamin D causes hypercalcemia in mice missing the 1α-hydroxylase gene,[419] arguing that 25(OH)D and other metabolites, when present in large amounts, are sufficient to cause hypercalcemia. Also, the weaker vitamin D metabolites may displace $1,25(OH)_2D_3$ from the circulating VDBP and increase the concentration of active, free $1,25(OH)_2D_3$.[420,421]

The hypercalcemia of vitamin D intoxication results both from increased intestinal absorption of calcium and from the direct effect of $1,25(OH)_2D_3$ to increase resorption of bone. Therefore in severe cases bisphosphonate therapy can be usefully added to the therapeutic regimen of hydration and omission of dietary calcium.

Sarcoidosis and Other Granulomatous Diseases

Sarcoidosis may be associated with hypercalcemia and, even more commonly, hypercalciuria.[422] Hypercalcemic patients have high levels of $1,25(OH)_2D_3$; the high level of $1,25(OH)_2D_3$ probably causes the hypercalcemia, although overproduction of bone-resorbing cytokines and PTHrP may contribute in some patients. As expected in $1,25(OH)_2D_3$-dependent hypercalcemia, intestinal absorption of calcium is increased and PTH levels are suppressed. Furthermore, the hypercalcemia and high levels of $1,25(OH)_2D_3$ fall upon treatment with glucocorticoids. The unregulated synthesis of $1,25(OH)_2D_3$, found even in an anephric patient, occurs not in the kidney but in the sarcoid granulomas. Removal of a large amount of granulomatous tissue can reverse hypercalcemia. Furthermore, isolated sarcoid macrophages can synthesize

$1,25(OH)_2D_3$ from 25(OH)D, as can normal macrophages stimulated with interferon-γ or after activation of toll-like receptors. Such macrophages express the gene encoding the identical 25(OH)D 1α-hydroxylase found in the kidney. The local synthesis of $1,25(OH)_2D_3$ by activated macrophages and activation of VDRs in those cells is a paracrine system that activates antibacterial mechanisms as part of the normal action of macrophages.[193]

The unusual increase in the numbers of activated macrophages in sarcoidosis leads, however, to elevations of blood calcium in these patients. These patients have unusual sensitivity to vitamin D and can become hypercalcemic in response to ultraviolet radiation or oral vitamin D intake. Abnormalities in calcium metabolism are usually found only in patients with active disease and large, clinically obvious total-body burdens of granulomas. Nevertheless, hypercalcemia can present in patients without obvious pulmonary disease. Furthermore, subtle abnormalities of vitamin D metabolism can be demonstrated even in patients with mildly active sarcoidosis.

Hypercalcemia is also associated with other granulomatous diseases, such as tuberculosis, fungal infections, and berylliosis and has been reported in Wegener granulomatosis, in acquired immunodeficiency syndrome (AIDS)–related *Pneumocystis jirovecii* infection, fat necrosis of the newborn,[423] and even in association with extensive granulomatous foreign-body reactions.[398] Patients with Crohn disease occasionally have hypercalcemia with elevations of $1,25(OH)_2D_3$ levels but often have elevated $1,25(OH)_2D_3$ levels with normal calcium levels and low bone mass, associated with increased production of $1,25(OH)_2D_3$ in intestinal macrophages.[424] In an analysis of 101 patients with hypercalcemia and high levels of $1,25(OH)_2D_3$ in Australia, half the patients had sarcoidosis and the others had hematologic malignancies, other infections, or other causes.[425]

CYP24A1 Loss of Function

Cyp24A1 encodes the vitamin D-24-hydroxylase, which is an important regulated inactivator of $1,25\text{-}(OH)_2D$. Homozygous loss of this gene leads to idiopathic infantile hypercalcemia, usually a severe disease presenting in early childhood. However, the spectrum of presentation is broad, and some patients present in late childhood and even adulthood, with hypercalciuria, kidney stones, and nephrocalcinosis.[426]

Hyperthyroidism

Mild hypercalcemia can result from thyrotoxicosis.[427] Blood calcium levels seldom exceed 11 mg/dL, but mild elevations are found in a quarter of patients. Patients have low PTH levels, low $1,25(OH)_2D_3$ levels, and hypercalciuria. The hypercalcemia is caused by a direct action of thyroid hormone to stimulate bone resorption.[428] β-Adrenergic blocking agents can reverse the hypercalcemia.[429]

Vitamin A Intoxication

Excess ingestion of vitamin A (retinol) results in a syndrome of dry skin, pruritus, headache from pseudotumor cerebri, bone pain, and occasional hypercalcemia. Hypercalcemia occurs only with the ingestion of 10 times the Recommended Dietary Allowance (RDA) (5000 IU/day). The identical syndrome can result from ingestion of the vitamin A derivatives isotretinoin (13-*cis*-retinoic acid [Accutane]) and tretinoin (all-*trans*-retinoic acid [Retin-A]), used to treat acne and acute promyelocytic leukemia.[430,431] Bones can show characteristic periosteal calcification on radiographs. The hypercalcemia is probably caused by the action of retinoids to stimulate bone resorption. The diagnosis is made by the association of a history of excess ingestion of retinoids with the characteristic syndrome and abnormal results of liver function tests; elevated vitamin A levels confirm the diagnosis. Treatment involves hydration and, if necessary, glucocorticoids or bisphosphonates.

Adrenal Insufficiency

Hypercalcemia occurs in the setting of adrenal insufficiency. Blood calcium is elevated partly as a result of hemoconcentration and increased albumin levels, but the level of ionized calcium can be increased as well.[432] PTH and $1,25(OH)_2D_3$ levels are low to low-normal.[433] The hypercalcemia in this study resulted from a combination of influx of calcium into the vascular space, probably from bone, combined with low renal clearance.

Thiazide Diuretics

Thiazide diuretics do not cause hypercalcemia by themselves, but they can exacerbate the hypercalcemia of primary hyperparathyroidism or any other cause of increased input of calcium into the bloodstream that is not suppressed by hypercalcemia. The mechanism of the hypercalcemia may involve the action of thiazide diuretics to increase proximal tubular calcium reabsorption as a secondary consequence of direct action of thiazides on the distal tubule.[434,435] Decreased renal clearance of calcium alone would be expected to raise blood calcium in the normal human only transiently because the transient hypercalcemia would be expected to suppress PTH secretion and lead to return of the blood calcium to normal. However, in the presence of primary hyperparathyroidism, sarcoidosis, excess calcium intake, or any other cause of high, fixed calcium load, thiazide administration will increase the level of calcium in blood.

As predicted by this model, thiazide administration leads to chronic hypercalcemia in patients with abnormal parathyroid physiology but not in normal subjects.[436] In primary hyperparathyroidism, thiazide administration exacerbates the hypercalcemia, and in hypoparathyroidism, thiazide administration facilitates the maintenance of normocalcemia when given in conjunction with $1,25(OH)_2D_3$ and calcium.

Milk-Alkali Syndrome

The triad of hypercalcemia, metabolic alkalosis, and renal failure can be the consequence of massive ingestion of calcium and absorbable alkali. This syndrome was first described when milk and sodium bicarbonate were used in large amounts to treat peptic ulcer disease. With the change in ulcer treatment to nonabsorbable antacids and suppression of acid secretion, milk-alkali syndrome became rare. In the last several years, however, the increased use of calcium carbonate to treat dyspepsia and osteoporosis has led to the reappearance of milk-alkali syndrome.[437,438] In most cases, a history of ingestion of several grams per day of calcium in the form of calcium carbonate can be elicited. Some reports suggest that less than 4 g of calcium daily can cause milk-alkali syndrome, fewer than previously estimated, perhaps because of greater ingestion of vitamin D than in the past.[439] The pathogenesis of the syndrome is not understood in detail but may well involve a vicious circle in which alkalosis decreases renal calcium clearance and hypercalcemia helps maintain alkalosis. Nephrocalcinosis, nephrogenic diabetes insipidus, decrease in GFR associated with hypercalcemia, and hypovolemia from vomiting all lead to renal failure, which can be severe. PTH levels, measured with currently available two-site assays, are invariably low in hypercalcemic patients, as are levels of $1,25(OH)_2D_3$. After clearance of the calcium by hydration

or dialysis, if necessary, renal function generally returns to normal, unless the disorder has been severe and longstanding.

Immobilization

Immobilization can lead to bone resorption sufficient to cause hypercalcemia. The immobilization is usually caused by spinal cord injury or extensive casting after fractures, though it can occur in settings such as Parkinson disease.[434] Hypercalcemia after trauma requiring immobilization is common, when studied prospectively, and is usually asymptomatic.[440] Hypercalcemia of immobilization occurs predominantly in the young or in patients with other reasons for a high rate of bone turnover, such as Paget disease or extensive fractures. Hypercalciuria and substantial bone loss are more common than hypercalcemia. After spinal cord injury, the hypercalciuria is maximal at 4 months and can persist for more than 1 year. PTH and $1,25(OH)_2D_3$ levels are suppressed; bone biopsies show increased resorption and decreased formation of bone. Bisphosphonates and denosumab have been used to reverse the hypercalcemia and hypercalciuria of immobilization.

Renal Failure

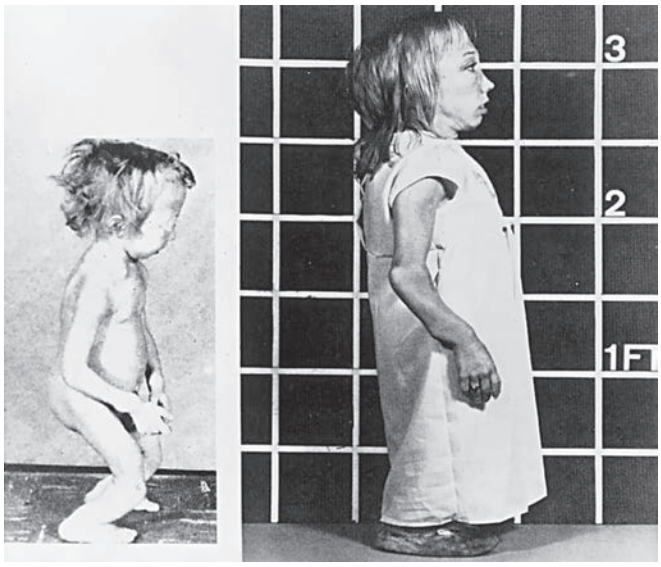
Following rhabdomyolysis, during the oliguric phase of acute renal failure, severe hypocalcemia can result from acute hyperphosphatemia and calcium deposition in muscle.[441] PTH levels are high, in response to the hypocalcemia. In the diuretic phase that follows, hypercalcemia can occur. The hypercalcemia results from the mobilization of the calcium deposits and, in a fraction of patients, from associated high $1,25(OH)_2D_3$ levels sometimes seen.[442]

In chronic renal failure, hypercalcemia can result from tertiary hyperparathyroidism or may appear during therapy of aplastic bone disease associated with low PTH levels and sometimes with aluminum toxicity.

Williams Syndrome

Williams syndrome is a developmental disorder in which supravalvular aortic stenosis is associated with elfin facies and mental retardation.[443] Hypercalcemia can occur transiently in the first 4 years of life, though, as a group, Williams patients have higher than normal calcium levels, usually at the top of the normal range.[444] Affected hypercalcemic infants have been found to have increased intestinal absorption of calcium and associated elevations of $1,25(OH)_2D_3$ that fall to normal as the blood calcium normalizes.[445] Levels of 25(OH)D are normal. The hypercalcemia can generally be controlled by dietary manipulation and, if needed, bisphosphonates.[446]

Molecular analysis has clarified the origin of the connective tissue component of Williams syndrome. Williams syndrome is a contiguous gene syndrome with deletions of one or several genes. Isolated supravalvular aortic stenosis is associated with deletion or translocation of the distal portion of the elastin gene. Williams syndrome, with more protean connective tissue abnormalities and mental retardation, is associated with large deletions that include the elastin gene and a gene encoding the protein kinase LIM-kinase 1. A gene within the Williams syndrome deletion region encodes a nuclear protein, Williams syndrome transcription factor, that is part of a large chromatin remodeling complex that can bind the VDR and influence the transcription of VDR-responsive genes.[447] For this reason, this gene is a strong candidate for the gene associated with transient hypercalcemia in this disorder. Genetic proof that this gene is responsible for the hypercalcemia and a connection between the gene and hypercalcemia, however, is lacking.



• **Fig. 29.27** A patient with Jansen metaphyseal chondrodysplasia at ages 5 years and 22 years. Note the short stature, characteristic facies, and misshapen metaphyseal region of long bones. (From Frame B, Poznanski AK. Conditions that may be confused with rickets. In DeLuca HF, Anastas CS, eds. *Pediatric Diseases Related to Calcium*. New York: Elsevier; 1980:269–289.)

Jansen Metaphyseal Chondrodysplasia

Jansen metaphyseal chondrodysplasia is a rare disease in which affected persons present in childhood with short stature and hypercalcemia (Fig. 29.27). Blood chemistry studies suggest hyperparathyroidism, with high calcium, low-normal phosphate, high $1,25(OH)_2D_3$, high alkaline phosphatase, and high urinary hydroxyproline levels, but PTH levels are suppressed.[448] A generalized defect in endochondral bone formation results from abnormally organized chondrocytes in growth plates. Metaphyses appear disordered and rachitic on radiographs. The bones may show signs of osteitis fibrosa cystica. Constitutive activation of the PTH/PTHrP receptor, caused by five different point mutations in three different amino acid residues in the transmembrane domains of the receptor, explains the findings in this disorder.[104,449,450] The abnormalities on serum chemistry studies result from PTH-like actions of the receptor in bone and kidney; one patient has been noted to have increased blood levels of FGF23.[451] The growth plate disorder results from PTHrP-like actions of the receptor on the growth plate.

Approach to the Hypercalcemic Patient

The diagnostic approach to the hypercalcemic patient is strongly influenced by the clinical setting and the knowledge that primary hyperparathyroidism is at least twice as common as all other causes combined (Table 29.2). These considerations are particularly significant in the patient who seems otherwise well and in whom the hypercalcemia is detected incidentally or is mild, stable, or known to be of long duration (i.e., years). Among outpatients referred to endocrinologists for evaluation of hypercalcemia, for example, more than 90% are found to have primary hyperparathyroidism. In ill or hospitalized patients, malignant disease is the cause in more than 50% of cases. The differential diagnosis is seldom complicated, however, because malignant hypercalcemia usually presents in the context of advanced, clinically obvious disease.

TABLE 29.2 Causes of Hypercalcemia

Parathyroid-Dependent Hypercalcemia

Primary hyperparathyroidism
Tertiary hyperparathyroidism
Familial hypocalciuric hypercalcemia
Lithium-associated hypercalcemia
Antagonistic autoantibodies to the calcium-sensing receptor

Parathyroid-Independent Hypercalcemia

Neoplasms
- PTHrP dependent
- Other humoral syndromes
- Local osteolytic disease (including metastases)

PTHrP excess (nonneoplastic)
Excess vitamin D action
- Ingestion of excess vitamin D or vitamin D analogues
- Topical vitamin D analogues
- Granulomatous disease
- Williams syndrome

Thyrotoxicosis
Adrenal insufficiency
Renal failure
- Acute renal failure
- Chronic renal failure with aplastic bone disease

Immobilization
Jansen disease
Drugs
- Vitamin A intoxication
- Milk-alkali syndrome
- Thiazide diuretics
- Theophylline

PTHrP, Parathyroid hormone–related protein.

Because hypercalcemia usually is first detected as an elevation of total serum calcium, it is important to distinguish hemoconcentration or rare instances of calcium-binding paraproteinemia or thrombocythemia-associated hypercalcemia (due to release of intracellular calcium in vitro) from a true increase in serum ionized calcium (Fig. 29.28). The presence of hypercalcemia should be confirmed by direct measurement of ionized calcium, and total calcium should be repeated, together with albumin, globulin electrolytes, blood urea nitrogen, creatinine, and phosphate. Especially when hypercalcemia is mild, it is prudent to repeat the serum total or ionized calcium measurement at least twice, preferably fasting and without venous occlusion, before proceeding with more costly studies directed at its cause.

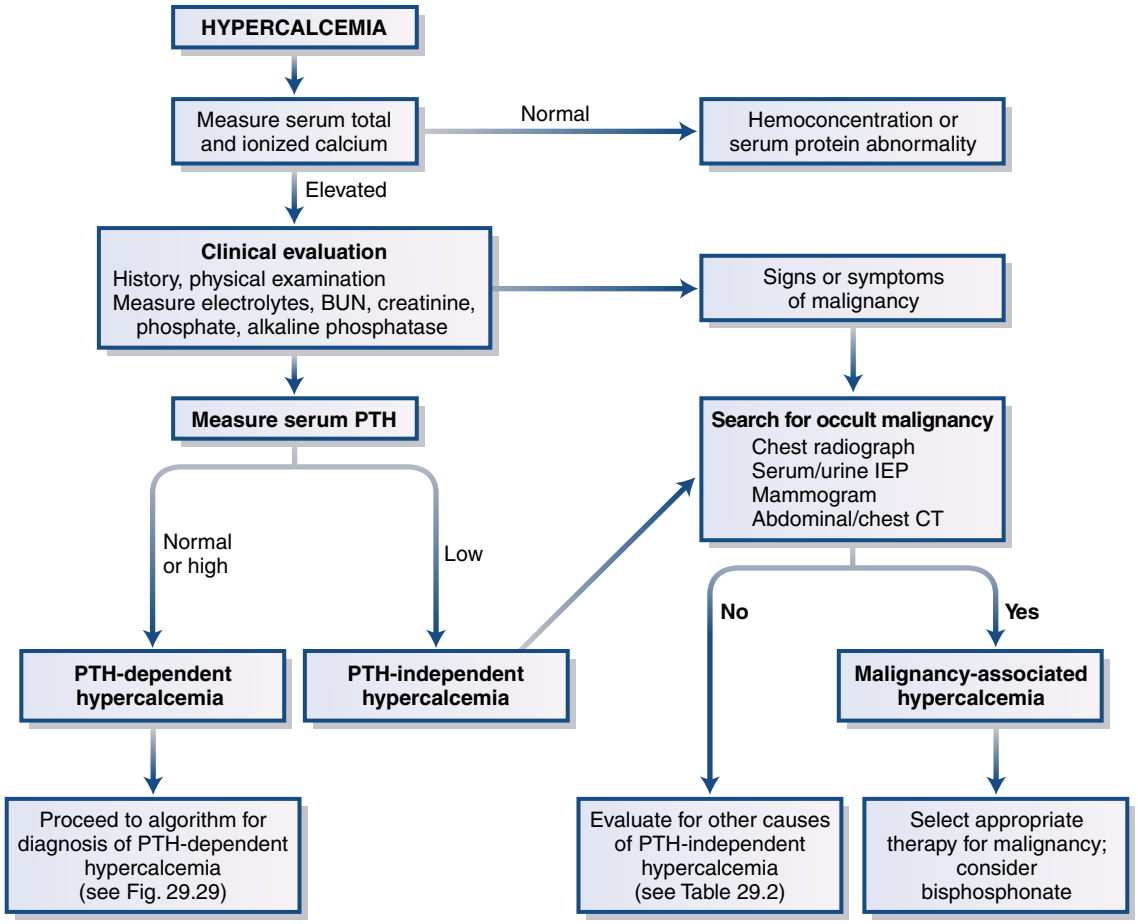
A careful history and physical examination, combined with efforts to assess chronicity by seeking prior results of routine multichannel serum chemistry determinations, most often will point to the likely diagnosis. Serum phosphate often is low in hyperparathyroidism, but as this often is true also of PTHrP-secreting malignancies, the presence of hypophosphatemia is not helpful in distinguishing these possibilities. When serum phosphate is normal or high despite correction of dehydration, the possibility of PTH-independent or PTHrP-independent hypercalcemia should be considered more strongly, however. Elevations in serum chloride and alkaline phosphatase, often observed in primary hyperparathyroidism, cannot be reliably used in the differential diagnosis of hypercalcemia. Important elements of the medical history of hypercalcemic patients include inquiries about kidney stones or fractures; weight loss; back or bone pain; fatigue or weakness; cough or dyspnea; ulcer disease or pancreatitis; ingestion of vitamins, calcium preparations, lithium, or thiazides; dates of most recent mammograms and chest radiographs; and a family history of hypercalcemia, kidney stones, ulcer disease, endocrinopathy, or tumors of the head or neck. Because malignancy is a common cause of hypercalcemia and may occur concomitantly with primary hyperparathyroidism, clinical findings strongly suggestive of malignancy should be acted upon by proceeding directly to a search for an underlying tumor, regardless of serum PTH.

The single most important test in the differential diagnosis of hypercalcemia is the measurement of serum PTH, preferably in a two-site assay specific for the intact, biologically active molecule (see Fig. 29.19). New PTH assays have been introduced that ignore long circulating fragments of the hormone, which lack the amino-terminal residues required for activity at the PTH/PTHrP receptor, but whether these new assays will be more useful than standard "intact PTH" assays remains to be established.[452,453] A consistently elevated serum PTH in the presence of true hypercalcemia always is abnormal and almost always indicates the presence of primary hyperparathyroidism. The exceptions that also can be associated with elevated PTH levels are FHH, autonomous parathyroid secretion complicating secondary hyperparathyroidism (tertiary hyperparathyroidism), lithium-associated hyperparathyroidism, and, very rarely, ectopic PTH secretion by a malignant neoplasm or antagonizing autoantibodies directed against the CaSR in patients with other autoimmune disease(s)—an acquired condition that mimics FHH.[385,386]

Diagnosis of primary hyperparathyroidism is complicated, however, by the fact that some patients fail to exhibit both hypercalcemia and elevated iPTH. In up to 10% of patients with hypercalcemia and primary hyperparathyroidism, PTH levels may fall within the normal range (high normal) with current PTH assays. Such PTH levels are inappropriate in the face of hypercalcemia, however, and support the diagnosis of PTH-dependent hypercalcemia. In fact, many such patients will manifest frankly elevated serum PTH if retested, especially if dietary calcium is restricted beforehand. As noted earlier, some patients may present with serum calcium in the high-normal range (>10 mg/dL) together with an elevated or high-normal PTH. This may be discovered incidentally in an otherwise asymptomatic subject or in the course of evaluating recurrent urolithiasis or osteopenia. Those with persistently high-normal serum calcium and high-normal iPTH should be retested at intervals and, meanwhile, given a provisional diagnosis of hyperparathyroidism and evaluated accordingly.

In patients with PTH-dependent hypercalcemia (Fig. 29.29), calcium and creatinine should be measured in a 24-hour urine collection and simultaneous serum sample to measure total urinary calcium output (mg/day) and the clearance ratio of calcium (Ca)/creatinine (Creat) in urine (U) and serum (S): $U_{Ca}/S_{Ca} \times S_{Creat}/U_{Creat}$). A daily calcium excretion of less than 100 mg per day, or a clearance ratio less than 0.01, should prompt consideration of FHH, especially in patients younger than 40 years, or those with a family history of FHH, or patients whose serum iPTH levels are within the normal range. A urinary calcium excretion greater than 4 mg/kg per day or clearance ratio greater than 0.02 effectively excludes FHH. In FHH, serum phosphate is normal or slightly low, serum magnesium may be slightly high, and serum $1,25(OH)_2D_3$ is normal or low (unlike in primary hyperparathyroidism).

A definite diagnosis of FHH, as in the MEN syndromes, may be provided by confirming the presence of mutations in the relevant genes, although such studies are not invariably informative



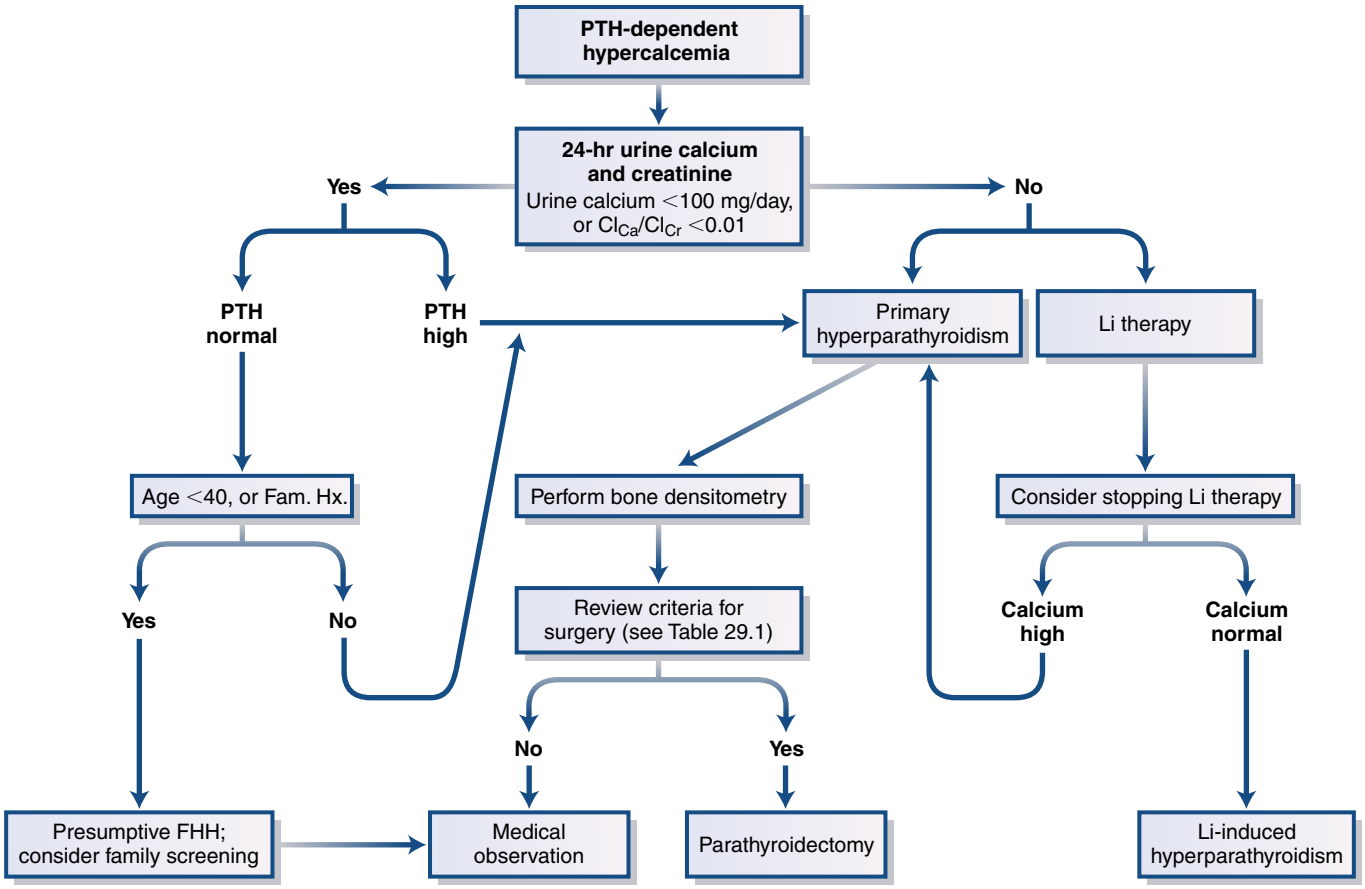
• **Fig. 29.28** Approach to the management of the hypercalcemic patient. *BUN,* blood urea nitrogen; *CT,* computed tomography; *IEP,* immunoelectrophoresis; *PTH,* parathyroid hormone.

(presumably because of mutations in introns and other unchecked regions) and usually are unnecessary. The identification of *RET* gene mutations is now an essential part of the management of families with MEN2 because this information most effectively guides the decision for preventive thyroidectomy to prevent medullary cancer of the thyroid. In contrast, the identification of *MENIN* gene mutations has not yet led to any effective preventive strategies, thus genetic analysis may be useful only for genetic counseling in families with MEN1. Even for this purpose, the incomplete ascertainment of mutations limits the effectiveness of such analysis.

In patients with suspected lithium-induced hyperparathyroidism, a trial off lithium, if feasible clinically, may confirm the diagnosis or indicate the presence of persistent primary hyperparathyroidism. Patients with primary hyperparathyroidism should undergo bone densitometry, preferably at sites rich in cortical and trabecular bone (i.e., forearm or hip and lumbar spine, respectively) to assist in the decision about surgery. Those younger than 40 years of age or having a family history of hypercalcemia (or other MEN manifestations) should be evaluated for these syndromes as well. Patients not meeting criteria for parathyroidectomy should be followed medically, as should those with FHH. In rare patients with CaSR-blocking autoantibodies, hypercalcemia may respond to glucocorticoids.[386]

A low or undetectable serum PTH level signifies the presence of nonparathyroid hypercalcemia and should prompt a detailed evaluation for malignancy or other causes of PTH-independent hypercalcemia (see Table 29.2). Breast and lung cancers alone account for over 50% of all malignancy-associated hypercalcemias. Mammography, chest radiography with or without CT, abdominal CT, and serum and urinary immunoelectrophoresis are among the more useful tests for detecting the cause of nonparathyroid hypercalcemia. Although humoral mechanisms, especially secretion of PTHrP, are implicated in the pathogenesis of most cancer-associated hypercalcemias, bone metastases are common, particularly in breast cancer. Technetium-99m bone scanning therefore generally is useful for detecting this syndrome and identifying bones vulnerable to fracture. The utility of serum PTHrP measurements probably is limited to the unusual situation in which serum PTH is suppressed but an underlying malignancy cannot readily be demonstrated. PTHrP-associated hypercalcemia can occur rarely during pregnancy and lactation, via secretion from benign neoplasms, or in association with lymphoid hyperplasia in lupus erythematosus or human immunodeficiency virus (HIV).[398]

In the absence of evident malignancy, unusual causes of hypercalcemia should be sought.[398] Vitamin D and vitamin A intoxication can be excluded by measurement of serum 25(OH)D and retinoids, respectively. Elevated $1,25(OH)_2D_3$ and hypercalcemia may occur in several settings, including sarcoidosis and other granulomatous diseases, B-cell and T-cell lymphomas (including AIDS-associated lymphomas), and uncommonly, in Crohn disease, in neonatal subcutaneous fat necrosis syndrome, or in epithelial neoplasms such as lung cancer. Very rarely, patients



• **Fig. 29.29** Approach to the management of the hypercalcemic patient with parathyroid hormone–dependent hypercalcemia. *Cl,* clearance; *Fam. Hx.,* family history; *FHH,* familial hypocalciuric hypercalcemia; *Li,* lithium; *PTH,* parathyroid hormone.

with severe idiopathic hypercalciuria and excessive absorption of dietary calcium may manifest mild, dietary-dependent hypercalcemia. Overtreatment of hypoparathyroidism or other conditions with oral $1,25(OH)_2D_3$ or topical use of analogues of the active metabolite in psoriasis should be obvious from the history. Because hypercalcemia and hypercalciuria are observed in up to 10% and 30%, respectively, of patients with thyrotoxicosis, measurement of serum thyroid-stimulating hormone (TSH) may be helpful, especially in older patients who may be less overtly symptomatic. Adrenal insufficiency and pheochromocytoma usually are accompanied by characteristic clinical features, but a definite diagnosis may be sought with appropriate studies. Granulomatous diseases are among the more common disorders that underlie initially unexplained hypercalcemia.

Causes of Severe Hypercalcemia

The need for urgent therapy of acute, severe hypercalcemia, usually defined as a serum calcium concentration greater than 14 mg/dL (>3.5 mmol/L), is unusual. This is because most patients with hypercalcemia have primary hyperparathyroidism, in which hypercalcemia is typically chronic and mild. Episodes of acute, severe hypercalcemia may occur occasionally in primary hyperparathyroidism (parathyroid crisis), usually in patients with large parathyroid adenomas and very high PTH levels. The severe hypercalcemia in this setting may be triggered by dehydration due to diarrheal illness, protracted vomiting or diuretic therapy, recovery from major surgery, immobilization, ingestion of large amounts of oral calcium salts, hemorrhage or rupture of a cystic parathyroid neoplasm, or parathyroid carcinoma.

Most often, acute, severe hypercalcemia is encountered in patients with underlying malignancy, in whom accelerated bone resorption dramatically increases the filtered load of calcium. The ensuing profound hypercalciuria impairs renal tubular sodium reabsorption, which induces progressive extracellular volume depletion, reduces GFR, impairs renal calcium clearance, and further aggravates the hypercalcemia. In many such patients, elevated circulating levels of PTHrP compound the problem by mimicking the action of PTH to enhance distal tubular calcium reabsorption.

Clinical Features of Severe Hypercalcemia

The indications for urgent therapy of hypercalcemia usually relate more to the presence of clinical symptoms of hypercalcemia than to the absolute level of serum calcium, although few clinicians would hesitate to treat patients in whom total serum calcium exceeded 14 mg/dL (>3.5 mmol/L). Many patients with previously mild hypercalcemia become symptomatic when serum calcium concentrations exceed 12 mg/dL (>3 mmol/L). It is important to remember that hypoalbuminemia may mask significant elevations of ionized calcium. The most common symptoms of severe hypercalcemia are referable to disturbances of nervous system and gastrointestinal function—fatigue, weakness, lethargy, confusion, coma (rarely), anorexia, nausea, abdominal pain (rarely

TABLE 29.3 Treatment of Severe Hypercalcemia

Type of Therapy	Usual Dose	Frequency
Rehydration	2–4 L/day of 0.9% NaCl IV	qd × 1–5 days
Furosemide	20–40 mg IV (after rehydration)	q12–24 hr
Pamidronate	60–90 mg IV over 2–4 hr	Once
Zoledronate	4 mg IV over 15–30 min	Once
Denosumab	60 mg SC	Weekly
Calcitonin	4–8 IU/kg SC	q12–24 hr
Gallium nitrate	200 mg/m^2 IV over 24 hr	qd × 5 days
Glucocorticoids	200–300 mg hydrocortisone IV 40–60 mg prednisone PO	qd × 3–5 days qd × 3–5 days
Dialysis		

IV, Intravenous; *PO,* orally; *q,* every; *qd,* every day; *SC,* subcutaneous.

due to pancreatitis), and constipation. Polyuria, nocturia, and polydipsia commonly are present also.

Bone pain is often present but is usually due to underlying metastatic disease. Cardiac arrhythmias may occur, particularly bradyarrhythmias or heart block; digitalis toxicity may be potentiated; and ST-segment elevation responsive to treatment of the hypercalcemia may be seen. Patients who suffer a fatal outcome from acute severe hypercalcemia may manifest coma, hypotension, acute pancreatitis, acute renal failure, widespread soft tissue calcification, heart failure, or venous thrombosis, particularly of the renal veins.

Management of Severe Hypercalcemia

The first decision to be made in the management of acute, severe hypercalcemia is whether or not to treat the problem at all. This may become an issue for the patient with an untreatable, widely disseminated malignancy, when all other approaches to controlling the neoplasm have been exhausted, and the patient has chosen not to have complications treated. Otherwise, as noted earlier, patients who are symptomatic or have serum calcium levels above 14 mg/dL ordinarily should be treated aggressively. Treatment most often entails rehydration and administration of a bisphosphonate intravenously (Table 29.3). Calcitonin can be useful as a temporary measure early in therapy, and glucocorticoids or dialysis may be indicated in some patients.[125]

Volume Repletion

When treatment is indicated, the first priority is to correct the extracellular volume depletion that almost invariably is present, usually by infusing isotonic saline at a rate of 2 to 4 L/day. The aggressiveness with which the individual patient is rehydrated must be considered in relation to both the patient's volume status and the risk of precipitating or aggravating congestive heart failure or ascites. Diuretics, particularly thiazides, should be discontinued. The use of furosemide or other potent "loop" diuretics to promote calciuresis may exacerbate extracellular volume depletion if used too early in the course of treatment. In light of the availability of highly effective alternatives for the therapy of hypercalcemia, such drugs probably are best avoided, except in circumstances in which vigorous rehydration fails to improve severe hypercalcemia or might precipitate congestive heart failure. In any case, prolonged use of saline-induced calciuresis without the early introduction of an effective antiresorptive agent is ill advised and ultimately futile.

Bisphosphonates

Intravenous bisphosphonates rapidly inhibit bone resorption and currently are the agents of first choice in managing severe hypercalcemia that is known or suspected to be driven mainly by osteoclastic bone resorption.[125] Bisphosphonates should not be used in patients with milk-alkali syndrome, for example, in whom they are likely to induce posttreatment hypocalcemia.[454] Pamidronate and zoledronate are approved by the Food and Drug Administration (FDA) for treatment of hypercalcemia of malignancy and are the most widely used agents in the United States, although ibandronate and clodronate have been successfully used elsewhere. These drugs generally are well tolerated, although local pain or swelling at the infusion site, low-grade fever 1 to 2 days after the infusion, transient lymphopenia, and mild hypophosphatemia or hypomagnesemia may occur. Serum calcium usually declines within 24 hours and reaches a nadir within 1 week following a single infusion, at which point calcium levels may be normal in 70% to 90% of treated patients. Intravenous bisphosphonates may be nephrotoxic, but clinical data to guide their use in patients with renal insufficiency are not yet available. Most clinicians will use the standard dose (see Table 29.3)—perhaps at half or less of the usual rate of administration—in patients with moderate renal insufficiency (GFR >30 mL/minute), which is common in the setting of severe hypercalcemia. In patients with more severe renal insufficiency, bisphosphonates probably are best avoided and dialysis may be a more appropriate alternative (see later). The duration of the response to intravenous bisphosphonate treatment is quite variable, ranging from 1 or 2 weeks to several months. Depending on clinical circumstances, repeated courses of therapy may be indicated and effective.

Denosumab

Denosumab, a monoclonal antibody directed against RANKL, has been shown to be effective in managing malignancy-associated hypercalcemia, including patients refractory to bisphosphonates.[455] Denosumab offers an alternative to intravenous bisphosphonates in patients with renal failure, but like bisphosphonates, denosumab carries a small risk of osteonecrosis of the jaw. Typical dosage is 60 mg given subcutaneously every week for a month, followed by 60 mg per month.

Calcitonin

Calcitonin, which directly inhibits osteoclast function, may be used with other antiresorptive agents to achieve more rapid control of severe hypercalcemia. Calcitonin rarely produces a decline in serum calcium of more than 1 to 2 mg/dL, and its efficacy typically is limited to a few days at most, possibly because of receptor downregulation in target cells of bone and kidney. Its major advantages are a more rapid onset of action than bisphosphonates (several hours) and its potential to augment renal calcium excretion directly. Calcitonin generally is well tolerated, although transient nausea, vomiting, abdominal cramps, flushing, and local skin reactions may occur.

Other Approaches to Treatment of Severe Hypercalcemia

Because of their potential toxicity, other antiresorptives such as gallium nitrate, plicamycin (mithramycin), and intravenous

phosphate (in patients with severe hypophosphatemia) have largely been abandoned in the treatment of severe hypercalcemia, although a randomized trial demonstrated that gallium nitrate may be more effective than bisphosphonate in controlling hypercalcemia of malignancy.[456] Oral or enteral phosphate repletion is appropriate for patients with significant hypophosphatemia (<2.5 mg/dL), provided that serum phosphate and renal function are closely monitored. Intravenous or oral glucocorticoids should be considered early in patients with suspected vitamin D–dependent hypercalcemia, including those with lymphoma or granulomatous disease. The response to glucocorticoids may be more delayed than that to bisphosphonates. Successful treatment of hypercalcemia in Crohn disease with infliximab has been reported.[457]

In patients with severe renal insufficiency, with or without complicating heart disease, in whom saline rehydration and associated calciuresis may not be feasible and bisphosphonates probably are best avoided, dialytic therapy against a low-calcium or zero-calcium dialysate may be the most appropriate tactic. In patients with known primary hyperparathyroidism and intercurrent severe hypercalcemia (parathyroid storm), urgent parathyroidectomy (following initial medical stabilization) should be considered.

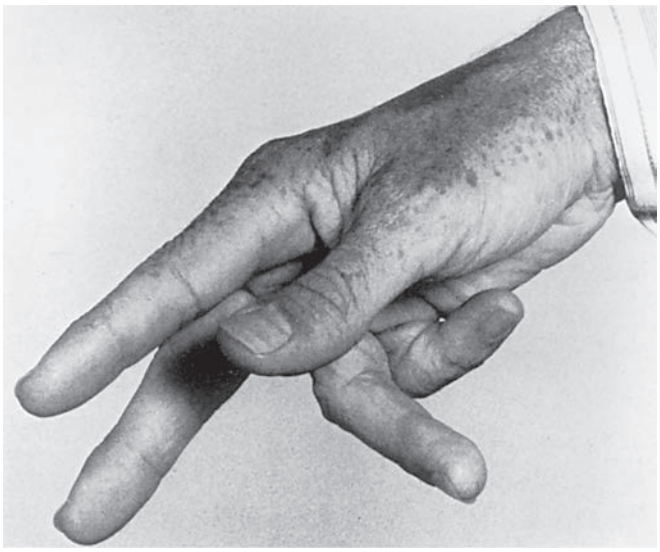
Novel approaches to the treatment of severe hypercalcemia currently are in development. One available therapy for parathyroid carcinoma is the calcimimetic cinacalcet, which may be effective in some patients,[458] and monoclonal antibodies directed against PTHrP could prove useful in controlling PTHrP-dependent hypercalcemia.[459]

Hypocalcemic Disorders

Clinical Presentation

The predominant clinical symptoms and signs of hypocalcemia are those of neuromuscular irritability, including perioral paresthesias, tingling of the fingers and toes, and spontaneous or latent tetany. Tetany can be elicited by percussion of the facial nerve below the zygoma, resulting in ipsilateral contractions of the facial muscle (Chvostek sign) or by 3 minutes of occlusive pressure with a blood pressure cuff resulting in carpal spasm, which, on occasion, can be very painful (Trousseau sign) (Fig. 29.30). The usefulness of these signs in diagnosing hypocalcemia and in following therapeutic responses cannot be overemphasized.

Electrocardiographic abnormalities also result from hypocalcemia, including prolonged QT intervals and marked QRS complex and ST-segment changes that may mimic acute myocardial infarction or conduction abnormalities. Ventricular arrhythmias are a rare complication of hypocalcemia, although congestive heart failure, corrected by normalization of serum calcium, has been reported. In profound hypocalcemia or during acute falls in serum calcium, grand mal seizures or laryngospasm also may be observed. Chronic hypocalcemia is associated with milder symptoms and signs of neuromuscular irritability and may even be asymptomatic. Longstanding hypocalcemia associated with hyperphosphatemia (observed with PTH deficiency or resistance) may lead to calcification of the basal ganglia[460–463] and occasionally, extrapyramidal disorders. In addition, mineral ion deposits in the lens may lead to cataract formation. Chronic hypocalcemia, when associated with hypophosphatemia, as in vitamin D deficiency, is associated with growth plate abnormalities in children (rickets) and defects in the mineralization of new bone (osteomalacia) (see Chapter 31). These findings are not seen in the hypocalcemia of hypoparathyroidism. Severe symptomatic hypocalcemia is an emergency that requires immediate attention to prevent seizures and death from laryngospasm or cardiac causes.



• **Fig. 29.30** Trousseau sign. (From Burnside JW, McGlynn TJ. *Physical Diagnosis*. 17th ed. Baltimore: Williams & Wilkins; 1987:63.)

Total calcium in serum includes both the free (biologically active) and protein-bound components; the major binding protein is albumin (discussed earlier). Therefore measurements of total calcium cannot be interpreted without concurrent measurement of albumin. Studies of hypoalbuminemic patients with cirrhosis have led to a formula for correction of total calcium based on concurrent albumin levels (a decrease in calcium of 0.8 mg/dL for every 1-g/dL decrease in albumin). No formula has proved to be accurate, however, for assessment of calcium in acutely ill patients. This probably relates to the variety of factors that may increase protein binding and decrease the fraction of total calcium present as the free ion, including alkalosis, elevated circulating free fatty acids, and lipid infusions. Consequently, ionized calcium should be measured when the diagnosis of hypocalcemia is considered in the setting of acute illness and severe hypoalbuminemia.

Chronic hypocalcemia is most often due to deficiency of PTH or $1,25(OH)_2D_3$ or to resistance to the biologic effects of these calcium-regulating hormones (Table 29.4).

Parathyroid-Related Disorders

Hypocalcemia associated with parathyroid dysfunction can be differentiated from other causes of hypocalcemia by routine laboratory tests. Serum calcium is low owing to lack of PTH-mediated bone resorption and urinary calcium reabsorption. Serum phosphate is increased owing to impaired renal clearance. Serum $1,25(OH)_2D_3$ is low because PTH and hypophosphatemia stimulate the renal 25(OH)D 1α-hydroxylase. Consequently, $1,25(OH)_2D_3$-mediated intestinal calcium absorption is markedly decreased, further exacerbating the hypocalcemia. PTH levels measured using sensitive two-site PTH assays (see Fig. 29.20) are usually low or undetectable but may be inappropriately normal if some degree of PTH production is preserved. Elevated levels of PTH are found in syndromes associated with resistance to the biologic effects of PTH.

Congenital or Inherited Parathyroid Disorders

Several rare syndromes associated with congenital or inherited hypoparathyroidism appear sporadically or in a variety of

TABLE 29.4 Causes of Hypocalcemia

Parathyroid-Related Disorders

Absence of the Parathyroid Glands or of PTH

Congenital
- DiGeorge syndrome
- X-linked or autosomally inherited hypoparathyroidism
- Autoimmune polyglandular syndrome type I
- PTH gene mutations

Postsurgical hypoparathyroidism
Infiltrative disorders
- Hemochromatosis
- Wilson disease
- Metastases

Hypoparathyroidism post radioactive iodine thyroid ablation

Impaired Secretion of PTH

Hypomagnesemia
Respiratory alkalosis
Activating mutations of the calcium sensor or *GNA11*

Target Organ Resistance

Hypomagnesemia
Pseudohypoparathyroidism
- Type I
- Type II

Vitamin D-Related Disorders

Vitamin D deficiency
- Dietary absence
- Malabsorption

Accelerated loss
- Impaired enterohepatic recirculation
- Anticonvulsant medications
- CYP3A4 mutation

Impaired 25 hydroxylation
- Liver disease
- Isoniazid
- *CYP2R1* mutation

Impaired 1α-hydroxylation
- Renal failure

Vitamin D–dependent rickets, type I
Oncogenic osteomalacia
Target organ resistance
- Vitamin D–dependent rickets, type II
- Phenytoin

Other Causes

Excessive deposition into the skeleton
- Osteoblastic malignancies
- Hungry bone syndrome

Impaired bone resorption
- Vitamin D deficiency
- Bisphosphonates
- RANKL inhibition

Chelation
- Foscarnet
- Phosphate infusion
- Infusion of citrated blood products
- Infusion of EDTA containing contrast reagents
- Fluoride

Neonatal hypocalcemia
- Prematurity
- Asphyxia
- Diabetic mother
- Hyperparathyroid mother
- Vitamin D–deficient mother
- Infantile malignant osteopetrosis

HIV
- Drug therapy
- Vitamin D deficiency
- Hypomagnesemia
- Impaired PTH responsiveness

Critical illness
- Pancreatitis
- Toxic shock syndrome
- Intensive care unit patients

CYP2R1, Cytochrome P450, family 2, subfamily R, member 1; *CYP3A4*, cytochrome P450, family 3, subfamily A, member 4; *EDTA*, ethylenediaminetetraacetic acid; *GNA11*, G protein subunit alpha 11; *PTH*, parathyroid hormone; *RANKL*, receptor activator of nuclear factor κB ligand; *X*, X chromosome.

inheritance patterns, suggesting multiple causes. Mutation of a parathyroid-specific transcription factor, glial cells missing homolog B (GCMB) (chromosome 6p23), which is expressed in the PTH-secreting cells of the developing parathyroids, has been shown to be a cause of familial hypoparathyroidism in humans and mice.[44] The genetic abnormality responsible for X-linked, recessive hypoparathyroidism has been identified as a deletion/insertion of DNA near the *SOX3* gene at Xq26-Xq27.[47] Analyses of a second kindred implicated mutations in the *FHL1* (four and a half LIM domains 1) gene as the molecular basis for the phenotype.[464]

In a number of diseases, hypoparathyroidism is associated with multiple abnormalities in embryonic development in the neck/chest region. DiGeorge syndrome occurs sporadically and is associated with an embryologic defect in the formation of the third, fourth, and fifth branchial pouches, resulting in the absence of parathyroid glands. DiGeorge syndrome may in fact be a neurocrestopathy, since ablation of the premigratory cephalic neural crest in chick embryos produces the same phenotype.[465] The contribution of homeobox genes to parathyroid development and their potential relationship to DiGeorge syndrome also has been demonstrated by the absence of thymic and parathyroid tissue, accompanied by cardiac and craniofacial abnormalities, in mice lacking the homeobox gene *hoxa3*.[466] DiGeorge syndrome is often associated with other congenital abnormalities in a syndrome referred to by the acronym CATCH 22 (*c*ardiac defect, *a*bnormal facies, *t*hymic hypoplasia, *c*left palate, *h*ypocalcemia, and *22*q11 deletions).[467] Microdeletion of 22q11.21–q11.23 and a t(2;22)(q14;q11) balanced translocation suggest that a gene at chromosome 22q11 may be pathogenetic in this syndrome. Recent studies identified the *TBX1* gene as being the locus affected since point mutations in this gene result in DiGeorge syndrome.[463] A number of cases of DiGeorge and velocardiofacial syndromes have been shown to have no detectable abnormality at 22q11, but rather terminal 10p deletions or interstitial 10p13/10p14 deletions, suggesting that two loci may be critical for development of branchial pouch structures. Terminal deletions of 10p accompanied by hypoparathyroidism can be further subdivided into DiGeorge critical region II (10p13-14) and a more telomeric region (10p14-10pter), wherein mutation of the transcription factor GATA3 causes the syndrome of hypoparathyroidism, sensorineural deafness, and renal anomaly (HDR).[468] Studies in murine models have demonstrated that GATA3 regulates the expression of GCM2 (the murine homolog of GCMB) and is essential for the

development of parathyroid glands.[469] The genetic basis for Kenny-Caffey syndrome type 1, characterized by hypoparathyroidism, extreme short stature, mental retardation, and dysmorphism, is mutations in the chaperone protein, TBCE, which is required for the proper folding of α tubulin and the formation of α-β tubulin heterodimers.[470] In contrast, autosomal dominant Kenny-Caffey syndrome type 2, characterized by short stature, hypoparathyroidism, and osteocraniostenosis, is caused by mutations in *FAM111A,* a gene of unknown function with protease motifs.[461]

Familial hypoparathyroidism is seen in conjunction with mucocutaneous candidiasis, Addison disease, and other immune disorders in autosomal recessive autoimmune polyglandular syndrome, type I, caused by mutations in the autoimmune regulatory gene (*AIRE*)[471,472] (see Chapter 43). NALP5 has been identified as a parathyroid-specific antigen in affected patients.[473] Hypoparathyroidism may also be observed in association with mitochondrial myopathies such as mitochondrial trifunctional protein deficiency[474] and the Kearns-Sayre syndrome.[475] Other inherited forms of hypoparathyroidism may be observed as an isolated defect or may present with other features such as lymphedema, dysmorphism, and renal and cardiac abnormalities.

Abnormalities in the PTH Gene

Specific defects have been found in the PTH gene in a small number of kindreds affected by congenital hypoparathyroidism. These include point mutations in the signal peptide[4,5] and in an intron border, leading to aberrant splicing[476] as well as a homozygous mutation in exon 2 of the PTH gene, leading to premature termination of the transcript.[477] A homozygous mutation in amino acid 25 of PTH(1-84) has also been described in familial hypoparathyroidism.[478]

Destruction of the Parathyroid Glands

The most common cause of chronic hypocalcemia is postsurgical hypoparathyroidism. This may occur after removal of all parathyroid tissue during thyroidectomy and radical neck dissection for malignancies or after inadvertent interruption of the blood supply to the parathyroid glands during head and neck surgery. Transient hypoparathyroidism, attributed to reversible damage to the remaining normal glands, is common after parathyroidectomy; permanent hypoparathyroidism may occur after vascular or surgical injury or inadvertent removal of all parathyroid tissue. Rarely, transient hypoparathyroidism may follow spontaneous infarction of autonomous tissue in primary hyperparathyroidism.[479] Hypoparathyroidism is a rare complication of radioactive iodine ablation of the thyroid gland for Graves disease.[480]

Hypoparathyroidism also can occur as a result of infiltrative diseases of the parathyroids. This is seen in diseases of iron overload such as hemochromatosis and in patients with thalassemia major who have been heavily transfused.[481] Copper deposition in Wilson disease[482] may also cause parathyroid dysfunction. Metastatic disease to the parathyroids can cause hypoparathyroidism, but rarely, presumably because of the need for four-gland involvement before significant hypoparathyroidism is observed.

Impaired PTH Secretion

Impaired secretion of PTH from the parathyroid glands can lead to functional hypoparathyroidism. This is commonly seen in profound hypomagnesemia,[483] in which target organ resistance to PTH can also occur. Both of these abnormalities are reversible upon magnesium repletion[484,485] (see "Disorders of Magnesium Metabolism").

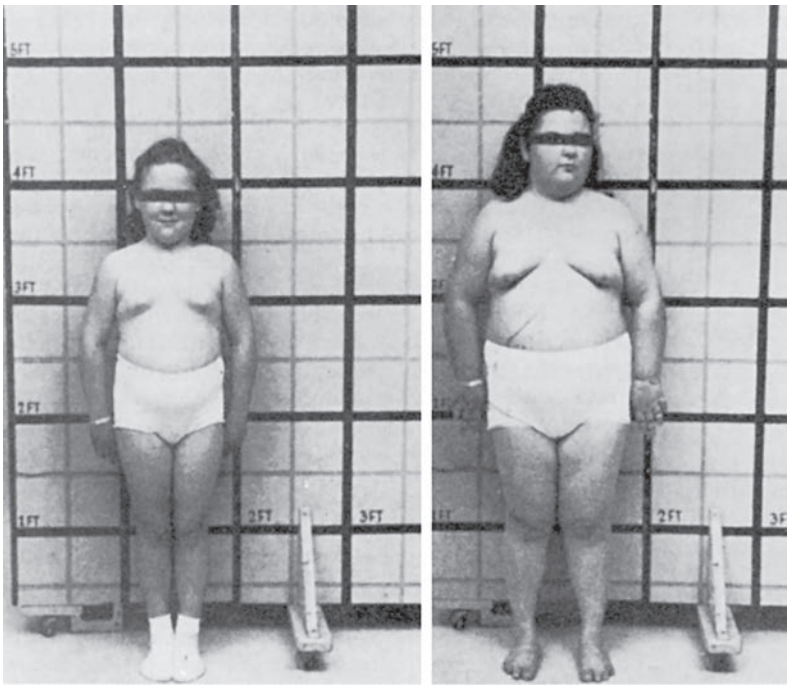
Chronic respiratory alkalosis leads to hyperphosphatemia and decreased ionized calcium levels accompanied by impaired renal calcium resorption and inappropriately normal PTH levels.[486] This biochemical phenotype suggests both an abnormality of PTH secretion and renal resistance to PTH. Acute alkalosis in dogs also suppresses PTH secretion.[487]

Activating mutations in the CaSR cause autosomal dominant hypocalcemia type 1 (ADH1) associated with inappropriately normal PTH levels. This syndrome can also be seen in patients with activating antibodies directed against this receptor.[488] ADH2 has been shown to be caused by gain-of-function mutations of the G protein subunit α11 (*GNA11*).[381] The clinical syndrome is variable; patients present with hypocalcemia and seizures, whereas their affected relatives may be only subsequently diagnosed with asymptomatic hypocalcemia.[14] Unlike patients with inactivating mutations of the calcium sensor, homozygously affected individuals with ADH1 do not appear to have a more severe phenotype. The presence of hypercalciuria in these patients makes medical management uniquely challenging. Treatment with vitamin D metabolites often results in a marked increase in renal calcium excretion, associated with renal calcification and resultant renal impairment. Based on these observations, it has been suggested that asymptomatic individuals be left untreated and that the goal of therapy in individuals with symptomatic hypocalcemia be solely to relieve symptoms, not to achieve normocalcemia. Treatment with calcium and vitamin D metabolites should be accompanied by the use of thiazide diuretics to decrease urinary calcium excretion as well as ensuring adequate urinary volume to decrease urinary calcium concentration.

Pseudohypoparathyroidism

The idiopathic and inherited forms of PTH resistance are referred to as pseudohypoparathyroidism (PHP). There are two main types of PHP: type I, which is characterized by impaired cAMP and phosphaturic responses to PTH, and type II, where the cAMP response is preserved but the phosphaturic response is not. The first cases of documented PTH resistance were described by Albright in 1942. The patients were hypocalcemic, hyperphosphatemic, and exhibited a number of features now called Albright hereditary osteodystrophy (AHO). These features include short stature, rounded face, foreshortened fourth and other metacarpals, obesity, and subcutaneous ossifications (Figs. 29.31 and 29.32). PTH administration to these patients failed to provoke a phosphate diuresis or an increase in serum calcium. It was subsequently demonstrated that hypocalcemic patients with features of AHO had elevated PTH levels and that PTH infusions failed to stimulate renal production of cAMP. Failure of stimulation of cAMP production suggested a defect in the PTH receptor or in its cAMP-mediated signal transduction. The measurement of cAMP in the urine following an infusion of synthetic PTH(1-34) can be used to establish the diagnosis of PTH resistance.[489]

The variable presence of AHO and renal resistance to PTH has led to the subclassification of pseudohypoparathyroidism (Table 29.5). Type IA is characterized by AHO and diminished $G\alpha_s$ activity (~50% of normal). The diminished $G\alpha_s$ activity has been demonstrated in several tissues, including kidney, fibroblasts, transformed lymphocytes, platelets, and erythrocytes. Decreased amounts of $G\alpha_s$ mRNA (50%) are present in fibroblasts of many patients with PHP1A, and inactivating mutations involving exons 1 to 13 of *GNAS*, the gene encoding $G\alpha_s$, have been identified in numerous kindreds and individual patients. Epigenetic changes like those seen in PHP1B (to come) play an etiologic role in a subset of patients with PHP1A.[490] Mutations in *PRKAR1A, PDE3A,* and *PDE4D* have also been found in subjects with acrodysostosis and clinical findings similar to those in patients with AHO without GNAS abnormalities; however, most of these patients have no or only mild PTH resistance.[491]

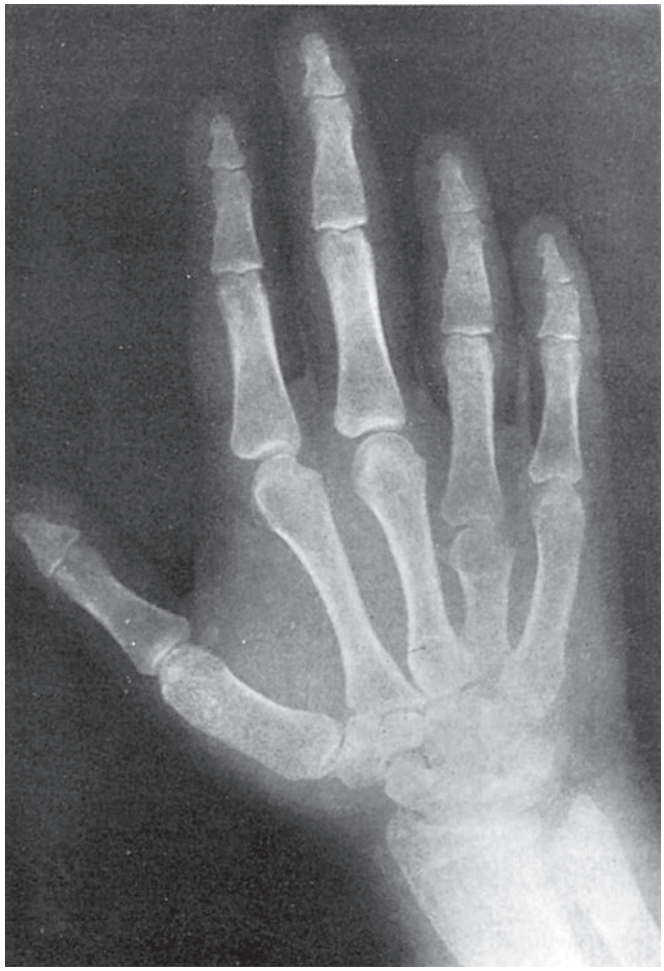


• **Fig. 29.31** Daughter *(left)* and mother *(right)* with pseudohypoparathyroidism and Albright hereditary osteodystrophy.

Impaired cognition is seen in approximately half of the patients with PHP1A and appears to be related to the $G\alpha_s$ deficiency rather than to chronic hypocalcemia, since patients with other forms of hypocalcemia do not have cognitive impairment. The $G\alpha_s$ deficiency in PHP1A may be associated not only with PTH resistance but also with resistance to other hormones such as TSH, glucagon, and gonadotropins, resulting in thyroidal and gonadal dysfunction. Paradoxically, two unrelated males with both PHP1A and gonadotropin-independent precocious puberty have been described. The $G\alpha_s$ point mutation found in these individuals is thought to lead to a protein that is unstable at 37°C and therefore confers renal resistance to PTH. At the lower temperature of the testes, however, the protein is not degraded. In this setting, the stable but mutated protein is constitutively active and stimulates the Leydig cell in a manner similar to the skeletal effects of the G_s mutations in McCune-Albright syndrome (see Chapter 26).[492]

Individuals with PHP1A also exhibit a decrease in resting energy expenditure[493] and a decrease in insulin sensitivity,[494] which may contribute to the development of diabetes.

Pseudo-pseudohypoparathyroidism (pseudo-PHP) is a term used to refer to individuals with the phenotype of AHO but with normal biochemical parameters. Patients with pseudo-PHP often are found in the same kindreds as those with PHP1A, but never in the same sibship, and they invariably inherit the same *GNAS* mutations involving the $G\alpha_s$-encoding exons found in their PTH-resistant relatives.[495] When patients inherit the *GNAS* mutation from their father, they exhibit pseudo-pseudohypoparathyroidism; when they inherit the mutant allele from their mother, they exhibit PHP1A.[496,497] This pattern, in which the phenotype depends on the parent of origin, is termed *genetic imprinting*; mice with targeted ablation of *Gnas* also display such imprinting.[498] In addition to the AHO phenotype, inheritance of paternal mutations involving *GNAS* exons 1 to 13 leads to severe intrauterine growth retardation suggesting a role for an mRNA transcribed from the paternal GNAS locus in fetal growth and development.[499]

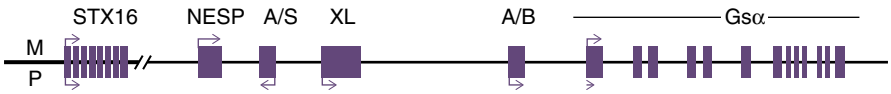


• **Fig. 29.32** Radiograph of the hand from a patient with pseudohypoparathyroidism and Albright hereditary osteodystrophy. Note the shortened fourth metacarpal.

TABLE 29.5 Types of Pseudohypoparathyroidism

Disorder	Urinary cAMP Response to PTH	Urinary PO_4 Response to PTH	Other Hormonal Resistance	AHO	Pathophysiology
Pseudohypoparathyroidism IA	Decreased	Decreased	Yes	Yes	$G\alpha_s$ mutation or imprinting abnormality
Pseudo-pseudohypoparathyroidism	Normal	Normal	No	Yes	$G\alpha_s$ mutation
Pseudohypoparathyroidism IB	Decreased	Decreased	Rare	No	GNAS1 locus imprinting abnormality
Pseudohypoparathyroidism IC	Decreased	Decreased	Yes	Yes	$G\alpha_s$ function normal, imprinting abnormality
Pseudohypoparathyroidism II	Normal	Decreased	No	Rare	Vitamin D deficiency or myotonic dystrophy

AHO, Albright hereditary osteodystrophy; *cAMP*, cyclic adenosine monophosphate; *GαS*, G protein alpha subunit S; *GNAS1*, guanine nucleotide-binding protein, alpha-stimulating activity polypeptide 1; *PO_4*, phosphate; *PTH*, parathyroid hormone.



• **Fig. 29.33** The GNAS locus. A schematic representation of the GNAS locus is shown, with the *black boxes* indicating exons for STX16, NESP55 *(NESP),* the antisense *(A/S)* NESP55 transcript, $XL\alpha_s$ *(XL),* A/B and $G\alpha_s$ *(Gsα).* The start site of and direction (sense vs antisense) of transcription is indicated by the *arrows*. Genes that are maternally transcribed (M) are indicated by *arrows* above the relevant genes, whereas those that are paternally transcribed (P) are indicated below. The expression of $XL\alpha_s$, A/B, and A/S is from the paternal allele, whereas the maternal NESP55 transcript is expressed. Deletions from exon 4 to 6 or, less commonly, from exon 2 to 4, along with associated introns, in the region containing the STX16 gene are associated with imprinting abnormalities in GNAS. The *arrowhead* below the Gsα locus indicates that only the maternal allele is expressed in the renal tubules.

The observation of a phenotype in a heterozygous loss of function mutation affecting $G\alpha_s$ is in contrast to the findings in mice with targeted deletions of the other Gα genes ($G\alpha_{i2}$, $G\alpha_o$, $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{13}$), in which a phenotype is observed only in the homozygous state.[500] The fact that the *GNAS* gene is imprinted in certain tissues has partly resolved the dilemma of this dominant phenotype. Notably, mice with targeted ablation of one *GNAS* exon 1 fail to express $G\alpha_s$ mRNA in the renal cortex when the mutant gene is inherited from the mother but have normal expression in the cortex when the mutant gene is inherited from the father. No such imprinting pattern is seen in the inner medulla; this correlates with the mice (and patients) exhibiting PTH, but not vasopressin, resistance.[498]

Pseudohypoparathyroidism type IB (PHP1B) presents with hypocalcemia, high PTH levels, and failure of PTH infusions to increase urinary cAMP production. However, in most patients it is neither accompanied by any of the clinical features of AHO nor associated with abnormal $G\alpha_s$ levels in fibroblasts. Although mild TSH resistance is frequent,[501–503] renal resistance to PTH is the most prominent feature of PHP1B; therefore several investigators had postulated that this syndrome was due to an isolated abnormality of the PTH receptor. However, a search for mutations in the coding exons of the receptor gene failed to reveal a functional receptor abnormality in those affected.[449] Subsequently, a family with a homozygous mutation in the PTH receptor coding sequence was identified, with some manifestations of PTH resistance and cognitive difficulties, but without functional testing of the mutant receptor.[504] Further studies are needed to clarify the role of the receptor mutation in this family's presentation. The target organ manifestations of PHP1B are variable, with some affected individuals having manifestations of PTH overactivity in bone and PTH resistance in the proximal tubule of the kidney. Cultured osteoblast-like cells from a patient with this disorder demonstrated normal cAMP responsiveness to PTH, despite the lack of renal responsiveness.[505]

The locus responsible for the familial, autosomal dominant forms of PHP1B resides on chromosome 20q13.3,[506] the same region that contains the *GNAS* gene encoding $G\alpha_s$. The methylation defect found most consistently in these PHP1B patients is a loss of methylation at exon A/B, which leads to decreased $G\alpha_s$ expression in the proximal renal tubule leading to the hormone resistance observed.[507] While the familial forms (15–20%) exhibit autosomal dominant inheritance,[508,509] the disease is otherwise inherited with the imprinting characteristic of PHP1A. Autosomal dominant PHP1B is caused in most cases by heterozygous maternal deletions within STX16 or GNAS, which are associated with loss-of-methylation (LOM) at exon A/B either alone or loss of methylation at three maternal GNAS promoters, respectively. The disease-causing mutations for the sporadic form of PHP1B remains unknown (except for a few cases with paternal uniparental disomy of 20q[501]), but it is the most frequent form of the disease and is also characterized by imprinting abnormalities of the *GNAS* locus.[502,507,510] The *GNAS* locus gives rise to multiple transcripts (Fig. 29.33), including $G\alpha_s$, which is biallelically expressed in most tissues, but only the maternal transcript is expressed in the renal proximal tubules, thyroid, gonads, pituitary, and possibly a few other tissues. In contrast, the XLαs, A/B, and antisense (A/S) transcripts are paternally expressed, whereas the NESP55 transcript is maternally expressed. Linkage analyses in multiple kindreds had pointed to deletion of a 3-kb region, approximately

220 kb centromeric of *GNAS*, in most families, in addition to an overlapping deletion in another family.[511,512] These deletions remove several *STX16* exons, which encode syntaxin-16, a protein that plays a role in intracellular trafficking, as well as associated introns. Although the development of PHP1B correlates with maternal inheritance of this gene defect and loss of methylation at the distant *GNAS* exon A/B, *STX16* itself is not imprinted.[512] Other deletions in this region, including that of DNA encoding NESP55, a chromogranin-like neurosecretory peptide, and of two noncoding *GNAS* antisense exons have also been found in autosomal dominant PHP1B.[512–514] Maternal inheritance of these deletions causes abnormal imprinting in this locus.

While most sporadic cases of PHP1B are not associated with *STX16* or NESP55/AS deletions, they are uniformly associated with impaired methylation of *GNAS* exon A/B. In addition to the methylation defects in the A/B region, gain of methylation at NESP55 and loss of methylation of XL and of the promoter of the A/S transcript are found. Some sporadic PHP1B cases share the same maternal *GNAS* haplotype with an unaffected sibling and an unaffected daughter, thus excluding the *GNAS* region as the disease-causing locus.[515] It is likely that an additional genetic locus underlies the methylation abnormalities in both sporadic and some familial PHP1B cases. Whether a common locus will be identified as the genetic basis for the majority of these PHP1B cases remains to be determined.

Several patients with AHO and PTH resistance have been found to have normal $G\alpha_s$ activity; this subgroup has been designated *PHP1C*. However, these patients have mutations involving the carboxy-terminal region of $G\alpha_s$ that lead to normal levels of $G\alpha_s$ activity when assayed in erythrocytes but defective activation by receptors.[516] Additional analyses of methylation patterns of A/B, XL, A/S, and NESP55 in 26 patients with normal $G\alpha_s$ activity demonstrated abnormal methylation patterns in at least one of these differentially methylated *GNAS* regions in six individuals, raising the possibility that these individuals represent sporadic PHP1B cases.[517]

In PHP2, PTH infusions increase urinary cAMP normally; however, PTH does not elicit a phosphaturic response.[518] This syndrome, like PHP1B, lacks signs of AHO, and no familial cases have been described. The age of onset of patients with this disorder is variable, ranging from infancy to senescence, suggesting that it is an acquired defect or that the biochemical phenotype may be unmasked by intercurrent abnormalities. A subset of patients with myotonic dystrophy display the biochemical features of PHP2, the degree of PTH resistance correlating with the degree of expansion of the pathogenic CTG repeats in the myotonin protein kinase gene. A similar biochemical phenotype can also be observed in vitamin D deficiency.[519] Minagawa and colleagues reported cases of three neonates with no signs of rickets and with normal levels of vitamin D who presented with transient PHP2 that resolved at about 6 months of age.[520] They postulated that PTH responsiveness is subject to maturation during fetal and neonatal development. Several unrelated patients with acrodysostosis, displaying a skeletal phenotype similar to AHO and a biochemical phenotype consistent with PHP2, were found to have heterozygous mutations in *PRKAR1A,* the gene encoding the regulatory subunit of PKA. Mutation in this gene impairs the protein kinase A response to cAMP stimulation, consistent with the normal urinary cAMP response to PTH observed in PHP2.[521] PHP2 therefore seems to reflect a heterogeneous clinical disorder associated with defects in PTH responsiveness distal to cAMP or involving a separate signal transduction pathway.[522]

Unlike the proximal tubules, which show resistance to PTH in PHP1A and PHP1B, bone-forming osteoblasts isolated from patients with PHP1A and PHP1B[505] show a normal cAMP response to PTH. This suggests that the hypocalcemia in PHP is not secondary to skeletal resistance but is a consequence of the renal resistance to PTH and the associated low levels of $1,25(OH)_2D$. Thus hypocalcemia occurs in the face of relatively normal bone and renal distal (hypocalciuric) tubular responses to PTH.[523] The lack of activation of vitamin D results in diminished intestinal calcium absorption and osteomalacia, both of which further exacerbate the hypocalcemia. Deficiency of $1,25(OH)_2D$ and the resultant hypocalcemia can, in turn, impair the phosphaturic but not the urinary cAMP responses to PTH[524]; therefore it is imperative that studies to confirm the diagnosis of PHP2 be performed in normocalcemic patients who have normal vitamin D status. Recent investigations have demonstrated that, despite the presence of secondary hyperparathyroidism, region-specific bone density is not reduced in PHP1A, and total body bone mineral density is greater than normal.[525] Patients with PHP1B have been reported to have skeletal changes consistent with hyperparathyroid bone disease that resolve with treatment.[526,527] Additionally, two brothers with PHP1B due a *STX16* deletion were found to have osteosclerosis with lumbar Z-scores of +5.4 and +4.9 associated with increased cortical width, and bone formation rate on trabecular and endocortical surfaces on iliac crest biopsy.[528]

Vitamin D–Related Disorders

Hypocalcemia secondary to vitamin D deficiency or resistance to the biologic effects of $1,25(OH)_2D_3$ is easily differentiated from the hypocalcemia of hypoparathyroidism by routine clinical and laboratory evaluation. The primary cause of hypocalcemia in vitamin D deficiency is decreased intestinal absorption of calcium. In the setting of normal renal function, the hypocalcemia of vitamin D deficiency, unlike that of hypoparathyroidism, is accompanied by hypophosphatemia and increased renal phosphate clearance. This increase in phosphate clearance is a direct result of compensatory (secondary) hyperparathyroidism. The hyperparathyroidism is a consequence of the hypocalcemic stimulus to PTH secretion, as well as the stimulation of PTH gene expression and parathyroid cell proliferation caused by hypocalcemia (see "Parathyroid Hormone Biosynthesis"). Therefore measurement of serum phosphate and PTH are very useful in distinguishing these disorders from hypoparathyroidism. The secondary hyperparathyroidism results in increased calcium mobilization from the skeleton, increased renal reabsorption of calcium, and increased renal 1α-hydroxylation of 25(OH)D. In severe vitamin D deficiency, the increased levels of PTH no longer lead to increased bone resorption, perhaps because osteoclasts do not resorb unmineralized osteoid.

In profound vitamin D deficiency the level of $1,25(OH)_2D_3$ is usually low, but in moderate vitamin D deficiency the stimulation of the renal 1α-hydroxylase by PTH can result in a normal or even elevated $1,25(OH)_2D_3$ level. These high levels of $1,25(OH)_2D_3$ reflect the action of PTH on the renal 1α-hydroxylase. The ineffectiveness of the high levels of total $1,25(OH)_2D_3$ to normalize serum calcium may be explained by increased binding of this metabolite to vitamin D binding protein when the levels of 25(OH)D are very low or may be a consequence of impaired local activation of 25(OH)D.

Vitamin D Deficiency

Because the two sources of vitamin D are the diet and cutaneous synthesis after ultraviolet (UV) irradiation, lack of solar irradiation

and decreased intake or impaired absorption of vitamin D can lead to vitamin D deficiency. As the population has become increasingly educated about the risks of skin cancer from solar irradiation, the avoidance of long periods of intense sun exposure and the use of high solar protective factor (SPF) sunblocks have resulted in increased reliance on dietary sources of vitamin D. Based on the Institute of Medicine (USA) 2010 report, the recommended daily allowance for vitamin D is 600 IU from 1 to 70 years of age and 800 IU for those over 70. However, increased supplementation may be required to maintain vitamin D sufficiency during pregnancy,[529] in those with underlying medical conditions, as well as the elderly and the obese.[530,531] Genetic variants in vitamin D metabolizing enzymes, including CYP2R1 and CYP24A1, in the VDR and in vitamin D–binding protein also impact the vitamin D intake required to achieve normal levels.[532,533] Vitamin D is present in many food sources, both vegetable and animal. In addition, many prepared foods, especially cereal products, are fortified with vitamin D. Although dairy products have been fortified with vitamin D as well, the actual amount of vitamin D provided does not correlate well with the purported content.[183] The vitamin D derived from vegetable sources is vitamin D_2 and that from animal sources is vitamin D_3. These two forms of vitamin D are metabolized identically and are used to fortify foods.

The Institute of Medicine has defined vitamin D "sufficiency" as a level greater than 50 nmol/L (>20 ng/mL); however, higher levels may be required to optimize intestinal calcium absorption in those with disorders such as intestinal disease, short bowel syndrome, intestinal bypass, and obesity as well as in the elderly. Although elderly, homebound individuals are at high risk, several studies have demonstrated that vitamin D deficiency is prevalent in the general population (as reviewed in Thomas and Demay[534]). The clinical relevance of this vitamin D deficiency has been confirmed by a study demonstrating that vitamin D administration (800 IU/day) to an ambulatory elderly population decreases serum PTH levels as well as the incidence of hip fracture.[535] Similarly, a meta-analysis of randomized clinical trials performed between 2011 and 2015 supports the use of calcium and vitamin D for the prevention of fractures.[536] Malabsorption also remains an important cause of vitamin D deficiency in all age groups. Because vitamin D is a fat-soluble vitamin, its absorption is dependent upon emulsification by bile acids. Any cause of fat malabsorption or short bowel syndrome (including bypass surgery for morbid obesity) can result in vitamin D deficiency, therefore malabsorption should be ruled out in patients with low 25(OH)D levels.

Accelerated Loss or Inactivation of Vitamin D

25(OH)D and $1,25(OH)_2D_3$ are secreted with bile salts and undergo enterohepatic circulation, therefore intestinal disease may also result in vitamin D deficiency due to excessive losses. Increased metabolism of vitamin D, leading to low blood levels of 25(OH)D, is seen in individuals given anticonvulsant medications and antituberculous therapy. Phenobarbital, primidone, phenytoin,[537] rifampin, and glutethimide[538] have all been reported to accelerate the hepatic inactivation of vitamin D.

Two unrelated subjects with early-onset rickets and impaired responsiveness to vitamin D metabolites were found to have a mutation in CYP3A4. This mutation dramatically increased the ability of CYP3A4 to inactivate vitamin D metabolites.[539]

Impaired 25-Hydroxylation of Vitamin D

The vitamin D that is absorbed undergoes 25-hydroxylation in the liver, therefore severe hepatic parenchymal damage can result in 25(OH)D deficiency. Clinically, severe vitamin D deficiency as a consequence of liver disease is rare, since the degree of hepatic destruction necessary to impair 25-hydroxylation is incompatible with long-term survival. However, isoniazid has been shown to decrease the 25-hydroxylation of vitamin D.[540]

Studies in individuals with mutations in CYP2R1 have identified this gene as the principal 25-hydroxylase in humans. The clinical and biochemical presentations and therapeutic responses support an inherited 25-hydroxylation defect.

Impaired 1α-Hydroxylation of 25-Hydroxyvitamin D

The final step in the activation of vitamin D is the hydroxylation of 25(OH)D by the renal 1α-hydroxylase to yield $1,25(OH)_2D_3$. Renal parenchymal damage therefore can result in deficiency of the active metabolite of vitamin D. Impaired 1α-hydroxylation is observed once creatinine clearance decreases to approximately 30 to 40 mL per minute. Unlike with liver failure, with renal failure, dialysis permits long-term survival, therefore deficiency of $1,25(OH)_2D_3$ as a result of impaired renal 1α-hydroxylation is a common and important clinical entity. The metabolic consequences of chronic renal failure on the parathyroid glands and the skeleton are complex. Impaired renal 1α-hydroxylation leads to decreased intestinal absorption of calcium, resulting in hypocalcemia. The diminished phosphate clearance associated with renal failure leads to elevated levels of blood phosphate and consequently increases in circulating FGF23; this, in turn, further lowers levels of $1,25(OH)_2D_3$ and calcium. The resultant secondary hyperparathyroidism increases release of calcium and phosphate from bone; however, because of the renal insufficiency, PTH does not have a phosphaturic effect. As a result, serum phosphate rises further. Oral phosphate binders are used to lower blood phosphate, including calcium-containing antacids and the phosphate-binding exchange resin, sevelamer. Calcium administration also attenuates the hypocalcemic stimulus to parathyroid secretion. $1,25(OH)_2D_3$ therapy is critical for the absorption of this calcium and should be administered early in the course of renal failure (when the creatinine clearance falls below 30–40 mL/minute) to avoid the development of secondary hyperparathyroidism, with careful monitoring to avoid hypercalcemia. Once secondary hyperparathyroidism has developed, pharmacologic doses of $1,25(OH)_2D_3$, delivered intravenously or orally, or calcimimetics[541] may be required to suppress PTH gene transcription and parathyroid cellular proliferation.

Decreased levels of $1,25(OH)_2D_3$ may also be observed in patients taking ketoconazole and in ADHR, X-linked hypophosphatemia, and TIO, diseases associated with high FGF23 levels (see Chapter 31).[190]

A rare heritable defect of vitamin D activation has been described in several kindreds. Biochemically, pseudo-vitamin D deficiency rickets (PDDR) is characterized by hypocalcemia and secondary hyperparathyroidism. The only metabolic abnormalities that differentiate it from dietary vitamin D deficiency are the presence of normal or elevated levels of vitamin D and 25(OH)D accompanied by low levels of $1,25(OH)_2D_3$.[542,543] The disease is inherited in an autosomal recessive fashion and presents in infancy with rickets, osteomalacia, and seizures.[544] Cloning of the 1α-hydroxylase gene confirmed that mutation of this gene is the molecular basis for the disorder and, as expected, physiologic replacement doses of 1α-hydroxylated metabolites of vitamin D result in clinical remission.[545]

Target Organ Resistance to 1,25(OH)$_2$D$_3$

A second rare inherited disorder, characterized by resistance to the biologic actions of 1,25(OH)$_2$D$_3$, has been described in several kindreds. This disorder, referred to as hereditary vitamin D–resistant rickets (HVDRR) is also characterized by autosomal recessive inheritance. Its biochemical presentation, with hypocalcemia, hypophosphatemia, and secondary hyperparathyroidism, resembles that of vitamin D deficiency, but it is accompanied by elevated levels of 1,25(OH)$_2$D$_3$. The molecular basis for this disease is mutation of the vitamin D receptor gene, resulting in impaired target organ responsiveness. Most of the mutations that have been described involve the DNA-binding domain of the receptor. These mutations result in a decreased affinity of the receptor for its response elements on target genes leading to impaired regulation of these genes. Mutations in the hormone-binding and nuclear receptor coactivator–binding domains of the receptor have also been described in kindreds with HVDRR.[546]

The clinical presentation of HVDRR is variable; however, most patients present in infancy with rickets, hypophosphatemia, and seizures, although presentation in late adolescence has also been described. Alopecia totalis, developing in the first 2 years of life, is present in some kindreds. The finding of alopecia in mice with VDR mutations[35] confirms that alopecia is due to disruption of the VDR gene.

Because of target organ resistance to the active metabolite of vitamin D, there is no ideal treatment for HVDRR. Pharmacologic doses of vitamin D—25(OH)D, 24,25(OH)D, and 1,25(OH)$_2$D$_3$—have been administered in an attempt to overcome this target organ resistance[547] with variable effects. In those patients in whom the hypocalcemia and osteomalacia are resistant to such therapeutic interventions, parenteral calcium infusions have been used to heal osteomalacic lesions.[209] Studies in VDR-ablated mice have demonstrated that maintenance of normal mineral ion homeostasis prevents all the complications of VDR ablation except alopecia.[208,210] Based on these observations, patients with VDR mutations should be treated early and aggressively to prevent skeletal abnormalities and parathyroid hyperplasia. Lifelong therapy is usually required, although spontaneous remissions off therapy have been described. The pathophysiology of the spontaneous remissions is not well understood since the underlying genetic defect still exists. It is likely that these so-called remissions reflect compensated calcium homeostasis once the needs of the growing skeleton are met. In support of this hypothesis is a report of a relapse in a pregnant woman, followed by a remission postpartum.[548]

Phenytoin causes target organ resistance to the biologic effects of 1,25(OH)$_2$D$_3$, in addition to its acceleration of the hepatic catabolism of vitamin D metabolites. Phenytoin has been shown to impair intestinal calcium absorption in vivo in rats[549] and impair PTH and 1,25(OH)$_2$D$_3$-mediated bone resorption in vitro. Combination chemotherapy with 5-fluorouracil and low-dose leucovorin has been reported to cause hypocalcemia in 65% of patients, associated with an acute decrease in plasma 1,25(OH)$_2$D$_3$ levels.[550]

Other Causes of Hypocalcemia

Excessive Deposition Into the Skeleton

Excessive deposition of calcium into the skeleton can occur in association with osteoblastic metastases, with chondrosarcomas,[551] or in the hungry bone syndrome. This syndrome presents as prolonged hypocalcemia, hypocalciuria, and hypophosphatemia post parathyroidectomy for primary hyperparathyroidism (see "Primary Hyperparathyroidism"). The hypocalcemia is a consequence of remineralization of a skeleton that has been subjected to the bone resorbing effects of PTH over a prolonged period. Hungry bone syndrome can also be observed after treatment of other diseases that are associated with excessive bone resorption. It has been described following radioactive iodine treatment of a patient with Graves disease.[552]

Impaired Resorption

Hypocalcemia can be observed as a consequence of administration of drugs that impair osteoclast-mediated bone resorption, including bisphosphonates and denosumab. Vitamin D deficiency is thought to be a significant risk factor for hypocalcemia associated with antiresorptive therapy.

Chelation

Decreases in ionized calcium have been reported with foscarnet, a pyrophosphate analog that is used as an antiviral agent,[553] perhaps because of complex formation between ionized calcium and the drug.

Hyperphosphatemia, due to phosphate administration or rapid destruction of soft tissue (i.e., rhabdomyolysis, chemotherapy of hematologic malignancies), may produce profound hypocalcemia by directly complexing and precipitating calcium in bone or soft tissues, by inhibiting bone resorption, and by blocking renal synthesis of 1,25(OH)$_2$D$_3$ (see "Hyperphosphatemia").

Massive infusions of citrated blood products may cause hypocalcemia, presumably because citrate complexes calcium in the recipient's plasma.[554] Large doses of ethylenediaminetetraacetic acid (EDTA)–containing radiographic contrast dyes have also been reported to cause hypocalcemia. Hypocalcemia, due to complexes of calcium and fluoride, has been reported with hydrofluoric acid burns[555] or ingestion.[556]

Neonatal Hypocalcemia

Neonatal hypocalcemia is seen in infants of hyperparathyroid mothers, infants of diabetic mothers, in premature infants, and in infants with birth asphyxia. The cause of hypocalcemia in infants of diabetic mothers is likely multifactorial. Prematurity per se does not account for the higher incidence.[557] The response of premature infants and infants of diabetic mothers to exogenous PTH suggests that functional hypoparathyroidism may, in part, account for the increased hypocalcemia in these two populations.[557,558] The hypocalcemia in infants of hyperparathyroid mothers is presumably secondary to the maternal hypercalcemia that, in turn, suppresses fetal parathyroid function.[559] Neonatal hypocalcemia is also seen in offspring of vitamin D–deficient mothers and in infantile malignant osteopetrosis.[560,561]

HIV

Hypocalcemia is sixfold more prevalent in HIV-infected patients than in the general population.[562] Although hypocalcemia is often a consequence of antiretroviral and antibiotic/antimycotic therapy, vitamin D deficiency and hypomagnesemia are also common in patients with AIDS. Impaired parathyroid responsiveness to hypocalcemia has also been documented (see Chapter 44).

Critical Illness

Hypocalcemia is commonly seen in critically ill patients and is thought to be a reflection of parathyroid gland suppression, failure

to activate vitamin D, calcium chelation or sequestration, and/or hypomagnesemia. However, an increased basal level and secretory response of PTH to lowering of serum calcium has been observed in some septic and nonseptic intensive care unit (ICU) patients, emphasizing the multifactorial origin of the hypocalcemia.[563] There was a correlation between cytokine levels and hypocalcemia in this and other studies, suggesting that these inflammatory agents may play a role in redistribution of calcium to the intracellular or other pools. Interleukin 1 (IL1) has been shown to increase the expression of the calcium-sensing receptor on parathyroid cells and lower PTH secretion and blood calcium in rats injected with the cytokine.[564] Severe acute pancreatitis is often associated with hypocalcemia and is a negative prognostic indicator. The hypocalcemia occurs shortly after the onset of pancreatitis and is associated with an increase in PTH levels, suggesting that parathyroid function is normal. It has long been thought that this hypocalcemia is secondary to deposition of "calcium soaps" consisting of calcium and fatty acids. Supporting this hypothesis, studies in a patient with a pancreatic fistula have demonstrated hypocalcemia (4.3 mg/dL) in the setting of high levels of calcium (26 mg/dL) and fatty acids in ascitic fluid.[565] Subsequent studies in a rat model have supported this finding and demonstrated that oleate has a high binding capacity for calcium.[566] However, other investigations in a porcine model of experimental pancreatitis have demonstrated that hypocalcemia does not occur if the animals are subjected to thyroidectomy prior to the induction of pancreatitis.[567] This finding suggests a role for calcitonin in the development of hypocalcemia with acute pancreatitis, although several clinical studies have documented normal calcitonin levels in hypocalcemic individuals with pancreatitis.[568] Severe hypocalcemia with hypercalcitoninemia and hypophosphatemia has been reported in the toxic shock syndrome sepsis and in critically ill patients.[569] As in acute pancreatitis, this hypocalcemia is usually accompanied by increases in serum levels of PTH, and the degree of hypocalcemia is a negative prognostic indicator. The mechanism of hypocalcemia in these patients is likely to be heterogeneous and has not been clearly defined.

Treatment of Hypocalcemia

Acute hypocalcemia is an emergency that requires prompt attention. If symptoms of neuromuscular irritability are present and carpopedal spasm is elicited on physical examination, or if electrocardiogram (EKG) changes are present, treatment with intravenous calcium is indicated until the signs and symptoms of hypocalcemia subside. Approximately 100 mg of elemental calcium should be infused over a period of 10 to 20 minutes (Table 29.6). If this is not sufficient to alleviate the clinical findings of hypocalcemia, an infusion of 100 mg per hour can be given to adults for several hours with close monitoring of calcium levels. In hypocalcemia associated with hypomagnesemia, magnesium replacement also is required. Magnesium should be given intravenously, 100 mEq over 24 hours in the acute setting. Because most of the parenteral magnesium is excreted in the urine, oral magnesium oxide should be instituted as soon as possible to replete body stores. Special caution and reduced doses are necessary when administering magnesium to patients in renal failure (see "Magnesium Disorders").

The treatment of hypocalcemia should be directed at the underlying disorder. In all cases, replacement with exogenous calcium (1–3 g elemental calcium daily, from food sources or oral supplements) should be instituted. Calcium carbonate is the least expensive formulation, but it requires acidification for efficient absorption. This becomes important in patients with achlorhydria and those in whom gastric acid production is being suppressed with pharmacologic agents. Notable in this respect is the acid buffering capacity of calcium carbonate. Because of this, it is recommended that patients take their calcium carbonate supplements in divided doses of 1 g or less. In these cases, the calcium should be taken with food or citrus drinks to promote maximal absorption.

In cases of vitamin D deficiency or resistance, the metabolite of vitamin D chosen depends on the underlying disorder. If impaired renal 1α-hydroxylation is present, such as in renal failure, hypoparathyroidism (or PTH resistance), or the vitamin D–dependent rickets syndromes, metabolites that do not require this modification should be administered (calcitriol 0.25–1 μg/day or doxercalciferol 2.5–5 mg/day). If decreased intake or increased losses are the problem, vitamin D should be administered and the treatment directed at the underlying disorder. Initial repletion of stores can be undertaken with 50,000 IU of vitamin D daily for 1 to 3 weeks, followed by a daily maintenance dose that significantly exceeds the RDA. In patients with resistance to vitamin D, such as those on phenytoin, 5000 IU daily or more may be required as maintenance therapy. The use of sublingual vitamin D has been shown to effectively replete levels in a patient with malabsorption due to Crohn disease.[570] In those without chronic conditions, once treatment of the underlying disorder and repletion of body stores have been addressed, 800 to 1200 IU should provide sufficient maintenance therapy.

Patients should be monitored closely to assess both response to therapy and to prevent therapeutic complications. Serum calcium should be monitored frequently (daily in profound hypocalcemia, weekly in moderate hypocalcemia) for the first week to month of therapy. Concomitant with resolution of hypocalcemia, one should observe a decline in the serum PTH level as secondary hyperparathyroidism resolves. Measurement of serum PTH and assessment of 24-hour urinary calcium excretion should be performed within 2 to 4 weeks of institution of therapy. The urinary calcium measurement reflects the effect of therapy on the patient's ability to absorb calcium and the net uptake of calcium by bone. A low urine calcium indicates poor adherence to a regimen, poor absorption of calcium, or increased uptake by bone. In addition, the urine calcium provides important information on which to base therapeutic modifications to avoid nephrolithiasis. Once normalization of serum and urinary calcium and a decrease in PTH levels are observed, a transition from aggressive replacement therapy to maintenance therapy should be undertaken to prevent hypercalcemia and nephrolithiasis. These same parameters should be monitored 1 and 3 months after a dose change to assess the effect of the therapeutic intervention. Monitoring of the alkaline phosphatase can also be performed at this time. Alkaline phosphatase levels may actually increase soon after starting treatment because of healing of the osteomalacic lesions; however, by 3 to 4 months after institution of therapy, a clear downward trend should be observed. Alkaline phosphatase and PTH values may remain elevated for 6 to 12 months after institution of therapy; they should not be a cause for alarm provided they are declining and the other parameters suggest therapy is effective.

The treatment of hypoparathyroidism is similar to that of vitamin D deficiency with the exception that these patients have impaired renal 1α-hydroxylation of 25(OH)D and therefore require treatment with 1α-hydroxylated metabolites. PTH(1-84) has also been approved for therapy. This therapy controls hypocalcemia with lower urine calcium excretion than with calcium

TABLE 29.6 Therapeutic Mineral Ion Preparations

				AVAILABLE FORMULATIONS					
				ORAL PREPARATIONS			PARENTERAL PREPARATIONS		
		MINERAL ION CONTENT			MINERAL ION CONTENT			MINERAL ION CONTENT	
Compound	MW[a]	mg/g	mmol/g	Compound	mg/g	mmol/g	Compound	mg/g	mmol/g
Calcium									
Ca carbonate	100	400	10.0	1250 mg[b]	500 mg	12.5 mmol			
Ca phosphate	310	383	9.6	1565 mg	600 mg	15.0 mmol			
Ca acetate	158	253	6.3	668 mg[b]	167 mg	4.2 mmol			
Ca citrate	498	210	6.0	950 mg[b]	200 mg	5.0 mmol			
Ca lactate	218	130	4.6	650 mg[b]	84 mg	2.1 mmol			
Ca glubionate		64	1.7	5 mL	115 mg	2 mmol			
Ca gluconate	430	93	2.3	1000 mg[b]	93 mg	2.3 mmol	10% soln	93 mg/10 mL	2.3 mmol/10 mL
Ca gluceptate	488	82	2				22% soln	90 mg/5 mL	2.3 mmol/10 mL
Ca chloride	147	273	6.8				10% soln	273 mg/10 mL	11.2 mmol/mL
Magnesium									
Mg oxide	40	603	24.8	400 mg[b]	241 mg	9.9 mmol			
Mg gluconate	450	54	2.2	500 mg	27 mg	1.1 mmol			
Mg chloride	203	120	4.9	535 mg	64 mg	2.6 mmol	20% soln	24 mg/mL	1 mmol/mL
Mg sulfate	246	99	4.1				50% soln[b]	49 mg/mL	2 mmol/mL
Phosphorus[c]									
Na/K phosphate (neutral)				Capsule	250 mg	8.1 mmol			
K phosphate (neutral)				Capsule	250 mg	8.1 mmol	soln	94 mg/mL	3 mmol/mL
Na phosphate (neutral)							soln	94 mg/mL	3 mmol/mL

[a]Molecular weights (MW) shown are for the usual chemical form, including water molecules (e.g., $MgSO_4 \bullet 7\ H_2O$).

[b]Other formulations exist. Those shown are among those approved in the United States.

[c]Phosphate preparations contain buffered mixtures of monobasic ($H_2PO_4^-$) and dibasic (HPO_4^-) ions; the phosphorus content therefore is specified in millimoles. Oral phosphates contain 7 mEq sodium and potassium per capsule (Na/K form) or 14 mEq potassium per capsule (K form). Parenteral solutions typically contain 4 mEq of sodium or potassium per milliliter.

MW, Molecular weight; *soln,* solution.

Data from *Drug Facts and Comparisons.* St. Louis: Facts and Comparisons; 1995.

and calcitriol therapy, but it is expensive and requires parenteral administration. Oral calcium and 1α-hydroxylated vitamin D metabolites therefore remain the mainstay of therapy. Monitoring of serum and urinary calcium should be performed as in the treatment of vitamin D deficiency. Therapy in these patients is lifelong, therefore careful monitoring is required to avoid renal or hypercalcemic complications. The aim of therapy should be to maintain serum calcium in the low-normal range without causing frank hypercalciuria. Because PTH plays an important role in renal calcium reabsorption, difficulties are often encountered attaining these therapeutic goals. In such cases, renal calcium losses can be minimized by the addition of a thiazide diuretic to the treatment regime. Replacement therapy with PTH(1-34) has also been shown to be effective in treatment of hypoparathyroidism.[571]

One of the frustrations often encountered in treating patients with hypoparathyroidism is the fluctuating response to a seemingly stable therapeutic regimen. Episodes of hypercalcemia are occasionally observed without any discernible cause. Because of this, serum calcium should be monitored every 3 months to permit temporary withdrawal of $1,25(OH)_2D_3$, should a hypercalcemic trend be observed. Fortunately the half-life of this metabolite is short, so that discontinuation for a few days to a week with resumption of a lower dose is usually efficacious.

All patients receiving vitamin D metabolites and calcium need to be aware of potential therapeutic complications. Importantly, the mild symptoms of hypercalcemia should be emphasized to the patient. It is essential that these patients be aware that their calcium should be monitored more frequently during intercurrent illnesses that may affect the absorption of calcium or their hydration status, to prevent the development of hypocalcemia or severe hypercalcemia.

TABLE 29.7 Causes of Hyperphosphatemia

Impaired Renal Phosphate Excretion

Renal insufficiency
Familial tumoral calcinosis
Endocrinopathies
- Acromegaly
- Hypoparathyroidism
- Pseudohypoparathyroidism

Heparin

Increased Extracellular Phosphate

Rapid Administration of Phosphate (Intravenous, Oral, Rectal)

Phosphate salts
Fosphenytoin
Liposomal amphotericin B

Rapid Cellular Catabolism or Lysis

Catabolic states
Tissue injury
- Hyperthermia
- Crush injuries
- Fulminant hepatitis

Cellular lysis
- Hemolytic anemia
- Rhabdomyolysis
- Tumor lysis syndrome

Transcellular Shifts of Phosphate

Metabolic acidosis
Respiratory acidosis

Disorders of Phosphate Metabolism

Hyperphosphatemia

Serum phosphate levels are controlled primarily by the rate of proximal renal tubular phosphate reabsorption, which is due, in turn, to the integrated activity of the major sodium-dependent cotransporters (NaPi-IIa and NaPi-IIc). The latter are strongly downregulated by parathyroid hormone and FGF23, both of which are stimulated by phosphate. Thus absent extraordinary filtered loads of phosphate, the capacity of normal kidneys to excrete phosphate is not easily exceeded. Consequently, the occurrence of hyperphosphatemia usually signifies impaired renal functon, hypoparathyroidism, defective FGF23 action, a huge flux of phosphate into the extracellular fluid, or some combination of these factors (Table 29.7).

The most common cause of hyperphosphatemia is acute or chronic renal failure in which GFR is so reduced that the usual daily load of phosphate cannot be excreted at a normal level of serum phosphate, despite maximal inhibition of phosphate reabsorption in the remaining functional nephrons. In hypoparathyroidism (or PHP), serum phosphate may rise to levels as high as 6 to 8 mg/dL because of loss of the tonic inhibitory effect of PTH on phosphate reabsorption, although elevated FGF23 levels may prevent even further increases in serum phosphate.[572] The hyperphosphatemia of hypoparathyroidism is only partly due to the absence of PTH per se. Hypocalcemia may further impair phosphate clearance in this setting, and correction of hypocalcemia by treatment with vitamin D metabolites and oral calcium may reduce serum phosphate, for example, even though PTH levels remain low.[573]

Other circumstances in which renal tubular phosphate excretion is decreased, in the absence of renal failure, include acromegaly,[574] chronic therapy with heparin, and familial tumoral calcinosis.[575] Familial tumoral calcinosis can result from inactivating mutations in either FGF23 or the *O*-linked glycosyl transferase GalNAc-T3, which glycosylates FGF23 at its cleavage site targeted by furin-like proteases, thereby suppressing this cleavage.[576] In the absence of GalNAc-T3, FGF23 is cleaved at an accelerated rate.[577–579] The choice of FGF23 assay is important therefore for diagnosing tumoral calcinosis. The responsible FGF23 and GalNac-T3 mutations may render the molecule more susceptible to proteolyic degradation, such that the blood levels of (inactive) carboxyl fragments may be quite high in contrast to low levels of (bioactive) intact FGF23.[578] Affected patients may display focal hyperostosis; large, lobulated periarticular ectopic calcifications, especially around shoulders or hips; hyperphosphatemia due to increased renal tubular reabsorption of phosphate; increased serum $1,25(OH)_2D$ despite normal or low serum PTH; and increased intestinal calcium absorption, consistent with the elevated serum $1,25(OH)_2D$ concentration. The disorder may present in childhood or adulthood, is more common in African Americans, and is lifelong, with a tendency for the tumoral calcifications to progress at affected sites. In contrast to the elevated serum $1,25(OH)_2D$, hyperphosphatemia is not a constant feature of tumoral calcinosis, although it tends to be most severe in those with prominent calcifications. Despite their chronic hyperphosphatemia, secondary hyperparathyroidism does not develop in these patients, presumably because of the high $1,25(OH)_2D$ levels and intestinal hyperabsorption of calcium. Treatment is problematic, although some success has been reported with phosphate-binding antacids, calcium deprivation, calcitonin, and acetazolamide therapy.[580]

Hyperphosphatemia may result from overly rapid administration of therapeutic phosphate preparations or phosphate-rich drugs (fosphenytoin, liposomal amphotericin B), especially if renal function is compromised[581] or from rapid shifts of phosphate out of cells, most often provoked by mechanical injury or metabolic insult. Most cases of hyperphosphatemia associated with intestinal phosphate loads have involved children who received phosphate-containing laxatives or enemas or older adults with impaired renal function receiving phosphate-based cathartics in preparation for colonoscopy.[582] Hyperphosphatemia due to cytolytic release of intracellular phosphate can be quite dramatic, with serum phosphate concentrations up to or exceeding 20 mg/dL. This disorder was described initially as a complication of rapid induction chemotherapy for certain hematologic malignancies (tumor lysis syndrome), although it also may occur from cellular injury associated with trauma, hyperthermia, overwhelming infection, hemolysis, rhabdomyolysis, or metabolic acidosis.[583] Rarely, apparent hyperphosphatemia may reflect measurement artifact caused by paraproteins in myeloma.[584]

Most often, hyperphosphatemia is mild and asymptomatic, although chronic hyperphosphatemia is an important factor in the development of secondary hyperparathyroidism in progressive renal failure. The clinical manifestations of acute, severe hyperphosphatemia are related mainly to those of the accompanying hypocalcemia, caused by formation of insoluble calcium phosphate precipitates. Thus tetany, muscle cramps, paresthesias, and seizures may occur, and they may be compounded by other metabolic disturbances (hyperkalemia, acidosis, hyperuricemia) that frequently coexist. Generalized precipitation of calcium phosphate into soft tissues may produce organ dysfunction, notably renal failure.[582]

Therapeutic options for hyperphosphatemia are limited. Volume expansion may be helpful to improve GFR in acute syndromes. Identification and removal of any exogenous sources of phosphate are important, and phosphate-binding aluminum hydroxide antacids may be useful in limiting intestinal phosphate absorption and chelating phosphate secreted into the intestine. Hemodialysis is the most effective approach and should be considered early in severe hyperphosphatemia, especially in the tumor lysis syndrome and particularly if symptomatic hypocalcemia cannot be adequately treated for fear of inducing widespread soft tissue calcification.

Hypophosphatemia

Etiology

Hypophosphatemia may result from one or more of three general mechanisms (Table 29.8): increased urinary losses due to decreased net renal tubular phosphate reabsorption; rapid shifts of phosphate from extracellular fluid into the intracellular space or the mineral phase of bone; or rarely, severe and selective deprivation of dietary phosphate, as may occur with chronic ingestion of large amounts of nonabsorbable aluminum-containing antacids. Fasting or starvation does not lead directly to hypophosphatemia, apparently because phosphate is mobilized from catabolized bone and soft tissue in amounts sufficient to maintain serum phosphate, even during prolonged caloric deprivation.[585] Starvation does induce phosphate deficiency and therefore predisposes to subsequent hypophosphatemia upon refeeding.[586]

Chronic hypophosphatemia usually can be traced to ongoing renal phosphate wasting. Elevation of serum PTH for any reason (other than renal failure), as in primary hyperparathyroidism or secondary hyperparathyroidism due to vitamin D or calcium deficiency, results in inhibition of tubular phosphate reabsorption and fasting hypophosphatemia. Phosphate clearance also is increased in PTHrP-associated hypercalcemia of malignancy, although when such patients develop severe hypercalcemia, hypophosphatemia may be masked initially by underlying volume depletion and compromised GFR. Therapy with the tyrosine kinase inhibitors imatinib and nilotinib appears to cause hypophosphatemia, at least in part by inhibiting both osteoblast and osteoclast formation, lowering serum calcium, and stimulating secondary hyperparathyroidism.[587–589] When PTH secretion is compromised by severe hypomagnesemia, rapid intravenous administration of magnesium alone, without concurrent attention to coexisting hypocalcemia, can provoke massive phosphaturia and hypophosphatemia in patients with underlying phosphate depletion.

The discovery that gain-of-function mutations in FGF23 cause autosomal dominant hypophosphatemic rickets inaugurated a new era in understanding of phosphate homeostasis.[590–592] Elevated FGF23 also occurs in autosomal recessive hypophosphatemia (ARHP), which is caused by mutations in the genes encoding dentin matrix protein-1 (DMP1) and FAM20C. DMP1 is expressed in osteocytes and presumably regulates local production of FGF23.[593] FAM20C is a secreted kinase that phosphorylates FGF23 near the furin cleavage site; this phosphorylation is required for inactivating cleavage by a furin-like protease. Immunoassays for FGF23[594] have pointed to elevated circulating FGF23 as at least one "phosphatonin" responsible for reduction of phosphate reabsorption and serum $1,25(OH)_2D$ levels in the more common disorder, X-linked hypophosphatemic rickets (XLH), in the rare but distinctive TIO and epidermal nevus syndromes, and in the approximately 50% of patients with McCune-Albright syndrome (fibrous dysplasia of bone) who manifest hypophosphatemia.[590–592,595–597] These disorders share a common biochemical phenotype, which may include a more generalized proximal tubular dysfunction, with modest proteinuria and aminoaciduria. Serum calcium usually is normal or low-normal, urinary calcium often is low, PTH is normal or only slightly elevated, and $1,25(OH)_2D$ is inappropriately normal. The clinical picture is dominated by weakness, bone pain, and other features attributable to the associated rickets or osteomalacia (see Chapter 31). Increased FGF23 also may play a role in impaired phosphate reabsorption seen in the 20% or so of patients with calcium kidney stones and idiopathic hypercalciuria who exhibit fasting hypophosphatemia,[598] although a few such patients may harbor mutations in the NaPi-IIa sodium phosphate cotransporter.[599]

Renal phosphate clearance may be impaired in the context of a more generalized renal tubular disorder such as Fanconi syndrome(s) or others associated with systemic diseases such as amyloidosis, Wilson disease, or cystinosis (see Table 29.8). In addition to NaPi-IIa mutations, inactivating mutations in the NaPi-IIc cotransporter, also expressed in the proximal tubule and known to be regulated by both PTH and FGF23, have been shown to account for the rare disorder known as *hereditary hypophosphatemic rickets with hypercalciuria*, in which primary

TABLE 29.8 Causes of Hypophosphatemia

Reduced Renal Tubular Phosphate Reabsorption

Excess PTH or PTHrP

- Primary hyperparathyroidism
- PTHrP-dependent hypercalcemia of malignancy
- Secondary hyperparathyroidism
  - Vitamin D deficiency/resistance
  - Calcium starvation or malabsorption
  - Imatinib
  - Rapid, selective correction of severe hypomagnesemia

Excess FGF23 or Other "Phosphatonins"

- Familial hypophosphatemic rickets (XLH)
- Autosomal dominant hypophosphatemic rickets (ADHR)
- Autosomal recessive hypophosphatemia (ARHP)
- Tumor-induced osteomalacia syndrome (TIO)
- McCune-Albright syndrome (fibrous dysplasia)
- Epidermal nevus syndrome
- Following renal or hepatic transplantation
- Idiopathic hypercalciuria

Intrinsic Renal Disease

- Fanconi syndrome(s), other renal tubular disorders
  - Cystinosis
  - Amyloidosis
  - Hemolytic uremic syndrome
  - Magnesium deficiency
  - Wilson disease
  - Multiple myeloma
  - Heavy metal toxicity
  - Rewarming or hyperthermia
- NaPi-IIa mutations
- NaPi-II2c mutations (HHRH)

Other

- Poorly controlled diabetes, alcoholism
- Hyperaldosteronism
- Following partial hepatectomy
- Following renal transplantation

Continued

TABLE 29.8 Causes of Hypophosphatemia—cont'd

Drugs or toxins

Ethanol	High-dose estrogens
Acetazolamide, other diuretics	Ifosfamide
High-dose glucocorticoids	Cisplatin
Bicarbonate	Suramin
Toluene	Foscarnet
Heavy metals (Pb, Cd)	*N*-methylformamide
Calcitonin	Bisphosphonates
Tenofovir	Paraquat

Shifts of Extracellular Phosphate Into Cells or Bone

Acute Intracellular Shifts

Intravenous glucose, fructose, glycerol
Insulin therapy for hyperglycemia, diabetic ketoacidosis
Catecholamines (epinephrine, albuterol, terbutaline, dopamine)
Thyrotoxic periodic paralysis
Acute respiratory alkalosis, salicylate intoxication, acute gout
Gram-negative sepsis, toxic shock syndrome
Recovery from acidosis, starvation, anorexia nervosa, hepatic failure
Rapid cellular proliferation
- Leukemic blast crisis
- Intensive erythropoietin, G-CSF therapy

Accelerated Net Bone Formation

Following parathyroidectomy
Osteoblastic metastases
Treatment of vitamin D deficiency
Antiresorptive therapy of severe Paget disease

Impaired Intestinal Phosphate Absorption

Aluminum-containing antacids

FGF23, Fibroblast growth factor 23; *G-CSF,* granulocyte colony-stimulating factor; *HHRH,* hereditary hypophosphatemic rickets with hypercalciuria; *PTH,* parathyroid hormone; *PTHrP,* parathyroid hormone–related hormone.

renal tubular phosphate wasting causes appropriate elevation of serum $1,25(OH)_2D$ and resulting hypercalciuria.[600,601] Other causes of impaired renal tubular phosphate reabsorption include the osmotic diuresis associated with poorly controlled diabetes, alcoholism, hyperaldosteronism, and exposure to any of a wide variety of drugs or toxins (see Table 29.8). The pathogenesis of phosphate wasting that often follows partial hepatectomy or renal transplantation remains unclear, but humoral mechanisms seem to be involved.[602,603]

Rapid egress of extracellular phosphate into cells is the cause of hypophosphatemia that develops acutely during administration of intravenous glucose, insulin therapy for hyperglycemia, administration of catecholamines (pressors or bronchodilators), thyrotoxic periodic paralysis, profound respiratory alkalosis, refeeding syndrome in the wake of severe acidosis or starvation, recovery from acute hepatic failure (in which hypophosphatemia is a recognized favorable prognostic factor[604]), or other circumstances involving rapid cellular proliferation such as leukemic blast crisis or responsiveness to hematopoietic growth factors. Hypophosphatemia in these situations is most pronounced when there is underlying phosphate depletion, as in hyperparathyroidism or vitamin D deficiency, or following prolonged malnutrition, alcoholism, or glycosuria. Accelerated uptake of phosphate into cells is particularly common in postsurgical, burn, or trauma patients, in whom it may be promoted by high levels of circulating catecholamines and exacerbated by concurrent respiratory alkalosis, fever, volume expansion, sepsis, and hypokalemia. Situations of greatly accelerated net bone formation, such as hungry bone syndrome occurring immediately following parathyroidectomy for primary or tertiary hyperparathyroidism, during initial treatment of severe vitamin D deficiency or Paget disease, or in occasional patients with extensive osteoblastic bone metastases, may manifest hypophosphatemia as well as hypocalcemia.

Clinical Features

The clinical significance of hypophosphatemia depends on the presence and severity of underlying phosphate depletion. Unfortunately the status of the total-body phosphorus pool, and more particularly the critical intracellular pool, is reflected only indirectly by the concentration of phosphate in the extracellular fluid, which contains less than 0.5% of body phosphorus. Thus, although serum phosphate concentrations generally are used to characterize hypophosphatemia as severe (<1–1.5 mg/dL, <0.3–0.5 mmol/L), moderate (1.5–2.2 mg/dL, 0.5–0.7 mmol/L), or mild (2.2–3 mg/dL, 0.75–1 mmol/L), serum phosphate may be normal or even high (depending on renal function) in the presence of profound intracellular phosphate deficiency. Conversely, it may be low when intracellular phosphate is relatively normal, such as following a sudden movement of extracellular phosphate into cells.

The prevalence of severe hypophosphatemia among hospitalized patients overall is less than 1%, whereas mild or moderate hypophosphatemia may be detected in 2% to 5%.[605] Hypophosphatemia is recognized most often in critically ill patients, alcoholics or other malnourished individuals, decompensated diabetics, and those with acute infectious or pulmonary disorders.[605]

The clinical manifestations of severe hypophosphatemia are protean. Among the most common are various neuromuscular symptoms, ranging from progressive lethargy, muscle weakness, and paresthesias to paralysis, coma, and even death, depending on the severity of the phosphate depletion. Confusion, profound weakness, paralysis, seizures, and other major sequelae generally are limited to those with serum phosphate concentrations below 0.8 to 1 mg/dL.[606] Biochemical evidence of muscle injury is observed within 1 to 2 days in over one-third of patients whose serum phosphate concentrations fall to less than 2 mg/dL.[607] Overt rhabdomyolysis also may occur, especially in the setting of chronic alcoholism with underlying malnutrition and phosphate depletion.[608,609] However, by the time this is recognized the serum phosphate often has been raised by the large amounts of cellular phosphate released from damaged muscle. Reversible respiratory failure due to respiratory muscle weakness may preclude successful weaning from ventilatory support.[610,611] Left ventricular dysfunction, heart failure, and ventricular arrhythmias may result from profound hypophosphatemia but may not be significant if serum phosphate is greater than 1.5 mg/dL.[612] Correction of moderate hypophosphatemia (<2 mg/dL) in patients with septic shock led to a significant increase in blood pressure as well as left ventricular function and arterial pH.[612] Hematologic sequelae of severe hypophosphatemia include hemolysis, platelet dysfunction with bleeding, and impaired leukocyte function (phagocytosis and killing).[613] Erythrocytes demonstrate increased fragility; altered membrane composition, rigidity, and microspherocytosis; and reduced levels of ATP and 2,3-diphosphoglycerate (2,3-DPG).[613] The reduction in erythrocyte 2,3-DPG impairs oxyhemoglobin

dissociation and thereby may reduce oxygen delivery to tissues. This problem, together with accelerated hemolysis, may provoke a substantial increase in cardiac output. The blockade in cellular glycolysis becomes demonstrable at levels of serum phosphate between 1 and 2 mg/dL.[614] Glucose intolerance and insulin resistance also have been demonstrable in these patients.[615]

Treatment

Hypophosphatemia appears most often in acutely or critically ill individuals. Accordingly, it often is difficult to discern whether hypophosphatemia is responsible for features of the multiple organ dysfunction commonly encountered in this population. For example, although depression of intracellular high-energy organophosphates has been demonstrated during treatment of diabetic ketoacidosis and phosphate repletion leads to more rapid recovery of erythrocyte 2,3-DPG concentrations, opinion is divided as to whether phosphate therapy in this setting hastens recovery, prevents complications, or reduces mortality rate.[616,617] Nevertheless, because severe hypophosphatemia has been associated, in a variety of clinical settings, with serious neuromuscular, cardiovascular, and hematologic dysfunction that is at least partially reversible with phosphate repletion, most now agree that one should adopt a relatively low threshold for treatment.[612]

The decision to correct hypophosphatemia urgently should be guided by the estimated severity of the cellular phosphate deficit, the presence of signs or symptoms suggestive of phosphate depletion, and the overall clinical status of the patient. The presence of renal insufficiency (a risk for iatrogenic hyperphosphatemia), concomitant administration of intravenous glucose (alone or as a component of hyperalimentation solutions), and the potential for aggravating coexistent hypocalcemia also should be considered.

Limited data are available from clinical trials to predict the appropriate dose and rate of phosphate administration. In patients without severe renal insufficiency or hypocalcemia, administration of intravenous phosphate at rates of 2 to 8 mmol/hour of elemental phosphorus over 4 to 8 hours frequently corrects hypophosphatemia without provoking hyperphosphatemia or hypocalcemia.[605,618–620] Suggested guidelines based on serum phosphate are shown in Table 29.9. It is essential that serum calcium and phosphate be monitored every 6 to 12 hours during and after phosphate therapy, both to detect untoward consequences and because many patients require additional infusions for recurrent hypophosphatemia within 24 to 48 hours of apparently successful repletion.[619] Less acute or severe hypophosphatemia should be managed with oral (or enteral) phosphate supplements if possible, generally given as a total of 1 to 2 g a day (as elemental phosphate) of neutral sodium or potassium phosphate in divided doses three to four times a day (see Table 29.6). In many patients, however, oral phosphate therapy is limited by gastrointestinal symptoms such as nausea or diarrhea.

TABLE 29.9 Urgent Therapy of Hypophosphatemia[a]

Factors to Consider

Severity of hypophosphatemia
Likelihood of underlying phosphate depletion
Clinical condition of the patient
Renal function
Serum calcium
Concurrent parenteral therapy (glucose, hyperalimentation)

GUIDELINES			
Serum PO_4 (mg/dL)	Rate of Infusion (mmol/hr)	Duration (hr)	Total PO_4 (mmol)
<2.5	2	6	12
<1.5	4	6	24
<1	8	6	48

[a]Rates shown are normalized for a 70-kg person. Most formulations available in the United States provide 3 mmol/mL of sodium or potassium phosphate.

PO_4, Phosphate.

Disorders of Magnesium Metabolism

The fourth most abundant extracellular cation, magnesium, like calcium, plays a critical physiologic role, particularly in neuromuscular function but also as a component of the mineral phase of bone. Intracellular magnesium is crucial for normal energy metabolism, as a cofactor for ATP and numerous enzymes and transporters, which is reflected in the rather global clinical effects that accompany disorders of magnesium homeostasis. Hypomagnesemia and hypermagnesemia are among the most common electrolyte disturbances; one or the other of these abnormalities is observed in as many as 20% of hospitalized patients and even more frequently (i.e., 30–40%) among those admitted to ICUs.[621]

Hypermagnesemia

Magnesium homeostasis is achieved mainly through highly efficient regulation of tubular magnesium reabsorption in the loop of Henle.[1] Because normal kidneys can readily excrete even large amounts of magnesium (i.e., 500 mEq/day), high filtered loads of magnesium rarely cause hypermagnesemia except in patients with significant renal insufficiency.[622] Increased magnesium loads in such cases may arise from ingestion of large amounts of oral magnesium salts, typically given as cathartics or antacids, or from extensive soft tissue ischemia or necrosis in patients with trauma, sepsis, cardiopulmonary arrest, burns, or shock[622] (Table 29.10). Hypermagnesemia may result from parenteral administration of magnesium salts, such as when magnesium is used to treat preeclampsia or as a tocolytic.[623] The infants of such hypermagnesemic mothers may manifest transient hypermagnesemia as well, along with parathyroid suppression and neurobehavioral symptoms.[624,625] The use of oral magnesium preparations as laxatives may lead to hypermagnesemia if absorption is increased by intestinal ileus, obstruction, or perforation.[626]

The most prominent clinical manifestations of hypermagnesemia are vasodilatation and neuromuscular blockade, which may involve both presynaptic and postsynaptic inhibition of neuromuscular transmission.[627] Signs and symptoms generally do not appear unless the serum magnesium exceeds 4 mEq/L.[622] Hypotension, often refractory to pressors and volume expansion, may be one of the earliest signs of progressive hypermagnesemia.[627,628] Lethargy, nausea, and weakness, accompanied by reduction or loss of deep tendon reflexes, may progress to stupor or coma with respiratory insufficiency or quadriparesis at serum concentrations in excess of 8 to 10 mEq/L. Gastrointestinal hypomotility or ileus is common. Facial flushing and pupillary dilatation may be observed. Hypotension may be complicated by a paradoxic relative bradycardia, and other cardiac effects may be evident, including

TABLE 29.10 Causes of Hypermagnesemia

Excessive Magnesium Intake

Cathartics, antacids, enemas
Dead Sea drowning
Parenteral magnesium administration
Magnesium-rich urologic irrigants
Intestinal obstruction or perforation following magnesium ingestion

Rapid Mobilization From Soft Tissues

Trauma
Shock, sepsis
Cardiac arrest
Burns

Impaired Magnesium Excretion

Renal failure
Familial hypocalciuric hypercalcemia

Other

Adrenal insufficiency
Hypothyroidism
Hypothermia

prolongation of the PR, QRS, and QTc intervals, appearance of heart block, and ultimately asystole as serum concentrations approach 20 mEq/L.

Hypermagnesemia activates CaSRs in the parathyroids, thereby suppressing PTH secretion,[629] and in the renal distal tubules, thereby reducing tubular calcium and magnesium reabsorption. Severe hypocalcemia opposes the effect of hypermagnesemia on PTH secretion so that serum PTH may remain within the normal range but still inappropriate for the level of serum calcium.[630]

Successful treatment of hypermagnesemia requires identification and interruption of the source of magnesium, together with measures to increase clearance of magnesium from the extracellular fluid. Use of magnesium-free cathartics or enemas to accelerate clearance of ingested magnesium from the gastrointestinal tract, together with vigorous intravenous hydration, generally have been successful in reversing hypermagnesemia. Refractory cases, especially those with advanced renal insufficiency, may require hemodialysis. Intravenous calcium (100–200 mg) infusions have been advocated as an effective antidote to hypermagnesemia, and there are examples in which this approach has apparently been successful, at least temporarily.[622,627,631]

Hypomagnesemia

Hypomagnesemia may occur because of impaired intestinal magnesium absorption or, more commonly, because of excessive gastrointestinal losses due to diarrhea, preprocedural bowel preparation, or prolonged drainage. Most often, hypomagnesemia reflects defective renal tubular reabsorption of magnesium, although rapid shifts into cells, other extrarenal losses, or incorporation into new bone may occur (Table 29.11). Because only 1% of the body's magnesium content is present in extracellular fluid, measurements of serum total or ionized magnesium concentration may not adequately reflect total-body magnesium or the magnesium status of the intracellular compartment in critical tissues such as muscle.[632] Thus patients with deficiency of tissue magnesium may fail to manifest overt hypomagnesemia[633] but may exhibit abnormal retention (i.e., >50% in 24 hours) of infused magnesium, a maneuver that may be used to assess magnesium status.[634]

Etiology

Intestinal Causes of Hypomagnesemia

Selective dietary magnesium deficiency does not occur, and it is remarkably difficult, in fact, to induce magnesium depletion experimentally by feeding magnesium-deficient diets, probably because renal magnesium conservation is so efficient. Large amounts of magnesium may be lost in chronic diarrheal states (this fluid may contain >10 mEq/L magnesium) or via intestinal fistulas or prolonged gastrointestinal drainage.[635] More commonly, magnesium becomes trapped within fatty acid soaps in disorders associated with chronic malabsorption.[636] Investigation of a rare autosomal recessive disorder, hypomagnesemia with secondary hypocalcemia (HSH), led to the identification of the transient receptor potential channel protein TRPM6 in the form of a hetero-oligomer, with the closely related channel protein TRPM7 as a key molecular mediator of intestinal (and renal tubular) transepithelial magnesium transport.[637]

Renal Causes of Hypomagnesemia

Roughly 60% of renal magnesium reabsorption occurs in the thick ascending limb of Henle loop, and another 5% to 10% is reabsorbed in the distal tubules.[1] Investigation of the pathogenesis of several genetic disorders associated with renal magnesium wasting has identified key pathways of magnesium reabsorption at these sites (see Table 29.11). Thus in familial hypomagnesemia with hypercalciuria and nephrocalcinosis, loss-of-function mutations in the claudin 16 gene encoding the paracellin-1 protein (or in the related gene, claudin 19), a component of the tight junctions between adjacent epithelial cells, selectively impair paracellular magnesium (and calcium) reabsorption in response to the (lumen-positive) transepithelial voltage gradient.[57,638]

In Bartter syndrome(s), inactivating mutations in any of several transporters involved in sodium chloride reabsorption in the ascending limb cause salt wasting, compromise the voltage gradient, and similarly impair paracellular magnesium and calcium reabsorption.[639–641] In autosomal dominant hypocalcemia, mutations causing increased sensitivity of CaSRs to cationic agonists cause hypomagnesemia, as well as hypocalcemia, through inappropriate CaSR-dependent suppression of PTH secretion and of renal tubular cation reabsorption.[642]

In Gitelman syndrome, inactivating mutations in the luminal thiazide-sensitive NaCl cotransporter (NCC) expressed in the distal convoluted tubules lead to sodium chloride and magnesium wasting, in this case with hypocalciuria.[639–641,643] The manner whereby impaired NCC activity compromises (transcellular) magnesium reabsorption in this segment is unclear, although NCC-knockout mice (or normal mice treated with thiazides) display reduced distal tubular expression of the TRPM6 channel protein required for normal magnesium transport across the apical membrane.[434]

Mutations in the FXYD2 γ-subunit of the distal tubular basolateral Na^+/K^+-ATPase similarly impair salt and magnesium reabsorption at that site and account for some, but not all, cases of isolated renal magnesium wasting.[644,645] An autosomal recessive form of renal hypomagnesemia is caused by inactivating mutations in the EGF gene, which likely also explains why hypomagnesemia may complicate use of cetuximab, a monoclonal antibody directed against the EGF receptor.[646] Another genetic syndrome featuring renal magnesium wasting and hypocalciuria (as in Gitelman syndrome), and thus presumably involving a defect in distal tubular function as well, in association with hypertension and hypercholesterolemia, is linked to a mutation in mitochondrial tRNA DNA.[647] Other rare genetic causes of distal tubular

TABLE 29.11 Causes of Hypomagnesemia

Impaired Intestinal Magnesium Absorption

Hypomagnesemia with secondary hypocalcemia
Malabsorption syndromes

Increased Intestinal Magnesium Losses

Protracted vomiting or diarrhea
Bowel preparation (procedures, surgery)
Intestinal drainage or fistulas

Impaired Renal Tubular Magnesium Reabsorption

Genetic Magnesium-Wasting Syndromes

Bartter syndrome(s)
Familial hypomagnesemia with hypercalciuria and nephrocalcinosis
Autosomal dominant hypocalcemia
Gitelman syndrome
Isolated renal magnesium wasting
Hypomagnesemia with hypertension and hypercholesterolemia
Hypomagnesemia with secondary hypocalcemia

Acquired Renal Disease

Tubulointerstitial disease
Postobstruction, acute tubular necrosis (diuretic phase)
Renal transplantation

Drugs and Toxins

Ethanol
Digoxin
Diuretics (loop, thiazide, osmotic)
cis-Platinum
Cyclosporine
Tacrolimus
Cetuximab
Interleukin 2
Pentamidine
Aminoglycosides
Foscarnet
Amphotericin B

Endocrine and Metabolic Abnormalities

Extracellular fluid volume expansion
Hyperaldosteronism (primary, secondary)
Inappropriate antidiuretic hormone secretion
Diabetes mellitus
Hypercalcemia
Phosphate depletion
Metabolic acidosis
Hyperthyroidism

Other

Hypothermia
Sézary syndrome
Acute brain injury
Hydrogen fluoride burns

Rapid Shifts of Magnesium Out of Extracellular Fluid

Intracellular Redistribution

Recovery from diabetic ketoacidosis
Refeeding syndrome
Correction of respiratory acidosis
Catecholamines
Thyrotoxic periodic paralysis

Accelerated Net Bone Formation

Following parathyroidectomy
Osteoblastic metastases
Treatment of vitamin D deficiency
Calcitonin therapy

Other Losses

Pancreatitis
Blood transfusions
Extensive burns
Excessive sweating
Pregnancy (third trimester) and lactation

magnesium wasting involve mutations in K channels (Kv1.1 or Kir4.1) that disrupt voltage gradients needed for efficient magnesium reabsorption.[648]

Most often, renal magnesium wasting is attributable to an acquired abnormality in tubular magnesium reabsorption. In normal subjects, magnesium reabsorption is virtually complete within several days of instituting experimental dietary magnesium deficiency, even before serum magnesium has declined substantially.[649] Thus the finding of more than 1 mEq per day of urinary magnesium in a frankly hypomagnesemic patient indicates a defect in renal tubular magnesium reabsorption. The causes of acquired primary tubular magnesium wasting include various tubulointerstitial disorders, recovery from acute tubular necrosis or obstruction, renal transplantation, various endocrinopathies, alcoholism, and exposure to certain drugs (see Table 29.11).

Hypomagnesemia or magnesium depletion due to subnormal renal reabsorption may complicate a variety of endocrinopathies, including hyperaldosteronism, hyperthyroidism, and disorders associated with hypercalcemia, hypercalciuria, or phosphate depletion.[634] In primary hyperparathyroidism, PTH stimulates increased tubular magnesium reabsorption, but this action is opposed by a direct tubular effect of hypercalcemia. As a result, serum magnesium in primary hyperparathyroidism generally is normal or only slightly reduced.[650] In hypoparathyroidism, serum and urinary magnesium are low. The magnesium depletion in hypoparathyroidism is consistent with loss of both the magnesium-retaining renal action of PTH and the stimulatory effect of $1,25(OH)_2D$ on intestinal magnesium absorption.[651]

Diabetes is among the most common disorders associated with hypomagnesemia.[652,653] The severity of the hypomagnesemia in diabetics correlates with indices of glycosuria and poor glycemic control,[654] suggesting that urinary losses of magnesium on the basis of glycosuria may partly explain the magnesium depletion. Rapid correction of hyperglycemia with insulin therapy causes magnesium to enter cells and may further lower the extracellular magnesium concentration during treatment.

Alcoholism is another very common clinical setting in which hypomagnesemia occurs. Magnesium depletion in alcoholism may result in part from nutritional deficiency of magnesium, overall caloric starvation and ketosis, and gastrointestinal losses due to vomiting or diarrhea, but an acute magnesuric effect of alcohol ingestion likely plays the major role.[655] This effect of alcohol is most evident during the rising limb of the blood ethanol curve and may be related to transient suppression of PTH secretion.[655] Other factors that may contribute to hypomagnesemia in alcoholism include pancreatitis, malabsorption, secondary hyperaldosteronism, respiratory alkalosis, and elevated plasma catecholamines, which increase intracellular sequestration of magnesium.[634]

A number of drugs have been identified as causes of defective renal tubular magnesium reabsorption and hypomagnesemia.[634] These drugs include diuretics (especially loop diuretics), digoxin, cisplatin, cetuximab, pentamidine, cyclosporine, tacrolimus, interleukin 2, aminoglycosides, foscarnet, and amphotericin B. Most often, drug-induced hypomagnesemia is mild and reversible, particularly when it is associated with diuretic therapy. In over half of patients treated with cisplatin, hypomagnesemia occurs within days or weeks, and roughly half of those who develop it exhibit persistent hypomagnesemia many months or even years later. The median duration of hypomagnesemia in cisplatin-treated patients is about 2 months, but recovery has been observed for up to 2 years after treatment.[656] Cisplatin may induce a more global nephropathy and azotemic renal failure, but the magnesium wasting appears to be an isolated functional abnormality.

Other Causes of Hypomagnesemia

Magnesium, like phosphate, is a major intracellular ion, and significant shifts of magnesium from the extracellular compartment therefore may occur during recovery from chronic respiratory acidosis or acute ketoacidosis, after refeeding, following administration of hyperalimentation solutions, and in response to elevations of circulating catecholamines.[634] Other rapid losses of extracellular magnesium may occur during periods of greatly accelerated net bone formation (after parathyroidectomy, during recovery from vitamin D deficiency, with osteoblastic metastases) or with large losses due to pancreatitis, cardiopulmonary bypass surgery,[657] massive transfusion,[658] extensive burns, excessive sweating, pregnancy, or lactation.

Consequences of Hypomagnesemia

Most of the signs and symptoms of hypomagnesemia reflect alterations in neuromuscular function: tetany, hyperreflexia, positive Chvostek and Trousseau signs, tremors, fasciculations, seizures, ataxia, nystagmus, vertigo, choreoathetosis, muscle weakness, apathy, depression, irritability, delirium, and psychosis.[634] Patients usually are not symptomatic unless serum magnesium falls below 1 mEq/L, although occurrence of symptoms, such as intracellular magnesium, may not correlate well with serum magnesium. Atrial or ventricular arrhythmias may occur, as may various electrocardiographic abnormalities—prolonged PR or QT intervals, T-wave flattening or inversion, or ST-segment straightening.[634] Hypomagnesemia also increases myocardial sensitivity to digitalis intoxication.[659]

Hypomagnesemia evokes important alterations in mineral ion and potassium homeostasis that frequently aggravate the clinical syndrome. Magnesium-deprived humans or animals develop hypocalcemia, hypocalciuria, hypokalemia (due to impaired tubular reabsorption of potassium), and positive calcium and sodium balance.[649,660] Sustained correction of hypocalcemia or hypokalemia cannot be achieved by administration of calcium or potassium alone, respectively, whereas both abnormalities respond to administration of magnesium.[636,661]

The mechanism of hypocalcemia in this setting may be multifactorial. Inappropriately normal or low serum PTH, despite hypocalcemia, is common and indicates a defect in PTH secretion,[662] which is due to augmented signaling by CaSR-associated G proteins, normally inhibited by magnesium, within the parathyroid cell.[663] Other evidence indicates that hypomagnesemia also may impair PTH action on target cells in bone and kidney, although some have observed normal responsiveness; the issue remains controversial.[483,484,661,662,664]

Vitamin D resistance also is a feature of hypomagnesemic states.[665,666] This disorder appears to be due mainly to impaired renal 1α-hydroxylation of 25(OH)D, although tissue resistance to $1,25(OH)_2D$ also may play a role.[651,667] The serum $1,25(OH)_2D$ concentration usually is low during hypomagnesemia, which may result from magnesium depletion per se, parathyroid insufficiency, or coexistent vitamin D deficiency.[668–670] Deficiency of $1,25(OH)_2D$ probably is not the main cause of hypocalcemia in these patients, however, because hypocalcemia can be rapidly corrected (within hours to days) by magnesium therapy alone, well in advance of any increase in the serum $1,25(OH)_2D$ concentration.[668,669]

Therapy of Hypomagnesemia

Patients with mild, asymptomatic hypomagnesemia may be treated with oral magnesium salts (i.e., $MgCl_2$, MgO, $Mg[OH]_2$), usually given in divided doses totaling 40 to 60 mEq (480–720 mg) per day (see Table 29.6). Diarrhea sometimes occurs with larger doses but generally is not a problem. The gluconate form (54 mg magnesium per gram) is said to cause less diarrhea.[634] Patients with malabsorption or ongoing urinary magnesium losses may require chronic oral therapy to avoid recurrent magnesium depletion. Although intestinal magnesium absorption is severely impaired in renal failure,[671] oral magnesium must be administered with great caution in this setting, especially in patients receiving concomitant therapy with $1,25(OH)_2D$.

Symptomatic or severe (<1 mEq/L) hypomagnesemia, especially if complicated by hypocalcemia, usually signifies magnesium deficits of at least 1 to 2 mEq/kg and is best treated promptly with parenteral magnesium salts. The use of intramuscular $MgSO_4$ is to be discouraged, as the injections are painful and provide relatively little magnesium (2 mL of 50% $MgSO_4$ supplies only 8 mEq of magnesium, compared with typical magnesium deficits in excess of 100 mEq). Moreover, because unretained sulfate ions also may increase urinary calcium excretion, intravenous magnesium chloride or gluconate probably is the most logical approach to initial parenteral therapy for patients who also may be hypocalcemic. In adult hypomagnesemic patients with normal renal function, rates of infusion of 2 to 4 mEq per hour (i.e., 50–100 mEq/day) generally are needed to maintain serum magnesium in the range of 2 to 3 mEq/L.[630,662,666] Up to 100 mEq per day for 2 days can be safely administered without elevating serum magnesium above 4 mEq/L, whereas doses of 200 mEq per day may increase serum magnesium to 4.5 to 5.5 mEq/L and thus are excessive.[672] In patients with active seizures or other urgent indications, the infusion may be preceded by a slowly administered bolus of 10 to 20 mEq, followed by a higher rate of infusion (i.e., 10–15 mEq/hour) for the first 1 to 2 hours only. Patients with normal renal function can readily excrete over 400 mEq per day of magnesium in the urine without becoming hypermagnesemic, but even mild renal failure may greatly limit magnesium excretion. Therefore doses of magnesium supplements should be reduced twofold to threefold, and careful serial monitoring of serum magnesium should be performed in patients with compromised renal function.

It is important to appreciate that a large fraction of parenterally administered magnesium may be excreted in the urine, even in patients with profound magnesium deficiency. Many such patients will excrete as much as 50% to 75% of infused magnesium, whereas in normal subjects this approaches 100%.[636] Moreover, because equilibration of the intracellular and extracellular magnesium pools is relatively slow, it is generally necessary to continue magnesium therapy for 3 to 5 days to achieve adequate

repletion of the typical deficit of 1 to 2 mEq/kg. Because serum magnesium may become normal well before tissue stores are repleted, monitoring of urinary magnesium excretion is a more reliable measure of the approach to full repletion, especially after patients are switched to oral therapy.

The need for calcium, potassium, and phosphate supplementation should be considered in the usual clinical setting of hypomagnesemia. Vitamin D deficiency also frequently coexists and should be treated with oral or parenteral vitamin D or 25(OH) D. Use of $1,25(OH)_2D$ is not necessary, does not hasten recovery, and may actually worsen hypomagnesemia by suppressing PTH secretion and thereby promoting renal magnesium excretion.[673]

Initial parenteral magnesium therapy in hypocalcemic patients may produce dramatic hypophosphatemia via the rapid stimulation of PTH secretion. This is most likely to be problematic in those with underlying phosphate depletion (malabsorption, alcoholism, diabetes), in whom it may provoke acute neuromuscular dysfunction, and it may be avoided by concomitant intravenous calcium therapy.

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Osteoporosis: Basic and Clinical Aspects

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CHAPTER OUTLINE

KEY POINTS

- The functional significance of bone has been reassessed in light of recent discoveries. In addition to the well-known mechanical support and storage of mineral, mineralized mesenchymal tissue also exports peptides critical for regulation of circulating phosphate and whole-body energy metabolism. We now have a more complete picture of the skeleton and its role in maintaining mineral and metabolic homeostasis.
- Osteoporosis has a great impact on quality of life and mortality because of its negative impact on bone strength. In the past three decades, risk factors for fractures have been exhaustively scrutinized. Online algorithms are available to help predict long-term fracture risk and are a readily available and important tool for evaluating patients. In parallel, biochemical markers of bone remodeling and imaging techniques to evaluate bone metabolism and structure, respectively, are employed for early recognition of fracture susceptibility.
- Advances in our knowledge of mechanisms driving bone loss have accelerated development of cost-effective drugs for the prevention of fractures. Moreover, ongoing studies point to additional and more effective drugs for osteoporosis. Curiously, clinical awareness for diagnosing and treating osteoporosis remains underdeveloped; unfortunately, few individuals benefit from the level of knowledge and technology available to diagnose and manage the disorder.
- Unlike available therapeutic options for other chronic disorders, osteoporosis therapy is unique in that weekly, monthly, biannual, or even annual dosing may be sufficient to accomplish successful treatment.

Historical Context

Osteoporosis is a disorder characterized by reduced bone mass, impaired bone quality, and a propensity to fracture in both men and women. Previously, this disease was considered within the context of a syndrome identified by back pain, vertebral fractures, and reduced mineralization on plain radiographs. In the past, the approach to patients with these features focused on identifying secondary causes of low bone mass and treating fractures with orthopedic intervention and pain management.[1] However, with the advent of bone density measurements, the development of new treatment options, and greater public awareness, osteoporosis emerged as a primary disease manifested in multiple ways and suitable to be managed by aggressive prevention and intervention. During this period, significant progress was also made in understanding the complex pathogenesis of the disease.[2,3] Furthermore, there have been tremendous advances in delineating the role of bone remodeling in normal physiology, particularly in defining the process of peak bone acquisition.[4] Recently, the skeleton was also found to be an endocrine organ regulating metabolic homeostasis through the release of bone-specific peptides that modulate glucose transport, phosphate balance, and muscle function.

In addition to these developments, a consensus began to emerge concerning the strength of the association between low bone mineral density (BMD) and fracture risk and about the importance of qualitative aspects of the skeleton as additional risk determinants for fractures.[5,6] Newer imaging technology has provided a window into the microstructural world of bone, and these advances now allow investigators to better understand the many diseases and drugs associated with skeletal fragility.[7,8] Great strides have also been accomplished in understanding the epidemiology of this disease and the socioeconomic impact of fractures on both patients and society. The picture that has emerged is that osteoporosis is a disease with a significant degree of morbidity and mortality risks.[9,10] Advances in the field have led to the development of drugs capable of increasing bone mass and improving bone quality that have proven to be effective in reducing the incidence of fractures. Since 1995, when US Food and Drug Administration (FDA) approval for new antiosteoporotic drugs first began, there has been a significant decline in the rate of fracture in the US population.

Unfortunately, in the past few years, this auspicious trend has reversed, attracting great interest in what has been coined the "crisis in osteoporosis treatment."[11] Although the incidence of adverse events are rare, their significance has served to understate the vast fracture prevention benefits enjoyed by the majority of patients, leading to physician underprescription and patient reluctance to use the medication. Thus, a systematic approach to understanding and treating this disease lies in appreciating the physiology of skeletal remodeling and the processes by which the basic skeletal unit becomes disordered, as well as on the appropriate use of the tools available for diagnosis and management.

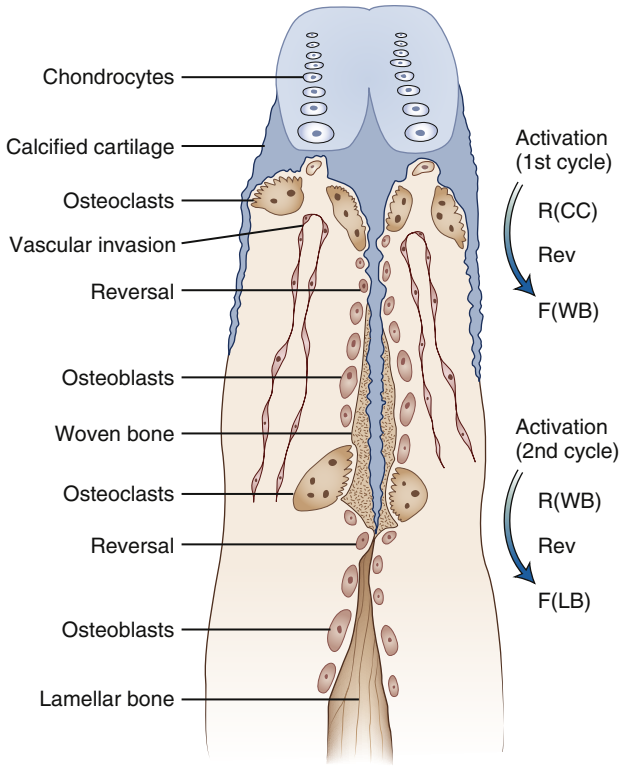
Skeletal Biology

Structure and Function of the Skeleton

The skeleton is one of the largest organ systems in the body, consisting of a mineralized matrix and a highly active cellular remodeling unit, composed of osteoblasts, osteoclasts, osteocytes, adipocytes, and lining cells. The most apparent function of the skeleton is to provide structural integrity for the organism that allows for a range of locomotor activity while concomitantly keeping additional body weight to a minimum. In addition to its structural function, the skeleton also serves as a mineral depot ensuring the maintenance of serum calcium and phosphate levels through normal remodeling processes and by secretion of bone-specific factors, such as fibroblast growth factor 23 (FGF23). Just as important, the skeleton is the home for hematopoiesis, maintaining a niche within trabecular bone elements that consists of osteoblasts, adipocytes, reticuloendothelial cells, sinusoids, and mesenchymal stromal and stem cells in a hypoxic environment. That niche provides progenitors that can respond to injuries at any site in the body for critical repair processes. Remarkably, the adult skeleton also harbors a huge adipose depot, composing 10% to 15% of all fat tissues in the body. Thus, it is clear that alterations in either the structural or metabolic functions of the skeleton have tremendous implications for the overall health of the organism.[12]

Embryology and Anatomy

Skeletal development begins early in embryonic life. The process starts with the condensation of mesenchymal cells that differentiate into a cartilaginous structure. Bone formation can then take place through one of two mechanisms: endochondral (i.e., through use of a cartilage framework and involving osteoblasts laying down true bone matrix on top of the cartilaginous matrix, followed by osteoclast-driven turnover of matrix and cells) or intramembranous bone formation, in which the mesenchymal precursors differentiate into bone-forming osteoblasts and lay down bone matrix without a cartilage template.[13] The growth of long bones and vertebrae involves endochondral bone formation. The cartilage cells in the growth plate proliferate and undergo hypertrophy; the hypertrophic chondrocytes then direct the mineralization of their matrix and, along with the action of osteoclasts, partly degrade their matrix. The cartilage is invaded by vessels, and the spicules of mineralized cartilage are covered by osteoblasts to form a cancellous or trabecular bone often called the *primary spongiosa.* These structures are resorbed and replaced by trabecular plates made up entirely of bone, called *secondary spongiosa* (Fig. 30.1). This process occurs at the ends of the long bones and in the bodies of the vertebrae.

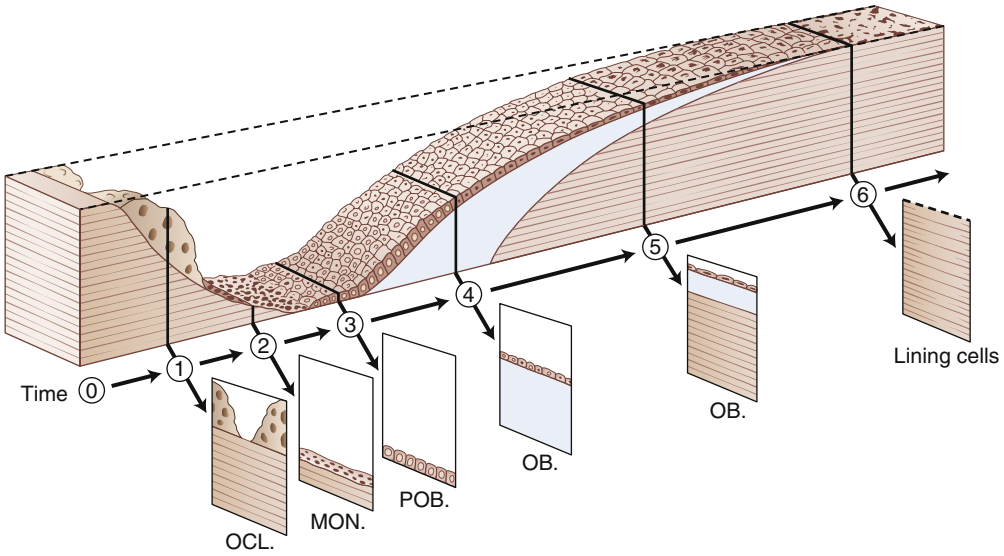


• **Fig. 30.1** Steps in endochondral bone formation. *CC,* calcified cartilage; *F,* formation; *LB,* lamellar bone; *R,* resorption; *Rev,* reversal; *WB,* woven bone. (Redrawn from Baron R. Anatomy and ultrastructure of bone. In: Favus MJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 2nd ed. New York, NY: Lippincott-Raven; 1993:3–9. Copyright 1993, American Society for Bone and Mineral Research.)

Intramembranous bone formation occurs next to the cartilage template in flat bones, such as the skull, scapula, and ileum, and on the outer surfaces of long bones, leading to periosteal apposition and expansion. Woven bone, with disordered fibrils of collagen I and a disorganized osteocyte network, is formed during the early stages of intramembranous acquisition but then becomes more organized as lamellar bone is produced by oriented layers of osteoblasts. As noted previously, the main difference between endochondral and intramembranous bone formation is that the latter does not use calcified cartilage as a direct template for osteoblasts.

Cortical bone is dense bone found in the shafts of long bones. It makes up 80% of the mass of the skeleton, determines its shape, and provides much of its strength. During longitudinal skeletal growth, endochondral and periosteal appositional bone formation determine the length and width of the bones.[4] New cortical bone is shaped by a process called *modeling*, in which osteoblast activity occurs uncoupled to osteoclastic bone resorption. Modeling leads to skeletal shape changes, which are critical for defining the strength of bone. Importantly, modeling is influenced by mechanical forces and is increased during the adolescent growth spurt.[4] As bones elongate, the wide cortex formed just below the growth plate must be sculpted by the modeling/resorption process to allow these bones to elongate while maintaining and extending the narrow tubular structure of the diaphysis.

Bone remodeling is an essential element of skeletal activity that provides skeletal stability and elasticity. It is the process that defines adult bone mass and maintenance (Fig. 30.2).



• **Fig. 30.2** Three-dimensional reconstruction of the remodeling sequence in human trabecular bone. *1*, Early bone resorption with osteoclasts (OCL). *2*, Late bone resorption with mononuclear cells (MON). *3*, Reversal phase with pre-osteoblasts (POB). *4*, Early matrix formation by osteoblasts (OB). *5*, Late bone formation with mineralization. *6*, Completed remodeling cycle with reversion to lining cells. (From Eriksen EF. Normal and pathological remodeling of human trabecular bone: three dimensional reconstruction of the remodeling sequence in normals and in metabolic bone disease. *Endocr Rev*. 1986;7:379–408. Copyright 1986 by The Endocrine Society.)

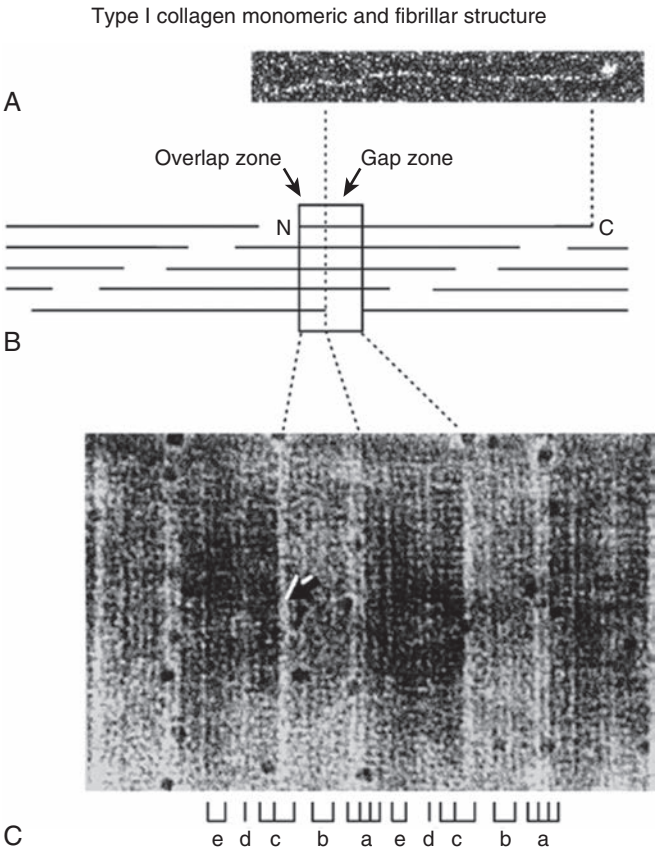
Remodeling is temporally orchestrated to maintain a balance between the amount of bone formed and the amount resorbed. Basic multicellular units (BMUs) carry out bone remodeling and consist of osteoblasts, osteoclasts, bone lining cells, and osteocytes. Remodeling is more active in cancellous or trabecular bone than in cortical bone.[14] In smaller animals, such as rodents, cortical bone can remain lamellar. In large animals and humans, lamellar cortical bone is gradually replaced through haversian remodeling to form cylindrical osteons. The initiation of bone remodeling is directed by endocrine, paracrine, and autocrine factors. The osteocyte, communicating by release of factors through tiny canaliculi, is thought to initiate the remodeling process, providing signals to both the lining cell and the osteoblast.[15] These cells can then signal to attract the osteoclast to the remodeling site where bone resorption occurs. This is followed by the release of matrix proteins that, in combination with osteoclast-derived factors, direct osteoblast differentiation, collagen synthesis, and ultimately matrix mineralization.

Bone Matrix and Mineral

The bone matrix consists of fibers of type I collagen laid down in layers that have various orientations, a portion of which in the mammalian skeleton may be disordered but still adds to the strength of the matrix (Figs. 30.3 and 30.4). The matrix contains several additional proteins, including other collagen types that may be important in the interaction of type I collagen with noncollagen proteins within the matrix. The noncollagen proteins, such as osteocalcin and several proteoglycans, represent about 10% of the total protein in bone and may direct the formation of fibers, mineralize bone, regulate the attachment of bone cells to its matrix, and play a role in the function of bone-forming and resorbing cells.

Protein composition of the matrix may vary, particularly between woven and lamellar bone.[16] The proteins range from the large cell-attachment proteins (e.g., thrombospondin, fibronectin), which have molecular masses higher than 400 kDa, to the small, vitamin K–dependent γ-carboxylated proteins (e.g., matrix Gla protein and osteocalcin), which are 6-kDa calcium-binding proteins. Osteocalcin can be incompletely or totally carboxylated depending on the number of glutamic acid sites within the molecule that are changed to γ-carboxylated glutamic acid by vitamin K–dependent enzymes; incomplete γ-carboxylation may represent the action of inhibitors such as warfarin or the action of decarboxylating processes. Undercarboxylated osteocalcin (GLU13-OCN) is released from the skeletal matrix during bone resorption (see later discussion). Some noncollagen proteins (e.g., biglycan, decorin, bone sialoprotein, osteopontin, osteoadherin) are highly acidic and play an important role in signaling and in the matrix of the hematopoietic niche. In addition to cell-attachment sequences, these proteins contain various amounts of carbohydrate and are called *glycoproteins* or *proteoglycans*. Noncollagen proteins of bone are often highly phosphorylated, which enables them to bind calcium, and thus may regulate mineralization. Genetic manipulations in experimental mouse models have provided important information on the function of noncollagenous proteins. For example, null mutations of the osteonectin gene lead to osteopenia in some studies, indicating that this matrix protein may be important for the maintenance of a normal bone structure.[17] However, deletion of the osteocalcin gene has been shown to increase bone mass.[18]

Osteocalcin null mice also have a striking body composition and insulin sensitivity phenotype.[19] Studies principally from the Karsenty laboratory, but now validated by other groups, have shown that GLU13-OCN is released from the skeletal matrix and can bind to a G protein–coupled receptor on the surface of beta cells and adipocytes.[20] This results in enhanced insulin production and greater glucose transport in adipocytes. Moreover, insulin itself can stimulate release of matrix GLU13-OCN. This activity requires the co-participation of osteoclasts, integrating bone remodeling in the regulation of insulin sensitivity. Insulin



• **Fig. 30.3** Type I collagen monomeric and fibrillar structure. (A) By rotary shadowing electron microscopy, procollagen (≅300 nm long) appears as a rope-like triple helix to which globular carboxy (C)-terminal (to the right) and amino (N)-terminal domains are attached. (B) Chapman's model of the collagen fibril. Diagram showing the arrangement of tropocollagen monomers within the collagen fibril, relative to the location of the overlap and gap zone fibril staining pattern. Tropocollagen molecules are shown as horizontal rods, and the polarity of all monomers in the fibril is indicated by *N* (NH2 terminus) and *C* (COOH terminus) markings on one monomer. (C) Electron micrograph of glutaraldehyde-fixed, heparin-gold type I collagen fibril complexes, in fibrils visualized by uranyl acetate staining. Letters below the micrograph show positions of positively stained fibril bands, following the accepted notation. Dotted lines between the molecular model in (B) and the electron micrograph show corresponding overlap and gap zones. The location of heparin-gold particles relative to the molecular structure of the fibril can be measured within each 67-nm period, beginning at the center of the left border of the overlap zone (origin, *arrow*), and extending to the center of the right border of the gap zone. Heparin-gold particles appear as circular dark objects present mainly in the "a" bands region of the fibrils. (From San Antonio JD, Lander AD, Karnovsky MJ, Slayter HS. Mapping the heparin-binding sites on type I collagen monomers and fibrils. *J Cell Biol*. 1994;125:1179–1188 by copyright permission of The Rockefeller University Press.)

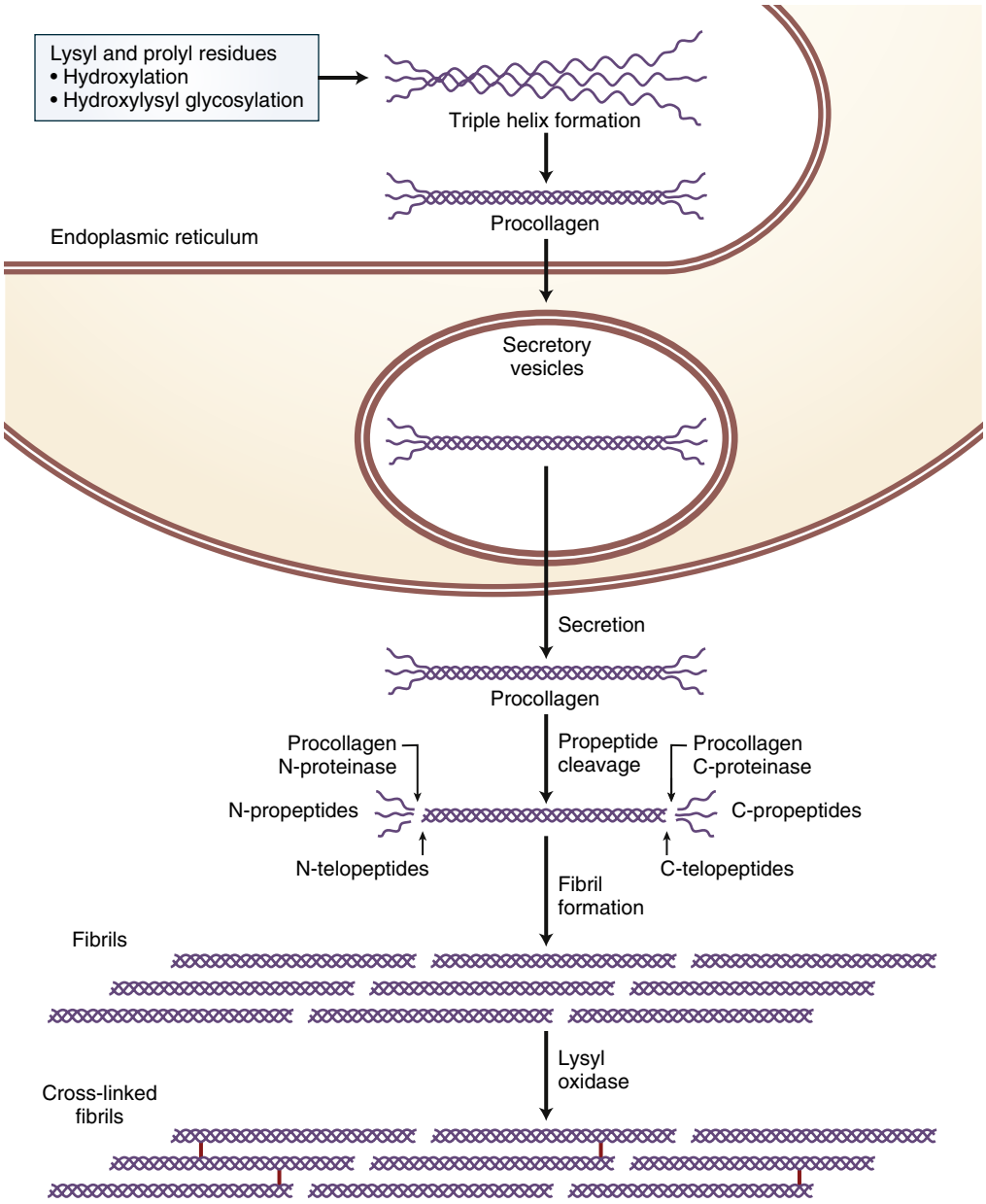
signaling in osteoblasts downregulates osteoprotegerin (OPG) via the forkhead box protein O1 (FOXO1), thereby enhancing osteoclastogenesis and ultimately greater bone resorption. The resultant increased osteoclastic activity creates the conditions necessary for acid-mediated decarboxylation of γ-carboxyglutamate residues[21,22] (Fig. 30.5). This was the first of several remarkable studies demonstrating the endocrine nature of the skeleton, in this case due to release of matrix proteins. Importantly, this finding led to even greater insights into the role of the skeleton in modulating energy metabolism.

Collagen Synthesis

Type I collagen is the most abundant protein of the bone matrix. It is a rigid, rod-like, insoluble molecule composed of two α1 chains and one α2 chain[23,24] (see Fig. 30.4). Collagen chains consist of repeating triplets of amino acids, with glycine in every third position and a high content of proline and lysine. The two α1 and the α2 collagen chains form a triple helix that is stabilized by the hydroxylation of proline and lysine residues and requires ascorbic acid. Collagen is synthesized as a soluble proprotein with large nonhelical extensions at the carboxy (C)- and amino (N)-terminal ends. Procollagen also contains C-terminal interchain disulfide bonds that help to initiate formation of the triple helical structure. Procollagen is released into the cisternae of the rough endoplasmic reticulum, packaged in the Golgi vesicles, and secreted extracellularly. The procollagen peptide ends are then removed by specific peptidases to produce mature insoluble collagen molecules, which are further stabilized by intramolecular and intermolecular cross-links. The major collagen cross-links are formed by lysine and hydroxylysine residues that ultimately form pyridinium ring structures (Fig. 30.6). In addition, nonenzymatic cross-links are formed as a result of reactions that create advanced glycation end products (AGEs), such as pentosidine. With advancing age, and particularly in the presence of diabetes mellitus, this process is more pronounced, compromising the structure and functional role of type I collagen in bone.[25]

Mineralization

Bone mineral is formed by small, imperfect hydroxyapatite crystals, which contain carbonate, magnesium, sodium, and potassium in addition to calcium and phosphate. Mineralization occurs by two distinct mechanisms, one outside the cell and catalyzed by alkaline phosphatase, and one in matrix vesicles that is accelerated by the enzyme phospho1.[26] Both enzymes are critical for adequate mineralization, the former acting to enhance the breakdown of pyrophosphate, an inhibitor of calcium-phosphate precipitation, and the latter to accelerate phosphate availability within the matrix by acting on phosphocholine and phosphoethanolamine. The initial mineralization of calcified cartilage and woven bone probably occurs by means of matrix vesicles.[26] These membrane-bound bodies are released from chondrocytes and osteoblasts, contain alkaline phosphatase, and can form a nidus for crystallization in the presence of adequate phosphate. In contrast, in lamellar bone, the collagen fibers are tightly packed, and matrix vesicles are rarely seen. Mineralization does not occur immediately after collagen deposition, and there is a layer of 10 to 100 μm of unmineralized osteoid between the mineralization front and the osteoblast. Changes in the packing of the fibrils and in the composition of the noncollagen proteins may be required for mineralization. A group of proteins, named SIBLINGs (small, integrin-binding ligand, N-linked glycoprotein), synthesized in osteocytes (i.e., osteopontin, dentin matrix protein 1 [DMP1], bone sialoprotein, and matrix extracellular phosphoglycoprotein [MEPE]), have a crucial role in the deposition of calcium into bone matrix. SIBLINGs combined with several endopeptidases, including phosphate-regulating endopeptidase homolog, X-linked (PHEX) modulate phosphate metabolism through the regulation of FGF23 synthesis, but also affect osteoclastogenesis and energy metabolism.[27,28] Mineralization of collagen fibrils begins in the *hole zones* of the calcium fibrils, where there is more room for inorganic ions to accumulate (see Fig. 30.3). Mineralization requires calcium, phosphate, and alkaline

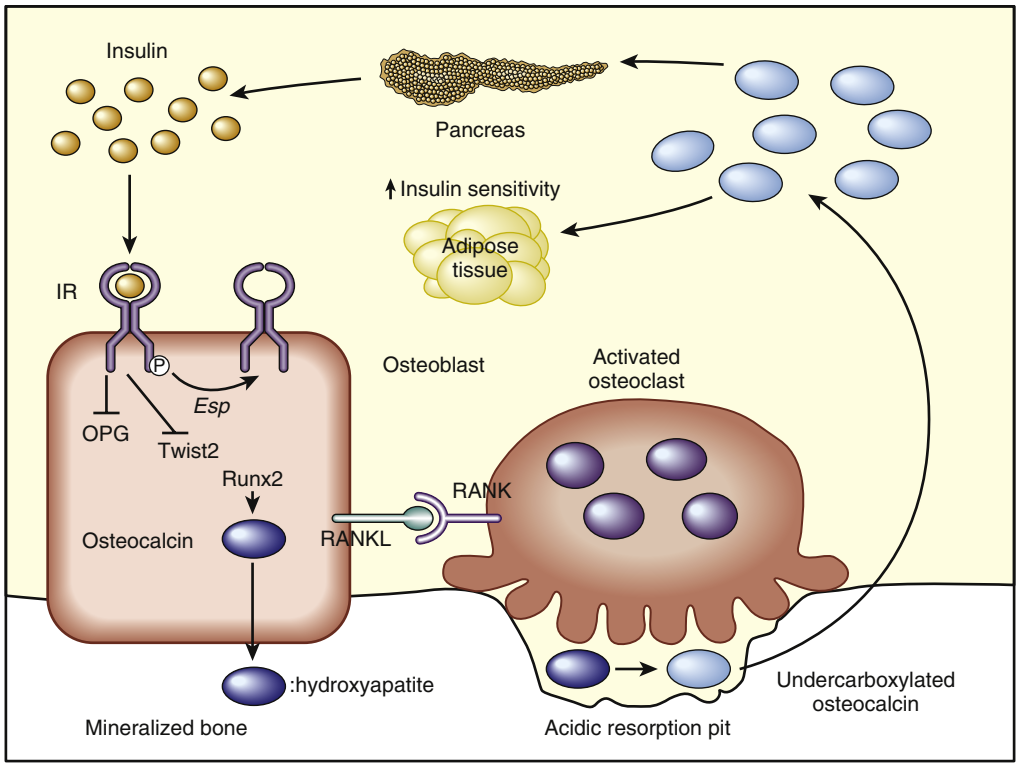


• **Fig. 30.4** Schematic diagram showing the different post-translational modifications and assembly of type I collagen into fibrils. (Modified from Myllyharju J, Kivirikko KI. Collagens, modifying enzymes and their mutations in humans, flies and worms. *Trends Genet.* 2004;20:33–43 and Viguet-Carrin S, Garnero P, Delmas PD. The role of collagen in bone strength. *Osteoporos Int.* 2006;17:319–336, with permission.)

phosphatase. This process is impaired in circumstances of vitamin D deficiency, very low calcium intake, hypophosphatemia, mutation in the gene encoding alkaline phosphatase, and importantly by the mineralization inhibitor pyrophosphate.[29] The characteristic feature of undermineralization on bone biopsy is greater osteoid (i.e., newly synthesized, nonmineralized collagen at the bone surface), and the most dramatic undermineralization occurs in the syndrome of hypophosphatasia, which is due to an absence of total nonspecific alkaline phosphatase. Recent work using a recombinant form of alkaline phosphatase has shown promising results by promoting complete mineralization of the skeleton and improvement in quality of life of patients with hypophosphatasia.[30]

Collagen Degradation by Osteoblasts and Osteocytes

As part of the bone remodeling process, collagen is cleaved and degraded by a group of proteases called *collagenases*. They are matrix metalloproteases (MMPs) that can initiate cleavage of collagen fibrils at neutral pH, and they are central to the process of collagen degradation, matrix breakdown, and bone remodeling. Three collagenases in bone have been described: collagenase 1 (MMP1), 2 (MMP8), and 3 (MMP13).[31] Human osteoblasts express the collagenase 1 and 3 genes (*MMP1* and *MMP13*). Resting osteoblasts secrete limited amounts of collagenase, and changes in the synthesis of collagenase correlate with changes in bone resorption. Collagenase plays a critical function in bone remodeling. Mice with deletions of the collagenase 3 gene or mutations of the



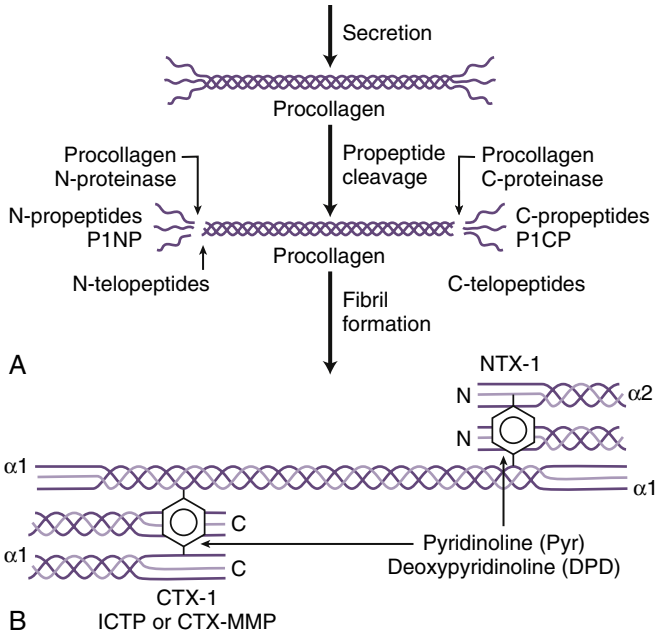
• **Fig. 30.5** Energy regulation and bone turnover by the insulin/osteocalcin Axis. A putative feed-forward regulatory loop ties bone turnover to energy regulation as proposed by Ferron and associates[22] and Fulzele and colleagues.[21] Insulin activates skeletal remodeling (i.e., increases bone formation by osteoblasts and resorption by osteoclasts), which in turn releases uncarboxylated osteocalcin from the skeletal matrix into the circulation. This enhances insulin secretion and increases the insulin sensitivity of adipocytes. A tyrosine phosphatase OST-PTP, which is encoded by the *Esp* gene, binds the insulin receptor (IR) and suppresses its activation through dephosphorylation. The transcription factor Twist2 is a critical downstream suppressor of osteoblast differentiation. Osteoprotegerin (OPG) is an osteoblast-specific inhibitor of receptor activator for nuclear factor κB ligand (RANKL), acting as a decoy receptor to block bone resorption. Hydroxyapatite is the mineral component of bone. (From Rosen CJ, Motyl KJ. No bones about it: insulin modulates skeletal remodeling. *Cell*. 2010;142[2]:198–200.)

α1[32] type I collagen gene have resistance to collagenase 3 cleavage and fail to resorb bone after exposure to parathyroid hormone (PTH).[33] The synthesis of collagenase by osteoblasts is regulated by hormones and by cytokines in the bone microenvironment that act by transcriptional and post-transcriptional mechanisms.[34] It should be noted that although cleavage by collagenase is an early and obligatory event, most degradation of collagen during remodeling is accomplished by osteoclasts that secrete protons and enzymes (e.g., cathepsin K) that solubilize mineral and degrade the matrix. Cleavage of collagen fragments leads to their excretion in the urine, in which sensitive assays can detect the N-terminal or C-terminal fragments (see later discussion).

Recent evidence points to the participation of osteocytes in aspects of remodeling. Osteocytes are terminally differentiated osteoblasts that are buried within the matrix of the skeleton but possess receptors for PTH and have metabolic activity. These cells are arranged in a functional unit termed the *osteon* that includes an elaborate dendritic network that communicates with the bone surface and probably participates in mechanical sensing. Osteocytes also are a rich source of receptor activator for nuclear factor κB ligand (RANKL), which enhances osteoclast differentiation, particularly during states of increased calcium demand, such as lactation, or immediately following estrogen deficiency. Moreover, osteocytes can secrete enzymes that directly degrade bone matrix in a process called *osteocytic osteolysis*, likely through elaboration of acid phosphatase and collagenases. Whether osteocytes also secrete alkaline phosphatase and participate in normal skeletal remodeling per se is still open to debate.

Bone Lining Cells, Osteoblasts, and Osteocytes

Bone is formed by *osteoblasts*, which are terminally differentiated cells that do not undergo mitosis and have many unique features (Fig. 30.7). Osteoblasts are derived from mesenchymal cells in the skeletal microenvironment.[35] Some osteogenic precursors may appear in the circulation, particularly during growth or following injury; they originate from skeletal tissue, and their contribution to bone formation is not certain. Osteoprogenitor cells, or preosteoblasts, replicate and differentiate into active osteoblasts that exhibit various phenotypic characteristics.[36] For example, osteoblasts in early development and during repair produce woven bone, whereas more mature osteoblasts produce lamellar bone. Osteoblast activity varies during bone formation. Some cells are tall and closely packed and produce a large amount of matrix in a small area; others are flatter and produce matrix at a slower rate over a larger area. Nevertheless, all differentiated osteoblasts share certain features. They are connected by gap junctions and contain a dense network of rough endoplasmic reticulum and a large Golgi complex, and they secrete collagen and noncollagen



• **Fig. 30.6** (A) During bone formation, the type I collagen molecule is synthesized as procollagen, and then amino (N)-terminal and carboxy (C)-terminal propeptides (P1NP and P1CP, respectively) are cleaved. The central part of the molecule, triple helix of collagen, is incorporated into bone matrix. (B) During bone resorption, different products of the breakdown of type I collagen are produced: cross-linked molecules (pyridinoline [Pyr], deoxypyridinoline [DPD]), C-terminal and N-terminal cross-linked telopeptides generated by cathepsin K (CTX-I and NTX-I, respectively), and C-terminal telopeptide generated by metalloproteinases (ICTP or CTX-MMP). (Redrawn from Szulc P, Kaufman JM, Delmas PD. Biochemical assessment of bone turnover and bone fragility in men. *Osteoporos Int.* 2007;18:1451–1461.)

proteins in an oriented fashion. Some products, such as osteocalcin, are synthesized almost uniquely by osteoblasts and osteocytes. A large proportion of the osteocalcin originating from osteoblasts is deposited in the matrix and subsequently released during bone remodeling. Hence, changes in serum levels of osteocalcin reflect bone turnover rather than bone formation per se. As noted previously, GLU13-OCN may serve as an endocrine hormone prompting insulin secretion and enhancing insulin sensitivity in peripheral tissues.

Mature osteoblasts have a finite capacity to produce matrix, and bone formation is sustained by the arrival of new populations of cells at the bone surface. The number and function of osteoblasts are determined by hormones, local growth factors, and cytokines. Some act as classic cell mitogens and increase the population of pre-osteoblastic cells, some determine their differentiation into mature osteoblasts, and others modify the function of mature cells or enhance osteocytic formation.[37] The ultimate fate of mature osteoblasts varies. They may die by apoptosis; they may become embedded in the matrix and become osteocytes; or they may be converted to flattened lining cells, which synthesize little protein and cover a large percentage of the surface of bone with a thin cytoplasmic layer (i.e., the bone lining cell) (see Fig. 30.7).

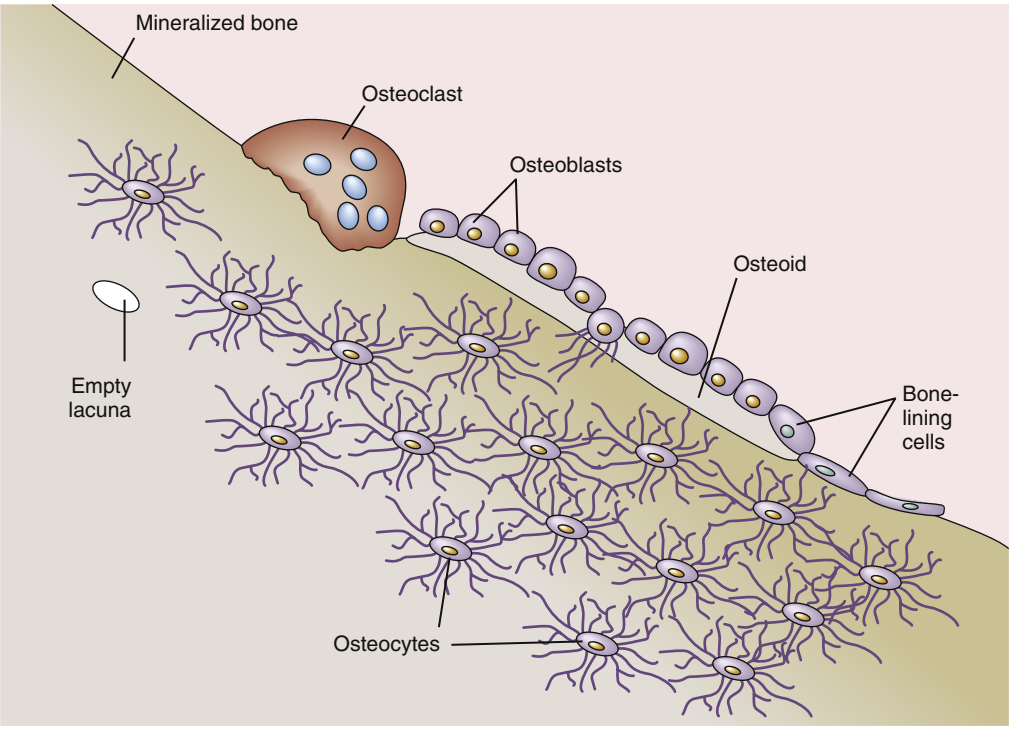
The bone lining cells are flattened and have a fibroblastic-like appearance. Recent work has begun to clarify the role of these lining cells as more active participants in the remodeling unit, in a manner similar to the osteocyte rather than purely quiescent cells. For example, bone lining cells express *osterix* (Sp7), which is a major transcription factor for osteoblast differentiation. In addition, these cells are in communication through small canaliculi with osteocytes buried within the matrix and express similar markers of differentiation. In response to PTH, which is a bone remodeling stimulant, bone lining cells differentiate into osteoblasts and thus become a pool of reserve cells necessary during accelerated turnover within the remodeling unit.[38] Bone lining cells also express stem-like genes that potentially indicate that there could be dedifferentiation of osteoblasts or that these cells are another reservoir for skeletal or even adipogenic progenitors (see later discussion).

Bone marrow stroma contain pluripotent cells that can differentiate into diverse cells of mesenchymal lineage, including osteoblasts, chondrocytes, and adipocytes[35] (Fig. 30.8). The ultimate cellular phenotype depends on factors present in the cellular microenvironment, the degree of hypoxia, the biochemical and metabolic signature of these cells, and their effects on intracellular signals and gene expression. The types and numbers of transcription factors are nuclear proteins that bind to DNA to regulate gene transcription. Some can determine the fate of undifferentiated cells, although the process is complex and includes metabolic determinants such as sufficient mitochondrial and glycolytic machinery.[39]

CCAAT/enhancer-binding proteins β and δ and peroxisome proliferator-activated receptor γ (PPARγ) play an essential role in the differentiation of cells toward adipocytes, whereas runt-related transcription factor 2 (RUNX2) plays a central role in the differentiation of cells toward osteoblasts.[40,41] Targeted disruption of the *RUNX2* gene results in disorganized chondrocyte maturation and a complete lack of bone formation due to an arrest of osteoblast development.[42]

Osterix (Sp7) is another transcription factor that is required for endochondral and intramembranous bone formation. It is regulated by RUNX2 and thought to be the next stage in osteoblast differentiation. *Sp7* null mice fail to develop a mineralized skeleton because of an arrest of late stages of osteoblast differentiation. Interactions between nuclear factors are common steps in the regulation of transcription and differentiation.[43] Osterix associates and acts cooperatively with a nuclear factor of activated T cells (NFAT), a transcription factor that regulates osteoblastogenesis and osteoclastogenesis.[44] CCAAT/enhancer-binding proteins can interact with RUNX2 and with the activating transcription factor (ATF)/cyclic adenosine monophosphate response element–binding protein family of proteins. ATF4 plays a central role in osteoblastic function, and its activity is regulated by a nuclear matrix attachment region binding protein, SATB2, which interacts with ATF4 and RUNX2 to regulate osteoblast differentiation.[45]

The conversion of osteoblasts to osteocytes involves a change in metabolic activity and the development of an extensive network of dendrite-like processes that communicate with those on adjacent osteocytes and cells of the bone surface (Fig. 30.9). Both osteoblasts and bone lining cells contain cell processes that are connected to underlying osteocytes through small canaliculi. After mineralization is complete and the osteocyte is encased in mineralized bone, these processes maintain connections among osteocytes and allow for at least two important features that are essential for the multicellular unit. First, the extended syncytium with its extensive canalicular network that allows rapid diffusion of small molecules from the marrow space, along with cell-cell junctions that allow transport from the cytoplasm of one osteocyte directly to that of another, is important for supporting the viability of the osteocytes. Second, it allows a constant exchange of information



• **Fig. 30.7** Microstructure of an actively remodeling trabecular bone surface. The osteoclast initiates the remodeling cycle by resorbing an area of bone matrix, immediately followed by osteoblast differentiation and osteoid (unmineralized bone matrix) production to replace the resorbed bone. During this process, a small fraction of osteoblasts differentiate further to become osteocytes, encasing themselves within the mineralizing bone matrix and joining the osteocyte network. Mature bone surfaces are populated with bone lining cells, whose origin and function remain unclear. (From DiGirolamo DJ, Clemens TL, Kousteni S. The skeleton as an endocrine organ. *Nat Rev Rheumatol.* 2012;8[11]:674–683.)

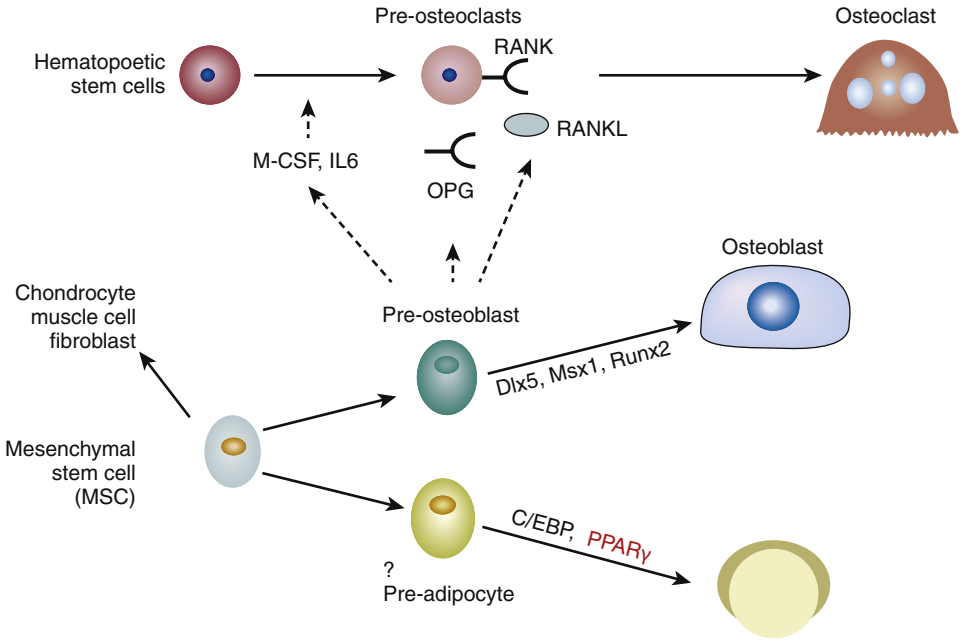
in the form of secreted factors between the endocortical surface and the matrix to regulate remodeling, as well as potential recruitment of precursors, such as the bone lining cells (Fig. 30.10; see Fig. 30.9).

Initially, osteocytes may continue to synthesize collagen and play a role in mineralization. Later, the major role of the osteocyte-osteoblast syncytium may be to sense mechanical forces.[46] Osteocytes probably sense bone deformation, as well as fluid shifts, and provide signals for the adaptive remodeling of bone size and shape.[46] One hypothesis is that small strains produce fluid shear stress in the canaliculi between osteocytes. This effect may result in intracellular signaling through changes in ion channels or in the production of biologically active molecules. Regions of bone microdamage contain apoptotic osteocytes, which may provide signals for the initiation of bone remodeling by osteoclasts and the consequent removal of damaged bone.[23]

Cells of the osteoblastic lineage are important for forming bone and for initiating bone resorption. Both mature osteoblasts and osteocytes may play a role in activating resorption. Most of the hormonal factors that stimulate bone resorption act on cells of the osteoblastic lineage. They stimulate the synthesis and perhaps release of RANKL and colony-stimulating factor 1 (CSF1), which are essential for osteoclastogenesis.[46,47] Osteoblasts also produce additional factors that regulate bone resorption, including cytokines, prostaglandins, and local growth factors. In cell culture, contact between osteoblastic cells and hematopoietic cells appears to be necessary for osteoclast formation (Fig. 30.11). Osteoblasts, as noted earlier, may also play a role in initiating bone resorption by releasing collagenases, other metalloproteinases, and plasminogen activator. These enzymes may remove the surface proteins of bone, which prevent the access of osteoclasts to the mineralized matrix. Osteoblasts also influence the development and maintenance of the marrow through their production of growth factors, cytokines, and chemokines that regulate the growth and development of hematopoietic cells.

Osteoclast Differentiation and Function

Osteoclasts are derived from hematopoietic progenitors and are myeloid in origin. Hematopoietic stem cells under the direction of cytokines and possibly cell-cell interactions express transcription factors that define their commitment to the osteoclast lineage (Fig. 30.12). CSF1 (i.e., macrophage CSF [M-CSF]) is the major cytokine regulating the replication and development in bone marrow of progenitor cells that are capable of differentiating into osteoclasts. Expression of the transcription factor SPI1 (formerly called *PU.1*) is also necessary for the osteoclast precursor cell to develop.[48] In bone marrow, the osteoclast precursor cell is multipotent and can differentiate into monocyte-macrophages, dendritic cells, or pre-osteoclasts.[49] The latter fuse to form highly differentiated, multinucleated osteoclasts that resorb bone. Osteoclasts also exhibit other remarkable characteristics, including a high number of mitochondria, the capacity to function in both oxygenated and hypoxic environments, and susceptibility to apoptosis.[50] Progression through the osteoclast pathway is influenced by multiple local and systemic hormones that may include 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], prostaglandins, and the cytokines interleukin 1 (IL1), IL6, and tumor necrosis factor (TNF). Osteomacs are macrophages in the bone remodeling unit and may be important for



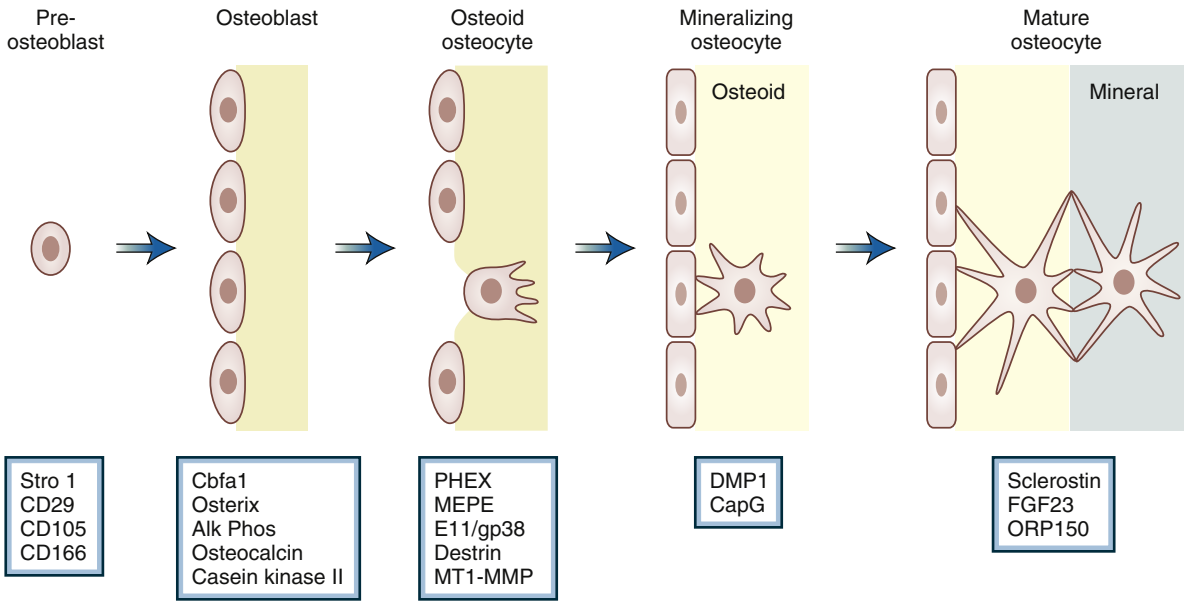
• **Fig. 30.8** For a mesenchymal stem cell to become an osteoblast, activation of several key factors, such as Runx2, BMP 2, TGFβ, and transcription factor Sp7 (osterix), are necessary, although the precise sequence of events in this cascade has not been fully clarified. In contrast, to achieve full adipocytic differentiation, there are two groups of critical factors already present in mesenchymal stem cells that need to be activated: C/EPBs α, β, and δ, and PPARs α, γ2, and δ. PPARγ2 activation by endogenous (e.g., prostaglandin J_2, long-chain and oxidized fatty acids) or exogenous (e.g., rosiglitazone) ligands dramatically shifts allocation of mesenchymal stem cells toward the adipocytic pathway and away from the osteoblast lineage. In vitro, this shift is characterized as an *either/or* allocation: *either* the cell becomes a fat cell *or* it becomes a bone cell, but not both. Inflammatory cytokines can be released from adipocytes, and circulating hormones such as leptin, adipsin, adiponectin, and resistin are also produced by fat cells. The *solid arrows* represent confirmed networks for regulation, and the *dashed arrows* represent potential regulatory pathways. BMP, bone morphogenetic protein; C/EBP, CCAAT/enhancer-binding proteins; Dlx5, distal-less homeobox 5; HSC, hematopoietic stem cell; IGF, insulin-like growth factor; IGFR, insulin-like growth factor receptor; IL, interleukin; M-CSF, macrophage colony-stimulating factor; Msx1, MSH homeobox homolog 1; OPG, osteoprotegerin (tumor necrosis factor ligand superfamily, member 11); PPAR, peroxisome proliferator-activated receptor; RANK, receptor activator of nuclear transcription factor κB (tumor necrosis factor receptor superfamily, member 11a); RANKL, RANK ligand (tumor necrosis factor receptor superfamily, member 11); Runx2, runt-related transcription factor 2; TGF, transforming growth factor. (From Rosen CJ, Bouxsein ML. Mechanisms of disease: is osteoporosis the obesity of bone? *Nat Clin Pract Rheumatol.* 2006;2[1]:35–43.)

development of a canopy over the remodeling unit and clearance of degraded proteins, as well as antigen presentation. Additionally, osteomacs may participate in the removal of "old" osteoblasts.

The nature of the osteoblast-lineage cell products, which directly regulate osteoclast formation and function, has been clarified.[47] The principal stimulator of osteoclast formation is RANKL, a member of the TNF protein superfamily. This protein was originally identified as a product of activated T lymphocytes, but it is also recognized as a critical stimulator of osteoclastogenesis from mesenchymal stromal cells, pre-osteoblasts, osteocytes, and hypertrophic chondrocytes. Production of RANKL in osteoblast-lineage cells is stimulated by essentially all agents that enhance osteoclast formation, including PTH, $1,25(OH)_2D$, prostaglandins, and many cytokines. Mice that are deficient in RANKL do not form osteoclasts and have osteopetrosis. In contrast, injection of RANKL into mice stimulates osteoclast formation and bone resorption but may also be associated with increases in marrow adiposity. RANKL is produced as a membrane protein; in activated T lymphocytes, RANKL is cleaved from the cell membrane and is released as a soluble factor.[51] It is unclear whether similar events occur in osteoblast-lineage cells, although there is some evidence that cleavage and release of soluble RANKL occurs in malignant cells that metastasize to bone.

OPG is a native inhibitor of osteoclastogenesis; it is a soluble receptor for RANKL that binds this ligand and prevents interaction of RANKL with its cognate receptor, RANK. OPG is produced widely. In bone marrow cultures, a number of stimulators of resorption, including PTH, $1,25(OH)_2D$, and prostaglandin E_2 (PGE_2), inhibit OPG production. For these factors, there is a reciprocal relationship between RANKL stimulation and OPG inhibition that causes activation of osteoclastogenesis and enhanced resorption. Mice that are deficient in OPG have osteoporosis, whereas mice that overexpress OPG have increased bone mass. These results, together with those for RANKL-deficient mice and mice injected with RANKL, demonstrate that osteoclast-mediated bone resorption is tightly regulated by the combined actions of RANKL and OPG.

The active receptor for RANKL is RANK, a member of the TNF receptor superfamily. Osteoclasts and their immediate precursor cells express RANK, and this expression is induced



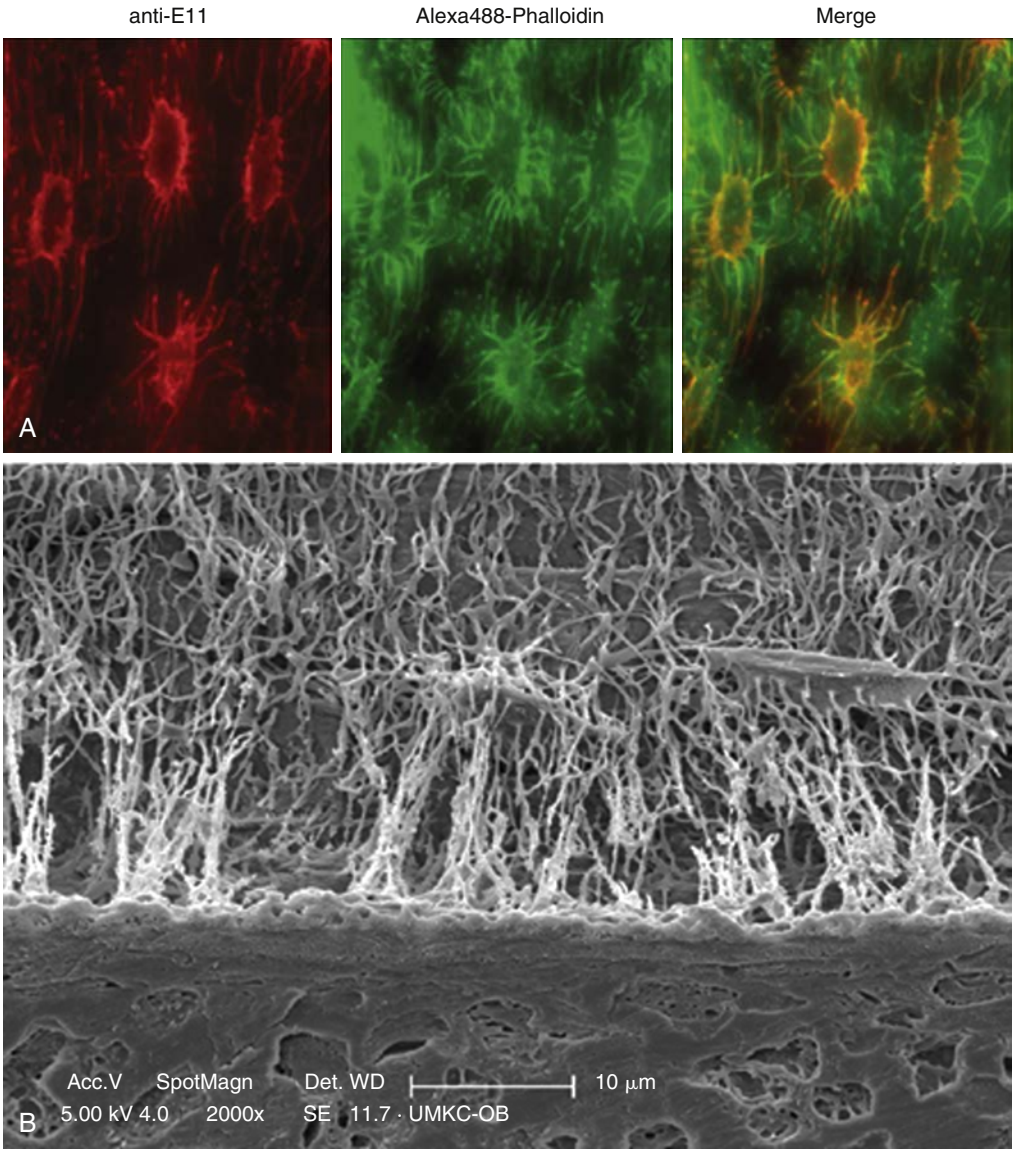
• **Fig. 30.9** Expression of markers during osteoblast-to-osteocyte ontogeny. The osteocyte appears to be the descendant of the matrix-producing osteoblast, which is a descendant of the mesenchymal stem cell known to express markers such as Stro1, CD29, CD105, and CD166. Matrix-producing osteoblasts express Cbfa1 and osterix, necessary for osteoblast differentiation, followed by alkaline phosphatase and collagen, necessary for the production of osteoid. Osteocalcin is produced by the late osteoblast and continues to be expressed by the osteocyte. By some unknown mechanism, some designated cells begin to embed in osteoid and begin to extend dendritic projections, keeping connections with already embedded cells and cells on the bone surface. Molecules such as E11/gp38 and MT1-MMP appear to play a role in dendrite/canaliculi formation, whereas molecules such as destrin and CapG regulate the cytoskeleton. Phosphate-regulating endopeptidase homolog, X-linked (PHEX), matrix extracellular phosphoglycoprotein (MEPE), and dentin matrix protein 1 (DMP1) regulate biomineralization and mineral metabolism, and fibroblast growth factor 23 (FGF23) regulates renal phosphate excretion. FGF23 is elevated not only in osteocytes from hypophosphatemic animals but also in those of normal rats. Sclerostin is a marker of the mature osteocyte and is a negative regulator of bone formation. ORP150 may preserve viability of this cell in a hypoxic environment. (Redrawn from Bonewald LF. The amazing osteocyte. *J Bone Miner Res.* 2011;26[2]:229–238.)

by M-CSF.[52] Binding of RANKL to RANK activates a series of intracellular pathways that activate NF-κB and mitogen-activated protein kinases, as well as NFAT and the activator protein 1 (AP1) family of transcription factors. The TNF receptor-associated factors (TRAFs), and particularly TRAF6, are ubiquitin E3 ligases that bind RANK intracellularly and are involved in RANK responses. Mice deficient in TRAF6, like those deficient in RANK, develop osteopetrosis. In addition to its effects on bone, the RANKL/RANK system is involved in lymphocyte function, barrier functions in the skin, and breast and lymph node development. Mature osteoclasts express RANK, and treatment of these cells with RANKL inhibits apoptosis and stimulates resorptive activity.[53]

In addition to RANKL, M-CSF is essential for osteoclast formation. Mice that are deficient in M-CSF have osteopetrosis and few osteoclasts.[54] In cultures of isolated osteoclast precursor cells, both M-CSF and RANKL must be present to form mature osteoclasts. M-CSF enhances RANK production in osteoclast precursors and inhibits apoptosis of osteoclast precursors and mature osteoclasts. The receptor for M-CSF, CSF1 receptor (CSF1R) (formerly designated C-FMS), is present on osteoclast precursors and mature osteoclasts.[52] Binding of CSF1R by M-CSF activates tyrosine kinase activity in the receptor, which initiates a series of intracellular downstream events.

A series of coactivator molecules is critical for osteoclast development. These molecules include members of the cytoplasmic immunoreceptor tyrosine-based activation motif (ITAM) family, namely Fc-receptor γ-subunit (FcRγ) and DNAX activation protein 12 (DAP12). ITAM proteins interact with receptor proteins in the cell membrane of osteoclast precursor cells. The search for receptors associated with these ITAM adaptors in myeloid cells has identified at least two candidates that associate with FcRγ (osteoclast-associated receptor [OSCAR] and paired immunoglobulin-like receptor [PIR] and two that associate with DAP12 (the triggering receptor expressed by myeloid cells 2 [TREM2] and the signal regulatory protein β1 [SIRPB1]). Mice that are deficient in FcRγ and DAP12 have osteopetrosis and deficient osteoclast formation despite their ability to express RANKL, RANK, M-CSF, and CSF1R.[44,55] This signaling pathway responds to ligands that have not yet been established genetically; together with RANK, it stimulates the accumulation of intracellular calcium, which is required for dephosphorylation of NFAT; this allows the transport of NFAT into the nucleus, where it acts as a transcription factor.

The formation of multinucleated osteoclast-like cells in vitro requires hematopoietic precursors and cells of the mesenchymal/osteoblast lineage present within the niche.[56] In vivo and in cultures with devitalized bone, mononuclear pre-osteoclasts attach to the bone surface and form multinucleated osteoclasts by fusion.[57] The accumulation of additional nuclei into osteoclasts by fusion probably continues while the cell is actively resorbing. The life span of the osteoclast is limited. As osteoclasts become inactive, they die by apoptosis. Hormones that enhance bone resorption may delay

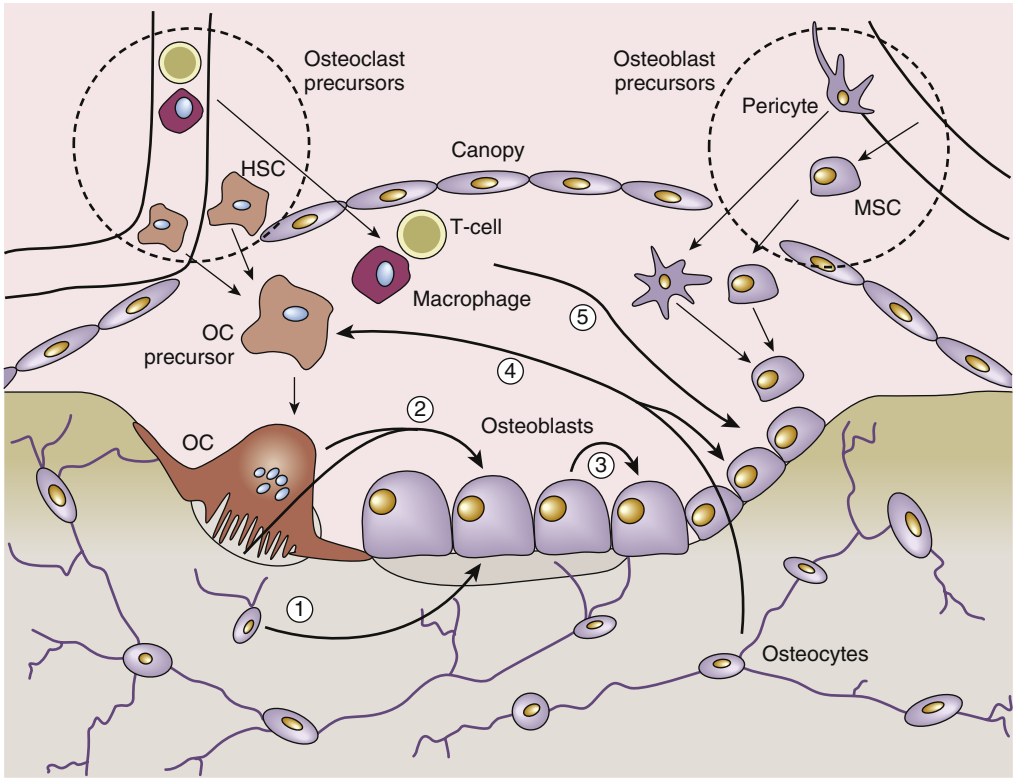


• **Fig. 30.10** (A) Visualization of early, embedding osteocytes. Using anti-E11 immunostaining and visualization of the actin cytoskeleton by alexa488 staining for phalloidin, one can visualize the embedding osteocyte and the early osteocyte in 12-day murine calvaria. The merged image shows that the majority of the E11 is on the cell surface and along the dendritic processes. In addition, if one looks closely, the dendrites that end on the cell surface have a bulbous tip of unknown function. This structure must interface with the cells on the bone surface. (B) An acid-etched resin-embedded murine sample showing an osteocyte lacuna sending canaliculi to the bone surface. Note the rough surfaces of canaliculi toward the bone surface and the smooth surface of canaliculi that project away from the bone surface, suggesting a difference between forming and formed canaliculi. Both sets of images demonstrate the complexity of this network and the interface of osteocytes with the bone surface. (The images in A are provided by Dr. Sarah Dallas, University of Missouri at Kansas City, MO; B, from Bonewald LF. The amazing osteocyte. *J Bone Miner Res.* 2011;26[2]:229–238.)

apoptosis, and inhibitors of resorption probably accelerate it. The mechanisms that limit the extent of osteoclastic resorption are incompletely understood and may involve inhibition by calcium ions, which accumulate under the osteoclast resorbing surface, or by local inhibitory factors, such as transforming growth factor β (TGFβ), which are released and activated during resorption.

The mature osteoclast is a unique and highly specialized cell (Fig. 30.13). It usually contains 10 to 20 nuclei, but giant osteoclasts with up to 100 nuclei can be seen in Paget disease and in giant cell tumors of bone. The large size of osteoclasts is probably essential for their resorptive function. The best evidence for this comes from studies of dendritic cell–specific transmembrane protein (DC-STAMP), because inhibition of this protein or its complete deficiency in mouse models results in the generation of only mononuclear osteoclasts that have impaired resorptive activity.[58]

The capacity of osteoclasts to resorb bone depends on their ability to isolate a region of the bone surface from the extracellular fluid and produce a local environment that can dissolve bone mineral and degrade matrix. The osteoclast must polarize and produce a basolateral membrane opposite the resorption space, which



• **Fig. 30.11** Intercellular communication pathways within the basic multicellular unit that comprise all steps of the bone remodeling process. *1*, Stimulatory and inhibitory signals from osteocytes to osteoblasts (e.g., Oncostatin M [OSM], parathyroid hormone–related peptide, and sclerostin). *2*, Stimulatory and inhibitory signals from osteoclasts to osteoblasts (e.g., matrix-derived transforming growth factor β and insulin-like growth factor 1, secreted CT-1, Sema4D, and S1P). *3*, Signaling within the osteoblast lineage (e.g., ephrinB2 and EphB4, Sema3a, parathyroid hormone–related peptide, OSM). *4*, Stimulatory and inhibitory signals between the osteoblast and osteoclast lineages (e.g., receptor activator for nuclear factor κB ligand, Sema3B, Wnt5a, and osteoprotegerin). *5*, Marrow cell signals to osteoblasts (e.g., macrophage-derived OSM, T cell–derived interleukins, and receptor activator for nuclear factor κB ligand). *HSC,* hematopoietic stem cell; *MSC,* mesenchymal stem cell; *OC,* osteoclast. (From Sims NA, Martin TJ. Coupling the activities of bone formation and resorption: a multitude of signals within the basic multicellular unit. *Bonekey Rep.* 2014;3:481.)

facilitates the excretion of resorption products (see Fig. 30.13). The resorbing apparatus consists of a central ruffled border area, which secretes hydrogen ions and proteolytic enzymes, surrounded by a clear or sealing zone in a structure called the *podosome*. The podosome contains filamentous actin linked to $\alpha_v\beta_3$ integrin, and it anchors the cell to the bone surface. The osteoclast attaches to bone through the interaction of integrins in the podosome with noncollagenous proteins such as vitronectin and osteopontin in the matrix.

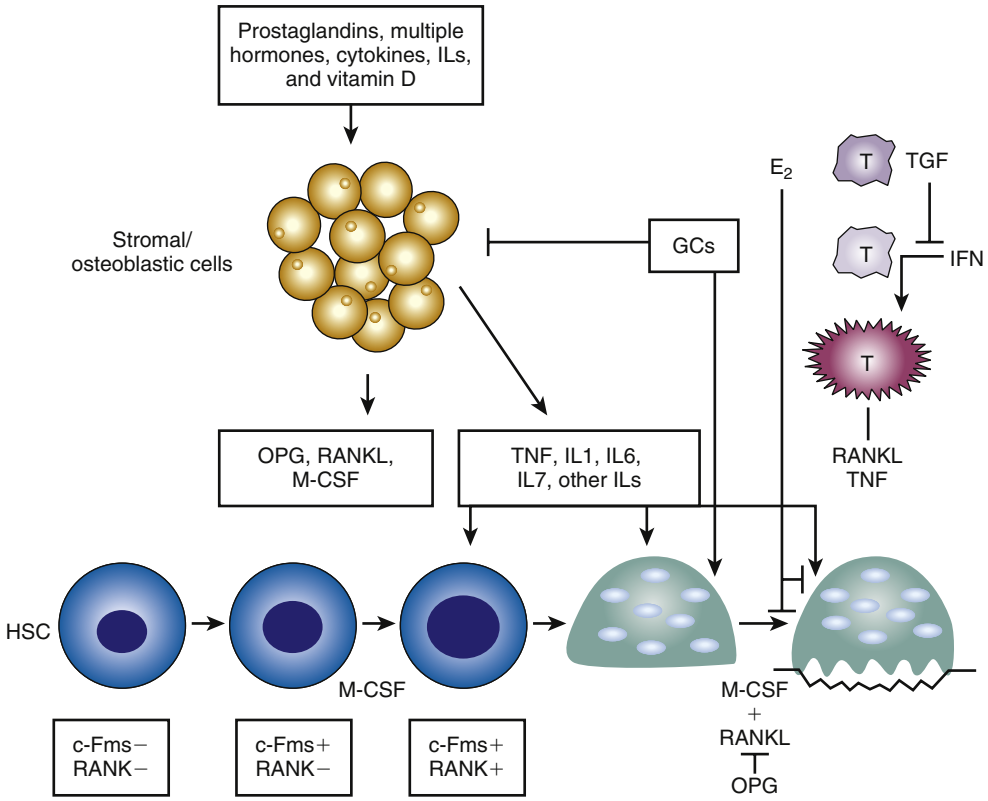
Acidification of the resorption space adjacent to the ruffled border membrane requires that osteoclasts have a vacuolar proton pump (H^+-ATPase [adenosine triphosphatase]) and a chloride channel that is charge coupled to H^+ secretion across the ruffled membrane to preserve electron neutrality.[59,60] These osteoclast H^+-ATPase pumps are similar to the vacuolar proton pumps that acidify intracellular organelles, but in the osteoclast they are exteriorized to increase the extracellular hydrogen ion concentration in the resorption space (see Fig. 30.13). The hydrogen ions dissociate from carbonic acid, which is synthesized by carbonic anhydrase II; the bicarbonate generated by this dissociation is removed from the cell by chloride-bicarbonate exchange at the basolateral membrane of the osteoclast. Ion pumps can transport the dissolved calcium from the bone surface through the cell to the extracellular fluid.

However, calcium can also reach the extracellular fluid directly if the sealing zone is disrupted. The proteolytic enzymes produced by the osteoclast include lysosomal enzymes and metalloproteinases.[61] Lysosomal proteases can degrade collagen at the low pH present in the ruffled border area. Cathepsin K is probably the most important of these.[62] Metalloproteinases, which are active at neutral pH, have also been detected at the resorption site.[63] The products of resorption are transported across the ruffled border membrane and excreted through the basolateral membrane of the osteoclast by a process called *transcytosis*.[64] In trabecular bone, osteoclasts characteristically resorb to a limited depth and then move laterally to produce irregular, plate-like resorption areas called *Howship lacunae*. In cortical remodeling, the path of directed resorption is longer, possibly because of renewal of osteoclasts from hematopoietic cells brought to the site through the haversian canal.

Bone Remodeling and Its Regulation

Overview of Remodeling

Adult bone mass is determined by two processes: acquisition of peak bone mass during adolescence and the subsequent bone loss

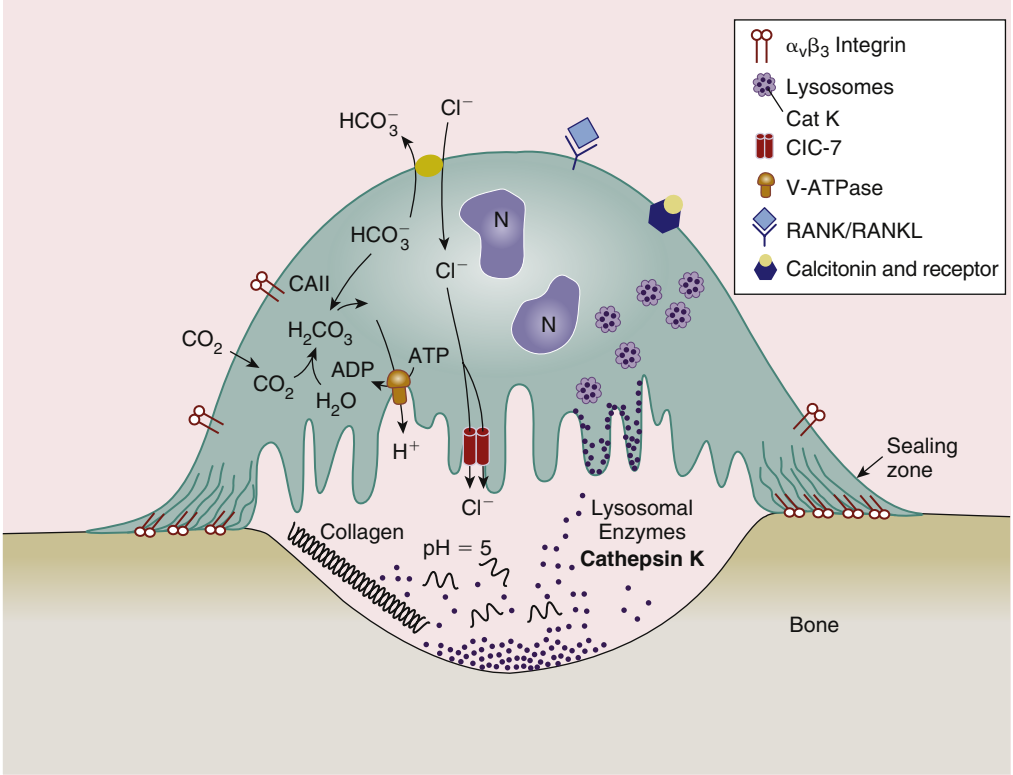


• **Fig. 30.12** Role of cytokines, hormones, steroids, and prostaglandins in osteoclast formation. Under the influence of other cytokines (data not shown), hematopoietic stem cells (HSCs) commit to the myeloid lineage, express c-Fms and receptor activator for nuclear factor κB (RANK), the receptors for macrophage colony-stimulating factor (M-CSF) and RANK ligand (RANKL), respectively, and differentiate into osteoclasts. Mesenchymal cells in the marrow respond to a range of stimuli, secreting a mixture of pro- and antiosteoclastogenic proteins, the latter primarily osteoprotegerin (OPG). Glucocorticoids (GCs) suppress bone resorption indirectly but possibly also target osteoclasts and their precursors. Estrogen (E_2), by a complex mechanism, inhibits activation of T cells, decreasing their secretion of RANKL and tumor necrosis factor α (TNFα); the sex steroid also inhibits osteoblast and osteoclast differentiation and life span. A key factor regulating bone resorption is the RANKL/OPG ratio. *IFN,* interferon; ILs, interleukins; *M-CSF,* macrophage colony-stimulating factor; *TGF,* transforming growth factor. (From Rosen CJ, ed. *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* 8th ed. Ames, IA: Wiley-Blackwell; 2013:2–33, Copyright 2013, American Society for Bone and Mineral Research.)

after maturity. Changes in bone mass result from physiologic and pathophysiologic processes in the bone remodeling cycle, and ultimately this can lead to skeletal fragility.[65] The most vulnerable periods in women are during accelerated linear growth in adolescence (ages 10–16 years) and later in life, usually immediately after menopause (ages 45–60 years). Male bone loss is much more gradual but is also determined by peak acquisition and age-related loss.

The bone remodeling cycle is a tightly coupled process whereby bone is resorbed at approximately the same rate as new bone is formed. BMUs comprise the remodeling unit of bone and, as noted previously, include osteoclasts, osteoblasts, osteocytes, and bone lining cells.[66] The bone marrow niche includes these cells plus hematopoietic elements, adipocytes, reticuloendothelial cells lining the sinusoids, and mesenchymal stromal cells (see Fig. 30.11). Activation of the remodeling cycle serves two functions in the adult skeleton: (1) to supply calcium acutely, as well as chronically, to the extracellular space, and (2) to provide elasticity and strength to the skeleton. When the remodeling process is uncoupled so that resorption exceeds formation, bone is lost. However, during peak bone acquisition, formation exceeds resorption, resulting in a net gain of bone. Remodeling is more pronounced in the trabecular skeleton (e.g., spine, calcaneus, and proximal femur), the most metabolically active compartment of bone. The trabecular elements are likely surrounded by a canopy that encloses the BMU and contains a capillary network for nutrient supply, as well as cells that can be identified as osteomacs. Although trabecular bone remodels more frequently than cortical bone, it is also extremely vulnerable to perturbations by local or systemic factors that can cause significant imbalances in bone turnover.

The bone remodeling cycle begins with activation of resting osteoblasts on the surface of bone, as well as the bone lining cells (see Fig. 30.11). The initial signal for remodeling has been actively debated, as has been the source of that signal (i.e., systemic or local). Microdamage or force changes sensed by the osteocyte may initiate the remodeling signal, although it is likely that local factors secreted by osteoblasts, osteoclasts, or lining cells could also begin the process. That initiating signal is followed by a series of secretory products originating from activated osteoblasts to osteoclasts and their precursors. These intercellular signals recruit and differentiate multinucleated cells from hematopoietic stem cells.[67] After osteoclast-induced bone resorption, matrix components

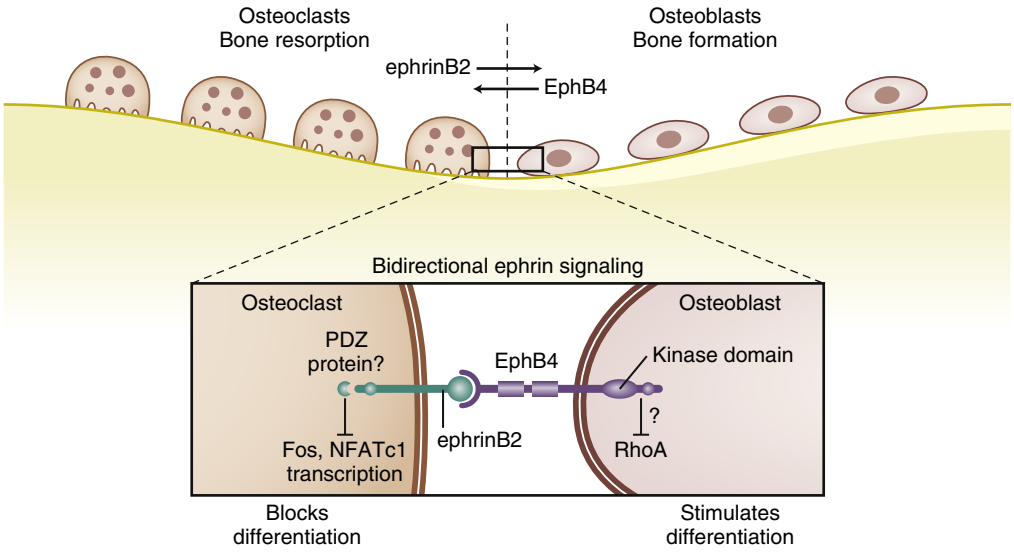


• **Fig. 30.13** Schematic representation of a resorbing osteoclast. The osteoclast acidifies the resorption lacunae by secreting H^+ and Cl^- ions for demineralization, and lysosomal cathepsin K for degradation of type I collagen. *ADP,* adenosine diphosphate; *ATP,* adenosine triphosphate; *ATPase,* adenosine triphosphatase; *CLC-7,* chloride channel 7; *RANK,* receptor activator of nuclear transcription factor κB; *RANKL,* RANK ligand. (From Rodan SB, Duong LT. Cathepsin K—a new molecular target for osteoporosis. *IBMS Bonekey*. 2008;5:16–24.)

such as TGFβ and insulin-like growth factor 1 (IGF1), as well as collagen, osteocalcin, calcium, and other protein and mineral components, are released into the microenvironment. Growth factors released by resorption contribute to the recruitment of new osteoblasts to the bone surface, which begin the process of collagen synthesis and biomineralization. In addition, cytokines released by activated osteoclasts such as bone morphogenetic protein (BMP) 7, Wnt 10b, and sphingosine 1-phosphate can stimulate osteoblastogenesis in reverse sequence.[68] Ephrins serving as both ligands and receptors also contribute to this bidirectional signaling mechanism[69] (Fig. 30.14). In healthy adults, as many as 2 million remodeling sites may be active at any given time, and it is estimated that nearly one fourth of all trabecular bone is remodeled each year. In general, resorption takes only 10 to 13 days, whereas formation is much more deliberate and can take upward of 3 months (see Fig. 30.2). Under ideal circumstances, by the end of the cycle, the amount of bone resorbed equals the amount reformed.

Although 80% of skeletal mass is cortical bone, the surface area of cortical bone is only about one fifth that of cancellous bone. Moreover, more osteoclast precursor cells are available in cancellous bone and on the endosteal surfaces of cortical bone. Consequently, turnover is greater on these surfaces than on periosteal bone, which normally undergoes little remodeling. However, subperiosteal resorption can be activated in hyperparathyroidism, and the periosteal surface contains pre-osteoblasts that may become active late in life and cause an age-related increase in the periosteal diameter of long bones.[70] This periosteal expansion may maintain bone strength and compensate for losses at the endosteal surfaces and in cancellous bone.[71]

Several key components of the remodeling cycle are susceptible to systemic and local alterations, which can lead to deleterious changes in bone mass. In particular, activation of remodeling via the osteoblast and recruitment of osteoclasts represent the two most vulnerable sites in the cycle. Often, with systemic changes in circulating hormones, such as estrogen deficiency, chronic PTH excess, or hyperthyroidism, the remodeling cycle is stressed such that coupling of the two processes cannot be maintained. This failure is due to the difference in timing between resorption, which generally occurs within a BMU over about 2 weeks, and formation, which can take upward of 12 weeks to fully mineralize newly synthesized matrix. Not surprisingly, a third cell altered in disease states is the osteocyte. Remodeling of the skeleton implies coupling of resorption to formation, and therefore no net change in bone mass. Because the osteocyte responds to mechanical loading or stress by communicating with bone lining cells and osteoblasts, these cells could also become damaged[72] (see Fig. 30.11). Indeed, osteocyte apoptosis may contribute to age-related osteoporosis either directly or through the elaboration of systemic peptides. Interestingly, remodeling may end with the osteocyte as well because it produces the protein *sclerostin*, which inhibits osteoblast activity by antagonizing the Wnt/lipoprotein receptor–related protein (LRP) 5 and BMP pathways.[15] Monoclonal antibodies



• **Fig. 30.14** Ephrin-Eph signaling and bone homeostasis. Osteoclasts are responsible for the resorption of a localized packet of bone, and when they cease their activity, a team of osteoblasts is attracted to the resorption site, where they proliferate, differentiate, and then re-form the packet of bone. **Inset,** Ephrin-Eph forward signaling from osteoclasts to osteoblasts may be responsible for driving the formation of the new bone packet, and ephrin-Eph reverse signaling may be responsible for the cessation of continued bone resorption by osteoclasts. (Redrawn from Mundy GR, Elefteriou F. Boning up on ephrin signaling. *Cell*. 2006;126[3]:441–443.)

that bind to sclerostin have been developed, and at least one is currently in phase III trials as a means of reducing the inhibitory effect of this protein on osteoblast differentiation, thereby increasing bone mass.[73,74]

As noted, uncoupled remodeling occurs during menopause because of estrogen deprivation or in response to endogenous PTH fluxes, cytokine stimulation, growth hormone surges, glucocorticoid excess, or changes in serum calcium. For the most part, estrogen deprivation remains one of the most common and critical elements in shifting resorption rates to a higher set-point. Although bone formation initially can "catch up," the length of time for each component of the remodeling cycle clearly favors resorption over formation, as the process of laying down new bone requires the interaction of several processes[72] (see Fig. 30.2). However, it is still unclear why falling estrogen levels, which is a universal event during the menopausal years, causes rapid bone loss in a relatively small percentage of women, although insidious bone loss has been clearly demonstrated for most, if not all, women who enter menopause.[75] Other factors such as peripheral conversion of testosterone to estradiol, which occurs to a greater degree in individuals who are obese, higher adrenal androgen production, and/or genetic determinants, as well as other local signals, may be important. Although the genetic causes of rapid bone loss have not been identified in humans, mice have strong heritable determinants that affect the rate of age-related and estrogen-deficient bone loss.[76,77] With advancing age, regardless of gender, a negative balance in bone remodeling is a natural occurrence when the number of senescent cells within bone increases. Remarkable changes are found in the chromatin and secretome profile of senescent cells. Their presence within the tissue microenvironment can be recognized by detecting increased expression of the biomarker p16Ink4a. Proinflammatory molecules are part of the repertory of paracrine and endocrine peptides secreted by senescent cells, producing a negative environment both locally and systemically. Osteocytes, the most abundant cells within bone tissue, can acquire the senescent phenotype. In experimental studies, these cells were removed by using the INK-ATTAC "suicide" transgene encoding an inducible caspase 8 expressed specifically in senescent cells. The investigators observed improvements in bone mass and microstructure, as well as in the biomechanical properties of bone. These results were reinforced by pharmacologic elimination of senescent cells, using a "senolytic compound," which also reversed bone deterioration in aged mice. Moreover, these results are in line with previous data suggesting that the removal of senescent cells potentially has beneficial effects on the cardiovascular system, insulin sensitivity, and frailty, opening the door for the development of new opportunities for preventing age-associated degenerative diseases.[78]

The nature of osteocyte-osteoblast-osteoclast interaction has been one of the most active areas of investigation (see Fig. 30.11). External signals (e.g., PTH, growth hormone, IL1, estrogen deprivation) to resting osteoblasts and stromal cells cause these cells to release cytokines (i.e., interleukins [e.g., IL1, IL6, and IL11], as well as M-CSF, TNF, and TGFβ) that enhance the recruitment and differentiated function of multinucleated giant cells destined to become bone-resorbing cells.[79] However, one of the most critical pathways in the osteoblast-osteoclast interaction scheme is the RANKL-OPG relationship.[47]

The OPG, RANKL, and RANK system, which affects osteoclast differentiation, in addition to the effects of M-CSF on osteoclast proliferation, provides the critical link between osteoclasts

and osteoblasts. It has also led to the synthesis of RANKL antibodies that, after successful human trials, have become therapeutic agents to inhibit bone resorption. Denosumab (Prolia) is the first monoclonal antibody against RANKL and was approved by the FDA, European regulatory agencies, and the Endocrinologic and Metabolic Drugs Advisory Committee for the treatment of postmenopausal osteoporosis, as well as for metastatic bone diseases, owing to its strong efficacy in reducing spine and hip fractures.[80] It is administered once every 6 months and suppresses bone resorption by 80% to 90%. Long-term follow-up of the original phase III trials reveals significant improvement in bone mass at both the spine and hip out to 10 years, with maintenance of antifracture efficacy and few, if any, adverse events.[81] After 10 years of treatment, the gain in bone mass was 21.7% in the lumbar spine, 9.2% in the total hip, and 9.0% in the femoral neck, with a continuous increase in BMD throughout therapy.[81] However, therapy withdrawal leads to a rapid inflexion in the BMD curve, implicating significant bone loss. The magnitude of reduction in BMD after a 17-month therapy gap was 8.1% and 8.4%, respectively, in the lumbar spine and the total hip, which corresponds to 35.5% of the total gain in the spine and 103.3% in the total hip.[82] Denosumab has also been approved for the treatment of women with breast cancer and skeletal metastases and is the only agent shown to reduce fractures in men undergoing androgen deprivation therapy for prostate cancer.[83,84]

The osteoblast functions not only to signal osteoclasts during remodeling but also to lay down collagen and orchestrate mineralization of previously resorbed lacunae in the skeletal matrix. These complex functions are tied to differentiation of mesenchymal stromal cells and bone lining cells, which become osteoblasts and rest on the surface of the remodeling space.[85] Recruitment of stromal cells into osteoblasts, rather than adipocytes, is a critical step in bone formation and requires a series of factors that enhance differentiation (see Fig. 30.8). One of the most important drivers of this process is RUNX2, a transcription factor that is essential for the early differentiative pathway of stromal cells toward bone and away from adipogenesis.[86,87] Regulation of RUNX2 and its downstream effects has become a major focus of work as investigators have begun to consider novel ways to enhance bone formation and reduce marrow adipogenesis. However, within that context, it is clear that pre-adipocytes or pre-osteoblasts may have significant plasticity such that the older paradigm illustrated in Fig. 30.8 may not truly represent the status of mesenchymal cells within the marrow niche.

During activation of resting osteoblasts, the mature cells synthesize collagen I; several minor collagens; the noncollagenous matrix proteins; and a series of growth factors, such as IGF1, IGF2, and TGFβ. These, in turn, are necessary for further recruitment of bone-forming cells.[88] In addition, osteoblasts deposit growth factors in the skeletal matrix, where they are stored in latent forms (e.g., TGFβ, IGF1, IGF2) and released during subsequent remodeling cycles. Osteoblast fate is determined within the remodeling sequence by several different systemic and local factors. Osteoblasts can further differentiate into osteocytes, become quiescent lining cells on the bone surface, or die through apoptosis.[85] As noted earlier, osteocytes may participate in bone resorption of the cortex by secreting RANKL to stimulate osteoclastogenesis and can also participate in osteocytic osteolysis, which may occur during lactation and other states of high calcium demand.[89]

The Wnt/β-catenin signaling pathway has emerged as a major regulator of bone formation and a potential mediator of the mechanostat. Wnts belong to a large family of proteins that bind to frizzled receptors and activate multiple pathways within the cell. When Wnts also bind to several coreceptors LRP5, LRP6, and probably LRP4, a so-called canonical signaling pathway is triggered.[90] Activation of this pathway occurs through a complex intracellular signaling pathway mediated by β-catenin, which translocates to the nucleus and stimulates the transcription of genes through cooperative interactions with TCF/Lef transcription factors. Sclerostin (the product of the *SOST* gene), the osteocyte-specific protein that inhibits bone formation and another important Wnt inhibitor, dickkopf 1 (Dkk1), work by binding to LRP5 and LRP6, thereby blocking Wnt signaling.[91]

Local Regulators of Remodeling

Characterization of local regulators produced within the bone itself represents a major advance in bone biology.[92,93] These local factors can be synthesized by bone cells or by adjacent hematopoietic cells and can interact with each other and with systemic hormones. They are critical in the repair of skeletal damage and in the response to mechanical forces.

Cytokines

The proinflammatory cytokines IL1α, IL1β, TNFα, and TNFβ are potent stimulators of bone resorption and inhibitors of bone formation; thus, they may play a role in the bone loss after estrogen withdrawal.[79] IL6 increases osteoclastogenesis in cell cultures and may mediate some of the resorbing activity of PTH. IL6 is synthesized by osteoblasts and immune cells, and its production is stimulated by PTH,[94] PGE_2, and other factors that increase bone resorption. There is also evidence suggesting that IL6 may modulate osteocyte communication toward osteoclasts.[95]

IL11, another member of the IL6 cytokine family, also stimulates resorption. LIF, also a member of the IL6 family, increases proliferation of osteoblast precursors but then slows their subsequent differentiation into mineralizing osteoblasts.[96] Inflammatory cytokines such as IL7, IL15, and IL17 stimulate bone resorption, whereas IL4, IL10, IL13, and IL18 inhibit bone resorption.[51,97–99] Interferon-β and interferon-γ inhibit resorption by blocking RANK signaling pathways.[100] IL10 is an inhibitor of osteoclastogenesis and bone resorption.[101] IL15 and IL17 stimulate resorption, whereas IL18 is inhibitory through its ability to increase production of granulocyte-macrophage CSF (GM-CSF).[102] Interferon-β and interferon-γ inhibit resorption by blocking RANK signaling pathways.[100] In addition to direct effects, responses to cytokines can be blocked by inhibitors such as the IL1 receptor antagonist and the soluble TNF receptor, or they can be enhanced by activators such as the soluble IL6 receptor.

TGFα and Epidermal Growth Factor

These peptides stimulate bone resorption through the same receptor and act by prostaglandin-dependent and -independent pathways. TGFα and epidermal growth factor (EGF) are potent mitogens in bone that probably act on mesenchymal and hematopoietic precursors.[103,104] The EGF family of peptides regulates cell migration and adhesion, playing an important role in the early recruitment of osteoblast progenitors to the remodeling site. Signaling in pre-osteoblasts by the EGFs is via the Cdc42/Rac network.[105] Studies in vitro, using immortalized and primary bone marrow stromal cells, reported that EGF receptor signaling enhances mechanotransduction, suggesting that the EGF system may act as a mechanosensitizer in bone marrow stromal cells.[106] TGFα is produced by neoplasms and may play a role in the increased bone resorption that occurs in certain malignancies.

Prostaglandins

Prostaglandins are potent regulators of bone cell metabolism and are synthesized by many cell types in the skeleton.[107] Prostaglandin production in bone is regulated by effects of local and systemic hormones and mechanical forces on the inducible cyclooxygenase 2. Increased prostaglandin production may contribute to the increase in bone resorption that occurs with immobilization, the enhanced bone formation seen with impact loading, and the bone loss after estrogen withdrawal. Many of the hormones, cytokines, and growth factors that stimulate bone resorption also increase prostaglandin production. Prostaglandins have biphasic effects on bone formation. Stimulation of bone formation is seen in vivo, and inhibition of collagen synthesis occurs in osteoblast cultures. Bone cells produce PGE_2, $PGF_{2\alpha}$, prostacyclin, and lipoxygenase products (e.g., leukotriene B_4), which may also stimulate bone resorption.

Peptide Growth Factors

Skeletal cells synthesize a variety of growth factors that regulate the replication, differentiation, and function of bone cells. These growth factors are not synthesized only by skeletal cells, and some are present in the systemic circulation and can act as local and systemic regulators of bone remodeling. Skeletal cells also synthesize growth factor binding proteins, which regulate the activity and storage of specific factors and their interactions with other proteins in the extracellular matrix.[92]

Fibroblast Growth Factors

FGFs form a large family of polypeptides characterized by their affinity to glycosaminoglycan heparin-binding sites.[108] FGF1 and FGF2, which have been studied extensively, have mitogenic properties for cells of the osteoblastic lineage, although eventual differentiation into mature osteoblasts does not occur in the presence of the FGF family of peptides.[109] In fact, FGF2 inhibits Wnt signaling and the synthesis of IGF1, which results in a decrease in osteoblastogenesis and in osteoblastic function.[110,111] In vivo experiments have confirmed this action of FGF, and mice overexpressing FGF2 are osteopenic.[112] However, studies in *Fgf2* null mice indicate that FGF is necessary for osteoblast formation, possibly because of its early effects on cell replication.[113] FGF can stimulate bone resorption by prostaglandin-dependent and -independent pathways.[114] The FGFs bind to a series of four FGF receptors that have intrinsic tyrosine kinase activity. In vertebrates, the 22 members of the FGF family range in molecular mass from 17 to 34 kDa and share 13% to 71% amino acid identity. Among vertebrate species, FGFs are highly conserved in both gene structure and amino acid sequence. FGFs have a high affinity for heparan sulfate proteoglycans and require heparan sulfate to activate one of four cell-surface FGF receptors. During embryonic development, FGFs have diverse roles in regulating cell proliferation, migration, and differentiation. In the adult organism, FGFs are homeostatic factors and function in tissue repair and response to injury. When inappropriately expressed, some FGFs can contribute to the pathogenesis of cancer.

FGF19, FGF21, and FGF23 are unique in that they bind to FGF receptors that heterodimerize with α- or β-Klotho. FGF23 is secreted by osteocytes and has been shown to regulate phosphate homeostasis by inhibiting vitamin D 1α-hydroxylase activity in the kidney, thereby suppressing the production of $1,25(OH)_2D$. In addition, FGF23 promotes phosphate loss in the renal tubule, acting as an endocrine hormone produced by bone cells. Disorders in FGF23, including excess production or impaired degradation, result in several syndromes that are characterized by hypophosphatemia and rickets/osteomalacia. FGF21 is produced in the liver and in adipocytes. It is an important counterregulatory hormone that stimulates PPARα and oxidation of fatty acids.[115] FGF21 is high during calorie restriction, malnutrition, and lactation. It can potentially cause browning of white adipocytes, but in mice it has been shown to stimulate bone resorption. Recently, FGF21 has been shown to induce significant bone resorption, and the FGF21 null mice have very high bone mass.[116] Increased production of IGFBP1 may mediate bone resorption, at least in part, by increasing the production of FGF21.[117,118]

Platelet-Derived Growth Factors, Vascular Endothelial Growth Factors, Hypoxia-Inducible Factors, and Reactive Oxygen Species

Platelet-derived growth factor (PDGF) was originally isolated from human platelets, and four members of the *PDGF* gene family have been identified: *PDGFA*, *PDGFB*, *PDGFC*, and *PDGFD*.[119] These peptides signal through the PDGFR family of receptors. Vascular endothelial growth factor (VEGF) shares a high degree of sequence homology with PDGF, and these factors are often referred to as members of the PDGF/VEGF family.[120]

PDGFs must form homodimers or heterodimers to exhibit activity. PDGF-AA, -AB, and -BB are the isoforms studied more extensively in skeletal cells, and they exert similar biologic actions. The primary function of PDGF in bone is the stimulation of cell replication, and PDGF impairs osteoblast differentiation and function.[121] PDGF also stimulates bone resorption. In mice, null mutations of *Pdfga* or *Pdfgb* and their receptors cause embryonic lethality or perinatal death, not allowing the study of the function of PDGF in the postnatal skeleton.[122] Although skeletal cells express products of the *Pdfga*, *Pdfgb*, and *Pdfgc* genes, the major source of PDGF is the systemic circulation, and skeletal cells become exposed to PDGF after platelet aggregation. PDGF receptor 1a (PDGFR1a) is expressed on numerous progenitor cells in the hematopoietic niche, including early osteoblasts and adipocytes.

VEGFA is essential for angiogenesis, and *VEGFA* and VEGF receptor genes are expressed by chondrocytes and osteoblasts.[123] VEGFA is essential for blood vessel formation and vessel invasion into cartilage during the process of endochondral bone formation and for chondrocyte survival during skeletal development.[124] Importantly, VEGFA is also required for intramembranous bone formation and osteoblastic maturation.[125] Osteoblast expression of PDGF and VEGF is regulated by other locally derived growth factors.

Hypoxia-inducible factors (HIFs) are produced locally in response to low oxygen tension in the hematopoietic niche and are transcription factors that mediate pathways that lead to angiogenesis, including production of VEGF. HIFs also induce genes essential for glycolysis, an essential biochemical pathway for ATP generation in the niche. During fracture repair, it has been demonstrated that more bone is produced in a mouse model with constitutively active HIF1α in osteoblasts. Conversely, mice deficient in HIF1α in osteoblasts had defective bone healing.[126]

Reactive oxygen species (ROS), which are free radicals that include peroxides, are products of cell metabolism, particularly oxidative phosphorylation. These products are inactivated by several enzymes that serve to prevent mitochondrial and cell damage. In several mouse models of aging, changes that occur in bone turnover and cell metabolism were associated with increases in ROS in bone cells.[127] Suppressing ROS through antioxidant therapy can

reverse some of the effects on bone from the loss of sex steroids.[128] However, ROS generation may be important to maintain a certain level of metabolic activity, and differences in ROS production may be critical to determining the fate switch between osteoblasts and adipocytes.

Insulin-Like Growth Factors

IGFs are mitogens that also can increase the differentiated function of the osteoblast, supporting mineralization and bone formation.[129] Animal models have demonstrated that both systemic and locally synthesized IGF1 contribute to bone formation.[130,131] Transgenic mice overexpressing IGF1 have increased bone mass, whereas *Igf1* null mice exhibit decreased bone formation, reduced mineralization, and decreased cortical bone.[132] IGF1 stimulates bone turnover by increasing osteoclastogenesis and bone remodeling.[133] Both IGF1 and IGF2 are synthesized by bone cells and are stored in the bone matrix, but IGF1 is a more potent stimulator of osteoblastic function.[129] Six IGF-binding proteins have been identified in bone and in the circulation (e.g., IGFBP1 through IGFBP6). IGFBPs can inhibit or enhance IGF responses, depending on their local concentration relative to IGFs and the presence of proteases that can cleave the IGFBPs. IGFBP2 has been shown to be synergistic in its actions on osteoblasts with IGF1 via signaling through the pleiotropic receptor that phosphorylates PTEN (phosphatase and tensin homolog).[134] PTH and PGE_2 are major inducers of skeletal IGF1 and IGFBP2 synthesis, and glucocorticoids suppress IGF1 transcription.[129] Thus, IGFs can mediate selected effects of these hormones on bone formation.

Transforming Growth Factor β

TGFβ belongs to a family of closely related polypeptides with various degrees of structural homology and important effects on cell function that are only expressed in mammals.[135] Skeletal cells express TGFβ1, TGFβ2, and TGFβ3. TGFβ has complex and somewhat contradictory actions in bone cells. TGFβ can stimulate osteoblastic cell replication and bone formation, but it does not favor osteoblastic differentiation.[136,137] The effects of TGFβ depend on the target cell and experimental conditions. The actions of TGFβ on bone resorption have been a source of controversy. TGFβ has a biphasic effect on osteoclastogenesis, but it decreases bone resorption.[138] Targeted disruption of the mouse *Tgfb1* gene is lethal but does not result in abnormal skeletal development.[139] TGFβ is secreted as a latent high-molecular-weight complex consisting of the C-terminal remnant of the TGFβ precursor and a TGFβ-binding protein.[140] The biologically active levels of TGFβ depend on changes in its synthesis and in its activation from its latent form.

BMPs and Wnt Proteins

BMPs are members of the TGFβ superfamily of polypeptides, and they were originally identified because of their ability to induce endochondral bone formation. BMPs are expressed by osteoblasts and play an autocrine role in osteoblastic differentiation and function.[92] The fundamental function of BMPs is the induction of osteoblastic cell differentiation, endochondral ossification, and chondrogenesis.[92,141] The genesis and differentiation of osteoblasts and osteoclasts are coordinated events, and BMPs also induce osteoclastogenesis and osteoclast survival.[142]

BMP activity is regulated by a large group of secreted polypeptides that bind and limit BMP action. These extracellular BMP antagonists prevent BMP signaling. Extracellular BMP antagonists include noggin, follistatin, myostatin, twisted gastrulation, the chordin family, and the Dan/Cerberus family of proteins.[92] These molecules can also bind to one or two activin receptors, ACVR2A and ACVR2B. Myostatin, a TGFβ superfamily member, is a negative regulator of muscle growth acting through ACVR2B. Inhibitors of these signaling peptides and their receptors may result in enhanced muscle mass and in some cases increased bone mass.[143]

The Wnt family of secreted cysteine-rich glycoproteins, like BMPs, plays a critical role in directing osteoblastogenesis. In skeletal cells, many Wnt family members use the canonical Wnt/β-catenin signaling pathway.[144] In the absence of Wnt, the proteins axin, adenomatous polyposis coli (APC), and β-catenin form a complex that facilitates the phosphorylation and degradation of β-catenin. Binding of Wnt family proteins to specific frizzled membrane receptors and their coreceptors (LRP5 and LRP6) leads to the stabilization of β-catenin. This allows β-catenin to be translocated to the nucleus, where it can regulate the transcription of target genes. The Wnt/β-catenin signaling pathway is central to osteoblastogenesis and bone formation, and Wnt and BMPs act in concert to regulate cell differentiation. Deletions of Wnt or β-catenin genes result in the absence of osteogenesis and of skeletal tissue, and inactivating mutations of Wnt coreceptors result in osteopenia.[145] For instance, either the loss-of-function or gain-of-function mutation in LRP5 results in skeletal diseases respectively characterized by either low bone mass (osteoporosis pseudoglioma)[146] or high bone mass.[147] Wnt/β-catenin signaling induces OPG, and through this mechanism, Wnts are negative regulators of osteoclastogenesis.[148,149] Wnt activity, like that of BMPs, is controlled by extracellular antagonists and intracellular signaling proteins.[93] Extracellular antagonists such as sclerostin and Dkk1 prevent interactions between Wnt family members and coreceptors, although others, like soluble frizzled-related proteins, bind Wnts directly and block their actions. These antagonists limit Wnt signaling, which decreases osteoblast function and bone mass.[144,150] Conversely, deletion of sclerostin in mice produces a high bone mass phenotype.[151] Sclerostin, made predominantly by osteocytes, is regulated by systemic factors such as PTH and by mechanical forces on bone. Precisely how circulating versus local sclerostin regulates bone formation and resorption is an important unanswered question, particularly because circulating sclerostin levels have been shown to have a negative or positive relationship to bone mass and fractures in normal individuals and patients with type 2 diabetes mellitus (T2DM).[152,153]

Systemic Hormones and Bone Remodeling

Remodeling is activated by both systemic and local factors. Changes in mechanical force can activate remodeling to improve skeletal strength, and remodeling removes and repairs bone that has undergone microdamage. This occurs particularly in cortical bone and may explain the fact that remodeling is sustained in the aging skeleton.[154] However, loss of osteocytes with age may impair this response.[155] Systemic hormones influence bone remodeling to regulate the movement of mineral from bone to the extracellular fluid to maintain serum calcium levels and to sustain linear growth. During pubertal growth, bone modeling and remodeling intensify and correlate with serum levels of IGF1, supporting the role of growth hormone as a major mediator of skeletal formation.[156] In addition to the growth hormone/IGF1 axis, it is now apparent that testosterone and estradiol must be present during peak acquisition to attain the highest possible BMD. Mineralization of newly formed bone is assured by activation of vitamin D 1α-hydroxylase in the kidney leading to greater calcium

absorption and higher $1,25(OH)_2D$ levels. Hence, modeling and epiphyseal closure are both modulated by systemic hormones that are both calcium regulating and growth centric.

Calcium-Regulating Hormones

Parathyroid Hormone

PTH acts on bone to stimulate resorption but does not act on osteoclasts in the absence of cells of the osteoblastic lineage. PTH receptors are abundant on osteoblasts, bone lining cells, and osteocytes, but not on osteoclasts.[157] PTH acts on osteoblasts to cause cell contraction; to induce immediate-early response genes, including c-Fos and the inducible form of prostaglandin G/H synthase (i.e., cyclooxygenase), and to increase the synthesis of local mediators, IGF1 and IL6.[157,158] High concentrations of PTH in vitro inhibit the expression of type I collagen, but intermittent administration of PTH in vivo or in vitro can stimulate bone formation.[159] PTH induces the production of RANKL and inhibits the production of OPG by cells of the osteoblast lineage, thereby increasing osteoclastogenesis and thus bone resorption. In some circumstances, PTH increases proliferation of cells of the osteoblast lineage and decreases their death by apoptosis.[157] PTH also induces 1α-hydroxylase activity in the kidney, thereby enhancing the active form of vitamin D (see later discussion). In osteocytes, PTH not only stimulates production of RANKL but also inhibits production of sclerostin and increases synthesis of FGF23. In this way, PTH uses osteocytes to regulate bone remodeling and systemic calcium/phosphate homeostasis. Genetic deletion of the PTH/PTHrP receptor (PTH1R) gene in mesenchymal stem cells of mice has a great impact on bone phenotypes (i.e., low bone formation, increased bone resorption, and high bone marrow adipose tissue [MAT]).[160] In addition, the same study showed that intermittent PTH administration to control group mice reduced bone MAT significantly.[160] These results suggest that PTH also drives progenitor cells differentiation into osteoblasts.

Vitamin D

The hormonal form of vitamin D, $1,25(OH)_2D_3$, is necessary for intestinal calcium and phosphorus absorption and therefore for mineralization. This form of vitamin D also has effects on the skeleton, but its physiologic role in bone remodeling is not clear.[161] By increasing RANKL production on osteoblasts or osteoblast progenitor cells, vitamin D is a potent stimulator of osteoclast formation in cell culture. High concentrations increase osteocalcin synthesis by osteoblasts and inhibit collagen synthesis and mineralization in vitro.[162] Lower concentrations may increase bone formation, although not to the extent seen with intermittent administration of PTH. Recent studies using conditional deletions of the vitamin D receptor (VDR) in bone and intestine provide further insight into the physiologic role of vitamin D on the bone remodeling unit. Intestinal deletion of the VDR mimics a low-calcium diet and is associated with bone loss related to increased bone resorption and decreased bone formation. However, serum calcium is preserved.[162] But in the osteocyte and mature osteoblast-specific *Vdr* null mice, there is no alteration in circulatory levels of calcium, phosphorus, PTH, and $1,25(OH)_2D$, as well as no bone phenotype. Moreover, mice missing the VDR only in the intestine have a delay in mineralization through the actions of high levels of $1,25(OH)_2D_3$ on osteoblastic cells.[162] These data provide a strong mechanistic rationale for active vitamin D action on remodeling: high $1,25(OH)_2D$ levels due to increased $1,25(OH)_2D$ bind to the VDR on osteoblasts and stimulate RANKL production leading to increased bone resorption as a way to protect the body against low serum calcium. It further slows entry of calcium into the skeleton by suppressing mineralization in vitro and in vivo, thereby preserving essential functions of the mammal.

Calcitonin

Calcitonin inhibits bone resorption by acting directly on the osteoclast, but it appears to play a smaller role in the regulation of bone turnover in adults.[163] Bone mass is not greatly altered in patients with medullary thyroid carcinoma, who have an excess of calcitonin production, or in athyreotic patients receiving adequate thyroid hormone replacement, who have low calcitonin levels.[164,165] Bone turnover is increased in patients with medullary thyroid carcinoma.[166] Mice with a deletion of the gene for calcitonin/calcitonin-related polypeptide α (*CALCA*), which is responsible for the production of calcitonin and its alternate transcript calcitonin gene–related peptide, have increased bone mass and enhanced rates of bone formation.[167] In contrast, mice with a deletion of only the calcitonin gene–related peptide have decreased bone mass.[168] These results imply that calcitonin influences both bone formation and bone resorption. However, the mechanisms by which calcitonin affects bone formation remain unknown.[169]

Other Systemic Hormones That Influence Remodeling

Growth Hormone

Deficiency and excess of growth hormone have marked effects on skeletal growth, as noted previously.[170,171] Growth hormone increases circulating and local levels of IGF1, which mediates many of the skeletal effects of growth hormone. Exogenous growth hormone and IGF1 increase bone remodeling presumably by directly acting on osteoblasts, although IGF1 is also present on osteoclasts, and recombinant IGF1 stimulates bone resorption. Osteocytes also possess IGF1,[172] and one can speculate that it might play an important role in regulating phosphate homeostasis via FGF23 during critical growth phases. Growth hormone stimulates cartilage growth, probably through an increase in local and systemic IGF1 production and possibly by direct stimulation of cartilage cell proliferation because low levels of growth hormone receptors are present in chondrocytes.

Glucocorticoids

Glucocorticoids exert profound effects on bone remodeling.[173] Glucocorticoids decrease the intestinal absorption of calcium and have the potential to induce osteoclastogenesis and bone resorption because they increase the expression of RANKL and CSF1 in osteoblasts.[174] However, the most significant effect of glucocorticoids is their ability to suppress bone remodeling through depletion of the osteoblastic cell population.[175,176] Glucocorticoids inhibit the replication of osteoblast precursors and their differentiation into mature osteoblasts. This effect occurs in part because they suppress Wnt signaling and factors necessary for osteoblastic differentiation.[177] In a recent study, it was observed in humans that glucocorticoids have diverse effects in the serum levels of two Wnt signaling antagonists: a progressive decrease in Dkk1 with a significant increase in circulatory levels of sclerostin.[178] Glucocorticoids induce the apoptosis of osteoblasts and osteocytes, contributing to the decrease in bone-forming cells.[179] Glucocorticoids inhibit the differentiated function of the osteoblast and bone formation. This results from direct effects of glucocorticoids on the osteoblast and suppression of IGF1 transcription.[180]

Thyroid Hormones

Thyroid hormone signaling is mediated by thyroid hormone receptor (TR), a ligand-dependent transcription regulator molecule. TR is encoded by the thyroid hormone receptor α (*THRA* or c-erbAα) and β (*THRB* or c-erbAβ) genes, which have different isoforms for which distribution is tissue- and age dependent.[181,182] The TRβ1 and TRα1 isoforms are expressed in bone, but previous studies suggested that TRα1 is the major mediator of thyroid hormone effects in the skeleton.[183] However, the net effect of thyroid hormone is complex and depends on the circumstances.[184,185] In children, hyperthyroidism is associated with increased bone mineralization and epiphyseal maturation, and hypothyroidism results in decreased growth.[186] But in adults, hyperthyroidism is associated with bone loss. Thyroid hormones are crucial for cartilage growth and differentiation and enhance the response to growth hormone. Thyroid hormones increase bone resorption and turnover, although their effects on bone formation are less clear.[187] Coupled with their effects on bone resorption, thyroid hormones increase the transcription of collagenase and gelatinase by osteoblasts.[188] As thyroid hormones increase bone remodeling, thyroxine (T_4) may also increase bone formation. Thyroid hormones also have indirect effects on skeletal metabolism by suppressing the synthesis of thyroid-stimulating hormone (or thyrotropin), which can inhibit osteoclast formation and survival and as a consequence suppressed bone resorption.[187,189,190] However, it is still debated whether thyroid-stimulating hormone (or thyrotropin) has direct effects on osteoblasts and osteoclasts.

Insulin

Normal skeletal growth depends on an adequate amount of insulin.[191] Excess insulin production by the fetuses of mothers with uncontrolled diabetes results in excessive growth of the skeleton and other tissues, possibly through its actions on IGF1. Poorly controlled diabetes mellitus leads to impaired skeletal growth and mineralization. Children and adolescents with type 1 diabetes mellitus (T1DM) are at increased risk for decreased bone mineral acquisition and accumulation of AGEs.[192] T2DM is associated with normal bone mass but increased skeletal fragility in part due to enhanced cortical porosity and increased AGEs in the matrix. In vitro, insulin at physiologic concentrations selectively stimulates osteoblastic collagen synthesis by a pretranslational mechanism. Insulin can mimic the effects of IGF1, although only at supraphysiologic levels.[109] Mice deficient in insulin receptor substrate 1, a major substrate of insulin and IGF1 receptor tyrosine kinases, exhibit impaired differentiative osteoblast function, low-turnover osteopenia, and an impaired response to PTH, documenting the central role of insulin and IGF1 signaling in the maintenance of bone remodeling.[193] More recently, insulin has been shown to stimulate osteoblastic function and bone resorption leading to greater release of GLU13-OCN (see Fig. 30.5), which in turn causes greater insulin sensitivity and enhanced insulin synthesis in the islet cells. The effect of insulin on glucose transport in osteoblasts is still controversial, although deletion of the insulin receptor in osteoblasts, using the osteocalcin Cre promoter, results in low bone mass, obesity, and insulin resistance. In humans, the ultimate effect of insulin resistance on bone still is to be elucidated, but there is much clinical evidence suggesting that it does not have detrimental effects on bone mass. Both pandemic (i.e., obesity) and rare (i.e., generalized congenital lipodystrophy) conditions linked to insulin resistance are associated with high bone mass.[194,195] In addition, there is no consensus in the literature about the association between visceral adipose tissue and bone mass.[196] In commonality, insulin deficiency and insulin resistance are associated with excessive formation of AGEs, which are involved in the development of classic diabetic micro- and macroangiopathy. AGEs may also impact bone microarchitecture through effects on collagen cross-linking, major post-translational modifications of collagen that influence bone strength. Collagen cross-links can be formed by lysyl hydroxylase and lysyl oxidase–mediated enzymatic immature divalent cross-links and mature trivalent pyridinoline and pyrrole cross-links, and cross-links can also be produced by glycation- or oxidation-induced nonenzymatic cross-links (AGEs), such as glucosepane and pentosidine. The last may impair mineralization and the capacity of bone to repair itself from microdamage. This mechanism can be a common link to bone fragility, which is common in both T1DM and T2DM patients.[197,198]

Gonadal Hormones

Estrogens and androgens are critical for skeletal development and maintenance. Bone cells contain estrogen and androgen receptors, but it has been difficult to demonstrate direct effects of gonadal steroids on bone formation or resorption in cell and organ culture. Gonadal hormones are crucial for the pubertal growth spurt, and estrogen is necessary for epiphyseal closure.[199] In addition, gonadal steroids contribute to skeletal gender differences. Men exhibit larger bones due to androgen stimulation of periosteal formation, whereas estrogen has an inhibitory effect.[200] Skeletal gender dimorphism impacts on bone biomechanical properties and, at least in part, explains the higher incidence of osteoporotic fracture in women.[201] Deficiency of estrogen or androgen increases bone resorption in vivo, partly by increasing the local synthesis or sensitivity to cytokines, such as IL1 and IL6 or TNFα; to prostaglandins; and through direct actions on estrogen receptors in osteoclasts. Androgens can increase bone formation in vivo.[202] The effect of estrogens on bone formation is less clear, depending on the animal model and the dose of estrogen. The absolute rate of bone formation is increased in estrogen deficiency states because of an increase in bone remodeling. However, estrogen deficiency causes bone loss, implying a relative deficiency in bone formation. In other words, in the condition of hypoestrogenism, the increase in bone formation is not of the same magnitude as the enhancement in bone resorption.

Epidemiology of Osteoporosis and Fractures

Over the past 25 years, our understanding of fractures and their causes has significantly evolved and along with it, the definition of the disease osteoporosis. The current definition of *osteoporosis* was developed by an international consensus development committee in 1993 as "a disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk."[203] This definition reflects our understanding of the link between low bone quantity, compromised bone quality, and increased fracture risk. Although hip, vertebral, and wrist fractures are most commonly associated with osteoporosis, risk of other fractures is also increased.

Fractures

Hip Fractures

Proximal femur fractures are a major cause of morbidity and fatality and occur much more frequently in older people, increasing

the costs of osteoporosis. Most commonly, these fractures occur in the femoral neck or intertrochanteric regions and require surgical repair. Increased risk of falls together with decreases in bone strength account for the increased risk with increasing age. Morbidity and mortality rates associated with hip fractures are substantial. The mortality rate within 1 year of the fracture is between 5% and 20%.[204] They are a major source of disability and loss of independence. For example, it is estimated that of those living independently before a hip fracture, only 50% are able to do so 1 year after the hip fracture.[205] After a hip fracture, there is also a substantial risk of other fractures, including a second hip fracture, and it is therefore important that further assessment and possible treatment be considered in patients with fractures at the hip (and other sites).[206,207]

Vertebral Fractures

Vertebral fractures are the most common manifestation of osteoporosis.[208] These fractures occur in a range of severities. Mild fractures, only some of which may be symptomatic, may only be apparent via radiographic or other types of imaging but are still, in aggregate, associated with clinical symptoms.[209,210] However, severe vertebral fractures can be accompanied by significant acute back pain that may persist for many weeks or indefinitely. Long-term consequences of vertebral fractures, particularly accumulation of multiple vertebral fractures, are substantial, leading to height loss, kyphosis, increased disability, decreased pulmonary function, substantial chronic back pain, and diminished overall quality of life.[211] There is also increased fatality associated with vertebral fractures.[212] Severity of these consequences increases with increasing severity and number of these fractures. Although they can be caused by trauma, they are often associated with little or no trauma.

Vertebral fractures have often been referred to as the hallmarks of osteoporosis and tend to occur at younger ages than other fractures. Vertebral fractures increase the future risk of additional vertebral fractures by 5 to 10 times and are associated with a much higher risk of nonvertebral fractures, including hip fractures.[213,214] Therefore, like hip fractures, they should lead to further assessment (e.g., dual-energy x-ray absorptiometry [DXA]) and consideration of osteoporosis prevention and treatment. This is particularly true of incidental findings noted on chest and lumbar radiographs for other reasons. Often, vertebral fractures reported on radiographs are asymptomatic, and a history of trauma cannot be ascertained. This does not diminish the importance of the fracture with respect to future risk or associated morbidity.

Wrist Fractures

Fractures of the distal radius are common in postmenopausal women and are associated with osteoporosis. However, in general, they have less morbidity and fewer costs than hip or spine fractures. Their incidence rises in women at menopausal age but then rises no further with increasing age. This pattern may be explained by different reactions to falling with age. Men have a much lower incidence of wrist fracture.

Other Types of Fractures

In addition to hip, spine, and wrist fractures, a large number of other fracture sites, such as the arm, lower leg, humerus, and ribs, are common and have been shown to have significant morbidity and mortality risks.[215] Furthermore, there is increasing evidence that the risk of most, if not all, of these fracture types are increased in those with low BMD or osteoporosis[216] and can be reduced by osteoporosis treatment.[207,217–219] A recently published study evaluated the relationship of mortality within a group of individuals with nonhip, nonvertebral fractures with or without comorbidities.[9] The mortality rate attributable to any fracture without comorbidity was 9.2% in women and 5.3% in men. Moreover, comorbidity independently worsened the mortality rate; for instance, a woman showing both humeral fracture and one comorbidity had a similarly reduced 5-year survival to that of a woman with a hip fracture and no comorbidities.[9]

TABLE 30.1 Diagnostic Categories for Osteoporosis Based on Measurements of BMD and Bone Mineral Content

Category	Definition
Normal	BMD ±1 SD of the young adult reference mean
Low bone mass (osteopenia)	BMD >1 SD and <2.5 SD lower than the young adult mean
Osteoporosis	BMD >2.5 SD lower than the young adult mean
Severe osteoporosis (established osteoporosis)	BMD >2.5 SD lower than the young adult mean in the presence of one or more fragility fractures

BMD, Bone mineral density; *SD*, standard deviation.

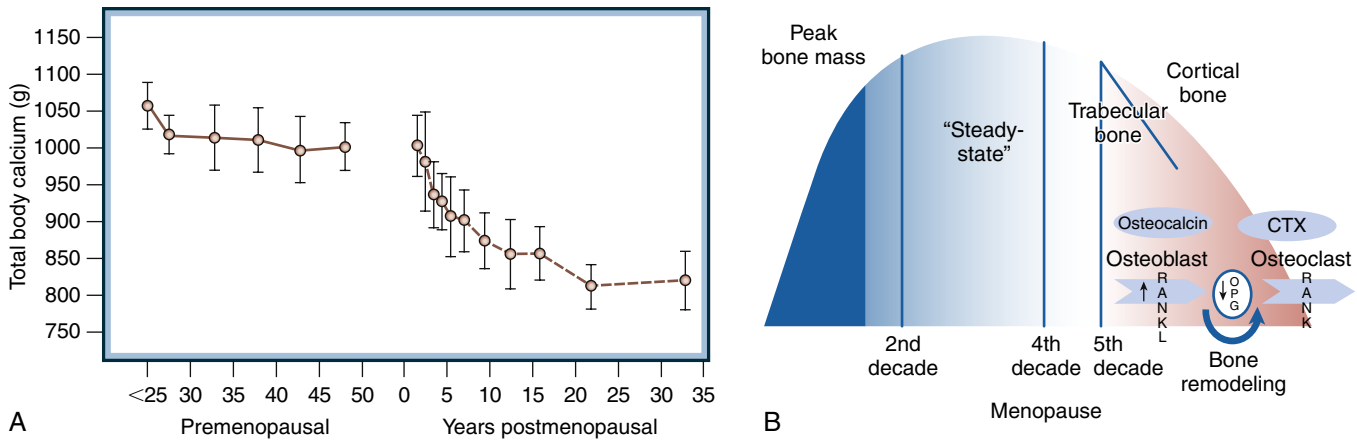
Clinical Assessments of Osteoporosis

Dual-Energy X-Ray Absorptiometry

DXA is the standard method for assessing the presence and extent of osteoporosis. DXA is most often performed at the spine and hip, although other sites (whole body, distal radius) can also be assessed. Although not a true density, it provides an areal density of mass per area in units of grams per square centimeter. The International Osteoporosis Foundation and other organizations have developed a classification system for DXA values called *T-scores* that classify the measurement (Table 30.1). T-scores are calculated by comparing a specific value with a normative reference range for patients of the same gender and ethnicity. The T-score is the number of standard deviations (SD) below young normal values. A value below −2.5 SD is usually considered osteoporotic. Clinically, usually three regions are used for diagnosis: BMD at the total hip, the femoral neck, and the lumbar spine.

DXA values at the hip generally peak at about age 30 to 40 years and then begin to decline (Fig. 30.15). The decline is somewhat accelerated at menopause for a few years and then becomes accelerated again after about age 65 or 70 years in women.[220] Spine BMD measurements can be useful; however, in older women and especially men, they can be confounded by osteophytes, aortic calcification, degenerative disease, and other conditions that increase the apparent spinal BMD and therefore are less reliable in those older than 65 to 70 years.

A large number of studies (many of them prospective) have consistently shown that DXA BMD is strongly predictive of future risk of fracture. Hip BMD is predictive of various types of both nonvertebral and vertebral fracture but is particularly strongly predictive of hip fractures. Although the risk of fracture increases significantly with decreasing BMD, several studies have verified that



• **Fig. 30.15** (A) Bone mass development, maintenance, and loss: trabecular bone loss starts earlier and is more intense than in cortical bone. (B) The decline of total body calcium with years since menopause. *CTX,* cross-linked telopeptide of type I collagen; *OPG,* osteoprotegerin; *RANK,* receptor activator of nuclear transcription factor κB. (Redrawn from Tella SH, Gallagher JC. Prevention and treatment of postmenopausal osteoporosis. *J Steroid Biochem Mol Biol.* 2014;142:155–170.)

osteoporotic fractures occur across a wide spectrum of BMDs. Most likely, these events are related to bone quality, a component not captured by DXA measurements. The World Health Organization definition is a guideline for interpreting DXA results, not a definitive way of diagnosing osteoporosis. Therefore, DXA is the primary imaging tool used in diagnosing and treating osteoporosis. It can be used in conjunction with other risk assessment tools that combine other risk factors with DXA (see later discussion). DXA measurements have been somewhat standardized across different types of scanners, although follow-up measures to assess change should be performed on the same scanner if at all possible.

Trabecular Bone Score

Approved by the FDA in 2012, the trabecular bone score (TBS) is now incorporated into clinical practice for the estimation of fracture risk. The TBS is an optional tool added to the menu of DXA equipment, which provides gray-scale textural analysis of spine DXA images, utilizing variograms of two-dimensional projection images. The TBS is a surrogate measurement of trabecular microarchitecture, and there is evidence showing correlation between the several parameters of quantitative computed tomography (QCT) and TBS values. The strongest association is observed at lumbar spine trabecular volumetric BMD, whereas in other sites the relationship between TBS with bone microarchitectural parameters has more variation. Several studies show convergent results that the TBS is an independent predictor of fracture in osteoporotic postmenopausal women, as well as in men. A large prospective study performed in Manitoba, Canada, which included more than 29,000 postmenopausal women followed for almost 5 years, reported the accuracy of the TBS in predicting fracture. Moreover, it demonstrated that the combination of BMD with TBS assessment provides a better estimate of fracture risk than either evaluation by itself.[221] Similar results were obtained in smaller samples in the United States (Osteoporotic Fractures in Men Study [MrOs]) and in Japanese populations who were followed for 10 years.[222] The TBS has also been shown to enhance the power of fracture prediction obtained by the use of the algorithm FRAX, which was designed to estimate a 10-year probability of fracture.[223]

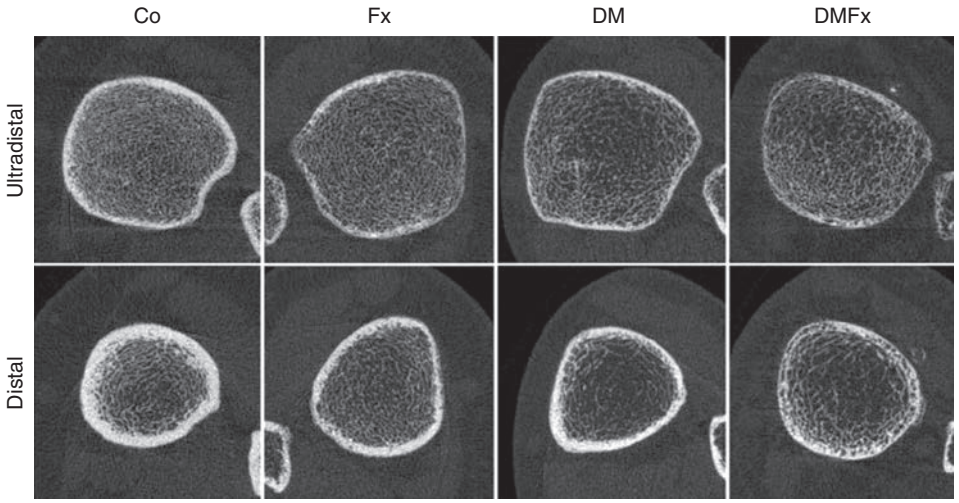
Quantitative Computed Tomography

Other imaging modalities have been studied and may be used for osteoporosis assessment, although DXA remains the preferred method, when available. QCT can be performed at the hip and spine, and studies have shown that it can predict fracture risk.[219,224,225] Several versions of software[226,227] are commercially available for analysis of QCT scans. One advantage of QCT is that it can provide specific measurements of trabecular and cortical BMD. However, QCT has the disadvantages (compared with DXA) of higher radiation exposure, less standardization across computed tomography scanners, and higher cost. Peripheral QCT (pQCT) and high-resolution pQCT have been developed and can currently measure bone density and bone microarchitecture (in the case of high-resolution pQCT) in the radius and tibia. Although these measurements can be helpful in the research context to help understand the impact of diseases such as diabetes and medications such as bisphosphonates on bone microarchitecture and quality, there is little evidence that they have advantages in clinical practice for predicting fracture risk compared with DXA (examples of such measurements are shown in Fig. 30.16).

Other measurement tools to assess risk include ultrasound of the fingers, calcaneus, and distal extremities and magnetic resonance imaging of the radius. Ultrasound has predictive value for fracture risk, but longitudinal assessments have not proved helpful clinically.[228] Magnetic resonance imaging provides significant insight into trabecular and cortical microstructure but is basically a research tool because of expense and time on the machines.

Bone Turnover Markers

The balance between bone resorption and formation is an important contributor to bone loss and osteoporosis. Bone turnover markers (BTMs) can provide an assessment of resorption and formation and might therefore be useful in understanding these imbalances within individual patients, prediction of fracture risk, and the effect of treatment. Bone formation can be assessed by several biochemical markers, including bone-specific alkaline phosphatase (BSAP), total procollagen type 1 N-terminal propeptide (P1NP), and osteocalcin. Bone resorption has traditionally been



• **Fig. 30.16** Representative high-resolution peripheral quantitative computed tomography images of the ultradistal (*above*) and distal (*below*) tibia: shown are the mid-stack tomograms for the Co (*left*), Fx (*left-center*), DM (*right-center*), and DMFx (*right*) groups. Major cortical porosity can be seen in DMFx (*right*). *Co,* controls; *Fx,* nondiabetic fracture patients; *DM,* diabetic patients without fractures; *DMFx,* diabetic patients with fractures. (From Patsch JM, Burghardt AJ, Yap SP, et al. Increased cortical porosity in type 2 diabetic postmenopausal women with fragility fractures. *J Bone Miner Res.* 2013;28[2]:313–324.)

assessed with urinary N-terminal cross-linked telopeptide (NTX) but is now more commonly assessed by serum C-terminal cross-linked telopeptide (CTX), both being collagen cross-linked peptides assessed with antibodies (Table 30.2).

There are several potential clinical applications of BTMs. However, all of them must take into account the high analytic variability (both within individual and between assays and laboratories) for these markers.[229] Important sources of variation within individuals include circadian variation, food intake, exercise, seasonal variation, diseases such as renal impairment, and recent fractures.[230] For predicting fracture risk, there is controversy about whether BTMs predict fracture independently of BMD and other risk factors, with some studies showing a positive relationship; however, others have not shown that BTMs independently predict fracture.[231–233] Because BTMs change dramatically and quickly with both antiresorptive and anabolic treatment, it has been suggested that they may be of some use in monitoring therapy. Although the value of this application is controversial, they could have some value for a clinician with patients whose self-reported compliance may be unreliable.[234–236] They are more commonly used in specialty practices where they can be helpful in defining secondary causes of osteoporosis. BTMs are particularly useful when measured in groups of patients during the development of new medications. In the research setting, these measurements can provide early assessments of potency of drugs, their effects over time, and assist in determining optimal doses.[237,238]

Bone Biopsy

Bone biopsy is used clinically to assess dynamic and static indices related to bone remodeling. It is rarely used clinically to diagnose osteoporosis but can be useful to assess the degree of mineralization (e.g., to exclude osteomalacia), rates of bone formation or resorption, and the overall status of remodeling (e.g., to exclude adynamic renal bone disease). Universal criteria can then be applied to the indices obtained after labeling bone by timed tetracycline administration.[239] Transiliac biopsies can be performed by physicians who specialize in osteoporosis medicine or by orthopedic surgeons and requires local anesthetic for a core biopsy, which generally requires a 7.5-mm trephine core for bone histologic diagnosis and histomorphometry. Mineral apposition rate, mineralizing surface, bone formation rate, eroded surfaces, number of osteoblasts and osteoclasts, and osteoid volume/bone volume all can be determined from a single biopsy, but only after serial tetracycline labeling, which is required to measure the distance between two mineralization fronts. Labeling intervals vary somewhat but generally are for 3 days at the beginning (days 1–3) and for 3 days 21 days later, using 200 mg of demeclocycline three times per day. Several commercial laboratories provide analysis of biopsies, although turnaround time can vary from 1 to several months. Not infrequently, however, bone biopsies in age-related or postmenopausal osteoporosis are normal, and hence the diagnostic specificity is low.

Fracture Epidemiology

Some of the central features of the epidemiology of fractures for the three primary osteoporotic fractures are illustrated in Fig. 30.17. These data show very clearly the exponential increase in women in hip and spine fractures. For example, an average Caucasian woman at age 50 years has about a 15% to 20% annual risk of hip fracture that increases still further beyond age 80 years. Wrist fractures show a different pattern in women with an increase at the time of menopause but then no further increase with time. In men, the exponential increase in hip and spine fractures with age is parallel to that for women. However, importantly, the age-specific risk of hip and spine fractures in men is much lower than that in women (approximately 50%), highlighting the key role of gender in the epidemiology of osteoporotic fractures. Wrist fracture incidence in men is much lower than in women at all ages.

TABLE 30.2 Markers of Bone Turnover

Marker	Tissue of Origin	Specimen	Analytic Method	Remarks
Bone Formation				
Bone-specific alkaline phosphatase (BAP)	Bone	Serum	Electrophoresis, precipitation, IRMA, EIA, ECMA	Specific product of osteoblasts; some assays show up to 20% cross-reactivity with the liver isoenzyme (LAP)
Osteocalcin (OC)	Bone, platelets	Serum	RIA, IRMA, EIA	Specific product of osteoblasts; many immunoreactive forms in blood; some may be derived from bone resorption
C-terminal propeptide of type I procollagen (PICP)	Bone, soft tissue, skin	Serum	RIA, EIA	Specific product of proliferating osteoblasts and fibroblasts
N-terminal propeptide of type I procollagen (PINP)	Bone, soft tissue, skin	Serum	RIA, EIA	Specific product of proliferating osteoblast and fibroblasts; partly incorporated into bone extracellular matrix
Markers of Bone Resorption				
Collagen-Related Markers				
Hydroxyproline, total and dialyzable (Hyp)	Bone, cartilage, soft tissue, skin	Urine	Colorimetry HPLC	Present in all fibrillar collagens and partly in collagenous proteins, including C1q and elastin Present in newly synthesized and mature collagen (i.e., both collagen synthesis and tissue breakdown contribute to urinary hydroxyproline)
Hydroxylysine-glycosides	Bone, soft tissue, skin, serum complement	Urine, serum	HPLC, EIA	Hydroxylysine in collagen is glycosylated to varying degrees, depending on tissue type Glucosylgalactosyl-OH-Lys in high proportion in collagens of soft tissues, and C1q Galactosyl-OH-Lys in high proportion in skeletal collagens
Pyridinoline (PYD)	Bone, tendon, cartilage, blood vessels	Urine, serum	HPLC, EIA	Collagens, with highest concentrations in cartilage and bone; absent from skin; present in mature collagen only
Deoxypyridinoline (DPD)	Bone, dentin	Urine, serum	HPLC, EIA	Collagens, with highest concentration in bone; absent from cartilage or skin; present in mature collagen only
C-terminal cross-linked telopeptide of type I collagen (ICTP, CTX-MMP)	Bone, skin	Serum	RIA, EIA	Collagen type I, with highest contribution probably from bone; may be derived from newly synthesized collagen
C-terminal cross-linked telopeptide of type I collagen (CTX-I)	All tissues containing type I collagen	Urine (α/β), serum (β only)	EIA, RIA	Collagen type I, with highest contribution probably from bone; isomerization of aspartyl to β-aspartyl occurs with aging of collagen molecule
N-terminal cross-linked telopeptide of type I collagen (NTX-I)	All tissues containing type I collagen	Urine, serum	EIA, RIA, CLIA	Collagen type I, with highest contribution from bone
Collagen I alpha 1 helicoidal peptide (HELP)	All tissues containing type I collagen	Urine	EIA	Degradation fragment derived from the helical part of type I collagen, $\alpha 1$ chain, AA (620-633); correlates highly with other markers of collagen degradation, no specific advantage or difference in regard to clinical outcomes
Noncollagenous Proteins				
Bone sialoprotein (BSP)	Bone, dentin, hypertrophic cartilage	Serum	RIA, EIA	Acidic, phosphorylated glycoprotein, synthesized by osteoblasts and osteoclast-like cells, laid down in bone extracellular matrix; appears to be associated with osteoclast function
OC fragments (uf-OC, U-Mid-OC, U-Long-OC)	Bone	Urine	EIA	Certain age-modified fragments are released during osteoclastic bone resorption and may be considered an index of bone resorption

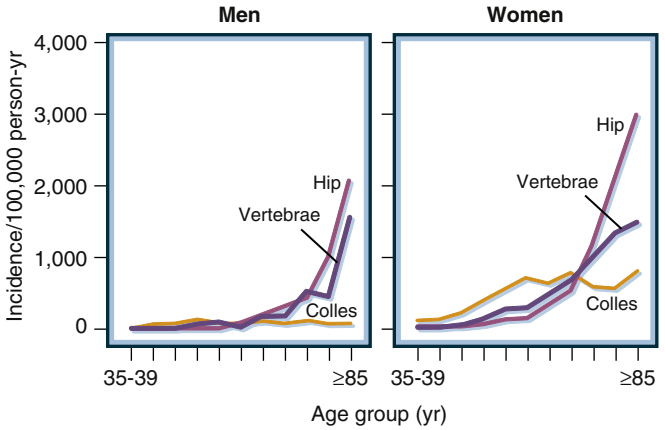
Continued

TABLE 30.2 Markers of Bone Turnover—cont'd

Marker	Tissue of Origin	Specimen	Analytic Method	Remarks
Osteoclast Enzymes				
Tartrate-resistant acid phosphatase (TRAcP)	Bone	Blood plasma	Serum colorimetry, RIA, ELISA	Six isoenzymes found in human tissues (osteoclasts, platelets, erythrocytes); band 5b predominant in bone (osteoclasts)
Cathepsins (e.g., K, L)	K: Primarily in osteoclasts; L: macrophage, osteoclasts	Plasma, serum	ELISA	Cathepsin K, a cysteine protease, plays an essential role in osteoclast-mediated bone matrix degradation by cleaving helical and telopeptide regions of collagen type I; cathepsin K and L cleave the loop domain of tartrate-resistant acid phosphatase and activate the latent enzyme; cathepsin L has a similar function in macrophages; tests for measurement of cathepsins in blood are presently under evaluation

BAP, Serum bone alkaline phosphatase; *BMD,* bone mineral density; *β-CTX-I,* epitope of C-terminal cross-linked telopeptide of collagen type I; *CLIA,* chemiluminescent immunoassay; *C-terminal,* carboxy-terminal; *CTX-MMP,* C-terminal cross-linking telopeptide of type I collagen generated by matrix metalloproteinases; *DPD,* deoxypyridinoline; *ECMA,* electrometric immunoassay; *EIA,* enzyme immunoassay; *HPLC,* high-performance liquid chromatography; *ICTP,* carboxyterminal type I collagen telopeptide; *IRMA,* immunoradiometric assay; *N-terminal,* amino-terminal; *PICP,* C-terminal propeptide of procollagen type I; *PINP,* N-terminal propeptide of procollagen type I; *PTH,* parathyroid hormone; *PTHrP,* parathyroid hormone–related peptide; *PYD,* pyridinoline; *RIA,* radioimmunoassay; *TAP,* serum total alkaline phosphatase; *ufOC,* urinary fragments of osteocalcin; *U-Long-OC,* long N-terminal fragment; *U-Mid-OC,* midmolecule epitope of the OC.

Adapted from Seibel MJ. Biochemical markers of bone turnover: part I: biochemistry and variability. *Clin Biochem Rev.* 2005;26(4):97–122.



• **Fig. 30.17** Age-specific incidence rates for hip, vertebral, and Colles fractures in Rochester, Minnesota. (From Cooper C, Melton LJ. Epidemiology of osteoporosis. *Trends Endocrinol Metab*. 1992;3:224. Copyright 1992 by Elsevier Science, Inc.)

Ethnicity and culture also play a key role in the epidemiology of osteoporosis (Fig. 30.18). For hip fractures, those of Caucasian ethnicity are at higher risk, Hispanics and Asians are at medium risk, and African Americans are at lowest risk. However, these general relationships can often be more subtle. For example, in Asians, there is evidence that rates are very low in areas with traditional lifestyles and that they increase substantially with increasing urbanization and adoption of Western lifestyles.[240] Rates of spine fractures are more challenging to assess, but many studies suggest that these rates vary less geographically than do rates of hip fracture.

Another key epidemiologic factor that interacts with age, gender, and ethnicity is weight or body mass index (BMI). There are studies showing that low lean mass is an independent predictor of incident fractures in a cohort of 65-year-old community dwellers.[241] In addition to the obvious mechanical connection between muscles and bones, other factors are important determinants of this association, including molecular, genetic, and endocrine interaction.[242] In general, higher BMI individuals are at lower risk of hip and spine fractures, owing to several factors, including a larger number of fat cells that produce greater amounts of estrogen, which is protective, and the higher amount of padding in higher BMI patients, which produces a larger distribution of the forces in the event of a fall. Although some data suggest that individuals who are obese have a higher risk of fractures, most studies support the tenet that a higher BMI is protective for most types of fractures, the exception possibly being lower limb fractures. Very importantly, those at low BMI are at particularly high risk of hip fracture for several reasons, one of which may be that low BMI, particularly in the elderly, may be a sign of frailty.[243,244]

Clinical Risk Factors and Their Combination With BMD

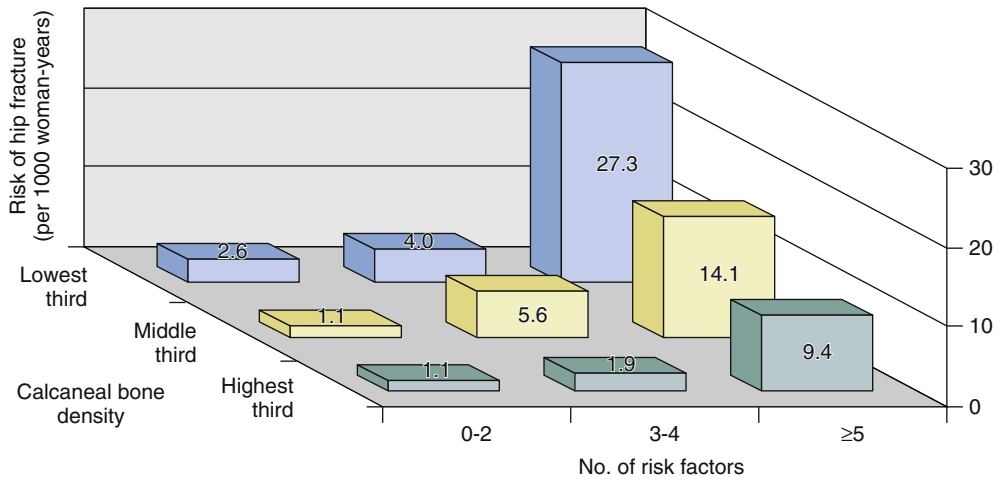
Several other clinical risk factors (in addition to BMD, age, gender, and race) have been consistently associated with fracture risk (Table 30.3; Fig. 30.19). Most important of these is a history of fracture since age 50 years or a mother's (or father's) history of hip fracture.[245] Other risk factors, including cigarette smoking, excessive alcohol consumption (more than two drinks per day), rheumatoid arthritis, and glucocorticoid use, also have some association with risk.[244,246] Compromised neurologic or muscular function is a strong risk factor, probably through an increased likelihood of falls. There is also a growing awareness that diabetics, despite a generally high BMI, are at higher risk of fracture and that this risk at the same BMD is higher than that for nondiabetics.[247] Surprisingly, some recent studies have suggested that obesity may be a risk factor for fracture in some settings: in the MrOS study, it was suggested that at the same BMD, elderly men who were obese were at higher risk than those who were not obese.[248]

There is a growing appreciation that fracture risk, like heart disease risk, is multifactorial and that these risk factors work in combination to increase risk.[244,246] More recently, risk assessment tools predicting 5- or 10-year risk of fracture from combinations of BMD and risk factors together have been developed. The most widely used and available is the FRAX fracture risk

• **Fig. 30.18** Age-standardized hip fracture incidence rates in women and men according to country. Countries are organized by continent or geographic region: Europe (*dark pink*), North America (*green*), Asia (*light blue*), Middle East (*yellow*), South America (*purple*), Oceania (*dark blue*), and Africa (*red*). *UK,* United Kingdom; *USA,* United States of America. (Redrawn from Cauley JA, Chalhoub D, Kassem AM, Fuleihan Gel-H. Geographic and ethnic disparities in osteoporotic fractures. *Nat Rev Endocrinol*. 2014;10[6]:338–351.)

TABLE 30.3 Different Combinations of Relevant Fracture Risk Factors Used to Calculate an Individual's Probability of Fracture in 10 Years

FRAX	Q-Fracture Score	Garvan
Age	Age	Age
Sex	Sex	Sex
Weight	Weight	
Personal history of fracture (vertebral inclusive)		Personal history of fracture
Parental history of hip fracture	Parental history of hip fracture	
Current smoking	Current smoking (graded)	
Glucocorticoid (prednisolone equivalent 5 mg for ≥3 months)	At least two prescriptions for systemic corticosteroids in the 6 months before baseline	
Rheumatoid arthritis	Rheumatoid arthritis	
Secondary osteoporosis, type 1 diabetes mellitus, osteogenesis imperfecta in adults, hyperthyroidism, hypogonadism, chronic malnutrition, and liver disease	Secondary osteoporosis, cardiovascular disease, type 2 diabetes mellitus, asthma, chronic liver disease, gastrointestinal conditions likely to result in malabsorption (e.g., Crohn disease, ulcerative colitis, celiac disease, steatorrhea, blind loop syndrome), thyrotoxicosis, primary or secondary hyperparathyroidism, Cushing syndrome	
Alcohol ingestion (>3 units/day)	Alcohol ingestion (>3 units/day) At least two prescriptions for tricyclic antidepressants in the 6 months before baseline At least two prescriptions for hormone replacement therapy (in women) in the 6 months before baseline	
	History of falls before baseline	History of falls
Bone mineral density (optional)		Bone mineral density

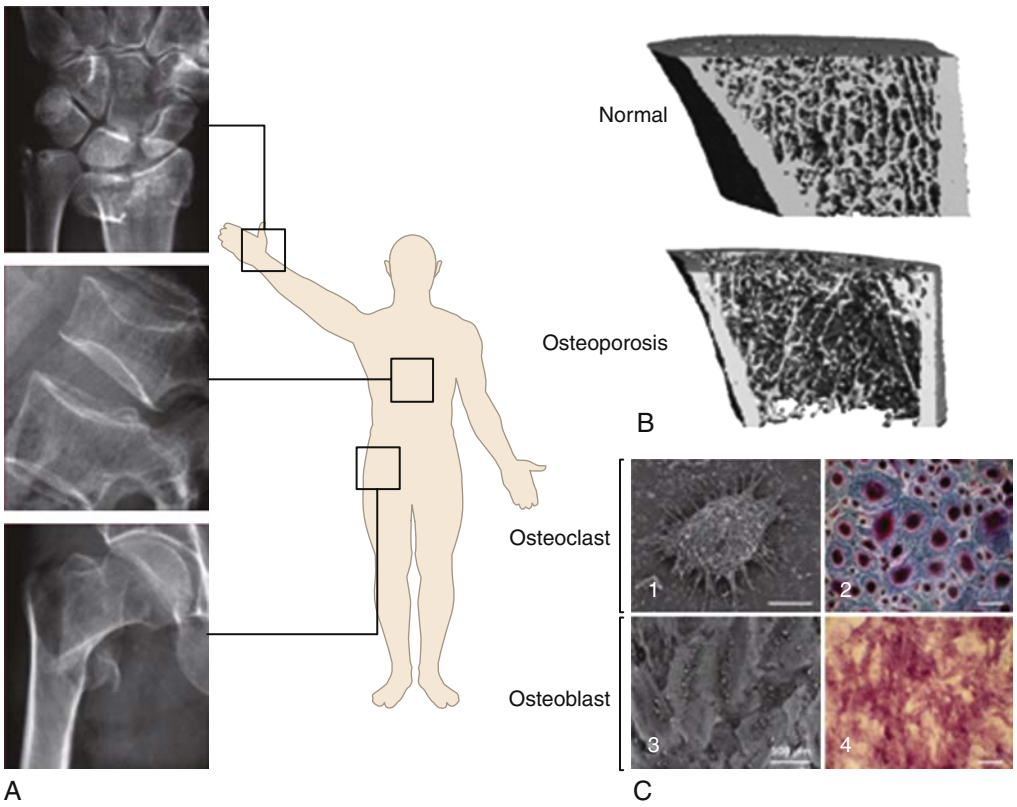


• **Fig. 30.19** Annual risk of hip fracture according to the number of risk factors and the age-specific calcaneal bone density. (Redrawn from Cummings SR, Nevitt MC, Browner WS, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. *N Engl J Med*. 1995;332:767–773. Copyright © 1995 Massachusetts Medical Society. Reprinted with permission.)

assessment tool, which includes the risk factors in Table 30.3 and has been implemented with country-specific versions[249] for predicting risk of hip and major osteoporotic fractures. Other risk assessment tools have also been proposed and include different or fewer risk factors.[250] Clinical guidelines for treatment have been developed incorporating these tools (discussed later; see Table 30.3).

Prevalence of Osteoporosis and Incidence of Fractures in the Population

Estimates of the prevalence of osteoporosis have depended on the working definition of this disease and the appropriate diagnostic criteria, and they have been based on either the prevalence of low BMD or fracture incidence.[251–253] Basing a definition solely on BMD became increasingly possible after the development and



• **Fig. 30.20** Osteoporosis at a glance. Osteoporosis is a systemic skeletal disease in which bone resorption exceeds bone formation and results in microarchitectural changes. (A) Fragility fractures typically involve the wrist, vertebrae, and hip. (B) Microcomputed tomography shows marked trabecular thinning of osteoporotic bone compared with normal bone. (C) Microscopic views of bone-resorbing osteoclasts and bone-forming osteoblasts: *1*, osteoclast with its distinctive morphologic appearance; *2*, tartrate-resistant acidic phosphatase staining of multinucleated osteoclasts; *3*, multiple osteoblasts on mineralized matrix; *4*, alkaline phosphatase staining of osteoblasts. (From Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. *Lancet.* 2011;377[9773]:1276–1287.)

clinical availability of DXA. In 1992, the World Health Organization set a cutoff point of 2.5 SD below a young normal mean value for BMD as a tool for estimating osteoporosis prevalence and comparing across populations[254] (see Table 30.1). The use of DXA in larger epidemiologic studies in women (e.g., the Study of Osteoporotic Fractures) and men (the MrOs study), and particularly in population-based samples (e.g., the National Health and Nutrition Examination Study), has provided estimates of the proportion of people with osteoporosis by BMD.[255–257] Estimates of overall prevalence of osteoporosis vary greatly but place the number close to 40 million Americans.[251,258]

Based on fracture incidence, currently most estimates suggest that there are approximately 0.3 million hip fractures per annum in the United States and 0.6 million hip fractures in Europe.[252,253] Best estimates are that more than a million American postmenopausal women will suffer a spine fracture in the course of a single year.[251,258] Considering the importance of fracture from an individual's point of view, a 50-year-old Caucasian woman has approximately a 15% to 20% lifetime risk of hip fracture, a similar lifetime risk of wrist fracture, about a 16% lifetime risk of clinical spine fracture, and approximately a 50% risk of at least one osteoporotic fracture.[259–261] Men have about one third the lifetime risk of hip and spine fractures as women but a much lower risk of wrist fracture. Combining osteoporosis prevalence based on either the presence of osteoporotic fractures (i.e., vertebral compressions, wrist fractures, hip fractures, or humerus/tibial fractures) or low BMD, it is estimated that more than 40 million Americans are afflicted with osteoporosis.[262] Worldwide, there are approximately 9 million fractures per year.[263]

Although numbers of fractures are likely to increase in the United States and Europe, this increase will be much more striking in the developing world, particularly in Asia and Latin America, because of an intersection of several trends including increasing population size, increasing life span (into the high-fracture-risk ages), and adoption of a more Western lifestyle (particularly decreasing activity in daily living).[264,265] This increase will result in a consequent increase in need for health care resources. Interestingly, although hip fractures are the most costly individual fractures, the overall costs of other types of fractures together may be greater than that for hip fractures.[266]

Pathogenesis of Osteoporosis

Osteoporosis is a complex disease with a multifactorial origin (Fig. 30.20). The cardinal feature of this disease is fractures usually accompanied by enhanced skeletal fragility. Although the primary focus of this chapter is on the endocrine and metabolic aspects of this disease, it should be noted that falls cause fractures and that one of the primary causes of osteoporotic fractures is falling. Falls are also multifactorial, particularly in older individuals, because

primary muscle weakness, neurologic disorders, drugs, vitamin D deficiency, balance problems, and cardiovascular events such as syncope all can cause falls. Hence, primary prevention of osteoporosis also mandates specific steps to reduce the chance of any fall for whatever reason. Noted in this section are causes of imbalance in the bone remodeling unit that lead to secondary bone loss and subsequent reduction in BMD, enhancing skeletal fragility.

Gonadal Deficiency

Estrogen

Altered bone remodeling is at the heart of the osteoporosis syndrome and can take many forms. Historically, the importance of estrogen in maintaining calcium homeostasis via coupled remodeling in the postmenopausal woman was first established by Fuller Albright in 1947.[267] Since that time, much evidence has accumulated from randomized intervention trials demonstrating that gonadal steroid replacement (estrogen with or without progesterone) reduces bone turnover and increases bone mass, thus protecting the skeleton from fractures.[202,268] However, these data provide only indirect evidence that estrogen levels are important as pathogenic components of the osteoporosis syndrome. More recent studies provide stronger evidence of the association between low estradiol concentrations and low bone mass. Several investigators have demonstrated that the lowest estradiol levels in postmenopausal women (i.e., <5 pg/mL) are associated with the lowest BMD and the greatest likelihood of fracture.[269] In addition, several studies have shown that males with osteoporosis have lower serum levels of estradiol than do age-matched men who do not have low bone mass.[270] Moreover, there are now several case reports describing mutations in genes encoding either aromatase or the estrogen receptor, producing a phenotype of severe osteoporosis in men.[271,272] In the former case, estrogen replacement therapy in those men resulted in a marked increase in spine and hip BMD. In both situations, the lack of functional estrogen, despite normal to high levels of testosterone, resulted in severely low BMD.[199]

Although declining estradiol levels contribute to the osteoporosis syndrome, the precise molecular events or sequences that result from changes in ambient hormonal concentrations are not clear. In some animal models, estrogen deprivation is associated with a marked increase in IL6 synthesis from stromal and osteoblastic cells. This is consistent with experimental findings demonstrating that estrogen regulates the transcriptional activity of the IL6 promoter.[273] However, results in other studies are conflicting. In other experimental paradigms, changes in TNF, IL11, and IL1 can be associated with increased bone resorption.[274–276] RANKL has been identified as a major regulator of osteoclast differentiation, and increases (both locally and systemically) likely contribute to the rapid increase in osteoclastogenesis after estrogen withdrawal. Thus, it seems likely that several cytokines, working in concert with RANKL, are active during estrogen deprivation, and each can accelerate the process of bone resorption. Osteoclasts express estrogen receptors, and some evidence suggests that direct actions of estrogen on osteoclasts are important as well. Enhanced bone resorption eventually leads to bone loss from estrogen deprivation because bone formation rates cannot keep up with the high rates of bone resorption[277] (see Fig. 30.15).

Androgens

In contrast to the plethora of studies on uncoupled bone remodeling and bone loss with low estradiol levels, there are fewer studies relating androgen deprivation to bone loss in both men and women. Androgen receptors are present on osteoblasts, and testosterone and dihydroxytestosterone both stimulate osteoblast differentiation.[278] Testosterone may also increase skeletal and circulating IGF1. However, both in vitro and in vivo studies in men have yielded conflicting results with respect to bone resorption. Like estrogen, androgens can regulate the IL6 promoter, and in experimental animals, orchiectomy has been associated with increased IL6 production and bone loss.[279] Men who undergo androgen deprivation therapy after prostate cancer lose bone rapidly as a result of increased bone resorption. Similarly, hypogonadal men, either due to primary or secondary insufficiency, have lower bone density values than control men. Because, unlike estradiol, testosterone can stimulate bone formation, this may be an additional factor that contributes to bone loss when absent in men.[280]

Low testosterone levels that are found in postmenopausal women have not been causally associated with rapid bone loss, but women with greater body weight have greater capacity to enzymatically convert testosterone to estradiol, which may contribute to the protective effect of obesity on bone mass, at least in women. Notwithstanding, because in men with chronically low androgen levels have been associated with low bone mass, testosterone replacement can be used to both enhance BMD and increase lean body mass.[280] However, as noted, estradiol levels in men may be a more important risk factor for fracture than androgen levels. In sum, the combination of both estrogen and androgens probably determines peak bone mass and maintenance of BMD in both men and women. In elderly men, estradiol levels may be essential for maintaining trabecular bone mass. To underline that tenet, in one randomized trial using a gonadotropin-releasing hormone analogue (to suppress gonadotropins) with and without an aromatase inhibitor in men, changing estrogen levels were associated with protection of the trabecular skeleton from bone resorption, whereas circulating androgen levels were more related to markers of bone formation than those of resorption.[202] In another study, Almeida and colleagues[281] found that in rodents, protection of cortical bone mass by estrogens is mediated via estrogen receptor α. In contrast, the androgen receptor of mature osteoblasts was indispensable for the maintenance of trabecular bone mass in male mammals but was not required for the anabolic effects of androgens on cortical bone.[278,282]

In men, conditions other than hypogonadism can contribute to bone loss and fractures. These states include chronic alcoholism, glucocorticoid excess, smoking, and idiopathic hypercalciuria. In the first two cases, low testosterone levels probably contribute to the pathogenic features of osteoporosis syndrome, whereas hypercalciuria due to renal loss probably causes bone loss through secondary hyperparathyroidism. Less frequent but still important secondary causes of osteoporosis in men must also be considered independent of androgen levels, and these include gluten enteropathy, primary hyperparathyroidism, thyrotoxicosis, multiple myeloma, lymphomas, or granulomatous diseases, all of which can present with multiple fractures and low bone mass[283] (see Table 30.3).

Age-Related Bone Loss

In women, bone loss is accelerated immediately after menopause. However, recent studies demonstrate that markers of bone resorption are also very high later in life. In particular, women in their 80s and 90s have been noted to lose bone at a rate of greater than 1% per year from the spine and hip[220] (see Fig. 30.15). Contrary to earlier studies, it is now evident that the older woman who is not

as physically active, and is not taking estrogen, is at extremely high risk of bone loss and subsequent fractures. The pathogenesis of this process is multifactorial, although dietary calcium deficiency, leading to secondary hyperparathyroidism, certainly plays some role. The average calcium intake of women in their eighth and ninth decades of life is now estimated to be between 800 and 1000 mg/day. If vitamin D intake is also suboptimal and serum levels of 25-hydroxyvitamin D are less than 20 ng/mL, or 50 nmol/L, secondary hyperparathyroidism may occur, although there are other causes in the elderly for increases in PTH, including a low glomerular filtration rate and low calcium intake.[284] PTH stimulates osteoblasts and provokes the remodeling sequence including the elaboration of several cytokines that accelerate bone resorption. Unfortunately, in most elders, bone formation is not enhanced, although the reasons for this are not entirely clear. Overall, the secondary hyperparathyroidism coupled with high $1,25(OH)_2D$ (see later discussion in the Vitamin D section) leads to further uncoupling in the bone remodeling cycle and significant bone loss. Furthermore, among elders with poor calcium intake who live in northern latitudes, seasonal changes in vitamin D levels, lowering levels below 20 ng/mL, might aggravate bone loss.[284,285] Whether increased bone loss is an independent risk factor for future fractures in the elderly remains somewhat controversial, necessitating further studies to define such a risk.

Many older individuals already have established osteoporosis, so coincidental vitamin D deficiency due to poor intake, absent sunlight exposure, or impaired conversion of vitamin D to its active metabolite can result in osteomalacia and aggravate preexisting osteoporosis.[286] Osteomalacia can lead to dramatic changes in skeletal microarchitecture, as was recently demonstrated in a cohort from Germany.[287] Trabecular and cortical changes seen in that study led to microcracks and greater skeletal fragility. Priemel and associates[287] reported that more than 50% of elders who presented with a hip fracture were vitamin D deficient. Combining vitamin D deficiency with inadequate calcium intake enhances the likelihood of rapid bone loss in a very susceptible population. Despite these findings, elevated PTH levels in older women have been associated with bone loss in some studies but not in other studies. In elderly individuals, it has been reported that PTH levels are closely correlated with increased synthesis of IGFBP4, which suppresses IGF action on bone cells and may increase sclerostin secretion.[288,289] Because IGF1 is an important growth factor for osteoblasts, it is conceivable that PTH downregulates IGF activity during states of relative calcium or vitamin D deficiency. This would shift the remodeling balance toward preserving intravascular calcium concentrations while inhibiting new calcium incorporation into the skeletal matrix. This response makes teleologic sense, although further studies are needed to assess whether serum IGFBP4 is a reliable marker of calcium deficiency in older individuals.[289] In sum, there is little doubt that calcium and vitamin D insufficiency can cause accelerated bone loss in the elderly, although the threshold for low vitamin D and poor calcium intake is still actively debated. Other concomitants of aging, such as accumulation of ROS (seen in aged mice) and other causes of damage to bone cells, appear likely to contribute to age-associated osteoporosis as well.[290]

Secondary Osteoporosis

The division of osteoporosis into primary and secondary forms is somewhat arbitrary. For example, patients with diseases that lead to hypogonadism early in life are considered to have *secondary osteoporosis*, whereas osteoporosis in women with natural menopause and older men with low sex hormone levels is called *primary osteoporosis*. Moreover, patients may have a combination of primary and secondary forms. Although most postmenopausal women and older men do not have a definable secondary cause, those who do can be treated more effectively. This possibility should be considered for every patient. There are many causes of secondary osteoporosis (Table 30.4), only a few of which are discussed here.

Glucocorticoid-Induced Bone Loss

The most common cause of secondary osteoporosis is glucocorticoid-induced bone loss, which is often a result of pharmacologic doses of steroids used to treat inflammatory or autoimmune disorders. Generally, it is considered that glucocorticoids have a dose-dependent effect on the skeleton such that longer duration and higher doses of steroids are most likely to cause bone loss and fractures. However, there clearly are subsets of individuals who are more or less sensitive to the skeletal effects of high doses of glucocorticoids. As a clinical rule, those individuals with a cushingoid appearance and fat redistribution phenotypes almost always have low bone mass and fractures. Notwithstanding, BMD measurements are indicated in patients on long-term glucocorticoids for both preventive approaches and treatment decisions.[291,292]

As noted previously, high circulating levels of glucocorticoids have a significant impact on bone acquisition and maintenance. In 1932, Harvey Cushing recognized the syndrome of endogenous steroid excess, which included marked osteopenia and fractures.[293,294] Long-term exposure to pharmacologic doses of glucocorticoids results in significant bone loss and enhanced marrow adipogenesis as marrow stromal cells differentiate down the fat lineage. In addition to having direct effects on the osteoclast and osteoblast, glucocorticoids also induce secondary hypogonadism and hyperparathyroidism, impaired vitamin D metabolism, muscle atrophy, and hypercalciuria. All of these factors contribute to a rapid and sustained loss of bone during the first few months of steroid therapy.[295,296] The addition of other immunosuppressants, such as cyclosporine, has been shown to aggravate bone loss by further increasing bone resorption. Because the number of organ transplants has increased exponentially over the past decade, the prevalence of post-transplantation osteoporosis has risen substantially. Steroid-induced osteoporosis is now considered the second most common cause of low bone mass in the general population and one of the most common causes of osteoporotic fractures.[297]

The effects of glucocorticoids on the skeleton are multifaceted and are particularly devastating because these agents cause uncoupling in the remodeling unit. Besides the indirect suppressive effects of glucocorticoids on the hypothalamic-gonadal axis, and inhibition of calcium absorption in the gut due to impaired $1,25(OH)_2D$ production, high doses of steroids can stimulate osteoclastogenesis, increase RANKL production, and decrease OPG. This situation results in higher rates of bone resorption. Additionally, glucocorticoids also have a strong negative effect on bone formation by suppressing expression of IGF1 in bone cells and by shifting marrow stromal cells into the fat lineage rather than down the osteoblast differentiation pathway.[292] It is presumed that just as fat redistribution is a clinical hallmark of Cushing syndrome in the supraclavicular and mediastinal area, enhanced adiposity in the bone marrow is a characteristic feature of steroid-induced bone disease, almost certainly as a function of increased stromal cell differentiation into adipocytes.[295] Bone strength is markedly compromised by dramatic uncoupling in remodeling, and bone loss can be rapid over a short period,

TABLE 30.4 Causes of Secondary Osteoporosis

Endocrine Disorders
Diabetes mellitus
Hyperparathyroidism
Hyperthyroidism
Cushing syndrome
Hypogonadism
Menstrual irregularity (even athletes)
Premature menopause
Low testosterone and estradiol levels in men
Hyperprolactinemia
Pregnancy and lactation

Autoimmune Disorders
Rheumatoid arthritis
Inflammatory bowel disease
Lupus erythematosus
Multiple sclerosis
Ankylosing spondylitis

Digestive and Gastrointestinal Disorders
Celiac disease
Inflammatory bowel disease
Weight loss surgery
Gastrectomy

Hematologic/Blood Disorders
Leukemia and lymphoma
Multiple myeloma
Sickle cell disease
Blood and bone marrow disorders
- Plasma cell dyscrasias: multiple myeloma and macroglobulinemia
- Myeloproliferative disorders: polycythemia

Thalassemia

Neurologic/Nervous System Disorders
Stroke, Parkinson disease, and multiple sclerosis
Spinal cord injuries

Mental Illness
Depression
Eating disorders

Cancer
Breast
Prostate

Connective Tissue Disorders
Osteogenesis imperfecta
Ehlers-Danlos syndrome
Marfan syndrome
Menkes syndrome

Drug-Induced Disorders
Glucocorticoids
Heparin
Anticonvulsants
Methotrexate, cyclosporine
Luteinizing hormone–releasing hormone agonist or antagonist therapy
Proton pump inhibitors
Aluminum-containing antacids

Other Diseases and Conditions
AIDS/HIV
Chronic obstructive pulmonary disease
Female athlete triad
Kidney disease
Liver disease
Organ transplant
Poliomyelitis and post-polio syndrome
Poor diet, including malnutrition
Weight loss
Lipidoses: Gaucher disease
Scurvy

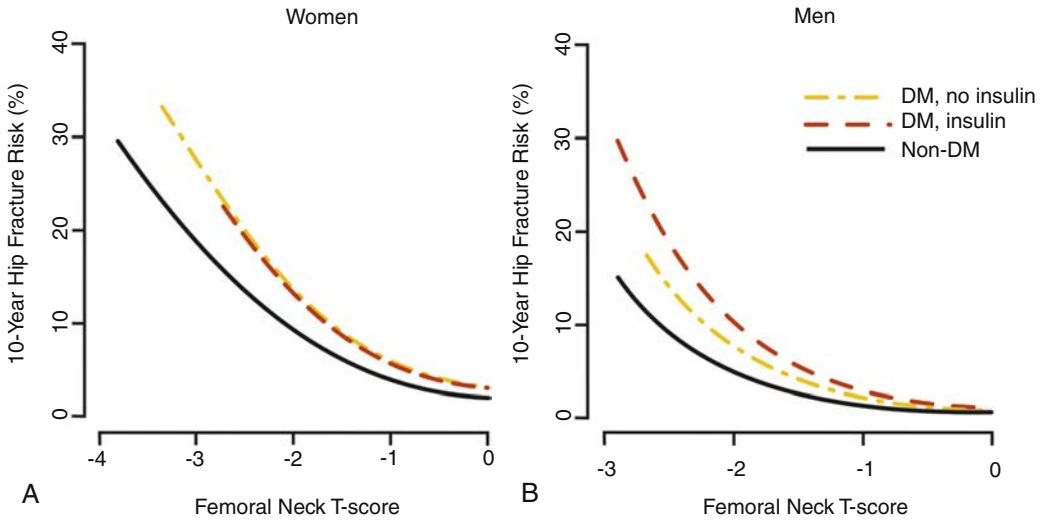
AIDS, Acquired immunodeficiency syndrome; *HIV*, human immunodeficiency virus.

particularly with high doses of glucocorticoids. Although there is no true dose-dependent effect on bone resorption, it is thought that prednisone doses as low as 5 mg/day may increase fracture risk.[298] Indeed, in this syndrome, baseline BMD is not predictive of fractures and can often be normal even in the presence of ongoing resorption and recurrent fractures.[299] Trabecular bone suffers the most in this syndrome, and spine DXA is the most sensitive indicator of bone loss. Markers of bone turnover are not helpful in the management of these patients.

Therapy for steroid-induced bone loss centers on treating the underlying disease and reducing the dose of glucocorticoids to the lowest possible regimen. Barring that, several interventions have been shown to retard bone loss and prevent fractures. Adequate calcium and vitamin D intake is critical for every patient receiving glucocorticoids. However, these measures alone are not sufficient. Regulatory approval for the prevention and treatment of glucocorticoid-induced osteoporosis has been consistent for the group of bisphosphonates, including alendronate, risedronate, and zoledronic acid.[300–303] These drugs are administered either weekly or cyclically to prevent bone loss and reduce the risk of fractures. Some anecdotal data support the use of gonadal steroids in this condition, but clearly bisphosphonates are superior. In a randomized trial, PTH (20 μg of teriparatide subcutaneously daily for 18 months) significantly increased hip and spine bone mass density and reduced the number of new vertebral fractures (7.2% to 3.4%) but not other fractures compared with alendronate.[304] More studies are needed to establish its long-term efficacy in this disease, particularly because secondary hyperparathyroidism is a frequent accompaniment of steroid-induced osteoporosis.

Osteoporosis Associated With Diabetes Mellitus

Diabetes mellitus includes a group of heterogeneous metabolic disorders that in common show hyperglycemia. The association between diabetes mellitus and osteoporosis underpins several relevant aspects. First, energy metabolism integrates the whole body; as such, severe disorders affecting energy metabolism do not spare any single system or tissue. Second, the endocrine regulation of bone and energy metabolism is not restricted to classical glands and neural regulation. Au contraire, there is an intricate mutual regulation between energy metabolism and mesenchymal tissues, including bone.[305] Last, bone fragility in diabetes mellitus represents another aspect of the heterogeneity of diabetes mellitus;



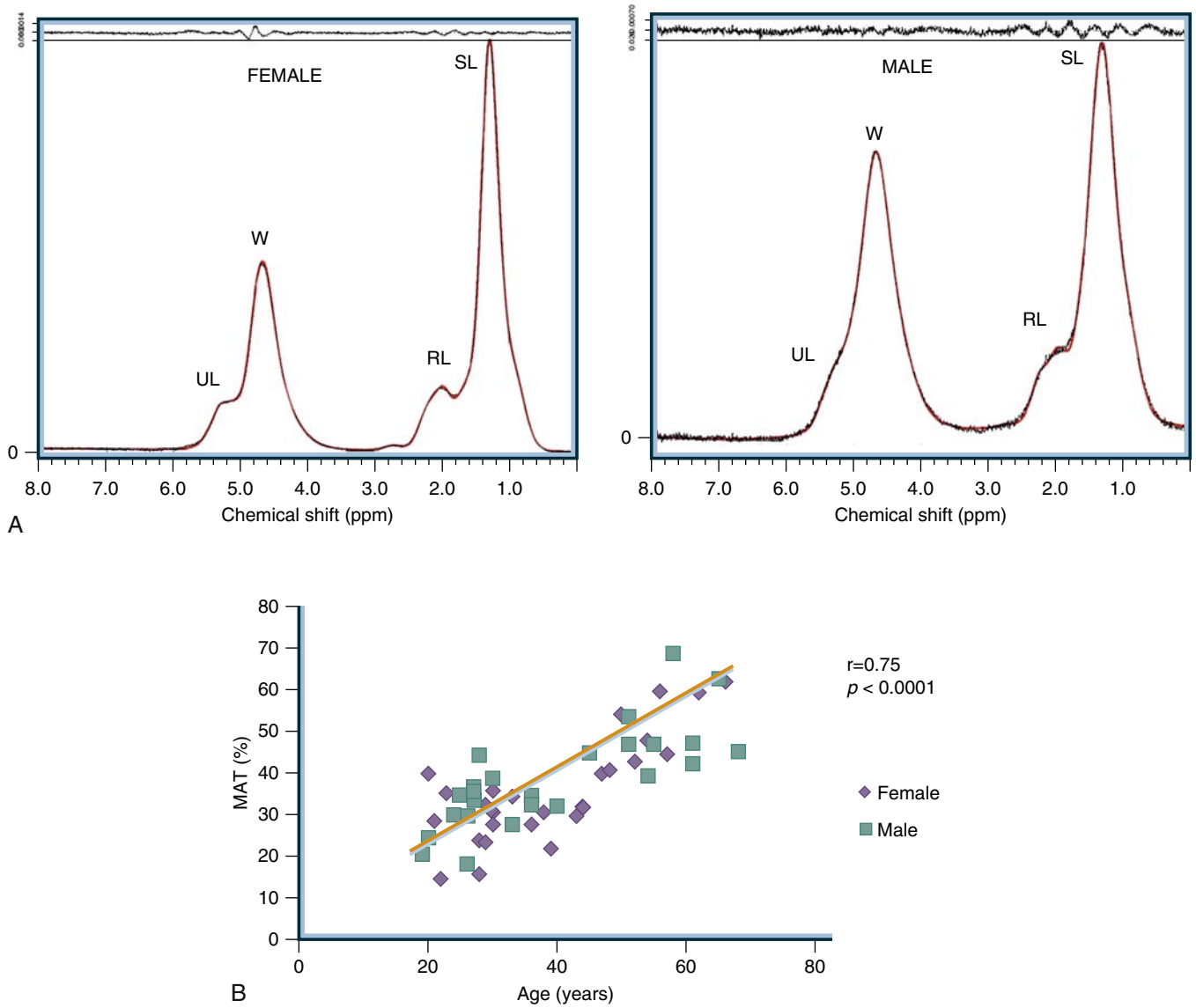
• **Fig. 30.21** Femoral neck bone mineral density T-score and 10-year fracture risk at age 75 years by diabetes status and insulin use. Estimated 10-year cumulative fracture risk at age 75 years in women (A) and men (B), calculated using the Cox proportional hazards regression model baseline survival function raised to the power of the relative hazard for each combination of diabetes group and T-score. *DM,* diabetes mellitus. (From Schwartz AV, Vittinghoff E, Bauer DC, et al. Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. *JAMA.* 2011;305[21]:2184–2192.)

striking differences exist between bone disorders in T1DM and T2DM. T1DM is associated with bone loss and was rapidly recognized as a risk factor for osteoporosis and bone fractures. However, bone mass is preserved or increased in T2DM, meaning that bone quality instead of quantity is the primordial mechanism affecting bone strength in this disorder.

Findings from epidemiologic studies in recent decades indicating dramatic increases in the prevalence of T2DM worldwide mirror the evolving numbers of US residents diagnosed with this metabolic disorder. The "Diabesity" (obesity and T2DM) epidemic has been referred to as the biggest epidemic in human history.[306] T2DM accounts for approximately 90% of all cases of diabetes. Globally, the number of people with diabetes is estimated to be 415 million, affecting more than one third of the US population. Therefore, the magnitude of the importance of osteoporosis in diabetes mellitus has only recently been fully recognized when it was demonstrated that the risk of fracture is increased not only in T1DM but also in T2DM. The delayed inclusion of T2DM among the risk factors of fracture was certainly influenced by consistent documentation that T2DM does not affect bone mass. Although there are studies suggesting that the rate of bone loss is increased in T2DM, in all probability bone quality, rather than quantity, is primarily affected by this disorder. At the same level of BMD, individuals with T2DM have increased fracture risk when compared with normoglycemic individuals[247] (Fig. 30.21).

Several findings support the increased bone mass observed in T2DM. First of all, obesity, which is the central determinant for the emergence of insulin resistance and hyperglycemia, has a positive effect on bone mass.[307] Bone remodeling is typically reduced in diabetes mellitus.[308] Moreover, a clinical study described a negative association between bone mass and the serum levels of osteocalcin in T2DM. Most likely, these results mean that bone turnover is not only reduced, but it also exerts a protective effect on bone mass in T2DM.[194] Hyperinsulinemia is another element involved in the protection of bone mass in T2DM. The striking difference in the secretion of growth factors and adipokines between T1DM and T2DM certainly is another point to be taken into consideration. T1DM is usually associated with low serum levels of IGF1, whereas this is not part of the hormonal alterations in T2DM. However, T2DM predominantly exhibits an inflammatory profile in adipokine secretion by the white adipose tissue that is represented by increased levels of leptin, chemerin, resistin, TNF, and interleukins, whereas adiponectin is reduced. Leptin and adiponectin have complex effects on bone, and there are still no conclusive results about their ultimate effects on bone.[305]

The paradoxical increase in fracture risk in T2DM has respectively challenged researchers and clinicians to unveil mechanisms determining bone fragility and expedite the identification of individuals at risk, as well as the best therapeutic options to prevent fracture in this population. Using an animal model mimicking T2DM, structural alterations in collagen and deterioration in bone strength were found coincidently with the onset of hyperglycemia.[309] The analysis showed an increase in AGEs, whereas a decrease in immature enzymatic collagen cross-links occurred in diabetic animals.[309] Under clinical investigation, it was observed that the urinary excretion of pentosidine, an AGE, was associated with vertebral and hip fracture in postmenopausal women.[310] Advances in imaging techniques allowed the detection of alterations not previously captured by conventional methods. Long bone cortical porosity was described in individuals diagnosed with T2DM.[311,312] Although this finding was not confirmed in other studies, cortical porosity remains a potential alteration determining fracture susceptibility in individuals with T2DM.[313,314] Magnetic resonance has been successfully used to quantify MAT and the composition of lipids within marrow adipocytes[305] (Fig. 30.22A). In several conditions, such as anorexia nervosa, glucocorticoid therapy, and aging, there is an inverse relationship between bone mass and MAT (see Fig. 30.22B). The amount of MAT was similar in normal weight/normoglycemic, obese/normoglycemic and obese/diabetic individuals.[194] However, another study found that both fractures and T2DM are associated with alterations in the composition of lipids in MAT, namely increased saturated and decreased unsaturated lipids.[315] In accordance, individuals with diabetes who are diagnosed with fractures exhibit remarkable



• **Fig. 30.22** (A) 1H magnetic resonance spectroscopy allows the estimation of lipids composition in MAT. Recent studies called attention for the clinical relevance of this parameter, such as the association of saturated lipids with fracture in diabetic individuals. The top figure exhibits differences in unsaturated fat between male and female (female > male). (B) Age-related increase in marrow adipose tissue in a group of healthy controls: 53 individuals, 28 females and 25 males; age, female: 38.8 ± 13.2 years and male: 39.7 ± 15.6 years; weight, female: 60.4 ± 6.4 kg and male: 66.0 ± 8.0 kg; height, female: 1.65 ± 0.08 m and male: 1.71 ± 0.06 m; and body mass index, female: 22.1 ± 1.8 kg/m^2 and male: 22.3 ± 1.9 kg/m^2). *H,* hydrogen; *MAT,* marrow adipose tissue; *RL,* residual lipids; *SL,* saturated lipids; *UL,* unsaturated lipids, *W,* water. (Redrawn from de Paula FJ and Rosen CJ. Structure and function of bone marrow adipocytes. *Compr Physiol*. 2017;8[1]:315–349.)

accentuation of saturated lipids within MAT.[315] More recently, a clinical investigation, using an in vivo microindentation device, observed an alteration in a bone material property, namely the bone material strength index (BMSi). The studies also highlighted a negative association between BMSi and long-standing poor metabolic control, indicating a connection between bone disease and chronic diabetes complications.[313] Reinforcing these results, there was also an inverse association between BMSi and AGEs, as determined by skin autofluorescence.[313]

Altogether, BMD underestimates fracture risk in individuals with T2DM (see Fig. 30.21). Objectively, for a similar hip fracture risk, it was estimated that T2DM women and men have higher T-scores of 0.59 (95% confidence interval, 0.31–0.87) and 0.38 (95% confidence interval, 0.09–0.66), respectively, than normoglycemic individuals.[247] Awareness regarding the implication of osteoporotic fractures in individuals with diabetes has to be highlighted due to their increased morbidity and mortality rate, as well as the worse fracture outcomes in this population.

The intricate relationship between bone and energy metabolism aggregates issues concerning the potential repercussions of osteoporosis therapy on glucose metabolism, on one side, and the effects of diabetes management on bone mass and fracture occurrence, on the other. Osteoblasts express insulin receptor, and the experimental silencing of insulin receptor specifically in osteoblasts results in both decreased bone mass and impairment in glucose tolerance. Two research groups independently collected evidence

TABLE 30.5 Effects of Antidiabetic Agents on Bone Based on Clinical Investigation

	BONE BIOMARKERS			
Agent	Bone Formation	Bone Resorption	BMD	Fracture
Metformin	↓/=	↓/=	=/↑	↓/=
Sulfonylureas	↑/=	↓/=	ND	↓/=/↑
Thiazolidinediones	↓↓/=/↑	↑↑/=	↓↓/=	↑↑/=
Incretin (GLP1 analogue)	=	↓↓	↑/=	=
Incretin (DPP4i)	↓/=	=	–	↓/=
SGLT2 inhibitor	=	=/↑	=	=/↑
Insulin	=	=	=	↑

↑, increased; ↓, decreased; =, unchanged; *DPP4i*, Dipeptidyl peptidase inhibitor; *GLP1*, glucagon-like peptide 1; *ND*, no data; *SGLT2*, sodium-dependent glucose cotransporter 2.

Adapted from Palermo A, D'Onofrio L, Eastell R, et al. Oral anti-diabetic drugs and fracture risk, cut to the bone: safe or dangerous? A narrative review. *Osteoporos Int.* 2015;26(8):2073–2089.

that uncarboxylated osteocalcin is an endocrine molecule originating in osteoblasts that modulates insulin secretion and sensitivity.[21,22] Initially produced in a carboxylated form, osteocalcin is the most abundant noncollagenous protein in the bone matrix and has a role in the mineralization process. The acidic environment created by osteoclasts to elicit bone resorption promotes osteocalcin decarboxylation. GPRC6A, a G-protein coupled receptor, has been identified as the pancreatic effector of uncarboxylated osteocalcin promoting beta-cell hyperplasia and insulin secretion, as verified in several experimental models.[316] Moreover, uncarboxylated osteocalcin seems to increase insulin sensitivity by stimulating adiponectin secretion in white adipose tissue.[317] However, inconclusive findings have been reported in clinical investigations related to the association between osteocalcin and insulin sensitivity, as well as glucose tolerance. For instance, individuals showing decreased circulatory levels of osteocalcin due to bisphosphonate and denosumab therapy do not exhibit increased incidence of diabetes.[318,319] Moreover, patients diagnosed with primary hyperparathyroidism exhibit insulin resistance despite an increased rate of bone remodeling and increased serum levels of osteocalcin.[320]

Antidiabetic drugs have diverse effects on bone, from beneficial to neutral or even detrimental effects[321] (Table 30.5). Thus, implementation of an appropriate strategy is necessary to preclude undesirable increase in fracture risk in these individuals. Thiazolidinediones are a group of drugs associated with adverse effects on bone mass and increased risk of fracture. Thiazolidinediones are exogenous agonists of PPARs that drive bone marrow mesenchymal stromal cells to differentiate into adipocytes, inhibiting osteoblastogenesis via decreasing Runx2 transcription factor, as well as IGF1 and Wnt signaling pathways. Insulin positively affects bone mass; notwithstanding, insulin therapy is associated with bone fracture. The mechanism determining bone fragility and whether it is mediated through hypoglycemia and increased risk of falls are still to be clarified. Metformin and sulfonylureas, however, seem to have neutral or beneficial effects on bone. Among the sodium-dependent glucose transporter 2 inhibitors, issues have been raised regarding the bone safety of canagliflozin.[322] There are several potential determinants of detrimental effect of sodium-dependent glucose transporter 2 inhibitors on bone, including hyponatremia-induced bone resorption.[323] In addition elevation in the serum levels of phosphate can stimulate the secretion of PTH and possibly FGF23, thereby leading to bone loss.[324] Experimental evidence indicates that incretins-based therapy (DDP4 inhibitors) and GLP1 receptor agonists (GLP1RA) have positive effects on bone. Osteopenia, increased osteoclast activity/numbers, and reduced bone strength are components of the bone phenotype in GLP1 receptor (GLP1R) knockout mice. Moreover, administration of GLP1RA reversed the bone loss in rats subjected to an experimental model of disuse osteoporosis.[325] Notwithstanding, there is limited clinical data about the bone effects of DPP4 inhibitors and GLP1RA; additional studies are needed to clarify the effects of the incretins-based therapy on fracture risk.

Factors That Impair Peak Bone Acquisition

Peak bone mass is acquired between the ages of 10 to 16 years. It is the zenith of bone acquisition and represents the sum of several processes including a marked increase in bone formation.[326] Boys tend to peak 2 years later than girls, and their BMD is higher than women at all skeletal sites. In part, this difference relates to a greater cross-sectional bone area in males than females.[326] Peak bone mass results from linear growth and consolidation of cortical and trabecular components. Acquisition is most rapid during the latter stages of puberty and coincides with maximum growth hormone secretion, high serum IGF1 levels, and rising levels of estradiol and testosterone. In addition, calcium absorption is maximum and skeletal accretion is optimal owing to higher levels of $1,25(OH)_2D$. These processes coalesce over a relatively short period to produce a bone mass that subsequently plateaus and then falls during later life. It is estimated that more than 60% of adult bone mass can be related to peak acquisition.[327] Hence, understanding the mechanisms responsible for low bone mass must include perturbations in peak bone acquisition.

There are several hormonal, environmental, and heritable determinants of peak bone mass, including estrogen/testosterone, growth hormone/IGF1, calcium/vitamin D, and unknown genetic factors. If any is perturbed, dramatic alterations in peak bone mass may occur, setting the stage for low bone density throughout life. Not surprisingly, gonadal steroids are essential not only to bone maintenance but also to acquisition. During puberty, estrogen and testosterone levels rise and contribute to consolidation of bone mass. Estrogen is also necessary for epiphyseal closure. Studies of males with an estrogen receptor mutation and men with an aromatase deficiency have established that estradiol is critical for

bone acquisition.[199,271,272] These young men share several phenotypic characteristics, including tall stature, unfused epiphysis, and very low bone mass. Hence, there must be a threshold effect for estradiol in men, and this effect must be time dependent. Similar conclusions can be drawn from studies in women. Acquired deficiencies in estrogen, such as occur with anorexia nervosa or chemotherapy-induced ovarian dysfunction, result in low peak bone mass and lead to subsequent risk for osteoporosis.[328,329] Similar findings have been noted in patients with untreated Turner syndrome and in men with Klinefelter syndrome.[330,331]

The timing of gonadal steroid surges is critical for bone acquisition because there is a relatively short window of time in which bone formation is favored and matrix synthesis is markedly enhanced.[332] That time window is likely to be less than 3 years and earlier in girls than boys. Probably the best study that addressed this issue comes from a retrospective analysis of men in their 30s who underwent late onset of puberty (i.e., at the age of 17 or 18 years) but were otherwise normal by full endocrine testing.[333] These men had significantly lower BMD than age-matched men who went through puberty at the normal time. These data suggest that both timing and quantity of gonadal steroids are critical for bone acquisition.

Pubertal surges of estrogen and androgens are also important for priming the growth hormone/IGF1 axis. Rising levels of both contribute to growth hormone surges that lead to increases in circulating and tissue expression of IGF1, an essential growth factor for chondrocyte hypertrophy and expansion. IGF1 may also be critical in defining the cross-sectional size of bone, a potentially important determinant of bone strength.[334] Once again, studies in individuals who are growth hormone deficient or resistant have established that low levels of circulating IGF1, especially during puberty, are associated with reduced bone mass.[170,335] In addition, recombinant human growth hormone replacement has been shown to restore linear growth and improve peak bone mass acquisition. Several studies in experimental animals, including inbred strains of mice, have established that IGF1 is important for bone acquisition and that the timing of IGF1 peaks coincides with maximal rates of bone formation.[336] Impairment in production of IGF1 due to acquired disorders (e.g., anorexia nervosa), malnutrition, delayed puberty, or diabetes mellitus can also impede peak bone acquisition.[337]

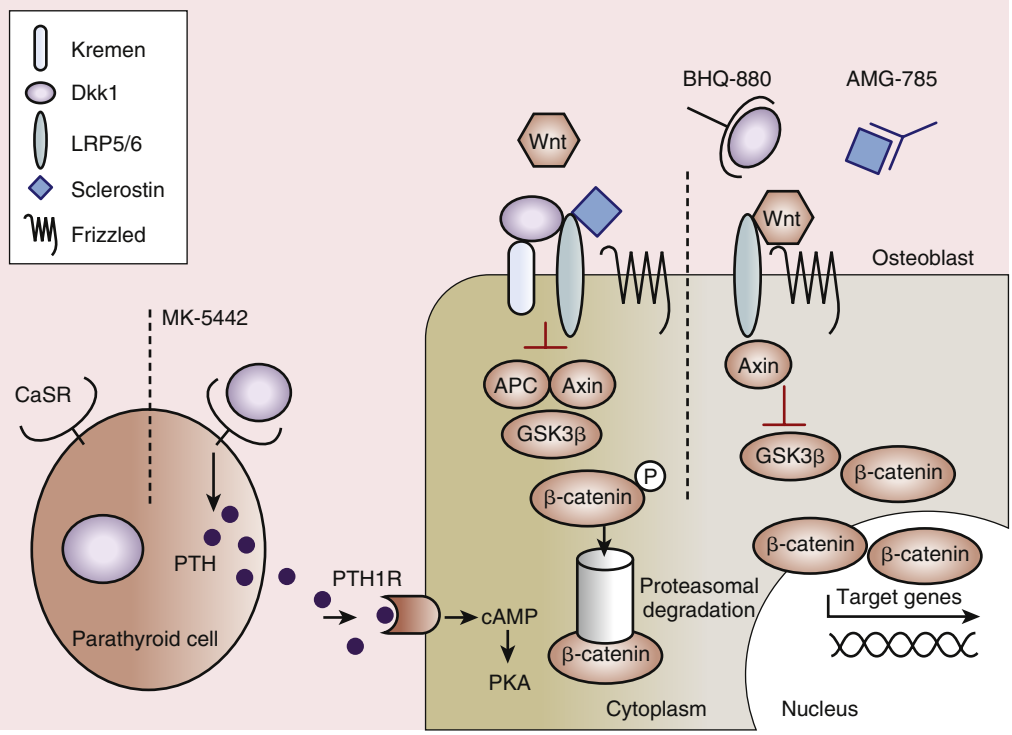
Hormonal abnormalities not only enhance bone resorption in older individuals but also may blunt the capacity of bone cells to maximize bone formation during adolescence. Clearly, hypogonadal boys and girls have impaired peak bone mass, resulting in low adult BMD.[330,331] One form of contraception, medroxyprogesterone, may reduce estrogen concentrations enough in the teenage girl to reduce her capacity to acquire peak bone mass.[338] Similarly, it seems likely, although not yet proved, that smoking during the teen years could impair osteoblast activity and flatten projected trajectories for peak bone acquisition.

To mineralize newly synthesized bone, calcium must become bioavailable to the skeletal matrix. In experimental studies in rodents and humans, it is clear that the several pools of available calcium are markedly enhanced during puberty. These sources include calcium efflux from the gastrointestinal tract and the calcium pool available for incorporation in the matrix. It is no coincidence that growth hormone surges not only increase IGF1 (thereby enhancing skeletal growth and matrix biosynthesis) but also result in increases in $1,25(OH)_2D$ (possibly via IGF1 induction of 1α-hydroxylase activity), the active metabolite of vitamin D, which markedly enhances calcium absorption from the gut.[339–341] Although there are no longitudinal studies in pubertal individuals with prolonged calcium deficiency, several randomized, placebo-controlled trials (RPCTs) in pubertal and prepubertal girls and boys have established that supplemental calcium can enhance BMD.[342,343] In a twin study in which one twin received calcium supplementation and one received placebo, radial BMD increased by as much as 5% after 3 years in the twins given calcium when compared with those given placebo.[343] This study suggests that there is significant gene-environmental interaction, and that even in those individuals with heritable determinants of low peak bone mass, calcium supplementation may provide an important and relatively simple means of protecting individuals from future osteoporotic fractures.

Genetic Factors That Determine Peak Bone Mass

Probably the most important determinant of peak bone mass, albeit one that has lacked clear definition, is the genetic contribution. As noted earlier, low peak bone mass may be the most important pathogenic factor in the osteoporosis syndrome of later life. Further, it appears that at least 50% of peak bone mass is determined by genetic factors.[344–346] Efforts to define heritable determinants of peak bone mass have been plagued by several issues that are also common to other complex diseases. They include the following: (1) a quantifiable phenotype, (2) heterogeneity within a given population under study, and (3) the polygenic nature of the disorder. In the postgenomic era, it has become clear that complex diseases, such as osteoporosis, cannot be settled by single nucleotide polymorphisms (SNPs). Instead, they are originated by a confluence of multiple genetic variations. Naturally, the interest has been driven to assess epistasis to unveil the complex network involved within multiple SNP interactions, as well as the influence of environmental factor into gene expression. Notwithstanding these barriers, it is now clear that BMD is an acceptable phenotype for defining heritable determinants. In addition, BMD is fully quantifiable and therefore is amenable to complex trait analysis. Moreover, BMD in the population is distributed in a gaussian manner, thereby allowing analyses at the extremes (<–2.0 SD or >2.0 SD) of the density distribution. Large homogeneous and heterogeneous populations are now being studied to ascertain genetic determinants of bone density in humans. Candidate genes identified by whole genome studies include RANKL, OPG, the VDR, collagen IA1, the estrogen receptor IL1, IGF1, and others. Depending on the cohort, the phenotype, and the number of individuals studied, there are likely to be hundreds of genes that contribute to individual variation in bone mass.[347] Indeed, in most large genome-wide association studies using validation cohorts to confirm candidate genes, the effect size of most noncoding SNPs is at best 1%. Notwithstanding, there has been a major effort to consolidate studies from around the world to increase power and detect rare variants that might provide even greater insight into genetic determinants, and importantly shed greater light on the biology of low bone mass. Twin studies examining discordant or concordant phenotypes are also helpful, as are sibling-pair studies, although the results have been disappointing.[76,347,348]

Originally, Johnson and colleagues[349] identified an extended family with very high bone density and fine mapped the locus to a region in chromosome 11. After several years of intense high-throughput analysis, the high-bone-density gene that was mutated in this family was identified as *LRP5*.[350] This member of the lipoprotein receptor family is important for binding Wnts, ligands critical for cell differentiation in several organisms. One year earlier, Gong and colleagues[351] identified mutations in the *LRP5* gene in several children with osteoporosis-pseudoglioma syndrome. The potential pathways that direct osteoblast function and



• **Fig. 30.23** Osteoblast physiology and potential therapeutic targets. The calcium-sensing receptor is antagonized by MK-5442 and triggers short bursts of PTH secretion. Binding of PTH to its receptor enhances osteoblast functions and bone formation. Presence of Wnt antagonists Dkk1 and sclerostin inhibits Wnt signaling. Dkk1 needs to form a complex with Kremen to bind LRP5/6, whereas sclerostin binds LRP5/6 directly. BHQ-880 and AMG-785 are antibodies for Dkk1 and sclerostin, respectively. After neutralizing Dkk1 and sclerostin, Wnt can bind to LRP5/6, which results in degradation GSK3β. As a consequence, β-catenin is stabilized, accumulates, and translocates into the nucleus, where it regulates transcription of osteoblastic genes. *APC,* adenomatosis polyposis coli; *cAMP,* cyclic adenosine monophosphate; *CaSR,* calcium-sensing receptor; *Dkk1,* dickkopf 1; *GSK,* glycogen synthase kinase 3; *LRP,* lipoprotein receptor–related protein; *PKA,* protein kinase A; *PTH,* parathyroid hormone; *PTH1R,* PTH 1 receptor. (From Rachner TD, Khosla S, Hofbauer LC. Osteoporosis: now and the future. *Lancet.* 2011;377[9773]:1276–1287.)

mineralization through LRP5, a coreceptor for the Wnt receptor, frizzled, have opened up new areas of investigation (Fig. 30.23). Moreover, natural antagonists to the Wnt/LRP5 signaling system, including sclerostin and Dkk1, have been studied using genetic engineering in mice. In the past 5 years, LRP5 has been studied extensively both in its function and its allelic effects through genome-wide association studies, as well as in translational bench work. Importantly, LRP5 polymorphisms have been strongly associated with BMD and fractures in large cohort studies, consistent with the central role of LRP5 in human skeletal biology. Hence, this pathway must be important in defining peak bone mass. However, it is also clear that because BMD is a polygenic trait, other genes are soon to be discovered.

In addition to the search for osteoporosis genes, intervention studies in adolescents have provided insight into the environmental impact on genetic determinants.[347] In another twin study from the Indiana group, the investigators found that as long as calcium supplementation continued during puberty, young boys could enhance their peak bone mass.[352] In a Swiss study, younger prepubertal girls supplemented with a protein product had a significant increase in spine bone density, as did a cohort of pubertal girls receiving a milk powder in England.[353] Remarkably, in the latter cohort, serum IGF1 levels also rose dramatically, providing further indirect evidence of a link between pubertal status, bone mass, and the growth hormone/IGF1 axis.[353]

Approach to Management of Osteoporosis

A comprehensive management plan for osteoporosis includes diagnosing those at highest risk, excluding secondary causes of low BMD, and selecting appropriate treatment. Screening by DXA to assess BMD at the hip and spine has been commonly recommended in most women older than 65 years and men older than 70 years,[354,355] and many, but not all, have this assessment. In addition, there is a growing appreciation that occurrence of a fracture, particularly one due to minimal trauma, should be a signal for more comprehensive risk assessment including BMD and has led to the explosion in development of fracture liaison services over the past 5 years and the emphasis on their importance by many groups.[263,354,356]

With increasing recognition of the importance of risk factors in addition to BMD, an assessment of risk should include both BMD and risk factors. These factors can be easily combined in the FRAX algorithm to attain an overall risk of fracture, which may be helpful, in addition to BMD alone, in making clinical decisions. FRAX scores are available online and have now been implemented within the software for most DXA scanners so that primary care physicians can easily obtain relevant diagnostic information in one report.

One commonly used algorithm in the United States is within the guidelines developed by the National Osteoporosis Foundation[354] (see Table 30.3), which strongly emphasize that patients

with a BMD T-score below –2.5 or a history of hip or spine fracture be considered for treatment. In these guidelines, FRAX is used for patients with BMD that is low but not in the osteoporotic range. In other countries, treatment decisions are based solely on risk, but consideration of treatment of those with BMD T-scores below –2.5, regardless of other risk, is well justified by many clinical trial results.[357–359]

Clinical decision making regarding pharmacologic therapy should consider several caveats. First, osteoporosis therapy can reduce fracture risk by as much as 50%, but this means that some people will continue to have fractures despite treatment. Second, lifestyle and pharmacologic interventions may be long-term commitments, and therefore cost, compliance, and safety must be factored into therapeutic decisions. Studies suggest that with weekly or monthly oral bisphosphonate therapy, more than 40% of individuals treated will not continue therapy beyond 1 year. Third, it is not uncommon for women with T-scores higher than –2.5 to have fragility fractures and therefore have osteoporosis by the consensus conference definition mentioned earlier.[198] In fact, in the National Osteoporosis Risk Assessment cohort of more than 140,000 postmenopausal women in the United States, almost one third of the women who had fractures also had BMD scores in the low BMD (T-score between –1 and –2.5) range.

General Measures

Diet

Calcium

Calcium supplementation should be an adjunct to drug treatments for women with established osteoporosis and must be part of any prevention strategy to ameliorate bone loss. Increased calcium intake reduces the secondary hyperparathyroidism often seen with advancing age and can enhance mineralization of newly formed bone. Evidence that calcium and vitamin D together or individually reduce fracture risk in the osteoporotic individual remains somewhat controversial. However, a recent meta-analysis of calcium and vitamin D intervention trials demonstrated a consistent, albeit small, increase in BMD and a reduction in nonvertebral fractures when 1200 mg of calcium is combined with more than 800 units of vitamin D.[360] Calcium supplementation alone has not been shown to reduce the incidence of nonvertebral fractures in high-risk women. Recently, a very large calcium intervention trial from the Women's Health Initiative (WHI) did not demonstrate hip fracture reduction with daily calcium supplements and 400 IU of vitamin D for all postmenopausal women, but for those older than 60 years, the risk reduction was statistically significant.[361] Interestingly, calcium supplementation in this cohort was associated with a 17% greater risk of kidney stones. Currently, an average total calcium intake of 1200 mg/day is still recommended by the Institute of Medicine (IOM) for all postmenopausal women. With intakes greater than 2000 mg/day, the risk of nephrocalcinosis certainly increases.

Vitamin D

Vitamin D is essential for skeletal maintenance and for enhancement of calcium absorption. Insufficiency of this vitamin is a growing problem; as many as two thirds of all patients who have hip fractures are classified as vitamin D deficient. Elderly people in chronic care living situations are particularly vulnerable. One large RPCT demonstrated a 43% reduction in hip fractures for nursing home residents, 84 years of age, who received calcium and vitamin D compared with those receiving placebo.[362] However, those subjects had significantly suppressed 25(OH)D levels and hence were likely to also have osteomalacia. In a large population-based study with calcium and vitamin D, supplementation had no effect on nonvertebral fractures,[361] although compliance and assessment of vitamin D levels were not sufficiently well documented to exclude an effect. At least one meta-analysis suggests that 800 IU/day of vitamin D plus 1200 mg of calcium per day is needed to reduce hip fractures by about 10%.[360] The IOM recommends an average intake of 600 IU/day, except for men and women older than 70 years, for which the IOM recommends 800 IU of vitamin D per day. The tolerable upper limit of vitamin D supplementation noted by the IOM was 4000 IU/day.

It should be noted that the US Preventive Services Task Force meta-analysis failed to show an effect of calcium and vitamin D on fracture risk.[363] And three systematic reviews showed that vitamin D alone did not affect BMD.[364–366] Thus, if there is an effect of vitamin D on bone mass and fracture, it probably is limited to older individuals who are at high risk and have low calcium intake. Besides the potentially positive effects of vitamin D supplementation on the skeleton, particularly in older women, vitamin D may reduce the risk of falling, although there continues to be significant controversy over the effect size, as noted in a recent meta-analysis. Moreover, it is uncertain whether the VDR expression level in adult muscle tissue is sufficient to mediate any direct effects. Therefore, for most individuals with osteoporosis, 800 IU/day of vitamin D is sufficient to maintain adequate levels of 25-hydroxyvitamin D. However, in those patients with low bone mass and insufficient or deficient 25-hydroxyvitamin D levels (i.e., <20 ng/mL), administration of 50,000 IU of ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3) given once weekly is a safe and effective way to restore vitamin D levels to the normal range. Moreover, high-dose bolus vitamin D supplementation to prevent fractures, falls, and or/cardiovascular disease should be avoided due to inefficacy (100,000 IU of cholecalciferol monthly),[367,368] or even an increased risk of falls and fractures (annual dose of 500,000 IU of cholecalciferol administered orally).[369] Upper levels of vitamin D are currently being reviewed to determine if there is toxicity at higher doses. The current upper limit of tolerability is set at 4000 IU per day. The US Preventive Services Task Force did not recommend screening of normal individuals for adequacy of vitamin D levels; however, among osteoporotic patients, measurement of at least one serum 25(OH)D level is considered to be standard of care, particularly for those individuals starting bisphosphonate therapy.

Vitamin D analogues have been used in the treatment of osteoporosis since the early 1980s. However, this remains a controversial area. High doses of $1,25(OH)_2D$ increase bone mass, but many patients develop hypercalciuria or hypercalcemia. At doses of 0.5 μg/day, $1,25(OH)_2D$ reduced the rate of both vertebral and nonvertebral fractures and increased bone density, but this finding was made in a very small randomized trial. Other studies have found little benefit with a narrow therapeutic window, particularly in relation to renal function and hypercalcemia. Currently, vitamin D analogues are not recommended for the routine treatment of osteoporosis. A subset of patients with renal insufficiency (chronic kidney disease ≥3 and high PTH levels) may benefit from supplementation with calcitriol, with very careful monitoring of serum PTH.

Physical Activity

Bed rest or immobility, particularly in elderly persons, can result in rapid bone loss. Moreover, the number of falls increases with age, and the number of falls that result in fractures also rises. A meta-analysis by the Cochrane Review Group demonstrated that

muscle strengthening, balance retraining, home hazard assessment, withdrawal of psychotropic medications, and use of a multidisciplinary risk factor assessment program are beneficial in protecting against falls.[370] An alternative approach is to reduce loads applied to the hip during a fall by padding. Hip protectors have been shown to reduce the risk of hip fractures in at least one population, although compliance is generally poor. A more recent study failed to demonstrate the efficacy of these devices in older women in an assisted living facility.[371] Regular physical activity, including aerobic, weight-bearing, and resistant exercises, is effective in increasing spine BMD and in strengthening muscle mass in postmenopausal women, but no large-scale studies have established whether these interventions reduce fracture risk.

Lifestyle

Other interventions, such as smoking cessation and reduction of alcohol intake, should be considered within the framework of an individual's preventive health strategy. However, studies to date have been inconclusive with respect to understanding how changes in these lifestyles affect overall fracture risk. Notwithstanding, some promising data support the use of Tai Chi to enhance balance and reduce falls and fractures.[370] The data on cigarette smoking and fractures are somewhat conflicted, although most investigators support the notion that smoking may impair peak bone acquisition in adolescence and could contribute to postmenopausal bone loss directly or via the development of chronic obstructive lung disease with hypoxia and hypercarbia.[372] Lifestyle also includes tools to manage activities of daily life with a particular focus on avoiding falls and eliminating barriers to ambulation.

Pharmacologic Approaches to the Treatment of Osteoporosis

Abundant evidence indicates that an aggressive intervention program can be successful in reducing fracture risk and in improving quality of life among postmenopausal women with preexisting osteoporosis. Several pharmacologic options are available, and they can be classified by their mechanism of action. The two major classes of osteoporosis drugs are (1) *antiresorptives* (i.e., agents that block bone resorption by inhibiting osteoclasts) and (2) *anabolics* (i.e., drugs that stimulate bone formation by primarily acting on osteoblasts).

Antiresorptive Agents

Antiresorptives inhibit bone resorption by suppressing osteoclast activity. Slowing the remodeling cycle allows bone formation to catch up to resorption, thereby enhancing matrix mineralization and stabilizing trabecular microarchitecture. The antiresorptives increase BMD and reduce fracture risk, but their efficacy varies.

Estrogen

Estrogen replacement therapy was long considered the cornerstone of therapy for postmenopausal women with osteoporosis. Studies in vitro and in vivo have supported the hypothesis that this hormone works by slowing bone resorption through inhibition of cytokine signaling from the osteoblast to the osteoclast, thereby increasing BMD. However, there is also compelling evidence from at least two groups that osteoclasts have estrogen receptors and that estrogen blocks apoptosis of osteoclasts.[373,374]

Estrogen treatment inhibits both cortical and trabecular bone loss, and BMD generally increases by 3% to 5% after 3 years.[375] There does not appear to be an additive effect from progesterone on bone mass in women also receiving estrogen. Conversely, progesterone is a necessary part of hormone replacement therapy in women with a uterus because it prevents the development of endometrial hyperplasia and carcinoma. In the WHI, estrogen and progesterone lowered hip fracture risk by one third.[268] Low-dose conjugated estrogens (0.3 or 0.45 mg/day) and ultralow-dose estradiol increase BMD and have been approved for the prevention of bone loss, but antifracture efficacy for these preparations has not been established. Discontinuation of estrogen results in measurable bone loss (3–5% in the first year), although controversy exists as to whether that translates into a greater fracture risk.

Significant concern has been noted about the nonskeletal risks associated with long-term estrogen and estrogen in combination with progesterone. Particularly troublesome is the increased risk of breast cancer with the long-term use of estrogen and progesterone. In the WHI, there was a 26% increase in risk of invasive breast cancer over a 5.2-year period of follow-up.[268] Hence, estrogen replacement is contraindicated in any woman with a history of breast cancer; yearly mammograms are indicated in all women receiving hormone replacement therapy. Previous case-control and retrospective studies suggested that estrogen could reduce the risk of coronary artery disease; however, in the WHI, the risk of myocardial infarction or death from coronary artery disease was 29% higher in women receiving combination therapy.[268] Thromboembolic disease is also increased more than threefold by hormone replacement therapy.[268] Hence, the use of estrogen or estrogen in combination with progestins for the prevention and treatment of osteoporosis has fallen dramatically. Moreover, the availability of newer and effective antiresorptive drugs for the treatment of osteoporosis has lessened enthusiasm for primary hormonal therapy in osteoporotic women.

Selective Estrogen Receptor Modulators

Selective estrogen receptor modulators, such as tamoxifen and raloxifene, also inhibit bone resorption by the same mechanisms used by estradiol. Both have been shown to reduce bone loss in postmenopausal women with breast cancer, but only raloxifene is approved by the FDA for the prevention and treatment of osteoporosis.

Both of these agents block the actions of estrogen on the breast but act like an estrogen agonist in bone; tamoxifen, but not raloxifene, has estrogen agonistic properties on the uterus and is associated with a greater risk of endometrial carcinoma with long-term use.[376] Both agents have been associated with a reduction in new cases of breast cancer when they are administered as prophylaxis for high-risk patients.[377] Low-density lipoprotein cholesterol levels are also reduced in patients receiving these selective estrogen receptor modulators. Raloxifene increases spine BMD slightly (as does tamoxifen) and lowers the risk of vertebral fracture by 40%, although it has no effect on nonvertebral fracture risk.[378] Hot flashes, leg cramps, and a greater risk of deep venous thrombosis can occur with raloxifene therapy. The recommended dose of raloxifene is 60 mg once daily.

Tissue selectivity with these selective estrogen receptor modulators and others being investigated is a subject of great scientific interest. Raloxifene and estradiol both bind to the same region of the estrogen receptor, but they induce different conformational changes in that receptor. Differing coactivating and corepressing proteins are recruited to these receptor-ligand complexes, and it is thought that these coactivators and corepressors ultimately determine the activity of the nuclear complexes. Because recruitment also depends on cell type, it is highly likely that significant tissue selectivity exists for these partners. Newer agents have been designed to facilitate particular complexes and rearrangements within the nucleus; they are being studied at both preclinical and clinical levels.

Bisphosphonates

Bisphosphonates are the most widely prescribed antiresorptives and are often considered first-line therapy for the treatment of severe postmenopausal osteoporosis. These drugs are carbon-substituted analogues of pyrophosphate that bind tightly to hydroxyapatite crystals. It is thought that these agents directly suppress resorption by inhibiting osteoclast attachment and enhancing programmed cell death. The first-generation bisphosphonates include etidronate and clodronate. Neither is approved for the treatment of osteoporosis in the United States, although etidronate is used off label and in Europe. The dose of etidronate is 400 mg/day for 2 weeks every 3 months. The drug has few gastrointestinal side effects, and vertebral fracture risk reduction is significant with this agent. Alendronate and risedronate, two second-generation nitrogen-containing bisphosphonates, are effective in suppressing bone resorption and increasing BMD. In RPCTs of postmenopausal women with established osteoporosis, alendronate and risedronate reduced vertebral, hip, and nonvertebral fractures by nearly 50%, particularly during the first year of treatment.[219,379] As with other antiresorptive drugs, increases in BMD with alendronate or risedronate account for a small fraction of their antifracture efficacy. Hence, follow-up DXA measurements may significantly underestimate fracture risk reduction. Recent clinical trials have shown that these drugs can be safely administered for at least 5 years without adversely affecting bone strength. Moreover, discontinuation of alendronate after 5 years results in minimal bone loss over the ensuing 5 years without a significant increase in fracture risk.[380] Both drugs have excellent safety profiles, although erosive esophagitis is a serious complication of all oral nitrogen-containing bisphosphonates. Once-weekly administration of alendronate has been shown to reduce the prevalence of drug-induced esophagitis, and currently both alendronate and risedronate are marketed as once-weekly treatments.

Two other bisphosphonates have been approved by the FDA and have reached the market since 2007: ibandronate and zoledronate. The former is given orally in a single monthly dose (150 mg) or intravenously every 3 months (3 mg).[381,382] Ibandronate suppresses bone resorption and reduces the rate of spine fractures by nearly 50%,[358,383] but its efficacy in nonspine fractures is somewhat less than that of alendronate or risedronate. Compliance with the once-monthly regimen is higher than with the weekly dosing, although long-term data are not encouraging that this effect persists. First-dose hypersensitivity can occur with ibandronate, and because it is a nitrogen-containing bisphosphonate, it is also associated with esophageal reflux. Zoledronate is also approved for the prevention and treatment of osteoporosis. It is administered as a single intravenous infusion over 15 minutes (5 mg) once yearly. Large randomized controlled trials have unequivocally established antifracture efficacy for hip, spine, and other nonspine fractures.[384] Recently, the FDA approved the use of zoledronate for prevention of osteoporosis by administration of the drug once every 2 years. Both newer bisphosphonates can cause side effects with the first dose, including joint pain, stiffness, and low-grade fevers. Generally, these do not persist with recurrent administration of the drug. However, the FDA has cautioned that zoledronic acid should be administered over an hour rather than over 15 minutes to lessen any risk, albeit small, of renal damage. In addition, it should not be used in patients with a reduced glomerular filtration rate (<30 mL/minute) and should be used with caution in the elderly. Intravenous zoledronate has been approved for the treatment of malignant hypercalcemia, multiple myeloma, and skeletal metastases.

Other bisphosphonates are available for off-label use or are being studied for the treatment of osteoporosis. Intravenous pamidronate has been available since the mid-1990s for the treatment of Paget disease and malignant hypercalcemia. It is currently also used to treat osteoporotic women who cannot tolerate oral bisphosphonates, although it has not been formally approved by the FDA, and its antifracture efficacy has not been established. The dose ranges from 30 to 90 mg given every 3 to 9 months. Acute and delayed-type hypersensitivity reactions can occur with this drug, and its use is contraindicated in patients who are vitamin D deficient because it can precipitously drop serum calcium—a concern that also applies to use of zoledronic acid and denosumab.

With widespread use of bisphosphonates for both prevention and treatment, two uncommon but serious adverse events have been associated with administration of this class of agents: subtrochanteric, or atypical, femur fracture and osteonecrosis of the jaw (ONJ).[385,386] With respect to the former, atypical femur fractures were found in some but not all studies to be associated with duration of bisphosphonate use and generally were noted in younger individuals relative to those affected by other types of hip fractures. Prodromal symptoms of hip or thigh pain and associated cortical thickening or beaking in the shaft of the proximal femur are risk indicators of these fractures, which with minimal trauma can have devastating consequences in terms of quality of life and mobility. Some guidelines recommend prophylactic rod placement to prevent fractures in high-risk individuals in the ipsilateral and contralateral femora. Currently, the prevalence of this fracture is not well established, although meta-analyses suggest that there is a causal association with bisphosphonate use.

The prevalence of ONJ is very low when bisphosphonates are used in the doses used to treat osteoporosis. When much higher doses are used to prevent the skeletal complications of cancer, ONJ is a substantial concern. However, in osteoporosis patients, the prevalence is estimated to be less than 1 in 100,000 patients exposed to oral or intravenous bisphosphonates who are otherwise healthy. ONJ has devastating effects on the mandible and can require prolonged antibiotic treatment and local oral care. Patients with dental procedures that invade bone, such as tooth implantation and tooth extraction, are at increased risk. Concomitant treatment with glucocorticoids likely enhances the risk, and infection often accompanies the necrosis.

Calcitonin

Calcitonin is a 32–amino acid peptide normally produced by the thyroid C cells. Osteoclasts have calcitonin receptors, and calcitonin can rapidly inhibit bone resorption. Salmon calcitonin is more potent than human and is the preferred treatment choice. Nasal and subcutaneous calcitonin are both approved for the treatment of postmenopausal osteoporosis. However, the evidence favoring a strong effect from this hormone on either bone loss or fracture efficacy is lacking. In an RPCT of women with postmenopausal osteoporosis, 200 IU/day of nasal calcitonin reduced vertebral fracture incidence by one third.[387] However, methodologic flaws in that trial have limited enthusiasm for this agent as a primary treatment for osteoporosis. In at least one placebo-controlled study, nasal calcitonin reduced the pain associated with new spine fractures. The recommended dose of nasal calcitonin is 200 IU/day, and that of subcutaneous calcitonin is 100 IU/day. Side effects are uncommon with intranasal calcitonin and include nasal stuffiness and flushing. With subcutaneous administration, nausea is not infrequent.

Strontium Ranelate

Strontium ranelate is orally administered and stimulates calcium uptake in bone while it inhibits bone resorption. It is thought to have some anabolic activity, although the precise mechanism

of action in the skeleton, where it is incorporated, is not known. In an RPCT of postmenopausal women with established disease, daily strontium reduced the risk of vertebral fractures by 40%.[388] However, a statistically significant effect on nonvertebral fractures was limited to a small subset of women in a post hoc analysis.[389] Recent data raised the safety issue of an increase in cardiovascular events among strontium users.[390] This drug was originally approved by European regulatory agencies but not by the FDA.

Strontium ranelate has been associated with increased risk of severe allergic skin reactions, venous thromboembolism, stroke, and heart ischemia. The European Medicine Agency has decided to restrict the use of strontium ranelate only to patients with severe osteoporosis who cannot be treated with other drugs approved for osteoporosis. The agency has also warned about the necessity of regular medical care and advised the discontinuation of therapy in those patients who develop heart or circulatory problems, including high blood pressure or angina. As expected, it has maintained the recommendation against using strontium ranelate in individuals with antecedent of cardiovascular disorders.

Denosumab

Denosumab is a fully human monoclonal antibody to the RANKL, the essential osteoclast-differentiating factor. The antibody inhibits osteoclast formation, decreases bone resorption, increases BMD, and reduces the risk of fracture. As noted previously, RANKL is a member of the TNF superfamily of ligands that is essential for the function of bone-resorbing osteoclasts. RANKL interacts with its receptor (RANK) on both osteoclast precursors and mature osteoclasts, and the RANKL-RANK interaction results in activation, migration, differentiation, and fusion of hematopoietic cells of the osteoclast lineage to begin the process of bone resorption. Denosumab blocks that activation by binding directly to RANKL. Unlike bisphosphonates, denosumab does not persist in the skeleton and hence needs to be administered once every 6 months to maintain its efficacy. In fact, discontinuation of denosumab can lead to a rebound increase in bone resorption but no increase in fractures.[391]

Clinical trials with denosumab, 60 mg once every 6 months, included the very large (>7000 postmenopausal women with low bone mass and fractures) FREEDOM (Fracture Reduction Evaluation of Denosumab in Osteoporosis Every 6 Months) trial. This trial, which was used for registration of the agent with the FDA, demonstrated that after 3 years, denosumab improved BMD of the spine and hip compared with placebo (9.2% vs. 0% and 4.0% vs. −2.0%, respectively).[80] In addition, biochemical markers of bone turnover were significantly reduced in patients taking denosumab. Importantly, fractures of spine were reduced by 70%, and nonvertebral fractures, including hip fractures, were also significantly reduced. The long-term extension of the FREEDOM trial revealed that at 6 years, BMD continued to improve and fracture risk remained low.[392] In males undergoing androgen deprivation therapy, denosumab reduced bone loss and prevented fractures. Denosumab was the first biologic approved by the FDA for the treatment of osteoporosis in both men and women. Surprisingly, long-term studies (in 2015, out to 8 years in the extension trial) have failed to show significant adverse events for this agent, although atypical femoral fractures occur.

Cathepsin K Inhibitors

Cathepsin K is a proteinase that is secreted by osteoclasts and results in bone degradation, primarily of type I collagen (see Fig. 30.4). In addition, it is also produced by cancer cells that metastasize to bone. Odanacatib, one cathepsin K inhibitor, has been tested in postmenopausal women with osteoporosis and is shown to be an effective suppressor of bone resorption. In addition, women receiving this agent in a large phase III registration trial showed significant increases in BMD of the spine and hip, as well as a reduction in spine and nonvertebral fractures, prompting premature discontinuation of the trial due to enhanced efficacy.[393] However, the safety profile for odanacatib is still not clearly defined, and the FDA has not yet approved this agent for use. Interestingly, women treated with odanacatib have suppressed bone resorption but no change or a slight increase in bone formation. This may be due to the finding that this agent blocks the breakdown of collagen but does not kill osteoclasts; hence, signals from osteoclasts to osteoblasts may be maintained, thereby preserving bone formation. If correct, this is one of the first drugs for osteoporosis that can uncouple remodeling in a positive manner. A correlative study examining the effect of odanacatib in the setting of bony metastases from breast cancer demonstrated that it suppressed a biochemical marker of bone resorption, N-terminal telopeptide, in much the way that zoledronic acid did.[394] In 2015, Merck released promising bone results from the LOFT (Long-Term Odanacatib Fracture Trial) study.[395] In comparison to individuals receiving placebo, patients treated with odanacatib showed the following reductions in relative risk rates: (1) 54% of new and worsening morphometric vertebral fractures, (2) 47% of clinical hip fractures, (3) 23% of clinical nonvertebral fractures, and (4) 72% of clinical vertebral fractures (all $p < 0.001$). In addition, the company recognized that adjudicated events of morphea-like skin lesions and atypical femoral fractures were a more frequent occurrence in the odanacatib group than in the placebo group.[396] Moreover, the number of adjudicated stroke events was more common in the odanacatib than in the control group. The sponsor, Merck, decided to remove odanacatib from consideration by the FDA.

Anabolic Agents

A new class of antiosteoporosis drugs was introduced in 2002. These so-called anabolic agents stimulate bone formation more than bone resorption. As such, these agents can enhance bone remodeling and contrast sharply with the antiresorptives, which slow bone turnover. PTH(1-34) (teriparatide) was the first of this class of drugs to be approved by the FDA. Previously, the prototypical anabolic drug was sodium fluoride, which saw widespread use in the 1970s and 1980s because of its ability to stimulate new bone formation. However, an RPCT in 1990 established that although there were dramatic increases in BMD, nonvertebral fracture risk actually increased.

Parathyroid Hormone

It has been known for several decades that PTH, when given intermittently, increases bone formation and bone mass in mammals.[397] The increase in bone turnover begins with a dramatic increase in bone formation that is followed later by a marked rise in bone resorption, maintaining the coupling in the multicellular unit. However, chronically high levels of PTH cause bone loss in the cortical compartment as noted for primary and secondary hyperparathyroidism.[398] The development of PTH as an anabolic agent centered on defining the intermittent nature of administration, which for teriparatide (human PTH[1-34]) was by daily subcutaneous injections. Teriparatide has been approved in virtually every country for the treatment of postmenopausal osteoporosis because it not only increases bone mass but also reduces fractures. In the largest RPCT using teriparatide in postmenopausal women

with severe osteoporosis, 20 μg/day of PTH, administered subcutaneously, reduced spinal and nonvertebral fractures by more than 50% while it substantially increased (i.e., 8%/year) lumbar BMD.[399] Similar findings were noted in men with osteoporosis who were treated for 11 months.[400] Unfortunately, the PTH trial in postmenopausal women was stopped after 20 months because of concerns related to the development of osteosarcoma in rats treated with high doses of PTH(1-34). However, retrospective studies have found no association between osteosarcoma and primary or secondary hyperparathyroidism, and only one case of osteosarcoma in PTH-treated patients has been reported from more than a million users. More recently, recombinant human PTH(1-84) has shown similar benefits and is approved for the treatment of hypoparathyroidism in the United States.[401] In Europe and Asia, but not in the United States, PTH(1-84) is approved for the treatment of postmenopausal osteoporosis. Currently, it is recommended that PTH therapy be limited to those individuals with moderate to severe osteoporosis and then given for only 2 years, based on a long-term toxicity study in rats demonstrating an increased risk of osteosarcoma.

Despite the appeal of using an anabolic with an antiresorptive, most evidence indicates that combinations of classes of drugs are not additive or synergistic. PTH plus bisphosphonates initiated together do not raise BMD more than PTH alone in either men or women.[402,403] However, PTH plus denosumab has been shown to increase BMD in the spine to 13% after just 1 year, a greater increase than that seen with other drugs.[404] That difference is maintained even after 2 years of combination therapy. Unlike with discontinuation of bisphosphonates, discontinuation of PTH can result in bone loss of 3% to 4% in the first year after PTH cessation. This posttreatment effect is prevented by adding an antiresorptive agent once PTH is stopped. In general, PTH is well tolerated, although nausea, flushing, hypotension, and mild but asymptomatic hypercalcemia (i.e., serum calcium <11 mg/dL) can occur. Cost and compliance have been limiting factors.

PTH-Related Protein

Since the approval of teriparatide in 2002, it took 15 years for the launching of a new bone anabolic drug in the US market. PTH-related protein (PTHrP) has been shown to work via the PTH receptor on osteoblasts to stimulate new bone formation in a manner analogous to PTH. PTHrP acts primarily as a paracrine growth/differentiation factor but also functions as a hormone during normal lactation and in the hypercalcemia of malignancy. However, when administered intermittently, PTHrP(1-36), an analogue of native PTHrP, increases BMD without significant hypercalcemia, similar to PTH.[405] Phase III trials with PTHrP(1-36) have been completed in Europe and show a significant reduction in both vertebral and nonvertebral fractures (including hip fractures). In April 2017, the N-terminal fragment of PTH-related peptide analogue (abaloparatide) received approval from the FDA for subcutaneous application in postmenopausal women with osteoporosis at high risk for fracture. Abaloparatide is appropriately indicated for those postmenopausal women with history of osteoporotic fracture, those harboring multiple risk factors for fracture, or patients showing failure or intolerance to other therapy options. The ACTIVE (Abaloparatide Comparator Trial in Vertebral Endpoints) study was a phase III, double-blind, randomized controlled trial developed in 10 countries. In comparison to placebo, after 18 months of therapy, abaloparatide significantly increased BMD in the lumbar spine (11.20% vs. 0.63%), femoral neck (3.60% vs. −0.43%), and total hip (4.18% vs. −0.10%). More importantly, the number of new morphometric vertebral fractures was significantly lower in the abaloparatide group (0.58% [n = 4] vs. 4.22% [n = 30] in the placebo group). In addition, the ACTIVE study reported a similar 80% reduction in vertebral fracture rate with abaloparatide compared with teriparatide. However, abaloparatide was associated with a significant reduction in nonvertebral fractures when compared with placebo—an effect not observed in teriparatide therapy. The occurrence of hypercalcemia was lower in women using abaloparatide (3.4%) than teriparatide (6.1%).[406]

Future Anabolic Agents

There is a significant appeal for the use of anabolic agents for the treatment of low bone mass and fractures in severely affected individuals (see Fig. 30.22). Bone remodeling is a highly regulated activity encompassing bone resorption and formation that dictates bone mass maintenance. Skeletal anabolic drugs stimulate bone formation, enhancing the number and function of bone-forming cells. Advances in knowledge of bone biology have allowed the recognition of paracrine peptides that represent local modulators of the differentiation of progenitor cells into osteoblasts. Wnt signaling is a positive driver of osteoblast differentiation, which is cautiously regulated by both intra- and extracellular mechanisms. In parallel, Wnt signaling inhibits osteoclastogenesis. Osteocytes, the terminally differentiated osteoblasts, have an important role in maintaining a constitutive inhibition of the Wnt/β-catenin canonical signaling pathway by secreting sclerostin. Mechanical loading on bone and PTH therapy stimulates bone formation by downregulating sclerostin. Osteopetrosis (i.e., sclerosteosis and van Buchem disease) can emerge due to a loss of function mutation in the *SOST* gene, leading to high bone mass. Those studies encouraged the development of monoclonal humanized antibody to sclerostin.

Monoclonal Antibodies to Sclerostin

As noted previously, sclerostin is produced by osteocytes and inhibits bone formation by blocking canonical Wnt signaling. *Sost* null mice have increased bone formation and high bone mass. It follows, therefore, that inhibition of sclerostin should enhance osteoblast function and improve bone mass. In animal models and in a phase I trial in healthy adults, administration of a sclerostin monoclonal antibody does increase bone mass. Similarly, in a phase II trial in postmenopausal women, all doses of a monoclonal antisclerostin antibody (romosozumab) increased bone density at the lumbar spine, total hip, and femoral neck.[73] In this 1-year trial, 419 postmenopausal women with low bone mass (T-score between −2.0 and −3.5 at the lumbar spine, total hip, or femoral neck) were randomly assigned to subcutaneous romosozumab (variable dosing once monthly or once every 3 months), an active comparator, versus alendronate (70 mg weekly), or subcutaneous teriparatide (20 μg daily), or placebo injections. The greatest increase in BMD was seen in the group receiving romosozumab (210 mg monthly; 11.3% compared with 4.1% and 7.1% in the alendronate and teriparatide groups, respectively).[73] Interestingly, there was a transient increase in bone formation markers and a more sustained decrease in bone resorption markers, a pattern that has not been seen among available osteoporosis therapies, again suggesting that it is possible to uncouple remodeling toward a more favorable balance. The suppression in bone resorption is most likely a function of reduced RANKL production due to inhibition of the Wnt/LRP5/6 signaling pathway. QCT examinations detailed that the positive effects of romosozumab occurred in

trabecular bone of the vertebrae and total hip, as well as in the cortical compartment of the vertebrae.[407] In accordance to the previous results, finite element analysis showed improved vertebrae and total hip strength in postmenopausal women treated with romosozumab.[408] The reported data suggested that romosozumab has beneficial effects in both trabecular and cortical compartments of bone. The head-to-head comparison between romosozumab and other agents showed that romosozumab has better performance in several aspects. Romosozumab therapy induced greater increase in BMD than alendronate (70 mg weekly) and teriparatide (20 μg daily). The improvement in bone strength, as assessed by infinite element analysis, was higher in osteoporotic women receiving romosozumab than teriparatide. Moreover, romosozumab potentially seems to have greater capability to enhance bone mass and volume in patients previously receiving a bisphosphonate.[409]

Romosozumab entered in a phase III trial but is not yet approved by the FDA.[348] Actually, the efficacy of romosozumab to alleviate fracture occurrences has been tested in two clinical trials. In the first, romosozumab (210 mg subcutaneously monthly) was compared with placebo for 1 year, then both arms changed to open-label denosumab for a year (60 mg biannually). In the whole period of 24 months, in 75% of patients, romosozumab/denosumab reduced the incidence of new vertebral fractures and had a statistically insignificant reduction in nonvertebral fractures.[410] In the second clinical trial, the authors defined the following design: monthly subcutaneous romosozumab (210 mg) or weekly oral alendronate (70 mg) administered in a blinded fashion for 12 months, followed by open-label alendronate in both groups. The romosozumab/alendronate group showed a 48% and 19% reduction, respectively, in incident vertebral and nonvertebral fractures compared with the alendronate-only group.[411] Necrosis of the jaw and atypical fractures have rarely been reported with romosozumab. In addition, there are concerns about serious cardiovascular events. During year 1, positively adjudicated serious cardiovascular adverse events were observed more often with romosozumab than with alendronate (50 of 2040 patients [2.5%] vs. 38 of 2014 patients [1.9%]). Safety studies and completion of the phase III trial will determine the place of this agent in treating osteoporosis.

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The complete reference list is available online at ExpertConsult.com.

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31

Rickets and Osteomalacia

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CHAPTER OUTLINE

KEY POINTS

- Rickets is a specific bone disorder of growing children and adolescents and is associated with characteristic skeletal deformities.
- Osteomalacia is a generalized softening of the bones regardless of age or cause and thus occurs both in children and adults.
- Worldwide nutritional vitamin D deficiency is the most common cause of rickets and osteomalacia. However, in countries where routine fortification of dairy and food products is implemented, genetic and acquired forms of rickets and osteomalacia are relatively more common.
- Calcium-deficiency rickets is a specific type of rickets that is mostly confined to certain geographic regions of the world. Calcium-deficiency osteomalacia in adults has not been reported.
- There are several types of genetic rickets and osteomalacia resulting in excess renal loss of phosphate due to inhibition of renal tubular reabsorption of phosphate.
- Several acquired forms of rickets and osteomalacia occur due to ectopic production of fibroblast growth factor 23, mostly by benign mesenchymal or skeletal tumors—the removal of which cures the bone disease.
- Several drugs, such as etidronate, sodium fluoride, and aluminum-containing antacids, as well as iron overload, directly inhibit the bone mineralization process, producing unusual types of histologic osteomalacia.
- The antiviral drugs tenofovir and adefovir, which are now widely used for the treatment of human immunodeficiency virus and hepatitis, cause renal tubular damage resulting in acquired forms of hypophosphatemic rickets and osteomalacia.
- Many rare bone disorders resemble either rickets on x-rays or osteomalacia on bone biopsy but do not respond to conventional doses of vitamin D therapy.

Introduction

Rickets is a specific bone disorder of the growing skeleton, thus occurring only in children and adolescents before the epiphyseal fusion has occurred, and is associated with characteristic skeletal deformities,[1] whereas osteomalacia is a generalized softening of the bones regardless of age or cause and therefore occurs in both children and adults.[2] Rickets is due to defective mineralization of both preosseous cartilaginous and mature osseous matrix resulting in subnormal linear growth, a consequence of the involvement of growth plates. In contrast, osteomalacia is due to defective mineralization of the mature lamellar bone. Of the four major metabolic bone diseases, osteoporosis is by far the most common (see Chapter 30), whereas rickets and osteomalacia combined are a distant second, followed by osteitis deformans (also known as Paget disease of bone; see Chapter 30). Osteitis fibrosa cystica, the typical bone disease of severe primary, secondary, or tertiary hyperparathyroidism, is the least common, and is rarely seen in the United States but still prevalent in other parts of the world where vitamin D deficiency is endemic[3,4] (see Chapter 29). The key contrasting features of these four common metabolic bone diseases are summarized in Table 31.1.

Soon after the discovery of vitamin D, rickets and osteomalacia became synonymous with any condition that could be cured by vitamin D therapy. However, with the recent discovery of fibroblast growth factor 23 (FGF23), and the understanding of its critical role in phosphate homeostasis and vitamin D metabolism[5,6] (see Chapter 29), several types of both genetic and acquired forms of rickets and osteomalacia are now better characterized with specific genotypic and phenotypic features[5–9] (see the specific sections that follow). These unusual FGF23-mediated rickets and osteomalacia do

TABLE 31.1 Contrasting Features of Four Major Metabolic Bone Diseases

Variable	Rickets and Osteomalacia	Osteoporosis	Osteitis Fibrosa	Osteitis Deformans
Basic abnormality	Defective mineralization of cartilage and bone	Insufficient replacement of normal lamellar bone	Replacement by woven bone and fibrous tissue	Abnormal woven bone
Prevalence	Second most common	Most common	Uncommon	Not uncommon
Serum calcium	Normal/Low	Normal	High/Very high	Normal
Serum phosphate	Normal/Low/Very low	Normal	Frequently low	Normal
Alkaline phosphatase	High	Normal/High	High/Very high	High
Parathyroid hormone	High	Normal/High	High/Very high	Normal
25-Hydroxyvitamin D	Low/Normal	Normal/Low	Frequently low	Normal/Low
Cortical thinning	Yes, except in X-linked hypophosphatemia	Sometimes	Yes	No
Vertebral deformities	Biconcave or cod fish like	Wedged/Compressed	Variable	Enlarged size
Long bone deformities	Bowing and pseudofractures	None, except fragility fractures	Brown tumors	Bowing and stress fractures

not respond to the conventional doses of vitamin D that cure nutritional vitamin D deficiency rickets and osteomalacia, and indeed many are resistant to even 10- to 100-fold the therapeutic doses of vitamin D used to treat vitamin D–deficiency rickets and osteomalacia. Furthermore, a variety of rare genetic bone disorders either resemble rickets on x-rays[10] or osteomalacia on bone biopsy,[11–14] but their pathogenesis is distinct from that of vitamin D–related or hypophosphatemic rickets and osteomalacia with variable or inconsistent response to therapy with vitamin D or its analogues.[12,13,15–17] In addition, a few rare acquired forms of osteomalacia are the result of adverse effects of a variety of drugs that either impair bone mineralization or inhibit renal tubular reabsorption phosphate and respond to the withdrawal of the offending drugs.

The following sections begin with a historical perspective of rickets and osteomalacia that is both instructive and informative, followed by epidemiology and demographics, process of normal and abnormal bone mineralization, pathogenesis, and clinical manifestations. The treatment strategies will be discussed in detail for nutritional-deficiency rickets and osteomalacia, but specific aspects as they relate to different types and forms of rickets and osteomalacia will be dealt within the specific sections. Where appropriate, reference will be made as it applies to management in children and adults.

Historical Perspective

Rickets

The earliest reports of rickets date back to the 17th century, with the first detailed contemporaneous descriptions by William Glisson and Daniel Whistler in 1645.[18] Glisson was also the first to recognize rickets as a separate entity from infantile scurvy, which often coexisted with rickets during that time. Even more interesting is that Glisson believed that rickets was neither congenital nor inherited,[18] although it is now clear that both types of rickets forms can occur. As early as in the middle of the 17th century, rickets was attributed to the industrial revolution and growing urbanization (presumably less sunlight exposure),[19] as well as to breastfeeding (breast milk has poor vitamin D content).[20] The temporal relationship between rickets and sunshine was not appreciated until the 19th century,[21] and the "proof of concept" that sunshine can cure rickets did not occur until the beginning of the 20th century.[18,19] Early 20th-century reports on rickets were exclusively from the regions of the world where vitamin D deficiency was endemic, numerically the most common, and almost all cases were due to nutritional vitamin D deficiency.[22–33] With the current policies of routine fortification of milk and other food products with vitamin D, nutritional vitamin D–deficiency rickets and osteomalacia all but disappeared from developed countries.[34–36] Consequently, genetic[37–40] and acquired[8,9,41–49] forms of rickets and osteomalacia are more prevalent in nonendemic regions of the world.[8,9,11,12,41–46,50]

Osteomalacia

The term *osteomalacia* originally referred to the generalized softening of the bones resulting in crippling deformities, and the most comprehensive report on its etiology and treatment dates back to 1896.[51] In earlier publications, various descriptive terms, such as *mollities ossium*,[52] *rheumatic*, *syphilitic*, *senile*, and even *neurotic osteomalacia*, were used to describe osteomalacia, implying that the bone disease was due to either infection or inflammation.[53] Because of the grotesque deformities involving long bones, even more descriptive terms, such as *boomerang bones* or *osteomalacia sclerotica*, were used to describe a peculiar disease seen rather exclusively in Australian Aborigines and in Sudanese populations.[54] Since osteoporosis was such a frequent accompaniment of osteomalacia, the term *osteoporomalacia* was often used in the 20th century.[55,56] The distinction between rickets that specifically involved metaphyses, and to a lesser extent epiphyses, and osteomalacia, which affected all of the bones, was recognized early in the 18th century,[51–53] and was reemphasized by Fuller Albright in his classic monograph.[57]

The histologic differentiation of osteomalacia from both osteoporosis and osteitis fibrosa was first made by Pommer in the late 19th century by examining cadaveric bones[58] and later confirmed

in humans by Fuller Albright with his elegant balance studies.[57] The first detailed tetracycline-based bone histomorphometric measurements were made by Harold Frost in 1966.[59] Although the original descriptions defined *rickets* and *osteomalacia* as distinct entities, the two terms are often used (and probably can be used, except that rickets occurs only in children) interchangeably. However, and perhaps more confusing, is that the descriptive terms have been extended to include various other conditions that resembled radiologic or bone histologic phenotypes of vitamin D–related disorders.[12–14,60] This is both unfortunate and unwarranted, as it may erroneously be misconstrued that conditions resembling rickets and osteomalacia will respond to vitamin D therapy; a recent review on the subject underscores this cautionary approach.[61]

Epidemiology and Demographics

Worldwide, nutritional deficiency of vitamin D and calcium is still the most common cause and type of rickets and osteomalacia.[15,34,40,62–73] Unlike the known prevalence, incidence, epidemiology, and demographics of osteoporosis (see Chapter 30), osteitis fibrosa (see Chapter 29), and osteitis deformans (see Chapter 30), precise estimates for the prevalence of rickets and osteomalacia are lacking. Recent reports suggest a rising trend in the prevalence of nutritional rickets worldwide, including the regions where routine fortification of dairy and other food products with vitamin D is practiced.[34,64–68,74–76] The most recent estimates of vitamin D–deficiency rickets ranged from 2.2 in 100,000 people in 1980 to 24 in 100,000 people in 2000.[64] Similarly, the prevalence of osteomalacia in adults due to vitamin D depletion may be rising because of the increasing rates of bariatric surgery for morbid obesity, which results in malabsorption of both vitamin D and calcium.[4,62,77] Although precise estimates on the prevalence or incidence of osteomalacia following bariatric surgery are lacking, it was the most common cause of osteomalacia at our institution in Detroit, Michigan.[4,77] A similar trend was observed following earlier, but now abandoned, intestinal bypass surgeries for obesity[4,77–79] and after gastrectomy (partial or total) for peptic ulcer disease, which is now performed rarely.[80–83] Rarity of vitamin D deficiency rickets and osteomalacia in developed countries[84–87] explains the relatively higher prevalence of genetic and acquired forms of rickets and osteomalacia in the Western world.[6–8,88,89] It is estimated that the most common genetic form of rickets and osteomalacia occurs 1 in 20,000 live births.[90] In the past decade, there has been a growing number of reports of osteomalacia related to the expanding use of the antiretroviral drugs tenofovir[9] and adefovir.[91] Although rickets in children due to antiviral drug therapy has not yet been reported, it is most likely because of the low prevalence of their use in the pediatric population. In addition, the incidence of rickets and osteomalacia due to anticonvulsants, antacids, and aluminum toxicity remains very low. Thus, rickets and osteomalacia not related to *nutrition* and *drugs* are most prevalent in the developed countries, whereas rickets and osteomalacia related to *vitamin D and calcium deficiency* are most common in developing countries.

Certain individuals and ethnic groups are particularly susceptible to the development of nutritional rickets and osteomalacia. Both secular and nonsecular tendencies of the populations, geographic locations, prevailing sunlight, and local dietary habits all contribute to the development of rickets and osteomalacia related to deficiency of vitamin D and calcium. Immigrants, particularly those with darkly pigmented skin or specific dietary habits (vegetarians, high phytate intake) moving to temperate zones with limited or reduced sunlight exposure are at risk of developing rickets and osteomalacia—so-called immigrant osteomalacia.[15] Because of the rarity of rickets and osteomalacia due to vitamin D deficiency in developed countries,[64,67,68,74,75] the diagnosis is often missed or delayed.[4,77]

Bone Remodeling and Mineralization

Bone remodeling is a necessary mechanism by which old bone is replaced by new bone throughout the life span of an individual and is sufficient to maintain integrity of the skeleton, both as an organ to serve its metabolic functions and as a structure for locomotion (see Chapters 29 and 30). In the course of normal bone remodeling, a moiety of old bone is removed and replaced by the same amount of normal lamellar bone in young adults, but in aging and disease, the replacement mechanism is not as efficient as it is in the young. A lesser amount of normal lamellar bone is replaced in osteoporosis (see Chapter 30), by a mixture of woven bone and fibrous tissue in osteitis fibrosa due to hyperparathyroidism (see Chapter 29), by an abnormal local production of woven bone in osteitis deformans, and by an unmineralized bone matrix (or osteoid tissue) in osteomalacia.[92] This fundamental difference in the nature of the replaced bone distinguishes osteomalacia from the most common metabolic bone diseases (see Table 31.1), as well as from rare bone disorders such as hypophosphatasia, fibrogenesis imperfecta ossium, and axial osteomalacia that resemble classical osteomalacia in one respect or another (see the Conditions That Resemble Rickets and Osteomalacia section).

Despite significant advances in our understanding of bone biology, very little is known about mechanisms that control or regulate the process of mineralization in bone. Why does biologic mineralization normally occur only in certain types of connective tissues but not in others, and how is the temporal and spatial regulation of matrix production and mineralization process accomplished? For proper and optimal mineralization of bone, at a minimum, two principal requirements must be met: synthesis of mature lamellar bone matrix by osteoblasts (see Chapter 30) and exposure of this newly synthesized lamellar bone matrix to optimal calcium × phosphate product provided by the mineral homeostatic system regulated by parathyroid hormone (PTH) and vitamin D (see Chapter 29). Any abnormality in either component will result in defective mineralization. In classical osteomalacia, deficiency of minerals, however produced, results in the accumulation of unmineralized bone matrix or osteoid. In contrast, in all other bone disorders that resemble osteomalacia (or "osteomalacia like"), the osteoid accumulation is a consequence of abnormalities outside of these two principal components. In hypophosphatasia, it is the enzyme deficiency, whereas in Paget disease of bone, fibrous dysplasia, fibrogenesis imperfecta ossium, and possibly osteogenesis imperfecta, it is the abnormal bone matrix, and in certain drug-induced osteomalacia (etidronate, fluoride, aluminum, and iron), it is the toxic effects of the drugs inhibiting matrix mineralization. Understanding the differences in the mechanism of osteoid accumulation in different diseases is critical for clinical management because disorders that resemble osteomalacia do not respond to vitamin D therapy as the term *osteomalacia* might imply.

Normal mineralization of bone matrix occurs in two stages. In the rapid phase, termed *primary mineralization*, 75% to 80% of the maximal mineral content is deposited within a few days to weeks. In the second and much slower phase, termed *secondary mineralization*, the mineral content of the bone increases further

to reach about 90% to 95% over a period of months. The remaining 5% to 10% represents the bone matrix that is newly formed but not yet mineralized. Accordingly, an osteoid surface greater than 15% of the bone surfaces, sometimes referred to as hyperosteoidosis, can be seen in conditions with high rates of bone turnover, such as immediately after estrogen depletion in postmenopausal women, hyperparathyroidism (primary or secondary), hyperthyroidism, and osteitis deformans. However, strictly speaking, the bone histologic features and osteoid indices in these high bone turnover diseases do not conform to the classical definition of osteomalacia (see the next section).

Definition and Histologic Evolution of Osteomalacia

Classical vitamin D deficiency osteomalacia, irrespective of its cause, evolves in three stages. The first stage is characterized by an increased bone remodeling due to secondary hyperparathyroidism (2°HPT), associated with increased osteoid surface and osteoid volume, *but not the thickness of osteoid*, and normal mineralization of bone. This represents the earliest bone histologic phenotype of vitamin D deficiency, designated as hypovitaminosis D osteopathy stage I (HVO I) or preosteomalacia[87] (Fig. 31.1). Similar bone histologic features can also be seen in patients with calcium malabsorption, but without vitamin D deficiency, designated as 2°HPT. However, the osteoid indices are much lower than in HVO I with some overlap (see Fig. 31.1). In both HVO I and 2°HPT, there is evidence of mainly cortical bone loss due to excess PTH secretion,[93] and the patients are usually asymptomatic at this stage but may present with fragility fractures. Serum levels of calcium and phosphate are normal, and the serum level of alkaline phosphatase is usually, but not always, elevated.[62,77] The serum 25-hydroxyvitamin D level is low (<10 ng/mL), and serum levels of PTH and 1,25-dihydroxyvitamin D are elevated. The increases in serum levels of alkaline phosphatase and 1,25-dihydroxyvitamin D respectively are related to increased bone turnover and increased 1α-hydroxylase activity in the kidney (see Chapter 29) as a consequence of 2°HPT. In addition, an irreversible PTH-mediated cortical bone loss may have already occurred.[93]

In the second stage, designated as HVO II, there is further accumulation of osteoid with increases in osteoid surface, osteoid volume, and *osteoid thickness* but with preservation of some mineralization as assessed by tetracycline uptake at the mineralization front.[87] Both serum PTH and alkaline phosphatase levels increase further, but serum 1,25-dihydroxyvitamin D levels may return to normal or low depending on the degree of vitamin D deficiency (as assessed by its substrate, 25-hydroxyvitamin D) and PTH elevation. The serum calcium level usually declines at this stage with low normal or frank hypocalcemia, and serum phosphate levels are usually low. Patients may be symptomatic with bone pain, muscle weakness, and fragility or pseudofractures.

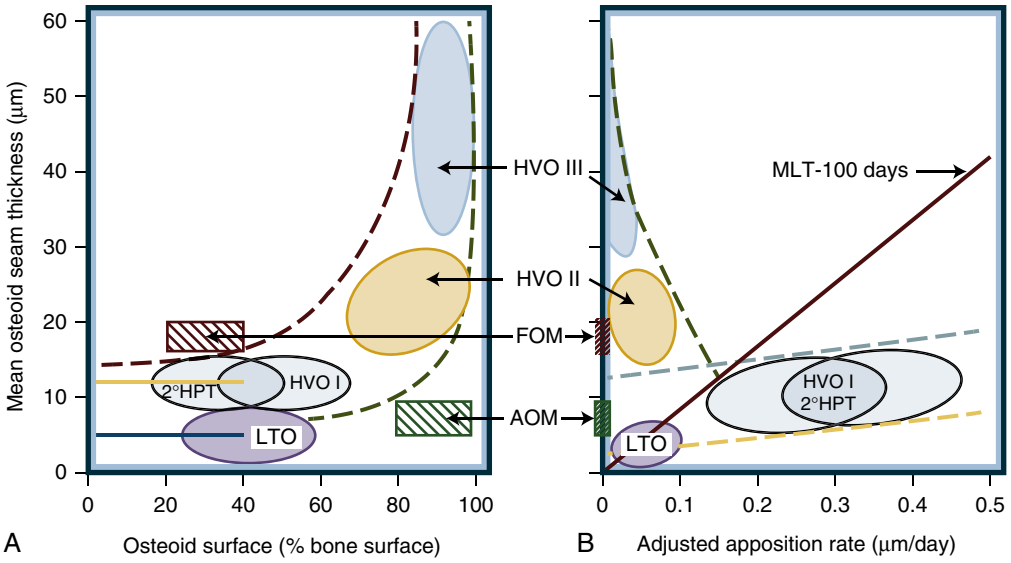
In the third stage, designated as HVO III, the mineralization of bone matrix ceases and osteoid accumulation continues to cover more than 90% of the bone surfaces. It is at this stage that hypocalcemia is invariable, as the osteoid covered bone is resistant to osteoclastic bone resorption, which is a necessary mechanism to maintain normal serum calcium levels. The extensive coverage of the bone surfaces with osteoid is perhaps a "protective mechanism" to prevent complete dissolution of bone. However, peritrabecular and bone marrow fibrosis, a feature of more severe hyperparathyroidism, occurs only in HVO III and can be demonstrated on bone biopsy.[4] Patients are almost always symptomatic at this stage, with diffuse bone pain, muscle weakness, and pseudofractures (Fig. 31.2), although an occasional patient may present primarily with muscle weakness without bone pain.[94] Osteomalacia defined by an osteoid thickness greater than 12.5 μm and a mineralization lag time of more than 100 days (see Fig. 31.1) conforms to the conventional clinical and radiologic descriptions of osteomalacia. Regrettably, no such data exist on the evolution of hereditary or acquired forms of hypophosphatemic osteomalacia, as most of those patients present clinically late in the course of their disease, corresponding to the third stage (HVO III) of vitamin D–deficiency osteomalacia.[87,92]

Pathogenesis of Rickets and Osteomalacia

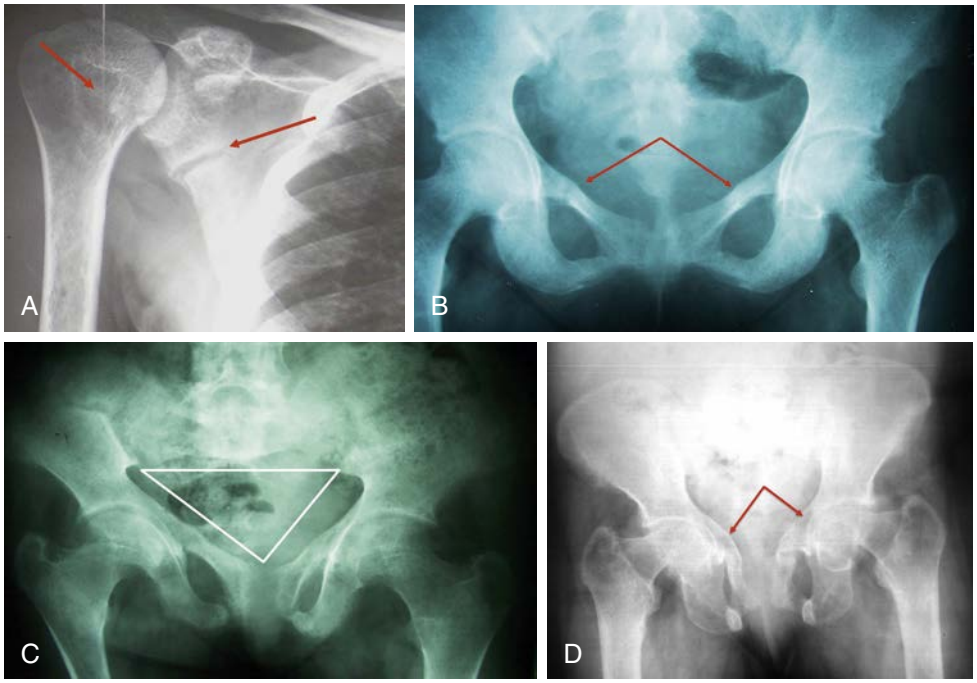
The three principal mechanisms by which rickets and osteomalacia develop are vitamin D depletion or deficiency, phosphate depletion or deficiency, and calcium deficiency—in that order of frequency.[62,95,96] Hypophosphatemia due to nutritional phosphate deficiency is a very rare cause of rickets or osteomalacia, although occasional cases have been reported in patients on prolonged total parenteral nutrition. Vitamin D deficiency or depletion, however produced (Table 31.2), if prolonged or left untreated, ultimately leads to rickets and osteomalacia.[97] Phosphate depletion, however caused (genetic, tumor induced, or acquired), is the second most common cause of rickets and osteomalacia, and the most prevalent type in parts of the world where vitamin D deficiency is not endemic. The two most common causes of hypophosphatemic rickets and osteomalacia are hereditary hypophosphatemic syndromes[5,7] and FGF23-secreting tumors.[8] Other less frequent causes of hypophosphatemic rickets and osteomalacia include prolonged use of phosphate-binding antacids,[41,98] as well as various genetic and acquired renal tubular defects[99] (Table 31.3). Even more uncommon, but histologically distinct from the vitamin D–deficiency and hypophosphatemic rickets and osteomalacia syndromes, are the atypical and focal osteomalacia caused by the toxic effects of drugs such as sodium fluoride,[100,101] etidronate,[102] aluminum, and iron, which directly inhibit bone mineralization[49,103] (Table 31.4; see Fig. 31.1).

Vitamin D deficiency can be extrinsic or intrinsic. Extrinsic vitamin D deficiency is due to deficient endogenous cutaneous production of vitamin D_3 or poor dietary intake (see Table 31.2). Inadequate exposure or avoidance of sunlight,[21,104] use of sun-protective lotions or sunscreens,[105,106] darkly pigmented skin,[107] excessive covering of the body with clothing for cultural reasons,[108,109] and aging[104] all contribute to the decreased production of vitamin D_3 or cholecalciferol from its precursor 7-dehydrocholesterol (see Chapter 29). Although inadequate intake of vitamin D as a cause of rickets and osteomalacia is rare in the United States, occasional cases or case series have been reported.[64,84,86]

Rickets and osteomalacia due to intrinsic vitamin D depletion (the descriptive term *depletion* for all intrinsic causes of vitamin D deficiency is probably more appropriate) is most commonly caused by impaired gastrointestinal absorption of vitamin D (and calcium)[110,111] as a result of intestinal disease, resection, or gastric bypass surgery (see Table 31.2). Vitamin D deficiency can also result from genetic or acquired causes of impaired or defective *vitamin D 25-hydroxylase* in the liver or impaired or deficient *25-hydroxyvitamin D 1α-hydroxylase* in the kidney and other target tissues, the necessary enzymes to convert the parent compound vitamin D to its biologically active metabolites,[112–118] or increased catabolism of vitamin D and its active metabolites to inert compounds.[119,120] Strictly speaking, rickets and osteomalacia due to



• **Fig. 31.1** Topographic depiction of stages of hypovitaminosis D osteopathy (HVO I, II, and III), atypical osteomalacia (AOM), and focal osteomalacia (FOM). For comparison, secondary hyperparathyroidism (2°HPT) without mineralization defect and low turnover osteoporosis (LTO) are shown. In (A), the location of the seven types of bone lesions is based on the relationship between osteoid thickness (y-axis) and the extent of bone surface covered by osteoid (x-axis). In (B), the location is based on the relationship between osteoid thickness (y-axis) and adjusted appositional rate (x-axis) as determined by tetracycline uptake (x-axis). In normal subjects, and in patients with 2°HPT, HVO I, and LTO, there is no relationship between osteoid thickness and osteoid surface until the osteoid surface exceeds greater than 50% to 60% of the bone surface (*straight solid horizontal lines*), after which the relationship becomes hyperbolic (*interrupted curvilinear lines*). By contrast, there is a positive relationship between osteoid thickness and the adjusted mineral apposition rate (*straight interrupted lines*) in normal subjects and in patients with 2°HPT, HVO I, and LTO (B). The *oblique interrupted line* indicates the reversal of this relationship in patients with more severe osteomalacia (HVO II and III), the cardinal feature of osteomalacia unlike all other conditions (2°HPT, LTO, AOM, and FOM). The *solid straight line* represents a mineralization lag time of 100 days (MLT, the time delay between matrix deposition osteoblasts and subsequent mineralization) that separates patients with and without osteomalacia. Locations are shown for clarity and simplicity. Note a significant overlap of 2°HPT, HVO I, and LTO. (Modified from Bhan A, Qiu S, Rao SD. Bone histomorphometry in the evaluation of osteomalacia. *Bone Rep*. 2018;8:125–134.)



• **Fig. 31.2** Radiologic manifestations of osteomalacia. (A) Pseudofracture in the scapula and a brown tumor in the proximal humerus. (B) Pseudofractures in both superior pubic rami. (C) Triradiate pelvis due to softening of the pelvic bones. (D) Bilateral protrusio acetabuli and deformed pelvis.

TABLE 31.2 Causes of Vitamin D–Deficiency Rickets or Osteomalacia, or Both

Extrinsic

Inadequate dietary intake of vitamin D
Decreased exposure or avoidance of sunlight
Use of sunscreens (especially a solar protective factor >8)
Excess clothing (veil/hijab)
?Increased or dark skin pigmentation

Intrinsic

Advancing age with decreased cutaneous production of vitamin D
Malabsorption due to various gastrointestinal disorders
- Gastrectomy (partial, total, or bypass procedure)
- Small intestinal disease, resection, or bypass
- Gluten enteropathy (Celiac sprue)
- Biliary cirrhosis (uncommon)
- Pancreatic insufficiency including cystic fibrosis (uncommon)
- Impaired or genetically defective vitamin D 25-hydroxylase enzyme
- ?Immaturity
- ?Neonatal hepatitis
- ?Cirrhosis of the liver
- Genetic defect (vitamin D–dependent rickets type 1B)
- Impaired or genetically defective 25-hydroxyvitamin D 1α-hydroxylase enzyme
- Genetic defect (vitamin D–dependent rickets type 1A)
- Chronic renal failure
- "Acquired" vitamin D deficiency
- Increased catabolism due to microsomal enzyme induction
- Anticonvulsants
- Calcium deficiency with secondary hyperparathyroidism

TABLE 31.3 Causes of Hypophosphatemic Rickets or Osteomalacia, or Both

Genetic

Autosomal dominant rickets
Autosomal recessive rickets
X-linked dominant rickets (X-linked hypophosphatemia)
X-linked recessive
Hypophosphatemic hypercalciuric hereditary rickets (or Dent disease)
Neurofibromatosis
Fibrous dysplasia
Genetic Fanconi syndrome (may have renal failure)

Acquired

Tumor-induced osteomalacia (most common acquired cause)
Renal tubular damage or nonfamilial Fanconi syndrome
- Paraproteinemia
- Wilson disease
- Galactosemia
- Tyrosinemia
- Glycogenosis
- Drug induced
  - Tenofovir and adefovir (second most common acquired cause)
  - Phosphate-binding antacids
  - Cadmium poisoning
  - ?Use of outdated tetracycline
  - ?Amphotericin

target organ resistance to vitamin D action[37,38,121–123] are neither a vitamin D deficient state nor a genetic defect in the vitamin D activation pathway (see Tables 31.2 and 31.3). Although excessive intestinal loss of 25-hydroxyvitamin D via enterohepatic circulation or renal loss of vitamin D and its metabolites due to heavy proteinuria (as in nephrotic syndrome) as a cause of vitamin D depletion has been proposed, the magnitude and scope of these mechanisms contributing to vitamin D depletion are not well characterized.[124–126]

Of all the intrinsic causes of vitamin D depletion, malabsorption of vitamin D is by far the most common cause of osteomalacia.[110] Both gluten enteropathy and Crohn disease have been associated with osteomalacia due to vitamin D depletion.[62,127–131] Although decreased bone mineral density (BMD), increased fracture risk, and growth retardation (possibly related to rickets) have been associated with inflammatory bowel disease,[132–134] osteomalacia due solely to inflammatory bowel disease has not been reported. Severe calcium malabsorption, malnutrition, or both, as seen in patients with inflammatory bowel disease, may lead to 2°HPT with the consequent cortical bone loss and increased risk of fragility fractures (see Chapter 30) but without vitamin D depletion severe enough to cause osteomalacia.[78,93,135] Total and partial gastrectomy, vagotomy and pyloroplasty,[80,81,136–139] intestinal resection,[125,140] and gastric or intestinal bypass surgery for morbid obesity[62,80,128,138,141,142] have all been associated with vitamin D depletion and osteomalacia (see Table 31.2). Because of the malabsorption of multiple nutrients, including calcium and vitamin D, the bone phenotype varies from simple osteopenia detected by BMD testing to osteoporosis with increased fracture risk, to frank osteomalacia on bone histomorphometry. The relative frequency of osteomalacia in patients with various gastrointestinal disorders or surgeries is not clearly established but may be as high as 50%.[4,77,128,135,143,144] Prolonged 2°HPT can occasionally lead to bone marrow fibrosis and hypercalcemic 2°HPT (or tertiary hyperparathyroidism; see Chapter 29).[4,130,145–147]

By contrast, hepatobiliary and pancreatic disorders are relatively less common causes of rickets and osteomalacia, although osteoporosis is very common in both kinds of disorders (see Chapter 30). Because of the large functional reserve, by inference adequate availability of vitamin D 25-hydroxylase enzyme activity, vitamin D depletion sufficient to cause rickets and osteomalacia is unlikely in patients with the usual types of parenchymal liver diseases.[148–152] Most often, additional factors, such as poor dietary vitamin D intake, antiviral drug therapy for hepatitis[153–156] (see the Drug-Induced Osteomalacia section), and coexistent primary biliary cirrhosis,[124,152,157–159] contribute to severe vitamin D depletion and osteomalacia. Immaturity and neonatal hepatitis are also rare causes of rickets in children[2,114] and osteomalacia in adults,[160] most likely related to defective or insufficient vitamin D 25-hydroxylase enzyme, although not conclusively documented. Despite a significant fat malabsorption and steatorrhea in patients with exocrine pancreatic insufficiency, rickets and osteomalacia are uncommon, but both rickets and osteomalacia have been reported in patients with cystic fibrosis.[161–165] Drugs that interfere with the 25-hydroxylation step in the vitamin D activation pathway are discussed in the section on drug-induced rickets and osteomalacia (see Table 31.6).

Calcium-Deficiency Rickets

Unlike nutritional vitamin D and phosphate deficiency, which cause both rickets and osteomalacia, only rickets has been convincingly documented as resulting from nutritional calcium deficiency without associated vitamin D deficiency as assessed by normal or low normal serum 25-hydroxyvitamin D levels.[1,96] Although much has been learned on the role of calcium nutrition in the pathogenesis of

TABLE 31.4 Relevant Abnormalities in Various Types of Vitamin D–Related Rickets and Osteomalacia

Variable	Vitamin D Deficiency	Vitamin D Dependency Type 1B	Vitamin D Dependency Type 1A	Vitamin D Dependency Type 2	Hypophosphatemic Vitamin D Resistant[a]
Basic defect	Nutritional/ malabsorption	25-Hydroxylase defect	1α-Hydroxylase defect	VDR defect	Excess FGF23
Gene locus	Not applicable	Chromosome 11p15.2	Chromosome 12q13.1	Chromosome 12q12-q14	Xp22.11
Enzyme defect	Not applicable	CYP2R1	CYP27B1	Receptor defect	*PHEX* gene defect
Serum calcium	Low/normal	Low	Low	Low	Normal/High
Serum phosphate	Normal/low	Normal/Low	Normal/Low	Normal/Low	Very low
Alkaline phosphatase	High	High	High	High	High
Parathyroid hormone	High	High	High	High	Normal/high
25-Hydroxyvitamin D	Low	Low/very low	Normal	Normal	Normal
1,25-Dihydroxyvitamin D	Variable	Low/low normal	Low	High	Low
Urine calcium	Low	Low	Low	Low	Normal/high
Vitamin D dose required to heal/ cure rickets[b]	1000–2000 IU daily Few weeks to months	10,000 IU daily Lifelong	10,000 IU daily Lifelong	100,000 units daily Lifelong	100,000 units daily Lifelong
Calcitriol dose required to heal/ cure rickets	0.04 μg/kg per day Few weeks to months	0.04 μg/kg per day Lifelong	0.04 μg/kg per day Lifelong	1–2 μg/day Lifelong	1–2 μg/day and oral phosphate Lifelong

[a]Previously referred to as vitamin D–resistant rickets because of the large doses of vitamin D required to heal rickets; the current name is X-linked hypophosphatemic rickets and osteomalacia.

[b]Shown only to illustrate comparative effective doses. Both calcitriol and alphacalcidol are now widely available and used to treat all vitamin D–dependent rickets and osteomalacia

CYP, Cytochrome P450; *FGF23*, fibroblast growth factor 23; *PHEX*, phosphate-regulating endopeptidase homolog, X-linked; *VDR*, vitamin D receptor.

osteoporosis (see Chapter 30), no case of osteomalacia in an adult due to calcium deficiency alone has been reported; the reasons for this discordant effect of calcium nutrition on the skeleton in children and adults is perplexing. A more severe 2°HPT over a relatively short period due to severe calcium malnutrition in a growing child may produce radiologic features similar to rickets—the so-called short-latency disease.[166] However, a milder 2°HPT over a longer period of time caused cortical bone loss, osteoporosis, and increased fracture risk—the so-called long-latency disease.[166] Further studies are needed to clarify this apparent paradox: why two different nutrient (calcium and vitamin D) deficiencies independently produce the same disease in children (rickets) but different diseases in adults (osteoporosis and osteomalacia).

Calcium malnutrition as a cause of rickets was first suggested in a child from San Francisco, California, who responded to calcium infusion, but the child also had aminoaciduria, and vitamin D deficiency was not conclusively excluded.[167] A similar case of rickets in an Italian child from Toronto, Canada, in whom both vitamin D deficiency and resistance were excluded with appropriate biochemical testing, probably represents the first unambiguous human case of true calcium-deficiency rickets.[168] A more comprehensive study on calcium-deficiency rickets in children from rural South Africa further characterized the condition as a separate entity,[169] with additional reports from Nigeria,[96,170] India,[171] Mongolia,[172] and Europe,[173] confirming the concept, but many children also had some degree of vitamin D insufficiency. A daily calcium intake of greater than 200 mg appears to be the lowest threshold for the risk of developing calcium-deficiency rickets independent of vitamin D nutritional status[1]; "wet-nursing" and prolonged breastfeeding, practices that are prevalent in some cultures, are other risk factors for calcium-deficiency rickets.[174,175] Rickets due to calcium deficiency tends to occur later in life than that due to vitamin D deficiency, with an average age at presentation of 4 years in Nigeria, but ranging from 4 to 16 years in other series.[1] Interestingly, calcium-deficiency rickets appears to be particularly prevalent in parts of the world with abundant sunshine.[96,170,171,176] Clinically, calcium-deficiency rickets differs from other forms of rickets, especially in adolescents who may have significant genu valgum without many end-plate deformities.[177] The rarity of calcium-deficiency rickets in developed countries may be related to a much higher dietary calcium intake and less prolonged breastfeeding.[176,178] Nevertheless, when a child is encountered with rickets and if the serum level of 25-hydroxyvitamin D is normal, think of calcium-deficiency rickets, particularly if serum calcium is low and PTH is elevated.

Phosphate-Deficiency/Depletion Rickets and Osteomalacia

Nutritional phosphate deficiency is a very rare cause of rickets and osteomalacia, as there is abundant phosphate in foods, fruits, vegetables, and dairy products. Since intestinal absorption of phosphate is mostly passive and quite efficient, it is very difficult to produce true nutritional phosphate deficiency in an otherwise healthy individual. The serum phosphate level is maintained within a narrow range by the kidney under the control of PTH and FGF23.[179,180] Hypophosphatemia, however, is not uncommon in hospitalized patients,[181] in patients with iron deficiency,[182] and in those receiving phosphate binders and antacids known to deplete phosphorus in the body.[41,43,45,98] Such hypophosphatemic states usually do not last long enough to produce rickets and osteomalacia. Consequently, almost all hypophosphatemic rickets and osteomalacia are either genetic or acquired (see later discussion).

Clinical Manifestations of Classical Rickets and Osteomalacia

The symptoms and signs of rickets and osteomalacia are primarily related to the musculoskeletal system. With few exceptions (discussed later in detail in the relevant sections), the clinical manifestations are similar regardless of their pathogenesis—nutrient deficiencies, genetic or acquired causes, tumor induced, or drug induced. Because rickets involves the growth plates affecting linear growth, short stature is common. When nutritional deficiencies begin in childhood, and if left untreated for prolonged periods, as usually occurs in vitamin D and calcium-deficient endemic regions, deformities of the skeleton are common. In long bones, rickets affects diaphysis (bowing), metaphysis (widening, fraying, and cupping), and epiphysis (irregular margins), whereas osteomalacia involves only the diaphysis of the long bones. If osteomalacia develops later in life without a history of rickets during infancy and childhood, as is common in clinical practice in developed countries, the clinical manifestations are subtle and resemble those of age-related osteoporosis (see Chapter 30). In general, the later the onset of osteomalacia, the more easily its clinical clues are missed and the more likely the symptoms are dismissed as aches and pains of aging. The most common presenting clinical symptoms are bone pain, muscle weakness and difficulty in walking, skeletal deformities, and fractures. Carpal and pedal spasms, muscle cramps, and seizures due to hypocalcemia are uncommon but mostly seen in children with rickets than in adults with osteomalacia. Triradiate pelvis, a rare complication of osteomalacia due to softening of the pelvic bones (see Fig. 31.2), may cause difficult or obstructed labor in childbearing women; however, it is far less common today, and its presence suggests severe disease.

Bone Pain

Bone pain in osteomalacia is diffuse, nondescript, dull aching, deep seated, and poorly localized, and at times can be debilitating.[85,183–185] It is felt more in the bones than in the joints and often is bilaterally symmetric. Because of its vague nature, bone pain is often misdiagnosed as tension headache (so-called osteomalacic cephalalgia), "angina" (chest pain due to pseudofractures in the ribs), rheumatism, and fibromyalgia.[186–188] The pain is persistent and gnawing, is aggravated by weight bearing or muscle contractions during attempted walking, and is rarely relieved by rest. The pain usually begins in the lower back and spreads to the pelvis, hips, thighs, upper back, and ribs but is nonradiating and is rarely felt below the knees unless fragility or pseudofractures are present in tibiae and fibulae. Bone tenderness can be elicited by pressure or percussion over the shin bones, squeezing of the forearm with a fist, lateral compression of the pelvis and rib cage, and posterior compression of the sternum. The propensity of pain to localize to the axial skeleton is probably related to an earlier and a greater accumulation of osteoid in the cancellous bone, whereas the appendicular skeleton, rich in cortical bone, is more subject to fragility fractures. The mechanism for bone pain is believed to be related to the stretching of the periosteum by the overhydrated unmineralized bone matrix. Bone pain almost never occurs in patients with osteoporosis in the absence of a fracture, but in osteomalacia, bone pain occurs with or without a fracture. A significant minority of the patients can be completely asymptomatic.[94]

Muscle Weakness

Proximal muscle weakness, especially in the lower extremities, is the most common muscular manifestation in osteomalacia, although weakness in the upper arms can also be demonstrated by physical examination. The severity of muscle weakness varies from subtle abnormalities detectable only on careful examination to severe debilitating disabilities mimicking more serious muscle disorders and rarely can cause total paralysis. In mild cases, the muscle weakness must be distinguished from the patient's reluctance to stand or walk for fear of aggravating the bone pain. Difficulty in rising from a sitting position or going up and down stairs without using the arms is quite specific. In advanced cases, classical waddling gait (walking like a duck), the result of a combination of muscle weakness and bone pain, is observed. With prolonged vitamin D depletion of increasing severity, as seen in endemic regions that are vitamin D and calcium deficient, a patient may become completely immobilized and bed bound because of profound weakness and excruciating bone pain, sometimes masquerading as a terminal illness.

Despite profound muscle weakness, muscle atrophy is uncommon, although mild muscle wasting with atrophy of the type II fibers has been reported occasionally. Hypotonia can be present, but fasciculation and clonus are absent. Deep tendon reflexes are normal or increased—all helpful clinical signs to distinguish the muscle weakness of rickets and osteomalacia from other types of muscle diseases and myopathies. Rarely, dilated cardiomyopathy that responds to vitamin D repletion has been reported in severe rickets.[189] Other muscular symptoms such as muscle cramps and spasms, tingling and numbness, and seizures (usually in children) occur when the serum calcium level falls below 6.0 mg/dL. Both the muscle weakness and atrophy are commonly attributed to hypocalcemia and 2°HPT, but both muscle abnormalities are also seen in patients with primary and tertiary hyperparathyroidism in whom the serum calcium level is elevated (see Chapter 29). Since muscle weakness is also a manifestation of hypophosphatemic osteomalacia in the absence of hyperparathyroidism or hypocalcemia, the relative contributions of hypocalcemia, hypophosphatemia, and hyperparathyroidism to muscular manifestations of rickets and osteomalacia is not entirely clear. In general, muscle weakness is more prominent in hypophosphatemic rickets and osteomalacia, whereas bone pain is more common in vitamin D–deficiency osteomalacia. Interestingly, despite a significant muscle weakness in patients with X-linked hypophosphatemia (XLH), bone mass, bone size, and estimated bone strength are normal or increased.[190,191]

Skeletal Deformities and Fractures

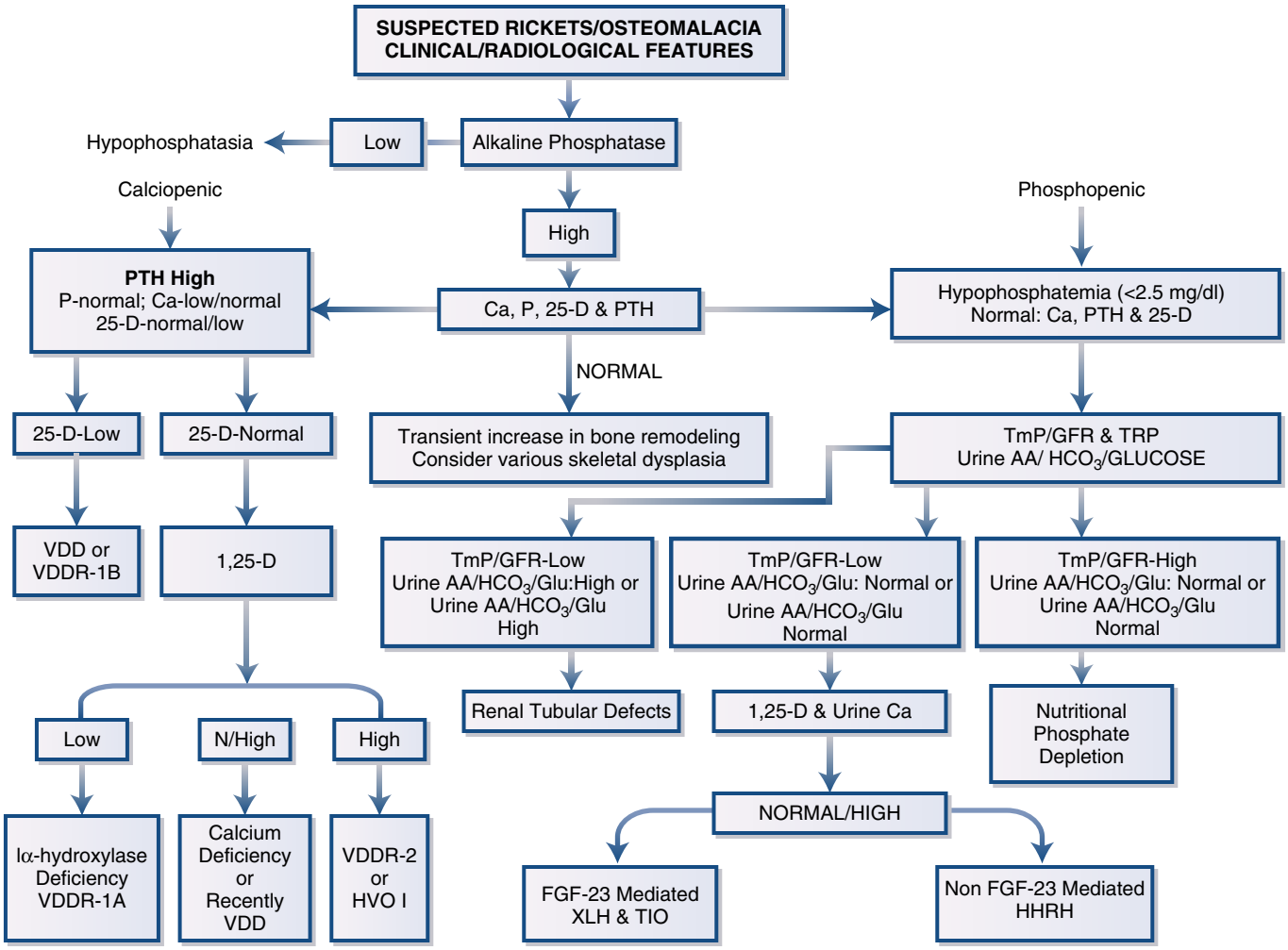
Skeletal deformities are common in children with rickets, vary with the age of presentation, and may remain permanent, whereas bone deformities are uncommon in adult-onset osteomalacia unless fractures have occurred. Infants present with open fontanelles, dolichocephaly, frontal bossing, rachitic rosary (due to consecutive pseudofractures of multiple ribs often bilaterally symmetric), Harrison sulcus (a visible horizontal line of depression at the level of the diaphragm due to weakness of the chest muscles), swollen wrist and ankle joints (due to widened metaphysis), and double malleoli. Once the child starts walking, bowing of the long bones, genu valgum, genu varum, and windswept deformity are seen. The skeletal deformities are usually more severe in genetic hypophosphatemic rickets and osteomalacia, and they predominantly involve lower limbs, resulting in a disproportionate short stature.[191] Fragility fractures are not uncommon, but Looser zones or pseudofractures, the diagnostic radiologic abnormalities (see Fig. 31.2), are more common in patients with rickets and osteomalacia. Pseudofractures are linear radiolucent bands perpendicular to the long axis of the bones, and are stress fractures that can extend to a complete fracture with separation or displacement with the pseudofractures that can progress to a complete fracture, usually in the subtrochanteric region of the femur or metatarsals—the greatest load-bearing bones. Rib fractures also commonly occur. The osteomalacic bones in children with rickets are soft rather than brittle, so fractures are less common. Conversely, when osteomalacia begins later in adult life, the usual types of fractures are more common.

Biochemical Changes

Relevant biochemical abnormalities at presentation of various forms of rickets and osteomalacia are summarized in Tables 31.4 and 31.5. Several excellent reviews have been published,[62,87] and only the salient abnormalities within each type and contrasting features between the types are briefly discussed in the following. In all types of rickets and osteomalacia, elevated serum alkaline phosphatase is the most frequent (~80–90%) and the earliest biochemical abnormality,[77] although an occasional patient may have normal alkaline phosphatase with histologically confirmed osteomalacia.[94] In general, hypocalcemia is a late biochemical manifestation, but it occurs earlier in the course of development of rickets in children than during the evolution of osteomalacia in adults. Mild to moderate hypocalcemia (serum calcium level of 7.0–8.5 mg/dL) is often asymptomatic unless it falls below the threshold for symptoms (usually <6.0 mg/dL). Like other types of biochemical abnormalities in clinical practice, it is the rate of change rather than the absolute value that determines the development of relevant symptoms. Most importantly, serum calcium is always normal in all forms of hypophosphatemic rickets and osteomalacia, except in certain types of renal tubular defects. The serum phosphate level is quite variable, lacks specificity, and is subject to diurnal variation, meal ingestion (and hence should be measured in the fasting state in the morning), renal function, and degree of serum PTH elevation—a known regulator of the serum phosphate level[179]; thus, serum phosphate in nutritional rickets and osteomalacia can be normal, low, and occasionally high, particularly in patients with more severe hypocalcemia.[94] By definition, the serum phosphate level is less than 2.5 mg/dL in all forms of hypophosphatemic rickets and osteomalacia. Although all patients with nutritional rickets and osteomalacia, by definition, have low 25-hydroxyvitamin D levels (usually <10 ng/mL), not all patients with low 25-hydroxyvitamin D levels necessarily develop rickets or osteomalacia.[62,87] This distinction is important in planning therapy, underscores the need not to equate low serum 25-hydroxyvitamin D level to osteomalacia, and avoids the often misused phrase "biochemical osteomalacia." In calcium-deficiency rickets, serum 25-hydroxyvitamin D is either normal or slightly reduced, although not to the same extent as in vitamin D–deficiency rickets and osteomalacia. An accelerated catabolism of 25-hydroxyvitamin D to its more polar and biologically active 1,25-dihydroxy vitamin D or to its inert metabolites contributes to the lower levels of serum 25-hydroxyvitamin D, sometimes referred to as conditional or obligatory vitamin D insufficiency. Serum levels of 1,25-dihydroxyvitamin depend on the stage in the evolution of nutritional rickets and osteomalacia, the availability of its precursor (25-hydroxyvitamin D), and the degree of PTH elevation; thus, the serum levels of 1,25-dihydroxyvitamin D can be high, normal, or low.[62,77,87,143] By contrast, serum 1,25-dihydroxyvitamin D levels are low, although not invariably, in hypophosphatemic rickets and osteomalacia.[8,88,89] Serum levels of PTH are always elevated in nutritional-deficiency (both vitamin D and calcium) rickets and osteomalacia, and the levels are normal in hypophosphatemic disorders regardless of the pathogenesis, unless vitamin D deficiency also exists. However, serum PTH levels rise progressively over time in patients with hypophosphatemic rickets and osteomalacia treated with long-term oral phosphate supplements and in some patients may even progress to hypercalcemic hyperparathyroidism that requires surgery.[192] A suggested clinical algorithm based on biochemical abnormalities in the evaluation of patients suspected of having rickets and osteomalacia is presented in Fig. 31.3.

Radiologic Imaging Features

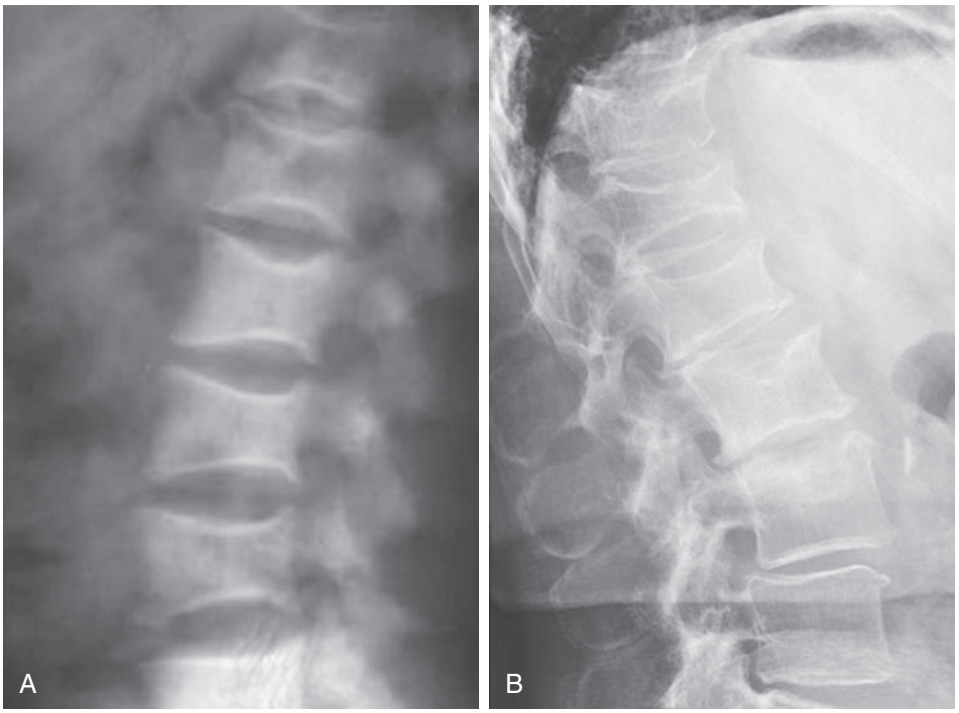
The major radiologic manifestations of nutritional rickets and osteomalacia are bone structural changes discernible on routine x-rays (now better defined on digital images), generalized decrease in apparent bone density on x-rays, vertebral deformities (Fig. 31.4), and pseudofractures (or Looser zones; see Fig. 31.2). Generalized thinning of cortices in the long bones is probably the earliest radiologic manifestation due to PTH-mediated endocortical bone resorption. Subperiosteal bone resorption (best seen on the radial aspect of the middle phalanges, metacarpals, and metatarsals) and brown tumors (osteitis fibrosa cystica; see Fig. 31.2) are seen in more advanced cases with severe hyperparathyroidism[193,194] (see Chapter 29). Although these latter bone abnormalities may resolve following vitamin D therapy, cortical thinning remains permanent and increases the fracture risk for the remainder of the patient's life.[93,195] Symmetrical biconcavity of vertebrae, referred to as cod fish vertebrae because they resemble vertebrae in cod fish, involves almost all vertebrae. "Fish-mouth" appearance of the intervertebral space, another structural change in the vertebral column, is the result of yielding of the soft vertebral bone to the pressure of the intervertebral discs (see Fig. 31.4A). This characteristic appearance of the vertebral column in osteomalacia is in stark contrast to the random anteriorly wedged or compressed asymmetrical vertebral deformities seen in patients with osteoporosis (see Fig. 31.4B; also see Chapter 30); when present, the cod fish vertebrae–like appearance of the spine is virtually diagnostic of osteomalacia (see Fig. 31.4A). The generalized apparent decrease in density of the bones on radiographs is manifested as decreased BMD by dual energy x-ray absorptiometry. Looser zones or pseudofractures are lucent



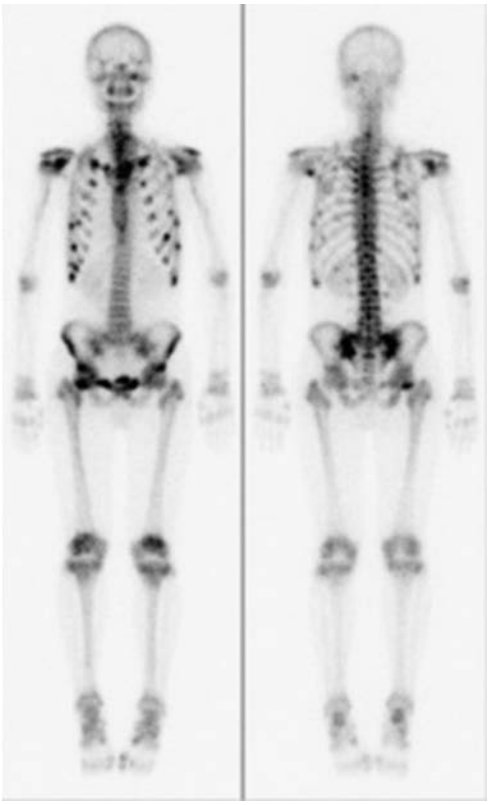
• **Fig. 31.3** Suggested clinical algorithm based on biochemical abnormalities in evaluating patients suspected of having rickets, osteomalacia, or both. Analytes: *Ca,* serum calcium; *P,* serum phosphate; *25-D,* serum 25-hydroxyvitamin D; *1,25-D,* 1,25-dihydroxyvitamin D; *PTH,* serum parathyroid hormone; *TmP/GFR,* tubular maximum for phosphate reabsorption/glomerular filtration rate; *HVO I,* hypovitaminosis D osteopathy stage I; *TRP,* tubular reabsorption of phosphate; *AA,* urine amino acid analysis; HCO_3, urine bicarbonate excretion; *Glu,* urine glucose. Conditions: *VDD,* vitamin D deficiency; *VDDR,* vitamin D–dependent rickets; *XLH,* X-linked hypophosphatemic rickets and osteomalacia; *TIO,* tumor-induced osteomalacia; *HHRH,* hereditary hypophosphatemic rickets with hypercalciuria.

bands (2–5 mm in width) perpendicular to the long axis of the bone or periosteum, often bilaterally symmetrical with sclerotic borders (Milkman syndrome); they occur more commonly in the ribs, pubic rami, and outer border of the scapulae (see Fig. 31.2), and less commonly at the inferomedial region of the proximal femurs and medial aspect of the shafts of the long bones. However, insufficiency fractures, sometimes inappropriately referred to as pseudofracture, can be seen in Paget disease of bone, hypophosphatasia, fibrous dysplasia, and atypical femur fractures due to long-term bisphosphonate therapy but usually occur (or begin) on the lateral cortex of long bones. Both insufficiency fractures and pseudofractures can progress across the shaft of the bone to a complete fracture. Looser zones or pseudofractures are caused by erosion of bone by nutrient arterial pulsation, which explains their specific medial cortical location, and represent the unhealed insufficiency type of stress fracture.

The generalized increase in radionuclide uptake throughout the skeleton, referred to as super scan, is specific for conditions associated with increased bone turnover but is more common in osteomalacia. Typically, there are no discrete focal abnormalities in the absence of pseudofractures, and the radionuclide uptake in the kidneys, in the absence of renal dysfunction, is either faint or absent, as most of the isotope is retained in the skeleton and very little is available for renal excretion (Fig. 31.5). When present, Looser zones are seen as "hot spots" on nuclear imaging (see Fig. 31.5). With a few exceptions, most radiologic features are similar among the various type of rickets and osteomalacia. Cortical thinning in long bones is not seen in XLH, and in fact, thick cortices are the rule rather than the exception. Similarly, enthesopathy is seen exclusively in XLH, but the underlying mechanisms for its development is not known. In most acquired forms of hypophosphatemic osteomalacia (tumor- and drug-induced or renal tubular defects), cortical thinning and decreased BMD may be seen, most likely the result of age-related bone loss considering that 2°HPT is not a feature of the genetic or acquired forms of hypophosphatemic disorders. Horizontal bands of endplate sclerosis of the vertebrae with relative lucency in the middle portion of the vertebral bodies (Rugger-Jersey spine, so named as the appearance resembles jerseys of rugby players) are sometimes observed in osteomalacia due to renal tubular acidosis but is a more characteristic feature of renal osteodystrophy (see Chapter 29).



• **Fig. 31.4** (A) Typical symmetrical biconcave deformities of all the vertebrae, sometimes referred to as cod fish vertebrae because they resemble the vertebrae of the fish. When present, it is diagnostic of osteomalacia of all types. (B) Typical random osteoporotic wedge and compression deformities in a postmenopausal woman with osteoporosis.



• **Fig. 31.5** Characteristic bone scan in a patient with osteomalacia. Note increased nuclide uptake throughout the skeleton with very little or no uptake in the kidneys as the radionuclide is all retained in the high remodeling skeleton. Also see multiple bilateral symmetrical uptake in the ribs (*left*) due to pseudofractures, often confused with metastatic disease.

Bone Mineral Density

BMD as assessed by dual energy x-ray absorptiometry is reduced at all of the relevant sites (lumbar spine, proximal hip, and forearm), usually with a greater deficit at the site of rich cortical bones in the forearms. By contrast, BMD is either normal or even increased at the lumbar spine in adults with XLH osteomalacia (see later discussion). A lower BMD in tumor-induced osteomalacia (TIO) is more likely age related. Although the patterns of BMD deficits are different in different types of osteomalacia, the findings are neither sensitive nor specific to differentiate osteomalacia from osteoporosis. Accordingly, in vivo tetracycline-labeled bone histomorphometry is the gold standard method to conclusively establish the diagnosis of osteomalacia in suspected cases.[87]

Treatment of Nutritional Rickets and Osteomalacia

Treatment of rickets and osteomalacia must be based on their pathogenesis. Since therapeutic approaches to treat and manage various types of rickets and osteomalacia (nutritional, genetic, and acquired) differ, the treatments that substantially differ will be discussed in the relevant sections in this chapter. In general, there is no "fixed-dose" or "one-size-fits-all" regimen to treat all varieties of rickets and osteomalacia. Several points deserve emphasis in the management of rickets and osteomalacia. First, most recommendations are largely based on personal preferences, clinical experience, and availability of suitable vitamin D preparations. Second, moderate to severe rickets and osteomalacia related to vitamin D and calcium deficiency, but not the hypophosphatemic variety, are frequently associated with hypocalcemia and thus require much higher doses of vitamin D and calcium supplements initially.

Third, it must be kept in mind that patient symptom relief is much faster (a few weeks to a few months) than the biochemical, radiologic, or histologic improvements, which may take a few months to years. Fourth, even after apparent "cure" of clinical, biochemical, radiologic, and bone histologic abnormalities, many patients remain at risk for fractures because of irreversible cortical bone loss. In some patients with long-standing vitamin D–deficiency rickets and osteomalacia, 2°HPT may persist[196] with continued bone loss for years and may even progress to hypercalcemic 2°HPT (or tertiary hyperparathyroidism) requiring parathyroidectomy.[146,197,198] This is analogous to the development of tertiary hyperparathyroidism after long-term oral phosphate therapy in patients with hypophosphatemic (genetic or acquired) osteomalacia,[192] and in patients on chronic maintenance dialysis[199] or after kidney transplantation.[200] Accordingly, the goals of therapy are not only to simply relieve symptoms but also to restore bone strength by mineralizing the excess osteoid and prevent bone loss by correcting 2°HPT.

Several dose regimens have been suggested, each with some merit,[201] but there is no perfect method. Whichever method is chosen, it must achieve the therapeutic goals, and which method to choose depends heavily on the urgency with which vitamin D repletion is needed, local standard of practice, availability of appropriate vitamin D preparations, cost, and potential for lapses in patient adherence. In symptomatic patients with moderate to severe rickets and osteomalacia, we recommend 50,000 IU of either ergocalciferol (vitamin D_2) or cholecalciferol (vitamin D_3) weekly for 8 to 12 weeks followed by a maintenance dose of 1000 to 2000 units daily. Despite the contradictory claims,[202,203] there is not much difference between vitamin D_2 and vitamin D_3 in replenishing depleted vitamin D stores,[203] and both vitamin D preparations are equally effective in treating rickets and osteomalacia. During follow-up, adjustments to the vitamin D dose should be made based on serum and urine levels of calcium, alkaline phosphatase, PTH, and achieved serum 25-hydroxyvitamin D levels, with target levels of 25-hydroxyvitamin D greater than 30 ng/mL and PTH in the reference range. Once achieved, a maintenance dose of 1000 to 2000 IU/day is recommended. Several vitamin D formulations (oral, sublingual, and injectable) are available, and most have comparable therapeutic efficacy, although no formal studies have been conducted. In addition, certain vitamin D metabolites (calcidiol, calcitriol, and alphacalcidol) are available in other countries, but only calcitriol is available in the United States. Perhaps calcidiol (25-hydroxyvitamin D_3), if available, is preferred because it has a shorter half-life (~2 weeks), which is an advantage should hypercalcemia develop during therapy, it can be measured directly to monitor treatment, and the dose can be adjusted based on serum levels. The other active metabolites (calcitriol and alphacalcidol) are not preferred in treating vitamin D–deficiency rickets and osteomalacia but have been used. The active vitamin D metabolites are a bit more expensive and do not provide any major advantage except for more rapid and effective suppression of PTH. Therefore, the use of calcitriol along with vitamin D is suggested in patients with more severe 2°HPT (PTH levels >500 pg/mL), in some patients with significant malabsorption of calcium due to celiac sprue or gastric bypass surgery, in patients with documented bone marrow fibrosis,[4] or in those with compromised kidney function. However, calcitriol should not be either the first-line or sole therapy for vitamin D–deficiency rickets and osteomalacia. Although the therapeutic efficacy of all formulations is comparable, the frequency with which they are administered differs.

In malabsorptive states, particularly in patients with small intestinal resection or gastric bypass surgery, higher doses of vitamin D (10,000–50,000 IU/day) may be required to replete vitamin D stores. Variable strengths of injectable preparations of cholecalciferol (300,000–600,000 units) are available in some countries but not widely in the United States and may be preferable in specific situations, such as in patients with significant malabsorption or poor compliance or intolerance to oral preparations. Compared with parenteral administration, the rise in serum 25-hydroxyvitamin D levels is rapid with oral preparations. Careful close follow-up is essential during the first few months (1–3 months) of treatment to monitor treatment-related adverse events, such as hypercalcemia, hypercalciuria, and renal dysfunction. Occasionally, high-dose vitamin D supplementation may unmask underlying primary hyperparathyroidism (see Chapter 29).

Vitamin D deficiency of sufficient severity to produce osteomalacia is invariably associated with decreased intestinal calcium absorption and negative calcium balance. Accordingly, oral calcium supplements in the form of calcium carbonate (or citrate) 1000 to 1500 mg/day in divided doses must be prescribed with vitamin D administration to accomplish not only clinical and biochemical but also radiologic and bone histologic responses. With effective therapy, symptoms of osteomalacia start improving within a few weeks, but complete disappearance of symptoms usually takes a few months and sometimes years.

Although nutritional rickets is mostly curable, osteomalacia is only treatable but not curable and requires long-term maintenance therapy largely dictated by the clinical and biochemical responses. Complete healing of osteomalacia and resolution of 2°HPT must be ensured before commencing antifracture therapy for the associated osteoporosis (see Chapter 30). Almost all patients with vitamin D deficiency due to gastrointestinal disorders, resection, or bypass require lifelong vitamin D supplements/therapy and regular follow-up to prevent recurrence of osteomalacia due to lapses in therapy. Nonadherence to long-term therapy is not uncommon because both the patient and sometimes the treating physician might assume that the condition is "cured" based on clinical symptoms, and therefore there is no need for continued therapy.

Rickets Due to Genetic Disorders of Vitamin D Metabolism

Vitamin D–Dependent Rickets Types 1A, 1B, and 2

There are three unique forms of rickets due to genetic disorders, resulting in the loss of function of the enzymes required for vitamin D biologic activation.[204] A unique case of osteomalacia resistant to high-dose vitamin D therapy was reported in 1979, perhaps the first documented case of osteomalacia due to possible defective vitamin D 25-hydroxylase,[160] although evidence presented was less than convincing, and no additional similar cases of osteomalacia in adults have been reported since. Severe rickets despite adequate vitamin D intake that responded only to high doses of vitamin D_2 in two siblings was probably the first example of rickets due to a possible genetic defect in the 25-hydroxylase enzyme.[114] Recent genetic studies suggest that mutations in the *CYP2R1* gene,[112,113] the principal 25-hydroxylase in humans, are responsible for the severe atypical form of vitamin D deficiency and rickets, although no mutations were found in another child with a similar phenotype.[118]

The genetic transmission appears to be both autosomal dominant and recessive, and this rare atypical form rickets is now designated as vitamin D–dependent rickets type 1B (VDDR1B).[205] The other two types—vitamin D–dependent rickets type 1A (VDDR1A) and vitamin D–dependent rickets type 2 (VDDR2)—are due either to a defective 25-hydroxyvitamin D 1α-hydroxylase, the critical enzyme required in the final step of vitamin D biologic activation,[204] or to an end-organ resistance to vitamin D action due to loss of vitamin D receptors,[206] respectively. Impairment of 1α-hydroxylase enzyme activity can also be acquired, as in chronic kidney disease, various renal tubular disorders,[99] or excess or ectopic production of FGF23, known to inhibit 1α-hydroxylase enzyme activity.[207] Genetic defect in the 25-hydroxyvitamin D 1α-hydroxylase enzyme, VDDR1A, or pseudo–vitamin D deficiency, is a rare autosomal recessive disorder due to mutations in the cytochrome P450 (*CYP27B1*) gene for the 1α-hydroxylase enzyme located on the chromosome 12q13.3, resulting in decreased serum levels of 1,25-dihydroxyvitamin D.[208,209] Rickets develops during the first year of life, and clinical features include growth failure, hypotonia, weakness, convulsions, tetany, open fontanels, and pathologic fractures, as well as oral and dental manifestations.[209] As can be predicted, the condition responds to the physiologic doses (0.04 μg/kg per day) of active vitamin D metabolite, 1,25-dihydroxyvitamin D or calcitriol, but requires much higher doses of the parent compound vitamin D (~10,000 units/day) to heal rickets—hence the name vitamin D–dependent rickets. Similar to VDDR1A, but even more resistant to vitamin D therapy, is due to defects in the vitamin D receptor, designated as VDDR2.[206] Children with VDDR2 have alopecia, a very unique feature that distinguishes VDDR2 from both VDDR1A and VDDR1B. However, the prevalence of alopecia is variable as is its extent of involvement ranging from alopecia of the head to alopecia of the entire body—"alopecia universalis." Interestingly, although the biochemical and radiologic abnormalities in VDDR2 respond to high-dose vitamin D or calcitriol and phosphate therapy, alopecia does not. Vitamin D receptors are lacking in fibroblasts of the skin.[210]

Biochemical and radiologic features of vitamin D–dependent rickets are similar to those seen in nutritional rickets, except serum 25-hydroxyvitamin D levels are normal in VDDR1A and VDDR2, and low or very low in VDDR1B (see Table 31.4). Because of the low or very low serum 25-hydroxyvitamin D levels, the biochemical findings in VDDR1B resemble those of nutritional vitamin D–deficiency rickets (see Table 31.4) and thus may be misdiagnosed as nutritional rickets. Family history, adequate vitamin D intake, lack of clinical and biochemical response to standard-dose vitamin D therapy, and lack of rise in serum 25-hydroxyvitamin D levels with vitamin D therapy should raise suspicion for the possibility of this very rare inborn error of vitamin D metabolism due to mutations in the *CYP27B1* gene.[205,211]

Both VDDR1A (due to 25-hydroxyvitamin D 1α-hydroxylase deficiency) and VDDR1B (due to vitamin D 25-hydroxylase deficiency) respond to physiologic replacement doses of calcitriol (0.04 μg/kg per day), just as nutritional rickets.[208] However, patients with VDDR2 require much higher doses of vitamin D or calcitriol because of end-organ resistance as a result of vitamin D receptor defects.[206] All children with vitamin D–dependent rickets regardless of the type require adequate calcium and phosphate supplements during therapy with calcitriol to achieve healing of the rickets and suppress 2°HPT. Hypercalciuria and hypercalcemia are potential complications during long-term therapy. All patients must be monitored closely, regularly, and throughout their lifetime.

Hereditary Hypophosphatemic Rickets and Osteomalacia

There are several types of hereditary rickets that in the past were collectively referred to as vitamin D–resistant rickets because of the very high doses of vitamin D required to cure rickets.[50,95,190,212] These rare types of rickets can be distinguished from the other types of rickets by the pattern of their inheritance, underlying genetic defects, and hypophosphatemia with normal serum calcium level, and if they are FGF23 dependent or independent.[6,7,95] The most common type of rickets and osteomalacia among them and clinically most relevant is XLH rickets and osteomalacia.

Autosomal Dominant and Recessive Rickets

Autosomal dominant hypophosphatemic rickets (ADHR) is caused by mutations in the *FGF23* gene,[212] whereas inactivating mutations in the dentin matrix protein (DMP1) and the ectonucleotide pyrophosphatase/phosphodiesterase 1 (*ENPP1*) genes are responsible for autosomal recessive hypophosphatemic rickets (ARHR) type 1 and type 2, respectively.[212,213] In patients with ADHR, there are mutations in several amino acids (Arg^{176} or Arg^{179}) resulting in resistance to proteolytic processing. In addition, the degree of hypophosphatemia correlates with the intact FGF23 levels.[95] In patients with FGF23-dependent hypophosphatemia due to mutations in the *DMP1* gene, there are deformities of the legs and short stature in childhood, similar to the clinical phenotype of nutritional rickets.[7,95] ARHR typically manifests during childhood with characteristic clinical features of rickets[7]; however, in adulthood, ARHR patients may manifest with bone pain, fatigue, muscle weakness, and repeated bone fractures.

X-Linked Recessive Hypophosphatemic Rickets

X-linked recessive hypophosphatemic rickets—a rare genetic form of hypophosphatemic rickets also known as the Dent disease complex—comprises of a group of heterogeneous inherited disorders characterized by a proximal renal tubular reabsorptive disorder of the Fanconi type.[50] It is caused by missense, nonsense, frameshift, and splicing mutations in genes located on the chromosome Xp11.22 and X25.[50,214,215] Two subtypes are known to exist: type 1 (~50–60% of cases), caused by an inactivating mutations in the chloride channel 5 (*CLCN5*) gene that codes for a chloride-proton exchanger, and type 2 (~15% of cases), by inactivating mutations in the oculocerebrorenal syndrome (*OCRL*) gene located on an X chromosome that codes for inositol polyphosphate 5-phosphatase OCRL-1.[50] Similar to other types of genetic or acquired forms of Fanconi syndrome,[9,156,216] Dent disease is associated with hypercalciuria with variable other proximal renal tubular dysfunction, nephrocalcinosis or nephrolithiasis, low-molecular-weight proteinuria, and progressive renal insufficiency, but only a minority of patients manifest rickets.[214] In addition to hypercalciuria and proteinuria, the disease may be associated with defective renal tubular reabsorption of one or more of the following solutes: glucose, phosphate, uric acid, potassium, bicarbonate, and amino acids. However, unlike all other forms of hereditary rickets, Dent disease is the only type of genetic rickets associated with nephrocalcinosis and nephrolithiasis, and it is the least common of all types of rickets.

X-Linked Hypophosphatemic Rickets and Osteomalacia

XLH (OMIM #307800) is an X-linked dominantly inherited disorder with an estimated prevalence of about 1 in 20,000 live births.[7] It is the most common form of hereditary

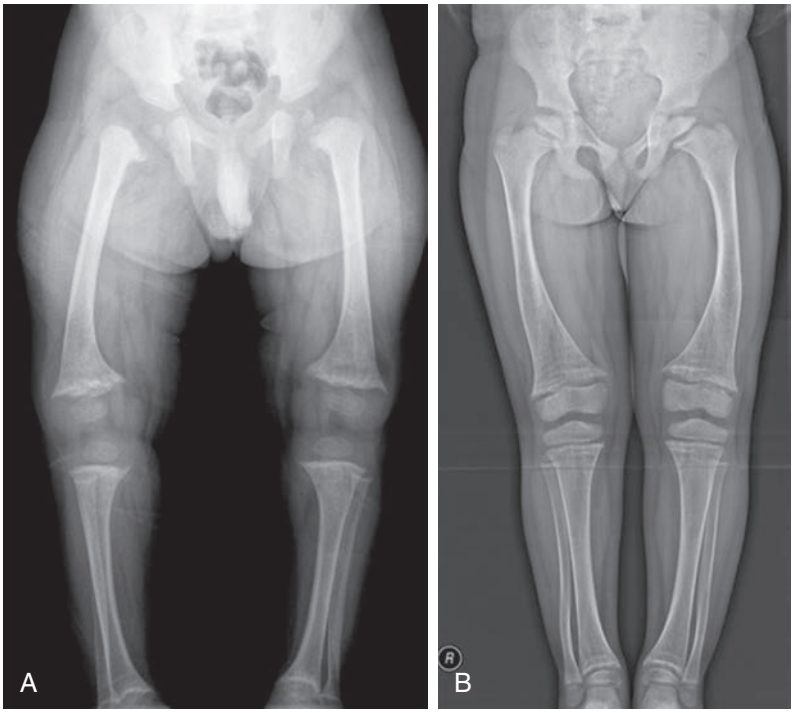
hypophosphatemic rickets, caused by an inactivating mutation in the phosphate-regulating gene with homologies to endopeptidases on the X chromosome (PHEX).[6,7,95,212] PHEX is expressed on the cell surface of bones and teeth. It is the most common form of FGF23-mediated hypophosphatemic rickets, but the substrate for PHEX remains largely unknown.[217] More than 300 types of mutations in the *PHEX* gene have been reported in patients with XLH, and the mutations may be de novo in some patients. The prevailing hypothesis is that the mutations found in the *PHEX* gene in patients with XLH are likely to enhance the production of FGF23 in bones, but the pathogenic implications to the clinical expression of the disorder are unclear.[7,95,212,217] Nevertheless, the unifying hypothesis proposed by Jan de Beur and Levine is most convincing and probably the most clinically relevant.[5] It is worthwhile to restate the concept: Although TIO, XLH, and ADHR all have overlapping phenotypical features, all three types of rickets share a common biochemical phenotype—*hypophosphatemia* as a result of defective renal tubular reabsorption of phosphate. Current evidence supports the notion that under normal physiologic conditions, the concentration of FGF23 in serum (and possibly other tissues) is regulated by PHEX-dependent proteolysis. However, conditions with excess circulating FGF23 concentrations or activity are associated with a marked depression in the proximal renal tubular reabsorption of phosphate and hypophosphatemia. When PHEX is inactive, as in patients with XLH, FGF23 is not degraded and accumulates in the circulation. In ADHR, missense mutations replace the key amino acids in FGF23 and render the protein resistant to proteolysis, thereby leading to increased circulating FGF23 concentrations or action. Similarly, ectopic overproduction of FGF23 by tumors causing TIO (see the following) may saturate the capacity of endogenous proteolytic enzymes, such as PHEX, to degrade FGF23. It is clear that FGF23 plays a central role in the pathogenesis of these disorders; however, the role of FGF23 in normal physiology and the mechanism(s) by which it regulates renal phosphate handling or calcitriol synthesis is not fully understood.

The clinical features of XLH are variable, with most patients presenting with rickets during childhood (Fig. 31.6). In childhood-onset XLH, skeletal deformities such as bowed legs and short stature are common. In adults, XLH may be discovered during a routine biochemical work-up revealing hypophosphatemia. Symptoms in adults resemble those seen in osteomalacia, such as bone pain, insufficiency fractures, muscle weakness that is often disabling, neurologic complications related to enthesopathy, and ectopic calcifications. In addition, dental disease, such as root abscesses, often develop due to a defect in dentin and enamel microdefects.[218]

Radiologic and Biochemical Findings

The radiologic features of all hereditary rickets are similar to those seen in nutritional rickets, except metaphyseal involvement is slightly asymmetrical and bowing is slightly more common (see Fig. 31.6). As adults, most patients are obese and manifest a disproportionate short stature with greater shortening of the lower extremities. However, none of these findings is specific enough to distinguish the type of rickets, but enthesopathy occurs almost exclusively in XLH and is almost never seen in other types of rickets and osteomalacia.

The most common and consistent biochemical findings are hypophosphatemia, renal phosphate wasting as assessed by tubular reabsorption of phosphate or tubular maximum for phosphate



• **Fig. 31.6** Nutritional vitamin D–deficiency (A) and X-linked hypophosphatemic (B) rickets in 5-year-old black and 5-year-old white children. Typical metaphyseal widening, fraying, and cupping at the ends of all of the long bones can be seen. Note the similarities, but a few subtle differences can be appreciated. Bowing is more pronounced and cortices are thicker in X-linked hypophosphatemic rickets (B) than in nutritional rickets (A).

reabsorption adjusted for glomerular filtration rate (GFR; TmP/dlGFR), and elevated serum alkaline phosphatase levels. Serum calcium, 25-hydroxyvitamin D, and PTH levels are characteristically normal in the untreated state. Serum FGF23 levels are inappropriately elevated in the context of chronic hypophosphatemia.

Although the constellation of clinical and biochemical findings is unmistakably diagnostic, the differential diagnosis of XLH includes nutritional rickets, metaphyseal dysplasia, physiologic bowing, and other forms of renal phosphate wasting disorders. In a patient with rickets with chronic hypophosphatemia and inappropriately elevated serum FGF23 levels, a diagnosis of FGF23-related hypophosphatemia is highly likely. Considering the possibility of de novo mutations in the *PHEX* gene, an absence of family history of rickets does not necessarily exclude XLH. Genetic testing is not a prerequisite for a clinical diagnosis of XLH.

Treatment of Hereditary Hypophosphatemic Rickets and Osteomalacia

Standard Treatments

The standard of practice for the treatment of XLH in children includes a combination of active vitamin D metabolites (calcitriol or alphacalcidol) and oral phosphate supplementation.[7] Skeletal deformities and growth retardation may improve with treatment but do not completely resolve. In adults, these same medications are used for symptom management and to improve impaired bone mineralization. In one observational study, treatment of adult patients with XLH reduced pain symptoms and improved fracture healing following orthopedic surgery but did not prevent or reverse enthesopathy.[219] In one prospective study, 6 years of treatment showed increases in bone resorption markers albeit without changes in spine and hip areal BMD.[220]

Novel Treatments

Recently, the use of burosumab, a recombinant human immunoglobulin G1 monoclonal antibody that binds to the FGF23 receptor and inhibits its activity, was evaluated in a phase 1 double-blind, placebo-controlled randomized clinical trial.[221,222] With a single dose, burosumab increased the TmP/GFR, and serum levels of phosphate and 1,25-dihydroxyvitamin D.[221,222] The peak serum phosphate concentration achieved was between 8 and 15 days and returned to the baseline within 50 days after the initial subcutaneous injection. In a subsequent phase 1/2 open-label, dose-escalation study, monthly burosumab resulted in a sustained improvement in TmP/GFR, and in serum phosphate and 1,25-dihydroxyvitamin D levels, with a favorable safety profile.[221,222] However, at present, it is not clear if these early clinical trials will have long-lasting effects on clinical and radiologic resolution of rickets and osteomalacia. Several clinical trials with burosumab in patients with both XLH and TIO are currently under way.

Long-Term Management

Treatment with active vitamin D metabolite, calcitriol, and oral phosphate supplements has the potential for adverse effects over the long term. Hypercalciuria with or without hypercalcemia may develop and may lead to nephrolithiasis, nephrocalcinosis, and impaired renal function. Use of oral phosphate supplements causes diarrhea and abdominal pain, which in turn leads to poor medication adherence. An unintended consequence of long-term (usually years) oral phosphate therapy is the development of 2°HPT, which may evolve into hypercalcemic 2°HPT (also referred to as tertiary hyperparathyroidism).[223–225] Several treatment strategies for tertiary hyperparathyroidism have been suggested depending on the degree of hypercalcemia, severity of hyperparathyroidism, and clinical status.[192,224] Cinacalcet, which produces a relative hypoparathyroidism, radiofrequency ablation of the tumor to reduce ectopic production of FGF23, and deliberate total parathyroidectomy have all been tried with variable success.[192]

Tumor-Induced Osteomalacia

TIO was first reported by Robert McCance in 1947,[226] but the first "proof of concept" was provided by Andrea Prader in 1959, who postulated the production of a "rachitogenic substance" by a "giant cell reparative granuloma of bone," the removal of which cured the young patient's rickets.[227] Interestingly, the discovery of FGF23 and characterization of its function as a major phosphate-regulating hormone did not occur until a few decades later.[228] The condition is conventionally referred to as osteomalacia, as most cases are detected in adults, but rickets in children has been reported.[226,227] Nearly 500 cases of TIO have been reported in the literature; the majority are adults with a mean age of 45 years at the time of diagnosis and a wide age range, and there does not appear to be any gender predilection.[8]

TIO is a rare paraneoplastic syndrome that clinically manifests with diffuse nonspecific bone pain, profound muscle weakness, and fractures.[8,88,89,229] The pathogenesis is related to the ectopic production of FGF23—a phosphatidic hormone secreted by small mesenchymal tumors.[228] The biochemical hallmark of TIO is the triad of hypophosphatemia due to renal phosphate wasting, inappropriately low or normal serum 1,25-dihydroxyvitamin D level, and elevated or inappropriately normal serum FGF23 level.[8,207]

FGF23, produced exclusively by osteocytes and osteoblasts,[6,230] exerts its effects on the kidney and parathyroid glands to maintain normal phosphate homeostasis and regulate 1,25-dihydroxyvitamin D synthesis and metabolism.[6,95,207] The kidney is the principal target for FGF23, where it regulates renal tubular reabsorption of phosphate and production of 1,25-dihydroxyvitamin D.[6,95,179,207] FGF23 inhibits both sodium-dependent phosphate reabsorption and 1α-hydroxylase activity in the proximal tubule, leading to hypophosphatemia and aberrant production and inappropriately low levels of 1,25-dihydroxyvitamin D. The combination of hypophosphatemia and "low" 1,25-dihydroxyvitamin D causes muscle weakness and mineralization defect, respectively.

TIO is usually caused by tumors of mesenchymal origin, referred to as phosphaturic mesenchymal tumor of mixed connective tissue variant, and rarely by other types of tumors, such as osteosarcoma, giant cell tumor, glomus tumor, small cell carcinoma of the lung, and adenocarcinoma of the colon.[8,88,89] These tumors tend to be small (often escaping clinical detection), slow growing,[231] and difficult to localize; about half of the tumors are located in the skeleton, and the remaining are located in soft tissues. Histology of the tumors reveals neoplastic spindled and stellate-shape cells with low nuclear and mitotic activity that stain for FGF23.[229] Although FGF23 is the predominant secretory product of the tumors and the most common phosphatonin, other phosphatonins such as frizzled-related protein 4, FGF7, and matrix extracellular phosphoglycoprotein have been described.

Patients with TIO usually present with long-standing and progressive debilitating symptoms that go undiagnosed for years, but an occasional patient may cruise along without necessarily suffering, except for the progressive slow growth of the tumor over

TABLE 31.5 Key Abnormalities in Various Types of Rickets and Osteomalacia

Variable	Vitamin D Deficiency	Vitamin D–Resistant Hypophosphatemic	Tumor-Induced Osteomalacia	Drug-Induced Osteomalacia
Basic defect	Nutritional/malabsorption	*PHEX* gene defect Defective catabolism of FGF23	Ectopic production of FGF23	Renal tubular damage Direct effect on bone mineralization
FGF23 levels	Not applicable	Usually high	Almost always high	High/variable
Serum calcium	Low/normal	Normal/high	Normal	Variable
Serum phosphate	Normal/low	Very low	Low/very low	Variable
Alkaline phosphatase	High	High	High	Variable
Parathyroid hormone	High	Normal/high	Normal	Variable
25-Hydroxyvitamin D	Low	Normal	Normal	Low/normal
1,25-Dihydroxyvitamin D	Variable	Low	Low	Low/variable
Bone mineral density	Low	Normal/high	Often low	Often low
Therapeutic strategy	Vitamin D + Calcium	Calcitriol and phosphate Burosumab	Resection of the tumor Other therapies (see text)	Discontinuation of offending drug

FGF23, Fibroblast growth factor 23; *PHEX*, phosphate-regulating endopeptidase homolog, X-linked.

decades causing minimal disability.[231] Symptoms can be nonspecific, and the most common complaints are bone pain, fatigue, muscle weakness, and multiple fractures. Many patients with TIO receive various erroneous diagnoses (rheumatologic, malignant, and even psychiatric) and, consequently, inappropriate treatments for years before the tumor causing osteomalacia is found.[88]

Laboratory evaluation of a patient suspected of having TIO should begin with measurement of serum phosphate, preferably in the fasting state. Since hypophosphatemia is uncommon in the ambulatory nonhospitalized patient, a serum phosphate level less than 2.5 mg/dL should raise suspicion in the right clinical setting. Elevated serum alkaline phosphatase supports the clinical suspicion. Other relevant biochemical abnormalities are summarized in Table 31.5. Once hypophosphatemia is detected and confirmed, further assessment of the renal tubular handling of phosphate (tubular reabsorption of phosphate and TmP/GFR: tubular maximum for phosphate reabsorption per unit of GFR; also see Chapter 29) should be performed, and a low value for either measurement clinches the diagnosis of a phosphate-wasting condition.

As in genetic hypophosphatemic disorders, serum levels of calcium, 25-hydroxyvitamin D, and PTH are normal in TIO, but similar to genetic disorders, 1,25-dihydroxyvitamin D levels are either inappropriately "normal" or low (see Table 31.5). Interestingly, serum levels of alkaline phosphatase are elevated in all varieties of hypophosphatemic rickets and osteomalacia despite low or absent bone remodeling, and the reason for this biochemical abnormality is not well understood.

Serum FGF23 levels are elevated in the majority of, but not all, patients with TIO and depend on the type of assay used (intact vs. C-terminal fragment).[232] Although an elevated level of FGF23 supports diagnostic suspicion, a normal serum FGF23 level does not exclude the diagnosis. A positive family history of rickets or osteomalacia, or presence of metabolic acidosis, makes TIO unlikely. In addition, serum FGF23 levels do not distinguish different types of hypophosphatemic rickets and osteomalacia. Similarly, tumors with demonstrable FGF23 expression by immunohistochemistry may not necessarily cause osteomalacia and are referred to as nonphosphaturic variants. Whether they represent a separate entity or simply the preclinical stage of the disease is unknown, because preoperative serum phosphate and FGF23 levels have not been reported in any of the so-called nonphosphaturic tumors.[229]

Radiologic and radionuclide manifestations of TIO are similar to those seen in other types of rickets and osteomalacia (see the following) with some exceptions: unlike genetic hypophosphatemic but similar to nutritional deficiency disorders, BMD is low, most likely the result of some combination of age-related bone loss and impaired bone mineralization due to hypophosphatemia. In addition, unlike XLH but similar to nutritional rickets and osteomalacia, enthesopathy is not seen in TIO, but fractures are common. Except for higher osteoid volume, which may remain high even after clinical "cure," bone histologic features are similar to other types of osteomalacia.[62,84,87]

Localization of the tumor can be quite challenging because most are small tumors, often found in obscure locations. A detailed physical examination followed by imaging is necessary. Since somatostatin receptors are expressed in many phosphaturic mesenchymal tumors of mixed connective tissue variant, an octreotide scan can help with tumor localization in about 50% of cases, especially when the extremities and skull are involved. 18F-fluorodeoxyglucose positron emission tomography is quite sensitive in localizing tumors but can lead to false-positive results. Gallium-DOTATATE positron emission tomography is an emerging imaging modality for tumors producing TIO, is now more widely available, and may be the imaging method of choice. Functional imaging can be supported by selective venous sampling with measurement of FGF23 to confirm the location of the tumor in cases in which the imaging studies suggest multiple lesions or the location of the single lesion poses a significant surgical risk.[8,89]

The treatment of choice for TIO is resection of the tumor, which results in clinical, biochemical, radiologic, and bone

histologic improvements. Wide surgical resection is essential to avoid tumor recurrence. Levels of serum phosphate and FGF23, which has a half-life of ~45 minutes, return to normal rapidly, often within 24 hours, after tumor resection, but healing of osteomalacia may take several months. Rarely, recurrences with metastasis can occur. If the tumors are not readily identifiable, or not amenable to surgical removal, lifelong medical therapy with oral phosphate and calcitriol is required. Oral phosphate in three to four divided doses with meals and calcitriol 0.5 to 1.0 μg/day in divided doses to maintain serum phosphates level at the lower end of the age-appropriate reference range is recommended. However, long-term oral phosphate therapy may lead to secondary and occasionally hypercalcemic 2°HPT (tertiary hyperparathyroidism) that requires surgical intervention.[192,225,233,234] Other potential treatment options in situations where the tumor cannot be surgically removed or localized, use of calcium-sensing receptor agonists, radiofrequency ablation of the tumor, or deliberate total parathyroidectomy can be considered.[192,235–237]

Drug-Induced Osteomalacia

Several drugs have been implicated in the pathogenesis of rickets and osteomalacia, and the most relevant drugs are listed in Table 31.6, along with the proposed mechanisms by which various drugs cause mineralization defects. Among them, nucleoside reverse transcriptase inhibitors (NRTIs), tenofovir and adefovir, are currently the most common causes of drug-induced rickets and osteomalacia.[9,156] Other drugs that cause rickets or osteomalacia, although less common, include anticonvulsants, aluminum-containing antacids, non-nitrogen containing bisphosphonates, and sodium fluoride.

Tenofovir and adefovir, adenosine analogue NRTIs, are now widely used in the treatment of human immunodeficiency virus and viral hepatitis infections. Sporadic Fanconi syndrome, renal failure, and osteomalacia have been reported in patients treated with this class of drugs.[9,156] Fanconi syndrome is a generalized transport defect in the proximal tubules, resulting in losses of glucose, phosphate, calcium, uric acid, amino acids, bicarbonate, and other organic compounds leading to hypophosphatemia, metabolic acidosis, and glycosuria.[216] Phosphate depletion due to renal phosphate wasting results in hypophosphatemic rickets and osteomalacia similar to the genetic and tumor-induced hypophosphatemic disorders (see later discussion). However, unlike other hypophosphatemic syndromes, serum FGF23 levels are characteristically normal in NRTI-related hypophosphatemia.[238] Studies have shown that a time interval between initiation of NRTI treatment and the development of Fanconi syndrome varies from 1 to 26 months, and the prevalence of osteomalacia is about 0.5% in patients receiving the drugs.[9,156] Concomitant use of ritonavir-boosted protease inhibitor increases NTRI toxicity because protease inhibitors increase intracellular concentrations of the drugs.[9,156,216] The exact mechanism of tenofovir-induced nephrotoxicity is not well understood, but it appears to be due to mitochondrial damage and alterations in human organic anion transporter 1.[216] Proximal renal tubular mitochondrial injury due to the inhibition of mitochondrial DNA polymerase results in decreased mitochondrial DNA replication and impairs molecular transport, vitamin D activation, and urinary acidification.[216] There is a modest decline in renal function that can become relevant in patients with preexisting renal dysfunction.[216] Discontinuation of the causative drug promptly corrects hypophosphatemia and renal dysfunction if detected early, but additional therapy with oral phosphate and vitamin D or its metabolites is required for patients with osteomalacia.[9,156]

TABLE 31.6 Causes of Drug-Induced Rickets and Osteomalacia

1. Drugs that cause renal tubular defects (Fanconi syndrome)
   - Nucleotide reverse transcriptase inhibitors
     - Tenofovir
     - Adefovir
     - Cidofovir
     - Protease inhibitor
     - Ritonavir
2. Drugs that produce "conditional vitamin D deficiency"
   - Cytochrome P450 enzyme inducers
   - (Increased catabolism of 25-hydroxyvitamin D)
   - Phenytoin
   - Phenobarbital
   - Primidone
   - Carbamazepine
     - Inhibits enzyme activity
   - Sodium valproate
3. Inhibition of 25-hydroxyvitamin D 1α-hydroxylase
   - Ketoconazole
4. Inhibition of osteoid mineralization
   - Aluminum
   - Fluoride
   - Iron
   - Etidronate

Anticonvulsants, phenytoin, primidone, phenobarbital, and rifampin induce the hepatic cytochrome P450 oxidase enzyme system, which increases the conversion of vitamin D to polar inactive metabolites in the liver, reducing the bioavailable 25-hydroxyvitamin D.[239,240] The resulting conditional vitamin D deficiency, if prolonged and severe, ultimately leads to rickets and osteomalacia.[46,239,241,242] The clinical manifestations, biochemical changes, and radiologic and bone histologic features are similar to those seen in patients with nutritional vitamin D deficiency and defective genetic 25-hydroxylase or VDDR1B (see later discussion). Treatment with vitamin D and calcium in the recommended doses for nutritional vitamin D deficiency is usually effective (see later discussion). Unlike in the case of NRTIs, there is no need to discontinue the drugs that caused the problem in the first place, but lifelong monitoring for vitamin D deficiency is required. Although both isoniazid and ketoconazole inhibit 1α-hydroxylase enzyme in the kidney, resulting in conditional vitamin D deficiency, cases of rickets and osteomalacia have not been reported.

In earlier times, a unique form of "vitamin D–resistant" osteomalacia related to the use of tap water in dialysate solution and aluminum-containing phosphate binders was seen exclusively in patients on maintenance hemodialysis.[243–245] Aluminum is preferentially deposited at the interface of mineralized and unmineralized (osteoid) bone, and uncouples matrix synthesis and its subsequent mineralization, resulting in excess osteoid accumulation.[246] With the use of deionized water, reverse osmosis, and abandonment of aluminum-containing phosphate binders, aluminum-induced osteomalacia has all but disappeared.[243,244,246] A similar form of osteomalacia has been reported with the use of sucralfate, another aluminum-containing antacid, particularly if used in conjunction with aluminum-containing phosphate binders or antidiarrheal preparations.[42]

Osteomalacia due to iron deposition is more complex.[247,248] Interestingly, both aluminum and iron co-localize in bone at the mineralized bone–osteoid interface, and some patients receiving

iron infusions develop FGF23-mediated hypophosphatemic osteomalacia.[247,248]

Drug-induced osteomalacia has rarely been reported with the use of etidronate and sodium fluoride, usually in high doses and over long periods, but no currently approved second-generation nitrogen-containing bisphosphonates have been reported to cause osteomalacia.[87] Neither etidronate nor sodium fluoride is approved for the prevention or treatment of osteoporosis (see Chapter 30) in the United States, but both drugs are approved for management of osteoporosis in other countries, including Canada. In addition, etidronate is used in high doses (up to 20 mg/kg per day) to treat rare bone and mineral disorders such as fibrous dysplasia, heterotopic ossification, and myositis ossificans with some success. Since the etidronate dose used in these conditions is much higher than the recommended dose (5 mg/kg per day) for osteoporosis and Paget disease of bone, the risk of osteomalacia exists; the exact scope of this complication is currently unknown.

Bone histologic features of drug-induced mineralization defects due to aluminum, iron, etidronate, and sodium fluoride differ substantially from the osteomalacia as defined earlier due to vitamin D and phosphate deficiency (see the earlier Definition and Histologic Evolution of Osteomalacia section). Defective bone mineralization due to aluminum overload, the osteoid accumulation is generalized, as in vitamin D deficiency and hypophosphatemic osteomalacia, but osteoid thickness is not increased and may even be thinner. This type of bone histologic abnormality is designated as atypical osteomalacia[87] (see Fig. 31.2). Bone turnover is extremely low, staining for aluminum is positive at the osteoid-mineralized bone interface establishing the diagnosis, and the bone lesion does not respond to vitamin D therapy. Osteoid accumulation in etidronate- and sodium fluoride–related osteomalacia is patchy with very thick osteoid seams randomly distributed throughout bone (both on bone surfaces and within the interstitial bone), which is designated as focal osteomalacia (see Fig. 31.2). A full exposition on the evolution of such bone lesions has recently been reviewed.[87]

Conditions That Resemble Rickets and Osteomalacia

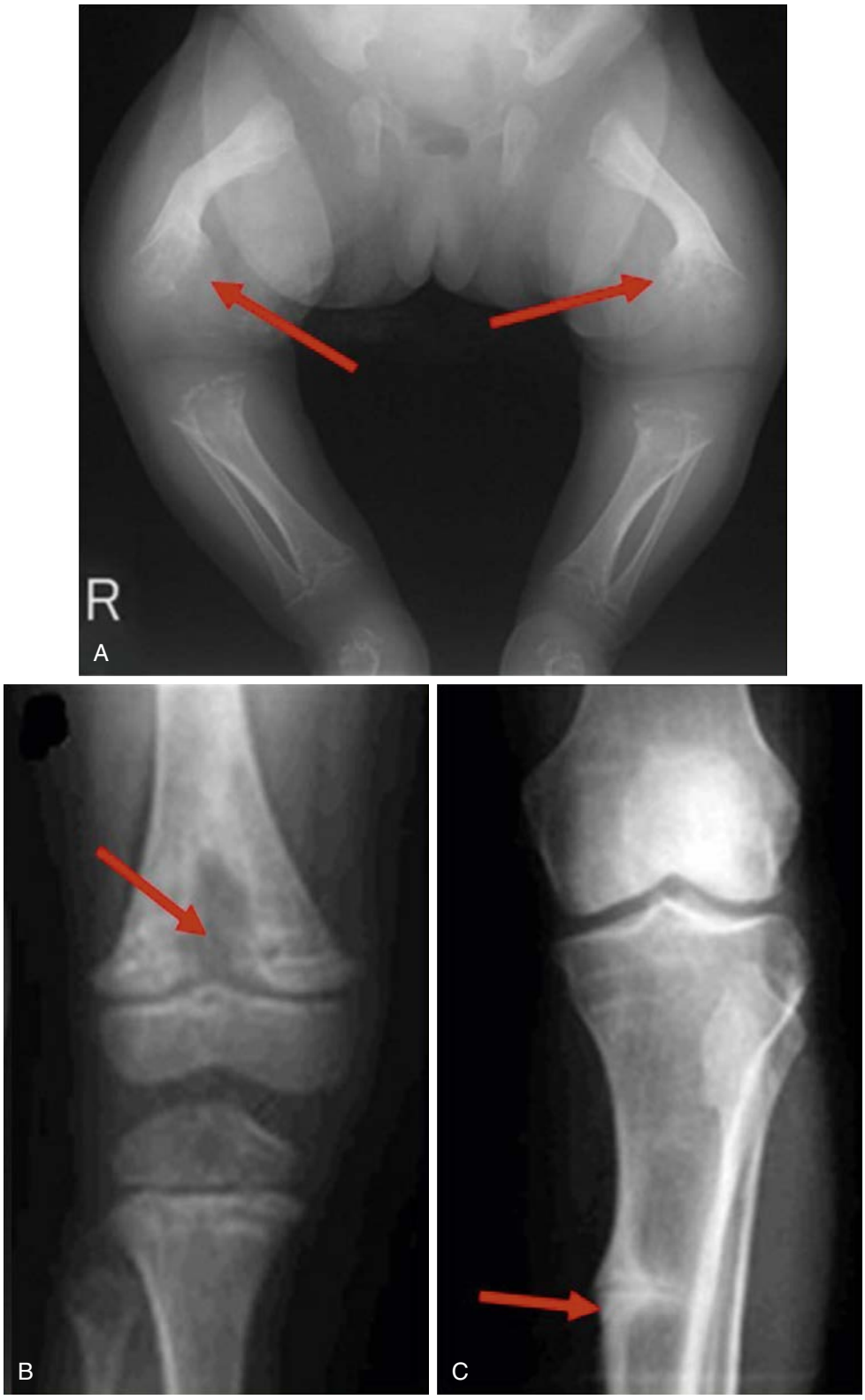
Several other bone diseases, some rather poorly characterized, resemble rickets and osteomalacia in one respect or another, but all have unique distinguishing features that separate them from classical rickets on x-rays or osteomalacia on bone histology. Much of the confusion has arisen because of the extension of the terms *rickets* and *osteomalacia* to include these other disorders without regard to the mechanisms by which the abnormalities occur. By not conforming to the strict definitions for *rickets* and *osteomalacia*, the terms lose their clinical relevance and might give a false impression that the conditions might respond to vitamin D administration. In fact, many of these conditions were once treated with large doses of vitamin D without much improvement in clinical, radiologic, or bone histologic features.

Pathogenesis of many of these rare bone disorders is currently poorly understood. The mechanism for the development of radiologic abnormalities and mineralization defect is different from that of classical rickets and osteomalacia. In most, but not all, conditions listed in Table 31.6, the abnormalities are either due to the effects of PTH excess on bone or to the defects in bone collagen matrix structure that does not mineralize normally.

In children with primary hyperparathyroidism, metaphyseal abnormalities resemble rickets or a child might have rickets due to both vitamin D deficiency and masked primary hyperparathyroidism. The radiologic abnormalities respond to parathyroidectomy. Similarly, in severe 2°HPT in children with end-stage renal disease, both "rickets" and "osteomalacia" have been noted. Any condition that increases bone remodeling inevitably increases the extent of osteoid surface (usually <50% of the bone surface) and by extension osteoid volume (usually >3–5% of bone volume), but osteoid thickness, the hallmark of mineralization defect in traditional osteomalacia, is always normal (<12 μm). Some have used the term *hyperosteoidosis* to describe this type of histologic abnormality, which is seen in conditions associated with increased bone remodeling, such as renal osteodystrophy, hyperthyroidism, primary hyperparathyroidism, and osteitis deformans (Paget disease of bone). However, the use of such a descriptive term as *hyperosteoidosis* does not serve any useful purpose and potentially confuses our understanding of these various disorders.

In certain very rare disorders, such as fibrogenesis imperfecta ossium and axial osteomalacia, various degrees of defective mineralization are seen, but they are due to abnormal collagen structure. Bone histomorphometric measurements in these disorders differ substantially from those of classical osteomalacia as defined earlier.

One rare bone disorder deserves special mention in the context of rickets and osteomalacia. Hypophosphatasia is now reasonably well characterized, and enzyme replacement therapy with asfotase alfa was recently approved for childhood onset of the disease. It is due to "loss of function" mutations in the gene that codes for the tissue nonspecific alkaline phosphatase. It is an autosomally inherited disorder with more than 300 different gene defects reported so far. Although radiologic and bone histologic findings may resemble rickets and osteomalacia, the condition is easily distinguished by the low serum alkaline phosphatase levels (<40 IU/L). Age-appropriate references ranges must be used for children and adolescents, and many laboratories now provide a lower limit for serum alkaline phosphatase ranges. As noted previously, the serum alkaline phosphatase is almost always elevated in vitamin D– and phosphate-deficiency rickets and osteomalacia. Bowing of long bones with insufficiency fractures resemble radiologic features of osteomalacia in adults, and metaphyseal and epiphyseal changes resemble those of rickets in children (Fig. 31.7A and B). The tongue-like lucent in the metaphyses of the long bones, especially in the distal femur, is characteristic of childhood hypophosphatasia (see Fig. 31.7B). However, cortical thinning, a feature of vitamin D–deficiency osteomalacia, is not seen in hypophosphatasia. In addition, pseudofractures are usually located on the lateral cortex of long bones, unlike the medial location in osteomalacia related to vitamin D and phosphate deficiency, but this feature lacks diagnostic specificity because it can sometimes be seen on the medial cortex (see Fig. 31.7C). Many adult patients with a milder form of the disease may present as having age-related osteoporosis, and the prevalence of hypophosphatasia in unselected patients with osteopenia and osteoporosis is unknown. Some have speculated that the pathogenesis of atypical femur fractures after long-term bisphosphonate therapy may be related to unrecognized hypophosphatasia, but convincing evidence is lacking. Nevertheless, it is clear that patients with hypophosphatasia should not receive bisphosphonate therapy for their "osteoporosis." The diagnosis is supported by high serum levels of vitamin B_6, pyridoxal phosphate, and inorganic pyrophosphate. Genetic testing for mutations is not mandatory, but any gene abnormalities should be interpreted with caution because not all carriers



• **Fig. 31.7** (A) Radiologic features of lower extremities in a child with hypophosphatasia. Note abnormalities in the distal ends (*arrows*) resembling nutritional and X-linked hypophosphatemia rickets (see Fig. 31.4). (B) Tongue-like lucent area (*arrow*) in the metaphysis of the distal femur, characteristic radiologic sign of hypophosphatasia. In addition, note abnormal metaphyseal regions resembling mild rickets (see Fig. 31.4). (C) Pseudofracture (*arrow*) in the tibia. Note the similarity of pseudofractures seen in vitamin D–deficiency osteomalacia (see Fig. 31.2).

manifest the disease. Enzyme replacement therapy with asfotase alfa is now available for childhood-onset hypophosphatasia. It is unclear how many patients with adult-onset hypophosphatasia are missed or misdiagnosed as "usual age-related" osteoporosis because the relevant reference ranges for the alkaline phosphatase have not yet been widely adopted by all clinical chemistry laboratories. One clinically relevant aspect of unrecognized adult hypophosphatasia is that it may contribute to the development of atypical femur fractures after long-term bisphosphonate therapy, although proof of this speculation is still lacking. Nevertheless, attention should be paid to lower alkaline phosphatase levels (<40 IU/L) in women with postmenopausal osteoporosis in whom antifracture therapy with a bisphosphonate or denosumab is considered (see Chapter 30).

Concluding Remarks

Rickets and osteomalacia are a group of disorders due to varied pathogenic mechanisms, but they all respond to administration of vitamin D or its metabolites, calcium, or phosphate supplements as appropriate, removal of a tumor producing ectopic FGF23, or administration of an antibody to FGF23. The resolution of clinical, biochemical, radiologic, and bone histologic abnormalities is usually complete in most cases, but lifelong therapy is needed in some forms of rickets and osteomalacia. In nutritional rickets and osteomalacia, 2°HPT may persist for months or years despite clinical improvement and confers an increased fracture risk due to irreversible cortical bone loss that has already occurred by the time of diagnosis. In a few patients, particularly those with hypophosphatemic rickets and osteomalacia, long-term oral phosphate therapy leads to hypercalcemic 2°HPT (or tertiary hyperparathyroidism) requiring parathyroidectomy. In most patients, the clinical response is excellent and gratifying, both to the patients and caring physicians. However, one must not lose sight of the fact that genetic rickets and osteomalacia, as well as TIOs, require lifelong follow-up for the development of therapy-related complications or malignant transformation of the tumors causing TIO.

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Kidney Stones

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CHAPTER OUTLINE

KEY POINTS

- Numerous factors, including sex, age, race, and the patient's geographic location, determine the prevalence of kidney stones.
- Kidney stones form when urine becomes supersaturated with respect to the specific components of the stone's constituents.
- A multitude of monogenic hereditary disorders that result in changes of either calcium handling at the level of kidney, bone, and gut, or calcium sensing at the calcium-sensing receptor on the parathyroid glands and renal tubular cells, can lead to hypercalciuria and stone formation.
- All patients, even those with a single stone, should undergo at least a basic evaluation to rule out a systemic etiology of stone formation.
- Increasing fluid intake is a simple measure that has considerable impact on reducing stone growth and new stone formation.

Nephrolithiasis is a common disorder with an incidence greater than one case per 1000 patients per year. In the year 2000, this resulted in nearly 2 million physician office visits with an estimated annual cost between $2.0 and $5.5 billion in the United States alone.[1–3] The prevalence in industrialized nations is approximately 7% in women and 11% in men and appears to be rising over time.[4,5] The incidence of nephrolithiasis peaks when patients are in their 30s and 40s, and the prevalence increases with age until about the seventh decade of life.[5,6] The prevalence of nephrolithiasis in the United States increased from nearly 3% in the late 1970s to nearly 5% in the 1990s, which is far faster than can be accounted for by alterations in our genome indicating that changes in our diet and/or environment are responsible, at least in part, for the increasing prevalence.[6]

Stones may be composed of calcium oxalate, calcium phosphate, uric acid, magnesium ammonium phosphate (struvite), or cystine, alone or in combination. A variety of pathogenic mechanisms determine the type of stone formed. Symptomatic stones tend to localize in the renal tubules and collecting system but are also commonly found within the ureters and bladder.[7,8] The recurrence rate of calcium oxalate stones is about 10% to 30% at 5 years and 50% at 10 years and higher for cystine, uric acid, and struvite stones.[9–11]

Kidney stones result in substantial morbidity. The severe pain of renal colic can lead to frequent hospitalization, shock wave lithotripsy, or invasive surgical procedures. Although rarely a cause of end-stage renal disease (ESRD), nephrolithiasis has been associated with chronic kidney disease (CKD) in various populations.[12–16] Patients with kidney stones have an increase in aortic calcification, and even mild CKD is associated with significant adverse cardiovascular events,[17–20] especially in men, and an increase in stroke, especially in women.[21] There is a significant decrease in bone mineral density leading to fracture in patients with nephrolithiasis.[22]

Insight into the mechanisms involved in stone formation can help direct appropriate therapy, which is known to significantly decrease the incidence of stone formation and its associated morbidity.

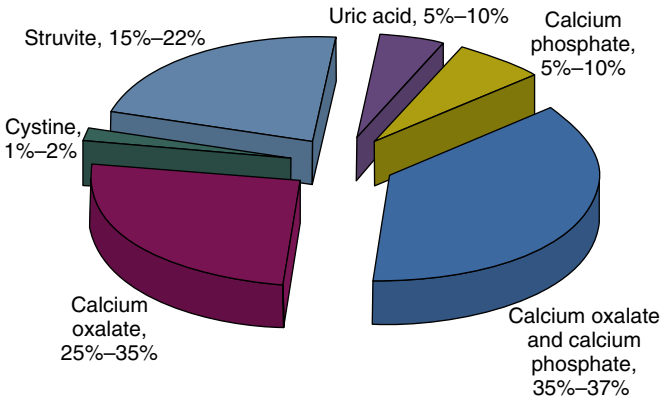
Epidemiology of Stone Formation

Numerous factors determine the prevalence of stones, including sex, age, race, obesity, and geographic distribution. Men are more likely to get nephrolithiasis than women.[5,6,23] In the United States, blacks, Hispanics, and Asian Americans are much less likely to have stones than whites.[5] Obesity is correlated with the risk for kidney stone formation.[24–26] Those weighing more than 220 pounds or having a body mass index (BMI) greater than 30 are more likely to form stones than those weighing less than 150 pounds or having a BMI between 21.0 and 22.9.[27] Geography also appears to influence stone formation in the United States, with a decreasing prevalence from south to north and, to some degree, from east to west.[23] The greater exposure to sunlight in the southeastern United States may be responsible for the increased rates of nephrolithiasis in that area. Sun exposure can lead to more concentrated urine by increasing insensible fluid losses due to sweating.[28,29] Although increased sun exposure should increase levels of serum 25-hydroxyvitamin D

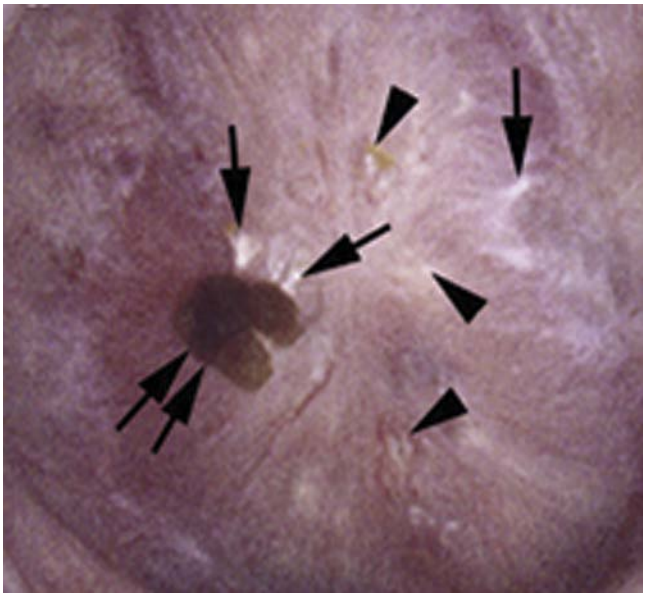
[25(OH)D], there is no evidence for a subsequent increase in the level of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], nor is there any evidence that hypercalciuria will worsen.

Along with geography, genetic predisposition can influence the type of stone formed.[28,30] For example, uric acid stones are seen in up to 75% of all cases in Mediterranean and Middle Eastern countries but constitute fewer than 10% of cases in the United States. By contrast, more than 70% of stones formed in the United States are calcium based.[31] Less common are magnesium ammonium phosphate (struvite or infection) stones, which account for about 10% to 25% of stones formed, and cystine stones, which are due to an autosomal recessive disorder and constitute only about 2% of all stones formed[6,30,32] (Fig. 32.1).

Diet and pharmacologic agents can also significantly impact stone formation. In a dramatic example, an outbreak of nephrolithiasis in Chinese infants was attributed to ingestion of melamine in infant formulas and milk powder. Melamine, intentionally added to raise the apparent protein content of the concentrates, led to formation of large particles in the kidney and resulted in many cases of nephrolithiasis and renal failure due to obstructive uropathy.[33–35] A variety of other dietary factors can have a significant impact on both formation and prevention of kidney stones (see the following discussion).



• **Fig. 32.1** Frequency of different types of kidney stones.



• **Fig. 32.2** An attached stone (*paired arrows*) is seen resting on a region of white plaque (*single arrows*) and intermixed with small areas of white (*single arrow*) and yellow plaques (*arrowheads*). (From Evan AP, Lingeman JE, Worcester EM, et al. Renal histopathology and crystal deposits in patients with small bowel resection and calcium oxalate stone disease. *Kidney Int*. 2010;78:310–317.)

Pathogenesis of Stone Formation

Physiology

Kidney stones form when urine becomes supersaturated with respect to the specific components of the stone.[7,8] Saturation is dependent on chemical free ion activities of the stone constituents. Factors that affect chemical free ion activity include urinary ion concentration, pH, and the combination of the constituent with other substances. For example, an increase in the urinary calcium concentration or a decrease in urine volume increases the free ion activity of calcium ions in the urine. Urinary pH can also modify chemical free ion activity. A low urinary pH increases the free ion activity of uric acid ions but decreases the activity of calcium and phosphate ions. Citrate combines with calcium ions to form soluble complexes and will decrease the free ion activity of unbound citrate and calcium. When the chemical free ion activities are increased, the urine becomes supersaturated (also termed *oversaturated*). In this setting, new stones may form and established stones may grow. In the event of decreased free ion activity, urine becomes undersaturated, and stones do not grow and can even dissolve. The equilibrium solubility product is the chemical free ion activity of stone components in a solution at which the stone neither grows nor dissolves.

Stones form through the processes of homogeneous or heterogeneous nucleation. In homogeneous nucleation, progressive supersaturation eventually results in formation of small clusters secondary to the aggregation of identical molecules. These clusters grow to form a permanent solid phase, or crystals. Heterogeneous nucleation refers to crystal formation on the surface of a different crystal type or on other dissimilar substances, such as cells. In vivo, this type of nucleation is more common than homogeneous nucleation because crystals form at a lower level of supersaturation in the presence of a solid phase.

The small crystals may then aggregate into larger clinically significant stones. Crystals generally anchor to renal tubular epithelium; this allows more time for growth. This anchoring of crystals occurs at the renal papillae, over areas of interstitial calcium phosphate present in the form of apatite termed *Randall plaques*[36–40] (Fig. 32.2). The apatite crystals appear to originate at the basement membrane of tubular cells in the thin loop of Henle and extend into the interstitium without damaging the cells themselves or filling the tubular lumens. A combination of apatite crystal and organic material extends from the loop of Henle tubular basement membrane to the papillary uroepithelial surface, where calcium oxalate crystals or other crystals can adhere and form stones. If the stone breaks this anchor to the urothelial surface, it will then be carried by the urine through the ureter and into the bladder. If the stone is small (generally <5 mm in diameter), it may pass with minor discomfort; however, if it has grown sufficiently, this migration may be extremely painful and, if the stone is of sufficient size, it may even completely obstruct the ureter leading to nonfunction of the unilateral kidney.

An important factor in the development of kidney stones may be the absence of adequate levels or activity of crystallization inhibitors in the urine.[8] Uropontin, pyrophosphate, citrate, and nephrocalcin are endogenously produced substances that have been shown to inhibit calcium crystallization. Differences in the

amount or activity of inhibitors are thought to account for the variability in stone formation among people with similar degrees of urinary supersaturation.[32]

Clinically, most physicians evaluate the lithogenic potential of the urine from stone formers by measuring the rate of excretion of the principal stone-forming elements in mass per unit time (e.g., milligrams or millimoles per 24 hours). It is clear, however, that the lithogenic potential of urine is better determined by the degree of supersaturation. Computer programs that calculate saturation from concentrations of various elements in the urine and the urinary pH are now available (e.g., Quest Diagnostics, Mayo Clinic, and Litholink are laboratories that measure urine ion excretion and calculate supersaturation) and more accurately determine the risk of stone formation. Any calculation of mean saturation underestimates the maximum supersaturation that may drive stone formation because of hourly variations in water and solute excretion throughout the day.

Diet

Dietary factors have a great influence on the concentration of excreted ions. Simply instructing patients to increase fluid intake appears to have a considerable impact on reducing stone growth and formation.[41–44] Renal calcium excretion is augmented by increased sodium excretion,[45–47] and hypercalciuric patients tend to have a greater calciuric response to a sodium load than control subjects.[48] Dietary sodium restriction with the consequent decrease in urinary sodium excretion reduces calcium excretion and lowers supersaturation with respect to calcium-containing kidney stones. Patients are counseled to limit their daily sodium intake to a maximum of 3000 mg (~130 mEq) to reduce hypercalciuria.[6,7,49]

A moderate reduction in animal protein (~1.0 mg/kg per day) is known to be beneficial in patients with nephrolithiasis. Animal protein contributes to stone formation via multiple mechanisms.[49,50] A mild metabolic acidosis develops when animal proteins are metabolized. To buffer the excess hydrogen ions, calcium is resorbed from bone, which leads to an increased filtered load of calcium.[51] Metabolic acidosis also directly decreases renal tubular calcium reabsorption, which further enhances hypercalciuria.[51] In addition, metabolism of amino acids contained in animal protein generates sulfate ions, which couple with calcium ions to form insoluble complexes.[51,52] Citrate, a base, acts as a urinary inhibitor of stone formation. Citrate forms soluble complexes with calcium and lowers calcium oxalate and calcium phosphate supersaturation. During metabolic acidosis, citrate is reabsorbed proximally, reducing the amount excreted in the urine.[52,53] Hypokalemia can also lead to reduced citrate excretion. An animal protein–induced reduction in urinary citrate can promote formation of both calcium oxalate and uric acid stones.[48,54] Indeed, the Dietary Approaches to Stop Hypertension (DASH) diet appears to reduce the risk of forming a kidney stone.[55–57]

Fructose has become a ubiquitous sweetener in American processed foods. In large food questionnaire studies, this sugar has been associated with a significant risk of developing nephrolithiasis. Although the mechanism is not known, fructose is the only carbohydrate that can increase uric acid production, and fructose metabolism may increase stone formation.[58]

Several studies have demonstrated the benefits of a diet containing an age- and gender- appropriate amount of calcium in patients with kidney stones.[42,49,50,59] Ingested calcium binds intestinal oxalate, reducing its absorption and consequent renal excretion.[42] In a long-term prospective trial, Borghi and colleagues[49] randomized hypercalciuric male stone formers to either a low-calcium diet or to a diet with a normal amount of calcium but low in sodium and animal protein. Both groups of men were instructed to restrict oxalate intake and drink 2 to 3 L of water daily. The group of men on a normal-calcium, low-sodium, and low–animal protein diet had a significantly lower recurrence of nephrolithiasis and a greater reduction in oxalate excretion and calcium oxalate supersaturation compared with the men on the low-calcium diet.[49]

Thus, patients should be maintained on an age- and gender-appropriate intake of calcium.[50] Dietary calcium restriction should be strongly discouraged because it not only increases risk of stone formation but also engenders a significant risk of bone demineralization and development of osteoporosis.[22,60,61] Note that although *dietary* calcium intake has been associated with a reduced incidence of kidney stones, calcium intake in the form of *supplements* can exacerbate stone formation in older women. The recommended dietary intake for men and women is 1000 mg of elemental calcium from ages 19 through 50 years and 1200 mg of calcium thereafter.[62] Teenagers should consume 1300 mg of calcium per day. Excess calcium should be avoided, because the combination of calcium and vitamin D supplementation has been shown to significantly increase the risk of kidney stones in postmenopausal women.[63]

Pathogenesis of Idiopathic Hypercalciuria

Idiopathic hypercalciuria (IH) is defined as excessive urinary calcium excretion in the setting of normocalcemia and the absence of secondary causes of hypercalciuria. IH is the most common cause of calcium-containing kidney stones. The disorder is familial; it was initially thought to exhibit an autosomal dominant pattern of inheritance but is almost certainly polygenic.[64–67]

The mechanism by which IH leads to hypercalciuria is not known. It has been postulated that IH comprises three distinct disorders: excessive intestinal calcium absorption, decreased renal tubular calcium reabsorption, and enhanced bone demineralization. In a genetic strain of hypercalciuric stone-forming rats, hypercalciuria appears to be due to an excessive number of enteric vitamin D receptors leading to a generalized disorder of calcium transport at all sites of calcium transport including the kidney, intestine, and bone.[67,68] In humans, recent observations also suggest that IH may be a systemic disorder of calcium homeostasis with dysregulation of calcium transport. An understanding of calcium homeostasis helps elucidate the potential mechanisms involved in IH.

Calcium Homeostasis

Urinary calcium homeostasis is regulated in the gastrointestinal (GI) tract, kidneys, and bone by the hormones parathyroid hormone (PTH) and $1,25(OH)_2D$ (also see Chapter 29 for additional discussion on calcium homeostasis). Approximately 99% of the calcium in the body is contained within the bone mineral. Daily bone resorption and bone formation, which in healthy, nonpregnant, nonosteoporotic adults should be equal, allow less than 1% of bone calcium to be exchanged with that in the extracellular fluid.

Both PTH and $1,25(OH)_2D$ at high concentrations stimulate release of calcium from the bone mineral through osteoclast-mediated bone resorption. Net calcium influx into the extracellular fluid is achieved by absorption from the GI tract, which occurs through $1,25(OH)_2D$-dependent and -independent mechanisms. Although PTH appears to have no direct effect on GI calcium

absorption, increased levels of the hormone stimulate production of $1,25(OH)_2D$, which in turn leads to enhanced absorption. Increased serum levels of calcium and $1,25(OH)_2D$ provide negative feedback to the parathyroid glands, resulting in reduced PTH secretion.

The roughly 60% of calcium in the extracellular fluid is not protein bound and is freely filtered by the renal glomeruli. Approximately 80% to 85% of this amount is passively reabsorbed in the proximal tubule. Most of the remaining calcium is reabsorbed in the thick ascending limb (TAL) of Henle and distal cortical tubules under PTH stimulation. Ultimately, these reabsorptive mechanisms result in a urinary calcium excretion that is less than 2% of the daily filtered load of calcium.[69] Except during pregnancy and lactation, in healthy, nonosteoporotic adults, urinary calcium excretion (and any calcium lost in sweat) precisely equals net intestinal calcium absorption.

Potential Mechanisms for the Development of IH

Dysregulation of calcium transport in the intestine, kidney, or bone can lead to hypercalciuria. For example, excessive calcium absorption by the GI tract leads to a transient increase in the serum calcium. This increase in serum calcium suppresses secretion of PTH that, along with the increased filtered load of calcium to the kidneys, results in hypercalciuria. Excessive $1,25(OH)_2D$ has a similar effect of increasing intestinal calcium absorption but also results in an influx of calcium into the extracellular fluid because of enhanced bone resorption. The result is hypercalciuria even in the setting of a low-calcium diet or an overnight fast. The excess $1,25(OH)_2D$ also suppresses PTH secretion, thereby further reducing renal tubular reabsorption of calcium.

If a primary defect in renal calcium reabsorption leads to hypercalciuria, there is a fall in the serum calcium concentration that stimulates synthesis of PTH and $1,25(OH)_2D$. Increased $1,25(OH)_2D$ results in enhanced intestinal calcium absorption and bone resorption. The renal loss of calcium persists even with a low-calcium diet or an overnight fast.

Hypercalciuria can also develop as a result of a defect in renal phosphate reabsorption. The resultant hypophosphatemia leads to enhanced $1,25(OH)_2D$ production, which stimulates intestinal absorption of phosphorus and calcium. The increased serum calcium and $1,25(OH)_2D$ suppresses PTH synthesis and release. The increased filtered load of calcium in the setting of suppressed PTH leads to hypercalciuria. Enhanced bone resorption due to excessive $1,25(OH)_2D$ increases the serum calcium concentration, which in turn suppresses PTH production further. The increase in the filtered load of calcium in this setting results in hypercalciuria.

Thus, there are several potential mechanisms for hypercalciuria. Do human or animal data support one mechanism above all others? From a clinical therapeutic standpoint, is it worth differentiating among the various potential mechanisms in each patient with suspected IH?

Human Data

Lemann[70] compiled the results of numerous calcium balance studies on patients with IH and normocalciuric control subjects and normalized the results for calcium intake. He found that intestinal calcium absorption was significantly higher in the subjects with IH.

Bushinsky and associates[8] and Coe and colleagues[71] also collected data from published metabolic balance studies, and compared net intestinal calcium absorption and urinary calcium excretion in hypercalciuric and normocalciuric adults. They also noted an increase in intestinal calcium absorption in subjects with IH but found that urinary excretion of calcium was increased to an even greater degree, thus placing many of these patients in net negative calcium balance. Although these data confirm that enhanced intestinal absorption of calcium probably plays a role in the pathogenesis of IH, the investigators could not clarify whether this is the primary defect or if it is secondary to another lesion, such as a primary dysregulation of renal tubular calcium reabsorption. Others suggested that the increase in intestinal calcium absorption, in combination with a decrease in renal calcium reabsorption, indicated a more generalized defect in calcium homeostasis. Nonetheless, the finding of enhanced calcium absorption makes enhanced bone resorption an unlikely primary mechanism of IH, because the increase in serum calcium concentration resulting from bone resorption would suppress $1,25(OH)_2D$-mediated intestinal calcium absorption.

In most published studies, patients with IH have higher serum levels of $1,25(OH)_2D$ than normocalciuric control subjects.[72–74] Kaplan and associates[74] determined that $1,25(OH)_2D$ levels were higher than control values in approximately one third of patients with IH and that intestinal calcium absorption was inappropriately high for the level of $1,25(OH)_2D$. These studies support either $1,25(OH)_2D$-mediated intestinal calcium absorption or a primary defect in renal tubular calcium reabsorption as a primary mechanism of hypercalciuria in IH.

PTH levels in patients with IH have been reported as normal or slightly lower than those in controls.[54,60] This finding argues against a reduction in renal tubular calcium reabsorption as the primary defect in IH, because with this mechanism, the hypercalciuria would lead to low serum calcium levels and stimulation of PTH secretion. This finding also does not support the hypothesis that elevated levels of PTH are the stimulus for the increased levels of serum $1,25(OH)_2D$ observed in many studies. It is, however, consistent with the other potential mechanisms for IH.

Bone mass in patients with IH has been assessed by several methods, including radiologic densitometry, quantitative computed tomography (CT), dual-energy x-ray absorptiometry, and single-photon absorptiometry, among others. Studies of patients with IH have generally shown a reduction of bone mineral density compared with values in controls.[22,54,60,75] The studies were unable to reveal a unifying mechanism for the mild reduction in bone mineral density. Altered $1,25(OH)_2D$ regulation would be consistent with this finding because the effects of $1,25(OH)_2D$ on bone resorption would be mitigated by the increased intestinal calcium absorption stimulated by the hormone.

Previously, it was considered important to determine whether a patient with IH tended to have excessive GI calcium absorption (absorptive hypercalciuria) or excessive renal excretion (renal leak).[76,77] Patients with excessive renal calcium excretion were prescribed thiazide diuretics, and those thought to have a predominantly absorptive defect were prescribed a low-calcium diet. Coe and associates[60] undermined the validity of this approach in a study in which 24 patients with IH and nine control subjects were given a low-calcium diet (2 mg/kg per day) for more than 1 week. Urine and blood tests revealed normal serum calcium levels, a mild decrease in PTH levels in the patients with IH, and no difference in $1,25(OH)_2D$ levels. The striking finding was that whereas all of the normocalciuric subjects excreted less calcium than they ingested on the low-calcium diet, 16 of the 24 subjects with IH had urinary calcium excretion that exceeded their intake. Thus, most of the patients with IH receiving a low-calcium diet were in net negative calcium balance. There was no clear demarcation

between the patients who excreted excessive amounts of calcium and those who did not. Instead, there was a smooth continuum of urinary calcium excretion among patients with and without IH that appeared not to be influenced by calcemic hormones. From a therapeutic standpoint, these findings have rendered obsolete both the need to clinically distinguish IH mechanisms in humans and also the prescription of a low-calcium diet in any of these patients. This approach to diet is important because a low-calcium diet can result in a dangerous reduction in bone mineral density, especially in women.[22,61,75,78] As mentioned earlier (see the Pathogenesis of Stone Formation section), a low-calcium diet also appears to increase recurrent stone formation.[42,49,59] Thus, there is no benefit, and additionally, there are several well-documented risks to advising a low-calcium diet to prevent recurrent stone formation in patients with IH.

Genetic Hypercalciuric Stone-Forming Rats

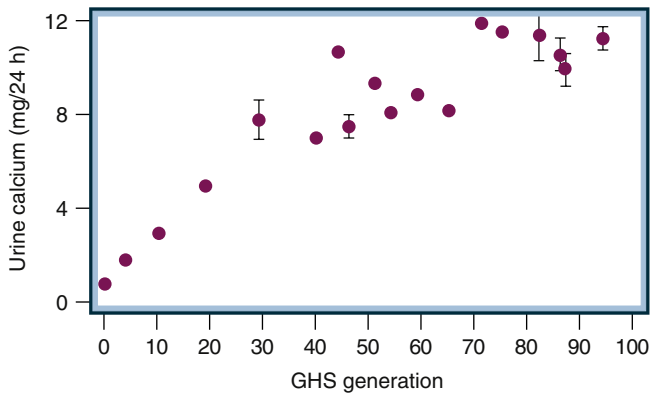
To more fully explain the mechanism of IH in humans, we have developed an animal model of this disorder.[67,79–85] Through more than 100 generations of successive inbreeding of the most hypercalciuric progeny of hypercalciuric Sprague-Dawley rats, we have established a strain of rats that excrete more than 10 times as much urinary calcium as control Sprague-Dawley rats (Fig. 32.3).

Compared with control Sprague-Dawley rats, the genetic hypercalciuric rats absorb far more calcium at lower dietary levels of $1,25(OH)_2D$.[86,87] When these hypercalciuric rats were fed a diet very low in calcium, their urinary calcium excretion remained elevated compared with that of similarly treated control rats, indicating a defect in renal calcium reabsorption or an increase in bone resorption, or both,[88] again similar to observations in humans.[60,89] Bone from these hypercalciuric rats released more calcium than the bone of control rats when exposed to increasing amounts of $1,25(OH)_2D$,[90] and their bone mineral densities were lower than control rats.[91] The administration of a bisphosphonate to the genetic hypercalciuric rats fed a low-calcium diet significantly reduced urinary calcium excretion.[92] In addition, a primary defect in renal calcium reabsorption was observed during clearance studies.[93] We have shown that besides the intestine, both the bone and kidney of the hypercalciuric rats have an increased number of vitamin D receptors and calcium receptors.[90,94,95]

Studies suggest the hypothesis that an increased number of vitamin D and/or calcium receptors may be the underlying mechanism for hypercalciuria in the rats.[96] Since the hypercalciuric rats have normal levels of serum $1,25(OH)_2D$, the higher levels of vitamin D receptors suggest that these receptors are undersaturated with $1,25(OH)_2D$. We tested the hypothesis that $1,25(OH)_2D$ would induce a greater increase in urine calcium in the hypercalciuric rats.[82] After injecting $1,25(OH)_2D$, urine calcium in both the controls and hypercalciuric rats increased; however, the increased urine calcium with $1,25(OH)_2D$ in the hypercalciuric rats exceeded that of controls, indicating that the increased number of vitamin D receptors in the hypercalciuric rats induces a greater biologic response. This increase in urine calcium must come from the intestine and/or bone. To determine whether the excess urine calcium was derived primarily from diet or from increased bone resorption, we asked whether $1,25(OH)_2D$ would increase urine calcium in genetic hypercalciuric stone-forming (GHS) rats fed a low-calcium diet.[83] On this low-calcium diet with $1,25(OH)_2D$, urine calcium in controls increased; however, urine calcium again increased significantly more in the hypercalciuric rats. In the hypercalciuric rats on the low-calcium diet with or without $1,25(OH)_2D$, urine calcium far exceeded daily calcium intake, implying a loss of bone mineral. To determine the role of bone resorption in the increased urine calcium in these hypercalciuric rats, we tested the hypothesis that a low-calcium diet, coupled with inhibition of bone resorption by the bisphosphonate alendronate, would eliminate the enhanced $1,25(OH)_2D$-induced hypercalciuria in GHS rats.[81] Alendronate eliminated the $1,25(OH)_2D$-induced increase in urine calcium in controls. However, in the hypercalciuric rats, alendronate decreased, but did not eliminate, the $1,25(OH)_2D$-induced hypercalciuria, suggesting that maximal alendronate cannot completely prevent the 1,25D-induced bone resorption in the GHS rats. These results confirm the role of increased bone resorption in the hypercalciuria of GHS rats. To study the effect of the increased number of vitamin D receptors on the osseous response to $1,25(OH)_2D$, we fed hypercalciuric and control rats ample calcium and injected $1,25(OH)_2D$ or vehicle daily.[80] With $1,25(OH)_2D$, there was a mineralization defect and a loss of bone mineral density in the hypercalciuric rats that exceeded changes in controls and contributed to increased hypercalciuria, suggesting that these bones would be more prone to fracture. The enhanced effect of $1,25(OH)_2D$ in the hypercalciuric rats indicates that the increased number of vitamin D receptors are biologically active and supports our hypothesis that in these hypercalciuric rats at baseline, their increased number of vitamin D receptors remain available for stimulation by exogenous $1,25(OH)_2D$.

These studies suggest that an increased number of vitamin D receptors in the hypercalciuric rats may be the underlying mechanism for their hypercalciuria, and perhaps this occurs in humans as well.[68,97,98] Circulating monocytes from humans with IH have been shown to have an increased number of vitamin D receptors.[99]

Thus, hypercalciuric rats appear to have a systemic abnormality in calcium homeostasis. They absorb more intestinal calcium, resorb more bone, and do not adequately reabsorb filtered calcium. Because every one of the hypercalciuric rats forms renal stones, we have described them as GHS rats.[68,97]



All data from published studies.

• **Fig. 32.3** Hypercalciuria in subsequent generations of genetic hypercalciuric stone-forming (GHS) rats. All data are from published studies.

Genetics of IH in Humans

The difficulty in ascertaining the genetics of IH arises, in part, from the numerous other factors that influence stone formation, such as diet, environment, and gender. Because half of patients with IH report a family history of stones and male patients often have fathers or sons with the disorder, inheritance is not believed to be recessive or X-linked.[100] A multitude of monogenic hereditary

disorders (see later discussion) can lead to hypercalciuria because they are caused by a variety of mutations resulting in changes in calcium handling at the level of kidney, bone, gut, and the calcium-sensing receptor in the kidneys and parathyroid glands. Given the evidence that IH is a complex trait with multiple pathways for developing the hypercalciuria phenotype, it is most likely a polygenic disorder, with heterogeneity of loci and possibly polygenic modifiers.[65,101] Lieske and associates[66] found that genetic factors appear to explain 20% to 36% of the interindividual variation in excretion of the ions critical to stone formation. Although attempts to diagnose the exact cause of IH in a particular patient might not be critical from a therapeutic standpoint, determining the etiology of IH in a particular family is essential for researchers attempting to clarify the genetics of IH.[8,65,66,85,102] Genome-wide association studies have found that genes associated with phosphate carrier NPT2a and NPT2c, claudin 14, aquaporin I, and others are associated with calcium nephrolithiasis.[65]

Other Genetic Causes of Stones and Nephrocalcinosis

Numerous monogenic disorders cause hypercalciuria leading to nephrolithiasis or nephrocalcinosis.[8,65,66,85,102] Disorders that lead to hypercalciuria by augmenting bone resorption include osteogenesis imperfecta type 1, multiple endocrine neoplasia type 1 syndrome with hyperparathyroidism, McCune-Albright syndrome, and infantile hypophosphatemia. Disorders that result in hypercalciuria because of excessive intestinal absorption of calcium include hypophosphatemia, Down syndrome, and congenital lactate deficiency. Others include autosomal dominant hypocalcemia (which is caused by an activating mutation of the calcium-sensing receptor), Lowe oculocerebrorenal syndrome, and Wilson disease. Next, we describe in more detail several disorders that result in hypercalciuria via their effect on genes expressed in the kidney.

X-Linked Hypercalciuric Nephrolithiasis (Dent Disease and Others)

Several families around the world have an X-linked disorder of proximal renal tubular dysfunction that is manifested by a variable combination of disorders including hypercalciuria, low-molecular-weight proteinuria, nephrocalcinosis or stones, hypophosphatemic rickets, and renal failure.[103–107] Some affected persons demonstrate evidence of defects in proximal tubular reabsorption of amino acids, glucose, or phosphate. PTH tends to be quite low and $1,25(OH)_2D$ high in the majority of patients. The abnormalities completely resolve in the patients who receive renal transplants, a finding that suggests a renal tubular disorder rather than a systemic process. In all families, the pattern of inheritance is consistent with an X-linked recessive disorder, with male patients affected to a greater extent than female patients. The latter are often minimally affected but transmit the disorder to half of their male offspring. Over time, the various disorders—X-linked recessive nephrolithiasis in the United States, Dent disease in the United Kingdom, X-linked recessive hypophosphatemic rickets in Italy, and low-molecular-weight proteinuria with hypercalciuria and nephrocalcinosis in Japan—have all been linked to mutations affecting the genes encoding the Cl^-/H^+ exchanger ClCN5 in the majority of patients and/or inositol polyphosphate 5-phosphatase (OCRL1), both on the X chromosome.[103–105] How defects in these genes lead to the array of these proximal tubular disorders is not yet understood.

Bartter Syndrome

Bartter syndrome is caused by at least five genetic mutations, predominantly autosomal recessive, that lead to sodium chloride wasting at the TAL of the loop of Henle.[37,38,64,83,105] Bartter syndrome is an autosomal disease caused by mutations of bumetanide-sensitive NKCC2 (the Na-K-2Cl cotransporter), ROMK (the renal outer medullary potassium channel), CaSR (the calcium-sensing receptor), and CLC-Kb (the voltage-gated chloride channel) or the CLC-Kb beta subunit barttin. These genes, all expressed in the TAL, cause a defect in sodium transport that leads to a reduction in the transtubular potential difference, resulting in a decrease in paracellular calcium reabsorption in the TAL. The ensuing reduction in intravascular volume also induces an aldosterone-mediated metabolic alkalosis. Bartter syndrome therefore resembles high-dose furosemide administration (that targets NKCC2) and differs from Gitelman syndrome in that hypercalciuria, nephrocalcinosis, and nephrolithiasis are seen with Bartter but not with Gitelman. An autosomal dominant form of Bartter results from a gain-of-function mutation in the calcium sensing receptor in renal tubular cells. This mutation leads to reduced calcium reabsorption and also hypocalcemia caused by low PTH levels. Therapy with vitamin D and calcium supplementation can exacerbate stone disease in this disorder.

Familial Hypomagnesemia With Hypercalciuria and Nephrocalcinosis

Familial hypomagnesemia with hypercalciuria and nephrocalcinosis is an autosomal recessive disorder that results in hypomagnesemia, hypercalciuria, nephrolithiasis, and distal renal tubular acidosis (dRTA). Polyuria and severe nephrocalcinosis also ensue, and progressive kidney failure is common by late childhood.[37,108–110] The genetic disorder results in defective production of either of the tight junction proteins, claudin-16 and claudin-19, that bind together to facilitate paracellular calcium and magnesium transport in the TAL, as well as renal sodium reabsorption. Currently, there is no effective treatment for the hypercalciuria or progression to kidney failure.

Distal Renal Tubular Acidosis

dRTA is caused by dysfunctional α-intercalated cells, resulting in defective acid excretion.[37,38,111–115] This inability to adequately acidify the urine results in metabolic acidosis, hypocitraturia, hypokalemia, hypercalciuria, nephrocalcinosis, and stones. The metabolic acidosis leads to resorption of both calcium and phosphate from bone. The increased filtered load of calcium and phosphate, along with the elevated urine pH and hypocitraturia, results in favorable conditions for calcium phosphate stone formation. Although there are secondary causes of dRTA, such as Sjögren syndrome and use of carbonic anhydrase inhibitors (e.g., acetazolamide), there are also several hereditary causes of dRTA.[116,117] Some are autosomal recessive and can also result in hearing loss; others are autosomal dominant. One form of dRTA that targets carbonic anhydrase II results in osteopetrosis and brain calcifications.[117] Patients with dRTA fail to lower their urine pH below 5.5 following ingestion of an acid load. Their urine citrate is extremely low despite mildly reduced or even normal serum bicarbonate levels.

Hereditary Hypophosphatemic Rickets With Hypercalciuria

Hereditary hypophosphatemic rickets with hypercalciuria is an autosomal form of hypophosphatemic rickets that is manifested clinically by hypophosphatemia secondary to renal phosphate wasting.[118–121] This disease is caused by mutations in solute carrier family 34, member 3 (SLC34A3), which is the gene that encodes

the sodium Na^+-dependent phosphate cotransporter 2c (NPT2c). These patients have hypophosphatemia-induced increase in levels of $1,25(OH)_2D$, which leads to increased intestinal calcium absorption and results in hypercalciuria. The bone pain, muscle weakness, limb deformities, and rickets resolve with administration of oral phosphate.

Primary Hyperoxaluria and Cystinuria

Primary hyperoxaluria and cystinuria are each discussed in the Therapy section.

Clinical Presentation and Evaluation

Kidney stones vary in clinical presentation, from those discovered asymptomatically on routine imaging to their painful passage through the ureters to large and obstructing staghorn calculi that can significantly impair renal function and even lead to ESRD.[6,7] The severity of stone disease depends on the pathogenic factors contributing to the rate of stone formation, as well as the stone type, size, and location.

In its most classic form, nephrolithiasis manifests as renal colic. This discomfort of abrupt onset intensifies over time into an excruciating, severe flank pain that resolves only with stone passage or removal. The pain often migrates anteriorly along the abdomen and inferiorly to the groin, testicles, or labia majora as the stone moves toward the ureterovesical junction. Gross hematuria, urinary urgency and frequency, nausea, and vomiting may be present. Nephrolithiasis can also result in a dull, poorly localizing abdominal pain. The probability of passing a kidney stone without intervention depends on its size and varies from about 70% for kidney stones 5 mm or less in size, whereas stones between 5 and 7 mm have a 60% chance, stones between 7 and 9 mm have a 48% chance, and stones 9 mm or larger have a 25% chance of passing spontaneously.[122]

Several studies, including a large meta-analysis, demonstrate that nephrolithiasis is associated with an increase in CKD that is independent of other risk factors, such as diabetes and hypertension found in stone formers; however, at least one other study did not find an increased risk of CKD.[123–127] A French study estimated the incidence rate of ESRD because of nephrolithiasis to be about 3.1 cases per million population per year,[128] whereas a Canadian study demonstrated that although only 0.8% of patients with ESRD had nephrolithiasis, any stone episode previously was associated with an increased risk of ESRD (hazard ratio 2.16).[129] The common reasons for loss of a single kidney in stone formers were staghorn calculi, high stone burden, infection, and ureteral obstruction.[130] However, no association between nephrolithiasis and incident CKD could be found in a recent study of more than 10,000 patients.[123]

The development of CKD from stone disease is thought to be from ureteral obstruction leading to parenchymal damage.[125] Most data are derived from animal models and suggest that unilateral ureteral obstruction causes intense renal vasoconstriction that reduces renal blood flow and glomerular filtration rate (GFR).[131] Brushite ($CaHPO_4$) stone formers have an increased risk of cortical fibrosis,[132] and the formation of Randall plaques in such patients was associated with duct plugging, collecting duct cell death, and inflammation.[133] Renal biopsy specimens in patients with staghorn calculi demonstrate extensive inflammation and macrophage infiltration.[134] Other stone-forming diseases like primary hyperoxaluria, cystinuria, and Dent disease have all been associated with crystal formation in the renal parenchyma that presumably triggers subsequent inflammation and CKD.[133]

Certain disorders can lead to diffuse renal parenchymal calcifications termed *nephrocalcinosis*.[6,38,115,135] The calcifications, usually calcium phosphate or calcium oxalate, may be present in the cortex or medulla. Among the most common causes of stone-related nephrocalcinosis are primary hyperoxaluria and medullary sponge kidney.

Metabolic Evaluation of Stone Formers

Although it is uniformly accepted that patients with multiple stones merit a thorough investigation into the cause of nephrolithiasis, the need for evaluation of the patient with a single stone is controversial. This is probably due to the difficulty in determining the cost-to-benefit ratio of stone evaluations and wide differences in reported rates of stone recurrence.

The National Institutes of Health has convened several consensus conferences to resolve such issues related to the prevention and treatment of kidney stones.[136] These panels determined that all patients, even those with a single stone, should undergo at least a basic evaluation to rule out a systemic etiologic mechanism. Patients with an increase in number or size of stones (metabolically active stones), all children, all non–calcium oxalate stone formers, and those in demographic groups not typically susceptible to stone formation warrant a more complete metabolic evaluation.[136]

The Basic Evaluation

Elements of the basic evaluation are listed in Table 32.1.[6,7]

History

In addition to the medical history typically obtained from new patients, the evaluation of the stone former includes a stone history and a thorough review of diet, fluid intake, and lifestyle. Specific laboratory studies and radiographic tests are also required.

Stone History

The stone history begins with a chronology of stone events: age of incidence of first stone, size and number of stones formed, frequency of passage, stone type (if known), and whether the stones occur equally in both kidneys or unilaterally. Also helpful is a report of the patient's symptoms with each episode, as well as the need for and response to surgical intervention.

This information is useful to judge not only the severity of the stone disease but also clues to the origin of the patient's nephrolithiasis. For example, nephrolithiasis that begins at a young age may be attributable to an inherited metabolic disorder such as primary hyperoxaluria or cystinuria. Large staghorn calculi that are difficult to eradicate and tend to recur despite frequent surgical intervention are more likely to be composed of struvite ($NH_4MgPO_4.6H_2O$) instead of calcium oxalate. Cystine stones are not disintegrated thoroughly with the use of lithotripsy, and alternative surgical modalities are generally required for stone removal. In patients who tend to form stones in only one kidney, the possibility of congenital abnormalities of that kidney, such as megacalyx or medullary sponge kidney, should be explored.

Medical History

Systemic disorders that can contribute to nephrolithiasis are sought in the medical history. For example, any disorder that can result in hypercalcemia, such as sarcoidosis or certain malignancies, may also lead to hypercalciuria. A variety of GI disorders associated with malabsorption (e.g., sprue, Crohn disease) can

TABLE 32.1 The Basic Evaluation of Stone Formers

- History
  - Stone history
  - Medical history
  - Family history
- Medications
- Occupation and lifestyle
- Diet and fluid intake
- Physical examination
- Laboratory tests
  - Urinalysis
  - Urine culture and sensitivity
  - Cystine screening
- Blood tests
  - Sodium, potassium, chloride, bicarbonate
  - Calcium, phosphorus, uric acid, creatinine
  - Intact parathyroid hormone if calcium is elevated or at the upper limit of normal
  - Tetrahydrodeoxycortisol, urinary free cortisol, and 25-hydroxyvitamin D levels as appropriate
- Stone analysis
- Radiology (choose appropriate study as indicated; see text)
  - Unenhanced helical (spiral) computed tomography
  - Kidneys, ureter, and bladder examination
  - Intravenous pyelography
  - Ultrasonography

Data from Monk RD, Bushinsky DA. Nephrolithiasis and nephrocalcinosis. In: Johnson RJ, Frehally J, Floege J, eds. *Comprehensive Clinical Nephrology*, 5th ed. Philadelphia, PA: Elsevier; 2015:688–702.

TABLE 32.2 Causes of Calcium Stone Formation

- Hypercalciuria
  - Cushing syndrome
  - Granulomatous diseases
  - Hypercalcemic disorders
  - Idiopathic hypercalciuria
  - Immobilization
  - Malignancy
  - Milk-alkali syndrome
  - Primary hyperparathyroidism
  - Sarcoid
  - Thyrotoxicosis
- Medications (see Table 32.4)
- Hyperoxaluria
  - Biliary obstruction
  - Chronic pancreatitis
  - Crohn disease
  - Dietary hyperoxaluria (urine oxalate secretion 40–60 mg/day)
  - Enteric oxaluria (urine oxalate 60–100 mg/day)
  - Jejunoileal bypass
  - Malabsorptive disorders
  - Primary hyperoxaluria types 1 and 2 (oxalate 80–300 mg/day)
  - Sprue (celiac disease)
- Hyperuricosuria (see Table 32.3)
- Hypocitraturia
  - Androgens
  - Exercise
  - Hypokalemia
  - Hypomagnesemia
  - Infection
  - Metabolic acidosis
  - Starvation
- Renal tubular acidosis (distal, type 1)
- Anatomic genitourinary tract abnormalities
  - Congenital megacalyx
  - Medullary sponge kidney
  - Tubular ectasia

Data from Monk RD, Bushinsky DA. Nephrolithiasis and nephrocalcinosis. In: Johnson RJ, Frehally J, Floege J, eds. *Comprehensive Clinical Nephrology*, 5th ed. Philadelphia, PA: Elsevier; 2015:688–702.

cause calcium oxalate nephrolithiasis on the basis of enteric hyperoxaluria. Patients with gout or insulin resistance are more likely to have uric acid stones[30,137] (Tables 32.2 and 32.3).

Family History

As noted earlier, several stone disorders are inherited, making the family history an important component of the basic evaluation. IH appears to be a familial disorder. Although the exact chromosomes and genes have not yet been identified, the pattern of inheritance is almost certainly polygenic.

Stones arising in childhood or young adulthood can be related to autosomal recessive disorders such as cystinuria and primary oxaluria. These genetic disorders are reviewed in the later sections on treatment of cystine and primary hyperoxaluria.

The high prevalence of uric acid stones in certain areas of the world is suggestive of both genetic and environmental risk factors. Genes that cause either excessively acidic urine or hyperuricosuria have been implicated.[30,37,65,66,78,135,138,139]

Medications

Medications can contribute to stone formation in several ways. Calcium-containing supplements, for example, can increase the amount of calcium absorbed and subsequently excreted.[63] Loop diuretics can directly promote renal tubular excretion of calcium and are associated with nephrocalcinosis in neonates who have received the drug.[140,141] Acetazolamide, a weak diuretic, induces a mild metabolic acidosis and alkaline urine, which are favorable conditions for the development of calcium phosphate stones. Other uricosuric medications, such as salicylates and probenecid, have been implicated in uric acid lithiasis.[142]

Certain crystals or stones can consist completely of precipitated medication. Such medications include intravenously administered acyclovir, triamterene, indinavir, and various sulfonamides, such as sulfadiazine. Oxalate is a metabolic end product of vitamin C, and large doses increase oxalate excretion and may predispose to stone formation[143,144] (Table 32.4).

Lifestyle and Diet

Occupation and lifestyle are aspects of the social history that can be relevant to stone formation. Surgeons and traveling salespeople, for example, tend to minimize fluid intake to avoid frequent micturition throughout the day. Insensible losses of fluid can also exacerbate nephrolithiasis and may be related to employment (e.g., construction work) or hobbies (running, gardening).

The evaluation proceeds with a thorough review of the patient's diet and fluid intake. Patients are asked to review what they eat at all meals and snacks. Particular attention is paid to ingestion of foods high in sodium (fast foods, canned foods, added salt or soy sauce) and the quantity of animal protein consumed (see later discussion). Patients are also asked to list four or five favorite foods or snacks to assess whether they may be consuming foods high in oxalate or purine as well. Many patients are erroneously counseled by physicians to avoid calcium-containing foods. As noted earlier, doing so is associated with increased risk of stone formation

TABLE 32.3 Factors Associated With Noncalcium Stone Formation

Uric Acid Stones

Cushing syndrome
Diarrhea
Diet high in animal protein
Excessive dietary purine
Excessive insensible losses
Genetic predisposition
Glucose-6-phosphatase deficiency
Gout
Hemolytic anemia
Hyperuricemia
Hyperuricosuria
Inadequate fluid intake
Inborn errors of metabolism
Insulin resistance
Intracellular to extracellular uric acid shift
Lesch-Nyhan syndrome
Low urine pH (<5.5)
Low urine volume
Malabsorptive disorders
Medications (see Table 32.4)
Metabolic syndrome
Myeloproliferative disorders
Obesity
Tumor lysis

Struvite Stones

Urease-producing bacteria
Proteus, *Pseudomonas*, *Haemophilus*, *Yersinia*, *Ureaplasma*, *Klebsiella*, *Corynebacterium*, *Serratia*, *Citrobacter*, *Staphylococcus*, and others
Never *Escherichia coli*—not a urease producer
High urine pH (~6.5)
Indwelling urinary catheter
Neurogenic bladder

Cystine Stones

Autosomal recessive trait
Excessive excretion of cystine, ornithine, lysine, and arginine
Low solubility of cystine (<250 mg/L)

Data from Monk RD, Bushinsky DA. Nephrolithiasis and nephrocalcinosis. In: Johnson RJ, Frehally J, Floege J, eds. *Comprehensive Clinical Nephrology*, 5th ed. Philadelphia, PA: Elsevier; 2015:688–702.

TABLE 32.4 Medications Associated With Renal Lithiasis and Nephrocalcinosis

Medications That Promote Calcium Stone Formation

Acetazolamide
Amphotericin B
Antacids (calcium and noncalcium antacids)
Calcium supplements
Glucocorticoids
Loop diuretics
Theophylline
Vitamin C
Vitamin D

Medications That Promote Uric Acid Lithiasis

Allopurinol (associated with xanthene stones)
Probenecid
Salicylates

Medications That Can Precipitate Into Stones or Crystals

Acyclovir (when infused rapidly intravenously)
Indinavir
Nelfinavir
Sulfonamides
Triamterene

Data from Monk RD, Bushinsky DA. Nephrolithiasis and nephrocalcinosis. In: Johnson RJ, Frehally J, Floege J, eds. *Comprehensive Clinical Nephrology*, 5th ed. Philadelphia, PA: Elsevier; 2015:688–702.

and may also result in bone demineralization, a grave concern in women with stones.[42,49,59]

Physical Examination

For most patients with nephrolithiasis, physical findings are normal. In some patients, however, the findings may reveal a systemic disorder related to the stone disease. An enterocutaneous fistula, for example, may be associated with Crohn disease, a common cause of enteric hyperoxaluria. A paraplegic patient with an indwelling catheter may be susceptible to frequent urinary tract infections with urease-producing organisms and consequent struvite stone formation. Hyperuricosuria and uric acid stone formation may be seen in patients with tophi related to gout.[6,7,73]

Laboratory Tests

Although valuable information is gleaned from the history and physical examination, it is often difficult to determine the metabolic cause of a patient's nephrolithiasis without laboratory data. The urinalysis is an easy and inexpensive test that provides a great deal of information. Often, the presence of different kinds of crystals can suggest the type of underlying stone (Fig. 32.4). Uric acid and calcium oxalate stones, for example, grow more favorably at an acidic pH, and a consistently high urinary pH may suggest calcium phosphate or struvite nephrolithiasis. The specific gravity, if high, can confirm suspicions of inadequate fluid intake.

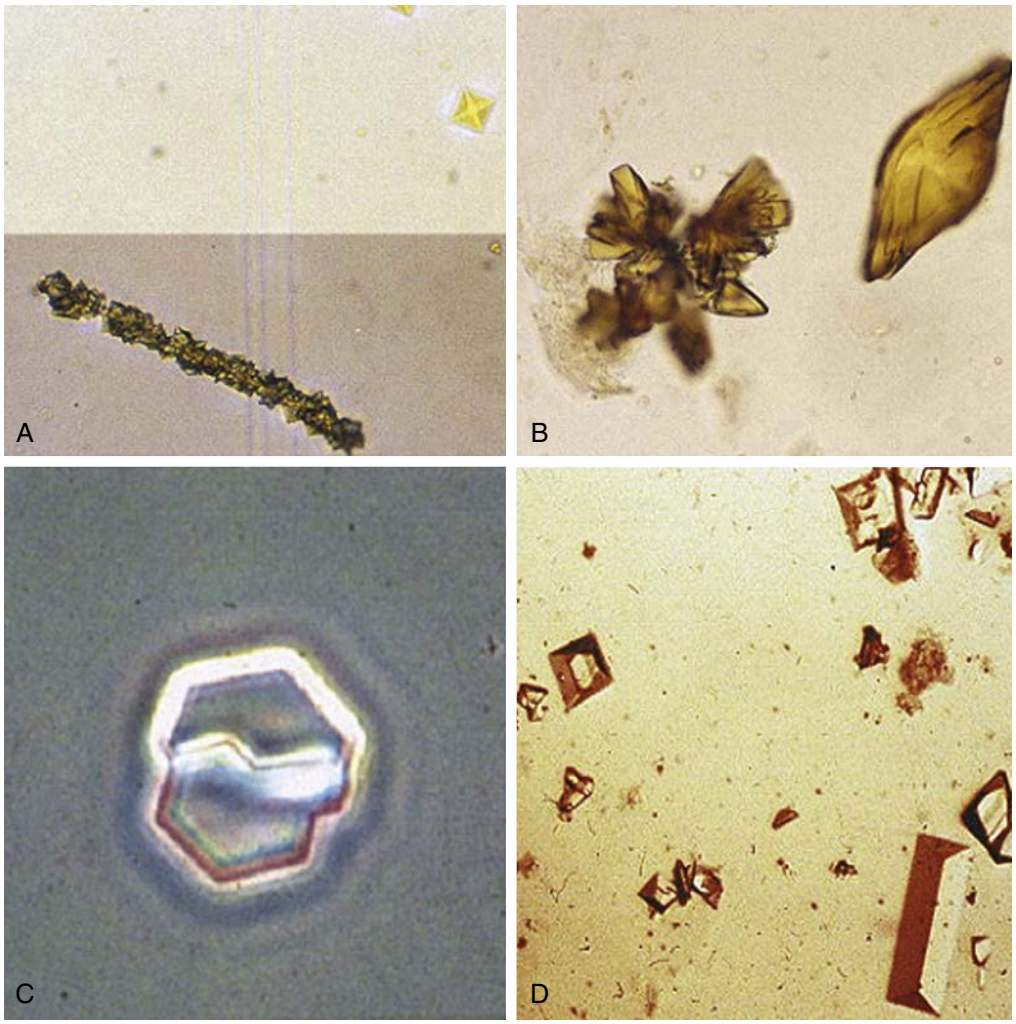
Hematuria is often present in active stone disease. Microscopic examination of the urine in this case might reveal characteristic crystals. Bacteria and pyuria noted in conjunction with a high urinary pH (~6.5) are characteristic of struvite stone disease. Urine specimens for culture should be obtained in this setting. Because enough urease may be produced to form struvite stones even when colony counts are low (~50,000 colony-forming units), the microbiology laboratory should be instructed specifically to identify the organism and check for urease-producing bacteria despite low colony counts.[145]

Qualitative cystine screening should be performed on a urine specimen. Urine turns purple-red when sodium nitroprusside is added to a specimen containing cystine at a concentration greater than 75 mg/L.[138]

Recommended blood tests in the basic evaluation include electrolytes (sodium, potassium, chloride, bicarbonate) uric acid, calcium, phosphorus, and serum creatinine to determine renal function.[73,78,111] If the serum calcium level is elevated or at the upper limit of normal, or if the serum phosphorus level is reduced or at the lower limit of normal, a serum intact PTH level is also determined to rule out primary hyperparathyroidism. Low serum bicarbonate levels suggest a hypocitraturic disorder such as renal tubular acidosis (RTA) or acetazolamide ingestion.

Stone Analysis

Stone analysis should be performed, whenever possible, in patients with a new history of nephrolithiasis or in patients with



• **Fig. 32.4** Crystals seen in urine of stone formers. (A) Calcium oxalate. (B) Urate. (C) Cystine. (D) Struvite.

long-standing stone disease who note a difference in clinical presentation or in the color, shape, or texture of any stone passed. Knowing the constituents of a stone can help the physician target certain elements of the medical history and specific urine studies. In most cases, the stone must be sent to an outside laboratory for examination. X-ray diffraction crystallography and infrared spectroscopy are currently the most accurate methods available for stone analysis.[32]

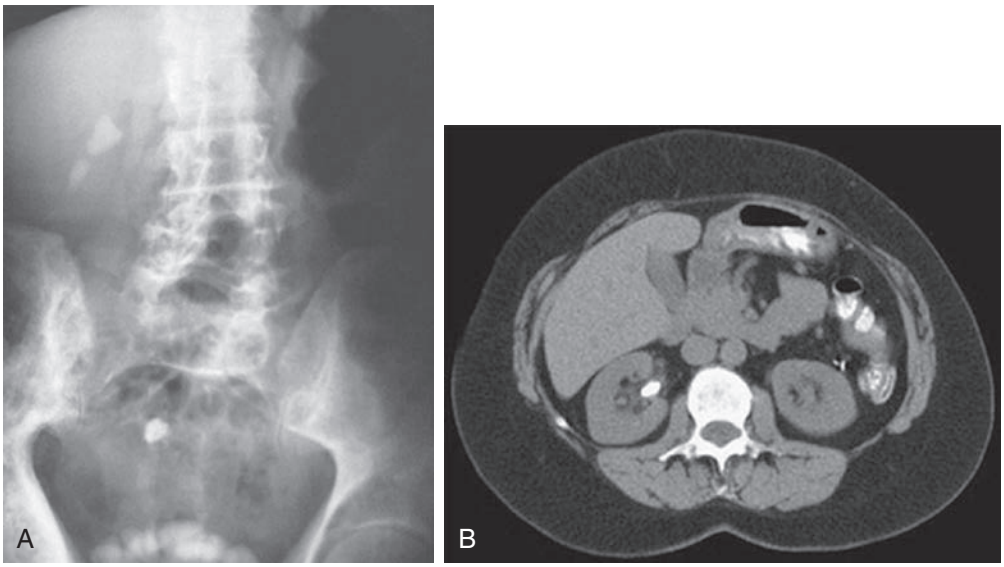
Radiologic Evaluation

Various radiologic tests can help determine the location and extent of the stone burden and might elucidate genitourinary abnormalities contributing to stone formation (Fig. 32.5). For acute renal colic, spiral (or helical) CT without contrast (unenhanced) has replaced intravenous pyelogram (IVP) as the optimal test for detection and localization of kidney stones. Helical CT has proved to be more sensitive (95%) and specific (98%) than IVP in detecting stones of all types in both the kidneys and ureters.[146] In addition, it can more accurately reveal causes of flank pain and hematuria not related to stones, and it circumvents the use of intravenous contrast material. Radiation exposure is a disadvantage of both CT and IVP, and the exposure to patients undergoing helical CT may be triple that of IVP. As such, it should be used judiciously, especially in young patients with frequent episodes of renal colic. Helical CT takes less time to perform, which is a potential advantage in an emergency department setting, but it is more expensive.[147–150] Some institutions are exploring the use of low-dose CT, which has about one third of the radiation exposure of conventional CT with a similar sensitivity and specificity.[146]

CT should be followed by a plain film (radiograph) of the abdomen that includes the kidneys, ureter, and bladder (KUB). Plain films can assist in determining stone composition. Stones composed of calcium, cystine, and struvite are radiopaque and visible on KUB, whereas radiolucent stones, such as those composed of uric acid and xanthine, are not.

Renal ultrasound is a useful test for patients who must avoid exposure to radiation or contrast, such as pregnant women and children. It is almost as sensitive (84%) but not as specific (53%) as spiral CT for detecting kidney stones.[151] Ureteral stones are very difficult to visualize on ultrasound. Emergency department patients with suspected nephrolithiasis who initially have ultrasonography have been shown to have a lower total radiation exposure than those initially evaluated with CT without any difference in serious adverse events, missed important diagnoses, pain scores, or subsequent emergency department visits or hospitalizations.[152,153]

IVPs are useful in detecting certain genitourinary abnormalities that can predispose to nephrolithiasis, such as medullary sponge kidney and caliceal abnormalities. Another advantage of IVP is that the osmotic diuresis generated by the administered contrast agent may aid in excretion of the offending stone during an episode of acute



• **Fig. 32.5** Renal stones on an abdominal radiograph and a computed tomography scan. (A) Radiolucent kidney stone can be seen on the kidneys, ureter, and bladder radiograph at the ureterovesical junction. (B) A large stone is seen in the renal pelvis of the right kidney.

renal colic. A major disadvantage of IVP is exposure to radiographic contrast material. Administration of contrast should be avoided in patients who are at high risk for developing nephrotoxicity from the contrast, such as the elderly; those with diabetes mellitus, proteinuria, or preexisting kidney disease; and patients with significant intravascular volume depletion. With the numerous advantages of CT and ultrasound, IVPs are rarely indicated.

Once a patient is known to have a certain type of stone, specific tests may be used in follow-up. For example, a patient known to have asymptomatic calcium stones can have a KUB test 6 to 12 months later to assess for any increase in stone size or number.[6,7,78] However, because of the radiation exposure, reimaging should be limited to patients in whom the results of the test will alter treatment. Little is gained in asymptomatic patients by checking for stone growth or movement if maximal dietary and pharmacologic therapy is already being prescribed.

The Complete Evaluation

The complete evaluation comprises the entire basic examination as well as a 24-hour urine collection to determine volume and levels of calcium, oxalate, citrate, sodium, urate, phosphorus, creatinine, and the urinary supersaturation with respect to the common solid phase components[6,7,111] (Table 32.5). Creatinine is used to assess the adequacy of the collection: men should excrete approximately 15 to 20 mg/kg of creatinine per day, whereas women should excrete 10 to 15 mg/kg of creatinine per day. Cystine should also be measured in patients known to have cystine stones or in whom prior urine studies have not determined if there is excessive excretion of this amino acid.

Patients should be instructed to collect their urine on a day when they perform usual activities and have their typical fluid and dietary intake. The first morning's urine specimen is discarded; following this, all urine for the next 24 hours (including the next morning's specimen) is collected in the container. The ideal 24-hour urine collection includes measurement and reporting of the daily excretion of the constituents listed in Table 32.5 and also reports supersaturation of calcium oxalate, calcium phosphate, and uric acid. Patients should be instructed to discontinue multivitamins approximately 5 days before the collection to prevent any antioxidant effect of the vitamins on the urine sample. In most cases, an acid or antibiotic is included in the collection container or added with the first urine sample as a preservative. Certain laboratories require various preservatives for the different factors measured. Physicians should ask their laboratory how many 24-hour urine

TABLE 32.5 Optimal 24-Hour Urine Values in Patients With Nephrolithiasis

Parameter	Value
Volume	>2.0–2.5 L
pH	>5.5, <7.0 (24-hour specimen not required)
Calcium	<300 mg or <3.5–4.0 mg/kg in men <250 mg or <3.5–4.0 mg/kg in women
Oxalate	<40 mg
Sodium	<3000 mg or <130 mEq
Uric acid	<800 mg in men <750 mg in women
Phosphorus	<1100 mg
Citrate	>320 mg
Creatinine	~15 mg/kg in men ~10 mg/kg in women to ensure adequacy of collection
Supersaturation of calcium oxalate	<5
Supersaturation of calcium phosphate	0.5–2.0[a] (ideally <1.0)
Supersaturation of uric acid	0–1[a]

[a]Ideal values can vary among laboratories that perform supersaturation analysis.

collections and which preservatives are required for the complete evaluation. Simplifying this process to a single urine collection in which all of the measurements are performed and resultant supersaturation calculated is available from several national laboratories and almost certainly improves adherence and perhaps accuracy of the calculation of supersaturation.[78,154]

Patients who require the complete evaluation are as follows: all children, nonwhite patients (demographic groups not typically prone to nephrolithiasis in the United States), non–calcium stone formers, and patients with metabolically active stone disease (metabolically active stones are those that grow in size or number within 1 year).[6,7]

Therapy

Surgical Treatment

Treatment of an acute episode of renal colic often involves surgical management for large stones that do not pass spontaneously. Stones less than 5 mm in size have a 68% chance of passing spontaneously, whereas those greater than 5 mm but less than 10 mm have a spontaneous stone passage rate less than 50%.[155] Most stones greater than 10 mm, and many greater than 5 mm in size, require surgical intervention for relief of renal colic, ureteral obstruction, or other symptoms of clinically active stone disease. With the advent of newer, less invasive urologic therapies, open surgical stone extraction is rarely performed. Current urologic therapy includes percutaneous nephrolithotomy (PNL), extracorporeal shock wave lithotripsy (ESWL), and ureteroscopic removal of stones (URS). The exact procedure used varies according to stone location in the kidney or ureter, size, composition, various patient factors, and surgical expertise.[155–158]

PNL involves placement of a large needle through the flank into the renal collecting system. The tract is dilated, and instruments are used to rupture and remove the stone. Although more invasive than URS and ESWL, PNL is more effective in removing large (>2 cm) or staghorn calculi, as well as stones that do not fragment well with lithotripsy. Large, infected stones, such as struvite stones, in which complete removal is desired, are best treated with PNL.[157,158] ESWL involves focusing sound waves from a lithotripter outside the body onto the kidney stone. The impulses fragment the stone into smaller stones, or "gravel," that can more easily be passed spontaneously. Newer-generation lithotripters do not require a water bath and often require less analgesia.[159] Kidney stones less than approximately 15 mm, proximal ureteral stones, upper and middle pole kidney stones, and those not composed of cystine or calcium oxalate monohydrate respond best to ESWL.[155,157–159] Because fluoroscopy is typically used to visualize radiopaque stones during the procedure, ESWL may be more complicated in patients with uric acid lithiasis. ESWL is relatively contraindicated in patients with coagulopathy and in pregnant women. It may be less successful in patients with a higher BMI, as a low skin to stone distance is necessary to achieve optimal success.[158,159]

Recent meta-analyses found that PNL is superior to ESWL for most ureteral stones because it results in longer stone-free rates and decreases the need for retreatment. However, it is associated with more, although generally minor, complications and longer hospitalizations.[160,161]

Ureteroscopy, the passage of a semirigid or flexible scope through the bladder and into the ureter, is a mainstay of surgical stone extraction for many ureteral stones, especially distal ureteral stones, and larger proximal stones. Endocorporeal lithotripsy can be added to URS to directly fragment visualized stones. One of the more commonly used devices is the holmium:YAG (yttrium-aluminum-garnet) laser lithotripter, which combines both pneumatic and ultrasound lithotripsy to fragment stones. This results in high stone-free rates following the procedure.[156,162] Complications of ESWL and URS include urinary tract infection; sepsis; ureteral stricture; ureteral injury; and steinstrasse, or "stone street," the linear accumulation of small stones blocking a ureter following fragmentation of a larger stone.

Medical Expulsive Therapy

Another form of therapy that has been shown to reduce the time to stone passage is medical expulsive therapy. Medical expulsive therapy may be utilized with ureteral stones that are less than 10 mm in diameter for up to 6 weeks if kidney function is normal, there is no evidence of infection or significant obstruction, and pain can be controlled. Several medications have shown benefit in reducing the time to stone passage and in assisting with passage of larger stones.[163] A recent trial found that tamsulosin (0.4 mg/day) and nifedipine (30 mg/day) were not effective at decreasing the need for further treatment to achieve stone clearance in 4 weeks.[164] However, meta-analyses have demonstrated a benefit for α-adrenergic blockers such as tamsulosin, terazosin (2–5 mg/day), or doxazosin (4 mg/day).[165,166] These agents act by reducing spasm of the ureteral smooth muscle and allowing ureteral peristalsis to more effectively move the stone through. The addition of corticosteroids may also assist with stone passage by reducing ureteral inflammation and swelling in the ureter where the stone is lodged. In a controlled study, these therapies were compared with control groups involving placebo[167] or standard therapy, such as antispasmodic therapy, nonsteroidal anti-inflammatory agents, or analgesics. In studies comparing α-adrenergic receptor blockers to calcium channel blockers, the α-adrenergic blocker tamsulosin was found in a few studies to result in higher stone passage rates and more rapid stone expulsion than nifedipine.[163,168,169] Both agents were generally well tolerated, although perhaps with less hypotension in the tamsulosin groups. The selective α-1A receptor blocker silodosin (8 mg/day) provided a significantly higher expulsion rate than tamsulosin for distal ureteral stones.[170] Frequent follow-up and occasional imaging with ultrasound is recommended to ensure that the patient remains free of complications while awaiting stone passage.

Medical Preventative Therapy

Medical preventative therapy is the mainstay of medical management, and the remainder of the chapter will focus on prevention of stone recurrence.

Nonspecific Therapy

Most patients, irrespective of stone type, are given general advice about fluid and dietary modification to prevent further stone formation.[6,7,111] These nonpharmacologic interventions, which include an increase in fluid intake, as well as restriction of dietary sodium and animal protein, can reduce the incidence of stone formation—a result termed the *stone clinic effect*.[42,171–173] In one study, such interventions resulted in a 40% decrease in stone recurrence over 5 years.[41]

The mainstay of nonspecific therapy involves dietary measures (see the Diet discussion in the Pathogenesis of Stone Formation section): increased fluid intake to raise urine volume to approximately 2 to 2.5 L, a reduction in sodium intake to less than 3000 mg/day (130 mEq), moderate reduction in animal protein ingestion to approximately 1.0 mg/kg per day, and perhaps eating certain fruits or juices high in citrate.[42,45,78,171,172,174,175]

Dietary calcium restriction is no longer recommended, because it not only can lead to a reduction in bone mineral content but also increases the rate of stone recurrence presumably by decreasing intestinal calcium oxalate absorption and increasing urinary oxalate excretion.[42] In retrospective studies of dietary intake, both women and men have been found to have reduced stone formation with increased dietary calcium ingestion. Calcium supplements, however, were associated with an increased risk of stones in women. Patients should therefore be advised to maintain an age- and gender-appropriate intake of dietary calcium, preferably without supplements.[42,49,59,63,176]

Specific Therapy Matched to Specific Pathogenesis

The optimal therapy for a patient with metabolically active stone disease is directed at the patient's particular metabolic abnormality.[8,177] Before medications for nephrolithiasis are prescribed, all patients should be treated with the nonspecific measures noted earlier. Prior to any therapeutic intervention, some clinicians assess the patient's existing stone burden with a radiologic examination (KUB, spiral CT, IVP, or ultrasonography). If stones are present, any subsequent passage of stones would not necessarily indicate therapeutic failure, because the patient may simply be passing the stones formed prior to therapy. However, this approach must be weighed against the expense and the radiation exposure associated with the radiologic examination. Our personal opinion is to not obtain baseline or follow-up radiographs in asymptomatic patients unless the results will alter subsequent therapy. The basic and complete evaluations help direct the clinician to the specific treatments discussed here.

Calcium Stones

Most kidney stones (~70%) contain calcium (see Fig. 32.1). More than one third of these are composed of calcium oxalate alone, and another 7% are composed of calcium phosphate alone. The rest are composed of a combination of calcium oxalate with either urate or calcium phosphate. The stones tend to be gray, brown, or tan and rarely grow larger than 1 to 2 cm.[78,178,179]

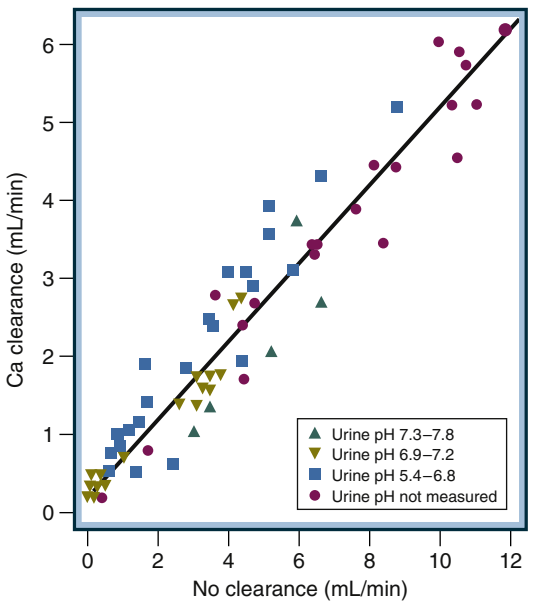
The main causes of calcium stone formation are hypercalciuria (excessive urinary calcium excretion), hyperoxaluria (excessive oxalate excretion), hyperuricosuria (excessive uric acid excretion), hypocitraturia (insufficient citrate excretion), RTA, congenital abnormalities of the genitourinary tract, and certain medications (see Tables 32.2 and 32.3).

Hypercalciuria. Patients with persistent hypercalciuria often benefit from a thiazide diuretic. This class of drugs is inexpensive and extremely effective at reducing urinary calcium excretion and stone formation.[44,78,180] To maximize the efficacy of thiazides, patients must consume a sodium-restricted diet. Urinary calcium excretion parallels sodium excretion (Fig. 32.6), and reducing sodium consumption is essential to reducing hypercalciuria. Whereas hydrochlorothiazide is commonly used for hypertension, chlorthalidone is favored for treating hypercalciuria because it has a longer half-life and requires only once-daily dosing. The starting dose is 25 mg and can be increased to 50 mg. In petite patients or those with low blood pressure, therapy can be initiated with 12.5 mg.

Side effects of thiazides include an increase in serum lipid levels and hyperglycemia. For patients in whom this is a concern, such as those with hypercholesterolemia, other cardiac risk factors, or elevated blood glucose levels, indapamide (1.25–2.5 mg) is an effective alternative.[181] This agent has less of an effect on serum lipids and blood sugar than thiazides.

Hypokalemia is another common side effect of thiazide therapy. Patients should be advised to increase their dietary intake of potassium-rich foods, and the potassium level should be checked 7 to 10 days after starting the medication. Hypokalemia can result not only in cardiac and neuromuscular problems but also in hypocitraturia—another risk factor for stone formation. The supplement of choice is potassium with a base, such as citrate or bicarbonate, as the accompanying anion. Potassium citrate is available as a liquid or as a wax-matrix tablet. The wax-matrix form is preferable because many patients find the liquid unpalatable. Patients with malabsorption disorders, however, may absorb potassium citrate better in the liquid form. Potassium citrate in the wax-matrix formulation is available as 5- and 10-mEq tablets; 20 to 40 mEq/day in single or divided doses is usually adequate supplementation. Determination of follow-up potassium and bicarbonate levels may be required for further dose adjustment. Because citrate is a base, metabolic alkalosis can result with this medication, especially when given with a thiazide diuretic. In such a situation, an alternative potassium supplement (e.g., potassium chloride) may be necessary. If hypokalemia persists or if large doses of supplemental medication are required, the patient might benefit from the addition of a potassium-sparing diuretic. Triamterene is generally avoided because it can precipitate into stones. Amiloride may be initiated at a starting dose of 5 mg or in a combination tablet with thiazide.

After 4 weeks of therapy with the new medication, the 24-hour urine collection should be repeated to assess the efficacy of therapy in reducing calcium levels. The 24-hour urinary sodium and citrate levels should also be measured. The thiazide dose may need to be increased to decrease calcium excretion to less than 3 to 4 mg/kg per day. If sodium excretion remains high in conjunction with elevated urinary calcium excretion, further dietary counseling aimed at reducing dietary sodium may be required. Additional



• **Fig. 32.6** Relationship between urinary calcium and sodium excretion. This study shows how urinary calcium excretion parallels urinary sodium excretion. There is almost a linear relationship between the urinary excretion of calcium and sodium, making sodium restriction in the diet imperative in the treatment of hypercalciuria. (From Walser M. Calcium clearance as a function of sodium clearance in the dog. *Am J Physiol*. 1961;200:1099–1104.)

potassium citrate may be required if urinary citrate or serum potassium levels remain low.[78,182]

Hyperoxaluria. Oxalate is produced predominantly by endogenous metabolism of glyoxylate and, to a lesser extent, by ascorbic acid. Some urinary oxalate is derived from dietary sources, such as rhubarb, cocoa, nuts, tea, and certain leafy green vegetables. Absorbed oxalate is excreted unchanged in the urine and raises urinary supersaturation with respect to calcium oxalate.[72,134,183,184] Hyperoxaluria, as the sole metabolic abnormality, accounts for the formation of only approximately 5% of all calcium-based stones, even though it is often present with other urinary abnormalities that lead to an increase in supersaturation.[37,185,186]

The three main causes of hyperoxaluria are excessive oxalate ingestion (dietary oxaluria), malabsorptive GI disorders (enteric oxaluria), and excessive endogenous production of oxalate related to a hepatic enzyme deficiency (primary hyperoxaluria).

Because ethylene glycol (used as antifreeze in automobiles) is metabolized to oxalate, nephrolithiasis, in conjunction with severe metabolic acidosis and renal failure, is often observed in patients after ingestion of ethylene glycol.[8]

Dietary Oxaluria. Dietary oxaluria results in urinary oxalate levels that are mildly elevated (40–60 mg/day). Many high-oxalate foods are fruits, vegetables, and nuts that are generally considered beneficial in most diets. In a retrospective analysis, patients consuming diets similar to the DASH diet have fewer stones than those who consume diets that are markedly different despite the DASH diet being high in oxalate.[187] The diet is also high in potassium and calcium and low in sodium, which are factors that may be more preventive in stone formation than oxalate is detrimental. Patients with dietary hyperoxaluria should be provided with a detailed list of high-oxalate foods to review (Table 32.6). How restrictive patients need to be with regard to the list is guided by urinary supersaturation and common sense, especially because many patients with stones may also have hypertension and diabetes mellitus and benefit from a diet high in fruits and vegetables.

TABLE 32.6 Foods High in Oxalate

Beans (green and dried)
Beer (draft, stout, lager, pilsner)
Beets
Berries (blackberries, blueberries, raspberries, strawberries, juice made from berries)
Black tea
Black pepper
Celery
Chocolate, cocoa
Eggplant
Figs, dried
Greens (collard greens, dandelion greens, endive, escarole, kale, leeks, mustard greens, parsley, sorrel, spinach, Swiss chard, watercress)
Green peppers
Lemon, lime, and orange peel
Nuts
Pecans, peanuts, peanut butter
Okra
Rhubarb
Sweet potato
Tofu

Data from Monk RD, Bushinsky DA. Nephrolithiasis and nephrocalcinosis. In: Johnson RJ, Frehally J, Floege J, eds. *Comprehensive Clinical Nephrology*, 5th ed. Philadelphia, PA: Elsevier; 2015:688–702.

Patients should be instructed to ingest calcium-containing foods, such as a glass of milk, when eating foods high in oxalate. The calcium in milk binds the dietary oxalate and may prevent its absorption.[37,42] In patients who have severe dietary hyperoxaluria with active stone disease, two or three calcium carbonate tablets (500–650 mg/tablet) may be prescribed with high-oxalate meals. However, this should be done cautiously given the association between calcium supplements and kidney stones in women in the general population (see the earlier Nonspecific Therapy section).

Enteric Oxaluria. Enteric oxaluria results in higher urinary oxalate levels (60–100 mg/day) than dietary hyperoxaluria. GI malabsorptive conditions associated with normal colonic function, such as Crohn disease, celiac sprue, jejunoileal bypass, chronic pancreatitis, and biliary obstruction, can lead to enteric oxaluria. In these disorders, malabsorbed fatty acids bind calcium in the intestinal lumen, making more "free" oxalate available for absorption in the colon. In addition, the colonic mucosa becomes more permeable to oxalate as a result of exposure to malabsorbed bile salts.[188–190]

The mainstay of treatment, whenever possible, is therapy for the underlying disorder. A gluten-free diet, for example, can significantly reduce hyperoxaluria associated with sprue. For other conditions (e.g., surgical short bowel syndrome), no specific therapy is feasible. In such cases, reduction of malabsorption and oxalate absorption may be achieved by instituting general therapy for steatorrhea, such as a low-fat diet, cholestyramine, and medium-chain triglycerides. As in patients with dietary oxaluria, an oxalate-restricted diet and calcium carbonate with meals should be prescribed.[42,191] Because of chronic diarrhea, these patients are at substantial risk for low urine volumes, hypocitraturia, hypokalemia, and hypomagnesuria. The acidic, concentrated urine also predisposes to development of uric acid stones.[192,193] Additional fluid intake must be stressed, and potassium citrate (the liquid form is generally better absorbed but poorly tolerated in these patients) and magnesium supplementation are often prescribed. Magnesium appears to be an inhibitor of stone formation and is supplied as magnesium oxide at 400 mg by mouth two times a day or magnesium gluconate at 0.5 to 1.0 g by mouth three times a day.[194]

Primary Hyperoxaluria. Primary hyperoxaluria (PHO) leads to nephrolithiasis because of hepatic enzyme deficiencies that lead to massive endogenous oxalate production and excretion in these patients.[135,195–198] PHO results not only in severe hyperoxaluria (80–300 mg/day) but also in widespread deposition of oxalate in numerous organs and tissues such as the heart, bone marrow, muscle, and renal parenchyma at a young age. Cardiomyopathy, bone marrow suppression, and renal failure can ensue. In type 1 PHO (80% of cases), the deficient hepatic enzyme is alanine glyoxylate aminotransferase (AGT), and deficiency is caused by one of several mutations found in the AGT gene, *AGXT*. In some patients with type 1 PHO, pyridoxine (vitamin B_6) can increase enzyme activity, thereby reducing oxalate production. In type 2 PHO (10% of cases) patients lack D-glycerate reductase and glyoxylate reductase due to mutations in the *GRHPR* gene. PHO type 3 is due to a defect in the *HOGA1* gene that encodes for mitochondrial 4-hydroxy-2-oxoglutarate aldolase and makes up the remaining cases of PHO.[199]

All patients with PHO should be treated with measures that reduce calcium oxalate supersaturation, such as ample fluid supplementation, potassium citrate, magnesium, and orthophosphate.[197] Orthophosphate is an effective inhibitor of calcium oxalate crystallization but should be avoided in patients with a GFR less than 50 mL/minute. The combination of pyridoxine and orthophosphate improved renal survival at 20 years from 20%

to 74% in patients with PHO I and II.[200,201] *Oxalobacter formingenes* is an enteric bacteria that relies on oxalate for its metabolism.[144,202] In small studies, probiotic supplementation of these bacteria to patients with PHO type 1 resulted in a slight reduction in urinary oxalate excretion.[203,204] Provision of these bacteria, if they become commercially available, may provide additional therapy in the treatment of PHO. PHO patients with renal failure might benefit from renal transplantation because dialysis is not as effective as a functioning kidney in oxalate removal. The general measures to treat PHO should be continued after renal transplantation to prevent rapid loss of the allograft caused by calcium oxalate deposition. Ultimately, for patients with type 1 PHO, liver transplantation can supply the missing AGT and is curative, especially if it is performed before the development of end-stage renal failure. Some patients require combined liver and kidney transplantation.[135,195,205]

Hyperuricosuria. Up to 15% of patients with hyperuricosuria have calcium stones. In contrast to patients with pure calcium oxalate stones, these patients typically have elevated urinary uric acid levels but normal urinary calcium and oxalate levels.[206–208] They also differ from patients with pure uric acid stones in that they tend to have a higher urinary pH.

The mechanism by which uric acid promotes calcium stone formation is unclear. The terms *heterogeneous nucleation* or *epitaxy* are used to describe the preferential formation of calcium oxalate crystals around a lattice of uric acid crystals present in the urine.[72,209,210] More recently, this mechanism has come into question. Grover and associates[211,212] have shown that the addition of sodium urate to urine or similar solutions increases calcium oxalate crystallization, with denser, more aggregated deposits, without the presence of urate crystals, and with no increase in calcium oxalate supersaturation. They attribute this to "salting out"—a process in which the solubility of electrolytes (or salts) in a solution is reduced (or the ion activity increased) by the addition of different electrolytes/salts. As such, the activity coefficient of calcium and oxalate would be increased not only by the concentrations of calcium and oxalate in the urine but also the urate concentration. This theory would explain why allopurinol is often an effective therapy for recalcitrant calcium oxalate nephrolithiasis, even in the absence of hyperuricosuria.[213,214] Another potential mechanism (but not borne out by studies) is that urate may reduce the concentration or the activity of urinary stone inhibitors.[215–217]

Whatever the mechanism, uric acid in the form of sodium urate is important in calcium oxalate crystal formation. Therapy has generally consisted of dietary purine restriction and increased fluid intake. If urinary uric acid levels remain uncontrolled with these measures, allopurinol, 100 to 300 mg/day, may be added.[206,207]

Hypocitraturia. Citrate, by combining with calcium to form a soluble complex, reduces calcium oxalate and calcium phosphate precipitation, thus acting as the most important inhibitor of calcium crystallization in urine.[8,72,177] In some patients, hypocitraturia is the principal metabolic abnormality found in the 24-hour urine collection. Risk factors for hypocitraturia include high protein intake, hypokalemia, metabolic acidosis, exercise, infection, starvation, and therapy with androgens or acetazolamide. Men tend to have lower urinary citrate concentrations than women, which may be responsible for the higher incidence of stone formation in men. Furthermore, women with nephrolithiasis have lower urinary citrate concentrations than non–stone-forming women.[218] Although citrate excretion below 320 mg/L per day of urine is defined as hypocitraturia, the risk of nephrolithiasis is a continuous function of urinary citrate concentration.[219]

Along with therapy for the underlying condition, such as moderating dietary protein intake, potassium citrate or potassium-magnesium citrate is prescribed, and both formulations are effective in preventing calcium stones, even in patients who are not hypocitraturic.[220] The potassium salt is preferable to sodium citrate because sodium excretion promotes calcium excretion in parallel and leads to hypercalciuria. Again, potassium citrate in the wax-matrix formulation is preferred to the liquid preparation because of increased palatability. Large amounts may be required (30–75 mEq/day) in divided doses to raise the urinary citrate concentration to more than 320 mg/day. Potassium and bicarbonate levels should be closely monitored, especially in patients with CKD. If metabolic alkalosis or hyperkalemia ensues, reduction of the dose may be necessary.[221,222]

Renal Tubular Acidosis. dRTA (type 1) is a disorder in which distal tubular hydrogen ion excretion is impaired, resulting in a non–anion gap metabolic acidosis and a persistently alkaline urine.[223,224] The acidosis leads to calcium and phosphate release from bone, as well as enhanced proximal tubular reabsorption of citrate and diminished tubular reabsorption of calcium.[8] The net result is an increased filtered load and excretion of calcium and phosphate, severe hypocitraturia, and an elevated urinary pH, all of which promote calcium phosphate precipitation. Nephrocalcinosis, or renal parenchymal calcification, is frequently seen in this setting.[6]

The 24-hour urinary citrate levels are commonly lower than 100 mg in patients with dRTA. Therapy consists of potassium citrate or potassium bicarbonate supplementation to treat both the metabolic acidosis and hypocitraturia. Large doses of these medications are often required: 1 to 3 mEq/kg per day in two or three divided doses.[178,195,206]

Nephrocalcinosis. Nephrocalcinosis is a process in which calcium is deposited in the renal parenchyma.[6] There are two forms: dystrophic calcification and metastatic calcification.

In dystrophic calcification, calcium deposition arises from tissue necrosis secondary to neoplasm, infarction, or infection. It may be seen in the setting of renal transplant rejection, renal cortical necrosis, chronic glomerulonephritis, ethylene glycol toxicity, acquired immunodeficiency syndrome–related infections, and Alport syndrome. In general, in dystrophic calcification, serum calcium and phosphorus levels are normal, and calcium phosphate deposition predominantly occurs in the renal cortex.

In metastatic calcification, patients often have elevated serum calcium and phosphate levels or an elevated urinary pH. Calcification in this setting occurs more commonly in the renal medulla. Common causes include RTA; primary hyperparathyroidism (or any disorder resulting in elevated serum calcium levels); medullary sponge kidney; papillary necrosis; PHO; hereditary hypophosphatemic rickets; and administration of acetazolamide, amphotericin B, oral sodium phosphate bowel preparations, and triamterene. PHO can result in both medullary and cortical calcifications.

Both medullary and cortical parenchymal calcifications are easily noted with ultrasonography and CT scanning, even before they can be detected on plain radiographs. Therapy consists of treating the underlying disorder whenever possible. Otherwise, measures aimed at reducing hypercalcemia, oxalosis, and hyperphosphatemia should be attempted.[38,114,225]

Uric Acid Stones

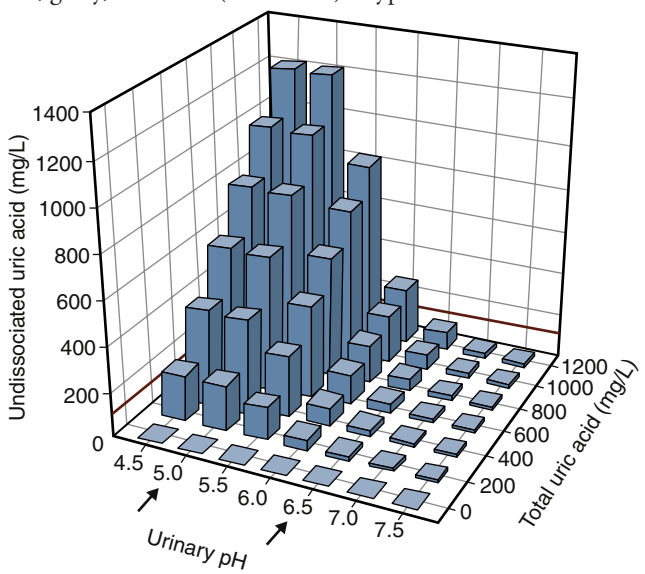
Uric acid lithiasis is far more common in Mediterranean countries than in the United States. However, the incidence of uric acid stones in the United States appears to be rising in parallel

with the epidemic of obesity. Obesity and the metabolic syndrome are associated with insulin resistance, which results in a very low urine pH.[30,226–228] Uric acid stones tend to be round, smooth, and yellow-orange. Because they are radiolucent, they are not visible on plain films but can be detected by ultrasonography or CT or as filling defects on IVP. Uric acid is a purine metabolite and is also found in large quantities within cells. Most patients with uric acid stones have a reduced urinary pH, whereas other less common causes are low urine volume or elevated urinary uric acid levels. Factors associated with uric acid stones are listed in Table 32.3.

A low urine pH is the major cause of uric acid nephropathy. The solubility of uric acid increases sixfold with an increase in urine pH from 5.3 to 6.5[207,226] (Fig. 32.7). Thus, conditions that lower urine pH tend to predispose patients to uric acid lithiasis. Loss of bicarbonate during chronic diarrheal diseases and the higher acid load of diets rich in animal protein lead to acidemia and contribute to an acidic urinary pH. In four studies, every patient with uric acid stones had a urine pH less than 6.0.[229–232] A low urinary pH leads to the formation of poorly soluble uric acid as opposed to the more soluble urate anion, thus predisposing to uric acid lithiasis, even when the total amount of uric acid being excreted is not above normal.[233]

Uric acid stone formers often have greater body weight and a higher incidence of insulin resistance and type 2 diabetes mellitus. Most of these patients have significantly lower urinary pH compared with non–uric acid stone formers. It is thought that insulin resistance leads to impaired ammoniagenesis and ammonium excretion, resulting in excretion of more urinary hydrogen ions with anions other than ammonia and at a lower urinary pH.[29,30,226,230]

Hyperuricosuria may be evident in patients who ingest large quantities of dietary purine or animal protein. Foods high in purine include organ meats, shellfish, certain fish, meat extracts, yeast, gravy, and stock (Table 32.7). Hyperuricemic disorders such as gout, myeloproliferative disorders, tumor lysis syndrome, and certain inborn errors of metabolism (e.g., glucose-6-phosphatase deficiency, Lesch-Nyhan syndrome) can also contribute to an increased urinary filtered load of uric acid. Certain medications such as salicylates and probenecid are hyperuricosuric as well and can predispose patients to uric acid lithiasis.[6,7,30,143,225]



• **Fig. 32.7** The pH and solubility of uric acid. The relationship between undissociated uric acid, total uric acid, and urinary pH is presented. The limit of solubility of undissociated uric acid is depicted by the *colored line* (~100 mg/L). Two hypothetical urine pHs are considered (*two arrows*). At low urinary pH (e.g., 5.0), even a modest amount of total urinary uric acid will exceed its solubility. At high urine pH (e.g., 6.5), even massive hyperuricosuria is well tolerated. (From Maalouf NM, Cameron MA, Moe OW, Sakhaee K. Novel insights into the pathogenesis of uric acid nephrolithiasis. *Curr Opin Nephrol Hypertens*. 2004;13:181–189.)

Therapy for patients with uric acid stones begins with nonspecific measures such as increasing fluid intake to maintain urine volume at about 3 L/day. A lowered animal protein diet is generally beneficial because the decreased endogenous acid production raises urinary pH.[51] Ideally, the urinary pH should be elevated to approximately 6.5 to 7.0, a level that can dissolve existing crystals and stones. However, care should be taken to prevent the urine pH from rising above 7.0 to minimize the risk of calcium phosphate lithiasis. A low-fructose diet may also be beneficial in reducing uric acid levels and hyperuricosuria (see the discussion in the Nonspecific Therapy section).[58]

Potassium citrate at doses of 30 mEq by mouth two times a day or more may be required to raise the urinary pH sufficiently to decrease supersaturation with respect to uric acid (see "Hypercalciuria" and "Hypocitraturia" on available potassium citrate preparations). If the urinary pH cannot be raised adequately despite high doses of potassium citrate or if the dose prescribed results in hyperkalemia, the carbonic anhydrase inhibitor acetazolamide may be initiated. Use of this medication results in an alkaline urine and mild systemic metabolic acidosis, a pattern similar to that in type 1 RTA. The urinary pH should be maintained at less than 7.0 to avoid calcium phosphate precipitation.[206] Prescription of nitrazine paper allows patients to monitor the urinary pH at various times of day and adjust their potassium citrate intake accordingly.

Patients with hyperuricemia are prescribed a low-purine diet to decrease uric acid production. Despite dietary intervention, hyperuricemia often persists, especially in patients with disorders of cellular metabolism. In this setting, allopurinol should be prescribed at a starting dose of 100 mg/day, increasing to 300 mg/day as needed.[72,234] Although sodium bicarbonate can effectively alkalinize the urine, it should be avoided because the additional sodium excretion encourages sodium urate formation, which may result in further crystal formation (see the earlier Hyperuricosuria section).

Struvite Stones

Struvite stones are also called *triple phosphate stones*, *magnesium ammonium phosphate stones*, and *infection stones*.[8] Although they make up only about 10% to 15% of all stones formed, most staghorn calculi (large stones that extend beyond a single renal calyx) are composed of struvite. The propensity of these stones to grow rapidly to a large size, to recur despite therapy, and to result in significant

TABLE 32.7 Foods High in Purine

Organ meats: brain, heart, kidney, liver, sweetbreads
Meat extracts: bouillon, consommé, stock, gravy
Meat: beef, chicken, goose, lamb, pork
Shellfish: clams, mussels, scallops, shrimp, oysters
Fish: anchovies, fish roe, herring, mackerel, sardines, and others
Certain vegetables: asparagus, cauliflower, kidney beans, lentils, lima beans, mushrooms, peas, spinach

Data from Monk RD, Bushinsky DA. Nephrolithiasis and nephrocalcinosis. In: Johnson RJ, Frehally J, Floege J, eds. *Comprehensive Clinical Nephrology*, 5th ed. Philadelphia, PA: Elsevier; 2015:688–702.

morbidity (and potential mortality) has also led to the appellation "stone cancer." Infection with urease-producing bacteria must be present for these stones to form, and therefore severe renal infections, as well as sepsis and loss of renal function, can develop. Factors associated with struvite stones are listed in Table 32.3.

In contrast to other stone types, struvite stones occur with a higher incidence in women than in men, largely because of the increased susceptibility of women to urinary tract infections.[235] Other groups at risk for development of struvite stones because of urinary stasis or infection include elderly people and patients with neurogenic bladders, indwelling urinary catheters, spinal cord lesions, or genitourinary abnormalities. Even without a stone analysis, struvite stones should be suspected in patients with large stones, an alkaline urinary pH (~7.0), and the presence of urease-producing urinary bacteria. Early detection and therapy are essential to prevent great potential morbidity.[145]

Urease-Producing Bacteria. The formation of struvite stones depends on the presence of both ammonium ions and an alkaline urinary pH—conditions met clinically only through the actions of urease-producing bacteria. Ammonium, magnesium, and carbonate apatite [$Ca_{10}(PO_4)_6CO_3$] in the urine combine with phosphate, which is present in its trivalent form in this setting.[8]

Numerous bacteria, both gram negative and gram positive, as well as *Mycoplasma* and yeast species, have been implicated in urease production. Bacteria species in which urease is frequently isolated include *Proteus*, *Haemophilus*, *Corynebacterium*, and *Ureaplasma*. *Escherichia coli*, despite its frequent role as a urinary tract pathogen, has not been shown to produce urease and thus is not implicated in the genesis of struvite stones. Urease production adequate to stimulate stone formation may be present despite low bacterial colony counts. For this reason, the microbiology laboratory should be asked specifically to perform identification of bacteria and determine sensitivities even if colony counts are lower than 100,000 colony-forming units. If no bacteria are isolated but a urease producer is suspected, special cultures should be ordered for *Ureaplasma urealyticum*, which is a mycobacterium that tends to be a fastidious grower on regular culture media.[236]

Therapy for Struvite Stones. To eradicate struvite stones, early and aggressive medical and urologic management is required.[237] Appropriate antibiotic therapy is essential but must be combined with long-term bacterial suppression and complete surgical or medical stone removal. ESWL is often adequate for fragmentation of stones smaller than 2 cm, but percutaneous nephrostolithotomy, or a combination of the two procedures, is usually required for larger stones.[238] Antibiotics should be continued on the basis of cultures of any stone fragments retrieved. After approximately 2 weeks of antibiotic therapy, when the urine culture is sterile, the dose of antibiotic should be halved. Suppressive antibiotics should continue at this dose until monthly surveillance cultures remain sterile for 3 consecutive months. At this point, antibiotics may be discontinued as long as surveillance urine cultures are obtained monthly for 1 year.[236,239]

In addition to antimicrobial therapy, medical treatment may involve urease inhibition and chemolysis. In chemolysis, the kidney is irrigated with an acidic solution through a nephrostomy tube or ureteral catheter. Although rarely used today with the advent of less invasive surgical techniques, this procedure may be useful in the dissolution of residual stone fragments. Ten percent hemiacidrin, the solution most commonly used, is composed of carbonic acid, citric acid, D-gluconic acid, and magnesium at a pH of 3.9. The use of chemolysis has been controversial because high mortality rates have been reported in the past.[240,241] The morbidity and mortality were mainly due to sepsis from instrumentation, local bacterial or fungal infections, and uroepithelial irritation rather than toxicity from the agents. When used as an adjuvant to surgical removal, chemolysis leads to lowered stone and infection recurrence rates.[240,242,243] The safety of the procedure remains in question due to the variety of techniques, stone burden, and comorbidities reported in older literature, but with close monitoring of serum magnesium levels, intrapelvic pressures, infection, and obstruction to flow, it may have a supporting role in the treatment of large struvite stones.[236,240,244]

Urease inhibition has been shown to retard stone growth and prevent new stone formation.[245,246] It does not decrease bacterial counts and cannot eradicate existing stones. Combined with antimicrobial therapy, it serves primarily as palliative care for patients who cannot undergo definitive surgical management. The agent most commonly used is acetohydroxamic acid (AHA). These medications require adequate renal clearance for therapeutic efficacy and are contraindicated in patients with a GFR less than 60. CKD increases the incidence of side effects of these medications, which are numerous and limit their use. Side effects that result in discontinuation of the drug include neurologic symptoms, GI upset, hair loss, hemolytic anemia, and rash. Fortunately, the side effects all resolve with discontinuation of the drug. AHA is also teratogenic. The starting dose of AHA is 250 mg by mouth two times a day. If it is well tolerated for about 1 month, the dose is increased to 250 mg by mouth three times a day.[236]

Cystine Stones

Cystinuria, which is not to be confused with the more serious and debilitating disorder cystinosis that results in extensive intracellular cystine accumulation, is an autosomal disorder that may be recessive or dominant with incomplete penetrance.[247] The disorder is due to mutations of the *SL3A1* gene on chromosome 2 or to mutations of the *SCLC7A9* gene on chromosome 19, both resulting in decreased renal tubular reabsorption and excessive urinary excretion of the dibasic amino acids cystine, ornithine, lysine, and arginine.[248] The genetic defect would probably go unnoticed were it not for the low solubility of cystine of approximately 300 mg/L. Factors associated with cystine stones are listed in Table 32.3.

People with no tubular defect in cystine transport excrete approximately 30 to 50 mg of cystine per day that dissolves in the normal daily urine volume. Patients heterozygous for this condition excrete about 400 mg/day, whereas homozygotes often excrete more than 600 mg/day.[138] Even though the solubility of cystine increases significantly when urine pH is greater than 6.5, the excessive amounts produced in this condition leads to cystine crystals precipitating and aggregating as cystine stones.

Stones usually develop in patients within the second or third decade of life. The stones can grow to a large size and can appear as staghorn calculi or multiple stones. They are radiopaque because of the sulfur content of cystine. The disease should be suspected in any patient with stone onset in childhood, frequent recurrence of nephrolithiasis, and a strong family history of the disease. The presence of the classic hexagonal cystine crystals in the urine can verify the diagnosis. Because these crystals might not be evident in dilute or alkaline urine, qualitative screening with the sodium nitroprusside test better confirms the presence of cystinuria at a concentration greater than 75 mg/L. Quantitative cystine measures with a 24-hour urine sample should follow to determine the risk of stone formation and to guide therapy.

Therapy for Cystine Stones. The aim of treatment is to lower the urinary cystine concentration below the limits of solubility

(~300 mg/L). Patients are advised to drink large quantities of fluids. A patient with a cystine excretion of 750 mg/day, for example, should ideally drink enough fluid to increase urine output to more than 3 L/day. Large quantities of milk should be avoided because dairy products and foods high in protein contain large amounts of methionine, an essential amino acid that is a precursor of cystine.[249] Because cystine is more soluble at a higher pH, juices are encouraged because they tend to alkalinize the urine. Potassium citrate (see the Hypercalciuria and Hypocitraturia sections for details) is also prescribed to maintain the urinary pH between 6.5 and 7.0.[250]

Approximately 50% of cystine stones are mixed stones. Patients with cystinuria often have other metabolic defects such as hypercalciuria, hypocitraturia, and hyperuricosuria. Therefore, a complete 24-hour urine collection for all stone-forming elements is necessary to treat nephrolithiasis fully in this setting. Restricting dietary sodium is also beneficial.[251]

If these measures are inadequate in controlling stone formation or if the urinary cystine concentration is too high to make adequate fluid intake practical, chelating agents called *cystine-binding thiol drugs* (CBTDs) may be added. Increasing urine pH increases the efficacy of thiol drugs.[250] D-Penicillamine is a CBTD that reduces the cystine concentration by forming a more soluble compound with cystine.[252] However, this medication is associated with numerous serious side effects that limit its use. Other CBTDs, such as tiopronin, α-mercaptoproprionyl glycine, and bucillamine, are now available that reduce the cystine concentration with fewer side effects.[138,253–255]

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Physiology of Insulin Secretion

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CHAPTER OUTLINE

KEY POINTS

- Beta-cell function is central for glucose homeostasis: insulin secretion is finely regulated by multiple mechanisms to ensure appropriate hormone delivery under changing short-term conditions (e.g., variable nutrient intake) and longer-term circumstances (e.g., obesity, insulin resistance, pregnancy).
- At a cellular level, the current model features different populations of cytoplasmic insulin granules based on their ability to undergo rapid exocytosis. An immediately releasable pool underlies the fast insulin secretion phenomena, and its exocytosis is triggered by membrane depolarization and calcium influx consequent to glucose metabolism. The amplifying role of glucose contributes to sustained insulin secretion and likely involves changes in granule status.
- The insulin secretion response is polymorphic, and its in vivo study requires different tests to assess different response modes.
- The main stimulus for insulin secretion is glucose. Measurement of plasma C-peptide concentrations during an oral (OGTT or mixed meal) or intravenous (hyperglycemic clamp, and graded glucose infusion) glucose tolerance test coupled with mathematical modeling allow recapitulating the main insulin secretion characteristics that are relevant to both normal and impaired glucose tolerance.
- In an oral glucose tolerance test, the insulin secretion excursions parallel those of glucose concentration, thereby defining a dose response relating the two variables, which is the most important beta-cell response mode. Additional modes include an anticipatory secretion phase at rising glucose and potentiation over time.
- An important longer-term insulin secretion adaptative response is the insulin secretion increase with insulin resistance.
- Reduced beta-cell glucose sensitivity and increased absolute insulin secretion characterize the early stages of glucose intolerance and predict progression to overt diabetes.

Introduction

The endocrine pancreas is made up of roughly 3 million islets scattered through the exocrine tissue, for a total weight of about 1 g (under 3% of the pancreas volume).[1] The human islet is populated by beta cells intermingled with α cells, δ cells, γ cells, and ε cells, secreting glucagon, somatostatin, pancreatic polypeptide, and ghrelin, respectively. Beta cells are the most abundant cell type (composing about 60% of islet cells) and represent a higher proportion of all endocrine cells in smaller, compared with larger, islets. Small islets also have a higher insulin content and are in closer contact with blood vessels, thereby representing a functionally distinct subpopulation. The spatial arrangement of cells within the islet is also important for islet function. Although beta cells are irregularly distributed in human islets, when stimulated by glucose, membrane potentials and cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_c$) show a complex pattern of oscillations in both single beta cells and whole islets.[2] Furthermore, gap junctions provide connectivity, which may be devolved to small clusters of cells with pacemaker properties.[3] The origin and regulation of intrinsic electrical oscillations and hyperspecialized beta-cell subsets are still under investigation, but the heterogeneity of islets, and of beta-cell subsets within them, adds levels of complexity to well-known intra-islet interactions among different cell types (e.g., paracrine inhibition of glucagon secretion by insulin, inhibition of glucagon and insulin secretion by somatostatin). Moreover, endogenous

islet regeneration may occur not only by neogenesis from ductal cells and beta-cell replication but also by transdifferentiation of α cells[4] or δ cells[5] following extreme beta-cell loss.[6] Therefore, a novel concept emerging in beta-cell science is that function is not only the result of mass and quality of beta cells but of the architecture and structural integrity of the islet as an organ.

Beta cells express a large number of transporters and receptors on their plasma membrane; their simultaneous or sequential ligand engagement following a variety of stimuli is eventually integrated into a given secretion rate.[7] Like neurons, whose response to multiple synaptic inputs is an action potential, the beta cell modulates its secretory response in phase and amplitude (i.e., in time course and amount) to precisely fit the stimulus.[8] The overwhelming complexity of stimulatory and inhibitory signals and their intracellular transduction and modulation can be operationally reduced to two main physiologic domains: a triggering pathway, starting at the cell membrane level, and an amplifying pathway, which is mostly intracellular.[9] Thus, glucose enters beta cells via the isoform 2 of the glucose transporter (GLUT2) and is metabolized through the glycolytic pathway and, subsequently, in mitochondria. The first rate-limiting step in this process is the phosphorylation of glucose to glucose-6-phosphate. This reaction is mediated by the enzyme glucokinase, which, by determining the rate of glycolysis, functions as the glucose sensor.[10] Glucose metabolism raises adenosine triphosphate (ATP) production, leading to closure of the ATP-sensitive potassium (K_{ATP}) channel and membrane depolarization. This causes Ca^{2+} entry through the voltage-dependent Ca^{2+} channel and elevation of $[Ca^{2+}]_c$, which initiates exocytosis of the hormone from readily releasable granules. The increase in ATP production is likely due both to nicotinamide adenine dinucleotide production at the glycolytic step of glyceraldehyde-3-phosphate oxidation and to pyruvate oxidation in the tricarboxylic acid cycle in mitochondria. The increase in ATP not only closes K_{ATP} channels but also serves as a major permissive factor for movement of insulin granules and for priming of exocytosis.

The biology of K_{ATP} channels is relevant for beta-cell physiology. These channels include sulfonylurea receptors (SURs) and potassium inward rectifiers (Kir6.1 and Kir6.2), which assemble to form a large octameric channel. In the beta cell, the SUR1/Kir6.2 pairs constitute the K_{ATP} channel, which controls the flux of potassium ions. Importantly, the opening of these channels can reset the resting membrane potential below the threshold for activation of voltage-gated Ca^{2+} channels, thereby aborting insulin secretory bursts. This occurs when plasma glucose levels are low or on insulin stimulation, in the latter case, installing an autocrine control of insulin secretion.[11] Mutations in both components of the beta-cell K_{ATP}—SUR1 (encoded by the *ABCC 8* gene) and Kir6.2—have been shown to lead to hypersecretion of insulin, resulting clinically in either a recessive form of familial hyperinsulinemia or persistent hyperinsulinemic hypoglycemia of infancy.

The amplifying pathway also requires glucose metabolism but is K_{ATP} channel independent. In signal amplification, cyclic adenosine monophosphate (cAMP) plays an important role. This second messenger is generated at the plasma membrane from ATP and potentiates glucose-stimulated insulin secretion, particularly in response to glucagon, glucagon-like peptide 1 (GLP1), and glucose-dependent insulinotropic peptide (GIP). The cAMP–dependent pathways appear to be particularly important in the exocytotic machinery.

More recently, additional molecules have been shown to act as glucose sensors independent of glucose metabolism.[12] In rodent systems, these are cell-surface receptors identified by blocking glucose utilization following the application of a glucose stimulus. They also work by changing the synarchic messengers cAMP and $[Ca^{2+}]_c$. These additional glucose sensors have tentatively been defined as heterodimers of an atypical sweet taste receptor (T1R3) and a calcium-sensitive receptor (CaSR). Their role in human beta cells and in vivo insulin secretion is still under investigation.

Neural Regulation of Beta-Cell Function

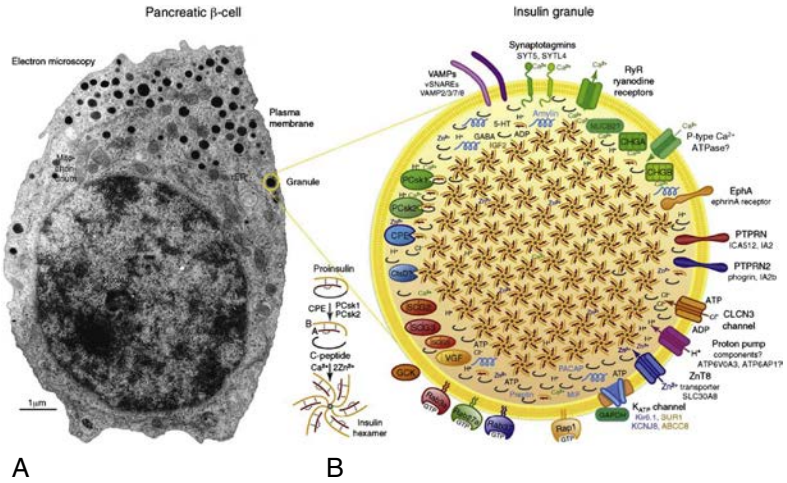
The brain provides supplemental control of insulin and glucagon secretion via efferent nerves penetrating the pancreatic islet.[13] Sympathetic stimulation (through α2 receptor–mediated norepinephrine release) inhibits insulin secretion and potentiates glucagon secretion,[14] whereas parasympathetic stimulation (through M3 muscarinic-mediated acetylcholine release) enhances insulin and glucagon release.[15] These autonomic circuits emanate from hypothalamic regions that differentially control hormone release. In particular, experimentally lowering glucose sensing in glucokinase-expressing neurons of the arcuate nucleus results in deficient glucose-stimulated insulin secretion and glucose intolerance, whereas the same manipulation in the lateral hypothalamic area results in enhanced glucose sensitivity and glucagon response to hypoglycemia.[16] The relative contribution of the brain/islet axis to whole body glucose homeostasis and counterregulation is difficult to quantitate in humans; however, it is interesting that in rodents, islet grafts under the kidney capsule[17] or in the anterior chamber of the eye[18] undergo timed peri-islet and intra-islet reinnervation, a striking example of neuroplasticity.

Beta-Cell Mass

Beta cell mass is the net balance between positive factors, including islet neogenesis, beta-cell proliferation, and beta-cell hyperplasia, and negative factors such as beta-cell apoptosis and dedifferentiation.[6] Despite being a terminally differentiated cell type, β cells are not entirely postmitotic. In humans, most beta-cell neogenesis from ductal epithelial progenitor cells occurs preterm; together with a neonatal burst of β-cell replication (from pancreatic progenitor and/or ductal precursor cells), the full complement of beta-cell mass is established within the first 5 years of life. Thereafter, beta-cell proliferation is very low, with the average lifetime of beta cells extending to about 25 years.[19] However, at the islet periphery, a specialized microenvironment or neogenic niche harbors a population of transcriptionally immature (virgin) beta cells, which constitute a lifelong reservoir of new beta cells.[20] Such cells have an intermediate phenotype between α-cells and mature beta cells and can transdifferentiate to one or the other depending on prevailing signals, such as increased insulin demand (as in obesity, extreme beta-cell loss or pregnancy) or stressors (e.g., hyperglycemia, oxidative overload as in the transition from normal glucose tolerance to type 2 diabetes [T2D]).[4-6] Of note, replicating beta cells upregulate hundreds of proliferation-related genes, but not genes involved in beta-cell function (glucose sensing and insulin secretion), suggesting that the quiescence-proliferation transition involves global amplification of gene expression, except for a subset of tissue-specific genes.[21] Efforts to catalog the transcriptional program of replicating beta cells are key not only to account for the physiologic plasticity of islet cell types but also to identify molecular pathways of beta-cell generation for potential transplant-based therapies.

Beta-Cell Insulin Content

Despite its small size, the endocrine pancreas has a large functional reserve, as pancreatic insulin content has been estimated to be in the range of 200 to 250 units (a 10-day supply for a healthy lean



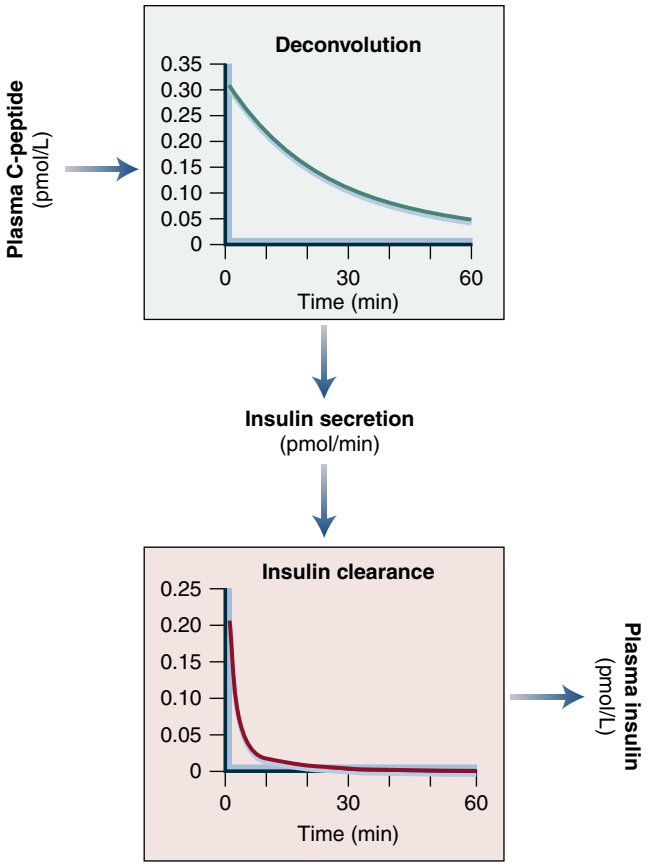
• **Fig. 33.1** (A) Electron micrograph of a human beta cell. The electron-dense spherical bodies in the cytoplasm are insulin granules, some in close proximity of the plasma membrane. Mitochondria and endoplasmic reticulum are also visible. (B) An insulin granule is blown up to schematically highlight its complex structure: in addition to insulin hexamers, receptors, ion channels, transporters, and other proteins are located in the vesicle membrane and within the granule. Also indicated is the maturation of the insulin prohormone proinsulin to insulin (A and B chain) and C-peptide catalyzed by carboxypeptidase E (CPE) and proconvertases (PCsk1 and PCsk2). *5-HT*, 5-Hydroxytryptamine (serotonin); *GABA,* γ–aminobutyric acid; *IAPP,* islet amyloid polypeptide; *IGF2*, insulin-like growth factor 2; *INS,* insulin; *MIF,* macrophage migration inhibitory factor; *PACAP,* pituitary adenylate cyclase-activating polypeptide. (From Suckale J, Solimena M. The insulin secretory granule as a signaling hub. *Trends Endocrinol Metab*. 2010;21[10]:599–609.)

adult). Within the beta-cell cytoplasm, insulin is packaged in 5000 to 8000 secretory granules spatially distributed in age-distinct populations.[22] As shown in Fig. 33.1, each granule (300–350 nm in diameter) contains an electron-dense core composed of tightly packed crystals of insulin hexamers stabilized by one calcium and two zinc ions.[23] Granules are not just delivery depots but dynamic structures containing many proteins, small molecules, and ions in the lumen, as well as several transmembrane proteins, channels, and membrane-associated proteins. Granule turnover and trafficking is a highly regulated process involving relationships with the cytoskeleton, intracellular insulin degradation, mobility (dynamic, restricted, or nearly none), docking, and fusion with the plasma membrane (mediated by soluble N-ethylmaleimide–sensitive factor attachment protein receptor [SNARE] proteins).[22,23] Functionally, the established facts are that (1) only a small fraction (much less than 1%) of granule insulin is secreted in response to acute in vitro glucose stimulation; (2) granule half-life is less than 5 days, with intracellular degradation starting already within about 3 days; and (3) younger granules are fewer but more mobile than older granules even if they come from deep in the cytoplasm and therefore form a readily releasable pool. Which features of secretory granule dynamics—age, position in the cell, and molecular signature—are critical for which aspect of in vivo insulin release dynamics and which steps may be altered in the diabetic state remain to be determined.

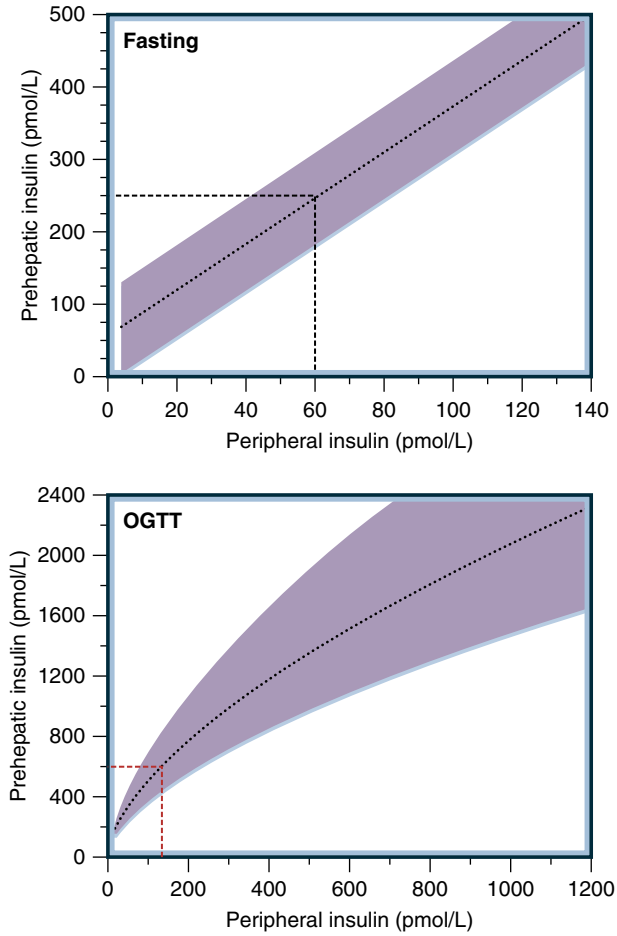
Insulin Secretion Versus Plasma Insulin

Circulating insulin concentrations are a function of secretion and clearance, which is complicated by the appearance of the hormone first into the portal circulation and by substantial hepatic clearance. Insulin secretion normally cannot be measured directly due to the inaccessibility of the portal circulation. Thus, when assessing insulin secretion in vivo, methods based on the measurement of C-peptide currently are state of the art. The principles of this method are that (1) C-peptide is co-secreted with insulin in equimolar amounts as a consequence of proinsulin cleavage, (2) C-peptide is not extracted by the liver, and (3) C-peptide clearance—half of which occurs through the kidney[24]—is approximately constant in any given individual. By this approach, insulin secretion is calculated using a mathematical procedure called *deconvolution*,[25] which reconstructs the pancreatic insulin secretion rate (in pmol/min) as it occurs before hepatic insulin degradation,[26,27] and can be reliably estimated from individual anthropometric data.[28] Thus, transducing plasma C-peptide measures into plasma insulin concentrations involves two catabolic processes (and computational steps) in series with one another (Fig. 33.2). Considering that about 15% of renal C-peptide uptake is excreted intact into the urine (the remainder being degraded),[24] measurements of the ratio of urinary C-peptide to creatinine have been shown to be reasonably well correlated with postprandial plasma C-peptide levels[29] and may therefore be used as indicators of residual beta-cell function in patients with type 1 diabetes.[30]

Because plasma insulin kinetics are fast, insulin secretion rates are quickly reflected in plasma insulin concentrations, which therefore are the prime and most widely used proxy for insulin secretion. However, insulin clearance may vary during stimulation of insulin secretion and may be lower or higher depending on the metabolic status. Hence, insulin concentrations may distort the actual time course of insulin secretion. Insulin clearance occurs through two principal routes depending on the site of entry of the hormone into the circulation: (1) peripheral (or exogenous) insulin clearance ($pMCR_I$), which can be determined experimentally during a euglycemic hyperinsulinemic clamp as the ratio of exogenous insulin infusion rate to arterial plasma insulin concentration at steady state, and (2) prehepatic (or endogenous) insulin



• **Fig. 33.2** The pancreatic insulin secretion rate is reconstructed by deconvolution of plasma C-peptide concentrations by the C-peptide disappearance rate (*green curve*). Secreted insulin then undergoes fast clearance (*red curve*), eventually yielding plasma insulin concentrations.



• **Fig. 33.3** Relationship between calculated prehepatic and measured peripheral plasma insulin concentrations in 1123 normoglycemic subjects (mean fasting plasma glucose of 90 mg/dL [5.0 mmol/L]) after an overnight fast (*fasting*) and during the absorption of a 75-g oral glucose load (OGTT). Note the linear relationship between prehepatic and peripheral insulin levels in the fasting state and the curvilinear relationship during the OGTT, indicating saturation of insulin degradation. The fasting range is plotted on the OGTT range (*dashed lines*). *OGTT,* oral glucose tolerance test. (Data from Ferrannini E, Balkau B, Coppack SW, et al. Insulin resistance, insulin response, and obesity as indicators of metabolic risk. *J Clin Endocrinol Metab*. 2007;92[8]:2885–2892.)

clearance ($eMCR_I$). Direct experimental determination of $eMCR_I$ is hampered by the difficulty of gaining access to the portal vein where pancreatic insulin is secreted. However, endogenous insulin clearance can be calculated as the ratio between endogenous insulin secretion—reconstructed from C-peptide deconvolution—and arterial plasma insulin concentration at steady state.

If both $eMCR_I$ and $pMCR_I$ are known from separate experiments, their fractional difference—for instance, ($eMCR_I$ – $pMCR_I$)/$eMCR_I$)—is an estimate of fractional hepatic insulin extraction. The fraction of portal insulin that is removed by the liver in its first pass is about 65%, ranging between 50% and 70%.[31,32] Once in the systemic circulation, insulin recirculates to, and is further cleared by, the liver and, to a lesser extent, by skeletal muscle and the kidneys. The overall contribution of the liver (first pass plus recirculation) is therefore dominant (approximately 80%). The physiologic characteristics of exogenous insulin clearance ($pMCR_I$ from insulin clamp studies) include large interindividual variability; saturation kinetics[31]; a substantial influence of genetic factors[33,34]; and, to a lesser extent, a negative effect of abdominal obesity and liver fat.[35,36]

By using the C-peptide method to calculate $eMCR_I$ and an estimate of hepatic plasma flow, one can reconstruct prehepatic (70% portal, 30% hepatic arterial) plasma insulin concentrations. As shown in Fig. 33.3, in individuals with normal glucose tolerance, fasting prehepatic insulin concentrations are related to peripheral insulin levels in an approximately linear fashion, with an average ratio of 4:1. In the fed state (e.g., after an oral glucose load), $eMCR_I$ is lower than in the fasting state as a consequence of saturation of liver extraction. The contribution of muscle is reduced,[37] whereas that of the kidney is increased,[31] although together they still contribute no more than 20% to overall insulin clearance. As a consequence, the ratio of prehepatic to peripheral insulin is progressively lower as pancreatic insulin release increases (see Fig. 33.3).

Multiple mechanisms appear to modulate insulin degradation. In mice lacking zinc-transporter 8 (ZnT8), which enriches the insulin vesicles in zinc, the clathrin-dependent insulin endocytosis in the hepatocyte is reduced; individuals with a single nucleotide polymorphism in the gene encoding for this transporter also display an increased insulin clearance during an oral glucose tolerance test (OGTT).[38] Neural inputs[39] and cellular redox state[40] may also influence insulin degradation, whereas gut hormones do not affect this process.[41] Many drugs may interfere with insulin degradation, especially at doses causing a degree of hepatotoxicity. Among antidiabetic agents, sulphonylureas are known to reduce

insulin clearance.[42] Whether this effect is a direct one on the degrading machinery or is secondary to saturation due to insulin hypersecretion is not known.

The role of insulin clearance in the maintenance of glucose homeostasis is complex. A reduction in insulin degradation is commonly interpreted as compensatory, with lower values being observed in individuals who are more insulin resistant and would take advantage of increased insulin levels. On the other hand, experimental animals in which the insulin-degrading enzyme is knocked out develop insulin resistance and diabetes.[43] Similarly, fasting hyperinsulinemia and mild degrees of insulin resistance develop in pancreas-transplanted patients with organ venous drainage into the systemic circulation as compared with those with portal drainage.[44] However, insulin clearance can influence glucose tolerance independently of other factors. For instance, an acute 30% increase in insulin clearance, produced by systemic nitric oxide inhibition, worsens glucose tolerance in normal glucose-tolerant persons.[45] Thus, it is difficult to cleanly dissect out saturation from a bidirectional association of insulin clearance and insulin action. The bulk of evidence, however, indicates that even after accounting for saturation of degrading capacity, insulin clearance is positively associated with insulin sensitivity regardless of the temporal sequence of changes in these two functions.

Characteristics of Insulin Secretion In Vivo

Insulin acts on several target metabolic pathways, such as lipids and amino acids, which also influence its secretion; however, the tightest physiologic feedback is with plasma glucose concentration. In vivo, beta-cell function must supply insulin to body tissues in amounts and time course apt to maintain plasma glucose within a narrow concentration range on a minute-by-minute basis. To this end, insulin output must handle acute challenges, such as size, composition, and appearance rate of meals, and adapt to long-term settings, such as changes in target tissue sensitivity to insulin. In comparison, insulin action, if equally complex at the cellular level, is functionally relatively stable within any given individual. In fact, when measured in vivo by a direct technique (the euglycemic hyperinsulinemic clamp), insulin sensitivity has been shown to vary by 30% to 80% during 24 hours of free living.[46] In most cases, insulin sensitivity can at best double with physiologic or pharmacologic intervention. In contrast, insulin secretion can vary manyfold in the same person in a matter of minutes, as occurs with a large mixed meal, or over years, as happens with weight gain.[47] For example, a lean, insulin-sensitive adult may need as little as 0.5 units of insulin to dispose of an oral load of 75 g of glucose over 2 hours, whereas an obese, insulin-resistant, glucose-intolerant person may require 45 units to perform the same task—an approximate 100-fold span.[48]

Given the highly polymorphic nature of beta-cell responses, absolute insulin secretion is a meaningful index of beta-cell function only in the context of a standardized stimulus (mainly glucose). For this reason, typical protocols employ a controlled stimulus, such as a hyperglycemic clamp (described in the following). When the stimulus is not standardized, the secretory response can be empirically normalized by calculating ratios between insulin (or insulin secretion) and glucose (absolute or incremental values or areas under the response curves). For instance, the time-honored insulinogenic index uses the ratio of 30-minute increments from baseline of insulin and glucose concentrations from an OGTT.

More elaborate approaches rely on mathematical models, which have been widely used to assess beta-cell function from clinical tests—in which glucose levels are not controlled—and also to gain insight into the mechanisms governing insulin secretion.[49] In general, these models relate insulin secretion rates to concomitant plasma glucose concentrations on the basis of a formal description of the secretory machinery. Requisites for such models are a simplified description of the system—to allow robust mathematical identifiability of its parameters—and a realistic congruity with known features of beta-cell function as emerging from cellular physiology.

Modes of Beta-Cell Response

There are multiple in vivo tests of beta-cell function (described later). Each in vivo test of insulin secretion reveals some aspect of beta-cell function; however, alone, none recapitulates the polymorphic nature of insulin's secretory response. The main modes of beta-cell response include (1) first-phase or acute insulin secretion, (2) glucose sensitivity, and (3) potentiation of insulin secretion. These three main features of insulin secretory response were already identified in isolated perfused rat islets more than four decades ago[50] and can be resolved by modern mathematical modeling from multiple types of stimulation.[49,51,52] First-phase insulin release is the sharp and short-lived peak of insulin secretion elicited by a brisk rise in glucose levels. Its magnitude depends on the size of the glucose stimulus and can be also represented as a function of the glucose rate of change (also called *rate sensitivity*, *anticipation*, or *derivative component*). First-phase insulin secretion is rarely observed under free-living conditions, although a biphasic response can be occasionally detected during an OGTT. Despite much investigation, the physiologic interpretation of the biphasic secretory response to a glucose step is still somewhat uncertain. Single-cell electrophysiologic studies have shown that acute exposure to high glucose enhances electrical activity synchronously with spikes in cytosolic calcium concentrations, which parallel insulin release both in terms of glucose dependency and time course.[53] Morphometric studies have shown that the beta cells harbors different populations of secretory granules, in different stages of maturation and in variable spatial array between the trans-Golgi and the plasma membrane, constituting a chain of secretory pools in dynamic exchange with one another.[54] First-phase insulin secretion contributes an estimated one tenth (about 3 nmol/m^2) of suprabasal secretion during a 2-hour OGTT; nevertheless, a prompt stimulation of insulin release as soon as glucose starts to rise is crucial to suppress endogenous glucose production and to prime tissue glucose uptake, thereby curbing subsequent glycemic excursions.[55]

Beta-cell glucose sensitivity measures the increase in secretion rate for any concomitant increase in plasma glucose concentration. This key mode of response determines the amount of insulin secreted during a meal and therefore is a major control of glucose tolerance. In fact, decreased glucose sensitivity is a hallmark of all forms of glucose intolerance.

Potentiation of insulin secretion is an intrinsic feature of beta-cell function. Potentiation occurs when the dose-response relationship between glucose level and insulin secretion is enhanced, as when prior exposure to glucose leads to a greater insulin secretion on subsequent exposure. Potentiation may be generated by a glucose "memory," incretins, or factors such as glucagon, cholinergic stimuli, other nutrients such as fructose, or drugs including sulfonylureas.[56]

From this description, it is clear that maintenance of glucose homeostasis depends not only on the absolute amount of insulin release but also on the time dynamics of the secretory response.

Other clinical tests reveal additional details of beta-cell function. For example, an arginine bolus superimposed on ramping glycemic plateaus explores the effect of an amino acid separately from that of glucose and has been used to estimate maximal secretory capacity.[57] Furthermore, many studies have gauged the influence on insulin secretion of different amino acids,[58] endogenous[59] or exogenous free fatty acids (FFAs),[60] incretin hormones[61] and neural stimuli.[62] These influences depend on stimulus quality, dose, route of delivery, duration (acute or chronic), combination with other stimuli, and simultaneous impact on insulin sensitivity. Ultimately, however, beta cells integrate all inputs and respond in a manner that can be analyzed in quantifiable terms of glucose sensitivity, potentiation, and rate sensitivity. Table 33.1 reports normative values of basic parameters of beta-cell function as measured in a large group of lean (body mass index ≤25 kg/m^2) healthy volunteers with normal glucose tolerance.

Insulin Secretion in the Fasting State

In healthy normoglycemic adults after an overnight (10–14 hours) fast, insulin secretion rates range widely, in rough proportion to the degree of obesity (and insulin resistance, see later discussion) and equally in women and men (Fig. 33.4). In addition to obesity, fasting insulin secretion values are also physiologically dependent on fasting plasma glucose levels. Thus, in hyperglycemic patients (e.g., individuals with impaired glucose tolerance [IGT] or overt T2D), fasting insulin secretion is generally higher than in normoglycemic subjects at each level of obesity.

When frequently sampled from portal vein blood, insulin concentrations oscillate, with detectable pulses at 5- to 14-minute intervals.[63,64] The pulsatility of insulin secretion is intrinsic to the islet and may reflect coupling between slow oscillations of glycolysis and faster oscillations involving Ca^{2+} fluxes.[65] Over 24 hours, slower ultradian cycles occur at 80- to 180-minute intervals; these can be entrained by small subthreshold changes in plasma glucose concentrations.[66] Pulsatile insulin secretion is disrupted in hyperglycemic states,[66,67] but its impact on insulin action on target organs (liver and peripheral tissues) is uncertain.

The ratio of proinsulin to insulin concentration (or the absolute proinsulin concentration adjusted for insulin) in a fasting plasma sample has been proposed as a marker of beta-cell function.[68] In epidemiologic studies, a higher ratio of proinsulin to insulin or proinsulin to C-peptide has been related to incident diabetes.[69]

TABLE 33.1 Insulin Secretion Parameters in Lean Persons With Normal Glucose Tolerance

	Mean	Median	25–75%
Fasting plasma glucose (mmol/L)	4.81	4.90	4.60–5.10
2-hour plasma glucose (mmol/L)	5.18	5.10	4.33–6.00
Fasting plasma insulin (pmol/L)	26	23	17–32
2-hour plasma insulin (pmol/L)	139	119	75–177
Fasting insulin secretion rate (pmol·min^{-1}·m^{-2})	61	58	46–72
Insulin secretion at 5 mM glucose (pmol·min^{-1}·m^{-2})	86	68	48–93
Total insulin output (2 hours) (nmol·m^{-2})	37	36	29–44
Beta-cell glucose sensitivity (pmol·min^{-1}·m^{-2}·mM^{-1})	150	125	91–181
Rate sensitivity (nmol·m^{-2}·mM^{-1})	1.06	0.69	0.01–1.43
Potentiation (ratio)	2.34	1.83	1.26–2.73
Insulin sensitivity (μmol·min^{-1}·kg$_{FFM}$·nM^{-1})	165	155	117–199

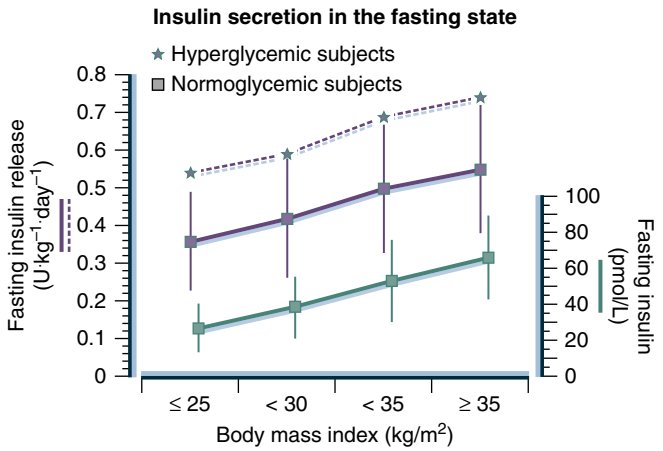
Data from 620 participants in the RISC study.[11]

Insulin Secretory Response to Intravenous Glucose

Various formats of intravenous glucose administration have been used to gauge beta-cell response independently of gastrointestinal influences.

Hyperglycemic Clamp and Biphasic Insulin Secretion

The hyperglycemic clamp protocol has been used in vivo, in the perfused pancreas, and in islet cultures. The rationale of this test is to expose beta cells to a square wave of hyperglycemia, whereby glucose concentration is abruptly raised and kept constant at a preset suprabasal level by means of a controlled variable glucose infusion. The insulin secretory response to this challenge



• **Fig. 33.4** Fasting plasma insulin concentrations and corresponding fasting insulin secretion rates (extrapolated to 24 hours) in 1123 normoglycemic subjects (mean fasting plasma glucose of 90 mg/dL [5.0 mmol/L]) by conventional category of obesity (lean, overweight, moderate, and severe obese). Plots are mean ± 1 standard deviation. Stars indicate mean values of fasting insulin secretion (corresponding insulin concentrations not indicated) in 289 subjects with impaired glucose tolerance or overt diabetes. (Data from Ferrannini E, Balkau B, Coppack SW, et al. Insulin resistance, insulin response, and obesity as indicators of metabolic risk. *J Clin Endocrinol Metab*. 2007;92[8]:2885–2892 and Ferrannini E, Gastaldelli A, Miyazaki Y, et al. Beta-cell function in subjects spanning from normal glucose tolerance to overt diabetes: a new analysis. *J Clin Endocrinol Metab*. 2005;90[1]:493–500.)

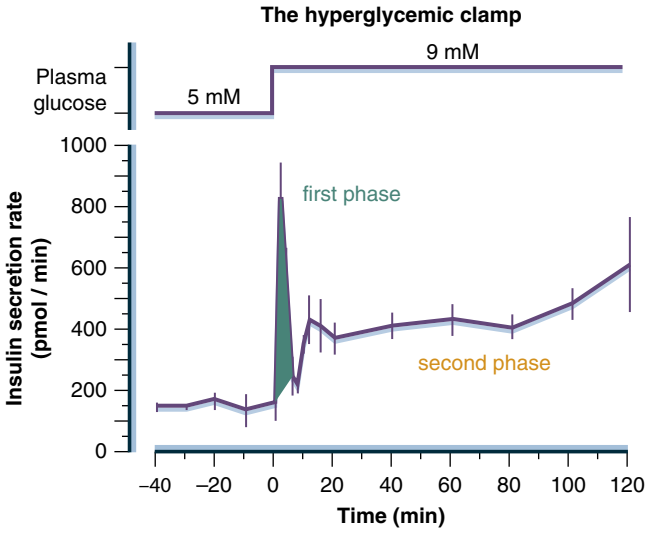
typically is biphasic, with an initial sharp insulin secretory burst lasting about 5 to 8 minutes (first-phase secretion), followed by a transient decrease and then a progressive slow increase, which continues as long as hyperglycemia is maintained (second-phase secretion) (Fig. 33.5).

The amount of insulin secreted during the first phase (also referred to as acute insulin response [AIR]) is dependent on the magnitude of the glucose rise; in a typical + 126 mg/dL (+7 mmol/L) hyperglycemic clamp, it is approximately 4 nmol per square meter of body surface area (~1 unit in a 70-kg adult), representing 10% to 15% of what is secreted per hour during the second phase. Although small, the amount of insulin secreted in the first phase is relevant for at least two reasons. First, although the secretory burst is apparent with a rapid elevation in glucose concentration, the underlying mechanisms appear to operate even for a more gradual rise in glucose concentration. These mechanisms are likely responsible for a response that is anticipated compared with what would be predicted solely on the basis of the dose response. As discussed later, this anticipation has relevant physiologic implications for glucose homeostasis. Second, attenuated first-phase insulin secretion is a very sensitive marker of early beta-cell dysfunction.[70] Impairment of first-phase secretion is already present in individuals at risk of developing diabetes[71] and is predictive of diabetes onset.[72] Because of these relevant characteristics, assessment of first-phase secretion has been widely used.

Higher glycemic plateaus elicit larger secretory responses. However, if multiple glucose steps are applied in sequence, the first-phase response is progressively attenuated while second-phase secretion increases in proportion to the height of the glycemic plateaus.[73] This paradoxical behavior may reflect an inhibitory influence of antecedent glycemia on the subsequent first-phase response or depletion of the pool of readily releasable insulin granules.[51,74]

Intravenous Glucose Tolerance Test

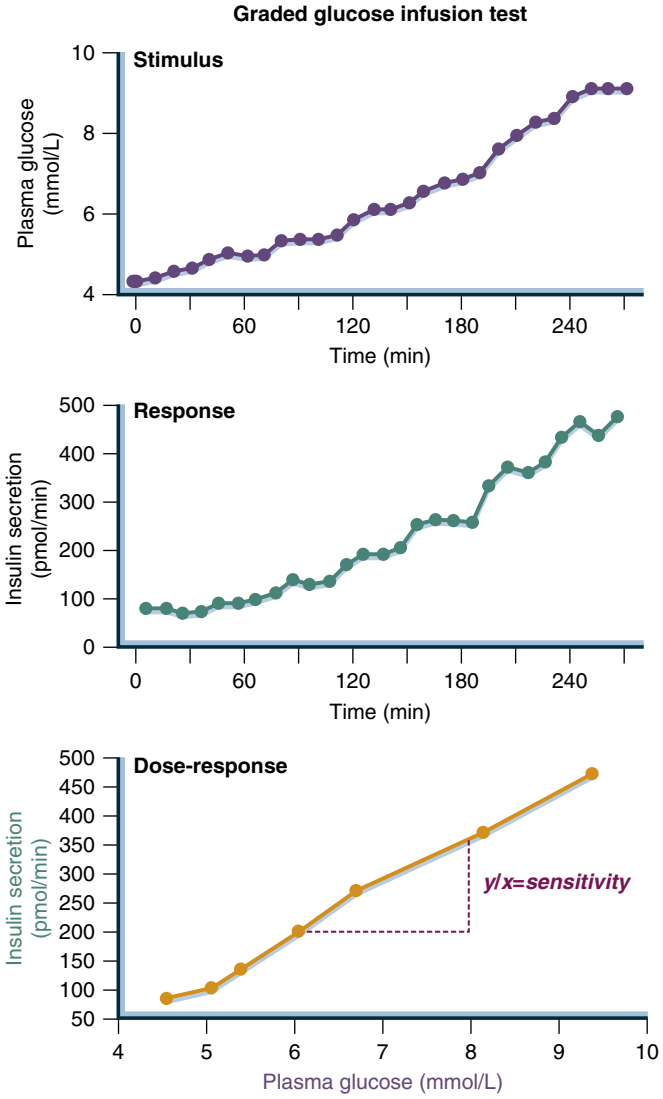
Another clinical test yielding the biphasic insulin response is the intravenous glucose tolerance test (IVGTT). Here, a glucose bolus standardized to body size is injected intravenously, and glucose, insulin and, possibly, C-peptide concentrations are measured. In contrast to the hyperglycemic clamp, glucose concentrations fall rapidly after the initial peak, and second-phase secretion is not sustained but transient with a multiphasic pattern.[25] Thus, the assessment of first-phase secretion is very similar between the hyperglycemic clamp and the IVGTT, whereas second-phase secretion on the IVGTT occurs at variable glucose and is thus less representative. Like the hyperglycemic clamp, the IVGTT has demonstrated the relevance of first-phase secretion as a hallmark of beta-cell function.



• **Fig. 33.5** Biphasic insulin response to a step increase in plasma glucose concentrations from 90 to 162 mg/dL (5–9 mmol/L). Plots are mean ± standard error of the mean. (Personal data.)

Graded Glucose Infusion Test and Beta-Cell Dose Response

In the graded glucose infusion test, glucose is infused at ascending rates to stimulate a progressive increase in insulin secretion. The plot of insulin secretion rates against plasma glucose concentrations represents the beta-cell dose response, whose slope quantifies the sensitivity of the beta cell to glucose (Fig. 33.6). In healthy persons, basal insulin secretion increases by five- to sixfold up to 180



• **Fig. 33.6** The slope (y/x) of the dose-response curve measures beta-cell glucose sensitivity to plasma glucose. Typical data in a normoglycemic subject. (Modified from Byrne MM, Sturis J, Polonsky KS. Insulin secretion and clearance during low-dose graded glucose infusion. *Am J Physiol*. 1995;268[1 Pt 1]:E21–E27.)

mg/dL (10 mmol/L) glucose; correspondingly, beta-cell glucose sensitivity is about 13 $mU \cdot min^{-1} mM^{-1}$ (80 $pmol\ min^{-1} mM^{-1}$). The relationship is fairly linear within this glucose concentration range and up to at least 360 mg/dL (20 mmol/L).[75]

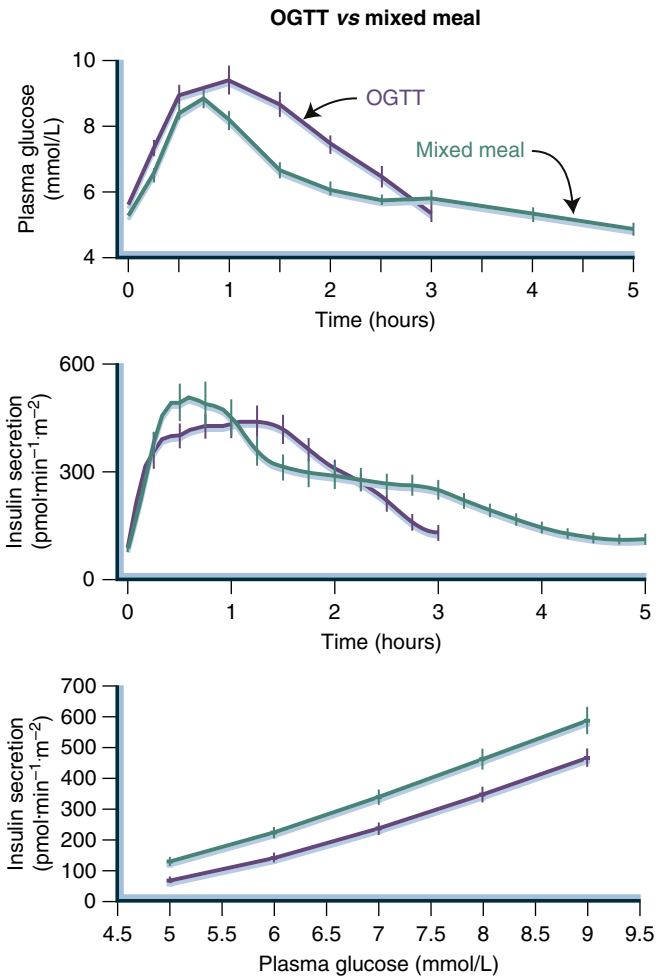
Slow Beta-Cell Response Modes and Adaptation Mechanisms

Under sustained, constant glucose stimulation, insulin secretion continues to rise slightly. This time-dependent potentiation of insulin secretion can be detected in the hyperglycemic clamp (see Fig. 33.5). Similarly, prolonged (2–4 days) exposure to mild hyperglycemia markedly enhances insulin secretion, with a steepening of the dose-response curve, as shown in hyperglycemic clamp[76] and graded glucose infusion studies.[75] Thus, the healthy beta cell is constitutively capable of potentiating its response when a chronic condition of mild hyperglycemia is induced by intravenous glucose administration.

Insulin Secretory Response to Oral Stimuli

When glucose is ingested, the biphasic pattern of insulin response seen with intravenous glucose is substantially spread out over the time of glucose absorption, with plasma glucose levels typically peaking at 0.5 to 1.0 hour and returning to baseline by 2 hours post ingestion. Insulin secretion rates rise and fall in close synchrony with plasma glucose levels. When a mixed meal containing the same amount of glucose (75 g as in a conventional OGTT) is ingested, glycemic excursions are smoothed out, but insulin secretion is enhanced due to the direct or indirect insulin-stimulating effect of protein and fat (Fig. 33.7). When four successive mixed meals with a high carbohydrate content are fed over a 14-hour period, insulin output appears to max out at 70 to 85 units in nondiabetic insulin-sensitive persons and at 140 to 170 units in insulin-resistant individuals, which highlights the large functional secretory reserve of the endocrine pancreas.[77] With consecutive nutrient loads, the plasma glucose and insulin secretory responses are attenuated during the second, as compared with the first, meal (Staub-Traugott effect); this phenomenon, which is also observed in patients with diabetes, is due to persistent suppression of endogenous glucose production by the hyperglycemia and hyperinsulinemia induced by the first load and to enhanced potentiation of insulin release.[78]

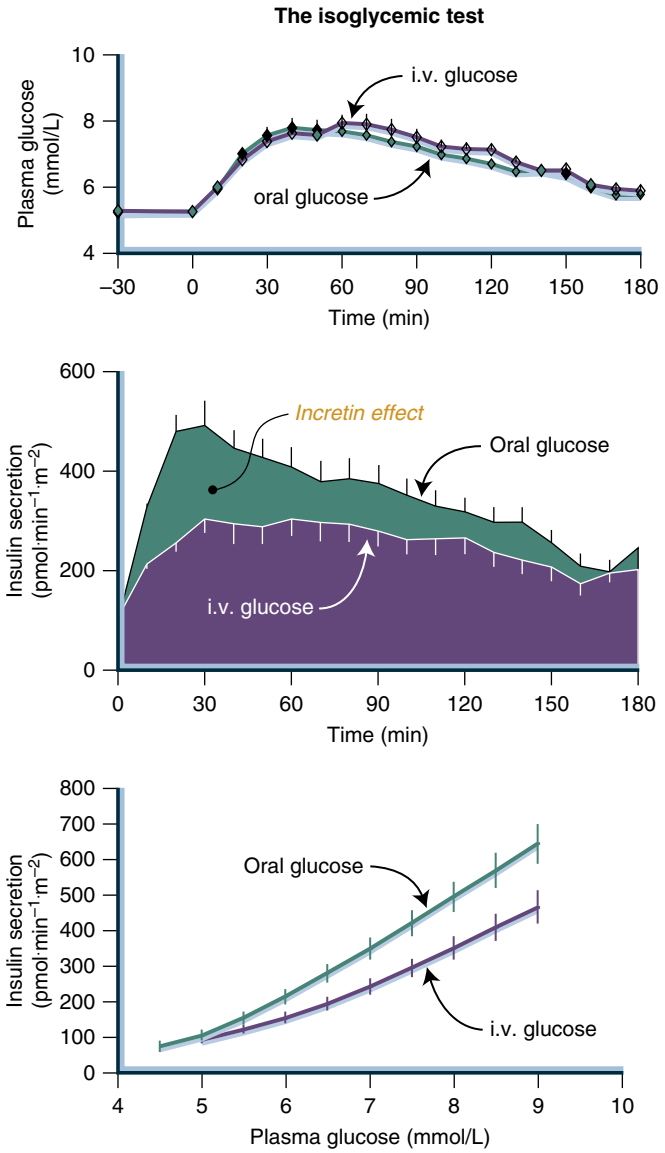
Notably, circulating insulin levels are reduced during starvation but quickly rebound on refeeding.[79] This transition, which is key to survival with the paleolithic hunter-gatherer lifestyle, requires a major, rapid adaptation of islet function. In rats subjected to prolonged (72 hours) fast, beta cells show marked degranulation and blunted in vivo insulin secretion. In contrast, autophagolysomal and lysosomal organelles are upregulated, the Golgi apparatus is expanded, and (pro)insulin biosynthetic capacity is enhanced. Within 4 to 6 hours of refeeding, proinsulin biosynthesis, cellular ultrastructure, in vivo insulin secretion, and glucose tolerance all recover, indicating a rapid replenishment of insulin secretory capacity.[80] Likewise, in obese nondiabetic individuals, plasma insulin concentrations drop by about 40% (and plasma glucagon levels rise) following a 14-day fast along with a 50% reduction in insulin sensitivity; 10 days of refeeding return all of these changes to baseline.[81] Thus, the beta cell protects against hypoglycemia during a prolonged fast but retains the potential



• **Fig. 33.7** Plasma glucose concentrations and insulin secretion rates in response to a standard (75-g) OGTT and to a mixed meal test (75 g of glucose plus 40 g of parmesan cheese and one 50-g egg, for a total of 500 kcal) in 22 normoglycemic subjects. The bottom panel shows the insulin secretion dose response to glucose: note the upward shift with the mixed meal versus oral glucose alone. Plots are mean ± standard error of the mean. *OGTT,* oral glucose tolerance test. (Personal data.)

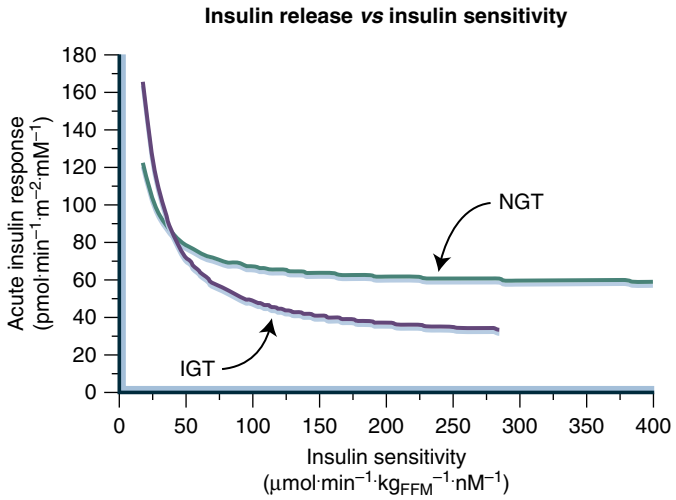
to efficiently increase insulin production on refeeding. With the present-day lifestyle, the fasting-feeding cycle is much shorter, but the coordinate regulation of beta-cell function and insulin sensitivity during the transition phase is the same. This is the basis for the recommendation that oral glucose (or mixed meal) tolerance tests be carried out in settings of an adequate carbohydrate load to prevent spurious glucose intolerance.

It has long been known that if glucose is ingested rather than infused, insulin secretion is higher at the same glucose levels.[82] GIP and GLP1, which are released by the intestinal epithelium in response to nutrient ingestion, are mainly responsible for the higher insulin release and have therefore been collectively termed *incretins*. The classical test to unveil and quantify the incretin effect is based on a standard OGTT and a separate test in the same subject, in which the time course of glucose concentration observed with the OGTT is reproduced by a controlled intravenous glucose infusion (also called *isoglycemic glucose infusion*).[83] As illustrated in Fig. 33.8, insulin secretion is considerably potentiated by the oral route of glucose entry, thereby resulting in an increase in slope of the beta-cell dose response, as estimated by modeling analysis.[56] The relative role of GIP and GLP1 in this



• **Fig. 33.8** Matching the plasma glucose response to oral glucose with a controlled i.v. glucose infusion reveals the incretin effect, namely the potentiation of insulin secretory response due to the oral route of glucose entry. The corresponding dose-response curves highlight the enhanced beta-cell sensitivity to oral, as compared with i.v., glucose. *i.v.,* intravenous. (Personal data.)

potentiation during physiologic stimulation, the interrelationships of the two hormones, and their relation to a host of other hormones secreted by islets (glucagon, somatostatin, ghrelin), as well as extrapancreatic tissues (leptin, obestatin), form a network whose nodes and links are difficult to dissect out in human experiments. The features of the in vivo incretin effect that are reasonably well established are the following: (1) GLP1 and GIP are released in phase with insulin and glucose concentrations; (2) when gastric emptying is accelerated (e.g., following gastric bypass surgery[47]), both the glucose and insulin peaks are anticipated and GLP1 levels are much higher if still synchronous with insulin; (3) glucose-induced and incretin-induced potentiation of insulin secretion—as resolved by modeling of isoglycemic experiments[56]—have different time courses and relation to glucose tolerance; and (4) strength of potentiation depends on the stimulus and the quality of beta-cell function.



• **Fig. 33.9** Nonlinear relationship between the acute insulin response to intravenous glucose (during an intravenous glucose tolerance test) and insulin sensitivity (separately measured by a euglycemic hyperinsulinemic clamp) in subjects with NGT (n = 1123) and individuals with IGT (n = 156). IGT, impaired glucose tolerance; NGT, normal glucose tolerance. (Modified from Mari A, Tura A, Natali A, et al. Impaired beta cell glucose sensitivity rather than inadequate compensation for insulin resistance is the domina;nt defect in glucose intolerance. *Diabetologia*. 2010;53[4]:749-756.)

Insulin Secretion and Insulin Sensitivity

A separate important aspect of beta-cell function is its relation to insulin sensitivity. In insulin-resistant states, more insulin needs to be secreted at the same glucose levels to maintain glucose tolerance. Evidence for this phenomenon dates back to the infancy of the insulin assay.[84] More systematic investigations have been carried out using the IVGTT, as this test allows the simultaneous assessment of the AIR and insulin sensitivity. The reciprocal relationship between AIR and insulin sensitivity likely reflects adaptation of the beta cell to impaired insulin action, which sets in as a steep increment in secretion as insulin sensitivity declines (Fig. 33.9). By fitting equilateral hyperbola on these IVGTT data, it has been proposed that a more appropriate index of intrinsic beta-cell function is the product of the insulin secretion and sensitivity indices, or disposition index.[85] The paradigm is commonly assumed to apply to other indices of insulin secretion, such as those obtained from an OGTT.[86] This, however, is only partly the case, as a similar relation of insulin sensitivity exists with postprandial insulin output (or fasting insulin secretion) but not with beta-cell glucose sensitivity.[87] Furthermore, in individuals with IGT, the curve is displaced as shown in Fig. 33.9, indicating that compensation is reduced until insulin sensitivity drops below approximately 50 $\mu mol \cdot min^{-1} \cdot kg_{FFM} \cdot nM^{-1}$, at which level AIR rises back to normal, very probably as a consequence of the mild hyperglycemia of these individuals.[88,89] The physiologic interpretation of these interrelationships is that insulin resistance raises the setpoint of beta-cell function, whereby absolute measures of insulin secretion (fasting, AIR, and postglucose) are chronically upregulated. However, the secretory mechanisms that control postprandial glucose excursions are less influenced by this form of adaptation. The anatomic counterpart of this phenomenon is the expansion of beta-cell mass in obese individuals,[90] in many of whom beta-cell glucose sensitivity and glucose tolerance are normal.

Genetic Influences on Insulin Secretion

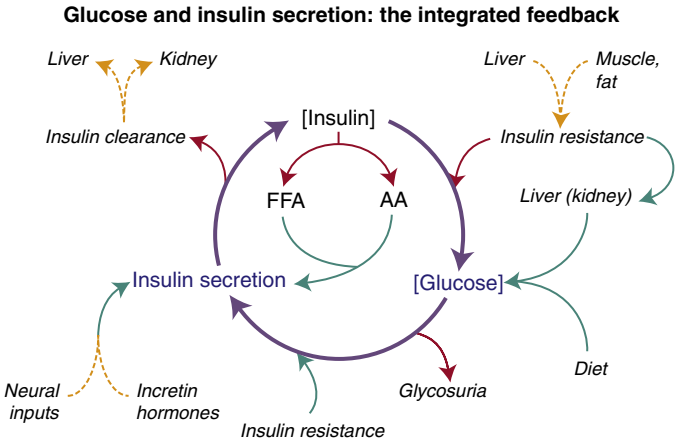
The large interindividual variability of beta-cell secretory outputs even in a very selected clinical phenotype (see Table 33.1) per se suggests that multiple influences are at play. Most of the more than 150 polymorphisms shown by genome-wide scans to be associated with diabetes or glycemic traits have been linked with defects in insulin secretion.[91] Even when tested in mendelian randomization studies,[92] the effect size of several of these risk variants is small, and they explain only a small proportion of the total heritability of T2D. Nevertheless, some interesting mechanistic information is emerging. For example, genetics variants of *TCF7L2*, a transcription factor that mediates the wingless/integrated (Wnt) pathway,[93] have been associated with impaired incretin-induced insulin secretion.[94] Other gene variants (*GIPR*, *WFS1*, *KCNQ1*) have also been shown to affect GIP- or GLP1-mediated insulin secretion.[95] In the future, it is likely that more and more knockout and knock-down experiments will add cellular and molecular granularity to these associations.

More detailed information on insulin secretion pattern is available in monogenic forms of diabetes, particularly in maturity-onset diabetes of the young (MODY). Mutations in one of at least six different genes (inherited by an autosomal dominant mode) result in hyperglycemia of variable severity in young, nonobese subjects (typically younger than 25 years) with a multigenerational family history of diabetes. As all these genes are expressed in beta cells, their mutation causes beta-cell dysfunction. In MODY 2, a heterozygous private mutation of the gene (*GCK*) encoding glucokinase results in partial enzyme deficiency and a loss of beta-cell glucose sensitivity, evidenced by a rightward shift in the dose response on a graded glucose infusion test.[96] Coexpression of the transcription factors (hepatic nuclear factor) *HNF1α*, *HNF1β*, and *HNF4α* controls gene expression during embryonic development and adulthood; in beta cells, they regulate the expression of the insulin gene and genes encoding proteins involved in glucose transport and metabolism.[97,98] Mutations in *HNF4α* (MODY 1) or *HNF1α* (MODY 3) also cause loss of beta-cell glucose sensitivity on a graded glucose infusion test[96] and rapidly progressing hyperglycemia. Interestingly, associated abnormalities include a deficient insulin secretory response to arginine and reduced glucagon release but not insulin resistance.[99] Mutations in other MODY-related genes (*insulin promoter factor 1* [or MODY 4], *HNF1β* [or MODY 5], and *neurogenic differentiation 1 transcription factor* [*BETA2*, or MODY 6]) expressed in beta cells likewise cause diabetes related to beta-cell dysfunction. Because these transcription factors are also expressed in other tissues (liver and kidney), the mutations produce a clinical phenotype in which islet dysfunction is associated with other abnormalities, particularly microvascular complications.

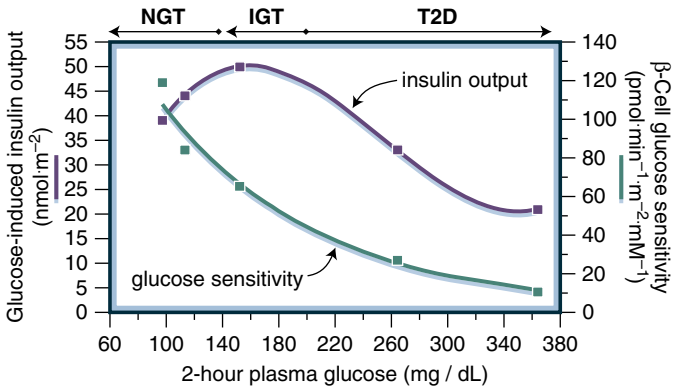
Insulin Secretion, Insulin Action, and Glucose Homeostasis

The information derived from clinical tests carried out across different conditions can be assembled into a simplified resume of beta-cell physiology (Fig. 33.10). Under most circumstances, the primary feedback is between glucose concentrations and insulin secretion rates: insulin decreases glucose and glucose increases insulin secretion. Several major processes modulate this feedback. Insulin secretion is potentiated by nonglucose substrates (FFAs and amino acids); incretin hormones; and, to a lesser extent, neurotransmitters. Insulin clearance—predominantly hepatic—translates secretion rates into circulating insulin concentrations; this step is modulated by an intrinsic saturation of liver-degrading capacity and by insulin resistance. Plasma insulin lowers plasma glucose by promoting tissue glucose uptake; this step is gated by insulin resistance. Plasma insulin also restrains lipolysis[100] and protein breakdown,[101] thereby lowering circulating FFAs and amino acids; this closes a secondary feedback loop modulating insulin secretion. Endogenous glucose production—enhanced by insulin resistance—and dietary carbohydrate independently contribute to plasma glucose levels, whereas glycosuria dampens them when the renal threshold for glucose reabsorption is exceeded. The cycle is completed by the stimulatory effect of glucose on insulin secretion, with a chronic effect of insulin resistance to raise the beta-cell set-point.

Diabetes and obesity can also be represented within this schematic: the presence and severity of insulin resistance affect insulin secretion by increasing baseline secretory activity and by incrementing the stimulatory signals (i.e., glucose, FFAs, and amino



• **Fig. 33.10** Integrated control of in vivo insulin secretion. The *square brackets* stand for plasma concentration, the *purple line* encircles the primary feedback, the *green arrows* indicate stimulation, and the *red arrows* indicate inhibition. *AA,* amino acids; *FFA,* free fatty acid.



• **Fig. 33.11** Median values of total insulin output over the 2 hours of a standard oral glucose tolerance test (*blue squares*) and beta-cell glucose sensitivity (*green squares*) are plotted against the 2-hour plasma glucose concentrations to highlight the different behavior of absolute insulin release and beta-cell function across progressive stages of glucose intolerance. NGT, normal glucose tolerance; IGT, impaired glucose tolerance; T2D, type 2 diabetes. (Data from Mari A, Tura A, Natali A, et al. Influence of hyperinsulinemia and insulin resistance on in vivo β-cell function: their role in human β-cell dysfunction. *Diabetes*. 2011;60[12]:3141–3147 and Ferrannini E, Gastaldelli A, Miyazaki Y, et al. Beta-cell function in subjects spanning from normal glucose tolerance to overt diabetes: a new analysis. *J Clin Endocrinol Metab*. 2005;90[1]:493–500.)

TABLE 33.2 Insulin Secretion Parameters in Individuals With NGT, IFG, IGT, and T2D

	NGT (n = 1189)	IFG (n = 33)	IGT (n = 140)	T2D.1 (n = 56)	T2D.2 (n = 56)
Fasting plasma glucose (mg/dL)	91	113	95	143	220
2-hour plasma glucose (mg/dL)	97	113	152	264	364
Fasting insulin secretion rate (pmol·min^{-1}·m^{-2})	68	86	96	117	104
Total insulin output (2 hours) (nmol·m^{-2}·h)	39	44	50	33	21
Beta-cell glucose sensitivity (pmol·min^{-1}·m^{-2}·mM^{-1})	118	83	64	26	10
Rate sensitivity (nmol·m^{-2}·mM^{-1})	0.83	0.47	0.81	0.34	0.14
Potentiation (ratio)	1.72	1.94	1.34	1.10	1.00
Insulin sensitivity (μmol·min^{-1}·kg$_{FFM}$·nM^{-1})	131	126	77	38	32

IFG, Impaired fasting glycemia; *IGT,* impaired glucose tolerance; *NGT,* normal glucose tolerance; *T2D,* type 2 diabetes.

Entries are median values. The T2D group is split at the median fasting plasma glucose concentration (T2D.1 and T2D.2).

acids). Genetic makeup (and epigenetic modifications), chronic hyperglycemia, and other acquired insults impair beta-cell secretory dynamics (glucose sensitivity and potentiation) and compromise islet structure and cellular phenotype through the expression of risk gene variants; glucose- and lipotoxicity; and, quite possibly, insulin resistance itself.[102-104]

An important concept is the opposite behavior of absolute insulin release and the glucose sensitivity of the beta cell across stages of glucose intolerance as reconstructed by physiologic modeling of OGTT data (Fig. 33.11). In the conventional diagnostic categories of impaired fasting glycemia and IGT, insulin release is increased (to compensate for the insulin resistance), whereas beta-cell glucose sensitivity is already markedly decreased and continues to decline monotonically throughout progressive hyperglycemia in overt T2D. In impaired fasting glycemia, for example, insulin output is increased by 15%, insulin sensitivity (on an insulin clamp) is only slightly impaired, but glucose sensitivity is 30% lower[105] (Table 33.2). Concordant with these cross-sectional data is the finding that beta-cell glucose sensitivity is a powerful negative predictor of incident T2D in nondiabetic cohorts above and beyond the impact of conventional risk factors (sex, age, body mass index, family history, etc.). In these models, insulin output typically features as an independent positive predictor[106] (it is reduced only in long-standing, severely hyperglycemic T2D patients). Also of note is that the model-derived potentiation factor picks up the incretin defect that is consistently observed in patients with T2D and, to a lesser extent, in IGT individuals. Thus, the pathogenesis and natural history of human diabetes can be construed as a recapitulation of the physiology of insulin secretion.

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34

Pathophysiology of Type 2 Diabetes Mellitus

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CHAPTER OUTLINE

KEY POINTS

- Type 2 diabetes mellitus (T2DM) is one of the most common health problems facing mankind and is a major public health problem. The International Diabetes Federation estimated in 2017 that 425 million people have diabetes worldwide and that by 2045 this number will rise to 629 million.
- T2DM is the predominant form of diabetes worldwide, accounting for 90% to 95% of cases globally.
- The pathogenesis of T2DM is complex and involves the interaction of genetic and environmental factors.
- Several environmental factors have been shown to play critical roles in the development of T2DM, particularly excessive caloric intake, a sedentary lifestyle, and increases in fat mass.
- The clinical presentation is heterogeneous, with a wide range in age at onset, severity of hyperglycemia, degree of obesity, and severity of other associated metabolic abnormalities.
- From a pathophysiologic standpoint, persons with T2DM consistently demonstrate three cardinal abnormalities:
  - Resistance to the action of insulin in peripheral tissues, particularly muscle, fat, and liver
  - Defects in insulin secretion, particularly in response to a glucose stimulus
  - Increased glucose production by the liver.
- Although more than 100 genetic variants have been linked with risk for T2DM, these variants collectively account for 5% to 10% of the overall familial risk for disease, suggesting the importance of epigenetic effects, as well as environmental influences.
- Insulin receptors are nearly ubiquitously expressed, yet the physiologic actions of insulin are highly tissue specific, promoting calorie storage in the postprandial state via pleotropic effects on growth, metabolism, and cell survival.

Epidemiology

Type 2 diabetes mellitus (T2DM) is the predominant form of diabetes worldwide, accounting for 90% to 95% of cases globally. An epidemic of T2DM is under way in both developed and developing countries. The International Diabetes Federation estimated in 2017 that 425 million people have diabetes worldwide and that by 2045 this number will rise to 629 million. Of those with diabetes currently, 79% live in low- and middle-income countries. The largest percentage of increases in diabetes cases are predicted for Africa, the Middle East, and Southeast Asia, where the greatest numbers of people are moving from low to middle income.[1] In the United States, the Centers for Disease Control and Prevention estimated in 2017 that 30.3 million people, or 9.4% of the population, had diabetes and that 7.2 million of them (23.8%) were undiagnosed. Based on fasting glucose or hemoglobin A_{1c} levels, they also estimated that 84 million people (34% of adults older than 20 years) had prediabetes and thus were at high risk of developing diabetes.[2]

The economic burden of diabetes is enormous. The International Diabetes Federation estimated that in 2017, diabetes-related health expenditures amounted to $727 billion.[1] The American Diabetes Association estimated that in the United States alone in 2017, diabetes accounted for $237 billion in direct health care expenditures and an additional $90 billion in lost productivity. Average expenditures per person, adjusted for age and gender, were 2.3-fold higher compared with the nondiabetic US population. The increased spending was driven by both the increased prevalence of the disease and the costs associated with care.[3] In addition, the high cost of diabetes medications, including insulin, leaves many patients in the United States unable to afford the medications they are prescribed.

TABLE 34.1 Epidemiologic Determinants of and Risk Factors for Type 2 Diabetes Mellitus

Genetic Factors
Genetic markers
Family history
Demographic Characteristics
Age
Ethnicity
Behavioral and Lifestyle-Related Risk Factors
Obesity (including distribution of obesity and duration)
Physical inactivity
Diet
Stress
Westernization, urbanization, modernization
Medications
Shift work
Metabolic Determinants and Intermediate-Risk Categories of Type 2 Diabetes
Impaired glucose tolerance
Insulin resistance
Gestational diabetes
Offspring of women with diabetes during pregnancy
Intrauterine malnutrition or overnutrition
Microbiome composition

Considerable information is available on the factors that are responsible for the development of T2DM, and these determinants are summarized in Table 34.1.[4,5] T2DM is thought to occur in genetically predisposed persons who are exposed to a series of environmental influences that precipitate the onset of clinical disease. Although T2DM is strongly associated with a high body mass index (BMI), there are clear differences in fat distribution and the degree of excess fat that is required to drive susceptibility to T2DM.[6] For example, individuals with high levels of visceral fat (central obesity) are clearly more insulin resistant and at higher risk for T2DM than those with high levels of peripheral, mainly subcutaneous, fat. Likewise, individuals from Southeast Asia are at high risk of developing T2DM at lower BMIs than other ethnicities due to greater proportional visceral adipose stores. Historically, when ethnicities prone to developing T2DM at lower BMIs migrate to the United States, they see weight gain and a rapid rise in the rate of T2DM with the change to a Western diet.[7,8] Not surprisingly, as many people in Asia adopt a more Western diet, the rates of T2DM increase even within those countries with previously low incidence rates, and there is no longer a migration effect because the diet had already changed.[9] In the United States, the prevalence of T2DM varies by race and ethnicity, with Native American populations having twice the rate of diabetes at 15.1% than non-Hispanic whites at 7.4%[2] (Fig. 34.1).

Historically, T2DM was viewed as a disorder of aging. Although this remains true today, the prevalence of obesity and T2DM in children has risen dramatically over the past decade. Thus, in the past, the overwhelming majority of children with diabetes had type 1 diabetes mellitus (T1DM), with only 1% to 2% of diabetic children considered to have T2DM or other rare forms of diabetes. Recent reports suggest that as many as 20% to 25% of children in the United States with newly diagnosed diabetes have non-T1DM, i.e., non–immune-mediated, forms of the disease. Most of these children have T2DM, but the rarer monogenic types are also being increasingly identified. Nevertheless, T2DM in children remains relatively rare, with an estimated prevalence of 5 cases per 10,000 children.[2,10]

Pathogenesis

The pathogenesis of T2DM is complex and involves the interaction of genetic and environmental factors. Several environmental factors have been shown to play a critical role in the development of the disease, particularly excessive caloric intake leading to obesity and a sedentary lifestyle (Fig. 34.2). Other environmental factors that impact obesity and T2DM development, including epigenetics, medications, inflammation, circadian rhythm disruptions, and the microbiome, also need to be considered. The clinical presentation of T2DM is heterogeneous, with a wide range in age at onset, severity of associated hyperglycemia, and degree of obesity. From a pathophysiologic standpoint, persons with T2DM consistently demonstrate three cardinal abnormalities:

- Resistance to the action of insulin in peripheral tissues, particularly muscle, fat, and liver (the classical tissues of insulin action).
- Defective insulin secretion, particularly in response to a glucose stimulus, although absolute levels may be high, low, or normal
- Increased glucose production by the liver leading to hyperglycemia in the fasting state.

In addition, people with T2DM may have hyperglucagonemia, alterations in incretin hormone secretion or action, accelerated lipolysis in the fat cell, increased renal tubular reabsorption, and abnormalities in central nervous system (CNS) regulation of metabolism.[11]

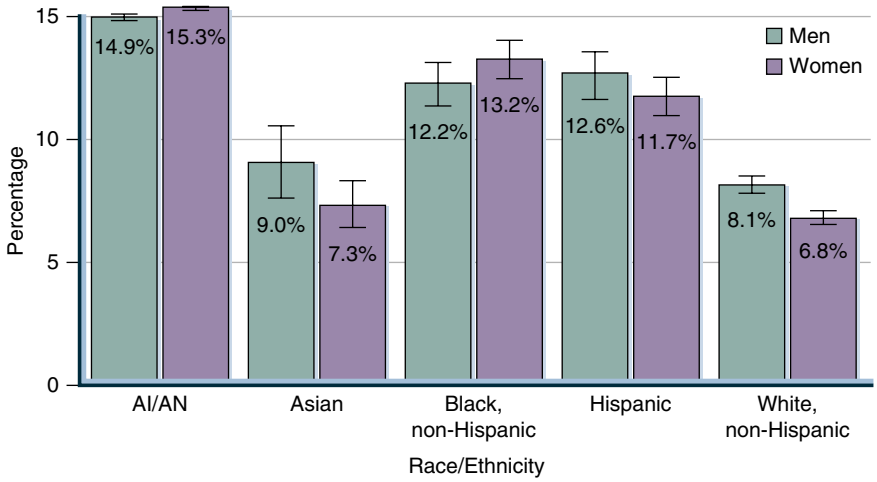
From a pathophysiologic standpoint, many argue that it is the inability of the pancreatic beta cell to adapt to the reductions in insulin sensitivity that precipitates the clinical onset of T2DM. What is clear is that the earliest detectable abnormality in those predisposed to T2DM is insulin resistance. Indeed, insulin resistance may precede T2DM by many years.[12] The most common factors that place an increased secretory burden on the beta cell are additional insulin resistance factors such as puberty, pregnancy, a sedentary lifestyle, and overeating leading to weight gain. An underlying genetic predisposition appears to be a factor in determining the frequency with which beta-cell failure occurs, although like T2DM itself, no single genetic alternation has been identified as the driver of beta-cell failure.

Genetic Factors in the Development of Type 2 Diabetes Mellitus

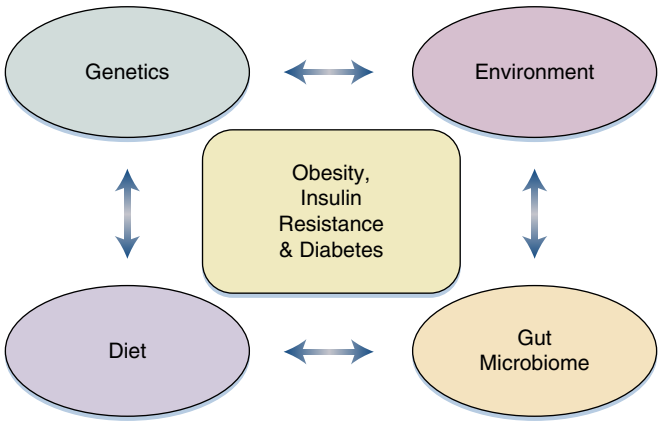
The precise way in which genetic, environmental, and pathophysiologic factors interact to lead to the clinical onset of T2DM is complex and may vary from individual to individual. The common forms of T2DM are polygenic in nature and are caused by an interaction between these genes and multiple environmental factors (known and unknown), as well as epigenetic factors. Therefore, it is not surprising that the individual genes involved in the common polygenic forms of T2DM have been difficult to identify and their contribution to disease development modest. The monogenic forms, although relatively uncommon, are important to recognize because they have led to important insights about normal physiology; if identified early, they may be important in selecting appropriate therapy. Several genes involved in monogenic forms have been identified and characterized.

Monogenic Forms of Diabetes Associated With Insulin Resistance

In the monogenic forms of diabetes, the gene involved is both necessary and sufficient to cause disease. In other words, environmental factors play little or no role in determining whether a



• **Fig. 34.1** Estimated prevalence of diabetes among US adults 18 years or older, age-adjusted data for 2013–2015. Error bars represent upper and lower bounds of the 95% confidence interval. AI/AN, American Indian/Alaska Native. (From Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2017. Available at https://www.cdc.gov/diabetes/data/statistics/statistics-report.html. Accessed October 3, 2018.)



• **Fig. 34.2** Genetics, the environment, diet, and the gut microbiome are among many interacting factors influencing the development of obesity, insulin resistance, and diabetes.

genetically predisposed person develops clinical diabetes, although there is some variation in penetrance of disease among individuals with the same genetic defect. The monogenic forms of diabetes are usually diagnosed in younger patients, often in the first two to three decades of life; however, if only mild, asymptomatic elevations in blood glucose occur, the diagnosis may be missed until later in life. The monogenic forms of diabetes can be divided into those in which the mechanism is a defect in insulin secretion and those that involve defective responses to insulin or insulin resistance. In this chapter, we will discuss the forms associated with insulin resistance.

Mutations in the Insulin Receptor

Numerous mutations have been identified in the insulin receptor gene in various insulin-resistant patients. Although there is a spectrum of disorders, at least three clinical syndromes caused by mutations in the insulin receptor gene have been described. *Type A insulin resistance* is defined by the presence of insulin resistance, acanthosis nigricans, and hyperandrogenism. Most often, these patients are identified as adolescents or young adults, often based on the acanthosis nigricans and/or signs of hyperandrogenism, rather than by their disturbances in glucose metabolism, even though insulin resistance can be very severe and accompanied by marked hyperinsulinemia.[13] Patients with *Donohue syndrome* (formerly called *leprechaunism*), however, have multiple early abnormalities, including severe intrauterine growth retardation, abnormal facies leading to the name of the syndrome, and death within the first 1 to 2 years of life.[14-16] Interestingly, the patients may have episodes of hypoglycemia despite their severe insulin resistance. *Rabson-Mendenhall syndrome* is associated with short stature, protuberant abdomen, and abnormalities of teeth and nails; pineal hyperplasia was a characteristic in the original description of this syndrome.[17]

These mutations impair receptor function by several different mechanisms. The majority of mutations associated with type A insulin resistance are in the intracellular tyrosine kinase domain, whereas in Donohue and Rabson-Mendenhall syndromes, the mutations are more frequently found in the extracellular domain, causing impairment in ligand binding, or the FnIII domains, which are key for receptor folding.[18] As noted earlier, the insulin resistance that is associated with these insulin receptor mutations is usually severe, manifesting in the neonatal period (e.g., Donohue and Rabson-Mendenhall syndromes), or it can occur in a milder forms in adulthood, and is often most easily detected biochemically by marked hyperinsulinemia and clinically by acanthosis nigricans often in the absence of obesity. Some individuals with insulin receptor mutations are able to remain normoglycemic due to massive elevations of endogenous insulin secretion, whereas others have presented with hyperglycemia that fails to respond to insulin therapy sometimes in doses exceeding 10,000 units/day.[13]

Lipodystrophic Diabetes

Lipodystrophic diabetes syndromes, which can either be genetic or acquired, are syndromes of severe insulin resistance associated with lipoatrophy (loss of fat) and lipodystrophy (loss and maldistribution of fat). These forms of diabetes are characterized by a paucity of fat, insulin resistance, and hypertriglyceridemia. The genetic forms can be divided into generalized lipodystrophies or partial lipodystrophies; however, within each of these categories, mutations in many different genes have been identified. The gene

mutations that cause lipodystrophy control a variety of functions including insulin signaling (AKT2, PIK3R1), caveolins (CAV1, PTRF), phospholipid biosynthesis (AGPAT2, PCYT1A), lipid droplet morphology (LMNA, BSCL2, ZMPSTE24), adipogenesis (CIDEC, PPARG), and lipolysis (LIPE, PLIN1).[19] Depending on the gene mutation and the particular mutation within the gene, the associated syndrome may be confined to loss of fat and the associated metabolic abnormalities or may cause a syndrome with a wider array of phenotypes. For example, mutation in the lamin A gene (*LMNA*) can cause either the face-sparing familial partial lipodystrophy commonly referred to as Dunnigan syndrome or through a different mutation in the same gene can cause mandibuloacral dysplasia syndrome, resulting in a partial lipodystrophy, accompanied by postnatal growth retardation, and craniofacial and skeletal malformations.[20,21] The generalized lipodystrophies are quite rare but easily diagnosed by the loss of subcutaneous fat throughout the entire body. These syndromes cause severe metabolic abnormalities, including severe fatty liver disease, sometimes leading to ascites and esophageal varices. Generalized lipodystrophy is also associated with very low levels of the adipose tissue–derived hormones leptin and adiponectin.[22] Leptin-replacement therapy improves glycemic control, decreases fatty liver disease, and decreases circulating triglyceride levels in patients with lipodystrophy and leptin deficiency.[23]

In contrast, the partial lipodystrophies are frequently missed, and thus the incidence is unknown. The phenotypes of the partial lipodystrophies vary significantly by mutation and gender. Leptin levels may be in the normal range or slightly below the normal range but do not show the elevations generally seen with obesity.[24] Diagnosis is made by clinical phenotype, with genetic testing generally only performed in research settings.

Acquired generalized lipodystrophy (also known as Seip-Lawrence syndrome) is a rare condition that may appear during childhood, adolescence, or young adulthood, characterized by fat loss affecting large areas of the body, initially usually the face, arms, and legs. Acquired generalized lipodystrophy is thought to be an autoimmune disorder with secondary destruction of the adipose organ, but definitive proof of autoimmunity is lacking.

Genetics of the Polygenic Forms of Type 2 Diabetes Mellitus

The common polygenic form of T2DM has complex pathophysiology, with genetic and environmental factors playing major roles. The phenotypic manifestations of the disease are also complex and include resistance to the action of insulin in the muscle, fat, and liver and defects in insulin secretory responses from the pancreatic beta cell. Together, these lead to reduced glucose uptake and increased hepatic glucose production. However, the primary defect or defects responsible for the development of the syndrome remain elusive. Indeed, with more than 100 genes linked to T2DM, the role of these genes and the nature of the gene-environment interactions that are ultimately responsible for development of the disorder in predisposed persons remain unknown.

Insulin resistance is present in persons predisposed to T2DM long before the onset of hyperglycemia, and this finding has been interpreted by some to indicate that insulin resistance is the primary abnormality responsible for the development of T2DM. Since some patients with severe insulin resistance can mount extreme hyperinsulinemic responses, it seems likely that in typical T2DM with less profound insulin resistance, defective beta-cell function is also present before the onset of T2DM, as impaired glucose tolerance (IGT) develops. In fact, altered insulin secretion has been reported in first-degree relatives of persons with T2DM who have normal plasma glucose concentrations.[12] Therefore, although there is still controversy about whether insulin resistance or abnormal insulin secretion represents the primary defect in T2DM, there is general consensus that both defects are present in essentially all subjects with the disorder at clinical presentation.

Over the past decade, there have been dramatic advances in understanding the role of genes in T2DM. Earlier genetic studies relied either on the candidate gene approach, in which the search for diabetes genes is dictated by the prevailing understanding of the pathways involved in glucose regulation, or on linkage studies, which involve identifying regions of chromosomal DNA that are shared to excess by affected family members. Although these approaches did allow identification of important diabetes genes, especially in monogenic forms of the disease, more recent studies have focused on the application of genome-wide associations (GWAS). These GWAS use an unbiased interrogation of the entire genome comparing cases and controls to determine which single-nucleotide polymorphisms (SNPs) are *associated* with disease. The location of the SNP can suggest associated genes in the region, but often SNPs are in noncoding intragenic regions, making the causative gene unclear. The genes that have been implicated in the pathogenesis of T2DM are depicted in Fig. 34.3. The following sections provide a summary of some of the genes that have strong evidence in the pathogenesis of T2DM.

Insulin Receptor Substrate 1 Gene

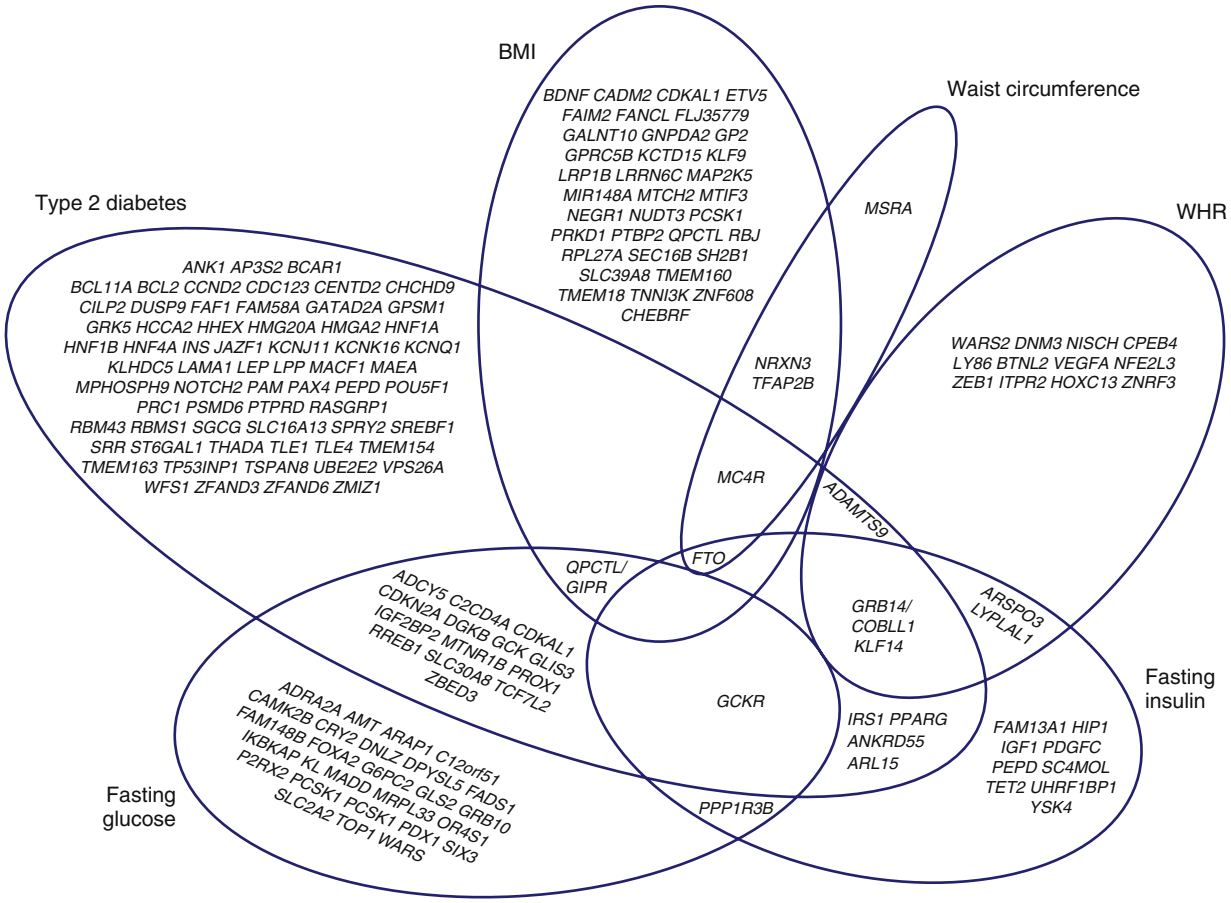
The first polymorphism identified for T2DM was Gly972Arg in *IRS1*, a key protein in the canonical insulin signaling pathway. This common polymorphism was detected by direct sequencing of 86 patients with T2DM and 76 controls, with three times as many cases present in cases than controls.[25] Subsequent studies demonstrated that this polymorphism results in impaired insulin-stimulated phosphatidylinositol 3-kinase signaling.[26] As genomic information expanded, it became clear that this polymorphism plays only a minor role in overall T2DM risk; however, this initial finding provided the first evidence of the genetic association with T2DM.

Transcription Factor 7–Like 2 Gene

Grant and colleagues[27] genotyped 228 microsatellite markers in Icelandic patients with T2DM and in control subjects. A microsatellite, DG10S478, in intron 3 of the transcription factor 7–like 2 gene (*TCF7L2*; formerly *TCF4*) was associated with T2DM. This was replicated in a Danish cohort and in a US cohort. Compared with noncarriers, heterozygous and homozygous carriers of the at-risk alleles (38% and 7% of the population, respectively) have relative risks of 1.45 and 2.41 for development of T2DM. Follow-up studies showed that specific polymorphisms in *TCF7L2* increase the risk of progression from IGT to T2DM, and this effect is associated with a reduction of glucose-induced insulin secretion.[28] The *TCF7L2* gene product is a high-mobility group box containing transcription factor previously implicated in colon cancer. In T2DM, it has been suggested to act through regulation of proglucagon gene expression in enteroendocrine cells by impacting on the Wnt signaling pathway. Of the common variants that determine diabetes risk, variants at the *TCF7L2* locus have the greatest impact. However, the diabetes-associated polymorphism marks an intronic variant, and how or if this affects expression or function of the *TCF7L2* mRNA or protein remains unknown.

KATP Channel Genes: *KCNJ11* and *ABCC8*

The beta-cell K_{ATP} channel is composed of two subunits, Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*). Kir6.2 is the pore-forming



• **Fig. 34.3** Venn diagram of the intersection between loci associated at genome-wide significance with type 2 diabetes mellitus, measures of adiposity, and glucose homeostasis. Genome-wide significant associations for six metabolic traits are shown. Gene symbols shown in the plot are by convention the closest gene and not necessarily the functional gene. *BMI,* body mass index; *WHR,* waist-to-hip ratio. (Adapted from Grarup N, Sandholt CH, Hansen T, Pedersen O. Genetic susceptibility to type 2 diabetes and obesity: from genome wide association studies to rare variants and beyond. *Diabetologia.* 2014;57:1528–1541.)

component of the potassium channel, whereas SUR1 is the sulfonylurea receptor that regulates opening of the channel. Opening of the channel results in potassium efflux from the beta cell, causing insulin secretion. Mutations in these genes are the most common cause of neonatal diabetes. Identification of these patients is important because these forms of neonatal diabetes are generally treatable with sulfonylureas rather than insulin.[29] The two genes are located adjacent to each other on chromosome 11, and SNPs within both genes are associated with an increased risk of diabetes.[30] A missense mutation Glu23Lys (E23K) in *KCNJ11* has also been associated with increased risk of typical adult-onset T2DM by an average of 13%, and the KK homozygote is at greatest risk (relative risk, 1.28).[31] A recent study suggests that, like neonatal diabetes caused by mutations in *KCNJ11* and *ABCC8*, polymorphisms in these genes, which increase the risk for T2DM, may be more readily treated with sulfonylureas.[32]

Peroxisome Proliferator-Activated Receptor γ Gene

PPARγ is a member of the PPAR subfamily of nuclear receptors. It is an important regulator of lipid and glucose homeostasis and cellular differentiation. Although PPARγ is most abundantly expressed in adipose tissue, it is also expressed in muscle and in the pancreatic beta cell. Targeted elimination of the receptor in muscle alters muscle fatty acid oxidation,[33] and knockout in the beta cell leads to a blunting of the normal increase in beta-cell mass that occurs on a high-fat diet.[34] Meta-analyses show that the common missense mutation Pro12Ala (P12A) in *PPARG* (the gene encoding PPARγ2) is associated with decreased risk for T2DM (estimated risk ratio for the alanine allele, 0.79).[35] People homozygous for the Pro12 allele are more insulin resistant than those with one Ala12 allele and have a 1.25-fold increased risk of diabetes. A second polymorphism, C161→T, has been linked to insulin resistance in Hispanic and non-Hispanic white women.[36] Loss of function mutations in PPAR cause familial partial lipodystrophy type 3 (FPLD3), and these patients may show dramatic improvement when treated with the thiazolidinedione PPAR agonists.[37]

Hepatocyte Nuclear Factor 4α Gene

Despite its name, HNF4A was the first discovered MODY (Maturity Onset Diabetes of the Young) gene, where it leads to abnormalities in insulin secretion.[38] HNF4α is also active in the liver, where it is upregulated by an alternative promoter from the pancreas and is important for gluconeogenesis. Patients with the risk alleles have increased hepatic glucose production, not impaired insulin secretion, confirming the liver-specific impact of the polymorphisms in the alternative hepatic promoter.[39] Several clinical studies have demonstrated that genetic variation in this liver-specific promoter for the *HNF4A* gene is associated with increased risk of T2DM.[39-42]

Kruppel-like Factor 14 (*KLF14*)

Kruppel-like factor 14 (*KLF14*) is a maternally imprinted transcription factor. Noncoding polymorphisms in the *KLF14* gene are associated with increased fasting insulin, waist-to-hip ratio, and T2DM.[43,44] Regulatory variants of *KLF14* appear to influence T2DM risk by causing a redistribution of fat from gynoid to visceral depots and increasing adipocyte size.[43] Accompanying in vitro experiments demonstrated that these changes are mediated, in part, by an increase in preadipocyte proliferation but impairment in lipogenesis. Interestingly, these effects only occur when the polymorphism is inherited from mother to daughter.

Diabetes Genes Identified by Genome-Wide Association Studies

GWAS continue to identify variants that determine genetic risk of T2DM and to define the genetic architecture of the disease. Fig. 34.3 depicts the intersection between loci associated at genome-wide significance with T2DM and five metabolic traits strongly associated with T2DM, including BMI, waist circumference, waist-to-hip ratio, fasting insulin, and fasting glucose. Note that there is substantial, but not complete, overlap in the loci significantly associated with these traits. For example, a variant in the gene *CREBRF*, found almost exclusively in the Samoan population, is associated with a large (1.4 kg/m^2) increase in BMI per risk allele and is also associated with a paradoxical decrease in odds ratio of 0.6 for the development of T2DM.[45] The mechanisms driving the increase in BMI but protection from T2DM are still to be elucidated but suggest that the increase in adipose tissue is confined to metabolically healthy subcutaneous tissues rather than pro-inflammatory visceral adipose tissue.

Based on these advances, the following general comments can be made regarding the genetics of T2DM:

1. The genes identified to date individually lead to a modest increase in the risk of diabetes. Persons with these individual polymorphisms have odds ratios between 1.10 and 1.45 when compared with individuals who do not have the at-risk polymorphisms.
2. The presence of multiple at-risk polymorphisms in a single individual substantially increases the risk of developing diabetes.
3. Although early GWAS suggested that a substantial proportion of the genetic variants associated with increased risk for diabetes were in genes that might alter insulin secretion, in recent studies the number of SNPs associated with decreased insulin sensitivity have increased, and at least some of these effects on insulin sensitivity appear to be independent of obesity.[43,46,47]
4. The majority of the SNPs associated with risk of T2DM appear to reside in noncoding regions of the chromatin. Studies have shown that some of these allelic variations exist in open chromatin regions alternatively called *stretch enhancers*,[48] which appear to be bound by proteins. Stretch enhancers across the genome are cell type specific and are located near and associated with increased expression of genes involved in cell-specification, as opposed to housekeeping, processes.
5. A large number of genes are associated with increased susceptibility to this disease. Whereas the total number of loci linked to T2DM is large, they account for a small proportion (estimated at no more than 5–10%) of the total genetic risk for diabetes in the population. In the search for this missing heritability, increasing attention is being given to the *rare variant hypothesis*, which states that common diseases are due to multiple, but rare, variants with large effects. Alternatively, there is new evidence that T2DM can be divided into multiple phenotypic subgroups with distinct genetic signatures that were difficult to identify when all of these subtypes were grouped together in previous studies.[49,50] Finally, the high familial risk of T2DM may be due to some element of shared environment or epigenetic effects (see the following discussion).

Epigenetic Risk of Diabetes

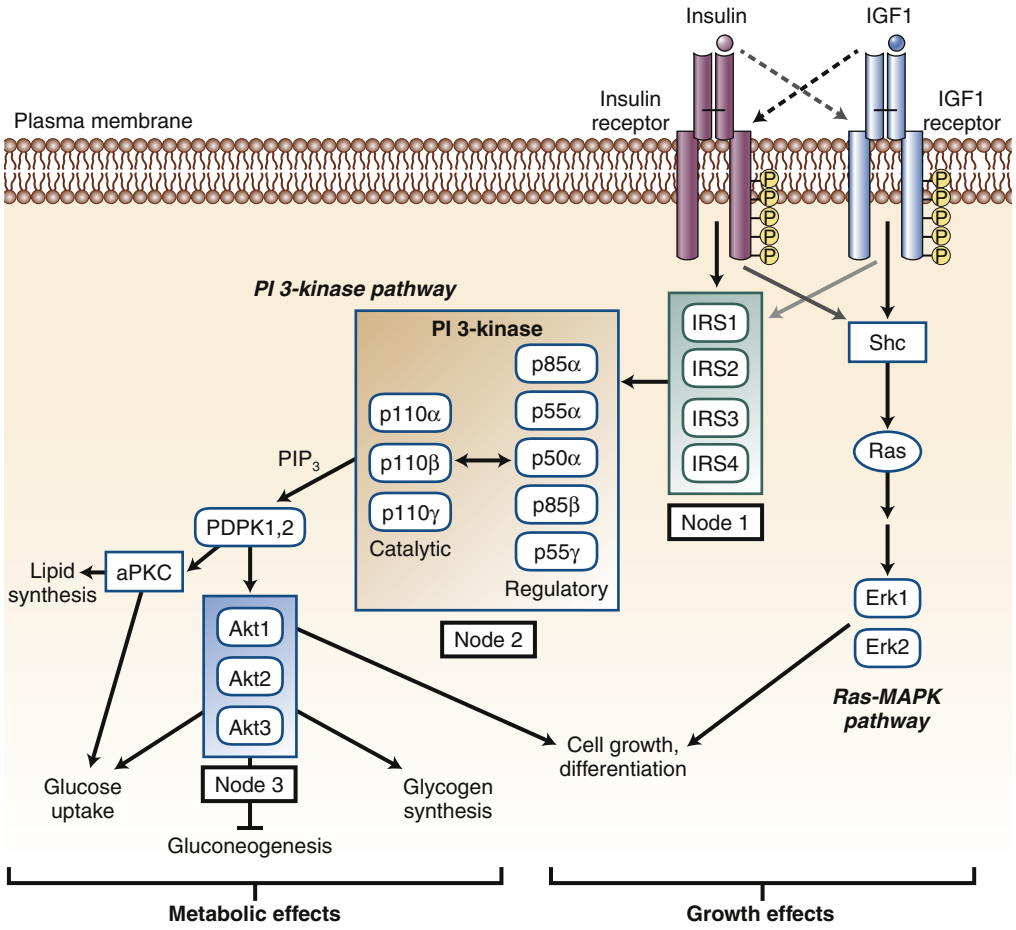
In addition to variations in the sequence of genes conferring risk of T2DM, epigenetic marks, which influence the expression of genes, can also influence disease risk. Detrimental epigenetic marks can be created in the developing fetus by both over- and undernutrition.[51,52] The idea that perinatal nutrition could influence the risk for obesity in offspring came from the observation of children born of pregnancies during the Dutch Hunger Winter, a period of starvation in the Netherlands during World War II. During this period, all Dutch citizens, including pregnant mothers, were restricted to as little as 400 to 800 calories per day. It was subsequently noted that the offspring of these pregnancies were more likely to become obese than same sex siblings conceived during normal food availability. This is attributed to epigenetic marks that persisted 60 years beyond the neonatal exposure.[53] Evidence of the same phenomena has been documented following several other famines around the world. These human observations have since been replicated in mouse models of undernutrition, which not only demonstrate the passage of obesity risk from mother to offspring, but that risk can be perpetuated for an additional generation by male offspring of undernourished mothers due to epigenetic changes to primordial sperm.[54] Similar to undernutrition, evidence also exists that overnutrition during pregnancy can cause epigenetic changes that predispose offspring to obesity and T2DM for multiple generations.[55,56]

Insulin Signaling

Insulin signaling is initiated through binding and activation of its tyrosine kinase cell-surface receptor. This initiates a cascade of phosphorylation and dephosphorylation events, generation of second messengers, and protein-protein interactions that result in diverse metabolic events in almost every tissue. Insulin receptors are ubiquitously expressed, yet insulin action is highly tissue specific due to the varied composition of downstream targets of insulin signaling. The insulin receptor consists of two insulin-binding α-subunits and two catalytically active β-subunits. Like insulin itself, the α- and β-subunits are derived from a single chain precursor or prereceptor. Once the subunits are formed, they are disulfide linked into an $\alpha_2\beta_2$ heterotetrameric complex. Insulin binds to the extracellular α-subunits, activating the intracellular tyrosine kinase domains of the β-subunits that phosphorylate intracellular partners on specific tyrosine residues.[57] On phosphorylation of the receptor, multiple classes of adapter proteins including insulin receptor substrates (IRSs), Src homology 2 domain containing (Src), growth factor receptor–bound protein (Grb), and others[58] can associate with the receptor and propagate the downstream signal resulting in modulation of cellular metabolism, stimulation of mitogenesis,[59] and receptor internalization.[60]

Downstream Events After Insulin Receptor Phosphorylation

IRS, phosphoinositide 3-kinase (PI3K), and Akt represent three critical nodes of insulin signaling that regulate a majority of the metabolic and transcriptional effects downstream of insulin activation (Fig. 34.4). The IRS proteins are critical adaptors that mediate



• **Fig. 34.4** Insulin signaling activates three critical nodes of regulation to mediate most of its metabolic actions across tissues. Node 1 contains the insulin receptor substrate (IRS) isoforms 1 through 4. Node 2 is phosphatidylinositol-3-kinase (PI3K), which is comprised of a p85 or p55 regulatory subunit and a p110 catalytic subunit. Node 3 contains three isoforms of Akt. These three signaling nodes regulate lipid and glucose metabolism, as well as cell growth and differentiation, and run parallel to the Ras-mitogen–activated protein kinase (MAPK) pathway with some crosstalk. *aPKC,* atypical protein kinase C; *ERK,* extracellular signal–regulated kinase; *IGF1,* insulin-like growth factor 1; *MAPK,* mitogen-activated protein kinases (MEK, MAPK/ERK); *PDPK,* phosphoinositol-3-phosphate–dependent kinase; *Ras,* rat sarcoma oncogene; *Shc,* SH3-containing protein.

many of the key metabolic actions of insulin across multiple tissues. IRSs have multiple functional domains, including pleckstrin homology (PH), phosphotyrosine binding (PTB), and Src homology (SH) domains that allow for interaction with phosphorylated tyrosine residues on the insulin receptor and the closely related insulin-like growth factor 1 (IGF1) receptor, allowing for docking with downstream effectors, such as PI3K.[61] There are four IRS isoforms (IRS1-IRS4), with IRS1 and IRS2 being ubiquitously expressed. Although there is evidence for functional overlap, IRS1 and IRS2 do display unique roles in growth and metabolism across tissues. Disruption of IRS1 in mice resulted in mild insulin resistance and growth retardation, whereas disruption of IRS2 resulted in beta-cell failure and secondary insulin resistance.[62]

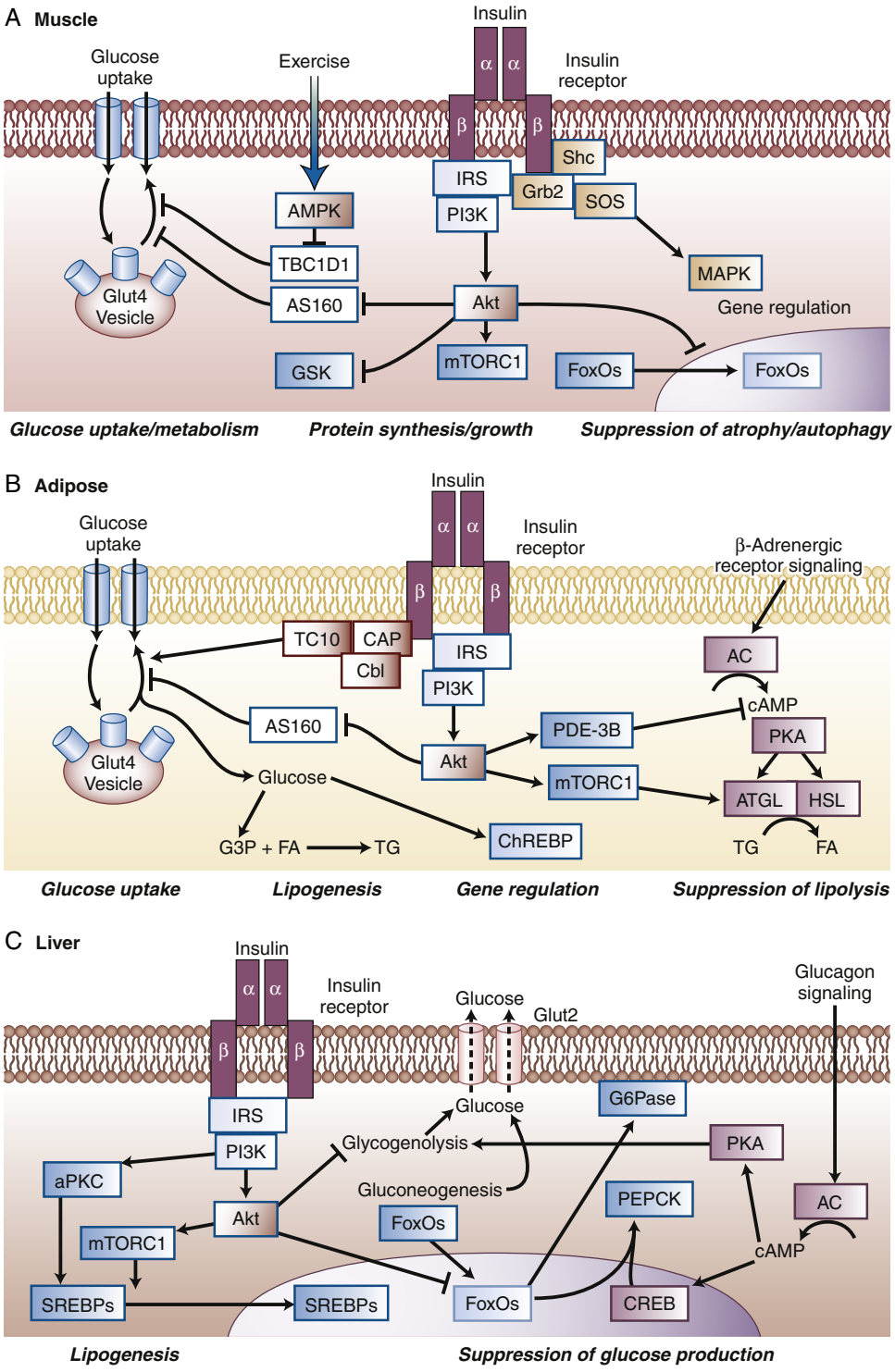
PI3K generates phosphatidylinositol 3,4,5-triphosphate (PIP3), which activates several PIP3-dependent serine-threonine kinases, such as PI-dependent protein kinases 1 and 2 (PDPK1 and PDPK2), which in turn activate Akt isoforms, atypical PKCs, wortmannin-sensitive and insulin-stimulated serine kinase, and others.

Akt kinase (also known as protein kinase B) exists as three distinct isoforms that are activated by phosphorylation on specific threonine and serine residues.[63,64] Full activation of Akt requires PDPK1-directed phosphorylation at threonine 308 (T308) in the kinase domain (KD) followed by phosphorylation at serine 473 (S473) in the hydrophobic motif (HM) of Akt by the rictor-containing complex, mTOR complex 2 (mTORC2).[64] Activated Akt has the ability to phosphorylate proteins that regulate lipid synthesis, glycogen synthesis, protein synthesis, and apoptosis. Disruption of Akt1 results in growth retardation,[65] whereas disruption of Akt2 results in insulin resistance and diabetes in mice.[66] Several investigators have examined the role of PI3K and Akt in persons with insulin resistance. Studies have shown a decrease in IRS-associated PI3K[67] and Akt[68] activity in insulin-resistant skeletal muscle; however, in some patients with reduced PI3K activity, there was normal activation of Akt.[69]

Tissue-Specific Actions of Insulin

Mechanisms of Insulin-Mediated Glucose Uptake in Muscle and Fat

A primary effect of insulin in skeletal muscle and adipose tissue is to stimulate glucose uptake by translocation of GLUT4 from an intracellular pool to the surface of cells[70] (Fig. 34.5A). Indeed,



• **Fig. 34.5** Tissue-specific insulin signaling. Insulin binds to the insulin receptor, allowing for docking and activation of multiple signaling molecules, most notably insulin receptor substrates (IRS), phosphatidylinositol-3-kinase (PI3K) and Akt, which can mediate different physiologic effects, depending on the tissue. (A) In muscle, insulin action increases glucose uptake/metabolism, promotes protein synthesis and suppresses atrophy, some of which can also be mediated by exercise. (B) In adipose, insulin increases glucose uptake and lipogenesis while suppressing lipolysis. (C) In liver, insulin promotes lipogenesis and counters the actions of glucagon, thereby suppressing glucose production. *AC,* adenylate cyclase; *AMPK,* adenosine monophosphate (AMP)–activated kinase; *aPKC,* atypical protein kinase C; *AS160,* Akt substrate of 160 kilodaltons (also TBC1D4); *ATGL,* adipose triglyceride lipase; *CAP,* Cbl-associated protein; *Cbl,* Cas-Br-M (murine) ectopic retroviral transforming sequence; *ChREBP,* carbohydrate response element–binding protein; *CREB,* cyclic AMP (cAMP) response element–binding protein; *FA,* fatty acid; *FoxO,* forkhead box O; *G3P,* glycerol-3-phosphate; *G6Pase,* glucose-6-phosphatase; *Glut,* glucose transporter; *Grb2,* growth factor receptor–bound protein 2; *GSK3,* glycogen synthase kinase 3; *HSL,* hormone-sensitive lipase; *MAPK,* mitogen-activated protein kinases (MEK, MAPK/ERK kinases); *mTORC1,* mammalian target of rapamycin complex 1; *PEPCK,* phosphoenolpyruvate carboxykinase; *PDE-3B,* phosphodiesterase 3B; *PKA,* protein kinase A; *Shc,* SH3-containing protein; *SOS,* son of sevenless; *SREBP,* sterol responsive element–binding protein; *TBC1D1,* tre-1/USP6 BUB2 cdc16 domain family member 1; *TC10,* small GTP-binding protein TC10; *TG,* triglyceride.

disruption of GLUT4 in muscle or adipose tissue causes an insulin-resistant phenotype.[71,72] Akt substrate of 160 kDa (AS160, also known as TBC1D4) and TBC1D1 are paralog Rab family guanosine triphosphatase (GTPase)-activating (Rab GAP) proteins that have been proposed to inhibit the translocation of GLUT4 to the plasma membrane through interaction with insulin-responsive aminopeptidase (IRAP).[73] AS160 activity is suppressed by Akt phosphorylation in response to insulin stimulation and mediates translocation of GLUT4-containing vesicles to the plasma membrane to increase glucose uptake in muscle and adipose tissue.[73,74] TBC1D1, however, is phosphorylated by adenosine monophosphate–activated kinase (AMPK) and appears to play a key role in exercise-regulated increases in muscle glucose uptake, which remain intact in patients with T2DM.[75-77] On phosphorylation of the Rab GAPs, the inhibition of GLUT4 translocation by AS160 and/or TBC1D1 is relieved, contributing to the increased glucose uptake. Although the composition and signaling pathways that converge on the intracellular GLUT4-containing vesicles to cause GLUT4 translocation are still not fully understood, it appears that the number of glucose transporters in skeletal muscle of insulin-resistant persons is not changed, but the ability of insulin to effect GLUT4 translocation is disrupted.[78-80] In adipose tissue, PI3K-independent mechanisms also play a role in mediating glucose uptake. Insulin receptors can associate with Cbl-associated protein (CAP) and the E3 ubiquitin-protein ligase Cbl to activate TC10, an actin remodeling enzyme.[81] This interaction occurs in lipid rafts on the plasma membrane, contributing to GLUT4 vesicle translocation in the absence of IRS-PI3K-Akt activation. A similar mechanism of actin remodeling to regulate GLUT4 trafficking occurs in striated muscle, but this is via Rac proteins and is not dependent on TC10.[82]

In addition to its regulation of glucose metabolism, insulin also has profound effects on protein homeostasis (proteostasis).[83] In muscle, insulin promotes hypertrophy by both enhancing protein synthesis and suppressing protein degradation pathways including proteasome and autophagy (see Fig. 34.5A). Insulin, via Akt, activates mTOR complex 1 (mTORC1) to promote protein synthesis and cellular growth.[84] At the same time, insulin suppresses forkhead box O (FoxO) transcription factor activation, a master regulator of protein degradation pathways.[83,85] Mouse models with muscle-specific deletion of insulin receptor, or both insulin receptor and IGF1 receptor, show profound muscle atrophy,[86] but the atrophy is completely rescued by deletion of FoxOs.[87] This increased protein degradation is also seen in rodent models of T1DM[88] and in patients with T1DM when insulin is withdrawn for as little as 8 hours.[89,90] Thus, insulin coordinates muscle protein turnover, and this regulation is dependent on suppression of FoxO-regulated protein degradation.

Insulin Regulation of Lipolysis and Lipogenesis

Insulin plays a critical role in regulating lipid storage. The liver is the primary site for de novo lipogenesis of fatty acids and triglycerides, whereas adipose tissue serves as the primary storage depot for triglycerides. Insulin signaling enhances lipid storage in adipose by both stimulating triglyceride synthesis and suppressing lipolytic triglyceride breakdown (see Fig. 34.5B). First, insulin stimulates rapid and potent increases in adipose tissue glucose uptake that is then converted into glycerol-3-phosphate, used to synthesize triglycerides from fatty acids. Fatty acid transport into adipose is also increased by insulin, which likely involves translocation of fatty acid transporters, including FATP1 and CD36,[91] and/or increases in lipoprotein lipase expression and activity.[92-94] In addition to providing substrates for triglyceride synthesis, glucose also activates carbohydrate response element–binding proteins to upregulate glycolytic and lipogenic genes in adipocytes.[95] This pathway to enhance lipid storage is critical for metabolic homeostasis, as overexpression of GLUT4 in adipose protects against insulin resistance caused by diet-induced obesity, but deletion of carbohydrate response element–binding protein prevents this effect.[95,96]

Insulin potently suppresses adipose tissue lipolysis, which, when combined with the enhanced lipid uptake in adipose, can significantly reduce circulating fatty acid content.[97] Insulin regulates lipolysis at multiple levels (see Fig. 34.5B). β-adrenergic signaling enhances cyclic adenosine monophosphate (cAMP)-induced protein kinase A activation, which in turn activates two of the principal lipases in fat cells: adipose tissue triglyceride lipase (ATGL) and hormone-sensitive lipase. Insulin activates PDE-3B, which reduces cAMP levels and inhibits protein kinase A–mediated activation of ATGL and hormone-sensitive lipase.[98,99] Two further mechanisms by which insulin suppresses lipolysis are by increases in ATGL mRNA and by increased expression of FSP27, a lipid droplet stabilizing protein.[100-102] These actions of insulin to suppress lipolysis are clinically important in T1DM because newly diagnosed T1DM patients show a rapid gain of fat during the first year of insulin therapy.[103,104]

Regulation of fat distribution by insulin is also important in metabolic health. A recent genomic analysis found strong associations between reduced adiposity in peripheral compartments, severe insulin resistance, and SNPs in 53 genes, among which insulin receptor (INSR), IRS1, and a regulatory subunit of PI3K (PIK3R1) were identified.[105] The 53 genes also correlated highly with familial partial lipodystrophy type 1 (FPLD1). These studies agree with data from rodent models that indicate deletion of insulin receptors in adipocytes leads to rapid decreases in adipose tissue mass, redistribution of body fat to liver, and lipodystrophic diabetes.[106-108] Thus, insulin regulation of lipolysis and lipogenesis in adipose plays a critical role in storage of lipids, maintaining whole-body insulin sensitivity, and preventing metabolic disease.

Insulin also regulates hepatic lipogenesis, the primary site of de novo lipogenesis in the body.[97] SREBP transcription factors are master regulators of lipogenic genes including fatty acid synthase, lipoprotein lipase, acetyl coenzyme A carboxylase (ACC), and stearoyl coenzyme A (CoA) desaturase isoforms, and are regulated by insulin at multiple levels in liver (see Fig. 34.5C). Through activation of atypical PKC isoforms λ/ζ (aPKC), expression of sterol regulatory element-binding transcription factor 1c (SREBP1c) is increased by insulin in liver.[109,110] Furthermore, SREBP1c requires proteolytic cleavage to enter the nucleus and activate transcription of lipogenic genes. This processing of SREBP1c is regulated by mTORC1 signaling downstream of the IRS-PI3K-Akt pathway,[111] and mTORC1 activation can also promote mRNA stability of SREBP1 target genes.[112] Studies in mice and humans with insulin receptor mutations show that reduced insulin signaling in the liver protects against hepatic steatosis.[113,114] However, patients with T2DM and metabolic syndrome have a paradoxical upregulation of hepatic lipogenesis and dyslipidemia that is observed in the context of muscle and adipose insulin resistance, altered CNS signaling to the liver, and increased nutrient supply. Although the exact mechanisms have not been elucidated, the upregulation of hepatic lipogenesis in insulin-resistant states is proposed to be due to either a selective postreceptor insulin resistance[113-115] or a combination of insulin resistance with altered substrate supply and extrahepatic signaling.[116-118]

Insulin Regulation of Hepatic Carbohydrate Metabolism

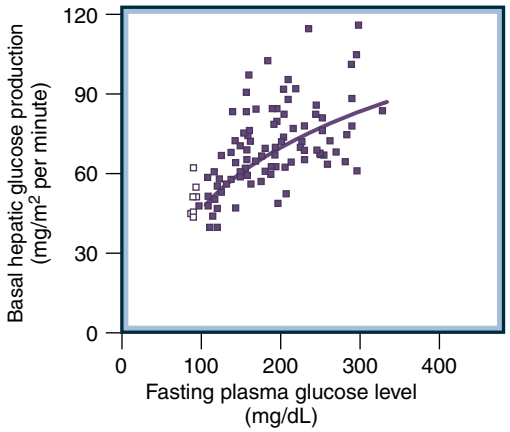
The production of glucose by the liver is regulated primarily by a balance between the actions of insulin to suppress glucose production

and glucagon to activate glucose production, with insulin being dominant over glucagon (see Fig. 34.5C). The sympathetic nervous system[119] and glucose autoregulation also play roles in hepatic glucose production but are probably less important.[120] The ability of insulin to reduce hepatic glucose output is an important mechanism for maintaining normal fasting blood glucose and normal glucose tolerance.[121,122] Insulin also suppresses glucose release from the liver by inhibiting glycogenolysis.[123] As with gluconeogenesis, the effect of insulin to suppress hepatic glycogenolysis is dominant over glucagon.[124] Glucagon increases glycogenolysis by activation of the classic protein kinase A cascade involving cAMP and also increases gluconeogenesis in part by increasing transcription of phosphoenolpyruvate carboxykinase (PEPCK) by means of cAMP response element–binding protein (CREB).[122,125]

Data suggest that the regulatory mechanisms triggered by cAMP are much more complex, with the CREB transcriptional coactivator, TORC2, playing an important role. TORC2 is specifically dephosphorylated in response to cAMP; this results in translocation of the TORC2 protein to the nucleus, allowing activation of CREB-dependent transcription of gluconeogenic enzymes.[126] In addition, CREB may increase transcription of PPARγ coactivator 1α (PGC1α), which serves as a critical coactivator of the transcription factor forkhead box protein O1 (FoxO1). FoxO1 plays a role in the transcriptional activation of various genes associated with gluconeogenesis, including PEPCK and glucose-6-phosphorylase.[125] Indeed, deletion of FoxO isoforms in the liver can restore glucose levels to normal in mice with insulin receptor knockout.[127,128] Deacetylation of transcription factors and coactivators can also regulate gluconeogenesis. The sirtuin, SIRT1, deacetylates PGC1α[129] and histone deacetylases and sirtuins can deacetylate FOXOs,[130,131] increasing their nuclear localization and interaction with HNF4A. The PGC1α/FOXO1/HNF4A complex is a potent activator of gluconeogenic gene transcription.[125]

Insulin decreases endogenous glucose production by direct and indirect mechanisms.[132] In its direct action, portal insulin suppresses glucose production by inhibiting glycogenolysis and gluconeogenesis by insulin receptor activation (see Fig. 34.5C). The indirect or peripheral effect of insulin in controlling glucose production by the liver is twofold. First, insulin profoundly decreases glucagon secretion by the alpha cell of the pancreas through systemic and paracrine effects.[133,134] The decrease in glucagon secretion decreases the activation of glycogenolysis and gluconeogenesis. The second important peripheral action of insulin is to decrease substrates for gluconeogenesis, such as alanine from muscle protein degradation,[83] and glycerol and free fatty acid levels, by suppressing lipolysis in adipose tissue[135] (see Fig. 34.5A,B). When the reduction in plasma fatty acids during a hyperinsulinemic clamp is prevented by infusion of triglyceride emulsions with heparin (which produces increased fatty acid levels through activation of lipoprotein lipase), insulin-mediated suppression of hepatic glucose output is reduced.[136] The suppression of glucagon secretion and the decrease in gluconeogenic substrate delivery to the liver are additive in reducing liver glucose production.[137]

Clinically, hepatic insulin resistance plays an important role in the hyperglycemia of T2DM,[138,139] and the impaired suppression of hepatic glucose output appears to be quantitatively similar to, or even larger than, the defect in stimulation of peripheral glucose disposal.[140] Defects in the direct effect of insulin to suppress hepatic glucose production in humans appear to be caused by a large rightward shift in the steep dose-response curve for insulin's inhibition of glycogenolysis.[141] There is a direct relationship between increased hepatic glucose output and fasting hyperglycemia[142] (Fig. 34.6). Insulin-mediated suppression of hepatic glucose output is impaired



• **Fig. 34.6** The relationship between fasting hepatic glucose output and fasting plasma glucose levels. *Open squares* represent nondiabetic control subjects; *closed squares* represent diabetic subjects. (From Maggs DG, Buchanan TA, Burant CF, et al. Metabolic effects of troglitazone monotherapy in type 2 diabetes mellitus: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med.* 1998;128:176–185. © American College of Physicians, used with permission.)

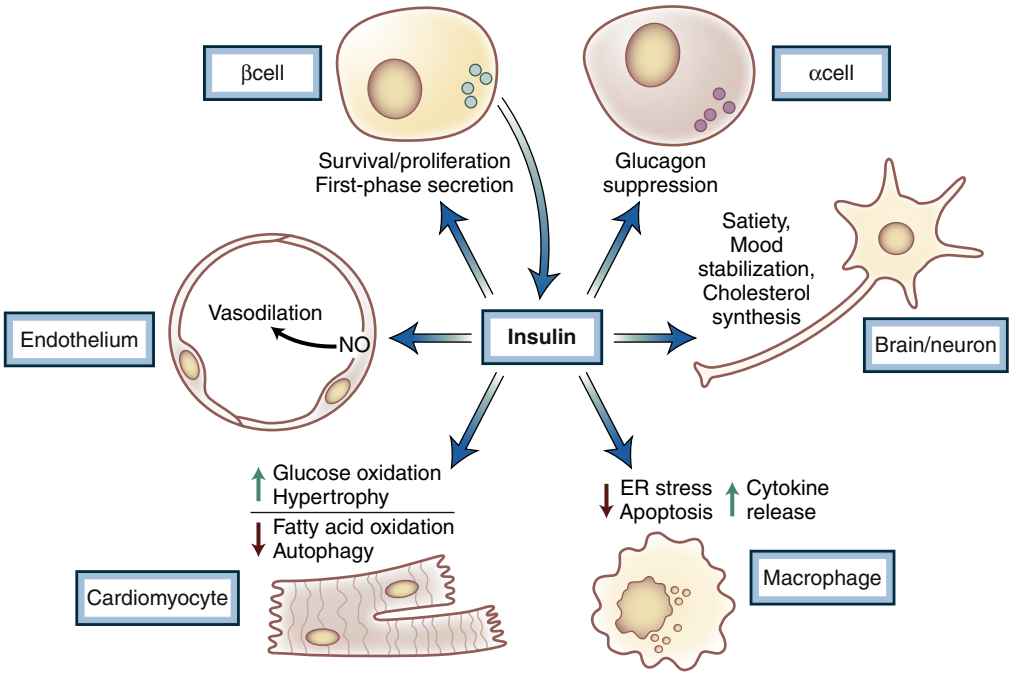
at both low and high plasma insulin levels in T2DM[140,143]; hepatic glucose production is elevated early in the course of the disease[144,145] but may be normal in lean, relatively insulin-sensitive T2DM.[146] Treatment of patients with metformin, which suppresses hepatic glucose production, results in improved glucose tolerance.[145]

Expanding Collection of Insulin-Sensitive Tissues

Although much of our understanding of insulin action on metabolism involves the insulin-sensitive metabolic tissues of muscle, liver, and adipose, insulin receptors are ubiquitously expressed and insulin action on other "nonclassic" tissues plays an important role in health (Fig. 34.7). Insulin signaling in beta cells is critical for adaptive survival and proliferation of these cells. Insulin secretion and beta-cell mass both increase in response to chronic mild hyperglycemia, and the ability to upregulate beta-cell mass influences the development of T2DM. Interestingly, when insulin receptors are deleted from beta cells, first-phase insulin secretion is lost and compensatory growth of beta cells is impaired in response to diet-induced obesity and hepatic insulin resistance,[147,148] indicating an unexpected autocrine loop. Furthermore, FoxO transcription factors, which are downstream targets of insulin action, are important for maintenance of beta-cell function and identity,[149] and beta cells appear to de-differentiate when FoxOs are deleted.[150] Evidence that beta-cell dedifferentiation occurs in insulin-resistant states in humans are emerging,[151] but the degree to which this phenomenon accounts for beta-cell failure in T2DM and whether this process can be intervened upon is still under investigation.

Insulin action on glucagon-producing alpha cells suppresses glucagon secretion and maintains glucose homeostasis. This was demonstrated by deletion of insulin receptors on alpha cells of mice, which leads to mild glucose intolerance, increased glucagon secretion, and progressive hyperinsulinemia,[134] all of which are features of metabolic syndrome and T2DM. Thus, insulin signaling on alpha and beta cells in islets is important for metabolic health and glucose homeostasis.

Vascular complications are a major contributor to morbidity and mortality in diabetes. Thus, it may not be surprising that vascular endothelium and cardiomyocytes are exquisitely insulin



• **Fig. 34.7** The expanding collection of insulin-sensitive tissues. Insulin receptors are ubiquitously expressed, and thus insulin controls pleotropic effects on numerous "nonclassical" tissues that play an important role in health. *ER,* endoplasmic reticulum.

responsive. In endothelial cells, insulin action promotes vasodilation via nitric oxide production, which is impaired in insulin resistant states.[152-154] Endothelial insulin sensitivity is also important for transendothelial transport of insulin to peripheral tissues,[155,156] and the relative permeability of the endothelial barrier in various tissues plays an important role in timing of insulin action in those tissues after a bolus of insulin.[157] Endothelial insulin signaling also plays a major role in the development of atherosclerosis. Insulin receptor knockout in endothelial cells caused a two- to threefold increase in atherosclerotic lesions in an atherogenic mouse model.[158] Conversely, enhancing downstream activation of insulin signaling by either overexpression of IRS or inhibition of FoxOs improves vascular endothelial function and can prevent atherosclerosis.[159,160] In cardiomyocytes, the insulin signaling cascade controls growth and metabolism in both physiologic and pathologic conditions.[161,162] The heart has a remarkable ability to utilize a wide variety of substrates for energy production, but under fasted states, fatty acids are preferentially metabolized. On insulin stimulation, glucose oxidation is increased and fatty acid oxidation is suppressed, but in insulin-resistant or diabetic states, this metabolic flexibility is lost.[163] Insulin also plays an important role in postnatal cardiac growth by suppression of autophagy.[164] These signals in the cardiovascular system integrate with insulin action in other tissues and metabolic flux to coordinate cardiovascular health. Disruption of the tissue cross talk during obesity and insulin resistance results in features of metabolic syndrome, which increase cardiovascular risk.[165]

Insulin can also affect cardiovascular risk by its action on immune cells, particularly macrophages. Macrophages play an important role in the development of atherosclerosis by formation of foam cells within atherosclerotic plaques, enhanced inflammation, and apoptosis leading to a necrotic core that is susceptible to rupture.[166] Mouse models with loss of insulin signaling in macrophages show mild protective effects on atherosclerotic lesion size[167] but also show worsening of the necrotic core, likely due to enhanced macrophage apoptosis from endoplasmic reticulum (ER) stress.[168] This observation also extends to obesity and aging models of atherosclerosis[169,170] indicating that insulin resistance in macrophages contributes to plaque progression in the context of metabolic syndrome.[171]

Insulin Signaling in the Central Nervous System

Although previously considered insulin independent, it is now clear that the brain is an important site for insulin signaling. Insulin action is not required for glucose uptake in the brain because it predominantly expresses the insulin-independent glucose transporters Glut1 and Glut3. The insulin receptor is expressed throughout the brain. Brain-specific knockout of the receptor in mice results in a variety of abnormalities including increased body weight, hypothalamic hypogonadism, increased hepatic glucose production, and a depression-like phenotype.[172] The impact of insulin signaling in the brain is cell-type specific. Although neuronal insulin receptor appears to be key for regulation of hepatic glucose production,[173] astrocyte insulin receptor signaling is vital for insulin-mediated effects on mood.[174] Activation of the brain insulin receptor can lead to improved synaptic transmission through cholesterol synthesis; proliferation through mitogen-activated protein kinase; axon growth and neuroplasticity through glycogen synthase kinase 3β (GSK3β); gene transcription and neuronal polarity through FOXO; and autophagy, protein synthesis, and neuronal plasticity through mTORC1.[175,176] Insulin entry into the brain shows regional differences and in some areas is dependent on a receptor-mediated transcytosis process.[157,177] Systemic insulin resistance results in lower than expected cerebrospinal fluid insulin levels due to decreased transport across the blood-brain barrier.[178] Brain-specific resistance to both insulin and IGF1 signaling in humans is associated with Alzheimer disease, but the significance of these findings to disease progression remains uncertain.[179]

The insulin receptor has two isoforms: IR-A is missing exon 11, whereas IR-B includes exon 11. Isoform A has a higher affinity for insulin-like growth factor 2 binding than isoform B. In contrast to most other tissues in the body, the brain predominantly expresses isoform A. Other unique aspects of brain insulin signaling include a unique glycosylation pattern of the brain insulin receptor and the propensity for insulin receptor to form heterodimers with the more abundant IGF1 receptor, which appears to favor IGF1 signaling over insulin signaling.[180] Greater understanding of the relative impacts of insulin and IGF1 signaling on the various cell types of the brain may prove critical as clinical trials are under way in testing the efficacy of insulin delivered to the brain intranasally to treat such diverse disorders as Alzheimer disease, depression, and obesity.

Insulin Resistance and the Risk of Type 2 Diabetes Mellitus

Insulin Resistance

The term *insulin resistance* indicates the presence of an impaired biologic response to either exogenously administered or endogenously secreted insulin. Insulin resistance is primarily manifested by decreased insulin-stimulated glucose transport and metabolism in skeletal muscle, impaired insulin suppression of adipocyte lipolysis, and impaired ability of insulin to suppress hepatic glucose output. However, given the pleiotropic actions of insulin, it is clear that insulin resistance could lead to disorders of multiple metabolic pathways involving amino acids, glucose, and lipid metabolism.

Insulin resistance can be measured using a variety of techniques. The gold standard for determining insulin sensitivity/resistance in an individual is a hyperinsulinemic euglycemic clamp. In this technique, developed by DeFronzo, a patient is given a constant infusion of insulin to produce hyperinsulinemia. A second infusion containing glucose is given concurrently and adjusted to produce euglycemia. Since the individual is in steady state, the glucose infusion rate represents the rate of glucose uptake/disposal into muscle, fat, and other tissues under hyperinsulinemic conditions (i.e., the insulin sensitivity of the patient).[181] When conducted with tracers, one can also measure the hepatic glucose production rate as a measure of liver insulin sensitivity. Because this technique is costly, invasive, and time consuming, alternative approaches have been developed to estimate insulin resistance, such as the steady-state plasma glucose method of Reaven[182] or the Bergman minimal model of glucose disposal assessed using a frequently sampled intravenous glucose tolerance test.[183] When coupled with insulin levels, one can also obtain the Disposition Index, which is a combined measure of insulin sensitivity and insulin secretion.[184,185] For large cohort studies or a simple measure in an outpatient setting, HOMA-IR (homeostatic model assessment of insulin resistance) can be calculated using just fasting glucose and insulin levels. It correlates favorably with a hyperinsulinemic euglycemic clamp with an R_s of 0.88.[186] Others favor the use of QUICKI (quantitative insulin-sensitivity check index), as an alternative mathematical model, also based on the reciprocal of the logarithm of HOMA-IR.[187]

Insulin sensitivity is influenced by several factors, including age, weight, ethnicity, body fat (especially intra-abdominal obesity), physical activity, dietary intake, gut microbiota, and medications. Substantial data indicate that insulin resistance plays a major role in the development of IGT and diabetes. Insulin resistance is a consistent finding in patients with T2DM, and resistance is present years before the onset of diabetes.[188-191] Prospective studies have shown that insulin resistance predicts the onset of diabetes.[189,190] There is also a strong influence of environmental factors on the genetic predisposition to insulin resistance and therefore to diabetes.[192,193]

The molecular mechanisms of insulin resistance are initiated by various pathophysiologic processes that feed back on the insulin receptor and its adapter proteins (Fig. 34.8). The insulin receptor β-subunit has been shown to undergo serine/threonine phosphorylation, which might decrease the ability of the receptor to autophosphorylate. The activities of several serine/threonine protein kinases that catalyze inhibitory phosphorylation of the insulin receptor or IRS proteins are elevated in animal models of insulin resistance and in insulin-resistant humans.[194,195] Interventions that decrease serine phosphorylation (inhibitory signal) of the insulin receptor result in increased insulin signaling.[196] Furthermore, enhanced tyrosine dephosphorylation by protein tyrosine phosphatases can attenuate insulin signaling. Two protein tyrosine phosphatases that have been shown to negatively regulate insulin signaling, PTP1B and LAR (leukocyte antigen related), have been reported to be elevated in insulin-resistant patients.[197,198] Conversely, disruption of PTB1B in mice resulted in a marked increase in insulin sensitivity and resistance to diet-induced obesity.[199]

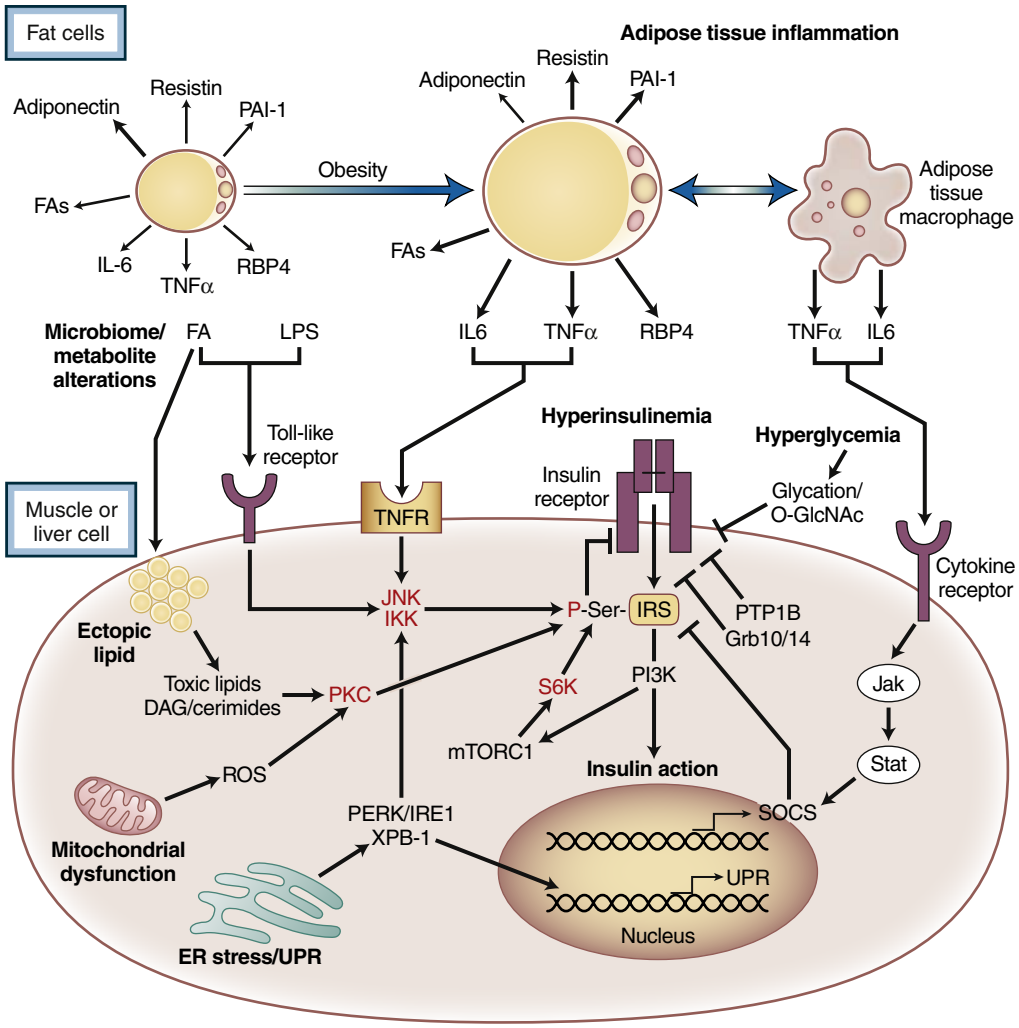
As mentioned previously, mutations in the insulin receptor are associated with rare forms of insulin resistance. In addition, individuals may develop autoantibodies to the insulin receptor, which induce severe insulin resistance (type B insulin resistance and acanthosis nigricans).[13,200]

Obesity and Type 2 Diabetes Mellitus

The association of obesity with T2DM has been recognized for decades (Fig. 34.9). Central (also referred to as intra-abdominal or visceral) adiposity is more strongly linked to insulin resistance and to several important metabolic variables, including elevated plasma glucose, insulin, total plasma cholesterol and triglyceride concentrations, and decreased plasma high-density lipoprotein cholesterol concentration, than is total adiposity.[201-207] Indeed, some studies have suggested that subcutaneous fat may actually be protective against insulin resistance.[208] As a result, the association between abdominal fat and glucose tolerance is independent of total adiposity.[209,210] The reason for the relationship between intra-abdominal fat and abnormal metabolism is not clearly defined, but several hypotheses have been proposed and may interact in this association. Abdominal fat is more lipolytically active than subcutaneous fat, perhaps because of its greater complement of adrenergic receptors.[211] In addition, the abdominal adipose store is resistant to the antilipolytic effects of insulin,[212] including alterations in lipoprotein lipase activity; this leads to increased lipase activity and a greater flux of fatty acids into the circulation, with the portal circulation receiving the greatest fatty acid load. Conversely, subcutaneous fat makes and releases more adiponectin, which is a beneficial adipokine. Finally, the high levels of 11β-hydroxysteroid dehydrogenase type 1 (HSD11B1) in intra-abdominal fat could result in enhanced conversion of inactive cortisone to active cortisol, resulting in increased local cortisol production. This might change adipocytes to increase lipolysis and alter the production of adipokines, which may directly modulate glucose metabolism.

Hyperinsulinemia and Insulin Resistance

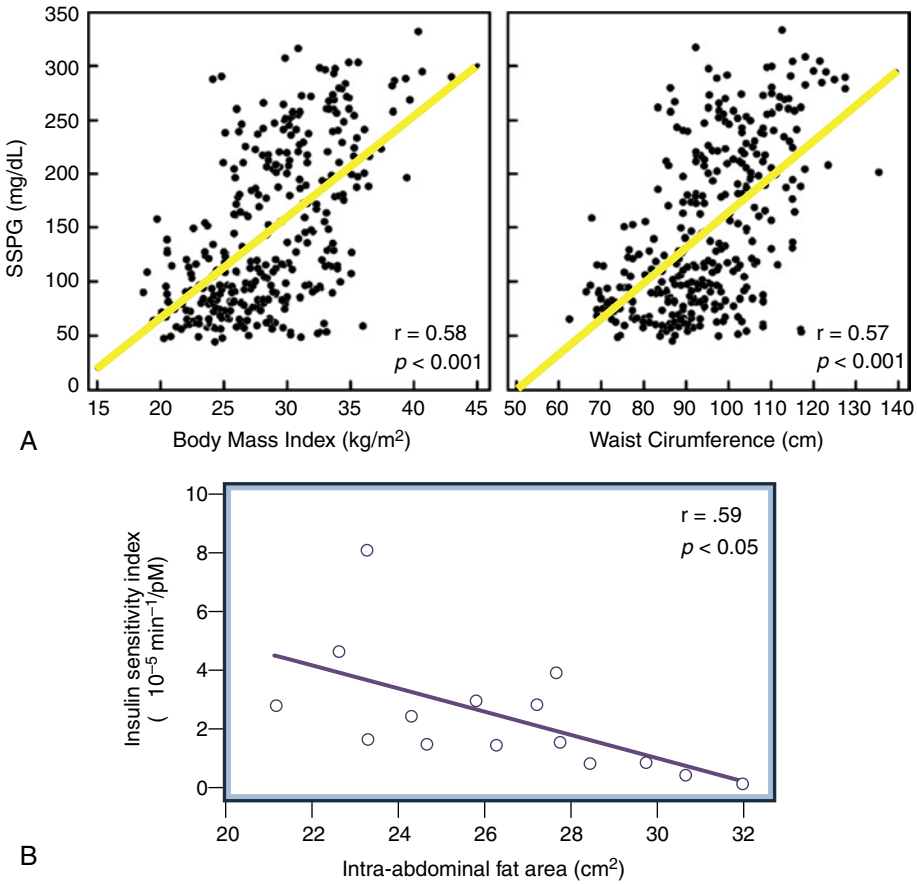
Hyperinsulinemia can cause insulin resistance. Elevated concentrations of insulin downregulates insulin receptors and desensitizes postreceptor pathways.[213] Del Prato and colleagues[214] showed that 24 and 72 hours of sustained physiologic hyperinsulinemia



• **Fig. 34.8** Cellular mechanisms of insulin resistance in overnutrition. Numerous environmental and pathologic insults can combine to impair cellular insulin signaling leading to insulin resistance. Overnutrition leads to adipose tissue inflammation, hyperinsulinemia, hyperglycemia, and microbiome/metabolite alterations in the body. These are associated with ectopic lipid accumulation, mitochondrial dysfunction, and endoplasmic reticulum (ER) stress/unfolded protein response (UPR) in insulin-sensitive tissues. A common mechanism of insulin resistance is activation of serine/threonine kinases (shown in red font) that phosphorylate insulin receptor and IRS proteins leading to feedback inhibition of these proximal signaling intermediates in the insulin cascade. Another mechanism is protein-protein interactions that decrease insulin receptor phosphorylation or inhibit its interaction with IRS proteins. *DAG,* diacylglycerol; *ERK,* extracellular signal–regulated kinase; *FA,* fatty acid; *Grb,* growth factor receptor-bound protein; *IKK,* IκB kinase; *IRS,* insulin receptor substrates; *IL6,* interleukin 6; *Jak,* janus kinase; *JNK,* c-Jun *N*-terminal kinase; *LPS,* lipopolysaccharide; *mTORC1,* mammalian target of rapamycin complex 1; *O-GlcNAc,* O-linked β-*N*-acetylglucosamine; *PI3K,* phosphatidylinositol-3-kinase; *PKC,* protein kinase C; *PTP1B,* protein tyrosine phosphatase 1B; *ROS,* reactive oxygen species; *S6K,* ribosomal protein S6 kinase; *Shc,* SH3-containing protein; *SOCS,* suppressor of cytokine signaling; *SOS,* son of sevenless; *Stat,* Signal transducer and activator of transcription; *TNFα,* tumor necrosis factor α.

in normal persons specifically inhibited the ability of insulin to increase nonoxidative glucose disposal in association with an impaired ability of insulin to stimulate glycogen synthase activity. Suppression of insulin secretion in obese, insulin-resistant persons results in increased insulin sensitivity.[215,216]

In cell systems, serine phosphorylation of IRS proteins inhibits insulin signaling and is mediated by a variety of kinases (see Fig. 34.8), including mammalian target of rapamycin (mTOR)/S6 kinase (S6K), which is downstream of the PI3K-Akt pathway. Feedback serine phosphorylation of IRSs on appropriate residues might increase ubiquitination and degradation IRS isoforms, which would result in decreased insulin signaling.[217] However, the mechanisms appear much more complex in vivo, because a mouse model with a serine to alanine mutation at 302 on IRS1, the prime target of mTOR-induced disruption of IRS signaling, did not prevent insulin resistance from mTOR activation in the liver.[218] Signaling via mTOR can also increase growth factor receptor bound protein 10 (Grb10) levels by phosphorylation.[219] Grb10 and Grb14 are negative regulators of insulin signaling, which act in key metabolic tissues including muscle and adipose,[220] and contain polymorphisms in humans that are linked to development of T2DM.[221] Extracellular signal–regulated kinase



• **Fig. 34.9** Relationships between body mass index, waist circumference, and insulin resistance measured by steady-state plasma glucose (SSPG) (A) or intra-abdominal fat and insulin sensitivity (B). (A from Farin MF, Fahim A, Reaven GM. Body mass index and waist circumference both contribute to differences in insulin-mediated glucose disposal in nondiabetic adults. *Am J Clin Nutr.* 2006;83:47–51; B from Kahn SE, Prigeon RL, McCulloch DK, et al. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects: evidence for a hyperbolic function. *Diabetes.* 1993;42:1663–1672.)

(ERK) has also been shown to feed back and inhibit IRS function and is downstream of insulin-mediated mitogen-activated protein kinase activation. Last, FoxO transcription factors regulate transcription of the insulin receptor and many inhibitors of Akt action, including Tribbles 3 (Trb3) and protein phosphatase 2A (PP2A).[222] Thus, activation of the insulin signaling cascade feeds back at the level of IRS and other insulin targets, with chronic activation from hyperinsulinemia itself impairing insulin signaling by chronic feedback inhibition.

Nutrient Overload and Insulin Resistance

Cells have developed several ways to sense incoming nutrients, including direct and indirect activation of transcription factors and protein kinases. Amino acids stimulate growth by direct activation of mTOR complex 1.[84] Fatty acids are sensed by several mechanisms, including cell surface receptors, such as toll-like receptors (TLRs), and by transcription factors, including PPARα and PPARγ.[223] Glucose itself can be converted to O-linked β-*N*-acetylglucosamine (O-GlcNAC) post-translational modifications that modify enzymes on Ser/Thr residues, acting similar to or opposing phosphorylation.[224] Many of these signals provide feedback inhibition on the insulin receptor cascade through Ser/Thr kinase activation (including S6K, c-Jun N-terminal kinase (JNK), and inhibitor of κB kinase (IKK).[217,225-227] However, chronic overnutrition can alter these normal cellular signals, causing insulin resistance (see Fig. 34.8). In a sense, the reaction of different tissues to obesity may be a relatively normal physiologic response to excess nutrient delivery, with prolonged activation leading to unintended and pathologic states that result in insulin resistance, inflammation, and even cell death.

Adipose Tissue and Insulin Resistance

To maintain metabolic homeostasis when nutrient intake exceeds energy expenditure, the excess calories must be used to increase cellular mass or it must be stored. Most excess nutrients, whether carbohydrate, protein, or lipid, are ultimately stored as triglyceride in white adipose tissue. If the storage capacity of adipose tissue is exceeded, lipids and other nutrients enter nonstorage tissues. This ectopic lipid accumulation occurs in myocytes, hepatocytes, vascular cells, and beta cells, and can produce toxic lipid metabolites (e.g., diacylglycerol or ceramides) that trigger activation of PKC isoforms among other adaptive and maladaptive cellular responses that lead to insulin resistance.[228] Indeed, some data suggest that the inability to expand fat mass in response to overeating may be an important factor in the development of insulin resistance.[229]

Adipocytes are more than storage cells. They regulate the uptake and release of fatty acids; participate in the glycerol–fatty acid cycle; release leptin and other hormones that signal the energy

status of the body; and secrete an ever-expanding number of cytokines that have hormonal, paracrine, and autocrine actions.[230] The adipocyte itself can be adversely affected by accumulation of excess nutrients, leading to events that can have adverse consequences on the body. As adipocyte surface area increases in obesity, there is increased expression of leptin, interleukin 6 (IL6), IL8, monocyte chemoattractant protein 1 (MCP1), and granulocyte colony-stimulating factor. These and possibly other cytokines attract pro-inflammatory macrophages (M1 type), which release factors such as tumor necrosis factor α (TNFα) that may have local and systemic inflammatory effects.[231]

In addition to energy-storing white adipose tissue, humans and other mammals have energy-burning brown adipose tissue. Brown adipose tissue is a thermogenic tissue that contains unique adipocytes with distinct morphology including multiloculated lipid droplets, increased mitochondrial content, and expression of the uncoupling protein 1 (UCP1) to uncouple electron transport and generate heat. Brown adipose tissue is activated by the sympathetic nervous system, which increases the mobilization and oxidation of fatty acids.[232] The content of brown adipose tissue in humans is negatively correlated with age and obesity, which may be a consequence or a contributor to insulin resistance in these conditions.[233] In humans, prolonged or repeated cold exposure can increase the mass and activity of brown adipose tissue in the neck and supraclavicular regions, as defined by the uptake of glucose, and can improve glucose homeostasis.[234] It has been recognized that rodents and humans also have beige or brite (brown in white) adipocytes,[235] which appear mixed into some white adipose tissue depots following cold or hormonal stimuli. Like brown adipocytes, beige adipocytes express UCP1; however, the developmental origin of these cells appears to be distinct from brown adipose tissue. The degree to which humans can increase beige adipocytes and how it may impact metabolism remains unknown but presents a potential therapeutic target.

Ectopic Lipid Accumulation

When the storage capacity of adipose tissue is exceeded, lipids accumulate in tissues such as muscle and the liver that are not well adapted to lipid storage, a process known as ectopic lipid accumulation. This leads to metabolic dysfunction in these tissues. In muscle, insulin-stimulated glucose uptake is inversely related to the amount of intramuscular triglycerides measured by biopsy, computed tomography, and magnetic resonance imaging , which can distinguish intramyocellular from extramyocellular fat.[236] First-degree relatives of T2DM patients have an increase in intramyocellular fat, and in this group there is also a correlation with insulin resistance.[237]

The mechanism for accumulation of triglyceride in the skeletal muscle of obese and insulin-resistant persons is probably related to mismatching of fatty acid uptake and oxidation. During resting postabsorptive conditions, about 30% of fatty acid flux in the plasma pool is accounted for by oxidation, and the remaining 70% of flux is recycled into triglyceride, indicating a physiologic reserve that exceeds immediate tissue needs for oxidative substrates. The uptake, transport, and metabolism of fatty acids are highly regulated processes, and alteration of the balance between uptake and oxidation in skeletal muscle leads to increased intramyocellular triglycerides. The increased lipolysis associated with obesity provides an increased amount of fatty acids presented to muscle, which again appears to activate PKC isoforms to feedback on insulin signaling intermediates.[238,239]

However, increased muscle triglyceride content is not invariably linked to insulin resistance. Indeed, exercise training is associated with increased muscle triglyceride content,[240] and chronic exercise increases both insulin sensitivity and the capacity for fatty acid oxidation.[241-243] The reason for this dissociation is not completely understood, but recent studies show that perilipin proteins PLIN2, PLIN3, and PLIN5 rapidly associate with lipid droplets in muscle from trained individuals but not sedentary individuals.[244] These data suggest that exercise training allows for improved lipid storage in muscle, thereby sequestering lipid intermediates and preventing fatty acid–induced insulin resistance, which is also seen with acute exercise.[245]

Lipid accumulation in the liver is a common feature of insulin resistance and T2DM often referred to as nonalcoholic fatty liver disease (NAFLD).[246] Enhanced substrate delivery to the liver in insulin-resistant states likely plays a key role in this lipid accumulation.[117,228] Enhanced fatty acid delivery from adipose tissue lipolysis and increased postprandial glucose concentrations due to muscle insulin resistance provide excess substrates for the liver that result in accumulation of acetyl-CoA and toxic lipid intermediates.[247] Humans with lipodystrophy and mouse models of lipodystrophy develop NAFLD and severe insulin resistance indicating that without the capacity to store lipids in adipose tissue, the liver becomes metabolically dysfunctional.[107,248,249] However, excess lipid intake is not the only way to develop NAFLD. Indeed, feeding mice excess glucose or fructose induces metabolic pathways in the liver that lead to NAFLD.[250,251] These data indicate that a combination of excess macronutrients and decreased adipose tissue storage promote lipid accumulation in the liver that is concurrent with insulin resistance.

Endoplasmic Reticulum Stress/Unfolded Protein Response

The ER functions in the post-translational processing of protein, including protein folding, maturation, quality control, and trafficking to other cellular compartments. As part of its quality control machinery, when the ER accumulates excess levels of unfolded or misfolded proteins, a distinct series of reactions occurs that slows overall protein synthesis while increasing the production of chaperones and other proteins that increase the fidelity of protein processing. Three pathways are commonly activated to mediate the unfolded protein response (UPR) and alleviate ER workload: RNA-dependent protein kinase–like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6). PERK phosphorylates eukaryotic translation initiation factor 2 (eIF2α), resulting in inhibition of most protein synthesis. IRE1 induces cleavage of X-box–binding protein 1 (XBP1) mRNA, which results in production of the active XBP1 transcription factor. XBP1, in combination with ATF6α, activates transcription to produce chaperones and proteins involved in ER biogenesis, phospholipid synthesis, and secretion.[252]

In states of overfeeding and obesity, there can be activation of the UPR in the liver, adipose tissue, pancreatic beta cells, muscle, and other tissues. The activation of UPR in response to overnutrition is thought to have several effects, including activation of the JNK and nuclear factor–κB (NF-κB)/inhibitor of κB kinase (IKK) pathways leading to decreased IRS1 activity; increased levels of endogenous inflammatory mediators; alteration in SREBP1-mediated transcription; reduction in hepatic gluconeogenesis; and, after prolonged activation, cellular dysfunction and apoptosis.[253]

Innate Immunity

The innate immune system was originally thought to be a cellular system that allowed discrimination of self and nonself to adapt cellular metabolism to fight microbial pathogens. However, this system is now recognized as a general response to cellular stress that activates

the inflammation and cellular repair systems. The innate immune system contains a series of pattern-recognition receptors to detect microbial motifs. These proteins, including TLRs and C-type lectins, are expressed on a variety of cell types, including macrophages, monocytes, dendritic cells, neutrophils, epithelial cells, and cells of the adaptive immune system. The pattern-recognition receptors detect extracellular and intracellular pathogen-related molecules, including lipids and nucleic acids, and initiate a stereotypical response, including NF-κB activation and activator protein 1 (AP1) transcription, which increases expression of cytokines and chemokines.

Activation of the innate pathway also increases the production of so-called inflammasomes—large, multisubunit protein complexes that are important in the control of caspase 1–mediated, post-translational maturation and secretion of interleukins, primarily IL1β, that have potent pro-inflammatory responses and are a risk factor for development of T2DM, perhaps through disruption of beta-cell function.[254]

Activation of the innate immune system in acute infection and obesity is associated with significant insulin resistance. In obesity, this is mediated in part by elevated levels of fatty acids. TLRs, especially TLR2 and TLR4, which normally respond to bacterial cell wall lipids to induce the innate inflammatory response, are also activated by circulating saturated fatty acids. Conversely, polyunsaturated fatty acids inhibit TLR signaling. Activation of TLRs in cells results in insulin resistance, whereas genetic disruption of the TLR4 receptor in mice is protective against fatty acid–induced insulin resistance.[227]

Mitochondrial Abnormalities

A decrease in oxidative capacity is seen in both humans and animals with insulin resistance, obesity, and T2DM.[255,256] Studies have suggested that increases in intramyocellular fat content in skeletal muscle associated with insulin resistance may be caused by alterations in mitochondrial mass. In one study, young insulin-resistant offspring of parents with T2DM demonstrated a 60% reduction in insulin-stimulated skeletal muscle glucose uptake compared with control subjects, and this reduction correlated with an increase of approximately 80% in intramyocellular lipid content.[257] The elevated intramyocellular lipid content was attributable to the 30% reduction in mitochondrial oxidative capacity, which may be due to the lower ratio of the mitochondria-rich oxidative type I fibers to glycolytic type II muscle fibers.

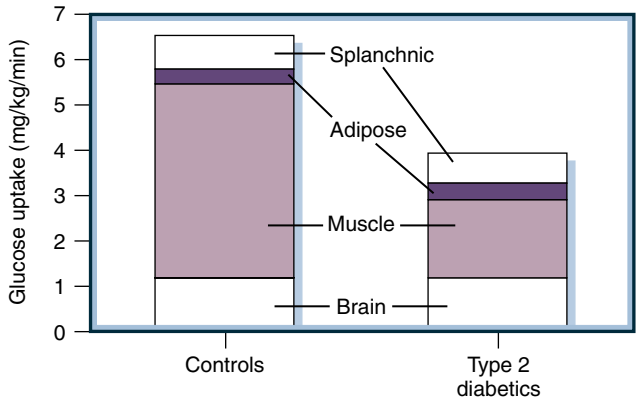
Decreased expression of nuclear-encoded genes that regulate mitochondrial biogenesis, such as PPARγ coactivators 1α and 1β (PGC1α and PGC1β, respectively), have been shown to be important for mitochondrial biogenesis and for fiber type selection during development.[258] PGC1α and PGC1β, and their target genes, were found to be downregulated in obese patients with IGT and T2DM.[259-261] The activity of the electron transport chain is reduced and intramyofibrillar mitochondria are smaller in patients with T2DM, and both of these measures correlate with severity of insulin resistance.[262] A study in which rats were bred for differences in oxidative capacity (determined by their intrinsic ability to run) suggests that the defects found in humans could have a genetic basis.[263] The group with poor aerobic capacity had several significant abnormalities, including obesity, insulin resistance, hypertension, and dyslipidemia. The metabolic decline also correlated with a reduction in mitochondrial gene expression and PGC1α in skeletal muscle, similar to that seen in humans. These data lead to the hypothesis that reversing the observed mitochondrial mass decline in muscle of T2DM patients may be a way to improve their metabolism. However, the potential beneficial effect of increasing mitochondrial mass by activation of PGC1α activity has been dampened by the finding that overexpression of PGC1α in mouse skeletal muscle does not improve metabolic status following a high-fat diet.[264]

Some studies have questioned the cause-effect relationship between alterations in mitochondrial mass/function and skeletal muscle insulin resistance. Rather than mitochondrial insufficiency being an inherited trait, these observed changes could be acquired. First, insulin itself can upregulate mitochondrial biogenesis, and withdrawal of insulin in patients with T1DM leads to reductions in mitochondrial gene transcription and adenosine triphosphate (ATP) synthesis.[265] Second, the observed reduction in ATP synthesis and tricarboxylic acid (TCA) cycle activity could be explained by reduced turnover of ATP in relatively sedentary individuals. In the absence of energy demand (e.g., exercise), the reduction in the adenosine diphosphate/ATP ratio would impair electron transport in the mitochondria, increasing levels of the reduced form of nicotinamide adenine dinucleotide (NADH), which would impair TCA cycle activity. This would result in the generation of reactive oxygen species due to elevated reducing pressure in the mitochondria.[266] Indicative of impaired oxidation in the mitochondria, mice fed a high-fat diet show a reduction in TCA cycle intermediates and an impairment of adequate β-oxidation of fatty acids, marked by increases in even-chained acyl-carnitine levels. Increases in plasma acyl-carnitines are found in obese, insulin-resistant individuals, and it appears that they are markers of resistance.[267,268]

Post-translational modification of mitochondrial proteins by acetylation (as well as succinylation and malonylation) of lysine residues on each protein has a marked effect on their activity and provides a potentially important mechanism for control of mitochondrial flux and insulin resistance.[269] In general, the acetylation events reduce mitochondrial enzyme activity. No enzymes promoting acetylation have been found. Rather, these appear to occur through pH-dependent, nonenzymatic chemical reactions wherein acetyl-CoA or other acyl-CoAs act as donors for exposed lysine residues. An increase in flux of nutrients in the mitochondria is expected to increase acetylation proportional to the steady-state levels of acetylation. An imbalance of formation to utilization, which occurs in overnutrition, would therefore be expected to inhibit further fuel utilization. The mitochondrial sirtuin, Sirt3, is the primary deacetylase of mitochondria[270] that is activated by nicotinamide adenine dinucleotide (NAD^+) and can deacetylate critical metabolic enzymes, including long-chain acyl-CoA dehydrogenase (LCAD) in the liver[271] and pyruvate dehydrogenase complex in muscle.[272] Similarly, the NAD+-dependent Sirt5 leads to desuccinylation and demalonylation of mitochondrial enzymes.[273,274] As ATP use increases, such as with exercise,[275] increased electron flow from NADH to support oxidative phosphorylation will result in increased intramitochondrial NAD^+, leading to Sirt3- and Sirt5-mediated protein deacylation and increased mitochondrial capacity for substrate utilization.

Skeletal Muscle Insulin Resistance

The primary site of glucose disposal after a meal is skeletal muscle, and the primary mechanism of glucose storage is through its conversion to glycogen.[276] In obesity, insulin resistance in skeletal muscle is manifested before abnormalities in insulin signaling in adipose tissue and the liver, possibly reflecting the relatively limited nutrient storage capacity of skeletal muscle. Studies using the hyperinsulinemic-euglycemic clamp technique have demonstrated that in insulin-resistant people (with and without T2DM), there is a deficiency in the nonoxidative disposal of



• **Fig. 34.10** Tissue uptake of glucose in nondiabetic and insulin-resistant diabetic subjects during a hyperinsulinemic-euglycemic clamp. (From DeFronzo RA. The triumvirate: beta-cell, muscle, liver—a collusion responsible for NIDDM. Lilly Lecture 1987. *Diabetes.* 1988;37:667–687.)

glucose related primarily to a defect in glycogen synthesis, which itself is secondary to a decrease in insulin-stimulated glucose uptake[277,278] (Fig. 34.10).

Elevated fatty acids predict the progression from IGT to diabetes,[279,280] although peripheral fatty acids might not be markedly elevated because of efficient extraction by the liver and skeletal muscle. Increased fatty acid flux to skeletal muscle related to increased visceral lipolysis has been implicated in the inhibition of muscle glucose uptake. The glucose–fatty acid cycle was originally proposed to account for the ability of fatty acids to inhibit muscle glucose utilization. Randle's group[281] hypothesized that fatty acids compete with glucose for substrate oxidation in isolated muscle. They found that increased fatty acid metabolism leads to an increase in the ratio of intramitochondrial acetyl-CoA to CoA and reduced nicotinamide adenine dinucleotide (NADH/NAD^+) ratios, which leads to inhibition of pyruvate dehydrogenase. The resulting increased intracellular mitochondrial (and cytosolic) citrate concentrations result in allosteric inhibition of phosphofructokinase, the key rate-controlling enzyme in glycolysis. Subsequent accumulation of glucose 6-phosphate inhibits hexokinase II activity, resulting in an increase in intracellular glucose concentrations and decreased glucose uptake.

More recent in vivo studies in humans suggest that the primary effect of fatty acids, at least in the presence of elevated insulin levels, is a decrease in glucose transport, as measured by a reduction in the rate of accumulation of intracellular glucose and glycogen using ^{13}C and ^{31}P nuclear magnetic resonance spectroscopy. In normal subjects, elevated fatty acids, achieved by infusion of triglyceride emulsions and heparin (which activates lipoprotein lipase resulting to release fatty acids into the circulation), resulted in a fall in intracellular glucose and glucose 6-phosphate concentrations that precede the fall in glycogen accumulation.[282,283] These results challenge the Randle hypothesis (which predicts a rise in intracellular glucose 6-phosphate concentrations) as the basis of the reduction in insulin sensitivity seen with elevated fatty acids. Similar decreases in glucose transport have been seen in patients with T2DM and in lean, normoglycemic, insulin-resistant offspring of T2DM parents.[284] These studies also find decreased activity of PI3K and increased activity of novel forms of protein kinase C, such as PKCθ, PKCδ, and others, that might, in part, mediate the effect of elevated fatty acids.[238,239]

Studies also suggest that PKC-mediated serine phosphorylation of the IKKβ subunit, leading to its degradation, and the unregulated translocation of NF-κB into the nucleus, might also be important to fatty acid–induced insulin resistance.[285] This is the mechanism by which high-dose aspirin and salsalate improve glucose metabolism in T2DM.[285,286] Disruption of the IKKβ inflammatory pathway by high-dose salicylate therapy in a small human trial resulted in improvements in insulin sensitivity.

Fatty Acid Metabolism in Skeletal Muscle

The uptake of fatty acid from the serum, where it is mostly bound to albumin, is mediated by at least three families of proteins: fatty acid translocase, plasma membrane fatty acid–binding proteins (FABPs), and fatty acid transport proteins.[287-289] The levels of the putative transport proteins are regulated by exercise,[290] correlated with body weight (at least in women), and can be modulated by insulin infusion.[291]

FABPs are capable of binding multiple hydrophobic ligands, including fatty acids, eicosanoids, and retinoids, with high affinity.[292] FABPs are thought to facilitate uptake of fatty acids and to promote subsequent intracellular transport to subcellular organelles.[293] There is a direct correlation between heart-type FABP content and oxidative capacity observed during development and among different muscle types.[294,295] In mice that have a disruption of the heart isoform[296] or the adipocyte isoform[297] of FABP, plasma fatty acid concentrations are significantly elevated and plasma glucose is decreased, suggesting a key role in normal regulation of fatty acid oxidation. Some,[297] but not all,[298] studies have shown a decrease in heart-type FABP in insulin-resistant humans.

Carnitine palmitoyltransferase 1 (CPT1) has been the subject of intense scrutiny for many years because of its central role in the balance between mitochondrial glucose and fatty acid metabolism, primarily because malonyl-CoA inhibits mitochondrial fatty acid uptake by inhibiting CPT1 in the liver.[299,300] A specific isoform contributes 97% of the CPT1 in muscle and has 100-fold lower sensitivity to inhibition by malonyl-CoA.[301] This lower sensitivity to malonyl-CoA inhibition suggests that the levels of CPT1 itself, as well as malonyl-CoA, may be important in the balance of uptake and oxidation of fatty acids. Evidence for this in skeletal muscle stems from the finding that overexpression of CPT1 in rodent muscle increased fatty acid oxidation and reduced intramyocellular triglyceride.[302] Furthermore, muscle CPT1 mRNA, as with other fatty acid–oxidizing enzymes, is regulated by PPARα activators, fat feeding, and exercise in rodents and is inversely correlated with obesity in humans.[303-306]

Long-chain fatty acids, after passing through the inner mitochondrial membrane as acylcarnitines, are metabolized at the surface of the inner mitochondrial membrane by CPT2 and the long chain–specific oxidation system consisting of very long chain acyl-CoA dehydrogenase (VLCAD) and the trifunctional protein oxidation complex (Fig. 34.11). Transfer of the acyl chain from carnitine to CoA, catalyzed by CPT2, is followed by one cycle of oxidation. This is catalyzed by VLCAD and trifunctional protein to yield a chain-shortened acyl-CoA that can recycle through the same oxidation system.[307] In vivo, four different acyl-CoA dehydrogenase enzymes catalyze the initial dehydrogenation of straight-chain fatty acids in mitochondria. Three of them—short-chain acyl-CoA dehydrogenase (SCAD), medium-chain acyl-CoA dehydrogenase (MCAD), and LCAD—are soluble enzymes located in the mitochondrial matrix as homotetramers. A fourth, VLCAD, is attached to the inner membrane as a homodimer. Their names derive from the length of the fatty acids that they process. The enzymes can act in tandem. VLCAD and LCAD shorten the long-chain fatty acids into medium-chain fatty acids that can then be processed by MCAD and SCAD.[308] The SCAD, MCAD, and LCAD monomers share a high degree of homology

but do not share homology with VLCAD. At least some of these enzymes can be regulated in humans by exercise training.[309]

UCP1 mediates the uncoupling of oxidative phosphorylation in brown adipose tissue for thermogenesis.[310] UCP2 and UCP3 have structural similarities to UCP1, but it is not clear that they are actually uncouplers of oxidative phosphorylation under physiologic conditions.[311,312] UCP3 mRNA is found primarily in skeletal muscle and in brown adipose tissue. UCP2 has a ubiquitous tissue distribution and may be involved in reactive oxygen species detoxification and CNS regulation of metabolism.[313] UCP2 and UCP3 mRNA levels have been correlated with different physiologic states, and numerous studies indicate that expression of UCP2 and UCP3 are stimulated by thyroid hormones and in the presence of high levels of fatty acids.[314] In humans, the levels of UCP2 and UCP3 mRNAs were upregulated by a high-fat diet, and the upregulation was more pronounced in humans with high percentages of type IIA muscle fibers.[315] In a small study, exercise training in humans increased mitochondrial oxidative capacity but did not change UCP2 or UCP3 levels.[316] Obesity itself was shown to be positively correlated with a splice isoform of UCP3.[317] A unique polymorphism in the promoter region of UCP3 correlated with expression of UCP3 in skeletal muscle.[318]

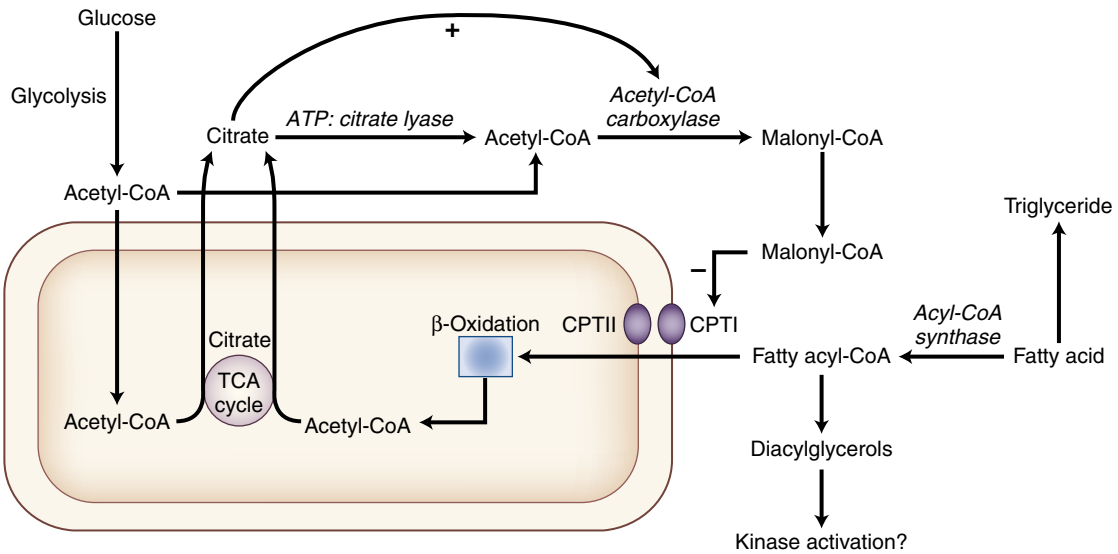
Glucose Influence on Skeletal Muscle Fatty Acid Metabolism

A concept that can help explain glucose elevations leading to the intramyocellular triglyceride accumulation associated with impaired insulin action is the central role of malonyl-CoA in regulating fatty acid and glucose oxidation[319] (see Fig. 34.11). Malonyl-CoA is an allosteric inhibitor of CPT1, the enzyme that controls the transfer of long-chain fatty acyl-CoAs into the mitochondria.[299,300,320] In the presence of elevated glucose and insulin levels, the TCA cycle is activated, resulting in an increase in citrate in the cytoplasm through increased malate cycling in the mitochondria. The increased citrate is converted to acetyl-CoA through citrate lyase and thus provides an indirect substrate for ACC. Citrate also allosterically activates ACC and makes ACC a better substrate for phosphatases that activate the enzyme.[321,322] Even in insulin-resistant skeletal muscle, glucose uptake into the skeletal muscle is higher than normal, especially at the elevated levels of glucose found in T2DM.[323,324] The glucose is shunted toward the glycolytic pathway, generating acetyl-CoA that can be converted to malonyl-CoA in the cytoplasm by the action of the highly regulated ACC. This has been demonstrated in humans where an infusion of insulin and glucose at a high rate leads to increases in the concentration of malonyl-CoA in skeletal muscle and to decreases in whole-body and, presumably, muscle fatty acid oxidation.[325]

ACC is also regulated by AMPK-mediated phosphorylation, which inhibits ACC basal activity and reduces ACC activation by citrate.[326] ACC then generates malonyl-CoA, which in turn allosterically inhibits CPT1 residing on the outer mitochondrial membrane, inhibiting uptake of acyl-CoA. The resulting buildup of long-chain acyl-CoAs and diacylglycerols is proposed to activate one or more PKC isoforms or other lipid-activated proteins, resulting in insulin resistance.[319] In support of this hypothesis, humans with obesity or T2DM show elevated intracellular malonyl-CoA levels associated with reduced ACC phosphorylation, impaired fatty acid oxidation, and accumulation of intramyocellular acyl-CoA and triglyceride, all of which can be reversed with a 3-month treatment with the insulin-sensitizer rosiglitazone.[327]

Circadian Rhythms, Obesity, and Insulin Resistance

Almost all mammals have a well-developed circadian cycle that is controlled by a complex, integrated network of transcription-translation feedback loops that work in a 24-hour cycle. A defined set



• **Fig. 34.11** Glucose effect on triglyceride metabolism. Increased uptake of glucose results in increased production of acetyl coenzyme A (acetyl-CoA) as a product of glycolysis. The increased tricarboxylic acid (TCA) cycle activity associated with oxidation of triglycerides and glucose increases the production of citrate, which is shuttled to the cytoplasm, activates the enzyme acetyl-CoA carboxylase (ACC) by allosteric mechanisms, and increases the susceptibility of ACC to phosphatases. This leads to increased ACC activity, converting acetyl-CoA to malonyl-CoA. Malonyl-CoA is a potent inhibitor of carnitine palmitoyltransferase (CPT) I on the outer mitochondrial membrane, which leads to accumulation of fatty acyl-CoAs in the cytoplasm. This may result in the production of signaling molecules that can increase the activity of kinases and other enzymes and lead to insulin resistance. *ATP,* adenosine triphosphate.

of genes establishes the circadian cycle, which sets behavioral and physiologic functions, including sleep-wake cycles, feeding behaviors, hormone secretion, and metabolism. The core circadian clock genes are present not only in the suprachiasmatic nucleus of the hypothalamus but also in nearly every cell of the body, where they activate specific sets of genes.[328] In mammals, the clock is driven by two transcription factors, CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like 1).[329] CLOCK and BMAL1 dimerize and bind the promoter region of target genes, including period (PER) and cryptochrome (CRY). The increased levels of these proteins promote heterodimerization and translocation to the nucleus, creating a negative feedback loop by suppressing transcriptional activity of CLOCK-BMAL1. In a second negative feedback loop, CLOCK-BMAL1 induce REV-ERBα (encoded by the nuclear receptor subfamily 1, group D, member 1 gene, *NR1D1*), which in turn suppresses BMAL1 transcription. Additional modulation of the activity and stability of these proteins is provided by post-translational modifications such as phosphorylation and ubiquitination.

There are significant epidemiologic associations in humans between reduction of sleep and increased rates of obesity and other metabolic disturbances, including T2DM.[330] Experimental sleep disruption can directly impair insulin action, alter secretion of leptin and ghrelin resulting in stimulation of appetite, increase inflammatory cytokine production, and create alterations in other cardiovascular risk factors. Alterations in normal feeding patterns that are attuned to circadian metabolism can change the relationship between nutrient appearance and nutrient-metabolizing enzymes. For instance, alterations in fatty acid appearance and lipoprotein lipase activity can lead to altered partitioning of lipids to vulnerable tissues, leading to lipotoxicity and decreased secretion of leptin, increasing appetite.[331] Obstructive sleep apnea, which combines sleep fragmentation and hypoxemia, is also a risk factor for insulin resistance and diabetes. There is growing evidence that glucose control in patients with T2DM patients can be improved by treating sleep apnea, although poor compliance to therapy is a major barrier to success.[332]

Role of the Gut Microbiome and Metabolome in Diabetes and Insulin Resistance

There is growing evidence that a major mediator of the effect of diet and other environmental factors in diabetes is the gut microbiome—or, more specifically, the bacteria that reside in the gastrointestinal tract.[333] In mammals, the gut microbiome is seeded at birth; it is reformed during infancy and then becomes relatively stable in adulthood, although it can be remodeled by diet, antibiotics, and a variety of disease states. Most gut microbiota are viewed as commensal (nonharmful) or mutualistic (providing benefit) to the host. These microbes play important roles in nutrient, xenobiotic, and drug metabolism, maintenance of gut mucosal integrity, protection against pathogens, and immunomodulation, and, through these basic functions, affect many aspects of normal physiologic function. Over the past decade, it has become clear that the gut microbiome can also contribute to obesity, diabetes, metabolic syndrome, and insulin resistance in both rodents[334-336] and humans,[337-340] and act as an integrator and mediator of some of the effects of genetics, diet, and bariatric surgery.[341-344] In mice, it has been shown that administration of low-dose antibiotics early in life may predispose to obesity and glucose intolerance by perturbing the development of a normal microbiome.[345]

The mechanisms by which gut microbiota may affect pathogenesis of diabetes, obesity, and insulin resistance are multiple (Fig. 34.12). Gut microbiota have major effects on intestinal barrier function, breakdown of otherwise indigestible dietary components, modification of bile acids and other substances, development of the gut, and education of the immune system.[333,346] These effects can lead to a release of bacterial proteins, endotoxins, and cytokines into the bloodstream,[347,348] as well as produce changes in hundreds of metabolic products, including bile acids, short-chained fatty acids, amino acids, and many other classes of molecules.[343,349-354] Together, these lead to tissue-specific metabolic dysregulation and immune activation, leading to insulin resistance and progression of diabetes pathogenesis. Several metabolites have been shown to correlate with insulin resistance in both mice[355] and humans,[356] and some, such as 2-aminoadipate, α-hydroxybutyrate, and *N*-acetylglycine, are suggested to be biomarkers of T2DM.[357-359] Further studies will be needed to determine exactly how gut microbiota affect insulin sensitivity and diabetes progression, and whether therapies that change gut microbiota can be used to treat or prevent T2DM. In this regard, several options are possible, ranging from administration of prebiotics (nutrients that change the composition of gut microbiota), probiotics (mixtures of bacteria themselves), and even transplant of a healthy microbiome by fecal transfer.

Special Conditions That Induce Insulin Resistance

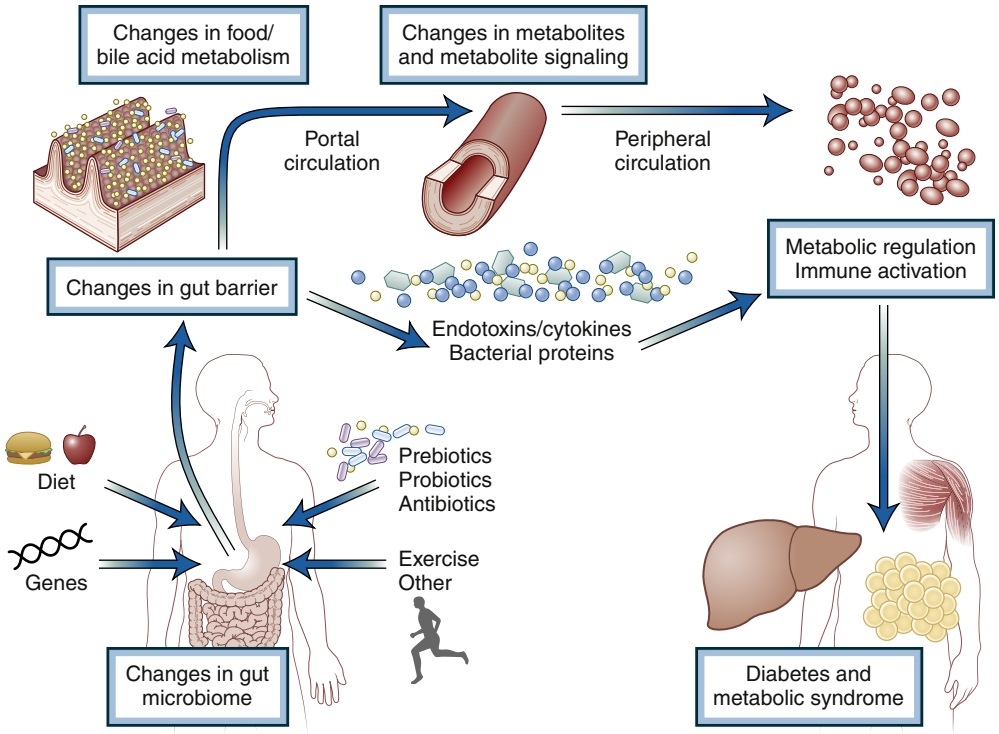
Gestational Diabetes

Diabetes diagnosed during pregnancy is categorized as either preexisting T2DM if diagnosed during the first trimester of pregnancy or gestational diabetes if diagnosed during the second or third trimester.[5] Although gestational diabetes generally resolves with the end of the pregnancy, it is considered a significant risk factor for the future development of T2DM.[360,361] Insulin resistance is a normal consequence of pregnancy. As the pregnancy progresses and insulin resistance builds, maternal insulin secretion increases by as much as 250% to compensate. When this compensation is inadequate, gestational diabetes develops.[362] Pre-pregnancy obesity is a major risk factor for the development of gestational diabetes.

The driver of insulin resistance in pregnancy is not simply the weight gain of pregnancy, as euglycemia generally returns within days of delivery. Rather, placenta-derived factors are believed to drive both the insulin resistance of pregnancy and the beta-cell expansion of pregnancy. Exactly what placental factors contribute remains debated because there are significant differences in beta-cell physiology between rodents and humans, and intervention studies of pregnant patients are challenging.[363] Placenta-derived growth hormone is one hormone that contributes to maternal insulin resistance but can account for only a portion of the change.[364] Other candidates that may contribute include placental lactogen[365,366] and placenta-derived pro-inflammatory exosomes.[367]

Drugs and Stress-Induced Insulin Resistance

In addition to the genetic and epigenetic risk factors discussed previously, there are many disease states and medications that can induce insulin resistance or a combination of insulin resistance and decreased insulin secretion leading to glucose intolerance (Table 34.2).



• **Fig. 34.12** The gut microbiome is impacted by our genes and environment. Changes to the gut microbiome may result in altered gut barrier function and food and bile acid metabolism, leading to immune activation and insulin resistance.

TABLE 34.2 Drugs and Stressors Associated With Insulin Resistance

Drugs

Glucocorticoids
Human immunodeficiency virus medications (all cause varying degrees of metabolic abnormalities)
Calcineurin inhibitors
Mammalian target of rapamycin (mTOR) inhibitors
Phosphoinositide 3-kinase inhibitors
Statins

Stressors

Pregnancy
Glucotoxicity
Surgery
Inflammation (from obesity or infection)
Overnutrition

Glucocorticoid-Induced Insulin Resistance

Cushing syndrome and exogenous glucocorticoid treatment have long been known to induce significant insulin resistance in humans. More than 80% of nondiabetic individuals with rheumatoid arthritis treated with greater than 30 mg/day of prednisone have an increase in their hemoglobin A_{1c}. The mechanism is multifactorial. There are rapid effects related to increasing hepatic glucose production, which occurs via increased gluconeogenesis, as well as acute insulin resistance in skeletal muscle, in part due to downregulation of IRS1.[368] Glucocorticoids also have tissue-specific effects in vivo, because 24-hour treatment of humans with hydrocortisone enhances adipose tissue insulin sensitivity while whole-body insulin sensitivity declines.[369] Tissue crosstalk is important in glucocorticoid-induced insulin resistance: a mouse model lacking glucocorticoid receptors specifically in adipose tissue retains some muscle insulin sensitivity in response to chronic dexamethasone.[370] Long-term glucocorticoid treatment also results in redistribution of fat from the periphery to the central compartment, increased lipolysis and elevations in triglyceride and fatty acid, and a reduction in insulin secretion. There is also increased muscle protein breakdown leading to muscle atrophy and alterations in skin integrity. These effects are due to a combination of modulation of transcriptional events by glucocorticoids and through inhibition of insulin and growth factor signaling.

Post-Transplant Diabetes Mellitus

Patients receiving organ transplants frequently develop IGT or post-transplant diabetes mellitus (PTDM) following the transplant, related to the immunosuppressive regimens. Most patients become transiently hyperglycemic immediately post-transplant due to the stress of surgery and high doses of corticosteroids; thus, diagnosis of PTDM should not be made until the patient has been discharged from the hospital and is on a stable immunosuppressant dose.[371] Consensus recommendations discourage the use of hemoglobin A_{1c} for PTDM screening during the first year following transplant due to significant changes in red blood cell turnover, which can result in missed cases. The doses and drug combinations used in transplant vary by organ but frequently include chronic use of medications that impair glucose metabolism, such as glucocorticoids, mTOR inhibitors (sirolimus and everolimus), and calcineurin inhibitors (tacrolimus and cyclosporine).

The primary mechanism for glucocorticoids and mTOR inhibitors is via induction of insulin resistance. Glucocorticoids

are discussed earlier. mTOR inhibitors induce insulin resistance via inhibition of the mTOR complex downstream of the insulin receptor (see Fig. 34.8). It seems that this inhibition is not enough to cause diabetes in low-risk patients but can induce diabetes in those with risk factors, particularly if used in combination with calcineurin inhibitors.[372] Calcineurin inhibitors appear to contribute to diabetes predominantly by decreasing insulin secretion.[373] This is a result of decreased insulin synthesis and reduced glucokinase activity.[374,375] There is also some evidence for increased insulin resistance in patients treated with calcineurin inhibitors; however, these data are mixed, possibly reflecting increased insulin resistance only in those with underlying risk factors.[372]

Inflammation and Inflammatory Cytokines

Obesity is associated with increased inflammation in adipose tissue, including macrophage infiltration and increased cytokine expression. Inflammation and cytokines drive insulin resistance.[376] This occurs through multiple mechanisms. Studies in humans and in animal models of obesity have identified decreases in the number and the kinase activity of insulin receptors and impairment in the activation of IRS1, PI3K, and Akt. These changes are driven, in part, by increased levels of cytokines, such as TNFα, which is made and secreted by adipocytes, as well as the infiltrating macrophages. TNFα administration reduces the kinase activity of the insulin receptor in rats[377] or in 3T3L1 adipocytes in vitro,[378] possibly by increased activity of JNKs and IKKs, and increased serine phosphorylation of the insulin receptor and IRS1.[379] High fat–fed mice with genetic ablation of TNFα production and rats treated with neutralizing antisera or soluble TNF receptors have increased kinase activity of the insulin receptor,[380,381] indicating increased insulin sensitivity. However, thus far, interventions to decrease TNFα action in humans have had little or no effect in reducing the insulin resistance of obesity or T2DM.[382,383]

Human Immunodeficiency Virus Infection

With the advent of effective medications to manage the infection, human immunodeficiency virus (HIV) infection has become a chronic disease. However, these medications are associated with significant metabolic risk. In a cohort of more than 3800 patients, there was nearly a doubling in the prevalence of T2DM in HIV versus non-HIV patients. A significant portion of this increase may be related to protease inhibitors used to treat chronic HIV. HIV-infected patients also have a 75% increase in the risk for acute myocardial infarction compared with uninfected patients, again related to protease inhibitor use. Other contributing factors to cardiometabolic risk were male sex, diagnosis of acquired immunodeficiency syndrome, responsiveness to antiretroviral treatment, and increases in CD4 T-cell counts.[384]

Both lipoatrophy and lipohypertrophy are seen in HIV-infected patients on antiretroviral therapy. Lipoatrophy is primarily associated with the use of the older thymidine analogue nucleoside reverse transcriptase inhibitors, whereas the contributors to lipohypertrophy are not as well understood.[385] In the setting of multidrug HIV treatment regimens and frequent changes to therapy, it is difficult to determine the relative contributions of individual drugs to metabolic syndrome or distinguish the drug effect from the metabolic consequences of adipose tissue redistribution.

There appear to be multiple mechanisms contributing to metabolic syndrome in HIV. The effects of the major HIV treatments are reviewed in detail by Srinivasa and Grinspoon[384] In brief, several protease inhibitors can inhibit glucose transport in vitro and in vivo, possibly through interaction with the insulin-responsive glucose transporter GLUT4 that could inhibit glucose uptake specifically in insulin-responsive tissue.[386] Mitochondrial abnormalities have been described in adipose tissue biopsy specimens obtained from HIV-infected patients with lipodystrophy, including reductions in mitochondrial number and oxidative function in adipocytes from patients treated with highly active antiretroviral therapy.[387,388] A direct effect of protease inhibitors on differentiation of adipocytes has also been described.[389,390] In adipose tissue of treated HIV patients, especially those with lipodystrophy, there is downregulation of the microRNA-processing enzyme Dicer. This is associated with decreased levels of circulating exosomal microRNAs, which may also contribute to insulin resistance.[391,392]

Treatment of HIV-associated lipodystrophy continues to be inadequate, although the incidence may be less with use of newer protease inhibitors. Switching from thymidine analogues has been shown to improve lipoatrophy in some patients.[393] Treatment with insulin-sensitizing thiazolidinediones has shown mixed results, with improvement in insulin sensitivity but little alteration in fat distribution.[394] Because of the increased risk of cardiovascular disease, treatment of hyperlipidemia is essential in these patients. Treatment guidelines do not differ from the general population; however, current cardiac risk predictors underestimate risk in HIV-infected patients.[395] Tesamorelin, a growth hormone–releasing hormone analogue, is approved to treat abnormal lipids associated with HIV lipodystrophy. The medication causes a modest redistribution of visceral fat with subsequent improvement in the lipid profile.[396]

Statins

The medications and disease states discussed earlier may have profound effects on insulin resistance and glycemia; however, there are other factors that will have much smaller, but potentially significant, impacts on a population basis. One example is the statin class of cholesterol-lowering drugs. These medications do not cause an appreciable change in blood glucose in individual patients, but large clinical trials have demonstrated an increased risk for developing T2DM in patients treated with a statin.[406] Given the multifactorial nature of insulin resistance and T2DM, it is likely that other medications will also increase the risk of T2DM in the right setting of genetic predisposition and environmental factors.

Glucotoxicity, Glucosamine

Hyperglycemia itself can cause insulin resistance, as well as decreased beta-cell function. In Pima Indians, the level of fasting glycemia is strongly correlated with insulin sensitivity.[397] This defect is primarily in skeletal muscle,[398] but its exact mechanism remains controversial.

On entry into cells, glucose is converted to glucose 6-phosphate, which has multiple metabolic fates. The hexosamine pathway is a relatively minor branch of the glycolytic pathway, encompassing less than 3% of total glucose used. Hexosamines, such as glucosamine, when incubated with adipose tissue, induce insulin resistance in fat cells[399] and in skeletal muscle.[400] Infusion of glucosamine into rats resulted in a dose-dependent increase in insulin resistance of skeletal muscle,[400] and transgenic mice that overexpress the rate-limiting enzyme glutamine–fructose-6-phosphate aminotransferase specifically in skeletal muscle acquired severe insulin resistance.[401] By a pathway that is unclear, glucosamine overproduction resulted in a disruption of the ability of insulin to cause translocation of GLUT4 to the cell surface.[402] Through its anti-insulin action, the hexosamine

pathway has been hypothesized to be a glucose sensor that allows the cell to sense and adapt to the prevailing level of glucose.[398]

Postoperative Hyperglycemia

Surgery is a significant stress to the body. This is driven by the surgical wound, anesthesia, and pain, all of which can stimulate the release of stress hormones, including cortisol, epinephrine, and norepinephrine. In addition, particularly following coronary bypass surgery, the use of pressors is common and can cause severe hyperglycemia. Postoperative hyperglycemia predicts mortality in nondiabetic patients following coronary bypass or general surgery.[403,404] Aggressive treatment of postoperative hyperglycemia, with a target blood glucose of between 140 and 180 mg/dL for both diabetic and nondiabetic patients, has been shown to reduce both wound infections and mortality following surgery.[5]

Epinephrine is one of the key mediators of postoperative hyperglycemia. This can be both endogenously derived and delivered through infusions. Epinephrine acts to increase blood glucose through several mechanisms. These include decreased insulin secretion and increased insulin resistance both in muscle and in the liver, where it leads to a failure to suppress hepatic glucose production.[405] When epinephrine infusions are used in an acute care setting, adjustments in the insulin infusion rate are generally necessary due to the rapid and substantial impact of this medication.

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35

Therapeutics of Type 2 Diabetes Mellitus

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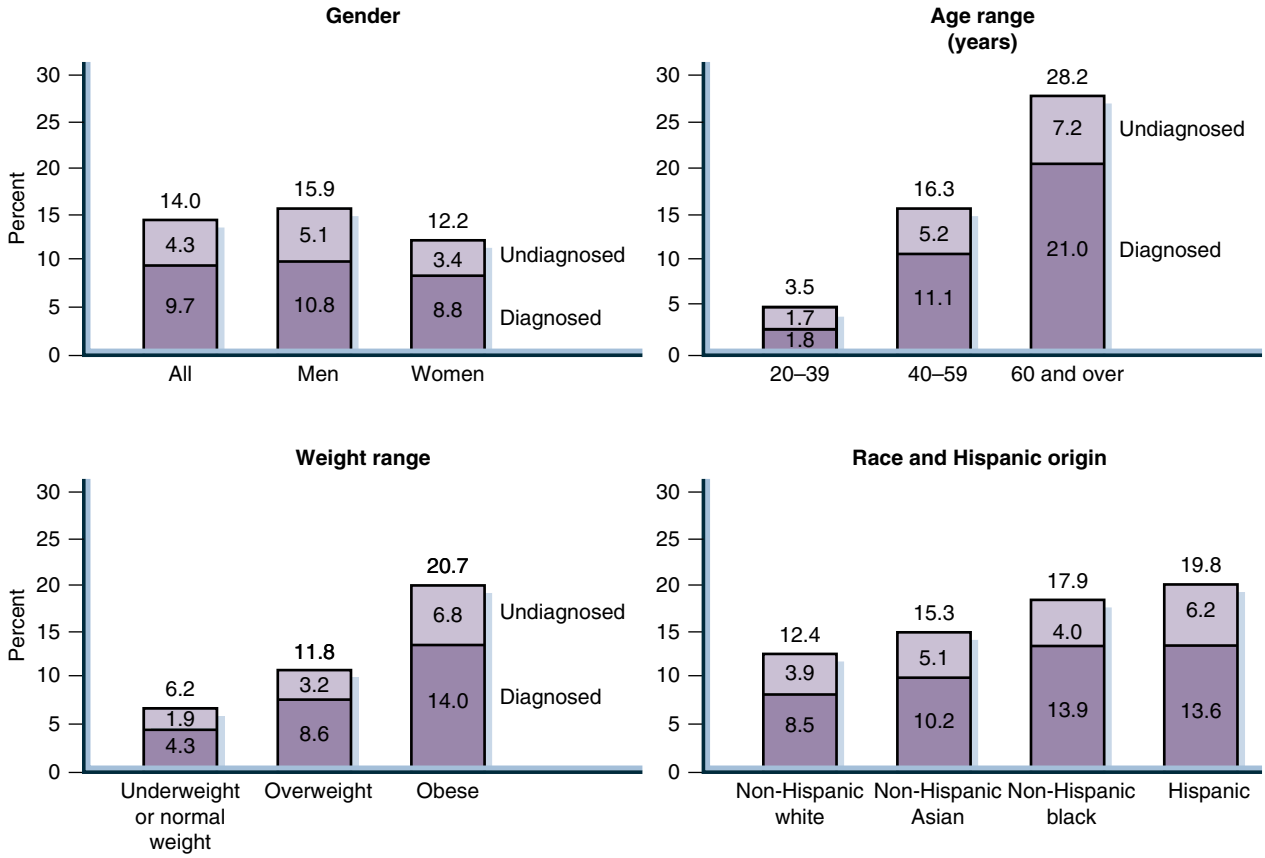
CHAPTER OUTLINE

KEY POINTS

- Type 2 diabetes mellitus (T2DM) is a major public health problem. The World Health Organization estimated in 2014 that 422 million people had diabetes worldwide, and a continuing increase is projected.
- T2DM is the predominant form of diabetes worldwide, accounting for 90% of cases globally.
- The pathogenesis of T2DM is complex and involves the interaction of environmental and genetic factors.
- Environmental factors that contribute to development of T2DM include excessive caloric intake and a sedentary lifestyle leading to obesity.
- Genetically, T2DM includes monogenic and polygenic forms, and some monogenic forms require a specific approach to treatment.
- The typical person developing T2DM is in late middle age and overweight or obese, but the clinical presentation is heterogeneous, with a wide range in age at onset, degree of obesity, and severity of associated hyperglycemia.
- Uncontrolled hyperglycemia is associated with eye, nerve, kidney, and cardiovascular complications that reduce both the quality of life and years of life expectancy.
- Fortunately, hyperglycemia is a modifiable risk factor.
- Attention should also be focused on additional cardiovascular risk factor management.
- Significant advances have been made in the treatment of patients with T2DM over the past 20 years, and a large number of treatment options are available.
- Metformin as initial therapy in combination with diet, exercise, and comprehensive diabetes education effectively lowers glucose with essentially no risk of hypoglycemia.
- If the response to metformin is inadequate initially or subsequently, one or more other agents can be added. There are six recommended second-line therapies: thiazolidinedione, sulfonylurea, dipeptidyl peptidase-4 (DPP4) inhibitor, sodium-glucose cotransporter (SGLT) inhibitor, glucagon-like peptide-1 (GLP1) receptor agonist, and basal insulin. Each has its advantages and disadvantages.
- A team approach to management greatly improves success, especially for patients with diabetes longer than 10 years and therefore requiring greater complexity and personalization of therapy.

Epidemiology

Type 2 diabetes mellitus (T2DM) is the predominant form of diabetes worldwide, accounting for 90% of cases. An epidemic of T2DM is underway in both developed and developing countries, although the brunt of the disorder is felt disproportionately in non-European populations. In the Pacific island of Nauru, diabetes was virtually unknown 50 years ago, but the prevalence among adults rose to nearly 40% before a recent slight decline. The highest prevalence rates remain in Micronesia at 23% to 35%.[1,2] It is projected that South Asia will see a 150% increase in adult diabetes from 2000 to 2025.[3] The International Diabetes Federation (IDF) projects that global prevalence will increase from an estimated 150 million in 2000 and 415 million in 2015 to nearly 600 million by 2035.[2,4] Along with this increase of prevalence, the economic burden of diabetes is expected to rise as well.[5] In the United States, the Centers for Disease Control and Prevention (CDC) has estimated that in 2016 14% of the adult population had diabetes, and 30.7% of those with diabetes were undiagnosed.[6] As shown in Fig. 35.1, among those with diagnosed diabetes, prevalence varied by ethnicity from 8.5% in non-Hispanic white to 10.2% in non-Hispanic Asian, 13.6% in Hispanic, and 13.9% in non-Hispanic black subpopulations.[6] Moreover, 33.9% of US adults (84.1 million) were estimated to have prediabetes and thus be at high risk for developing diabetes.[7] Based on epidemiologic



• **Fig. 35.1** Demographic factors, including gender, age, weight, and race, affecting prevalence of diabetes in adults in the United States in 2013–2016. (Data from Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2017. https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf.)

trends, for those born in the United States in 2000, the estimated lifetime risk of developing diabetes is 32.8% for men and 38.5% in women.[8]

The metabolic abnormalities of T2DM lead to illness, disability, and early mortality. In the United States, diabetes is the leading cause of blindness and accounts for at least 40% of end-stage renal disease. The risk of heart disease and stroke is commonly stated to be two to four times higher, and that of lower-extremity amputation is approximately 20 times higher for people with diabetes than for those without diabetes. Life expectancy is reduced by approximately 10 years in people with diabetes, and the hazard ratio for death is most pronounced in those having diabetes under age 55 years.[8–10] Although diabetes is considered the seventh leading cause of death in the United States, this figure is surely an underestimate because many illnesses identified as causes of death are in fact complications of diabetes. Only about 35% to 40% of those who die with diabetes have the disease listed anywhere on the death certificate, and 10% to 15% have it listed as the underlying cause of death. Rates of morbidity and mortality differ between countries for various reasons, but the overall patterns are very similar.

Thus, mainly through its long-term complications, T2DM causes a serious burden of illness, much of which might be delayed or prevented by treatment. There is evidence that rates of some complications of diabetes have indeed declined recently. Data from the United States suggest a reduction by 50% or more between 1990 and 2010 for myocardial infarction (MI), stroke, amputations, and death from hyperglycemic crisis, and end-stage renal disease declined approximately 30%.[11] Amputations experienced the greatest relative reduction. However, there are reasons for concern. Although incidence rates have decreased in the United States in the last decade, prevalence rates will continue to be high. More individuals are being diagnosed with T2DM at a younger age, and the complications of diabetes will be occurring more often in middle-aged adults with a prevalence that will cause a significant personal and societal burden.[10]

The economic effect of diabetes is enormous. Recent estimates suggest the worldwide cost of diabetes to be $1.31 trillion, with anticipated increases from 1.8% of the global gross domestic product to 2.2% by 2030, with total costs of $2.2 trillion.[5] Costs directly attributed to diabetes in the United States in 2017 were $327 billion with average expenditures per person, adjusted for age and gender, 2.3-fold higher than in the nondiabetic population.[12] These costs increased by 26% between 2012 and 2017. They result from the complications of diabetes, comorbid conditions, costs of medications, and the frequency of visits, as well as from missed work, reduced productivity, and premature mortality.[12]

Considerable information is available on factors underlying the development of T2DM.[13–18] Overt diabetes is thought to occur mainly in genetically predisposed persons who are exposed to various environmental influences that precipitate the onset of clinical disease. The syndrome consists of monogenic and polygenic forms that can be differentiated both on clinical grounds and in terms of the genes involved in pathogenesis. Greater adiposity

TABLE 35.1 Criteria for Diagnosis of Prediabetes and Diabetes

Measurement	Units	Diagnostic of Prediabetes	Diagnostic of Diabetes[a]	Comments
Fasting plasma glucose (FPG)	mg/dL mmol/L	100–125 5.6–6.9	≥126 ≥7	After no calorie intake at least 8 hours
2-hour plasma glucose (2-hour PG)	mg/dL mmol/L	140–199 7.8–11	≥200 ≥11.1	2 hours after 75 g oral glucose
Random plasma glucose	mg/dL mmol/L	Not applicable	≥200 ≥11.1	Without oral glucose but with classic symptoms
HbA_{1c}	% mmol/mol	5.7–6.4 39–47	≥6.5 ≥48	Measured in certified laboratory

[a]In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

Data from American Diabetes Association. Classification and diagnosis of diabetes. Standards of medical care in diabetes, 2018. *Diabetes Care*. 2018;41:S13–S27.

and restricted physical activity are important factors in determining the risk of developing T2DM. The disorder is more common in men, and the increased prevalence in certain racial and ethnic groups has already been mentioned.[6] As in the past the risk of onset of T2DM increases steadily with age, but in recent years the prevalence in children has risen dramatically, presumably due to increasing obesity. Previously, an overwhelming majority of children with diabetes had type 1 diabetes mellitus (T1DM), and less than 2% were considered to have T2DM or other rare forms of diabetes. More recent reports suggest as many as 20% to 25% of children in the United States with newly diagnosed diabetes have T2DM, with the proportions highest among children of non-European ancestry diagnosed between age 10 and 19 years.[19]

Diagnostic Criteria

The diagnosis of diabetes rests on demonstration of hyperglycemia. Because plasma glucose concentrations range as a continuum, the criteria are based on estimated thresholds for the complications of diabetes. The primary endpoint used to evaluate the relationship between glucose levels and complications is retinopathy. All three commonly used tests—fasting plasma glucose (FPG), 2-hour plasma glucose after a 75-g oral glucose load (2-hour PG), and glycated hemoglobin A_{1c} (HbA_{1c})—predict the presence of retinopathy and, by inference, define the glucose levels that are diagnostic of diabetes.[20] There is a relationship between elevated levels of all three markers and nephropathy, neuropathy, and cardiovascular disease as well as retinopathy.

Current criteria for the diagnosis of prediabetes and diabetes are shown in Table 35.1.[21]

When the present fasting glucose and glucose tolerance test parameters were defined for diagnosing diabetes in 1997 according to the levels associated with an increase in retinopathy, measurement of HbA_{1c} was recognized as a viable alternative but not recommended for diagnostic purposes because assays were not reliably standardized.[22] Current HbA_{1c} assays are standardized and have several technical advantages over the other currently used laboratory measurements of glucose. Furthermore, measures of fasting and post-challenge glucose concentrations in the same individual over time are less reproducible than those for HbA_{1c}. In one study, the coefficient of variation for measurements repeated in single individuals was 6.4% for fasting plasma glucose and 16.7% for 2-hour plasma glucose values, and less than 2% for HbA_{1c}.[20] Use of HbA_{1c} has the added advantage of simplicity in not requiring timing of collection, and it is generally a more stable measure of average glucose control. It has also been shown to be a good indicator of prediabetes with an ability to predict development of T2DM.[23,24] Nevertheless, the HbA_{1c} level is misleading in some situations, and discrepancies must be evaluated.[25–27]

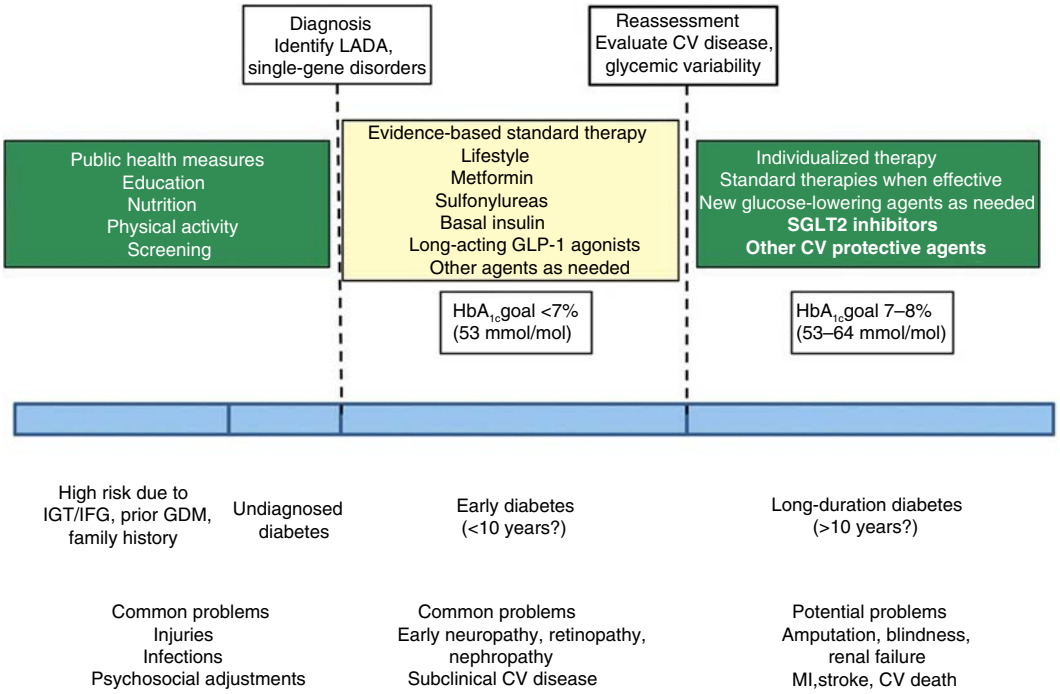
Although the oral glucose tolerance test (OGTT) is very useful for research, it is not recommended for routine use in diagnosing diabetes. It is generally inconvenient, and in most cases the diagnosis can be made by either an elevated fasting plasma glucose concentration, an elevated random glucose determination in the presence of hyperglycemic symptoms, or confirmed elevation of HbA_{1c}. The various glycemic parameters do not always correlate to make the diagnosis. It is possible to see a diagnostic elevation of plasma glucose while the HbA_{1c} is below 6.5% or vice versa. A confirmatory test by the same or a different method is usually required to confirm the diagnosis.[21]

General Approaches to Management

Over the past 20 years a transformation in population-based management of T2DM has occurred. Understanding of the lifetime trajectory of this illness has improved, and there is now greater urgency to identify individuals at risk, make the diagnosis in a timely way, and intervene systematically over time to improve medical outcomes while minimizing risks associated with therapy. The hyperglycemia that defines diabetes is accepted as a modifiable risk factor for microvascular (eye, kidney, and nerve) complications and, to a lesser degree, cardiovascular outcomes.[28–32] The variable but sometimes long delay between appearance of the metabolic abnormalities of diabetes and these complications is now more fully appreciated. Many additions to the available array of pharmacologic agents and monitoring devices have made it possible to lower glucose levels safely to nearly normal ranges in a majority of patients.

In this chapter we address the main features of the diagnostic strategies, treatment guidelines, lifestyle interventions, and pharmacotherapeutic agents before discussing clinical decisions and special considerations in management. The *Clinical Practice Recommendations* of the American Diabetes Association (ADA) is an excellent source of additional information on these matters. It is published each January as a supplement to the journal *Diabetes Care* and is available in a periodically updated version online.[33]

Fig. 35.2 illustrates the extended temporal path of T2DM and identifies some milestones that present opportunities for



• **Fig. 35.2** Overview of the natural history of type 2 diabetes (T2D) and opportunities for assessment and intervention. A schematic depiction of three stages of management of the natural history of T2D, noting several opportunities for improvement of management. *CV,* cardiovascular; *GDM,* gestational diabetes mellitus; *IFG,* impaired fasting glucose; *IGT,* impaired glucose tolerance; *LADA,* latent autoimmune diabetes of adulthood; *MI,* myocardial infarction; *SGLT2,* sodium glucose transporter 2. (From Zinman B, Skyler JS, Riddle MC, et al. Diabetes research and care through the ages. *Diabetes Care*. 2017;40:1302–1313.)

assessment and intervention.[34] This evolution of the disorder over time suggests division of interventions into three categories that are most relevant at specific intervals.

Public Health Measures

For the interval prior to diagnosis, public health interventions are possible. These may include administrative or regulatory efforts to educate families about risks of obesity and diabetes, promote healthful lifestyles, and restrain marketing and distribution of unhealthful foods. Efforts can be made to promote desirable lifestyle choices for at-risk individuals in the setting of primary medical care. A public health approach also includes screening to identify emergence of hyperglycemia as soon as possible.[35]

Primary Care Management

Individuals known to have prediabetes do not routinely require glucose-lowering pharmacotherapy, but attention to other modifiable cardiovascular risk factors is always appropriate.[36] (See the upcoming section regarding diabetes prevention in patients with prediabetes.) More specific medical interventions are needed at the time of diagnosis of overt T2DM.[37] Because the development of tissue complications is related to both the severity and duration of hyperglycemic exposure, the goal of therapy is to maintain glycemic control as close to normal levels as is safely possible from the start. Initial therapy includes enhanced lifestyle efforts together, in most cases, with pharmacotherapy.[33,38] Drug treatment can initially be given in a standardized and relatively simple way, based on medical evidence for both benefits and risks. This approach is most effective in the first 10 years after diagnosis.

Complex Diabetes Care

Progression of the pathophysiologic abnormalities underlying T2DM typically leads to increasing difficulty in controlling hyperglycemia over time. At the same time, an increasing burden of medical illness, both diabetes related and from other causes, adds to potential risks and may limit potential benefits of intensive therapy. By 10 years after diagnosis, progression of the disorder commonly leads to a need for multiple and often injected therapies, more personalized choices of therapies, and greater reliance on treatments with less well-understood long-term benefits versus risks. How to provide these more complex and time-consuming services is one of the dilemmas of health system management. In general, the best results are obtained by access to a specialized team approach.

Screening

As noted, the prevalence of T2DM in the United States and elsewhere continues to increase. The increasing burden of diabetes is driven by aging of the population; by population growth, particularly among ethnic groups with greater susceptibility to the disease; and by increasing obesity related to increasingly sedentary lifestyles and greater consumption of simple sugars, fats, and highly processed calorie-dense foods. Opportunistic screening in high-risk populations is recommended by professional societies and many insurers. In the United States the proportion of people with diabetes who have been diagnosed has increased from approximately 50% in the 1990s to about 70% today.[6] Nonetheless, undiagnosed T2DM is still common. People with undiagnosed T2DM have approximately twofold greater risk for coronary heart disease,

stroke, and peripheral vascular disease. Delay in the diagnosis of T2DM allows hyperglycemia to remain untreated for a period of time, resulting in an increase in microvascular and macrovascular disease. Affected individuals also have a greater likelihood of having dyslipidemia, hypertension, and obesity, all of which are modifiable risk factors for cardiovascular disease. Therefore it is desirable to screen individuals who demonstrate major risk factors for diabetes, as summarized in Table 35.2.

Recent modeling studies based on the US population suggest that universal screening coupled with guideline-based therapy for T2DM is cost effective when initiated between the ages of 30 and 45 and subsequently conducted every 3 to 5 years.[39] In a systematic review of 16 studies, people with an HbA_{1c} of 6% to 6.5% have a 25% to 50% likelihood of developing diabetes within 5 years, a 20-fold excess risk compared to those with an HbA_{1c} of 5%.[40] A more recent study of American Indians found similar predictive value for HbA_{1c} in children and adolescents.[23]

TABLE 35.2 Criteria for Testing for Prediabetes or Diabetes in Asymptomatic Adults

1. Testing should be considered in overweight or obese adults (body mass index ≥25 kg/m² or ≥23 kg/m² in Asians) who have one or more of the following risk factors:
   - First-degree relative with diabetes
   - High-risk ethnicity (e.g., African, Latino, Native American, Asian, Pacific Islander)
   - History of cardiovascular disease
   - Hypertension (blood pressure [BP] ≥140/90 mm Hg or using BP-lowering therapy)
   - High-density lipoprotein cholesterol <35 mg/dL (<0.90 mmol/mol) and/or triglycerides >250 mg/dL
   - Women with polycystic ovary syndrome
   - Physical inactivity
   - Other conditions associated with insulin resistance (e.g., severe obesity, acanthosis nigricans)
2. Patients with prediabetes—test yearly
3. Women with prior gestational diabetes—test at least every 3 years
4. All other people—begin testing at age 45
5. If results are normal—repeat testing at least every 3 years

Modified from American Diabetes Association. Classification and diagnosis of diabetes. Standards of medical care in diabetes, 2018. *Diabetes Care.* 2018;41:S13–S27.

Interventional Study Results

Prospective, randomized clinical trials have documented reduced rates of microvascular complications in patients with T2DM treated to lower glycemic targets. In the United Kingdom Prospective Diabetes Study (UKPDS),[28,41] patients with new-onset diabetes were treated with diet and exercise for 3 months, with an average reduction in HbA_{1c} from approximately 9% to 7%, still somewhat above the upper limit of the normal range. Patients with fasting plasma glucose remaining greater than 108 mg/dL (6 mmol/L) were then randomly assigned to two treatment policies. In the "conventional" intervention, participants continued lifestyle therapy alone. Pharmacologic therapy was initiated only if fasting plasma glucose reached 270 mg/dL (15 mmol/L) or the patient became symptomatic. In the "intensive" treatment arm, all patients were assigned to treatment with either a sulfonylurea or basal insulin as initial therapy, with the dose increased with the aim of maintaining fasting plasma glucose less than 108 mg/dL. Combinations of agents were used only if the patient became symptomatic or fasting plasma glucose rose to greater than 270 mg/dL. In a secondary study within the UKPDS a similar comparison was made between lifestyle alone and metformin as the intensive intervention for participants who were overweight or obese.[41]

As a consequence of the design, which aimed to test the effects of therapy with only one class of glucose-lowering agents, HbA_{1c} fell initially to about 6% but gradually rose to approximately 8% over 10 years of randomized treatment. During that time the median HbA_{1c} in the conventional treatment group was approximately 1 percentage point higher than with the active treatments. Hypoglycemia needing assistance in the group treated with a sulfonylurea or insulin occurred between 1% and 5% per year in the first few years, with the highest rates seen with glyburide. Weight gain was modest in all groups but higher in patients assigned to sulfonylurea or insulin and lower in those receiving metformin.[41] Associated with this improvement in glycemic control, there was a significant 25% reduction in the risk of microvascular complications (retinopathy, nephropathy, and neuropathy) in the group assigned treatment with a sulfonylurea or insulin (Table 35.3). Although there was also a trend toward a reduced rate of MI with sulfonylurea or insulin, it did not reach statistical significance ($p = 0.052$).[28] In the metformin part of the study, more consistent improvements were found (Table 35.4). Metformin treatment was associated with a nonsignificant 29% reduction

TABLE 35.3 Treatment Effects of Sulfonylureas or Insulin in the UKPDS

	YEAR 1997 END OF RANDOMIZED TREATMENT		YEAR 2007 AFTER 10 YEARS OF FURTHER OBSERVATION	
Aggregate Endpoints	Relative Risk Reduction	*p* Value	Relative Risk Reduction	*p* Value
Any diabetes-related endpoint	12%	0.029	9%	0.040
Microvascular disease	25%	0.0099	24%	0.001
Myocardial infarction	16%	0.052	15%	0.014
All-cause mortality	6%	0.44	13%	0.007

Data from UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet.* 1998;352:837–853; Holman RR, Paul SK, Bethel MA, et al. 10-Year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med.* 2008;359:1577–1589.

TABLE 35.4 Treatment Effects of Metformin in the UKPDS

	YEAR 1997 END OF 10 YEARS OF RANDOMIZED TREATMENT		YEAR 2007 AFTER 10 YEARS OF FURTHER OBSERVATION	
Aggregate Endpoints	Relative Risk Reduction	*p* Value	Relative Risk Reduction	*p* Value
Any diabetes-related endpoint	32%	0.0023	21%	0.013
Microvascular disease	29%	0.19	16%	0.31
Myocardial infarction	39%	0.010	33%	0.005
All-cause mortality	36%	0.011	27%	0.002

Data from UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet.* 1998;352:837–853; Holman RR, Paul SK, Bethel MA, et al. 10-Year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med.* 2008;359:1577–1589.

of microvascular disease, but there were statistically and clinically significant reductions of any diabetes-related endpoint (32%), MI (39%), and all-cause mortality (36%).

Similar reductions in microvascular events were observed in the Kumamoto study, a trial of somewhat different design and much smaller size. Japanese patients of normal weight with T2DM receiving insulin were randomly assigned to standard treatment or an intensive program of insulin therapy designed to achieve normal glycemia. The control group maintained HbA_{1c} values at approximately 9%, whereas the HbA_{1c} in the intensive therapy group was reduced to approximately 7%, and the separation was maintained for 6 years. Again, there was a modestly increased risk of hypoglycemia and weight gain, a reduction in microvascular complications, and a (not statistically significant) trend toward reduced rates of cardiovascular events.[42]

In 2008, three studies examining the effects of two levels of glycemic control on cardiovascular endpoints in T2DM were reported. Action to Control Cardiovascular Risk in Diabetes (ACCORD),[43] Action in Diabetes and Vascular Disease—Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE),[44] and the Veterans Affairs Diabetes Trial (VADT)[45] each randomized middle-aged and older individuals who were selected for having high risk for cardiovascular events. Participants randomized to intensive treatment in ACCORD and VADT aimed for an HbA_{1c} target of less than 6% using complex combinations of oral agents and insulin. In ADVANCE the HbA_{1c} target was 6.5% or less using a somewhat less intensive approach based on the addition of the sulfonylurea gliclazide. None of the trials demonstrated a statistically significant benefit in combined cardiovascular endpoints. In ACCORD a 22% increase in total mortality accompanied intensive therapy. Modest improvements in some microvascular endpoints were documented in all three trials. Some secondary analyses of these trials suggested that people without clinical cardiovascular disease and with shorter duration of disease and lower baseline HbA_{1c} had better outcomes with the more intensive glucose-lowering strategies. Meta-analysis of the randomized trials confirmed statistically and clinically significant microvascular benefits from intensive treatment. The same analysis suggested that an average HbA_{1c} reduction of 0.9% correlates with a 17% reduction in nonfatal MI and a 15% reduction in coronary heart disease without significant effects on stroke or all-cause mortality rate. However, there was significant heterogeneity in the results with respect to mortality rates across trials, the cause of which is still unknown.[46]

Continued follow-up of the UKPDS cohort, during which the difference in glycemic control was not maintained, demonstrated that the relative benefits of more intensive management of glucose noted at the end of 10 years of randomized treatment persisted during 10 more years of observation[29] (see Tables 35.3 and 35.4). By 20 years after enrollment, sulfonylurea or insulin treatment still showed a significant 24% relative risk reduction for microvascular outcomes, compared with the conventional intervention with lifestyle alone. For MI and all-cause mortality the relative risk reductions with sulfonylurea or insulin had become statistically significant, 15% ($p = 0.014$) and 13% ($p = 0.007$), respectively. Similarly, the risk reductions for MI and all-cause mortality in the metformin part of the UKPDS remained highly significant after 20 years, 33% ($p = 0.005$) and 27% ($p = 0.002$), respectively. This pattern suggests a "legacy effect" of initially good glycemic control, likely based on persisting changes of tissue structure in blood vessels and elsewhere.

Finally, two post hoc analyses of data from ACCORD have added further insights into the risks associated with intensive management in a high-risk population. First, an analysis of on-treatment HbA_{1c} showed that the individuals who accounted for the excess mortality in the intensive treatment group were not those who attained low HbA_{1c}, levels, but rather those whose average HbA_{1c} remained greater than 7% after starting treatment at a higher level.[32] Thus, aiming for an HbA_{1c} of less than 6% and achieving a target of less than 7% was not associated with excess mortality.[32] On the other hand, those individuals who were seeking the 6% target but failed to attain even 7% after a year of intensive treatment had clearly increased risk. The cause for increased risk in this subgroup remains unknown, but might be related to more advanced physiologic abnormalities or limitations in adherence. In another analysis, an index was calculated based on relationships between baseline HbA_{1c} and fasting glucose. The ACCORD participants in the top tertile of this index (with higher HbA_{1c} than would be predicted based on fasting glucose) had increased mortality and no improvement of the primary cardiovascular endpoint. On the other hand, those in the lower and middle tertiles had no increased risk of mortality and an approximately 25% benefit by the primary endpoint. Whether observations using this index reflect an altered rate of glycation in tissues or disproportionate

TABLE 35.5 American Diabetes Association Recommendations for Typical Target Ranges for Glycemic Control for Many Nonpregnant Adults

Measurement	Units	For Healthy Individuals	For high-Risk Individuals	Comments
Preprandial plasma glucose	mg/dL mmol/L	80–130 4.4–7.2	Not applicable	Fasting, before lunch, and before dinner
Peak postprandial glucose	mg/dL mmol/L	<180 <10	Not applicable	1 to 2 hours after any meal
Mean plasma glucose	mg/dL mmol/L	<154 <8.6	Not applicable	Computed from glucose profile values
HbA_{1c}	% mmol/mol	<7 <53	7–8 53–64	May need adjustment when mismatch between glucose and HbA_{1c} occurs

Data from American Diabetes Association. Glycemic targets: standards of medical care in diabetes, 2018. *Diabetes Care.* 2018;41:S55–S64.

daytime hyperglycemia, the analysis further suggests that benefits and risks of intensive treatment may differ between subpopulations of T2DM.[47]

Glycemic Treatment Targets

Whereas the glucose levels used for the diagnosis of diabetes were determined by their epidemiologic associations with complications of diabetes, the glycemic targets judged appropriate for treatment meant to reduce or prevent these complications have been derived from the results of the intervention studies described earlier in the chapter. All of these studies obtained HbA_{1c} levels averaging close to or below 7% during randomized assignment to intensive therapy.

Glycemic targets suggested by the ADA are presented in Table 35.5.[48] These guidelines suggest that the goal of treatment should generally be an HbA_{1c} value of less than 7%. Furthermore, the ADA suggests that lower targets may be pursued in selected patients, such as those with recent-onset disease, long life expectancy, and no significant cardiovascular disease, if they can be achieved without significant hypoglycemia or other adverse effects of treatment. The guidelines also recommend that a less stringent HbA_{1c} target, such as 7% to 8% or even higher in some cases, may be appropriate for patients with evidence suggesting high risk, including a history of severe hypoglycemia, limited life expectancy, advanced complications, or extensive comorbid conditions, as well as inability to achieve HbA_{1c} less than 7% despite the usual initial therapeutic efforts.[48] The American College of Endocrinology (ACE) has recommended an HbA_{1c} goal that is less than or equal to 6.5%, again with language suggesting individualization of targets.[38] It is agreed that treatment targets and tactics should be adapted to clinical circumstances and personalized to each patient's needs and preferences, to the extent possible.

With respect to fasting, premeal, or postprandial glucose, there is little experimental support for any particular targets for these measurements in the management of T2DM. The ADA recommendation to seek fasting and premeal plasma glucose levels between 80 and 130 mg/dL (4.4–7.2 mmol/L) was based on an estimate of the range of average glucose values that would be associated with a low risk of hypoglycemia and an HbA_{1c} less than 7%. The ACE fasting glucose target of less than 110 mg/dL (<6.1 mmol/L) is an effort to achieve a normal level. However, fasting and premeal glucose values consistently lower than 110 mg/dL would be expected to be associated with an HbA_{1c} of approximately 5.5%, and such levels are not safely attained by most patients as diabetes progresses.[49]

No published studies have demonstrated safety or improved clinical outcomes for a particular targeted level of postprandial blood glucose. The ADA treatment target for peak postprandial glucose levels is less than 180 mg/dL (<10 mmol/L) in part because such levels would be associated with an HbA_{1c} close to 7% and also because people without diabetes who consume a large evening meal can experience transitory elevations of glucose to that level.[50] The ACE has recommended a 2-hour postprandial glucose target of less than 140 mg/dL (<7.8 mmol/L) in an effort to achieve near-normal glycemia.[51] Consistent postprandial glucose values lower than 140 mg/dL would be associated with an average HbA_{1c} of approximately 5% and are very difficult to attain with current treatments except quite early in the course of T2DM.[49]

Monitoring of Glucose During Treatment

Hemoglobin A_{1c}

Measurement of HbA_{1c} provides an estimate of the average plasma glucose level over a period of 2 to 3 months preceding the test.[52] Use of this indicator of glycemic control not only facilitates clinical trials but also assists routine clinical management. When treatment is started or intensified, measurement at approximately 3-month intervals will reveal the success of the intervention. When treatment is established and glycemic control appears stable, testing one or two times a year is usually sufficient.[48] However, measurement of HbA_{1c} has limitations. Because HbA_{1c} reflects glycemia over a 3-month period, current glucose levels do not always correlate well with current HbA_{1c} measurements. Furthermore, individual patients differ somewhat in the relationship of HbA_{1c} to mean glucose concentrations, due to variations in erythrocyte survival or red cell turnover, hemoglobin variants, late kidney disease, or other factors.[26,27,53,54] Such a mismatch between glucose and HbA_{1c} values has sometimes been termed a "glycation gap." This glycation gap can be due to recent changes in glycemia and may warrant changes in therapy. Lower HbA_{1c} than expected based on blood glucose levels may occur with increased red cell turnover seen in a variety of circumstances, such as occult blood loss or iron treatment of iron deficiency anemia. The inappropriately low HbA_{1c} could be misconstrued as adequate control when intensification of therapy is indicated. Alternatively, low red cell turnover such as with untreated iron deficiency anemia could lead to the opposite conclusion. In many cases, however, the higher HbA_{1c}

level relative to the self-monitored blood glucose results is due to unrecognized high glucose levels such as postprandial or nocturnal times when patients typically are not testing. Comparison of HbA_{1c} values with other measures of glucose is advisable at some stage of management, so that individuals with differences of 0.3% to 0.4% or greater from predicted levels can be identified.[25] Moreover, HbA_{1c} does not assess daily patterns or day-to-day variations of glycemic control.

Self-Monitoring of Blood Glucose

Self-monitoring of capillary blood glucose (SMBG) with glucose-oxidase strips provides that information. Since introduction of SMBG 40 years ago, the convenience and reliability of test strips and meters for use in measuring point-in-time glucose values have improved greatly, and all currently available personal glucose meters are generally accurate.[55] Even so, recent studies have shown variable performance under conditions of daily use, highlighting the need for expertise and care in use of the information thus obtained.[56] At the present time SMBG is widely recommended to monitor the progress of therapy and to identify specific glucose patterns in T2DM. It is especially helpful for patients with T1DM and those with T2DM who use intensive insulin therapy. Testing before-breakfast glucose levels is essential for initiating and guiding titration of basal insulin when it is added to oral agents, and premeal testing is necessary to guide adjustment of further injections of rapid-acting insulin before meals.[57–59] Although clinical trials have not consistently shown SMBG can change outcomes in other groups of patients with T2DM when used in isolation,[60,61] diabetes self-management programs that include SMBG can be beneficial in controlling glucose and reducing complications.[62,63] Potential benefits of SMBG in non–insulin-treated patients include supporting educational concepts of nutrition and exercise, determining a daily glucose pattern to select the best pharmacologic tactics, documenting early responses to pharmacotherapy, and serving generally as a motivational tool.[48,62,64] Use of SMBG is important for all patients with a new diagnosis of diabetes for purposes of education, and it subsequently should be advised selectively according to the duration of diabetes, risks of hypoglycemia, and other factors.[65,66]

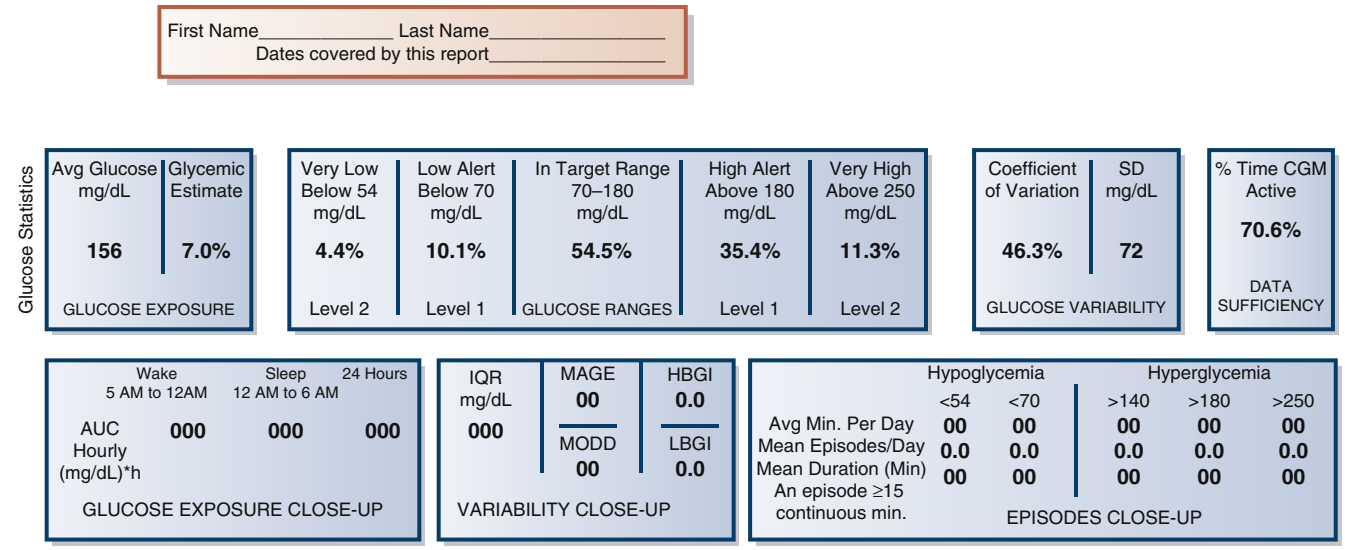
Use of SMBG is particularly recommended for patients with T2DM who are taking insulin or sulfonylureas because it can identify minimal or asymptomatic episodes of hypoglycemia, which may predict increased risk of more severe events. Severe hypoglycemia is relatively rare in T2DM, but it can have devastating consequences such as falls and other trauma, or a change in the ability of a patient to continue to live independently due to risk of confusion or loss of consciousness. Recognition of minor hypoglycemia is also important to avoid undue concern about nonspecific symptoms that may be due to other causes. Monitoring studies show that most symptoms in patients with T2DM are not related to hypoglycemia, but some patients are sufficiently concerned that they may consume extra calories in response to sweating, anxiety, or emotional distress without documenting low glucose levels by SMBG. Fear of hypoglycemia may also lead to lack of adherence to medications or inappropriate reductions of dose, and the ability to document actual glucose values can be reassuring.

The timing of SMBG that is most useful depends on various factors. One basic principle is that patients should periodically vary the time of day at which glucose is tested. For some patients glucose values are highest in the morning, whereas for others glucose is highest in the evening. Times of highest risk of hypoglycemia can vary as well. When glucose control is poor, concentrating on premeal glucose levels is adequate. Once premeal glucose levels are reduced to 120 to 130 mg/dL (6.7–7.2 mmol/L), adding some measurements 1 to 2 hours after meals is appropriate. This calls attention to the effect of diet and enables patients to understand how changes in meals, activity, and medications have significant influences on glycemic control. Postprandial testing also becomes important when the HbA_{1c} remains high despite attainment of reasonably good premeal glucose levels. In such circumstances, postprandial glucose increments can be responsible for the higher-than-expected HbA_{1c} and may require a change in therapy. In recently diagnosed diabetes and gestational diabetes, monitoring only 1 to 2 hours after meals can allow patients to assess the effect of lifestyle and pharmacologic efforts on postprandial glucose levels, which may be the leading glycemic abnormality in those settings.

Clinical trials have confirmed the benefits of structured use of SMBG in patients who have been educated on how to use the information.[67–69] For example, the Structured Testing Program (STeP) trial found use of 7-point daily glucose testing for 3 days each quarter increased the frequency of treatment adjustments and improved HbA_{1c} levels.[70] It is likely that the benefits of SMBG stem from assisting patients in controlling of their own therapy. If patients are aware of the glycemic targets associated with the outcomes they seek to achieve, SMBG enables them to evaluate their responses to therapy and actively participate in reaching their goals. Present meters are downloadable, and clinics should have software that allows reproducing a summary of experience since the last visit, a task once falling to the patients themselves. In some cases it is useful for patients periodically to keep a personal daily diary of SMBG, making them more aware of their results. Unfortunately, patients often fail to bring their meter or their handwritten log to the visit.[71] The importance of this information must be emphasized by the treating clinic. Providers should examine records of SMBG carefully at the time of visit, as it is frustrating for patients to put extensive effort into collection of data that are not taken seriously by their health advisors. In fact, it appears that the more attention that is given to the blood glucose results at a visit, the more frequent the monitoring by the patient and the lower the HbA_{1c}.[72] In some circumstances, such as recent changes in therapy, rapid deterioration of control, or worrisome hypoglycemia, SMBG should be communicated to a member of the health care team by telephone, fax, or privacy-protected email at shortened intervals to facilitate appropriate correction of the problem. Providing adequate financial support for such services may be challenging for health systems, but this is necessary to limit the burden placed upon providers. Similarly, it may also be challenging for patients and providers to keep current on the array of available equipment and supplies for glucose testing and handling of the resulting data. A useful resource in this regard is the annual *Consumer Guide*, which is published as the January issue of *Diabetes Forecast*, a magazine for laypeople with diabetes and their families.[73]

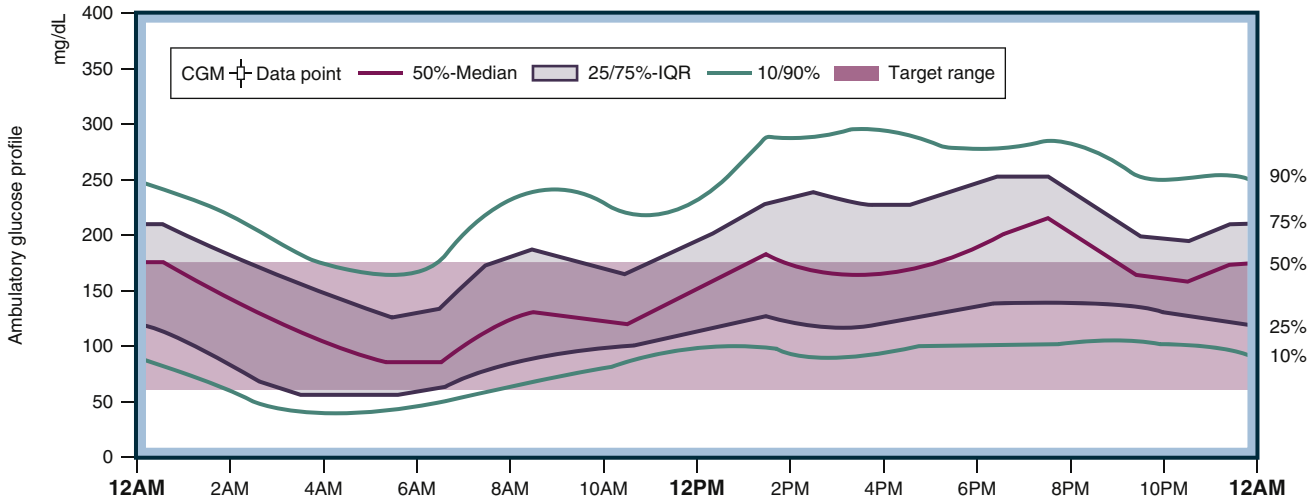
Continuous Glucose Monitoring

Recently, development of continuous glucose monitoring (CGM) systems has added a new dimension to tracking glycemic control. These devices sample subcutaneous interstitial fluids at frequent intervals and can report the results either in real time or upon demand. Their value in T1DM is well documented, and CGM is now frequently recommended for patients with T2DM who embrace newer technologies with the aim of reducing glycemic variability and hypoglycemia while seeking improved HbA_{1c}.[74]



Level 1 = Needs attention. Level 2 = Immediate action.

Curves/plots represent glucose frequency distributions by time regardless of date.



• **Fig. 35.3** An electronic ambulatory glucose profile report that visualizes the key continuous glucose monitoring metrics: (1) mean glucose; (2) hypoglycemia: clinically significant/immediate action required; (3) hypoglycemia alert/low/monitor; (4) target range; (5) hyperglycemia: alert/elevated/monitor; (6) hyperglycemia: clinically significant/very elevated/immediate action required; (7) glycemic variability; (8) estimated HbA_{1c}; (9) time blocks; (10) collection period; (11) percentage of expected readings; (12) hypoglycemia/hyperglycemia episodes; (13) area under the curve; (14) hypoglycemia/hyperglycemia risk; and (15) standardized continuous visualization. *AUC,* area under the curve; *Avg,* average; *IQR,* interquartile range; *MAGE,* mean amplitude of glucose excursions; *MODD,* mean of daily differences; *SD,* standard deviation. (Modified from Danne T, Nimri R, Battelino T, et al. International consensus on use of continuous glucose monitoring. *Diabetes Care*. 2017;40:1631–1640.)

The real-time CGM devices include audible alerts to inform the patient of higher trends as well as low or impending low glucose values. A smartphone may serve as the receiver, and appropriate apps will give real-time glucose information, updating every 5 minutes. Trend arrows are shown, and information can be shared with providers as well as family members or other supporting individuals. The intermittently scanned or "flash" CGM monitors only record glucose results when the reader is passed over the sensor that is in the interstitial space. However, once scanned, the reader will display the present glucose value as well as a rate of change arrow and a recording of the glucose trend over the last 8 hours. If the sensor is scanned every 8 hours, a continuous chart of the glucose trends can be viewed. Favorable effects of using CGM for patients with T2DM who are treated with intensive insulin regimens have also been validated.[75,76] Medicare covers CGM for use by people with T2DM on intensive insulin therapy. A strong rationale for this decision is that protection against hypoglycemia is of heightened importance in older patients.[77,78] Patients with T2DM requiring insulin may also benefit from CGM through promotion of desirable diet and exercise patterns, but the cost effectiveness of this application has not yet been fully assessed. Finally, CGM can provide accurate measurement of mean glucose levels, thereby validating HbA_{1c} values or, alternatively, identifying patients with a significant glycation gap.[25] Fig. 35.3 shows one

TABLE 35.6 American Diabetes Association Classification of Hypoglycemia

Levels of Hypoglycemia	Glucose Units	Criteria	Comments
Level 1 (Alert value)	mg/dL mmol/L	<70 and ≥54 <3.9 and ≥3	Important independent of symptoms, adjustment of therapy may be needed
Level 2 (Clinically significant)	mg/dL mmol/L	≤54 ≤3	Requires immediate action
Level 3 (Severe)	mg/dL mmol/L	Altered mental and/or physical status requiring assistance	Calls for reevaluation of regimen and/or targets

Modified from American Diabetes Association. Glycemic targets: standards of medical care in diabetes, 2018. *Diabetes Care*. 2018;41:S55–S64.

form of display that can be used to summarize and communicate a variety of kinds of clinically useful information provided by CGM systems.[79]

Hypoglycemia

Among the most important purposes of measuring glycemic patterns during treatment of T2DM, as with T1DM, is to identify hypoglycemia.[80,81] The definitions of hypoglycemia have recently been a topic of debate. Table 35.6 summarizes the ADA's most recent statements on this point.[82] Division of hypoglycemia into three levels is based on three kinds of observations. Under normal circumstances a decline of glucose concentrations below 70 mg/dL (<3.9 mmol/L) triggers physiologic responses of the so-called counterregulatory hormones: glucagon, epinephrine and norepinephrine, cortisol, and growth hormone. Symptoms of hypoglycemia may or may not result from these responses, but declines below this threshold, termed level 1 hypoglycemia, are considered important as alerts to allow protection against more worrisome declines of glucose. Level 2 hypoglycemia is defined as glucose documented at or lower than 54 mg/dL (3 mmol/L). Repeated declines to this range are considered clinically significant because they can cause blunting of compensatory hormone responses and loss of warning symptoms ("hypoglycemia unawareness"), and thereby increase the risk of more dangerous lows. Level 3 is considered severe hypoglycemia, defined as requiring assistance by another person, whether a friend or family member or a medical provider. This level of hypoglycemia in T2DM is strongly associated with risk of physical injury, cardiovascular events, and death. Although hypoglycemia can directly cause such events through falls and cardiac arrhythmias, this relationship with serious medical outcomes is thought to be partly related to an association of hypoglycemia with other causes of risk, including concurrent illness, inconsistent eating patterns, malnutrition and weight loss, physical frailty, and cognitive impairment. Nonetheless, level 3 severe events are markers of risk and call for both conservative glycemic management and renewed attention to other aspects of care.

Lifestyle Interventions

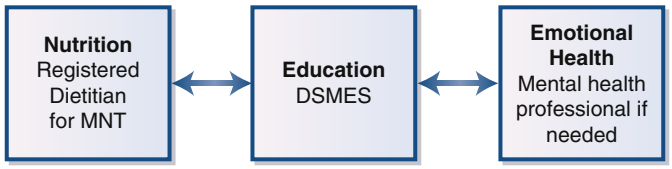
The leading lifestyle interventions for management of T2DM are medical nutrition counseling and exercise recommendations. Beyond these traditional pillars of lifestyle management there is growing attention to psychosocial concerns as well as interest in various forms of stress reduction, relaxation, and sleep management. The evidence base supporting such interventions has become much stronger recently, and reimbursement for these efforts has become more frequently available. They are most effective when presented as parts of a comprehensive plan of self-management with the goal of moving the focus of care from the provider to the individual with diabetes.[83–85]

Diabetes Self-Management Education and Support

Diabetes is a chronic, ever-present disease that places the patient in a unique position of self-care, requiring daily decisions and the ability to solve problems when they arise. The provider has little connection with day-to-day care, thus the patient needs the tools, confidence, and a plan for self-management. Professional services promoting this process are now described as diabetes self-management education and support (DSMES). The evidence for benefit of DSME is now quite strong.[86,87] The ADA together with the American Association of Diabetes Educators (AADE) and the Academy of Nutrition and Dietetics recommend that all individuals with diabetes should receive DSMES.[85] As illustrated in Table 35.7, it is recommended that DSMES be emphasized at four critical settings in the evolution of T2DM. These include the time of diagnosis, annual reassessments, when complicating factors arise, and the time of critical transitions in medical care or life circumstances. The importance of ongoing contact and reassessment has proven to be significant.[88] There is also consensus on a core group of considerations, methods, and barriers to address.[85] Unfortunately, there is evidence that DSMES services are underutilized. A review of a large commercial insurance database reported that only 6.8% of patients received DSMES within the first 12 months of diagnosis.[89] When subgroups were considered by ethnicity, socioeconomic groups, age, and other factors, the highest utilization subgroup received DSMES only 15% of the time. Why utilization is so low is not clear, but providers' unawareness of the significance and availability of this important resource are thought to be important factors. The AADE (telephone 800-TEAM-UP4) and the ADA (telephone 800-DIABETES) can provide information regarding diabetes educators and education programs in local areas of the United States.

DSMES refers to all activities and individuals that help patients turn knowledge into action. A summary of the necessary components of an effective DSMES program is provided in Table 35.8.[90,91] Health care providers are seldom adequately trained and often do not have the patient contact time to deal with all these issues alone. The need for a team approach has long been recognized in diabetes care and remains a critical ingredient for success. The providers (physicians and advanced practice providers) enrich their care with the help of nurses, dietitians, exercise specialists, behavioral specialists, pharmacists, podiatrists, and other medical and surgical specialists. Among

TABLE 35.7 Diabetes Self-Management Education and Support (DSMES) for Adults With Type 2 Diabetes: Algorithm of Care



FOUR CRITICAL TIMES TO ASSESS, PROVIDE, AND ADJUST DSMES

At Diagnosis	Annual Assessment	When Complicating Factors Arise	When Transitions in Care Occur
When primary care or specialist provider should consider referral			
Initial evaluation at diagnosis and to ensure both nutrition and emotional health are addressed	Needs review of knowledge, skills, behaviors Long-duration T2DM Change of regimen HbA_{1c} out of target range Unexplained hypoglycemia or hyperglycemia Planning pregnancy or pregnant For support in changing life situations For weight or nutritional concerns	Complex medication regimen Change in health conditions such as renal disease, stroke, glucocorticoid use, etc. Physical limitations such as visual impairment, dexterity issues Emotional factors such as anxiety, depression Basic living needs such as food access, housing, financial status	Rehabilitation, assisted living, living alone New medical care team Change of insurance Age-related changes affecting cognition, self-care, etc.

MNT, Medical nutritional therapy.

Modified from Powers MA, Bardsley J, Cypress M, et al. Diabetes self-management education and support in type 2 diabetes. *Diabetes Educ.* 2017;43:40–53.

TABLE 35.8 Key Components of Effective DSMES Programs

- Evidence based
- Individualized to the needs of the person, including language and culture
- Has a structured theory-driven written curriculum with supporting materials
- Delivered by trained and competent individuals (educators) who are quality assured
- Delivered in group or individual settings
- Aligns with the local population needs
- Supports the person and their family in developing attitudes, beliefs, knowledge, and skills to self-manage diabetes
- Includes core content—i.e., diabetes pathophysiology and treatment options; medication usage; monitoring, preventing, detecting, and treating acute and chronic complications; healthy coping with psychologic issues and concerns; problem solving and dealing with special situations (e.g., travel, fasting)
- Available to patients at critical times—i.e., at diagnosis, annually, when complications arise, and when transitions in care occur
- Includes monitoring of patient progress, including health status, quality of life
- Quality audited regularly

DMES, Diabetes self-management support and education.

From Davies MJ, D'Alessio DA, Fradkin J, et al. Management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care.* 2018. [Epub ahead of print.]

the commonly overlooked contributors to ineffective care of T2DM are the barriers created by psychiatric, neurocognitive function, and adjustment disorders, which are largely responsive to psychosocial therapies.[92] Modern diabetes management principles emphasize that the patients are at the center of the team and that the management plan is not dictated by the provider but ultimately determined by informed patients who may include personal health beliefs as well as cultural influences and other factors in their approaches to self-care.[83,85,93]

People other than medical professionals can contribute to DSMES. Group sessions provide added support as do programs that involve peer groups and health coaches without a traditional medical professional directly involved.[94–96] In the last decade we have also seen growing implementation of digital health and telehealth tools that improve communication and otherwise support patients in self-management.[97–102] Finally, the potential role of the community in which the patient lives and works in the diabetes self-care process is enormous; family, friends, employers, and health insurance providers may all be involved.[103] Peer support has proven benefits.

Medical Nutrition Therapy

Medical nutrition therapy (MNT) is an essential component of DSMES. Ideally all patients should have access to a registered dietitian-nutritionist (RDN) to facilitate it.[83,104–106] Recent guidelines recommend three to six MNT sessions in the first 6 months after diagnosis. Medical nutrition therapy has been shown to decrease HbA_{1c} by 0.3% to 2%. In a typically obese and older T2DM population the basic goals of nutrition therapy include weight reduction or maintenance, adequate glucose control, a nutrient-sufficient diet, and nutritional efforts to reduce cardiovascular risk by controlling blood pressure and lipid levels. Nutritional strategies, of course, require individualization based on the patient's clinical profile, personal and cultural factors, health literacy, motivation, and economic limitations. A summary of general nutritional principles for people with diabetes is shown in Table 35.9.[83]

Beyond the general principles, there are specific features of eating patterns that are relevant to the medical management of individual patients, and some of them are directly related to the success of treatment with insulin. It is very helpful to know how many meals the patient eats in a day, and at what times. Does this pattern differ from day to day? Are meals often missed, and if so why? What does the patient view as the primary challenge in healthful eating? Do family members eat with the patient and

TABLE 35.9 Major Nutritional Recommendations for Type 2 Diabetes

Topic	Recommendations
Effectiveness of nutrition therapy	• An individualized MNT program, preferably provided by a registered dietitian, is recommended for all people with type 1 or type 2 diabetes or gestational diabetes mellitus. • A simple and effective approach to glycemia and weight management emphasizing portion control and healthy food choices may be considered for those with type 2 diabetes who are not taking insulin, who have limited health literacy or numeracy, or who are older and prone to hypoglycemia.
Energy balance	• Weight loss (>5%) achievable by the combination of reduction of calorie intake and lifestyle modification benefits overweight or obese adults with type 2 diabetes and also those with prediabetes. Intervention programs to facilitate weight loss are recommended.
Eating patterns and macronutrient distribution	• There is no single ideal dietary distribution of calories among carbohydrates, fats, and proteins for people with diabetes; therefore macronutrient distribution should be individualized while keeping total calorie and metabolic goals in mind. • A variety of eating patterns are acceptable for the management of type 2 diabetes and prediabetes.
Carbohydrates	• Carbohydrate intake from vegetables, fruits, legumes, whole grains, and dairy products, with an emphasis on foods higher in fiber and lower in glycemic load, is preferred over other sources, especially those containing added sugars. • For people with type 1 diabetes and those with type 2 diabetes who are prescribed a flexible insulin therapy program, education on how to use carbohydrate counting and in some cases fat and protein gram estimation to determine mealtime insulin dosing is recommended to improve glycemic control.
	• For individuals whose daily insulin dosing is fixed, a consistent pattern of carbohydrate intake with respect to time and amount may be recommended to improve glycemic control and reduce the risk of hypoglycemia.
	• People with diabetes and those at risk should avoid sugar-sweetened beverages to control weight and reduce their risk for CVD and fatty liver and should minimize the consumption of foods with added sugar that have the capacity to displace healthier, more nutrient-dense food choices.
Protein	• In individuals with type 2 diabetes, ingested protein appears to increase insulin response without increasing plasma glucose concentrations. Therefore carbohydrate sources high in protein should be avoided when trying to treat or prevent hypoglycemia.
Dietary fat	• Data on the ideal total dietary fat content for people with diabetes are inconclusive, so an eating plan emphasizing elements of a Mediterranean-style diet rich in monounsaturated and polyunsaturated fats may be considered to improve glucose metabolism and lower CVD risk and can be an effective alternative to a diet low in total fat but relatively high in carbohydrates.
	• Eating foods rich in long-chain n-3 fatty acids, such as fatty fish (EPA and DHA) and nuts and seeds (ALA), is recommended to prevent or treat CVD; however, evidence does not support a beneficial role for the routine use of n-3 dietary supplements.
Micronutrients and herbal supplements	• There is no clear evidence that dietary supplementation with vitamins, minerals, herbs, or spices can improve outcomes in people with diabetes who do not have underlying deficiencies, and are not generally recommended. There may be safety concerns regarding the long-term use of antioxidant supplements such as vitamins E and C and carotene.
Alcohol	• Adults with diabetes who drink alcohol should do so in moderation (no more than one drink per day for adult women and no more than two drinks per day for adult men). • Alcohol consumption may place people with diabetes at increased risk for hypoglycemia, especially if taking insulin or insulin secretagogues. Education and awareness regarding the recognition and management of delayed hypoglycemia are warranted.
Sodium	• As for the general population, people with diabetes should limit sodium consumption to <2300 mg/day, although further restriction may be indicated for those with both diabetes and hypertension.
Nonnutritive sweeteners	• The use of nonnutritive sweeteners may have the potential to reduce overall calorie and carbohydrate intake if substituted for caloric (sugar) sweeteners and without compensation by intake of additional calories from other food sources. Nonnutritive sweeteners are generally safe to use within the defined acceptable daily intake levels.

ALA, Alpha-lipoic acid; *CVD,* cardiovascular disease; *DHA,* docosahexaenoic acid; *EPA,* eicosapentaenoic acid; *MNT,* medical nutrition therapy.

Modified from American Diabetes Association. Lifestyle management: standards of medical care in diabetes, 2018. *Diabetes Care.* 2018;41:S38–S50.

support the recommended nutrition goals? What beverages does the patient drink? How many alcoholic drinks does the patient take daily? This information should be reassessed periodically, with specific suggestions for changes that are achievable.

Problematic habits may become apparent. Intake of caloric beverages (e.g., juices) or very high glycemic index foods (e.g., cold cereal) might be perceived as healthful but can significantly affect glycemic control.[107,108] Finding foods that the patient may view as healthful due to being "low carb" while ignoring the high fat content or, conversely, foods chosen for being low in fat but having high carbohydrate content can provide opportunities for improvement. A pattern of snacking from dinner until bedtime or consistently overeating on weekends can create difficulties. Portion control techniques, variation in food preparation (e.g., fried vs. grilled), appropriate meal spacing, and mindful eating are important concepts to reinforce for the patient and the family, including a potential benefit for family members who are at risk of developing diabetes.[109] It has also become apparent that diabetes providers should consider food insecurity that is becoming increasingly recognized and relates to high HbA_{1c}.[110] Finally, it is important to track the success of nutrition goals determined in previous visits, setting new goals and potentially offering new resources where appropriate.[111]

Structured lifestyle programs can produce long-term weight loss of 5% to 7% of starting weight and[112] reduce the risk of developing diabetes.[113] Such systems emphasize education, reduced energy intake (especially fat), conscious decisions regarding carbohydrate intake, regular physical activity, and ongoing support from professionals or peers. Achieving a 5% weight loss more consistently yields significant metabolic benefits than lower levels of weight loss.[114] Focusing on a specific macronutrient distribution appears to be less important than reducing overall caloric intake and facilitating maintenance of the weight loss.[115,116]

As an example, it has been demonstrated in a population with T2DM of less than 6 years' duration that consistent application of a specific intervention program, in a primary care setting, can result in significant reduction of weight and HbA_{1c} over a 1-year period.[117] The same report showed glycemic improvement was proportional to the reduction of weight. Patients who lost less than 5% of body weight had insignificant HbA_{1c} reductions, similar to that in the control group. Of those in the intervention group who lost 5% to 10 % of body weight, 34% ended with HbA_{1c} below 6.5%. Among those losing more than 15%, 86% achieved this level of control. The reduction of HbA_{1c} was often accompanied by less use of pharmacologic agents as well. For more information on therapeutic weight loss, see Chapter 40.

Much controversy has surrounded the macronutrient makeup of the diet of people with T2DM. In general, the critical nutrient for glycemic consistency is carbohydrate. Glucose levels after meals are strongly influenced by the carbohydrate content of the diet. For patients with T2DM requiring prandial insulin, the carbohydrate-counting technique is used to facilitate consistent carbohydrate intake or to allow adjustment of insulin doses in response to changes in carbohydrates consumed.[118] However, evidence for the benefit of calculating insulin:carbohydrate ratios in T2DM over other methods of dose adjustment is less compelling than in T1DM.[118,119] For patients not using insulin, carbohydrates still affect postprandial glucose excursions, and consistency of carbohydrate intake is still relevant. However, other methods can be used to assess and modulate carbohydrate intake. These include the "plate method," carbohydrate exchange lists, and even simple portion control tactics.[119,120] Whereas the beta cell in T2DM has usually lost its immediate response to glucose, the second phase of insulin secretion is largely spared in T2DM and is in part driven by amino acids and fatty acids. Therefore including some protein and fat in each meal and snack is useful, and these macronutrient groups along with fiber content can attenuate the effect of carbohydrate on postprandial elevations. Although the carbohydrate content of typical nutrition plans for those with T2DM has generally decreased in the last decade, the optimal proportion of macronutrients is uncertain. Low carbohydrate diets, including ketogenic diets, have grown in popularity and are associated with decreased postprandial glucose and a reduction in HbA_{1c}.[121–123] Even so, the benefit in reducing HbA_{1c} may come more from the frequently associated weight loss as the evidence indicates carbohydrate content, independent of weight loss, does not significantly influence HbA_{1c}. In many cases the postprandial glucose decreases with lower carbohydrate intake, but fasting glucose may be higher. Long-term adherence to very low carbohydrate diets is difficult. The glycemic index refers to the glucose response to equal amounts of carbohydrates in various foods. This index has proven somewhat difficult to use for meal planning, and studies evaluating the benefit of lowering glycemic index have yielded mixed results.[107,108,124] As a practical measure, identifying high glycemic index foods in a patient's diet could help reduce high postprandial blood glucose patterns. Nutrition practice guidelines also recognize the benefit of SMBG in confirming the glucose response to food intake and success of nutritional interventions.[83,85]

Dietary fat is the nutrient that is most closely associated in epidemiologic studies with the risk of developing T2DM. Although dietary fats clearly have an impact on total caloric intake (related to their caloric density) and on circulating lipids, they have little effect on glycemia. Fat intake is a contributor to obesity and is the critical nutrient for cardiovascular risk management. It is recommended that people with diabetes (and everyone in general) consume a diet that is modestly restricted in calories (if overweight) and contains less than 10% of total calories as saturated fat and less than 10% as polyunsaturated fat.[85] Some advocate substituting foods high in monounsaturated fatty acids (i.e., seeds, nuts, avocado, olives, olive oil, and canola oil) for carbohydrate, but most patients do not find adequate variety in the monounsaturated fatty acid category and often overeat these high-calorie foods. Higher carbohydrate diets can raise postprandial glucose and triglycerides but are much less calorically dense than higher fat diets and have a higher thermic effect, both of which tend to promote weight loss.

Dietary protein similarly has little impact on glucose levels, although amino acids do promote insulin secretion. Metabolism of protein results in the formation of acids and nitrogenous waste, which can lead to bone demineralization and glomerular hyperfiltration. At least 0.8 g of high-quality dietary protein per kilogram of body weight is generally recommended. Restriction of protein intake to 10% to 20% of total calories minimizes potential adverse long-term effects of high protein intake. However, recent guidelines do not support the notion that dietary protein need be reduced in those with chronic kidney disease.[105] Likewise, there is no evidence for a distinction between the effects of vegetable-based versus animal-based protein sources in kidney function.

The evidence base for benefit of higher fiber intake is not very convincing. Still, there may be some benefit on HbA_{1c} and lipid profiles for those ingesting at least 15 g of fiber per 1000 kcal compared to lower fiber diets. Alcohol in moderation (up to one drink per day for adult women or two drinks per day in adult men) is not specifically recommended but is considered acceptable. An added risk associated with excessive intake of alcohol by people with T2DM, especially those who use sulfonylureas or insulin, is delayed hypoglycemia, which typically occurs at night when ability to recognize hypoglycemia can be impaired. Moderate red wine intake may result in mild improvement in some lipid parameters but seems to have little effect on glucose control. Nonnutritive sweeteners such as aspartame, ace-K, saccharin, stevia, or sucralose do not appear to impact lipid parameters, insulin secretion, or blood pressure independent of weight loss. They are deemed safe for use in T2DM if consumed within the FDA recommended daily intake amounts. Sodium restriction to less than 2300 mg per day is recommended, and lower sodium diets may have additional benefit in some individuals with hypertension.[85]

The roles of vitamins, trace minerals, and nutritional supplements in the treatment of diabetes are poorly understood. Some clinicians are convinced of the utility of magnesium, chromium, zinc, folic acid, pyridoxine, cyanocobalamin, vitamin A, vitamin C, vitamin E, vanadium, selenium, garlic, and other micronutrients. Clinical trial data regarding their safety and efficacy are inconclusive at best. Many patients are convinced that nutritional supplementation is healthful, and it is often counterproductive to engage in scholarly discussion of the nature of the evidence base for their decision. At a minimum, discussion should include the documented efficacy of more classic lifestyle and pharmacologic interventions and the idea that these efforts should not be ignored.[125]

Although various specific dietary strategies have their proponents, few data from long-term outcome studies are available to support routine use. For example, ketogenic diets or low carbohydrate diets have limited long-term evidence of benefit or risk at the present time. However, if a patient or practitioner wants to use a lower carbohydrate higher protein/fat reduced calorie diet, that choice may be associated with short-term improvements in glycemia, cardiovascular risk markers, and weight. The most validated meal plans in T2DM include the Mediterranean diet, the Dietary Approaches to Stop Hypertension (DASH), and plant-based diets.[115,126–128] A Mediterranean diet emphasizing use of monounsaturated and polyunsaturated fats may have the best experiment support for glycemic control, cardiovascular protection, and perhaps other outcomes.[127]

Physical Activity and Exercise

There is a substantial body of literature supporting exercise as a modality of treatment in T2DM. Exercise is associated with improved glycemic control, insulin sensitivity, cardiovascular fitness, and quality of life.[83,129,132] Aerobic exercise and resistance (strength) training both have positive impact on glucose control.[130] Self-directed structured exercise programs have been associated with mean HbA_{1c} reductions of 0.4% to 0.9%, with the largest reduction seen in those using combined aerobic and resistance exercise.[131,133] Supervised exercise is also effective but too expensive for many and less practical. In many cases the benefits in HbA_{1c} are not accompanied by weight loss. With this independent benefit it is clear why the physician can refer to exercise as a therapeutic modality equivalent to pharmacologic therapies. Improvements in glycemic control are usually apparent immediately, but the improvement in insulin resistance may not last more than 48 to 72 hours. It is often recommended that individuals strive for 150 minutes of moderate-intensity physical activity (50–70% of maximum heart rate) or 75 minutes per week of vigorous exercise (>70% of maximum heart rate) on at least 3 days per week with no more than 2 consecutive days without exercise. Both aerobic and resistance exercise are effective and recommended.[83,132,133]

The key concept is to promote an increase in activity using an approach similar to the one discussed for diet. Goals, methods, intensity, and frequency must be negotiated with patients with great sensitivity to recognizing barriers and helping patients discover solutions. The role of educators, exercise specialists, physical therapists, and social supports in this process is critical. The major role for the physician is to screen for complications (neuropathy, nephropathy, retinopathy, vascular disease) and discover ways for patients to be able to exercise safely.[132] It is also important for the physician to inquire about exercise patterns and potential barriers and consider implications of exercise on hypoglycemia at each clinic visit.

Although it has not been recommended to have all patients formally evaluated for ischemic heart disease with a stress test, conducting a careful history of potential signs and a clinical risk assessment is desirable to identify patients who may warrant additional testing or cautions. Patients at high risk for coronary artery disease should start with short periods of low-intensity exercise and increase the intensity and duration slowly as tolerated.[132] Development of symptoms of coronary ischemia, including dyspnea out of proportion with activity, calls for further evaluation and treatment. It is important to encourage patients not to overexert and to recognize exertional chest, jaw, or arm discomfort as well as palpitations and dyspnea as symptoms of cardiac dysfunction.

For the average patient with T2DM starting an exercise program it is best to start with low-level activity such as walking at a pace of 2 miles per hour. Initially it may be advantageous to start with shorter duration exercise sessions and build up the frequency and duration. Caution is important to avoid overuse injuries that then interrupt the exercise progression. Many different exercise programs are beneficial, and individualization is appropriate. For example, interval training appears to be effective but is not appropriate for all.[134] Aquatic exercise is beneficial and may be particularly useful for those with joint pain.[135] Walking after dinner can have a significant benefit on postprandial hyperglycemia.[136] Consideration of diabetes complications is imperative. For example, in the setting of proliferative diabetic retinopathy intense aerobic and resistance exercise should be avoided. With significant peripheral neuropathy, careful attention must be given to footwear, and weightbearing exercise should be limited. Daily foot inspection for foot ulcers is critical. Autonomic neuropathy requires particular cardiac cautions. Exercise does not appear to accelerate kidney disease but will increase albuminuria acutely and could cause false-positive albumin:creatinine ratios temporarily.[137]

In addition to scheduled exercise, all individuals should be encouraged to reduce sedentary time, particularly by breaking up extended amounts of time spent sitting.[137,138] It is recommended to have some activity break at least every 30 minutes to include brief walks, resistance exercise, or at least standing. This may also help prevent diabetes. Balance training and exercise improving flexibility may be helpful as patients get older, with the aim of decreasing falls and increasing mobility.[139]

Addressing Psychosocial Needs

Many psychosocial factors influence the ability of a patient with T2DM to practice effective self-management.[92] There is a complex interaction of factors such as economic stability, food insecurity, family support or conflict, intellectual abilities and learning skills, and the incessant need to consider the implications of timing and content of food intake, emotional stress, physical activity, and adherence to medications each day. Not surprisingly, patients often experience diabetes distress and depression, two separate but related diagnoses.[140] The incidence of diabetes distress over an 18-month period has been reported to range from 38% to 48%.[141] Diabetes distress screening tools have been developed and utilized in revealing studies where the level of distress impacts glycemic control, adherence to medication regimens, exercise behaviors, self-efficacy, and diet. There are also depression screening tools for use in clinical practice. When clinical depression is diagnosed, the patient should be referred to a mental health provider, preferably one with an understanding of diabetes and its treatment.[92] It is recommended that patients with diabetes be screened at least once yearly as depression is present in about 25% of screened patients, and appropriate treatment can greatly facilitate diabetes self-management and quality of life. Another psychologic comorbidity to be considered in T2DM is chronic anxiety, which often appears as fear of hyperglycemia, hypoglycemia, chronic complications, or injections. On occasion, management may be influenced by obsessive compulsive disorder resulting in inadequate nutrition or overtreatment of hypoglycemia. A few patients on intensive insulin may experience a posttraumatic stress disorder or panic disorder related to a severe episode of hypoglycemia.

The psychosocial aspects of diabetes care are relevant for the entire treatment team, emphasizing the importance of patient-centered care and a collaborative approach.[92] Therefore, in addition to a psychiatric history, the office visit should include basic

TABLE 35.10 Classes of Antihyperglycemic Agents for Type 2 Diabetes

Classes of Agents	Route of Delivery	Mechanism of Effect on Glucose	Basal Glucose Control	Prandial Glucose Control	Weight Control	BP Control	Short-Term CV Risk Reduction
Biguanide	Oral	Decrease hepatic glucose production	+++	+	++	+	+
Secretagogue	Oral	Increase insulin secretion	+++	++	Weight increase	↔	↔
Thiazolidinedione	Oral	Decrease insulin resistance	+++	++	Weight increase	+	?
DPP4 inhibitor	Oral	Increase insulin, decrease glucagon	++	+	↔	↔	↔
α-Glucosidase inhibitor	Oral	Delay carbohydrate absorption	+	+++	++	↔	+
SGLT inhibitor	Oral	Increase renal clearance of glucose, sodium	++	+	+++	++	+++
Bile-acid sequestrant	Oral	Delay carbohydrate absorption?	+	+	↔	↔	?
Dopamine agonist	Oral	Decrease insulin resistance	+	+	+	+	?
Insulin	Injection	Increase insulin availability	+++	+++	Weight increase	↔	↔
GLP1 receptor agonist	Injection	Increase insulin, decrease glucagon, slow gastric emptying	+++	+++	+++	++	++
Amylin receptor agonist	Injection	Decrease glucagon, slow gastric emptying	+	+++	+++	↔	?

CV, Cardiovascular; *DPP4,* dipeptidyl peptidase-4; *GLP1,* glucagon-like peptide-1; *SGLT,* sodium-glucose cotransporter.

questions on stress levels, family support structures, general mood and energy, and perceived barriers to self-management success. Recognizing that diabetes distress is so prevalent, the practice can facilitate stress reduction through one-on-one discussion and by providing resource lists or directly providing clinic-based support groups, exercise groups with support elements, and mindfulness training. When screening suggests a psychologic barrier beyond the capacity of the clinical practice, referral to a mental health specialist should be proposed to the patient in a sensitive, professional manner.

Glucose-Lowering Pharmacotherapy

A revolution in the treatment of T2DM since 1995 in the United States has been driven by the release of several new classes of drugs that independently address different pathophysiologic mechanisms that contribute to the development of diabetes. Antihyperglycemic agents have commonly been divided into those that improve the sensitivity of tissues to insulin, those that augment insulin availability, and those with other mechanisms of action. This classification has become less helpful as additional classes of agents have been introduced, some of them with multiple pharmacologic and clinical effects. Here we will present each class of oral therapy separately, and then describe the currently available agents that require injection or other means of delivery. Some important properties of various classes of drugs available for the management of T2DM are summarized in Table 35.10. This area has been the subject of extensive reviews.[33,49–51,78,118,125,142–148] In the following discussion, the principles outlined in these reviews are summarized, and a limited number of additional references are provided. In addition, when questions arise regarding the details of handling, dosing, and administering any of the agents available, it is advisable to consult the product information approved by regulatory authorities.

Oral Agents

Some clinically relevant features of the main currently available oral antihyperglycemic agents are summarized in Table 35.11.

Biguanides

Metformin is the only biguanide available in the United States. Phenformin was removed from the market in the 1970s because of deaths associated with lactic acidosis. Phenformin and buformin remain available in some countries, however. The precise mechanism of action of metformin is still not well understood.[149,150] Some studies suggest it activates adenosine monophosphate-activated protein kinase (AMPK), an intracellular signal of depleted cellular energy stores that has been implicated in stimulation of skeletal muscle glucose uptake and inhibition of hepatic gluconeogenesis, whereas more recent studies implicate inhibition of mitochondrial glycerophosphate dehydrogenase. Additional new evidence suggests much of metformin's effect is mediated by direct contact with the intestinal mucosa, provoking neural or hormonal signals to the brain or other tissues.[151] There is some evidence that it can influence the microbiome, and it increases glucagon-like peptide-1 (GLP1) release. It has been suggested the main clinical effect of metformin is to reduce hepatic gluconeogenesis and glucose production, but this does not account for all of its effects.[150] It less consistently improves insulin sensitivity in peripheral tissues. Because of its limited duration of action, it is usually taken at least twice daily, although a sustained-release formulation is available.

TABLE 35.11 Clinical Features of Commonly Used Oral Antihyperglycemic Agents

Classes and Specific Agents (Commercial Names)	Commonly Used Dosages	Contraindications	Side Effects	%HbA_{1c} Reduction as First or Second Therapy
Biguanide				
Metformin (Glucophage)	500–1000 mg BID	T1D, DKA eGFR <30	Nausea, diarrhea, abdominal pain	1 to 2
Metformin-ER	500–1000 mg BID	Severe cardiac, hepatic disease	Vitamin B_{12} deficiency	
Secretagogue				
Glipizide (Glucotrol)	5–20 mg BID	T1D, DKA	Hypoglycemia	1 to 2
Glipizide-ER	2.5–10 mg QD		Weight gain	
Gliclazide (Diamicron)	80–160 BID			
Gliclazide-MR	30–120 QD			
Glimepiride (Amaryl)	0.5–4 mg QD			
Glyburide (Micronase et al)	2.5–10 mg BID			
Repaglinide (Prandin)	0.5–2 mg TID			
Nateglinide (Starlix)	60–120 mg TID			
Thiazolidinedione				
Pioglitazone (Actos)	15–30 mg QD	T1D, DKA	Weight gain	0.75 to 1.5
Rosiglitazone (Avandia)	4–8 mg QD	Symptomatic heart failure	Edema Fractures	
DPP4 inhibitor[a]				
Sitagliptin (Januvia)	25–100 mg QD	T1D, DKA	Hypersensitivity	0.5 to 1
Vildagliptin (Galvus)	50 mg QD or BID			
Saxagliptin (Onglyza)	2.5–5 mg QD			
Linagliptin (Trajenta)	5 mg QD			
Alogliptin (Nesina)	6.25–25 mg QD			
α-Glucosidase inhibitor[b]				
Acarbose (Precose)	25–50 mg TID	T1D, DKA	Flatulence, diarrhea, abdominal discomfort	0.5 to 1
Miglitol (Glyset)	25–50 mg TID			
SGLT inhibitor				
Canagliflozin (Invokana)	100–300 mg QD	TID, DKA eGFR <30	Urinary frequency Urogenital infections Nausea, diarrhea Hypotension	0.5 to 1
Dapagliflozin (Farxiga)	5–10 mg QD			
Empagliflozin (Jardiance)	10–25 mg QD			
Ertugliflozin (Steglatro)	5–15 mg QD			
Bile-acid sequestrant				
Colesevelam (Welchol)	Six 625-mg tabs QD	T1D, DKA Pancreatitis, intestinal disease, hypertriglyceridemia	Constipation	0.5 to 1
Dopamine agonist				
Bromocriptine (Cycloset)	1.6–4.8 mg QD	T1D, DKA	Somnolence, dizziness, hypotension	0.5 to 1

[a]The DPP4 inhibitors listed here are approved in the United States and/or the European Union. Other DPP4 inhibitors are available in certain countries, including anagliptin (Suiny), evogliptin (Suganon), gemigliptin (Zemiglo), gosogliptin (SatRx), omarigliptin (Marizef), teneligliptin (Tenelia), trelagliptin (Zafatek), and vildagliptin (Galvus).

[b]Acarbose and miglitol are available in the United States and the European Union. Voglibose (Basen et al) is available in other countries.

BID, Twice daily; *DKA,* diabetic ketoacidosis; *eGFR,* estimated glomerular filtration rate; *ER,* extended release; *MR,* modified release; *QD,* once daily; *TID,* three times daily.

Because biguanides do not increase insulin levels, they are not associated with a significant risk of hypoglycemia. The most common adverse events are gastrointestinal: nausea, abdominal pain or bloating, and diarrhea. Up to a third of patients have some gastrointestinal distress, especially early in their course of treatment. This can be minimized by starting with a low dose once daily with meals and titrating upward over several weeks to fully effective doses. Sustained-release metformin is associated with less frequent and less severe upper gastrointestinal symptoms, but it can increase the frequency of diarrhea, which is overall less common. Most patients tolerate metformin adequately during long-term use. Perhaps related to clinical or subclinical gastrointestinal effects, metformin is associated with less weight gain than other antihyperglycemic agents and can be associated with a modest weight loss.

Metformin has been said to cause lactic acidosis, which is quite rare and occurs almost exclusively in patients who are at high risk for development of the condition independent of metformin therapy.[152] Metformin is not metabolized and is cleared only through the kidney. Prior to April 2016 the package insert stated that it was contraindicated in patients with renal insufficiency in an effort to avoid lactic acidosis. The revised package insert recommends obtaining an estimated glomerular filtration rate (eGFR) before

starting metformin and at least annually thereafter. Initiation is not recommended if the eGFR is below 45 mL/minute/1.73 m^2 and is contraindicated if the eGFR is below 30 mL/minute/1.73 m^2. Once treatment is established, one should consider a dose reduction if the eGFR is under 45 mL per minute, and metformin should definitely be stopped if the eGFR is less than 30 mL per minute.[153] Metformin remains contraindicated in patients with hepatic insufficiency and in the setting of alcohol abuse. Because some patients taking metformin develop significant vitamin B_{12} deficiency that might exacerbate peripheral neuropathy, supplementation with vitamin B_{12} (e.g., 1000 μg daily) or intermittent monitoring may sometimes be prudent.[154–156]

Both the glucose-lowering efficacy and the frequency of gastrointestinal side effects of metformin increase in the dose range from 500 to 2000 mg per day. The maximal daily dose of 2550 mg does not generally provide additional benefit beyond that seen at 2000 mg daily.[157] To minimize gastrointestinal side effects, dosage should usually be started with 500 mg once or twice daily. Newer formulations of metformin combined with other classes of oral antihyperglycemic agents have been developed to maximize glucose-lowering effectiveness with a single prescription through the synergy of two classes of agents with different actions.

Arguably, metformin has the best record among oral antihyperglycemic agents in medical outcome studies. As previously noted (see Table 35.4) overweight participants in the UKPDS who were randomly assigned to metformin had reduced rates of myocardial infarction and all-cause mortality that persisted long after cessation of randomized therapy.[41] While the number of participants using metformin in the UKPDS was relatively small, other studies have supported its protective effects.[158] A beneficial effect of metformin on cardiovascular complications through mechanisms independent of glycemic control is certainly plausible and is consistent with metformin-associated modest reductions in LDL, triglycerides, blood pressure, and procoagulant factors.

Based on its good tolerability, safety, and effectiveness together with evidence for medical benefits in controlled trials, it is generally recommended that metformin therapy be started in all patients with T2DM at or near the time of diagnosis of diabetes, provided no contraindications are present.[33,38,159]

Insulin Secretagogues

Along with biguanides, this class of oral agents has a long history of clinical use, and mechanisms have been carefully studied. Some characteristics of the most widely used insulin secretagogues are summarized in Table 35.11. Currently available secretagogues all bind to the sulfonylurea receptor-1 (SUR1), a subunit of the K_{ATP} potassium channel on the plasma membrane of pancreatic beta cells. The SUR1 subunit regulates the activity of the channel and also binds adenosine triphosphate (ATP) and adenosine diphosphate (ADP), effectively functioning as a glucose sensor and trigger for insulin secretion. Binding leads to closing of the channel, as do increases in intracellular ATP and decreases in ADP resulting from fuel metabolism. The membrane depolarization that ensues causes the opening of voltage-dependent L-type calcium channels. Subsequent calcium influx results in an increase in intracellular calcium, which leads to insulin secretion. A direct effect on insulin secretion, independent of ambient glucose levels, is seen with the first dose of any secretagogue. However, this direct effect wanes during continued occupancy of SUR1, while potentiation of the effect of current glucose levels continues.

Differences in pharmacokinetic and binding properties of the various agents contribute to their differing clinical effects, as do differences in their formulations and mechanisms of clearance from circulation. These properties are apparent clinically as differences in duration of action and likelihood of causing hypoglycemia. The best uses and also the relative risks versus benefits of the various secretagogues differ considerably.

Sulfonylureas

The sulfonylureas have been available since the 1950s. They are reliably effective in lowering glucose levels early in the natural history of T2DM when considerable beta-cell function is present.[160,161] Glimepiride and extended-release glipizide and gliclazide are generally the most widely used. These currently preferred sulfonylureas share several pharmacologic features. Each has a long duration of action after a single dose, allowing once-daily administration for most patients. Due to these longer profiles, continued SUR1 occupancy limits glucose-independent insulin release accompanying each subsequent dose, while continuing to potentiate glucose-dependent basal insulin secretion. The clinical effect of these agents is to lower fasting glucose levels with little effect on postprandial glucose levels, and relatively low risk of hypoglycemia compared with other secretagogues. Because none of these agents is strongly dependent on renal excretion, declining renal function has less direct effect on risk of hypoglycemia during their use. In contrast, chlorpropamide is a long-acting sulfonylurea that is cleared only by the kidney. Thus it can cause severe hypoglycemia when renal function becomes impaired, and largely for this reason it is now rarely used.

The shorter acting sulfonylureas include standard formulations of glipizide and glyburide. Taken twice daily they can be as effective as the longer-acting agents in overall glucose control. However, they have a greater effect on postprandial hyperglycemia and can cause daytime hypoglycemia when food intake is reduced. Glyburide has an active metabolite that can be cleared only by the kidney, and for this and other reasons it is associated with greater risk of hypoglycemia than other currently used sulfonylureas.[162]

There has been long-standing concern that sulfonylureas might cause increased arrhythmic cardiovascular events in patients with diabetes as a result of an effect on SUR2 subunits of the K_{ATP} complex in vascular and cardiac cells. Binding to SUR2 can blunt ischemic preconditioning, a normal cardioprotective mechanism. Epidemiologic studies assessing whether this effect confers increased cardiovascular risk in clinical practice differ in their conclusions, but more recent ones, including the newer sulfonylureas and accounting for confounding factors, are generally unable to confirm that risks of sulfonylureas outweigh potential benefits.[162] The ADVANCE trial, which used extended-release gliclazide as its dominant glucose-lowering strategy, did not show any evidence of cardiovascular toxicity.[44] The UKPDS similarly demonstrated long-term safety relative to lifestyle intervention for glyburide, glipizide, and chlorpropamide, which were all used in its main (sulfonylurea or insulin) treatment arm.[28] However, the individual sulfonylureas differ in pharmacologic studies of ischemic preconditioning, with this adverse effect confirmed for tolbutamide and glyburide and less apparent with glimepiride, glipizide, and gliclazide. Concern about cardiovascular risk is especially relevant to glyburide, an older sulfonylurea that continues to be prescribed despite its effects on ischemic preconditioning and tendency to cause hypoglycemia.

The maximum approved dose for the sulfonylureas is two to four times higher than the maximum effective dose. Initiating treatment with no more than a quarter of the maximal approved dose provides significant glucose lowering while limiting costs and adverse events. Small doses of the longer-acting modern sulfonylureas (e.g., 0.5–1 mg of glimepiride or 2.5 mg of extended-release glipizide) are often effective, particularly in patients receiving metformin concomitantly, and are almost always well tolerated. The leading

disadvantages of sulfonylureas are their tendency to cause hypoglycemia and usually modest weight gain. These unwanted effects may contribute to their more rapid loss of effectiveness than other agents, another observation best documented with glyburide.[163]

Glinides

Repaglinide is a member of the meglitinide family of insulin secretagogues, distinct from the sulfonylureas. It has a short half-life and a distinct SUR1 binding site. As a result of rapid absorption, it produces a faster and briefer stimulus to insulin secretion than the shorter acting sulfonylureas. It is typically taken with each meal and provides better postprandial control and generally less hypoglycemia and weight gain than glyburide. Repaglinide has a long residence time on the SUR1 and therefore some effect on fasting glucose, even though its pharmacologic half-life is quite short. Repaglinide is available in 0.5-mg, 1-mg, and 2-mg tablets. The maximum dose is 4 mg with each meal. As with the sulfonylureas, there is only a modest glucose-lowering advantage of high compared with moderate doses of repaglinide.

Nateglinide is a derivative of phenylalanine and is structurally distinct from both sulfonylureas and the meglitinides. It has a quicker onset and a shorter duration of action than repaglinide. Its interaction with SUR1 is fleeting. As a result, its effect in lowering postprandial glucose is quite specific, and it has little effect on fasting glucose. This provides both advantages (less overnight hypoglycemia) and disadvantages (less overall glucose-lowering effectiveness). Nateglinide is most appropriately used when glucose levels are modestly elevated in early diabetes or in combination with agents that control overnight glucose levels. It is available as 120-mg tablets and is taken with each meal. A 60-mg tablet is available but is not generally used except in patients with minimal hyperglycemia.

The rationale for using repaglinide or nateglinide to stimulate insulin secretion at mealtime to improve postprandial control without increasing the risk of overnight hypoglycemia is attractive. Furthermore, these newer agents demonstrate little binding to the vascular smooth muscle and cardiac SUR2 receptors. However, use of them has been modest, probably because of the need for multiple daily doses, higher cost than sulfonylureas, and lack of head-to-head comparative studies that demonstrate superiority over the newer sulfonylureas.

Thiazolidinediones

The thiazolidinedione class of drugs (TZDs, or glitazones) has engendered great interest and controversy since the first agent, troglitazone, was approved in 1997. Rare fatal hepatotoxicity was associated with troglitazone, and it was withdrawn from the US market in 2000. The currently available TZDs (pioglitazone and rosiglitazone) are considered safer. These agents bind to and modulate of the activity of a family of nuclear transcription factors termed *peroxisome proliferator-activated receptors* (PPARs), thereby initiating many downstream effects. They are associated with slow improvement in glycemic control over weeks to months in parallel with an improvement in insulin sensitivity and a reduction in free fatty acid levels.

Pioglitazone and rosiglitazone are equally effective in improving glycemic control and provide equivalent improvements in markers of insulin resistance and inflammation. No substantial evidence has linked the newer TZDs to hepatotoxicity, and a record of safety has been established in appropriate patients. The package insert nevertheless calls for liver function tests before beginning TZD therapy, and these agents are contraindicated in patients with active hepatocellular disease or unexplained serum alanine aminotransferase (ALT) levels greater than 2.5 times the upper limit of normal. Nonetheless, recent studies show a favorable effect of pioglitazone on nonalcoholic steatohepatitis, a common and otherwise difficult to treat condition.

Pioglitazone and rosiglitazone differ with respect to lipid effects. In a head-to-head study among dyslipidemic patients, pioglitazone reduced triglycerides by approximately 20%, whereas rosiglitazone increased triglycerides on average by 5%. Pioglitazone is associated with a modestly greater improvement in high-density lipoprotein (HDL) particle number and size and an improvement in low-density lipoprotein (LDL) particle size and number. Rosiglitazone was associated with an increase in LDL particle number and improved LDL particle size.[164]

Early epidemiologic evidence suggested that the ability of TZDs to improve insulin-sensitivity might lead to cardiovascular protective effects. This hypothesis was supported by a series of physiologic associations with TZD use: reduced carotid intimal/medial thickness, improvement of vascular endothelial function, improvements in dyslipidemia, lower blood pressure, and improved fibrinolytic and coagulation parameters. The hypothesis was tested in the PROactive (PROspective pioglitAzone Clinical Trial In macroVascular Events) study, a randomized, double-blind, placebo-controlled trial in 5238 patients with T2DM and documented macrovascular disease. Subjects were randomized to placebo or to 45 mg per day of pioglitazone and otherwise treated according to guidelines for hyperglycemia and major cardiovascular risk factors. The primary endpoint was the time from randomization to a broad set of macrovascular outcomes. Although this endpoint showed no significant improvement with pioglitazone, there was a marginally significant 16% reduction in a secondary, more restrictive cardiovascular composite endpoint. After extensive discussion of this controversial trial, it appears that pioglitazone therapy may be associated with some reduction of cardiovascular risk related to improvements in control of glucose, lipids, and blood pressure, but that these benefits are attenuated by an increased incidence of weight gain and heart failure.[165] Notably, in a recent placebo controlled study in nondiabetic patients with a recent ischemic stroke or transient ischemic attack, pioglitazone reduced the subsequent occurrence of stroke or myocardial infarction.[166]

The Rosiglitazone Evaluated for Cardiovascular Outcomes in Oral Agent Combination Therapy for Type 2 Diabetes (RECORD) trial was an open-label study that compared the effect of adding rosiglitazone versus either metformin or sulfonylurea to patients who had T2DM inadequately controlled with sulfonylurea or metformin. There was no difference in cardiovascular hospitalizations or death.[167] Subsequently, a controversy based on epidemiologic analyses developed as to whether rosiglitazone was associated with increased risk of myocardial infarction.[168] This dispute remains unresolved, but it caused a shift away from use of rosiglitazone.

Another potentially important attribute of the TZDs is an improvement in insulin secretory dynamics. Several trials have demonstrated that TZDs can delay the progression of dysglycemia to diabetes.[33,142] In addition, ADOPT (A Diabetes Outcome Progression Trial) found that patients with early diabetes had a slower rate of secondary glycemic failure when they were treated with rosiglitazone compared with metformin and glyburide. These outcomes were correlated with improved insulin secretion, although it remains unclear whether the improvement resulted mainly from direct effects on the beta cell as opposed to persisting reduction of insulin resistance.[163]

Despite these desirable effects, several adverse effects of the TZDs have raised concerns. These include weight gain, fluid retention, increased risk of bone fractures, and anemia. The weight gain has been shown to result both from extracellular fluid accumulation and an increase of subcutaneous but not visceral fat.[165] There are, in fact, desirable reductions in visceral, hepatic, and intramyocellular fat. The weight gain resulting from TZDs may not have the negative metabolic consequences that are generally ascribed to overweight and obesity, but it is nevertheless viewed negatively by most patients and practitioners. All patients prescribed TZDs should be counseled to enhance lifestyle efforts to limit weight gain.

With appropriate caution, relatively few patients will need to withdraw use of a TZD because of fluid retention. The patients most likely to experience edema are those using insulin and those with preexisting edema.[169,170] It is prudent to teach patients with preexisting edema how to assess pitting pretibial edema at home and to suggest they make a habit of checking nightly. If weight accompanied by increasing edema is noted, patients can be instructed to restrict sodium intake, to start a diuretic, or to increase their diuretic dosage as needed.

It is usually prudent to begin therapy with the lowest available dose, which in the case of pioglitazone is 15 mg daily. If after 3 months the glycemic response has been inadequate and significant edema has not developed, an increase to 30 mg may be considered. The highest approved dose, 45 mg, is less well tolerated. Many patients with mild edema respond to a thiazide diuretic or spironolactone. and combination therapy with a moderate-dose loop diuretic may be considered.[171] Fluid retention to the point of clinically apparent heart failure can occur, in which case the TZD should be discontinued. In the PROactive and RECORD studies, an excess of approximately 2% of patients treated with high-dose TZD required hospitalization for heart failure. There have been conflicting reports of worsened macular edema with thiazolidinediones.[172,173]

A more recent safety concern regarding TZDs is bone health. An excess of fractures has been reported during TZD use in both pharmacoepidemiologic studies and randomized trials, mainly in older women.[174,175] Whereas distal sites were primarily affected in these studies, loss of bone density in the lumbar spine may occur as well. Preclinical studies suggest that activation of PPARγ inhibits bone formation by diverting stem cells from the osteogenic to the adipocytic lineage. To minimize this risk, an assessment of risk factors and measurement of bone density should be considered before prescribing a TZD, especially for women.[176]

Finally, pioglitazone has been suspected of causing bladder cancer as a result of inconsistent preclinical and clinical observations. Large, long-term studies have not confirmed this association.[177] If pioglitazone is associated with bladder cancer, the absolute risk to an individual is low. However, current recommendations to avoid its use in patients with a history of bladder cancer seem prudent.

DPP4 Inhibitors

Oral glucose has a greater stimulatory effect on insulin secretion than does intravenous glucose at the same circulating glucose concentration. In humans this effect is mediated mainly by glucagon-like peptide-1, which is secreted in response to nutrients by intestinal L cells. Its binding to GLP1 receptors stimulates insulin secretion in a glucose-dependent fashion, inhibits inappropriate glucagon secretion, slows gastric emptying, reduces appetite, and improves satiety. Secreted GLP1 is very rapidly (1–2 minutes) inactivated in plasma by the enzyme dipeptidyl peptidase-4. A variety of pharmacologic techniques have been developed to harness the potential of GLP1 signaling to treat diabetes.[178,179] Several injectable drugs that are agonists for GLP1 receptors will be discussed later. Another approach is inhibition of DPP4, resulting in increased blood levels of endogenously produced GLP1 and other circulating peptides.

Five DPP4 inhibitors—sitagliptin, vildagliptin, saxagliptin, linagliptin, and alogliptin—are approved for clinical use in the United States and/or the European Union, and others are available in other countries. Some of their clinical characteristics are shown in Table 35.11. These agents increase fasting and postprandial levels of GLP1 about twofold. This elevation of GLP1 potentiates glucose-dependent insulin secretion and suppresses basal and postprandial glucagon. However, neither satiety nor the rate of gastric emptying is affected. The main clinical effect of DPP4 inhibitors is on fasting glucose levels, leading to HbA_{1c} reductions of 0.5% to 1%. They have no consistent effect on weight and no tendency to cause hypoglycemia when used alone or with metformin.[180]

All agents in this class are remarkably well tolerated, with an adverse effect profile similar to that of placebo. Postmarketing cases of pancreatitis have been reported, and DPP4 inhibitors are contraindicated for use by patients with a prior history. Specificity for DPP4 appears to be crucial, as inhibitors that are less specific for this enzyme been shown to have effects on immune function and cancer growth in animal studies. Further, the biologic effects of all the DPP4 inhibitors are complex, owing to their potential effects on clearance of other peptides that are substrates for this enzyme.[181] In cardiovascular outcome trials, there was an increased risk for heart failure hospitalization with saxagliptin and a numerical imbalance for alogliptin,[182–184] but no increased risk was demonstrated for sitagliptin.[185] It is not clear whether these findings represent an important clinical distinction, the play of chance, or differences in trial populations or conduct. Serious hypersensitivity reactions have been reported, but causality has not been substantiated because of the rarity of events.

Linagliptin is available in only one tablet size. For the other DPP4 inhibitors in current use, which are partially cleared by the kidney, the usual dose is the maximum marketed dose, and smaller doses are recommended in the setting of stage 3 or greater chronic kidney disease. The weight neutrality, lack of hypoglycemia, broad applicability, tolerability, and ease of use of DPP4 inhibitors are appealing properties. Consequently, they have been widely used despite their relatively weak glucose-lowering power that is partly dependent on retained beta-cell function.

α-Glucosidase Inhibitors

α-Glucosidase inhibitors (AGIs) slow the terminal step of carbohydrate digestion at the brush border of the intestinal epithelium. As a result, carbohydrate absorption is shifted more distally in the intestine and is delayed, allowing the sluggish insulin secretory dynamics characteristic of T2DM to catch up with carbohydrate absorption.

The two agents currently available in the United States are acarbose and miglitol. Voglibose is available in other countries. Use of AGIs in the United States has been limited by a number of factors, including the need to administer the medication at the beginning of each meal, flatulence as a common side effect, and only modest reductions in blood glucose levels. These factors should be balanced against the ability of AGIs to lower postprandial glucose without increasing weight or hypoglycemic risk.[186] There is also some evidence suggesting that acarbose improves cardiovascular outcomes better than most antihyperglycemic agents.[187]

TABLE 35.12 Treatment Effects of Empagliflozin and Canagliflozin in the EMPA-REG OUTCOME and CANVAS Program Studies

	EMPAGLIFLOZIN IN EMPA-REG OUTCOME		CANAGLIFLOZIN IN THE CANVAS PROGRAM	
Endpoints	**Hazard Ratio (95% CI)**	***p* Value**	**Hazard Ratio (95% CI)**	***p* Value**
Primary cardiovascular composite Cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke	0.86 (0.74–0.99)	0.04	0.86 (0.75–0.97)	0.02
Cardiovascular death	0.62 (0.49–0.77)	<0.001	0.87 (0.72–1.06)	NA
All cause death	0.68 (0.57–0.82)	<0.001	0.87 (0.74–1.01)	NA
Hospitalization for heart failure	0.65 (0.50–0.85)	0.002	0.67 (0.52–0.87)	NA
Progression of albuminuria	0.62 (0.54–0.72)	<0.001	0.73 (0.67–0.79	NA

CI, Confidence interval; *NA,* not available.

Data from Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med.* 2015;373:2117–2128; Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med.* 2017;377:644–657.

To maximize the potential for these agents to be well tolerated, the starting dose should be low (e.g., one-fourth of the maximum dose) and taken just once daily, with slow increases in dosage and frequency of administration over a period of weeks or months.

Sodium-Glucose Transporter Inhibitors

This is the newest class of glucose-lowering medications, first marketed for the treatment of T2DM in 2013.[188–190] These agents depend upon the kidney's central role in the regulation of glucose dynamics and fluid balance. Under normal conditions about 180 g of glucose is filtered from plasma at the glomerulus and reabsorbed in the proximal tubule. That quantity is equal to a fairly standard daily dietary intake of carbohydrate. In the setting of diabetes, glucose appearance in the proximal tubule increases, and glucose reabsorption by SGLT2 increases as well. This high-capacity low-affinity transporter is responsible for 90% of renal tubular glucose reabsorption. There is a second active transport molecule, SGLT1, which is a low-capacity high-affinity transporter responsible for approximately 10% of glucose reabsorption in the kidney but critically important for glucose absorption from the gut. Naturally occurring mutations in the SGLT2 gene cause a rare familial renal glycosuria syndrome associated with up to 170 g glucose lost in the urine daily, lesser rates of obesity and diabetes, and no evidence of long-term renal consequences. When diabetes is poorly controlled, more glucose enters the renal tubules than can be reabsorbed, and glucose appears in the urine. Because increased reabsorption of glucose by SGLT2 is accompanied by increased reabsorption of sodium, sodium balance may also be disrupted in poorly controlled diabetes. Given these renal dynamics, it is not surprising that drugs blocking SGLT2 have important physiologic and clinical effects. They reduce both fasting and postprandial glucose levels and promote modest weight loss through loss of calories as glucosuria. They also reduce blood pressure, at least in part by increasing sodium clearance and reducing extracellular fluid volume.

Four SGLT inhibitors are currently available in the United States for clinical use: canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin. All are relatively specific for inhibiting SGLT2, but others in development also have some effect on SGLT1. The available agents have been studied in T2DM as monotherapy, in combination with other oral antihyperglycemic agents, and with insulin, with results that are quite similar between agents.[191] Studies comparing SGLT inhibitors with metformin, sulfonylurea, and DPP4 inhibitors suggest that these agents are about as effective in HbA_{1c} reduction as the other oral agents when used in combination regimens. In 26-week studies these agents lead to approximately 2 to 3 kg more weight loss than placebo.

Beyond these desirable effects that were expected based on known mechanisms of action, this class of drugs also provides cardiovascular benefits. The Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients—Removing Excess Glucose (EMPA-REG OUTCOME) trial was a large randomized cardiovascular outcome study of empagliflozin. It showed unexpectedly good effects of this agent in T2DM patients who were selected for having high cardiovascular risk. Treatment with empagliflozin for a median time of about 3 years was associated with a 32% relative risk reduction of all-cause mortality, 38% reduction of cardiovascular mortality, and 35% reduction of hospitalization for heart failure. Favorable effects of empagliflozin on established renal disease were also found in this study.[192] A subsequent report of similar studies of canagliflozin described generally similar, although quantitatively less prominent, cardiovascular and renal results.[193] These findings are summarized in Table 35.12.

Demonstration of strong short-term cardioprotective effects of this class of drugs has generated much enthusiasm for their use, supported by relatively consistent findings, including real-world studies.[194–199] It has also led to follow-up studies that should provide more information regarding whether all drugs in this class have similar effects, and to what extent these observations are applicable to T2DM patients without established cardiovascular or renal disease. There is particular interest in the potential value of SGLT inhibitors for patients with heart failure, a cardiovascular complication of diabetes that is increasingly common.[199,200] The mechanisms underlying these cardiovascular and renal effects are not well understood. At present it seems likely that several factors contribute, including improvement of glycemic control, blood pressure, and weight control, together with improved regulation of fluid balance and/or increased availability of ketones as fuel for injured cardiac or renal tissues.

The most common side effects of this class are related to glycosuria and include urinary frequency, genital infections, and relatively rare episodes of lower urinary tract infections as well as dehydration and its consequences. Genital infections are usually related to yeast, occur in approximately 10% to 15% of women (about a fivefold increase of risk), and may recur.[201,202] Genital mycotic infections are much less common in men and predominantly are found in those who have not been circumcised. A cardiovascular outcome trial found increased lower extremity amputations with canagliflozin.[195] There is still uncertainty about this risk and whether it could involve other agents in the class.[195,196,203] Theoretic questions related to bone metabolism have been raised, but an increased risk of fractures has not been found in most completed clinical trials.[190,204]

Good renal function is essential for full efficacy of SGLT inhibitors. Initiation is not recommended when eGFR is below 45 mL/minute per 1.73 m^2, and use should be discontinued when eGFR is lower than 45 mL/minute per 1.73 m^2. Although acute renal insufficiency has been reported, presumably due to changes in renal perfusion, this class of medications shows potential for long-term renal protection as well.[205–207] An unexplained imbalance in bladder cancer without a change in total cancer or cancer fatality was reported in early development of dapagliflozin.[208,209] Dapagliflozin is the only agent in the class with a warning against use of the drug in patients with bladder cancer.

As a final concern, there have been reports of diabetic ketoacidosis occurring without markedly elevated glucose levels (euglycemic diabetic ketoacidosis) during treatment of presumed T2DM with an SGLT inhibitor. Although some of the individuals affected certainly had T2DM, some likely had unrecognized adult-onset T1DM. In many cases the event was preceded by a surgical procedure, an acute illness, or an inappropriate reduction of insulin dosage. The mechanisms underlying this adverse effect are only partly understood, but may include a tendency toward dehydration, elevation of circulating glucagon levels, and accelerated gluconeogenesis due to glucosuria during fasting.[190,210] Any patient treated with an SGLT inhibitor who experiences persistent or recurrent nausea, vomiting, or malaise, or who develops a metabolic acidosis, should be evaluated for the presence of urine and serum ketones, even if glucose levels are near normal.[211]

Colesevelam

Colesevelam is a second-generation bile acid sequestrant. It was observed to mediate modest reductions in glucose during the clinical development program. An expanded program in patients with T2DM resulted in approval for marketing of this drug as an adjunct for the treatment of diabetes. It provides an HbA_{1c} reduction of about 0.5% in addition to approximately 15% improvement in LDL.[212] HDL changes tend to be trivial. Triglycerides can increase by 5% to 20%. Gastrointestinal side effects affect 10% or more of patients but seldom lead to withdrawals. The mechanisms for the glycemic effects are not definitively known.[213]

Bromocriptine

A quick-release formulation of bromocriptine administered within 2 hours of rising in the morning has been developed and is approved for marketing in the United States. It is suggested that creating a circadian peak in central dopaminergic tone improves insulin sensitivity. Nausea is the most common adverse effect, occurring in about 30% of patients and leading to discontinuation in about 10% at highest doses. Lower doses are better tolerated. HbA_{1c} reductions are generally modest but have been reported to be as high as 1.2%. In a 1-year safety study, broad cardiovascular outcomes were improved 40% compared with placebo.[214] Recent studies suggest substantial efficacy in the setting of inadequate glycemic control on high-dose insulin therapy.[215,216]

Glucose-Lowering Agents Requiring Injection

In addition to the glucose-lowering therapies that are orally administered small molecules, there is a group of relatively larger peptide agents that must be administered by other means. At present, these are mostly delivered by subcutaneous injection, but delivery by inhalation, sublingual absorption, nasal spray, or other means may become more common in the future. At present, one formulation of inhalable insulin is available for use, and other products that can be delivered by means other than injection are under study. Because injected therapies require more active participation by the patient, or assistance by others, more education and support in their use is needed than with oral therapies.

Insulins

Insulin has been commercially available since the early 1920s and is arguably the best studied form of therapy for diabetes. It is essential for all patients with T1DM and a mainstay of treatment for T2DM. Subcutaneous injection of insulin can be used to supplement endogenous production of insulin both in the basal state, to modulate hepatic glucose production, and in the postprandial state, in which a surge in insulin release normally further suppresses hepatic glucose production and facilitates glucose uptake by muscle and fat. Almost all insulins currently used worldwide are human insulins synthesized by recombinant technology or synthetic analogues of the human molecule.[217,218] The various formulations differ largely in their pharmacokinetics, as shown in Table 35.13. As patents on insulin analogues expire, equivalent products that are manufactured by different companies are becoming available. These "biosimilar" insulins appear to have effects very like those of the originally patented versions.[219,220]

Insulins are grouped into shorter-acting versus longer-acting formulations. This distinction has practical importance, as these two categories of insulin are used quite differently. Both short-acting and long-acting insulins have profiles of action that vary by the size of the dose injected. With higher doses the onset and time to peak effect become more delayed and the total duration longer.

Long-Acting Insulins

Long-acting insulins are usually initiated before short-acting ones. In the past, formulations of human insulin were modified to prolong its action by slowing absorption. The most widely used formulations were ultralente insulin and protamine zinc insulin (PZI), both of which were very long acting, and lente insulin and neutral protamine Hagedorn (NPH), both considered intermediate acting. Ultralente, lente, and PZI are no longer available, but NPH human insulin is still commonly used. The time to onset, peak effect, and full duration of action of NPH are about twofold greater than those of regular human insulin, with an onset in 1 to 2 hours, a peak usually at 4 to 8 hours, and a duration of 12 to 16 hours. Insulin detemir is a long-acting analogue in which a fatty acid side chain is covalently bound to the insulin molecule, resulting in slowing of both absorption from subcutaneous tissue and clearance from circulation. Detemir's time of onset and duration of action are quite similar to those of NPH, but more consistent from injection to injection.[221] To reliably provide a sustained between-meal and overnight (basal) effect, both NPH and detemir must be taken twice daily, usually before breakfast and either before dinner or at bedtime. However, for patients with

TABLE 35.13 Clinical Features of Commonly Used Insulins

Types and Generic Names (Commercial Names)	Onset of Action (Minutes)	Time to Peak (Hours)	Duration (Hours)	Administration
Rapid Acting				
Aspart (Fiasp)	<5	0.5–1.5	3–5	Just before or just after meals
Aspart (Novolog)	10–20	0.5–1.5	3–5	0–15 minutes before or just after meals
Lispro (Humalog)	10–20	0.5–1.5	3–5	
Glulisine (Apidra)	10–20	0.5–1.5	3–5	
Short Acting				
Regular human (Humulin R, Novolin R)	30–45	2–4	4–8	15-30 minutes before meals
Intermediate Acting				
NPH (Humulin N, Novolin N)	60–120	4–8	12–20	Once or twice daily
Long Acting				
Detemir (Levemir)	60–120	6–10	16–24	Usually once daily
Glargine (Lantus, Basaglar)	60–120	No pronounced peak	~24	
Degludec (Tresiba)	60–120	No pronounced peak	Up to 72	
Premixed				
70/30 NPH/R (Humulin 70/30, Novolin 70/30)	30–40	4–8	12–20	Usually twice daily, 0–30 minutes before meals
75/25 Protamine-lispro/lispro (Humalog Mix 70/30)	10–20	4–8	12–20	
70/30 Protamine-aspart/aspart (Novolog Mix 70/30)	10–20	4–8	12–20	
50/50 Protamine-lispro/lispro (Humalog Mix 50/50)	10–20	4–8	12–20	
50/50 Protamine-aspart/aspart (Novolog Mix 50/50)	15–60	4–8	12–20	
Concentrated				
U-500 human regular (Humulin U-500)	30–45	6–12	12–24	Twice daily
U-200 degludec (Tresiba U-200)	60–120	No pronounced peak	>24	Once daily
U-300 glargine (Toujeo 300 U/mL)	60–120	No pronounced peak	Up to 72	Once daily

T2D who add detemir as basal insulin while continuing noninsulin agents to cover the day, once daily dosing can be sufficient for at least 40% of patients.[58]

Insulin glargine is a long-acting analogue that is soluble at acid pH but precipitates when neutralized in tissues after injection. The resulting gradual absorption leads to a duration of action that, at doses commonly needed in T2DM, usually provides more than 24 hours of basal insulin effect with relatively little peak-to-trough variation. This action profile results in consistently less risk of hypoglycemia than occurs with NPH insulin, and has led to use of insulin glargine taken once daily, either before breakfast or at bedtime, as a reliable way to initiate insulin therapy.

Insulin degludec and a more concentrated formulation (300 units/mL) of insulin glargine have recently been introduced, and both provide even longer and flatter profiles of action than detemir and glargine.[222,223] Further prolongation of action of degludec is due both to slower absorption after injection and to delayed clearance, whereas that of glargine 300 units/mL results entirely from an effect on absorption. The pharmacokinetics of these newer long-acting insulins result in even lower risk of hypoglycemia than with glargine, but their advantage over glargine in this respect appears smaller than that with glargine versus NPH.[224]

Short-Acting Insulins

Regular human insulin and the rapid-acting analogues (insulins lispro, aspart, and glulisine) are all administered by injection with meals to assist in control of postprandial hyperglycemia.[217,218] Regular insulin has an onset of action about 30 minutes after injection, a peak at 2 to 4 hours, and duration of 6 to 8 hours or longer at high doses. The three rapid-acting analogues all have an onset in 5 to 15 minutes, peak activity in approximately 1 hour, and duration of approximately 4 hours. A more rapidly absorbed formulation of insulin aspart with onset of action within 2.5 minutes has recently become available.[225] Other very rapid-acting insulin formulations and delivery technologies are being developed. Regular human insulin administered intravenously is immediately effective, with a half-life on the order of 10 minutes. Lispro, aspart and glulisine are approved in the United States for intravenous use but offer no advantage over regular insulin for this purpose and cost much more.

U-500 regular insulin has been available for decades, but its use has increased in the setting of severe insulin resistance.[226] This concentrated insulin has a delayed and prolonged action profile compared to U-100 regular.[227] It has been administered twice daily, three times daily, and by insulin pump with good efficacy.[228] Pen devices are now available for ease and safety.

One inhaled formulation of human insulin is available.[229,230] The device by which it is delivered is quite convenient to carry and use. The insulin-loaded microparticles produce rapid onset of effect with peak activity in 30 minutes (the t_{max} is about 10 minutes), much earlier than with lispro.[231] The time to peak is extended with higher doses, and the duration also varies from 90 minutes at lower doses to 180 minutes with 12 units. Inhaled insulin may be preferred by patients who are very averse to taking injections, and some patients prefer its more rapid action to help with postprandial glucose control, but the shorter duration can be problematic. The current formulation requires spirometry before initiation, at 6 months, and annually thereafter. This preparation is contraindicated in patients with asthma or chronic obstructive pulmonary disease, and there are advisory precautions for patients with active lung cancer and smokers. Its use is associated with cough or throat irritation in addition to the usual hypoglycemic risk, and is at present limited to 4-unit, 8-unit, and 12-unit doses resulting in limitations of dose adjustments.

TABLE 35.14 Clinical Features of Commonly Used Injectable Agents Other Than Insulin

Types and Generic Names (Commercial Names)	Administration	Main Effects	Contraindications	Side Effects
Short-acting GLP1 agonist				
Exenatide (Byetta)	5–10 μg BID before breakfast and dinner	Postprandial glucose control and weight loss	T1D DKA Pancreatitis History medullary carcinoma	Nausea, diarrhea, abdominal pain Pancreatitis?
Lixisenatide (Adlyxin)	10–20 μg QD before breakfast			
Long-acting GLP1 agonist				
Liraglutide (Victoza)	0.6–1.8 mg daily	Basal glucose control and weight loss	T1D DKA Pancreatitis History medullary carcinoma	Nausea, diarrhea, abdominal pain Pancreatitis?
Dulaglutide (Trulicity)	0.75 or 1.5 mg weekly			
Extended-release exenatide (Bydureon)	2 mg weekly			
Semaglutide (Ozempic)	0.5 or 1 mg weekly			
Fixed-dose GLP1/insulin combination				
Liraglutide/degludec (Xultophy)	Daily, titrated	Glucose and weight control	T1D DKA Pancreatitis History medullary carcinoma	Hypoglycemia Nausea, diarrhea, abdominal pain Pancreatitis?
Lixisenatide/glargine (Soliqua)	Daily before breakfast, titrated			
Amylin agonist				
Pramlintide (Symlin)	TID before meals in T1D or T2D requiring prandial insulin	Postprandial glucose control and weight loss	Confirmed gastroparesis	Nausea Abdominal pain Hypoglycemia

Premixed Insulins

Premixed insulin formulations provide greater convenience and accuracy of dosing than mixing of two formulations in a syringe by the patient at the time of administration, but at the expense of reduced flexibility of dosing. Premixed formulations available in the United States are 70/30 and 50/50 mixtures of NPH and regular insulin, a 75/25 and a 50/50 mixture of lispro insulin in its NPH-like formulation with insulin lispro, and a 70/30 mixture of insulin aspart with its NPH-like congener. Premixed insulins provide a single-peak action at about 4 to 6 hours after each injection, as expected from summation of the activities of their components. In a very instructive study, use of 70/30 insulin in T2DM showed greater improvement in glucose control but at the cost of significantly more hypoglycemia compared to basal insulin.[232]

Human Versus Analogue Insulins

Insulin analogues are clearly helpful in managing T1DM, which requires complete replacement of endogenous insulin and accurate matching of the profile of action to daily needs. The degree to which both long-acting and rapid-acting analogues offer advantages over NPH and regular human insulin for T2DM is less well established and a focus of debate. Among the issues is that both the list price and the direct cost to patients can be much higher for the analogues.[142,233,234] Both long-acting and rapid-acting insulin analogues have been shown in large studies to cause less frequent hypoglycemia than human insulins, but for some patients little difference may be found. The absolute risk of severe hypoglycemia in patients with T2DM is relatively small, approximately one-third to one-tenth as high as in similarly treated patients with T1DM. This risk can be further minimized with appropriate education of patients and expectant monitoring of glucose at times when hypoglycemia is most likely to occur, such as at night or during unplanned or strenuous activity. Allergies to currently available insulins are rare, as are chronic skin reactions, which include lipoatrophy and lipohypertrophy.

Insulin Delivery Devices

Newer insulin needles cause less discomfort than those previously available because of a finer gauge, shorter length, sharper points, and smoother surfaces. Improved insulin pen design has made teaching a patient to take insulin much easier and provides greater convenience and accuracy of dosing. Pen injectors capable of injecting larger doses, up to 160 units, are now available for U-300 glargine and U-200 degludec. Insulin pump therapy has been used in patients with T2DM but is not widely accepted as cost effective in routine practice.[235–237] Insulin pump delivery of U-500 regular insulin has been proposed for highly insulin-resistant T2DM but is still a relatively uncommon application.[226]

GLP1 Receptor Agonists

GLP1 agonists are homologs or analogs of human GLP1 that have significantly changed the landscape of T2DM therapy.[179,238,239] As with insulins, GLP1 agonists divide logically into two groups: those that are short acting and those with longer duration of effects. Important features of the available agents in this class are shown in Table 35.14.

Short-Acting GLP1 Agonists

The first to be developed was exendin-4, a short-acting agent. It is a naturally occurring component of the saliva of the Gila monster *(Heloderma suspectum)* and shares 53% sequence identity with GLP1 but is relatively resistant to degradation by DPP4. Exenatide is synthetic exendin-4 and was the first GLP1-based therapeutic agent to be approved for human use.[240,241] When injected subcutaneously, it has a peak of action at about 2 hours and total duration of no more than 6 hours. Like GLP1 itself, it potentiates insulin secretion in response to rising glucose levels, suppresses postprandial glucagon secretion, delays gastric emptying, and promotes satiety. With twice-daily injection before breakfast and before dinner it produces a reduction of approximately 1% in HbA_{1c}, largely through a reduction of postprandial glucose along

TABLE 35.15 Treatment Effects of Liraglutide and Semaglutide in the LEADER and SUSTAIN-6 Studies

Endpoints	LIRAGLUTIDE IN LEADER		SEMAGLUTIDE IN SUSTAIN-6	
	Hazard Ratio (95% CI)	*p* Value	Hazard Ratio (95% CI)	*p* Value
Primary cardiovascular composite Cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke	0.87 (0.78–0.97)	0.01	0.74 (0.58–0.94)	0.02
Cardiovascular death	0.78 (0.66–0.93)	0.007	0.98 (0.65–1.48)	0.92
All cause death	0.85 (0.74–0.97)	0.02	1.05 (0.74–1.5)	0.79
Hospitalization for heart failure	0.87 (0.73–1.05)	0.14	1.11 (0.77–1.61)	0.57
Progression of albuminuria	0.74 (0.60–0.91)	0.004	NA	NA

CI, Confidence interval; *NA,* not available.

Data from Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med.* 2016;375:311–322; Marso SP, Bain SC, Consoli A, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med.* 2016;375:1834–1844.

with modest weight loss (2–4 kg). With prolonged use, these effects can be associated with modest improvements in blood pressure and lipids. The most common adverse effect is nausea, which occurs in up to 50% of patients, is usually mild to moderate in intensity, occurs early in the course of therapy, and typically wanes over time. Nausea leads to withdrawal of therapy in about 5% of patients.

Lixisenatide is a more recently introduced agent that has properties similar to those of exenatide except for a slightly longer duration of action.[242] It has been approved for administration once daily before breakfast. When given in this way it reduces postprandial increments of glucose during the daytime but has much less effect overnight. Both exenatide and lixisenatide are recommended to be started at the lower available dose and advanced to full dosage after several weeks to limit side effects on initiating therapy.

Long-Acting GLP1 Agonists

Newer, longer-acting GLP1 receptor agonists include liraglutide, an extended-release formulation of exenatide, dulaglutide, and semaglutide.[179,239,243] Liraglutide is administered once daily without any restriction as to timing or relation to meals. The other long-acting agents are to be given once weekly. In general, they are associated with greater HbA_{1c}-lowering efficacy than short-acting exenatide and lixisenatide, owing to their greater effects on overnight fasting glucose levels.[179,239] However, they have much less effect on postprandial increments of glucose than the short-acting agents. They also are associated with a lower frequency of gastrointestinal side effects. All the various long-acting agents appear to be quite effective in lowering HbA_{1c}, although some differences are shown in early head-to-head trials.[244–247] Some evidence suggests that semaglutide can cause superior reduction in HbA_{1c} but also more nausea.[245,246,248] Studies under conditions of clinical practice have shown some differences from randomized clinical trials, suggesting that results of treatment can be influenced by differences in adherence to therapy.[249] There are some differences in the pen injectors used for delivering doses of the various agents, and instruction in their use is needed to optimize dosing and adherence.[250]

Weight loss is consistently seen and similar among the various GLP1 receptor agonists, although only liraglutide is currently approved for weight loss in patients with or without diabetes (see Chapter 40). Semaglutide appears to have slightly more weight loss potential when used at doses approved for diabetes in the United States.

Recently, large randomized trials examining cardiovascular safety of two long-acting GLP1 agonists—liraglutide and semaglutide—have shown significant cardiovascular benefit in populations selected for having high cardiovascular risk.[251–255] Details of these studies are shown in Table 35.15. The Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) study followed patients randomized to treatment with liraglutide or placebo for a median of more than 3 years. Significant reductions of risk were found for the primary composite cardiovascular endpoint (13%), cardiovascular death (22%), all-cause mortality (15%), and progression of albuminuria (26%).[252] The Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes (SUSTAIN-6) had a generally similar design but studied patients starting with somewhat worse glycemic control who received randomized treatment for about 2 years.[251] This study demonstrated somewhat greater reduction of risk for its primary cardiovascular composite endpoint (26%) compared with LEADER and showed a reduction of renal endpoints. However, no reduction of mortality was found. Neither LEADER nor SUSTAIN-6 showed a reduction of hospitalizations for heart failure. Up to now, these favorable outcomes have not been found with short-acting GLP1 agonists and there has been inconsistency in the specific benefits seen in trials of the other long-acting agents. Many of the variations could relate to the study designs and populations selected in those trials.

Hypoglycemia is not a direct effect of GLP1 receptor agonists but can occur when they are added to other agents. When one is added to prior therapy with a secretagogue, it is prudent to reduce to the minimum dose of secretagogue, at least initially. When a GLP1 receptor agonist is added to insulin, a 20% reduction of insulin dosage is advised unless HbA_{1c} is greater than 8%.

Concern about pancreatitis has emerged from postmarketing reports of GLP1 receptor agonists. A direct causal link has not been proved, although passage of gallstones accompanying weight loss remains a possible mechanism. It is recommended that drugs in this class be avoided in patients with a history of pancreatitis.[256] Exenatide is renally cleared and is contraindicated in the setting

of advanced kidney disease (eGFR <30 mL/minute/1.73 m^2). The other GLP1 receptor agonists do not share this feature with exenatide. Nevertheless, cases of acute renal failure have been reported in association with GLP1 receptor agonist therapy, usually in patients with chronic renal insufficiency who develop superimposed prerenal azotemia in the context of prolonged anorexia, nausea, and vomiting. To mitigate the risk of pancreatitis and renal failure, it is prudent to instruct patients treated with GLP1 receptor agonists to stop their medication if they develop nausea, vomiting, or abdominal pain of more than a few hours' duration and to seek medical attention if they are unable to keep down fluids after 4 hours.

Another safety concern is medullary thyroid cancer. No signal exists for this problem with GLP1-based therapy in humans, but a clear increase in the incidence of these tumors was observed in preclinical testing in rodents, although not in other animal models. It is suggested that these agents be avoided in those with a personal or family history of medullary thyroid cancer.

Amylin Receptor Agonists

Amylin is a neuroendocrine hormone that is cosecreted with insulin by pancreatic beta cells. A deficiency of amylin is therefore evident in patients with T1DM or T2DM, in parallel with insulin deficiency. Amylin and insulin have complementary actions in regulating plasma glucose. Amylin binds to brain nuclei and promotes satiety and reduces appetite. Through efferent neural pathways it mediates a decrease in the rate of gastric emptying and limits inappropriate glucagon secretion in a glucose-dependent fashion. By these means it regulates the rate of plasma glucose appearance from the gastrointestinal tract and the liver. Insulin concurrently regulates the rate of glucose appearance from the liver and stimulates glucose uptake in muscle and fat.

Amylin is relatively insoluble in aqueous solution and aggregates on plastic and glass. Pramlintide was developed as a soluble, nonaggregating amylin analogue, with similar clinical effects, that is administered by injection before meals. It is approved for use by patients with T1DM or T2DM requiring both basal and mealtime insulin.[257] Most of the effect on glycemic patterns is a reduction of postprandial increments, with little effect on overnight control. When pramlintide is added to insulin therapy with or without metformin or a sulfonylurea in T2DM, HbA_{1c} can be reduced by about 0.5 to 0.7 percentage points, and weight is variably reduced as well.[258]

Mild or moderate nausea, which wanes with continued therapy, is pramlintide's most common adverse effect. It is minimized by initiating therapy with 60 μg before meals, with an increase to the full 120 μg dose over 1 to 2 weeks as tolerated. Hypoglycemia is less frequent in patients with T2DM than in patients with T1DM and can be limited by reducing dosage of mealtime insulin by about 50% when initiating therapy, with the option of subsequently returning to higher doses as needed.[257] Oral medications that require rapid absorption for effectiveness should be administered either 1 hour before or 2 hours after injection of pramlintide. Coformulations of pramlintide with regular human insulin are being evaluated.

Practical Aspects of Treatment

A Team Approach

Considering the wide array of interventions available, and the need to adapt to differences between patients as well as changing needs over time, it is obvious that many treatment decisions will be needed for every patient. It is also clear that various kinds of providers with differing training and experience can contribute to making and implementing these decisions. No one provider can do it all, and the concept of a team approach to management of diabetes is well established. Teams may include nurses, nutritionists, pharmacists, educators, and physicians, all with specific interest and experience in diabetes. Although the previous discussion of DSMES describes in detail the services and processes required for care, it does not define specifically the kinds of providers needed. This is because different health systems use different models for providing DSMES, with different configurations of providers. However, in each setting a locally available group of providers should be identified who can function as a team with each individual patient. Providers specialized in other aspects of care commonly needed by people with T2DM—such as cardiology, nephrology, ophthalmology, and podiatry—also can contribute to the team effort. The overall goal of this collaboration is to ensure optimal management of overall metabolic control, other risk factors for complications of diabetes, and the complications themselves. Making the team concept a reality is a major challenge for health systems today.

The specialized team can assist at various points in the natural history of T2DM. As noted in Fig. 35.2 and Table 35.7, these include the time of diagnosis, a later time when injected therapies or use of devices such as CGM systems are needed, or whenever diabetes-related problems such as severe hypoglycemia, ketoacidosis, or repeated hospitalization occur or reoccur.

Identifying Pathophysiologic Subgroups

The time of diagnosis presents an opportunity to favorably influence the entire future experience of the patient. It is typically a teachable moment at which the patient can be introduced to DSMES and develop a long-term plan of management. It is also an opportunity to identify those individuals who do not have the most typical form of T2DM and adjust the plan accordingly. Several of the more common atypical subgroups are discussed in this section.

Latent Autoimmune Diabetes of Adulthood

The largest subgroup is that with diabetes of autoimmune origin, commonly called *latent autoimmune diabetes of adulthood* (LADA) or adult-onset type 1 diabetes. This group comprises up to 12% of patients diagnosed with diabetes after age 20.[259,260] Such individuals may have rapid deterioration of glycemic control prior to diagnosis, sometimes accompanied by prominent symptoms of hyperglycemia and weight loss, but these tend to progress less rapidly than in youth-onset T1DM. They frequently are not obese and may have other autoimmune disorders or a family history of these. Laboratory support can be provided by detection of anti–glutamic acid decarboxylase (GAD) antibodies or anti-islet cell antibodies, although lack of antibodies does not exclude an autoimmune process. Both family history and genetic markers are likely to be more consistent with T1DM, but there is heterogeneity and uncertainty in classifying individual cases.[261,262] Insulin has been considered the main therapy from the outset, but other therapies may be possible in those with slow progression. There is some evidence that early use of insulin may prolong beta-cell function and prevent a period of sustained hyperglycemia that could initiate chronic complications.

Pancreatic Diabetes

Another relatively common subgroup comprises patients with other disorders affecting the pancreas. Pancreatic diabetes has been referred to as type 3c diabetes and is underdiagnosed.[263,264] The leading causes include acute or chronic pancreatitis, pancreatectomy for cancer or pancreatitis, hemochromatosis, and cystic fibrosis. Despite widely differing pathogenesis, patients with pancreatic diabetes share a tendency to have significant deficiency of insulin secretion and prominent postprandial hyperglycemia, and may respond less well than expected to oral therapies.[264] In addition to insulin deficiency, there are significant decreases in glucagon secretion and in the effects of incretin hormones. As in the case of LADA, patients in this subgroup commonly need insulin at or relatively soon after diagnosis and quite frequently require both basal and prandial insulin. In some cases, due to the multihormonal loss, there is more glucose variability than is seen in T2DM or T1DM.[265]

Monogenic Diabetes

A smaller but increasingly recognized subgroup is that with monogenic diabetes. This is a heterogeneous group of heritable disorders, which are often diagnosed in childhood but sometimes present as diabetes diagnosed in adulthood.[266–268] A subset of this group of disorders has been commonly described as maturity-onset diabetes of the young (MODY). The proportion of newly diagnosed adults with one of these conditions is estimated to be between 1% and 2%. The diagnosis should be considered in atypical cases that might otherwise be diagnosed as T1DM or T2DM. A strong family history consistent with autosomal dominant inheritance with high penetrance is typical but not always present. A definitive diagnosis depends on genetic testing that can identify specific subtypes and thus assist in planning treatment. This approach to monogenic diabetes is one of the best present-day examples of precision medicine.

Two of these disorders deserve specific comment. One is a heritable deficiency of glucokinase (GCK-MODY) that alters glucose sensing in the beta cell.[269] It presents typically in youth as mild fasting hyperglycemia (e.g., 100–140 mg/dL) that is not progressive and causes little or no tissue damage over time. The HbA_{1c} may range from 5.6% to 7.8%. In many cases this disorder is not recognized until routine chemistry tests indicate elevated fasting glucose levels typical of prediabetes. It is often recognized in women during testing for gestational diabetes. Recognition of GCK-MODY is important mainly to avoid unnecessary treatment of the patient and other identified members of the family. Current evidence indicates that those with a GCK mutation will not develop chronic complications of diabetes, and treatment is not likely to render significant changes in glucose control. However, longer follow-up of larger numbers or individuals with this disease will be needed to be certain.[270]

In contrast, the most common form of monogenic diabetes, hepatic nucleocyte factor-1A (HNF1A), has direct clinical relevance.[271] It can present in adulthood with progressive hyperglycemia, more postprandial than fasting, that responds very well to sulfonylureas. Other monogenic forms of diabetes, such as HNF4α mutations, may also respond to sulfonylureas, which bind to the molecular complex on the surface of beta cells that is genetically affected. Successful responses to sulfonylureas may persist for extended periods of time. The possibility of sulfonylurea-responsive monogenic diabetes can be suspected when there is a prominent history of adult-onset but quite stable and slowly progressive diabetes on one side of the patient's family, often in the absence of obesity. Laboratory screening for these disorders is now more available than in the past but not always covered by insurance. Identification of affected families can avoid unnecessary or inappropriate therapy for many family members.

Standardized Versus Personalized Tactics

The remaining majority of patients with diabetes newly diagnosed in adulthood can be considered to have T2DM. Although some progress is being made in identifying subgroups based on clinical characteristics,[272] at the present time it is simplest to begin with relatively standardized, algorithmic approaches that will be described later. Moreover, even after separation of subgroups, T2DM remains physiologically heterogeneous and patients also differ markedly in their daily habits, preferences, and capacities for self-management. The metabolic defects leading to diabetes also tend to worsen, and the burden of diabetes-related and other infirmities increases over time. For all these reasons, whatever approach is first used will need to be personalized eventually. This expectation can from the start be shared with the patient, who should be enlisted to participate actively in making decisions beyond any initial algorithm.

A Standardized Initial Therapeutic Approach

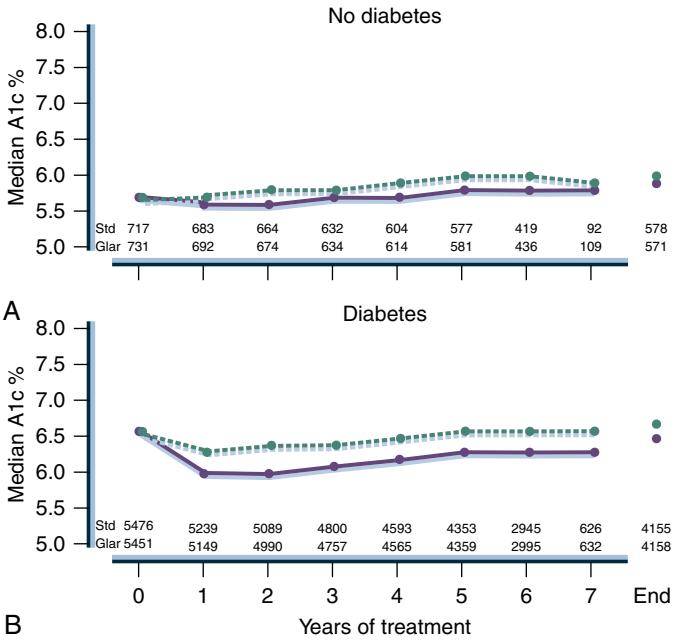
The most critical part of long-term glycemic management is continuing reassessment of glucose patterns and HbA_{1c} values to guide refinement of interventions. The goal is to maintain optimal control with the lowest doses of the fewest medications. In general, initial drug therapy should be considered from the time of diagnosis, along with lifestyle intervention, and by 5 years after diagnosis most patients require two or more drugs to maintain selected targets. Early in therapy it is usually desirable to add additional agents to prior therapies, rather than replacing one agent with another. Guidance from randomized trials, when available, is desirable in making therapeutic choices. A general approach to be considered in the absence of any patient-specific factors is suggested here.[142,159]

Metformin

Metformin is considered the default choice for initial drug therapy. Used in combination with diet, exercise, and a DSMES program it can usually provide impressive lowering of glucose with essentially no risk of hypoglycemia.[273] However, approximately 5% of patients will experience gastrointestinal symptoms with even low doses of metformin, and others should be prescribed metformin only with caution due to impaired renal function or serious cardiac, liver, or intestinal disease. If metformin is contraindicated or poorly tolerated, or when the response to it is inadequate after a trial of 3 to 6 months, various agents can be substituted or added. There are six recommended second-line therapies: sulfonylureas, TZDs, DPP4 inhibitors, SGLT inhibitors, GLP1 receptor agonists, and basal insulins. Each has advantages and disadvantages.

Stepwise Combination Therapy

Because of more than 60 years of experience, widespread current use, and clear evidence of benefit relative to risk from long-term randomized interventional studies, sulfonylureas and basal insulins are still appropriate considerations for second-line therapy. Addition of a sulfonylurea to metformin may serve as an example of the value of combination regimens that is relevant to many other combinations of agents in different classes. Because most classes act through distinctive mechanisms, their glycemic effects



• **Fig. 35.4** Long-term control of HbA_{1c} in the ORIGIN trial. Participants (n = 12,537) with dysglycemia (impaired glucose tolerance or impaired fasting glucose) or diagnosed T2DM on diet with or without one oral agent were randomized to treatment based on insulin glargine *(closed circles, solid lines)* or to sequential use of oral therapies *(open circles, dashed lines)*, seeking fasting plasma glucose 95 mg/dL (5.3 mmol/L) or less. Both regimens maintained HbA_{1c} levels close to 6.5% for the duration of the trial, shown for study participants with dysglycemia at study entry in (A) and for those with diabetes at study entry in (B). (Modified from the ORIGIN Trial Investigators. Characteristics associated with maintenance of mean A1C<6.5% in people with dysglycemia in the ORIGIN trial. *Diabetes Care.* 2013;36:2915–2922.)

are usually additive. Moreover, combining agents generally allows greater glucose lowering without increased side effects. In the case of metformin and the preferred sulfonylureas (glimepiride, long-acting glipizide, and gliclazide), more than half the maximal therapeutic effect is obtained at half maximal dosage. At the same time, the leading side effects—gastrointestinal symptoms with metformin and hypoglycemia with sulfonylureas—are more likely at higher doses. As a specific example, combining metformin 500 mg twice daily with glimepiride 1 mg daily is likely to provide greater therapeutic power and fewer side effects than full dosage of either alone. However, if metformin has previously been titrated to the maximally effective dose of 2000 mg daily and is well tolerated, there is no reason to decrease the metformin dose when adding a second agent, including sulfonylureas, unless renal function is impaired. Continuing metformin and a sulfonylurea when basal insulin is added similarly may allow continuation of good glycemic control while keeping insulin dosage and risk of hypoglycemia low.

The effectiveness of combination therapy approaches using these agents in the Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial is shown in Fig. 35.4.[274] This trial enrolled participants who had either dysglycemia (impaired glucose tolerance and/or impaired fasting glucose) or T2DM treated with diet and no more than one oral agent. Participants were selected for having high cardiovascular risk. They were randomly assigned to one of two regimens, each seeking fasting glucose no higher than 95 mg/dL (5.3 mmol/L). One regimen was based on glargine as basal insulin with continuation of any prior oral therapy, the other on conventional stepwise use of oral therapies with insulin added only if necessary. Participants were followed an average of about 5 years. Both regimens maintained HbA_{1c} levels close to 6.5% during the study, which for those with T2DM at entry was up to a total of 10 years after diagnosis.[275] Most participants in both randomized arms used only metformin, a sulfonylurea, or basal insulin, confirming the ability of these older agents to provide good glycemic control for many patients up to 10 years after diagnosis. No differences in medical outcomes between the two arms of the trial were observed other than more hypoglycemia in the group assigned to initial glargine therapy and less progression from dysglycemia to overt diabetes in that group.

Considerations in Personalizing Therapy

As the array of glucose-lowering therapies continues to grow, promising results of clinical trials using newer agents are providing additional guidance for therapy. As a result, personalizing drug therapy beyond traditional use of metformin, sulfonylureas, and basal insulin is increasingly common and very appropriate. Any of the classes of agents other than metformin may be considered as second-line therapy when there are specific reasons to do so.[276] In many cases, specific short-term clinical advantages may be judged to outweigh greater cost and lack of long-term experience. The rationale for the choice of therapy should be discussed with the patient, especially in the case of the newest agents.

Oral Agents and Injected Therapies Other than Insulin

The DPP4 inhibitors, the TZD pioglitazone, and long-acting GLP1 agonists are often used in combination with metformin. The glucose-lowering effect of DPP4 inhibitors is often stated to be less than that of sulfonylureas, but they are unlikely to cause hypoglycemia and have few symptomatic side effects.[277] Furthermore, it has been argued that some of the apparent difference in efficacy is due to considering older sulfonylurea studies where baseline HbA_{1c} was higher.[278,279] Pioglitazone is variably effective in controlling glucose and often causes weight gain and edema, among other side effects, but it improves peripheral insulin sensitivity and can reduce hepatic fat content in patients with nonalcoholic steatohepatitis (NASH).[280,281] It is most often prescribed in lower doses now when treating T2DM, although many of the original efficacy studies and the NASH studies have been performed with the 45-mg full dose. Fixed-dose combination formulations of metformin with a DPP4 inhibitor or pioglitazone can facilitate dosing.

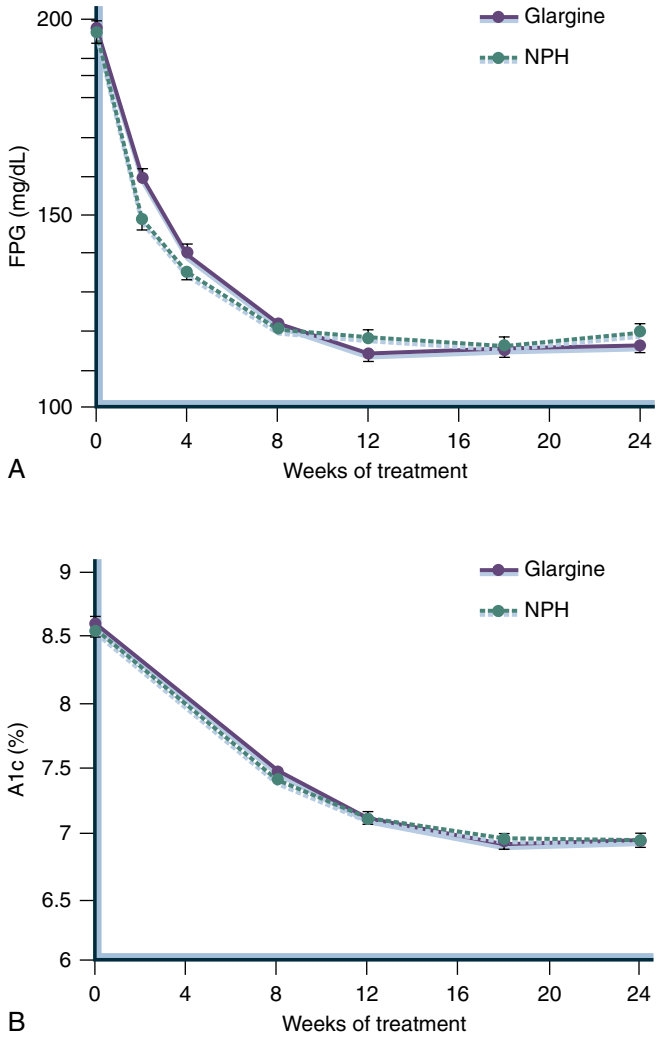
The injectable GLP1 receptor agonists are increasingly proposed as similarly effective alternatives to basal insulin for patients who are known to have high cardiovascular risk because of the cardiovascular protection shown with liraglutide and semaglutide.[251,252] However, they often cause gastrointestinal symptoms requiring thoughtful titration. Use of the long-acting GLP1 agents with once-weekly dosing may improve convenience and reduce symptoms, but even so gastrointestinal side effects can adversely affect adherence. Fixed-dose combinations of basal insulin with GLP1 agonists have been proposed as initial injectable therapy, and while they are reliably effective in reducing glucose the selection of proper doses and the titration process itself require specialized skills.[282,283] Because the mechanism of action of GLP1 agonists is similar to that of DPP4 inhibitors, there is no advantage to continuing the latter agents when an injected GLP1 agonist is started.

Following the studies showing cardiovascular benefits with empagliflozin and canagliflozin, early use of an SGLT inhibitor has been proposed for consideration for patients with established cardiovascular disease, especially when heart failure has been diagnosed or suspected. However, the glucose-lowering effect of these agents is only moderate, and because they are new the magnitude of risk from several side effects has not been fully explored.[198,284,285]

Basal Insulin

Uniquely among the classes of glucose-lowering therapies, use of insulin must always be personalized. The need to personalize dosing of insulin is driven by both physiologic and behavioral factors. The strategy of adding basal insulin when agents other than insulin are no longer successful is well established.[286,287] Some practitioners prefer to stop oral glucose-lowering agents when insulin is started, especially when using twice-daily premixed insulin, but most continue oral therapy while adding NPH or a long-acting analogue as basal insulin. NPH is usually given as a single injection at bedtime or twice daily, before breakfast and at bedtime. The long-acting analogues can be usually given once daily either in the evening or before breakfast, depending on the patient's preference. They are significantly less likely to cause hypoglycemia overall and especially at night, compared with human NPH insulin. The newest long-acting analogues, degludec and the U-300 formulation of glargine, offer a modest further reduction of risk of hypoglycemia compared with U-100 glargine, especially for patients using lower doses and those with prior difficulties with hypoglycemia.[221,223,288–291] There is evidence that hypoglycemia during initiation of basal insulin is associated with lower adherence and higher costs of care.[292] Delays in the initiation of basal insulin as well as its appropriate titration over time and the progression beyond basal insulin have all been characterized as clinical inertia.[293,294]

Personalization of insulin applies particularly to the dose required. The necessary daily dosage of basal insulin ranges between 10 and 200 units, and a given patient's requirement must be determined by titrating to a fasting glucose target. Treatment can be started either with a fixed daily dose of 10 units or with a dose calculated as 0.1 to 0.2 units per kilogram body weight.[159] Dosage should be increased at regular intervals, guided by SMBG before breakfast. Various titration schemes can be used with equal success.[295] Once-weekly titration by 2 to 4 units is commonly used, as is daily titration by a single unit every day until a target level of fasting glucose is attained. For patients continuing metformin alone or with one additional oral agent, a typical dose of basal insulin required to approach the fasting glucose target is 0.4 to 0.5 units per kilogram, but with wide variation between patients. In many clinical trials the titration target has been set at 100 mg/dL (5.5 mmol/L) or less, and early in the natural history of T2DM this is often attainable with little or no risk of hypoglycemia. However, when insulin is started after HbA_{1c} levels have increased to above 8%, an initial target of less than 120 mg/dL (<6.7 mmol/L) is safer and more realistic. The glucose goals and the importance of titration must be emphasized at each visit, as it has been shown that patients often do not remember the initial instructions in basal insulin dosing.[296] In most cases a patient-directed titration is safe and effective, even to fasting goals below 100 mg/dL.[297] Fig. 35.5 illustrates the responses of fasting glucose and HbA_{1c} to this approach to starting basal insulin with use of human NPH versus insulin glargine.[57] A stable level of fasting glucose can often be attained after 12 weeks of careful titration, with HbA_{1c} levels lagging slightly behind as expected. Failure to maintain HbA_{1c} close to 7% despite use of a regimen that includes properly titrated basal insulin calls for attention to postprandial hyperglycemia.



• **Fig. 35.5** Responses of fasting plasma glucose (A) and HbA_{1c} (B) after initiation and titration of neutral protamine Hagedorn (NPH) or glargine as basal insulin with continuation of prior oral therapies. (From Riddle MC, Rosenstock J, Gerich J. Insulin glargine 4002 study investigators. The treat-to-target trial: randomized addition of glargine or human NPH insulin to oral therapy of type 2 diabetic patients. *Diabetes Care*. 2003;26:3080–3086.)

Treating Postprandial Hyperglycemia

The greatest challenge to personalization is presented by therapy of postprandial hyperglycemia. Hyperglycemic exposure in untreated T2DM is quantitatively due more to overnight and between-meal (basal) hyperglycemia than to further elevations after meals. The predominance of basal hyperglycemia is more pronounced when overall control is poor and HbA_{1c} is above 8%.[298] Fortunately most glucose-lowering therapies are more effective in controlling basal hyperglycemia than limiting postprandial increments. Specifically, metformin, the long-acting secretagogues, basal insulin, DPP4 inhibitors, long-acting GLP1 agonists, and SGLT inhibitors have greater basal than postprandial effects. After initial therapy with one or several of these agents, most of the residual glycemic exposure is postprandial.[299] As beta-cell function declines over time, postprandial hyperglycemia becomes more prominent, and maintaining HbA_{1c} in a target range becomes more difficult. After

therapy of basal glucose has been optimized, the postprandial glucose excursion is generally highest after breakfast, and the highest postprandial glucose is after dinner.[300] At this stage of T2DM, which commonly is reached by 10 years after diagnosis, further therapy targeting postprandial control is needed. Use of prandial therapies demands consideration of the patient's eating habits, and these can differ markedly between individuals and in each patient from day to day.

In addition to dietary strategies—more frequent but smaller meals and selection of foods that are absorbed less rapidly and have less tendency to increase glucose—several drugs can be helpful. The short-acting secretagogues modestly limit postprandial increments of glucose when taken at low dosage two or three times a day with meals.[301,302] The alpha-glucosidase inhibitors reduce postprandial hyperglycemia significantly and have favorable effects on weight, but their use is often limited by gastrointestinal side effects.

Short-acting insulins are more effective and are the most widely used approach. Traditionally, a direct change from basal insulin alone to full basal-bolus therapy with injections prior to two or three meals has been recommended. Recent studies suggest that adding a single injection of short-acting insulin (at the morning meal or at the largest meal) is just as effective as basal-bolus therapy at this stage and is less likely to cause weight gain or hypoglycemia.[289,303,304] Initial dosage for the first prandial injection can be 4 to 6 units, with systematic titration upward by 1 to 2 units weekly, aiming for glucose prior to the next meal (or at bedtime if the dose is prior to dinner) approaching 120 mg/dL. When prandial insulin is added to basal insulin with continuation of one or more oral agents, optimal control often requires total daily insulin doses greater than 1 unit per kilogram of body weight daily. In the typical patient with insulin-resistant T2DM, there is usually little advantage to splitting basal insulin into two injections. If a second injection is needed it usually should be prandial insulin. As with basal-bolus insulin therapy in T1DM, close monitoring and effective DSMES are needed.

While adding prandial insulin has been a common practice when basal insulin is not adequately effective in T2DM, an increasingly popular new option is the addition of a GLP1 receptor agonist while continuing basal insulin. The shorter acting drugs in this class have the greater postprandial effect, but the longer acting ones—despite having less ability to control postprandial glucose excursions—may improve overall control sufficiently to equal the effect of added prandial insulin. Compared with addition of prandial insulin, addition of a GLP1 agonist has several advantages: Less SMBG is required, hypoglycemia is less likely, and weight gain will be avoided.[305]

Examples of Personalized Choices of Therapy

Fig. 35.6 illustrates four specific ways in which personalized choices of therapy may be applied.

Minimizing Cardiovascular Risks

Evidence supporting initial use of metformin for reducing cardiovascular risk has established this drug as the fundamental therapy for T2DM.[29,306] Recently, the quest for further therapy for patients with high cardiovascular risk has favored early use of GLP1 agonists and SGLT inhibitors in some settings. An expert group has recently suggested that GLP1 agonists with evidence of cardiovascular benefit should be preferred over insulin as the first injected therapy for T2DM in the setting of established atherosclerotic cardiovascular disease, provided they are locally available and affordable. Similarly, the expert group proposed use of an SGLT inhibitor with evidence for cardiovascular benefit as second-line therapy after metformin for patients with known or suspected heart failure or chronic kidney disease provided eGFR is not seriously reduced.[90] It is still unknown whether benefits of these two classes of drugs for these conditions can be extrapolated to all drugs in each class and whether primary protection against cardiovascular or renal events will be found in the larger numbers of T2DM patients with relatively low cardiovascular risk. More specific information as to which patients will derive the greatest cardiovascular benefit from these newer agents is needed and likely to become available soon.

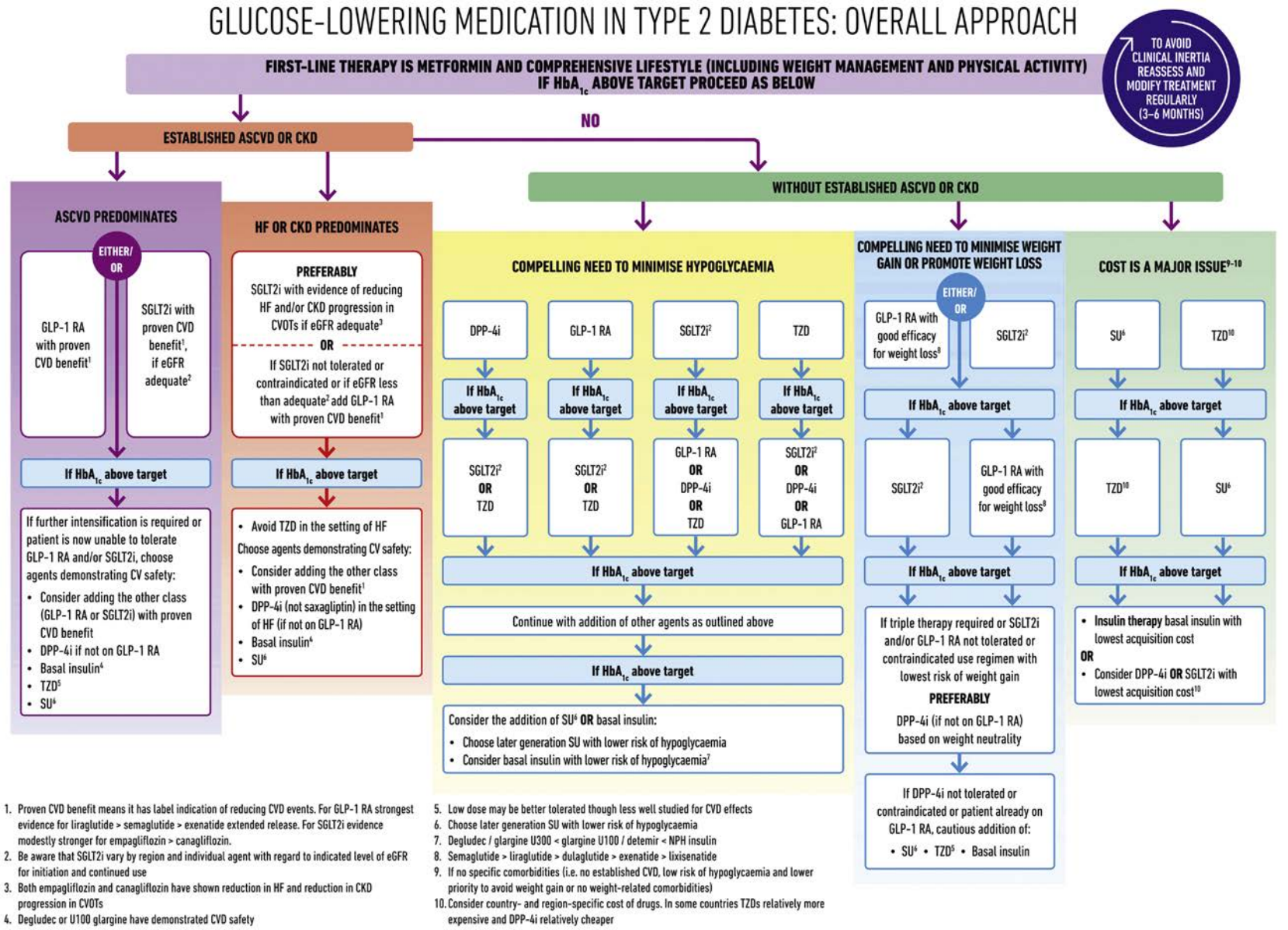
Sulfonylureas and insulin have been suspected of increasing cardiovascular risk. Although direct evidence supporting this contention is weak, the availability of other agents providing glycemic control allows these classes to be moved down the priority list for patients with known cardiovascular disease. It seems especially desirable to avoid use of prandial insulin, which is much more likely to cause weight gain and hypoglycemia than basal insulin. Pioglitazone increases the risk of heart failure in susceptible patients and thus is also less appropriate in this setting.

Minimizing Hypoglycemia

Hypoglycemia is of concern for reasons beyond its possible contribution to cardiovascular events. Older patients with a long duration of diabetes, especially those who have cognitive impairment or nephropathy, have greater risk of hypoglycemia-related falls or other injuries.[307,308] Fear of hypoglycemia can limit attainment of even conservative glycemic treatment goals, either due to therapeutic inertia on the part of the provider or incomplete adherence by the patient. A prior history of hypoglycemia requiring assistance by another person is a strong risk factor for future hypoglycemia. When these considerations apply, therapeutic tactics should be adjusted to avoid hypoglycemia. Metformin does not cause hypoglycemia when used alone, and drug choices after metformin with little or no additional risk include DPP4 inhibitors, GLP1 agonists, SGLT inhibitors, and TZDs.[90,309] Combination therapy using these agents could provide considerable glucose-lowering power with little risk of hypoglycemia. Should insulin eventually become necessary, the newer long-acting insulins will cause the lowest risk of hypoglycemia. Finally, prior occurrence of severe hypoglycemia may be sufficient reason to increase the HbA_{1c} target range to 7% to 8% or even higher.

Minimizing Weight Gain

Weight gain associated with the treatment of diabetes is of concern to most clinicians and can be an overriding issue for some patients. A strategy to minimize weight gain would emphasize diet and exercise and use metformin as initial therapy. Both GLP1 agonists and SGLT inhibitors are options as second-line therapy when weight is a major consideration. Surgical procedures that lead to both weight loss and improved glycemic control are beyond the scope of this chapter, but are increasingly considered when weight control is a primary concern in T2DM.[310–313] Because of their tendency to cause weight gain, sulfonylureas, pioglitazone, and prandial insulin are less desirable. Basal insulin is less likely to cause weight gain than prandial insulin and can be included in the regimen when necessary.



1. Proven CVD benefit means it has label indication of reducing CVD events. For GLP-1 RA strongest evidence for liraglutide > semaglutide > exenatide extended release. For SGLT2i evidence modestly stronger for empagliflozin > canagliflozin.
2. Be aware that SGLT2i vary by region and individual agent with regard to indicated level of eGFR for initiation and continued use
3. Both empagliflozin and canagliflozin have shown reduction in HF and reduction in CKD progression in CVOTs
4. Degludec or U100 glargine have demonstrated CVD safety
5. Low dose may be better tolerated though less well studied for CVD effects
6. Choose later generation SU with lower risk of hypoglycaemia
7. Degludec / glargine U300 < glargine U100 / detemir < NPH insulin
8. Semaglutide > liraglutide > dulaglutide > exenatide > lixisenatide
9. If no specific comorbidities (i.e. no established CVD, low risk of hypoglycaemia and lower priority to avoid weight gain or no weight-related comorbidities)
10. Consider country- and region-specific cost of drugs. In some countries TZDs relatively more expensive and DPP-4i relatively cheaper

• **Fig. 35.6** Glucose-lowering medication in type 2 diabetes: overall approach. *ASCVD,* atherosclerotic cardiovascular disease; *CKD,* chronic kidney disease; *CVD,* cardiovascular disease; *DPP-4i,* dipeptidyl peptidase-4 inhibitor; *eGFR,* estimated glomerular filtration rate; *GLP-1 RA,* glucagon-like peptide-1 receptor agonist; *HF,* heart failure; *NPH,* neutral protamine Hagedorn; *SGLT2i,* sodium glucose transporter-2 inhibitor; *SU,* sulfonylurea; *TZD,* thiazolidinedione. (From Davies MJ, D'Alessio DA, Fradkin J, et al. Management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care*. 2018;41(12):2669–2701.)

Minimizing Costs

For many patients, including large populations in developing countries and individuals with inadequate insurance coverage in developed countries, the cost of drugs limits management of diabetes. Despite the effectiveness of newer agents in clinical trials where they are provided free of charge, no therapy is successful when it is unaffordable. Fortunately, diet and exercise can be effective and are almost cost free. The least expensive drugs for the treatment of diabetes are metformin, sulfonylureas, pioglitazone, alpha-glucosidase inhibitors, and human insulin. There can be marked differences in price between regions and between pharmacies, and websites are available to find the best prices.[314] In the case of insulin, there is a name brand of human regular, NPH, and 70/30 premixed human insulin (ReliOn) that is generally available in the United States for approximately 10% of the list price of analogue insulins. Human insulin remains an effective option for treatment of T2DM, although its use may require twice-daily dosing of NPH, more consistent timing of meals, and slightly higher HbA_{1c} targets (e.g., 7.5–8%) to limit hypoglycemia. A recent statement by the World Health Organization (WHO) has noted and endorsed these considerations for treatment of T2DM in low-resource settings.[315]

Special Situations in Clinical Management

In addition to the principles of treatment described for most T2DM patients in the usual ambulatory setting, some specific therapeutic challenges deserve further comment.

Severe Hyperglycemia at Diagnosis

Some patients will come to diagnosis of T2DM with very poor glycemic control, even ketoacidosis.[316] Those who are especially ill, with serious dehydration, acidosis, impaired mental status, or serious concomitant illness will need hospitalization to receive intravenous fluids and insulin. Some will recover remarkably after in-hospital treatment, with substantial recovery of insulin secretion and tissue responsiveness to insulin. Those with the best metabolic recovery may maintain good glycemic control with lifestyle management and little or no oral therapy. How such patients differ from those who continue to need routine oral therapy or insulin is not entirely clear, but they have been described as having ketosis-prone diabetes.[317]

Many other patients will be diagnosed when they have poor glycemic control, with HbA_{1c} above 9%, but are not acutely ill. Several studies have suggested that intensive glycemic management in this setting may also lead to a substantial remission of diabetes by reversal of glucolipotoxicity in a significant number of patients.[318,319] After 2 to 4 weeks of treatment with oral combination therapy, basal insulin, or basal plus prandial insulin seeking nearly normal glucose levels, HbA_{1c} levels below 6.5% or 7% may persist for up to 1 year, even if pharmacotherapy is completely withdrawn. These proof-of-principle studies have not led to recommendations for this approach routinely in newly diagnosed T2DM but they do suggest that temporary use of insulin to attain excellent glycemic control soon after diagnosis is appropriate for some patients. It has also been proposed that those with very high HbA_{1c} at diagnosis, especially if accompanied by symptoms, could also be treated immediately with dual or triple therapy that may include basal insulin.[38] Multiple drug combinations without insulin are less well validated but may reduce HbA_{1c} to target levels more consistently than a usual single-agent approach. However, independent of the regimen used, rapid reduction of HbA_{1c} in patients who have had many months of poor control has potential risks. Aside from the risk of hypoglycemia from use of insulin by relatively inexperienced patients, there is potential for worsening of retinopathy or neuropathy accompanying overly rapid reduction of glucose. As a general rule, patients diagnosed with HbA_{1c} above 9% should seek a gradual improvement of control with HbA_{1c} below 7% within 6 months, with continuation of at least metformin after that.

Youth-Onset T2DM

The worldwide increase of obesity has led to diagnosis of T2DM in susceptible individuals at earlier ages than in the past. One consequence of this is an alarming increase of onset below age 20.[320] In some regions, the incidence of T2DM in children is approaching that of T1DM. Recent studies of T2DM in adolescence have demonstrated severe insulin resistance and initially high levels of insulin secretion but a relatively rapid decline of beta-cell function that is not slowed by metformin therapy.[321,322] At present there is little evidence as to how treatment of youths with T2DM can be improved, but it is evident that much greater effort to improve dietary and exercise patterns is needed in this population for both prevention and treatment.[323] While the ability of weekly GLP1 receptor agonists to conveniently control both glucose and weight suggests these agents may come to be important, their use in this setting is at present off label, and objective data are limited.

Pregnancy

Management of diabetes during pregnancy is beyond the scope of this chapter. However, because it is centrally important in the natural history of T2DM, some important features must be mentioned. Women with risk factors for diabetes often first develop overt hyperglycemia during pregnancy, otherwise known as gestational diabetes mellitus (GDM). After delivery, some continue to have sufficient hyperglycemia to justify the diagnosis of T2DM, but more revert to having normal glucose levels. For the latter group, the risk of reappearance of hyperglycemia consistent with the diagnosis of T2DM in the absence of pregnancy is 50% to 70% after 15 to 25 years, a risk that is increased 10-fold to 18-fold.[324,325] Consequently, periodic follow-up with testing of glucose or HbA_{1c} is indicated every 1 to 3 years to allow early detection. In addition to signaling a high risk of future development of diabetes, GDM poses more immediate risks to both mother and child. The likelihood of complicated delivery or neonatal complications is greatly increased by GDM. Furthermore, children with exposure to hyperglycemia in utero have increased risk of later obesity and/or T2DM.[326] This risk appears to be attenuated by good maternal glycemic control during gestation. Preconception counseling of women at high risk, routine screening for GDM during pregnancy, careful management of hyperglycemia during pregnancy, and systematic follow-up after delivery should be part of routine clinical practice. For women of childbearing age who already have diabetes, preconception planning and gestational management are even more urgently needed, especially to limit the risk of fetal malformations resulting from poor glycemic control in the first 10 weeks of pregnancy. Glucose targets during gestation are lower than advised in routine management of T2DM. The recommended target ranges are less than 95 mg/dL (<5.3 mmol/L) for fasting glucose, less than 140 mg/dL (<7.8 mmol/L) for 1-hour postprandial glucose, and less than 120 mg/dL (<6.7

mmol/L) for 2-hour postprandial glucose.[327] Careful nutritional therapy is essential, and additional treatment with insulin is often needed. Metformin or the sulfonylurea glyburide are sometimes used instead of insulin, but there are concerns about their safety, particularly with sulfonylureas.[327,328] For women with preexisting T2DM, insulin therapy is generally recommended during gestation. Whenever possible, an experienced specialized team should assist in management of diabetes during pregnancy.

Preventing Type 2 Diabetes Mellitus

The possibility that T2DM can be prevented in high-risk persons has been formally tested in a series of clinical trials reviewed elsewhere. Lifestyle intervention can provide a reduction of 30% to 60% in progression to diabetes over a period of 3 to 5 years. This is sustained and correlates with weight loss. However, the average sustained weight loss in these trials was modest, on the order of 5%.[329–331] Intervention with metformin was associated with a somewhat smaller reduction in progression to diabetes, although the benefit was similar to that of lifestyle in persons younger than 45 years of age, those with body mass index (BMI) greater than 35 kg/m^2, and those with an fasting plasma glucose level greater than 110 mg/dL (>6.1 mmol/L). Metformin had less effect in patients older than 60 years of age, those with a BMI of less than 30 kg/m^2, and those with a fasting plasma glucose of less than 100 mg/dL (<5.6 mmol/L). Metformin's ability to reduce progression to diabetes has been shown to be durable and cost effective.[332]

Acarbose has also been shown to reduce progression to diabetes without evidence of diminished efficacy in different subgroups. The TZDs provide sustained improvement of insulin sensitivity and can delay onset of diabetes. However, concern regarding possible long-term effects on weight, heart, and bones has limited the use of this class for prevention. GLP1 receptor agonists have also shown promise, but the expense and initial adverse effects preclude their promotion for this indication at present. Bariatric surgery has also been shown to reduce incident diabetes in more severely obese patients.

The success of the lifestyle interventions is impressive, showing that by using various techniques at-risk individuals can achieve physiologically relevant changes in body weight. It is unknown whether lifestyle plus medications provides even greater benefit. It seems reasonable to screen on the basis of the recommendations outlined earlier, while recognizing that patients with prediabetes (HbA_{1c} >5.7% and particularly ≥6%, fasting plasma glucose ≥100 mg/dL [≥5.6 mmol/L] and particularly ≥110 mg/dL [≥6.1 mmol/L], or impaired glucose tolerance) are the best candidates for preventive strategies. High-risk persons should be counseled about nutritional approaches to weight loss, instructed to increase physical activity, and monitored for progression of dysglycemia. Translation of the evidence found in the DPP study to practical expansion across the United States has been evolving through the CDC National Diabetes Prevention Program. The program includes a structured curriculum of 22 sessions over 1 year in a group setting or online programs delivered through a diverse group of public and private organizations. Each local program is certified through the CDC Diabetes Prevention Recognition Program to maintain standards, promote utilization, and document outcomes. Providers must increase identification of those with prediabetes or other high-risk characteristics and direct patients to the programs available in the community.[333,334] The status of drug therapy, notably with metformin, is less clear, in part because significant diabetes complications are unlikely to develop in the relatively short window of time during which fasting glucose levels increase from 100 to 126 mg/dL (5.6–7 mmol/L). On the other hand, metformin therapy brings little risk and offers substantial benefit for some individuals, notably those with BMI of 35 kg/m^2 or more, those under age 60 years, and women with previous gestational diabetes.[36] Therefore on balance, consideration of metformin therapy in patients who are at particularly high risk is recommended.[36]

Future Directions

Present-day management of T2DM is significantly more effective and easier for patients than in the past, and it promises to improve further. Development of new drugs and smart devices for tracking glucose levels and administering therapies continues and challenges our ability to evaluate and assimilate these new tools. Notable unmet needs concern controlling glucose after meals, maintaining metabolic control during acute illness and hospitalization, and limiting weight gain. However, it can be argued that the main limitation of therapy at present is not a lack of tools and treatments, but a variety of economic, psychologic, and organizational barriers to using them to full advantage.[335] Practical, cost-effective public health approaches are needed to slow the increase in incidence and prevalence of T2DM.[336] Screening for diabetes or prediabetes may be cost saving and should be made more efficient. Globally, there continues to be inequality in access to medical care for all conditions, including diabetes. Although the prognosis for people with diabetes has never been better, the greatest challenges they face relate to the complexity and cost of care. If both the means of managing diabetes and the organizational structure of health systems are improved simultaneously, the morbidity and mortality of T2DM may be greatly reduced in the near future.

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The complete list of references is available online at ExpertConsult.com.

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Type 1 Diabetes Mellitus

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CHAPTER OUTLINE

KEY POINTS

- Type 1 diabetes mellitus (T1DM) is a disorder resulting from a chronic autoimmune destruction of the insulin-producing pancreatic beta cells.
- Worldwide, the incidence of T1DM is increasing by 3% to 5% per year.
- The disorder is clearly polygenic (>50 loci impacting susceptibility have been identified to date), yet more than half of disease susceptibility for T1DM is provided by the major histocompatibility complex; these genes/loci have been combined into a "genetic risk score" to identity a given individual's genetic predisposition to this disease.
- The risk for the disease can be ascertained through a combination of immunologic, genetic, and metabolic markers of disease, with the natural history now defined by a series of "stages."
- There is a growing appreciation that T1DM, rather than being a singular disease, may be a heterogeneous disorder with a common phenotype at clinical presentation/diagnosis.
- The pancreas of T1DM patients possesses an islet immune infiltrate derived from a variety of immunologic phenotypes, is reduced in size and weight, and is subject to unusual exocrine features.
- Significant advances in terms of disease management, afforded by technological innovations related to insulin analogues, advanced diabetes technologies, and more, set the foundation for marked reductions in hemoglobin A_{1c}, enhanced diabetes care, and improved biomedical and psychosocial outcomes.
- Although there have been many advances in the management of T1DM, this disease remains associated with many acute- and chronic complications.
- Numerous metrics (costs, patient outcomes, etc.) demonstrate that specialists (i.e., endocrinologists) provide more effective care for those with T1DM than nonspecialists, with best results arising from multidisciplinary, individualized care.

Where to begin to tell the story of the disorder we now refer to as type 1 diabetes mellitus (T1DM) represents somewhat of a literary challenge. Does one begin with the earliest writings scripted thousands of years ago conveying the disease's symptoms, or is it more fitting to describe exciting works performed in the 1800s and early 1900s that defined both the anatomy of and physiologic roles for the pancreas and insulin-secreting beta cells?[1] Other pieces of literature initiate their narrative on T1DM by sharing an account of the fast-paced and ultimately Nobel Prize–winning efforts in the 1920s by Banting and Best,[2] when the ability to purify insulin from animal pancreata brought forward a means to sustain life to those who, in the absence of such an intervention, met an early demise.

We have elected to begin this chapter by building on the story of a German pathologist named Martin Schmidt,[3] who, in 1902, noted a small peri-islet cellular infiltrate on microscopic evaluation of the pancreas obtained at autopsy from a 10-year-old child with diabetes. This effort represented a key early investigation seeking to better define the pathogenesis of the disorder, a process that was continued by many over the ensuing century. Works shortly thereafter by Shields Warren[4] in the 1920s drew attention to the relationship between this infiltrate and the age of diabetes onset. The term used to define this pancreatic feature, *insulitis*, was coined in 1940 by yet another pathologist, Hanns von Meyenburg.[5] Studies of pancreatic disease over ensuing decades taught us much regarding this inflammatory lesion, including its

relative infrequency in older individuals diagnosed with diabetes, its association with reduction in beta-cell mass, the identification of *pseudoatrophic* islets (i.e., islets devoid of insulin-containing cells), a preferential targeting of insulitis for beta cells containing insulin, and many other findings eventually deemed seminal.[6,7] These conditions, when joined together with immunologic and genetic studies of living patients in the 1970s, noting the presence of self-reactive antibodies (i.e., autoantibodies) against islet cells in persons with T1DM and that disease susceptibility was associated with major histocompatibility complex (MHC) molecules known to influence immune system functions, formed three lines of evidence pointing to an autoimmune basis for T1DM.[8] Built on this foundation, this chapter will share current views on the autoimmune nature of T1DM (including its natural history and pathogenesis); emerging views on genetics, disease biomarkers, and epidemiology; increased information on the contribution of beta cells to their own demise; and perhaps most importantly, how treatments are advancing for this disease—all as we consider how this information is vital to the optimal diagnosis and optimal care of individuals with T1DM.

Diagnosis

A diagnosis of diabetes has historically included an elevated fasting blood glucose level, any glucose value higher than 200 mg/dL (11 mmol/L) with symptoms of hyperglycemia, or an abnormal 2-hour oral glucose tolerance test (OGTT).[9] American Diabetes Association (ADA) guidelines for the diagnosis of diabetes were modified in 2009 to include a hemoglobin A_{1c} (HbA_{1c}) value greater than 6.5%.[10] Under certain settings (e.g., obesity, racial status other than Caucasian) and particularly among adults, the diagnosis of T1DM versus type 2 diabetes mellitus (T2DM) can prove quite challenging. At present, the best criterion for separating the two disorders resides in laboratory identification of any one of a number of islet cell autoantibodies (also known as T1DM-associated autoantibodies; i.e., anti-insulin autoantibodies [IAA], anti–glutamic acid decarboxylase [GADA], anti–insulinoma-associated antigen 2 [IA2A], or anti-zinc-transporter 8 [ZnT8A]). Literally hundreds of studies over the past three decades have suggested that the presence of these autoantibodies provides high sensitivity for diagnosing persons with T1DM.[11] Indeed, more than 90% of Caucasian children presenting with diabetes express at least one of these four T1DM-associated autoantibodies.[12] In terms of disease specificity, T1DM-associated autoantibodies are typically positive in less than 1% to 2% of unaffected (i.e., non-T1DM) individuals, further validating their diagnostic utility. However, among African-American and Latino children and adolescents in the United States diagnosed with diabetes, almost one half lack any T1DM-associated autoantibody. Many patients from these ethnic minorities in the United States present clinically as if they have early-onset T2DM (e.g., mild ketosis, slow symptomatic onset), some have attendant risk factors such as obesity, and many lack human leukocyte antigen (HLA) alleles associated with T1DM. Increasingly diverse genetic admixtures, due to geographic migration and social changes (e.g., multiracial offspring), further contribute to diagnostic complexity.

In childhood and adolescence, two peaks of T1DM presentation occur: a smaller peak between 5 and 7 years of age and a larger peak at or near puberty.[13] Although most autoimmune disorders disproportionately target females, T1DM affects males slightly more than females. The incidence of T1DM varies with seasonal changes and birth month. Incidence of T1DM diagnosis is higher in autumn and winter, whereas being born in the spring is associated with an increased likelihood for T1DM.[14] Interestingly, the development of T1DM-associated autoimmunity (i.e., the formation of islet autoantibodies) in the months to years prior to the onset of symptomatic T1DM also shows a degree of seasonal synchronization.

The measurement and presence of T1DM-associated autoantibodies have also fueled much debate regarding the percentage of T1DM cases that are errantly misclassified as T2DM. Indeed, it is conceivable that 5% to 15% of adults diagnosed with T2DM may have T1DM, given the frequency of T1DM-associated autoantibodies in populations diagnosed with T2DM.[15] This is, in effect, a problem in health care provider recognition of the potential for T1DM disease in adult populations combined with a lack of widespread screening for such autoantibodies in settings in which screening would seem warranted. If this assertion is correct, given the vastly greater number of persons diagnosed with T2DM (relative to that of T1DM), the number of actual T1DM cases in a given population may be massively underestimated. This is therapeutically unfortunate because an accurate diagnosis of T1DM is vital; persons misdiagnosed as having T2DM when they indeed have T1DM experience higher HbA_{1c}, have risk of diabetic ketoacidosis (DKA) due to use of noninsulin therapies, and have accelerated progression toward micro- and macrovascular complications..

Efforts to differentiate adult-onset T1DM from T2DM (Table 36.1) have also resulted in a series of proposed new disease classifications, most notably *ketosis prone diabetes* and *latent autoimmune disease of adults*.[16] The lack of firm diagnostic criteria dampens enthusiasm for adopting these presumed new disease entities as novel categories for diabetes. It is also important to note that T1DM patients can manifest insulin resistance, a feature most often associated with T2DM. Specifically, T1DM patients (including those who are autoantibody-positive) may present with diabetes characterized by high serum levels of fasting insulin or C-peptide but loss of stimulated insulin secretion.

As noted previously, some Caucasian children (~10%) are devoid of a T1DM-associated autoantibody at disease diagnosis (i.e., time of hyperglycemic onset), raising questions as to whether such persons lost their autoantibody expression (or at least the ability to detect it with laboratory testing) by the time of diagnosis, or whether a diagnosis of T1DM is accurate. The notion of autoantibody loss versus the presence of a different form of diabetes is also difficult to ascertain because many have HLA alleles associated with susceptibility for T1DM; are not insulin resistant; present with DKA; and, with time, lose their C-peptide secretion. With appropriate laboratory testing, notation of such persons as T1DM patients negative for T1DM-associated autoantibodies can be an appropriate diagnosis. This said, advances in understanding the heretofore underappreciated disease classifications of monogenic diabetes, including those of maturity onset of diabetes in youth, warrant genetic testing of diabetes cases diagnosed in the very young (i.e., <1 year of age).[17]

Animal Models

Nonobese Diabetic Mouse Model

The nonobese diabetic (NOD) mouse is, without question, the most intensively studied animal model for T1DM.[18] The frequency of diabetes among different colonies ranges from 30% to 100% among female NOD mice, suggesting that housing conditions (i.e., environment) represent an important variable. Unlike human T1DM that has a relatively equal distribution among genders, a

TABLE 36.1 Characteristic Comparison of Type 1 Versus Type 2 Diabetes Mellitus

Characteristic	Type 1	Type 2
Nature *Very different*	Autoimmune disorder marked by destruction of insulin-producing beta cells and loss of insulin production	A disorder of insulin deficiency involving an interplay between both pancreatic and extrapancreatic contributions to disease
Symptoms *Partial overlap*	Rapid onset; very high to extremely high blood glucose levels; polyphagia; polydipsia, polyuria; ketoacidosis	Mild to moderate onset; modest to high elevations in blood glucose; mild polydipsia/polyuria; fatigue; visual changes/headache
Onset *Very different*	Sudden (symptoms for days to weeks)	Slower onset (symptoms for months to years)
Risk factors *Typically different but overlap*	Family history of autoimmune disease but particularly type 1 diabetes mellitus (10-fold increased risk vs. general population)	Overweight/obese; poor diet; sedentary lifestyle; ethnicity (higher in African Americans, Hispanics); family history of type 2 diabetes mellitus; history of gestational diabetes
Onset age *Typically different but overlap*	Typically early life through adolescence but can occur at any age	Typically adults but trending toward earlier age of onset
Treatment strategy *Typically different*	Absolute requirement for insulin (multiple daily injections or insulin pump); self-management lifestyle modification (monitor food types, exercise, etc.)	Dietary modifications and exercise alongside oral agents (for most); increasingly greater percentage of patients require insulin over time
Can it be prevented? *Very different*	Not at present (subject of major research efforts); future cases can be predicted by autoantibodies and genetics	Yes, for more than half of potential cases, with dietary modifications and exercise
Can it be reversed? *Very different*	Not at present (subject of major research efforts)	No, but for a limited few; patients can see disease managed and risk for complications reduced through diet modifications, exercise; growing evidence for disease improvements through combination therapies
Complications *Mostly similar, but some variation*	Acute emergencies of hypoglycemia and ketoacidosis leading to hypoglycemic unawareness; chronic effects of hyperglycemia can lead to retinopathy, nephropathy, neuropathy, cardiovascular disease, etc.	Acute emergencies of hypoglycemia and ketoacidosis leading to hypoglycemic unawareness; chronic effects of hyperglycemia can lead to retinopathy, nephropathy, neuropathy, cardiovascular disease, etc.

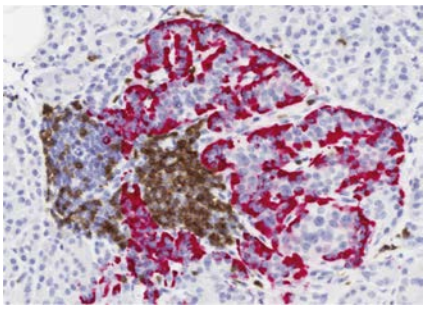
strong female bias with respect to disease exists in NOD mice. As with T1DM of humans, diabetes in the NOD mouse is polygenic with specific MHC class I and II molecules being central for disease pathogenesis[19] and many other genetic loci bearing relatively small contributions neither necessary nor sufficient for disease.[20] NOD mice, like humans, produce IAA before developing diabetes, although T cells, not autoantibodies, appear to mediate the beta-cell destruction. The insulitis lesion, the hallmark facet of T1DM, appears more intense and quantitatively obvious in NOD mice as compared with humans. Interestingly, a relative overrepresentation of T cells bears a receptor specific for the beta-cell antigen, insulin. Although a majority of studies have provided evidence for a central role of T-cell autoimmunity directed at insulin,[21] to be clear, there is considerable debate whether any given beta-cell antigen is primary (i.e., the first, major driver of disease development in an autoimmune response). Studies of islet beta-cell mass indicate that both destruction and replication/regeneration are present months before the onset of T1DM, with an acceleration of beta-cell destruction at disease onset.[22] A seeming plethora of published studies highlight the therapeutic potential from beta-cell replication/regeneration in NOD mice or other rodent strains.[23–25] However, recent convincing evidence suggests that in terms of beta-cell replication, rodent models differ dramatically from human physiology, with beta-cell replication being a rare event in humans from adolescence through adulthood.[26] One of the primary concerns for the NOD mouse relates to the relative ease by which the disease can be prevented or reversed.[18] One can observe clear therapeutic benefits in the NOD mouse (e.g., preserved C-peptide secretion), yet only a limited number these findings have been effectively translated to humans. Animal models (unfortunately) represent somewhat of an idealized situation characterized by features that are not reflective of the sociologic and lifestyle settings in humans, such as limited genetic diversity through selective inbreeding, housing in highly controlled environments devoid of pathogens, uniform diets, and several other facets. It remains unclear which of these or other differences underlie the generally disappointing translation.

Induced Models of T1DM

Diabetes, and even insulitis, can be induced in several strains of rodents with drugs that induce islet cell destruction and broadly activate immune responses, including those directed against beta-cell antigens. For example, the drug streptozotocin is directly toxic to islet beta cells. In high doses, it rapidly induces diabetes (i.e., hyperglycemia). In low doses, a more chronic diabetes develops that is likely to have some immunologic derivation, meaning pathogenesis tied to immune responses against beta cells.[27] Other models are used less frequently.

Histopathology

As noted earlier, T1DM in humans is characterized by selective destruction of beta cells within the pancreatic islets[7] (Fig. 36.1). Although certain exceptions exist, an overall assimilation of the



A

Islet cells in type 1 diabetes

- Insulitis (mixed mononuclear, adjacent or within islet)
- Loss of β cells (increase with disease duration)
- Hyper-expression of class I MHC
- β cell necrosis/apoptosis (?)
- Diminished insulin in remaining β cells
- β cell expression of interferon α

B

Pancreas in type 1 diabetes

- Decreased overall weight
- Atrophy of dorsal region
- Exocrine atrophy
- Hydrophic change (hypertrophy) (?)
- Composed of pseudoatrophic (glucagon staining) islets in type 1A
- Lobular loss of β cells
- Heterogeneous lobular insulitis

C

• **Fig. 36.1** Pathologic characteristics of the pancreas in T1DM. (A) Islet infiltrate (i.e., insulitis) seen in a patient with recent-onset T1DM. Immunohistochemistry shows the intra-islet presence of CD3-positive cells (*brown*) and glucagon-producing alpha cells (*pink*). Histologic features of islet cells (B) and gross pathologic characteristics of the pancreas (C) associated with the natural history of T1DM (i.e., preonset, onset, post onset). *MHC*, major histocompatibility complex; *T1DM*, type 1 diabetes mellitus. (From Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet*. 2014;383:69–82, used with permission.)

literature supports that T1DM patients with a disease onset at age 0 to 14 years and within 1 year of diagnosis show more inflamed islets (68%) and fewer islets with residual beta cells (39%) than in patients with onset at 15 to 39 years of age.[28] This finding suggests that a more vigorous autoimmune response occurs when disease develops in young children.

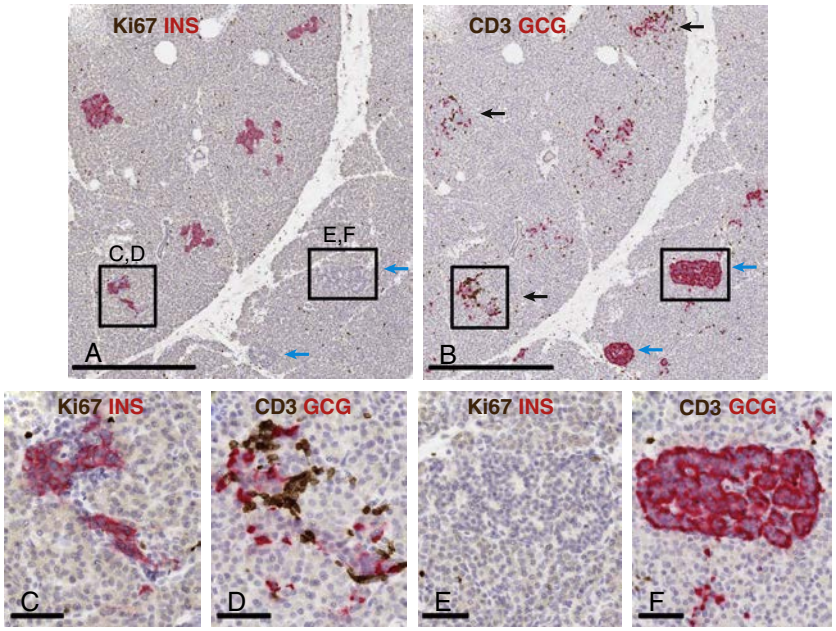
The immunotype of insulitic lesions shows a predominance of $CD8^+$ T lymphocytes and macrophages, but cells of other phenotypes are also observed (e.g., $CD4^+$, $CD20^+$, and $CD68^+$).[29,30] More recent studies have evaluated immunotype as a function of age, with younger age of onset associated with higher levels of $CD20^+$ B cells, $CD45^+$ cells, and $CD8^+$ T cells in insulitis lesions, alongside fewer insulin-positive islets.[28,31] Conversely, infiltrates with fewer $CD20^+$ cells are observed in T1DM patients who are older at onset and associated with lower levels of $CD45^+$ cells and $CD8^+$ T cells, as well as more insulin-positive islets. These same studies also show that islet $CD8^+$ T cells express T-cell receptors that bind MHC class I tetramers loaded with the beta-cell autoantigen G6P2 and other target peptides in recent-onset T1DM patients.[31] These studies are also in agreement with recent findings that the T cells invading pancreatic islets are, in fact, directed at beta-cell antigens.[30] In contrast to long-standing dogma, the presence of insulitis in human T1DM is quantitatively limited, with an expert panel establishing a standardized definition for this lesion of three islets, containing more than 15 $CD45^+$ cells, in a pancreas.[6]

Somewhat surprisingly, inflammation is also present in pancreatic exocrine tissue in T1DM. Specifically, chronic inflammation, including enhanced $CD8^+$ T-cell infiltration (and, to a lesser degree, $CD4^+$ and $CD11c^+$ cells), is present in the exocrine pancreas in T1DM subjects.[32] Other studies report a similar propensity for neutrophil invasion of the pancreas (along with decreased peripheral neutrophil counts) in T1DM.[33] The propensity of this organ for inflammation/pancreatitis induced by multiple factors (e.g., hypertriglyceridemia, virus infection, drugs) could be a result of a susceptibility gene that affects tissue-based inflammation or other facets that have, in the past, been considered "leakiness." Importantly, the inflammatory process in the exocrine pancreas appears to be subclinical, as most new-onset T1DM patients do not present with symptoms of pancreatic inflammation.

Recent studies assessing the persistence of insulin production in patients with long-standing T1DM (i.e., stimulated C-peptide production) using ultrasensitive assays have overturned the long-standing dogma that residual C-peptide secretion is rare.[34] Emerging histopathologic evidence supports this notion through identification of insulin-positive islets, albeit extremely limited in number, in patients with long-standing T1DM.[35] The non–insulin-producing cells of the islets (i.e., alpha, delta, pancreatic polypeptide; see Chapter 33) remain in patients with long-standing T1DM, and these remaining islets, lacking insulitis and beta cells, are termed *pseudoatrophic*. This information sets the stage for another remarkable feature of the pancreas of patients with new-onset T1DM, namely heterogeneity of islet lesions. Histopathologic examination of the same section of pancreas showed that a normal islet with no immune infiltrate can coexist with an islet containing beta cells with intense infiltration, as well as a pseudoatrophic islet that has no infiltrate (Fig. 36.2). This spottiness of the pathologic process is reminiscent of the destruction of areas of the skin in patients with vitiligo, in which melanocytes are destroyed in patches. Such heterogeneity of lesions may underlie the variable natural history of T1DM, in which the time period between initial beta-cell autoimmunity and overt symptoms can be one of months, years, or even many decades. Additionally, pancreata from non-T1DM but autoantibody-positive organ donors often show limited to no insulitis but have shown distinct histologic abnormalities, such as an increased proinsulin to insulin area ratio,[36] that match functional alterations. Such information has been considered to support the notion that T1DM is a relapsing/remitting disease,[37] with waves of immunologic destruction.

The islets of T1DM and T1DM autoantibody-positive nondiabetic patients overexpress class I HLA antigens, interferon-alpha, and potentially Fas molecules.[38,39] The specific means by which the immune system destroys beta cells is not known. Molecules such as Fas may be important because T cells expressing Fas ligand can induce apoptosis of beta cells. This said, more enthusiasm surrounds the notion that cytokines (e.g., interleukin 1 [IL1]) and $CD8^+$ cytotoxic T lymphocytes are likely to be major contributors to beta-cell destruction. With respect to what might induce these features, searches for viral particles and viral RNA within islets of patients with new-onset diabetes have largely been unrewarding. However, newer technologies and concepts should facilitate additional studies, and it is likely that there will be heterogeneity among patients.

Finally, reduced pancreatic weight has been demonstrated at autopsy or in organ donors with recent-onset T1DM, as well as in those with long-standing disease.[40] Reduced pancreatic volume has also been shown in living patients with T1DM through noninvasive imaging techniques.[41] Mechanisms underlying loss of pancreatic size or weight in T1DM are not well understood but have largely been hypothesized to result from loss of insulinotrophic



• **Fig. 36.2** Lobular variability in insulin⁺ Islets and insulitis. Islets were imaged from a 13-year-old patient with type 1 diabetes mellitus for 5 years (nPOD 6243). Serial paraffin sections were stained for Ki67 plus insulin (INS) (A, C, and E) and CD3 plus glucagon (GCG) (B, D, and F), and islets were subtyped as described in research design and methods. Five insulin⁺ islets (A) are seen in a lobule adjacent to a lobule with two insulin⁻ islets in the patient with type 1 diabetes mellitus (A and B, *blue arrows*). Three insulin⁺ islets had insulitis (B, *black arrows*), and both insulin⁻ islets did not have insulitis. One of the insulin⁺ islets with insulitis is shown at higher magnification (C and D), as well as an insulin⁻ insulitis⁻ islet (E and F). Few islet cells were Ki67⁺ (A and C), indicating no effect of insulitis on proliferating cell numbers. Scale bars: A and B, 500 µm; C–F, 50 µm. (From Campbell-Thompson M, Fu A, Kaddis JS, et al. Insulitis and β-cell mass in the natural history of type 1 diabetes. *Diabetes*. 2016;65[3]:719–731.)

effects on the exocrine pancreas, as islets constitute only 1% to 2% of the entire pancreatic volume (see the Pancreatic Exocrine Abnormalities in T1DM section).

Mechanisms of Beta-Cell Death in T1DM

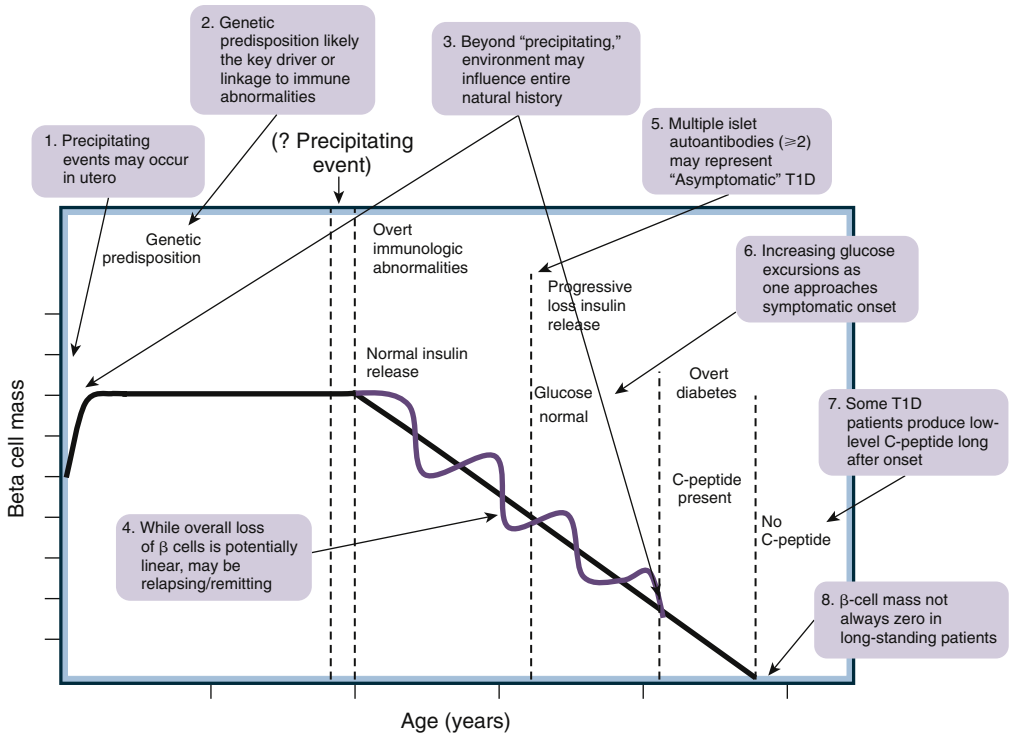
The mechanism(s) by which beta cells die in T1DM remains somewhat controversial. A majority of studies support necrotic cell death, mediated by cytotoxic T cells in response to self-antigens, as one likely mechanism. Evidence from studies of human T1DM pancreas noting the presence of CD8⁺ T cells and macrophages is consistent with this concept.[7] T cells can induce necrosis following release of cytolytic granules containing granzymes and perforin, which act on the beta-cell membrane. Ischemia resulting from impaired vascularity, a process that may result from hyperexpression of extracellular matrix materials (e.g., hyaluronan, hyaluronan-binding proteins) may also lead to necrotic cell death.[42] Additional mediators of necrotic cell death finding support in T1DM studies include reactive oxygen species, which are capable of inducing mitochondrial injury and loss of cell membrane integrity through oxidative DNA damage, lipid peroxidation, and protein damage.[43] Finally, necrosis of beta cells may release factors, including post-translationally modified antigenic peptides[44] or modifications of "normal" antigens (i.e., hybrid peptides[45]) that further stimulate the immune response and thereby perpetuate beta-cell death by necrosis.

Beyond necrosis, apoptosis has also been implicated for its role in beta-cell death in T1DM through activities largely linked to the janus kinases (JAK1, JAK2) and nonreceptor tyrosine-protein kinase (TYK2) pathways.[46] Immunity finds a link here, with apoptosis likely driven by cytokines, including tumor necrosis factor (TNF), that activate Fas/FasL, TNFR1, and TNFR2. Apoptosis can also be initiated through the B-cell lymphoma 2 (BCL2) pathway via cytokine deprivation or endoplasmic reticulum stress, leading to caspase activation. Apoptosis due to the activity of caspases leads to apoptotic cell morphology, nuclear fragmentation, and chromatin condensation—facets that have been observed in human T1DM pancreas. The notion of the so-called unfolded protein response (i.e., how a cell responds to the accumulation of misfolded proteins in the endoplasmic reticulum) and how it may modulate beta-cell inflammation and death in T1DM is also gaining interest in T2DM.[47]

In reality, beta-cell death in T1DM likely sees contributions from both of these mechanisms (i.e., necrosis and apoptosis). The dynamic glycemic fluctuations, ultimately including overt hyperglycemia seen in the period of pre-T1DM (see the Stages in the Natural History of T1DM section), support the notion of ever-increasing functional changes in the beta cells, possibly as a result of immunologic stressors or increased metabolic demand for residual beta cells.

Natural History of T1DM—Historical Concepts

Perhaps the most helpful and beneficial guide to studies pertaining to the natural history of T1DM was the model put forward in 1986 by a previous author of this chapter, the late Dr. George Eisenbarth.[48] In this model (Fig. 36.3), persons destined to develop T1DM were assumed to begin life with a full cadre of beta cells. A triggering insult, likely environmental, was proposed to initiate a process involving recruitment of antigen-presenting cells. The antigen-presenting cells would sequester self-antigens released by injured beta cells, followed by their transport to pancreatic lymph



• **Fig. 36.3** The natural history of type 1 diabetes mellitus—a 25-year-old concept revisited. A re-creation of the model as originally proposed in 1986 is tracked by the *black line*. Several additions and conjectures can be made to this model based on recent knowledge gains (*lavender line*). *T1D*, type 1 diabetes. (Redrawn from Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet*. 2014;383:69–82, used with permission.)

nodes, where they are subsequently presented to autoreactive T cells. These T cells, rogue constituents brought to life because of genetically driven failures of thymic deletion (i.e., central tolerance) combined with defects in mechanisms designed to induce peripheral immune tolerance, would come into play.[49] This toxic duo imparting lack of tolerance formation, again in the context of genetic susceptibility, would allow for migration of self-reactive T cells to islets, mediating beta-cell killing and promoting further inflammation.[50] Symptoms of the disease were thought to appear when 85% to 90% of pancreatic beta cells had met their demise. In the final stage of the model, the autoimmune process ends with the complete elimination of beta cells.

Although this model faithfully served as a guide for multiple decades, and certainly the majority of its aspects hold true to this day, some constituents are under states of correction (e.g., the aforementioned notion that C-peptide production is lost in T1DM at onset) or are still subject to intense investigation—facets that will be discussed throughout this chapter (see the "Natural History of T1DM-Emerging Concepts" section).

Natural History of T1DM From Long-Term Family and Population Studies

The major longitudinal clinical epidemiologic studies of T1DM have included BABYDIAB in Germany[51]; DIPP (T1DM Prediction and Prevention) in Finland[52]; DAISY (Diabetes Autoimmunity Study in the Young) in Denver, Colorado[53]; TEDDY (The Environmental Determinants of Diabetes in the Young) with US sites in the states of Colorado, Georgia, Florida, and Washington, as well as European sites in Germany, Sweden, and Finland[54]; T1DM TrialNet Pathway to Prevention, with more than 200 locations across the United States, Europe, Canada, Australia and New Zealand[55]; and SEARCH (the SEARCH for Diabetes in Youth), with study centers in the US states of South Carolina, Ohio, Colorado, California, and Washington.[56] These efforts, together with cross-sectional investigation of human pancreas tissues obtained through biobanks, such as the Network for Pancreatic Organ donors with Diabetes (nPOD),[57] have reshaped our understanding of the natural history of T1DM and human pancreas pathology with some of the seminal findings reviewed in Table 36.2.

A pivotal discovery was made from combined analysis of data generated in the BABYDIAB, DAISY, and DIPP studies. Namely, once two or more anti-islet autoantibodies are present in a given individual, the individual's 10-year risk for developing diabetes ranges from 60% in older seroconverters (>3 years) to 75% in those who seroconvert before 3 years of age.[11] Recent and ongoing analyses of longitudinal data generated from the DIPP and TEDDY cohorts are unveiling potential environmental influences (e.g., birth by cesarean section, gut microbiome, dietary fiber, early introduction of cow's milk or gluten to an infant's diet, vitamin D, antibiotic use in early life, vaccination, infections)[58–64] and novel biomarkers (e.g., gut microbiome, metabolomics, lipidomic and proteomic profiles) of eventual T1DM progression (see the Emerging Biomarkers of T1DM Natural History and Diagnosis section)[65] that might ultimately enable identification of the disease early in its pathogenesis. The hope is that prevention or delay of T1DM onset may one day be possible through early lifestyle adjustments or tailored intervention.

Genetics

It has long been recognized that diabetes is a heterogeneous group of metabolic disorders, with hyperglycemia representing a common feature. More recently, it has become apparent that T1DM is also heterogeneous as a disease entity. This conclusion is based on a series of observations including the age at disease onset, form of

TABLE 36.2 T1DM Clinical Research Consortia and Natural History Trials

Trial	Enrollment Criteria	Endpoint(s)	Clinical and Mechanistic Outcomes	Reference
BABYDIAB	Offspring of parent with T1DM	1. Anti-islet autoantibody seroconversion 2. T1DM onset	• 4% developed anti-islet autoantibodies by age 2 years • IAA first autoantibody, followed by GADA	51
DIPP T1DM Prediction and Prevention	Neonates and siblings with high-risk HLA from the general population	1. Anti-islet autoantibody seroconversion 2. T1DM onset	• 6% developed anti-islet autoantibodies by age 2 years • 46% of anti-islet autoantibody–positive children reverted to negative serostatus • IA-2A positivity conferred increased risk or protection	52, 393, 394
DAISY Diabetes Autoimmunity Study in the Young	Neonates with high-risk HLA from the general population and first-degree relatives with T1DM	1. Anti-islet autoantibody seroconversion 2. T1DM onset	10-year risk of progression to T1DM: • 15% with one anti-islet autoantibody • 70% with two anti-islet autoantibodies • 74% with three anti-islet autoantibodies • Anti-islet autoantibody seroconversion after 8 years of age was more common in African-American and Hispanic children than in non-Hispanic white children • Age of anti-islet autoantibody seroconversion was a major determinant of age of T1DM progression	53
TEDDY The Environmental Determinants of Diabetes in the Young	Neonates with high-risk HLA from the general population and first-degree relatives with T1DM	1. Anti-islet autoantibody seroconversion 2. T1DM onset	5-year risk of progression to T1DM: • 11% with one anti-islet autoantibody • 36% with two anti-islet autoantibodies • 47% with three anti-islet autoantibodies • IAA+ and GADA+ progressed equally to T1DM but IAA occurred first at a younger age	54, 395
T1DM TrialNet Pathway to Prevention (Formerly TN01 Natural History Study)	Children and adults with anti-islet autoantibodies from the general population and first-degree relatives with T1DM	1. T1DM onset	Risk of T1DM was associated with the following: • Number of anti-islet autoantibodies • Age • Hemoglobin A_{1c} • OGTT	396, 397
SEARCH SEARCH for Diabetes in Youth	Diagnosed diabetes (excluding gestational diabetes) in subjects <20 years of age from the general population at six sites across the United States	N/A	• Between 2002 and 2009, T1DM incidence increased by 2.7% in non-Hispanic white youth • Between 2002 and 2012, the annual rate of incidence increase was 4.2% in Hispanic youth versus 1.2% in non-Hispanic white youth • 30–50% of youth diagnosed with T1DM were also overweight or obese • 26% of anti-islet autoantibody–positive cases also had insulin resistance	56, 398
nPOD Network for Pancreatic Organ donors with Diabetes	Organ donors across the United States: 1. Diagnosed with T1DM 2. Diagnosed with T1DM and pancreas or pancreas/kidney transplant recipient 3. Nondiabetic, anti-islet autoantibody positive and younger than 30 years of age	N/A	• Insulitis is markedly different in human T1DM versus the NOD mouse model • Defined human insulitis as at least three islets with >15 infiltrating immune cells • Pancreas weight is reduced in T1DM • HLA class I is hyperexpressed in islets of the human T1DM pancreas • Insulin-expressing beta cells are reduced but still detectable in the human T1DM pancreas	7, 39, 40, 57, 399–401

GADA, Glutamic acid decarboxylase autoantibodies; *HLA,* human leukocyte antigen; *IAA,* insulin autoantibodies; *N/A,* not applicable; *NOD,* nonobese diabetic; *OGTT,* oral glucose tolerance test; *T1DM,* type 1 diabetes mellitus.

symptomatic presentation (i.e., rapid and ketotic vs. mild without ketosis), pancreatic pathologic changes, type and number of autoantibodies, and certainly the genetics.

Indeed, there are likely many genetic forms of T1DM, influenced to a major degree by the presence of specific forms of HLA class II molecules.[66] Beyond HLA, the form of T1DM is also influenced by the presence of abnormalities (immunologic, beta cell, etc.) fostered by the quantity and combinations of genes that influence these aberrancies. The number of specific genes that contribute to T1DM remains in flux, to some degree, as studies of loci validation in varying global populations are conducted. However, current estimates provided by genome-wide association studies (GWAS) support that more than 50 such loci exist, with the majority thought to influence immune responses.[67] Many of the

TABLE 36.3 Risk of Type 1 Diabetes Mellitus

Group	Childhood Annual Incidence
US general population	0.3% (15–25/100,000)
Offspring	1%
Sibling	3.2% (through adolescence); 6% lifetime
Dizygotic twin	6%
Mother	2%
Father	4.6%
Both parents	~10%
Monozygotic twin	50%, but incidence varies with age of index twin

HLA and non-HLA genes underlying T1DM susceptibility are similar in diverse countries, although specific alleles of those genes differ in their frequency. It is generally thought that specific combinations of HLA and non-HLA genes contribute to a loss of tolerance to self-antigens in beta cells, ultimately resulting in T1DM.

Beyond the polymorphisms seen in these HLA and non-HLA genes identified by GWAS, genetics studies have also been extremely helpful in identification and characterization of several monogenic forms of T1DM. Although these disorders often clinically continue to be classified as T1DM, such diseases should strictly be identified as *monogenic diabetes*. Genetic risk scores (GRSs) accounting for HLA and non-HLA risk alleles can discriminate T1DM from other forms of diabetes, including monogenic diabetes and T2DM[68–70] (see The Genetic Risk Score section).

Overview of Disease Prevalence

In the United States, the risk of childhood diabetes is approximately 1 in 300.[71] This risk is 15 times lower than the diabetes risk for a first-degree relative of a patient with T1DM (Table 36.3), which, in turn, is 150 times lower than the risk for a monozygotic twin of a patient with T1DM.[72] Although the risk of diabetes is much greater for relatives of patients with T1DM, it is important to realize that most persons (>85%) in whom T1DM develops do not have a first-degree relative with the disease. The incidence of sporadic cases results, in part, because almost 40% of persons in the general population carry high-risk HLA alleles for T1DM (see The Major Histocompatibility Complex section).

The highest known incidence of T1DM is observed in Finland and Sardinia. Finland now has an annual incidence approaching 60 per 100,000 children.[73] Since the 1950s, the incidence has increased almost fivefold, suggesting a dramatic environmental change (either an increase of causative factors or a decrease of protective factors), and it is doubling in many Western countries, including the United States, every 10 to 15 years (Fig. 36.4). Of note, the increased T1DM incidence in many countries has not been linear but rather fluctuating over the past several decades. In contrast, the disorder is quite uncommon in China, India, and Venezuela (~0.1 per 100,000 per year).

Twin Studies

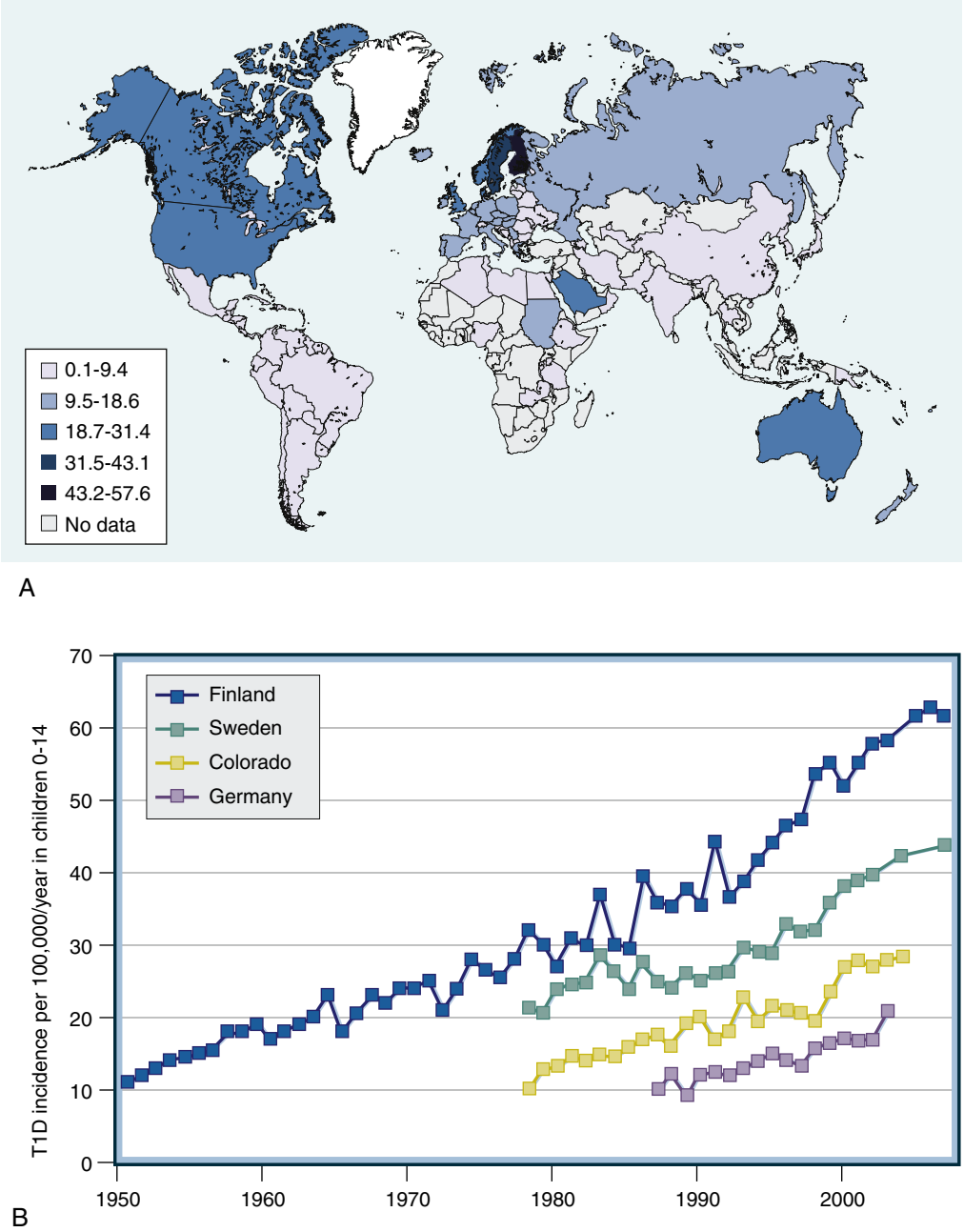
Twin studies of diabetes have made impressive contributions to our understanding of the disease. The study of monozygotic twins of patients with diabetes by Barnett and coworkers[74] contributed to the recognition of distinct forms of diabetes, initially termed *juvenile onset* and *adult onset*, later *insulin dependent* and *non–insulin dependent*, and now T1DM and T2DM. The concordance rates for monozygotic and dizygotic twins provide important information regarding genetic factors contributing to a given disease, because monozygotic twins share all germline-inherited polymorphisms or mutations, whereas dizygotic twins are similar to nontwin siblings of patients with a disease and have only half of their genes in common. For a locus that contributes to disease in a recessive manner, only one quarter of dizygotic twins would be homozygous to a sibling with diabetes at that locus, but all monozygotic twins would be homozygous for all recessive loci of their diabetic twin. As overall concordance rates of monozygotic twins for T1DM vary across studies, it is likely that T1DM is heterogeneous and that groups of monozygotic twins have different genetic causes for their diabetes. With such genetic heterogeneity, one would expect different concordance rates for different genetic causes.

Redondo and coworkers[75] analyzed prospective follow-up data from a large series of initially discordant monozygotic twins from the United Kingdom combined with a series from the United States. Progression to diabetes was identical for both series of twins. There was no length of time of discordance beyond which a monozygotic twin of a patient with T1DM did not have a risk of developing the disease. Nevertheless, the hazard rate for development of diabetes decreased as the period of discordance increased. There was also a marked variation in the risk of diabetes relative to the age at which the disorder developed in the index twin. With long-term follow-up, the overall rate of concordance for monozygotic twins exceeds 50%.[72] However, if T1DM developed in the index twin after age 25 years, the concordance rate by life table analysis in the study of Redondo and coworkers[75] was less than 10%. If diabetes developed in the index twin before age 5 years, the concordance rate was 70% after 40 years of follow-up. Therefore, environmental factors, random factors, and non–germline-inherited variations (e.g., imprinting, T-cell receptor polymorphisms, somatic mutations) likely contribute to lifetime diabetes risk. Interestingly, studies of dizygotic twins suggest that their risk of diabetes may not differ from that of nontwin siblings or, at most, may be increased by a factor of two as compared with the 10-fold increase for monozygotic twins.[76]

Genetic factors influence not only the development of diabetes but also the expression of anti-islet autoantibodies. For identical twins, the expression of anti-islet autoantibodies is tightly linked to the eventual progression to overt diabetes, and, true to form, monozygotic twins have a highly concordant prevalence for the presence or absence of anti-islet autoantibodies. Dizygotic twins much less often exhibit concordant positivity for anti-islet autoantibodies, and the prevalence is similar to that of nontwin siblings.[77]

The Major Histocompatibility Complex

The most important loci determining the risk of T1DM reside within the MHC on chromosome 6p21 and, in particular, the HLA class II molecules (DR, DQ, and DP).[66] The major determinants of T1DM susceptibility are DR and DQ molecules, and specific alleles of both HLA-DR and -DQ can either increase or decrease the risk of diabetes. Table 36.4 summarizes the diabetes risk associated with several DR and DQ haplotypes.[78] In addition, HLA class I loci (HLA-A, -B, and -C) influence disease, and it is likely that additional loci within or linked to the MHC influence immune function and contribute to risk.[79] The nomenclature for alleles of this region is somewhat daunting, but with definitions of



• **Fig. 36.4** Incidence of T1D in children age 0 to 14 years by geographic region and over time. (A) Estimated global incidence of TID, by region, in 2011. (B) Time-based trends for the incidence of TID in children ages 0 to 14 years in areas with high or high-intermediate rates of disease. T1D, type 1 diabetes. (Redrawn from Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet.* 2014;383:69–82, used with permission.)

several terms and a description of the basis for classification, it is comprehensible and quite important to understand.

As noted previously, the function of HLA molecules is to present peptides to T lymphocytes. Each HLA molecule is made up of two chains, and each chain is encoded by a separate gene. These molecules are extremely polymorphic in amino acid sequence. Each polymorphic variant of each chain is designated with a gene locus name (e.g., HLA-DRB1) followed by an asterisk (*), followed by two digits referring to the serologic specificity (from the time in diagnostic history when typing was performed with antibodies), followed by two digits for the specific allele (now determined with DNA-based typing), followed by a single digit to distinguish silent nucleotide polymorphisms (nucleotide differences that do not change the amino acid sequence). For example, the designated allele HLA-DRB1*04:05 has DR4 serologic specificity and is associated with high T1DM risk. There are hundreds of known alleles of HLA-DRB1. Each person inherits two HLA-DRB1 alleles, one from each parent. For HLA-DR alleles, usually only the DRB chain is specified, because the DRA chain is not polymorphic. Likewise, for the class I molecules (A, B, and C), only a single chain is specified, because the other chain, β_2-microglobulin, is minimally polymorphic.

Because HLA gene loci are in close proximity to each other on the sixth chromosome, a group of alleles is usually inherited as a unit, which is termed a *haplotype*. For example, the alleles HLA-A*01:01,

TABLE 36.4 Diabetes Risk of Representative HLA-DR and DQ Haplotypes

DRB1	DQA1	DQB1
High Risk		
0401 or 0403 or 0405	0301	0302 (DQ8)
0301	0501	0201 (DQ2)
Moderate Risk		
0801	0401	0402
0404	0301	0302
0101	0101	0501
0901	0301	0303
Moderate Protection		
0403	0301	0302
0701	0201	0201
1101	0501	0301
Strong Protection		
1501	0102	0602 (DQ6)
1401	0101	0503
0701	0201	0303

HLA-B*08:01, and HLA-DRB1*0301-DQA1*0501-DQB1*0201 constitute a common haplotype associated with diabetes risk probably related to the presence of HLA-DRB1*0301. When specific alleles of different genes are nonrandomly associated with each other on a haplotype (e.g., A1, B8, and DR3), the alleles are said to be in linkage disequilibrium. Linkage disequilibrium is not the same as linkage, although to have linkage disequilibrium, genes must be linked. Genes are linked when they are close together on the same chromosome and thus transmitted from parent to child as a haplotype group. If alleles of linked genes are nonrandomly associated with each other in a population, they are in linkage disequilibrium.

Two MHC haplotypes, one inherited from each parent, constitute an individual's MHC genotype. This genotype ultimately determines the MHC-encoded risk of T1DM. For HLA-DQ molecules, both of the chains (DQA and DQB) are polymorphic. This adds an important level of diversity in that the protein chains encoded by the alleles of one haplotype can combine with the chains encoded by the other haplotype. For example, persons with the highest-risk genotype HLA-DRB1*03:01-DQA1*05:01-DQB1*02:01 and HLA-DRB1*04:05-DQA1*03:01-DQB1*03:02 can produce four different DQ molecules: the expected DQA1*05:01-DQB1*02:01 and DQA1*03:01-DQB1*03:02 but also DQA1*05:01-DQB1*03:02 and DQA1*03:01-DQB1*02:01. Studies suggest that the DQA1*05:01-DQB1*03:02 combination determines enhanced diabetes risk for DR3/4 individuals. The DQ molecule DQA1*05:01-DQB1*02:01 is also called *DQ2*, and DQA1*03:01-DQB1*03:02 is called *DQ8*.

A common DQ molecule, HLA-DQA1*01:02-DQB1*06:02, provides dominant protection from T1DM and is termed *DQ6*. A DR allele, HLA-DRB1*14:01, also appears to provide dominant protection.[80] The protective haplotype HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 is present in 20% of the general population and in fewer than 3% of patients with T1DM.

In several, children from the general population or relatives of patients with T1DM have been HLA typed.[81,82] In the United States, 2.4% of newborns have the highest risk HLA-DR-DQ genotype for T1DM, namely DR3-DQ2 with DR4-DQ8 (DR3/4-DQ2/8 heterozygotes). Fifty percent of children younger than 10 years and approximately 30% of older children who develop T1DM have this highest-risk genotype. Approximately 1 of 16 children with the highest-risk HLA genotype from the general population progresses to T1DM (compared with a population risk of 1 per 300). Alternatively, 15 of 16 children from the general population who are DQ2/DQ8 heterozygotes do not develop T1DM. Studies indicate that newborn siblings of patients with T1DM who have DQ2 and DQ8 have a risk of expressing islet autoantibodies exceeding 40% by age 6 years, and 50% of these newborns will develop diabetes by age 10 years. Ninety-five percent of persons who develop T1DM have either DR3-DQ2 or DR4-DQ8, as does approximately 40% of the general population.

There are additional high-risk haplotypes that are not common, such as HLA-DQA1*04:01-DQB1*04:02. It has been proposed as a simple rule that the presence of aspartic acid at position 57 of the DQβ chain and arginine at DQα 52 is associated with T1DM risk.[83] There are many exceptions to this rule, and knowledge of the complete sequences (allele) rather than dependence on this rule is essential.

Other Loci

In impressive collaborative form, more than a decade of international GWAS efforts led to the definition of many loci that contribute to small but varying degrees toward the development of T1DM.[84] For example, polymorphisms of the cytotoxic T-lymphocyte–associated protein 4 gene contribute to Graves disease and apparently to T1DM in some, but not all, populations, with relative risks less than 1.3.[85] A locus associated with the IL2 receptor has a statistical association based on analysis of thousands of persons.[86] Sequencing in one region has also identified rare mutations of a gene influencing interferon-alpha induction.[87] A curated list of T1DM risk loci can be found at http://www.immunobase.org.

Although much of the attention toward T1DM genetics has resided on HLA, other loci have drawn attention for their potential contributions to disease risk, including those related to insulin. In 1984, Bell and colleagues[88] published their discovery that variations in the number of nucleotide repeat elements located upstream (also known as 5′) of the insulin gene were associated with the development of T1DM. The longest group of repeats was associated with decreased diabetes risk.[89] In a mechanism potentially related to the role of autoimmune regulator (AIRE) in the thymus, the protective insulin gene polymorphism is associated with greater insulin messenger RNA expression within the thymus.[90] Indeed, for both the AIRE mutation of autoimmune polyendocrine syndrome type 1 (APS1) and the effect of insulin gene mutations, the level of expression of insulin in the thymus may be critical.

Additionally, *PTPN22*, a gene encoding a lymphoid-specific phosphatase that influences T-cell receptor signaling, is the third confirmed gene (vs. mere loci) influencing T1DM risk.[91] The polymorphism associated with T1DM (Trp for Arg) blocks binding to a signaling kinase molecule, C-terminal Src kinase (CSK). Nevertheless, the relative risk associated with this polymorphism for T1DM and other autoimmune disorders, such as rheumatoid arthritis, is only 1.7. The variant associated with disease risk results in gain of function and decreased T-cell receptor signaling.[92]

Genetic and Immunologic Heterogeneity by Age of Onset

T1DM can develop at any age, from the neonatal period to the last decades of life. Given that identical twins can become concordant more than 40 years after the first twin develops T1DM, not all age heterogeneity can be ascribed to different genetic syndromes.[77] Nevertheless, there is an overall correlation between the age at which diabetes develops in one twin or sibling and the age of development of diabetes in his or her relative. Children in whom T1DM develops at an early age more often are DR3/4-DQ2/8 heterozygotes. In addition, there is evidence that class I HLA alleles (or other non–class II genes within the HLA region) can influence the age at diabetes onset; an example is the A24 allele.[93] At the other end of the age spectrum, there is evidence that the protective HLA allele DQA1*01:02-DQB1*06:02 is not as protective for young adults as it is for children.[94]

The most characteristic difference related to age at diabetes onset is the presence of higher levels of IAA in children who develop the disease at an early age (e.g., <5 years). The high levels and frequent positivity of IAA make measurement of these autoantibodies the best single marker for diabetes development in young children.[95] In those children in whom autoantibodies arise during the first 3 years of life, IAA often appear first. In contrast, GADA are more often positive in adults who develop T1DM. Seroconversion to IAA or GADA as the first anti-islet autoantibody has also been linked to HLA.[96] The correlation of levels of IAA and age at diabetes onset may be related to faster progression to diabetes in children with higher levels. However, such rapid progression occurs only if IAA are present with another anti-islet autoantibody (discussed in the Stages in the Natural History of T1DM section).

Monogenic Forms of Diabetes

The most common forms of diabetes, T1DM and T2DM, are polygenic, meaning that the risk of developing these disorders is related to multiple genes. Environmental factors (discussed later) also play a part in the development of polygenic forms of diabetes. However, additional and rare forms of diabetes, termed *monogenic*, result from mutations in a single gene. Monogenic forms of diabetes account for about 1% to 5% of all cases of diabetes in young individuals. So far, more than 20 genes have been linked to monogenic diabetes. In most cases of monogenic diabetes, the gene mutation is inherited; in the remaining cases, the gene mutation develops spontaneously. Neonatal diabetes (ND) and maturity-onset diabetes of the young (MODY) are the two main forms of monogenic diabetes (Table 36.5), with ND far less common than MODY.

Genetic testing can diagnose most forms of monogenic diabetes. If genetic testing is not performed, people with monogenic diabetes may appear to have one of the polygenic forms of diabetes (i.e., T1DM or T2DM). When hyperglycemia is first detected in adulthood, monogenic diabetes is often misdiagnosed as general T2DM. Importantly, some monogenic forms of diabetes can be treated with oral diabetes medications, whereas other forms require insulin injections. A correct diagnosis that allows the proper treatment to be selected should lead to better glucose control and improved health in the long term.

Neonatal Diabetes

ND is a monogenic form of diabetes that occurs in newborns through the first 6 months of life. It is a rare condition occurring in only 1 in 100,000 to 500,000 live births. Owing to lack of professional awareness (although at decreasing frequency) or lack of screening, ND can be mistaken for T1DM, a disorder that usually occurs later than the first 6 months of life. For approximately one half of those with ND, the condition is lifelong, yet for the remainder, the condition disappears during infancy but can reappear later in life (i.e., transient ND). Symptoms of ND mimic those of T1DM and include thirst, frequent urination, and dehydration. In severe cases, insulin deficiency can lead to DKA. In addition, fetuses with ND may not see normal growth in utero, with newborns being small for gestational age (i.e., intrauterine growth restriction). Following, some infants demonstrate protracted growth and weight gain, but appropriate therapy has the ability to improve and may in fact normalize growth and development.

Maturity-Onset Diabetes of the Young

Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes that usually first occurs during adolescence or early adulthood[97] but may remain undiagnosed until later in life, as MODY patients often are asymptomatic or demonstrate only mild hyperglycemia. Several different gene mutations have been shown to cause MODY[98] (see Table 36.5). MODY variants have been estimated to account for about 1% to 5% of all cases of diabetes in the United States.[99] Relatives of those with MODY are at increased risk for the disorder (e.g., children of a MODY parent have a 50% chance of inheriting the disease). MODY patients are generally not overweight. Although both T2DM and MODY can run in families, MODY pedigrees typically portray a family history of diabetes in multiple successive generations.

Autoimmune Polyendocrine Syndrome Type I (AIRE Gene Mutations)

Autoimmune polyendocrine syndrome type 1 (APS1) is rare (<500 cases worldwide), with an increased incidence in Finland and Sardinia and among Iranian Jews, but it has a worldwide occurrence. The disorders of the syndrome, such as T1DM, mucocutaneous candidiasis, hypoparathyroidism, Addison disease, and hepatitis (see Chapter 43 for a more detailed discussion beyond genetics herein), identify a unique syndrome, and patients with this group of disorders almost always have mutations (usually autosomal recessive) of the *AIRE* gene on chromosome 21, a gene that encodes a DNA-binding protein. This gene may play an important role in maintaining self-tolerance, as well as influence the expression of what immunologists term *peripheral antigens* (e.g., insulin) in the thymus. This notion becomes relevant in that it has been hypothesized that greater thymic expression of insulin and other tissue-specific antigens leads to T-cell tolerance and disease suppression. At the same time, the influence on insulin is not specific, as damage to the adrenal glands, parathyroid glands, and other organs underlie the major features of APS1 and are why candidiasis infections predominate.

X-Linked Polyendocrinopathy, Immune Dysfunction, and Diarrhea (Scurfy Gene)

The syndrome of X-linked polyendocrinopathy, immune dysfunction, and diarrhea (*XPID*, also termed *IPEX*) is associated with overwhelming neonatal autoimmunity, and most children die in the first few days of life or in infancy.[100] In this syndrome, lymphocytes invade multiple organs. It is associated with insulitis and beta-cell destruction, as well as lymphocytic intestinal inflammation with flattened villi and severe malabsorption. It is inherited as an X-linked recessive disease affecting only boys, with a frequent clinical history of lack of male births.

TABLE 36.5 Characteristics of Monogenic Diabetes

Type of Diabetes	Gene or Syndrome	Affected Protein	How Common?	Typical Age at Onset	Type of Inheritance or Mutation	Causes Intrauterine Growth Restriction?	Transient or Permanent?	Treatment
Neonatal Diabetes Mellitus (NDM) (Rare; Occurs in ~1 of Every 100,000–500,000 Live Births)								
Permanent Neonatal Diabetes Mellitus (PNDM) (50% of All Cases of NDM)								
PNDM	*KCNJ11*	Kir6.2	Most common type of PNDM	3–6 months	Autosomal dominant (10%) Spontaneous	Yes	Permanent (this gene also causes a transient form of NDM)	Treated with insulin in the past but often can be treated with oral sulfonylureas
PNDM	*ABCC8*	SUR1-sulfonylurea receptor 1	Rare	1–3 months	Autosomal dominant (12% of NDM cases) Spontaneous	No	Permanent (this gene also causes a transient form of NDM)	Treated with insulin in the past but often can be treated with oral sulfonylureas
PNDM	*GCK*	Glucokinase	Rare	1 week	Autosomal recessive	Yes	Permanent	Insulin
PNDM	*IPF1*; also known as *PDX1*	Insulin promoter factor 1	Rare	1 week	Autosomal recessive	Yes	Permanent	Treat to replace endocrine and exocrine pancreas functions
PNDM	*PTF1A*	Pancreas transcription factor 1 A	Rare	At birth	Autosomal recessive	Yes	Permanent	Treat to replace endocrine and exocrine pancreas functions
PNDM	*FOXP3*, IPEX syndrome	Forkhead box P3	Rare	Sometimes present at birth	X-linked	Yes	Permanent	Insulin
PNDM	*EIF2AK3*, Wolcott-Rallison syndrome	Eukaryotic translation initiation factor 2-alpha kinase 3	Rare	3 months	Autosomal recessive	Yes	Permanent	Insulin and treatment for associated conditions
Transient Neonatal Diabetes Mellitus (TNDM) (50% of All Cases of NDM)								
TNDM	*ZAC/HYMAI*	*ZAC*: pleomorphic adenoma gene-like 1 or PLAG1 *HYMAI*: hydatidiform mole–associated and imprinted transcript	Most common form of NDM	Birth to 3 months	Autosomal dominant Spontaneous	Yes	Transient	Initially, treat with insulin; reduce dosage as needed; when diabetes recurs, treat with diet modification and physical activity; may also require insulin
TNDM	*ABCC8*	SUR1 (sulfonylurea receptor 1)	Rare	Birth to 6 months	Autosomal dominant Spontaneous	Varies	Transient (this gene also causes a permanent form of NDM)	Oral sulfonylureas
TNDM	*KCNJ11*	Kir6.2	Uncommon cause of TNDM but most common cause of PNDM	Birth to 6 months	Autosomal dominant Spontaneous	Yes	Transient (this gene also causes a permanent form of NDM)	Oral sulfonylureas
TNDM	*HNF1 β* (beta); also known as *HNF1B*	Hepatocyte nuclear factor 1B	Rare	Birth to 6 months	Autosomal dominant (60%) Spontaneous	Yes	Transient	Insulin

Maturity-Onset Diabetes of the Young (MODY; 1–5% of All Cases of Diabetes in the United States)

MODY 1	*HNF4A*	Hepatocyte nuclear factor 4α (alpha)	Rare	Adolescence or early adulthood	Autosomal dominant	No	Permanent	For most, oral sulfonylureas; some patients may need insulin
MODY 2	*GCK*	Glucokinase	MODY 2 and MODY 3 account for about two thirds of all cases of MODY MODY 2 is the second most common form of MODY	Mild hyperglycemia may be present at birth; otherwise, early childhood	Autosomal dominant	Lower than normal birth weight can occur	Permanent	Diet modification and physical activity; medications usually not required; some patients do not require any treatment during childhood
MODY 3	*TCF1*	Hepatic nuclear factor 1α (alpha) or HNF1α (alpha) or HNF1A	MODY 3 is the most common form of MODY	Adolescence or early adulthood	Autosomal dominant	No	Permanent	Initially, treat with diet modification; can be treated with oral sulfonylureas; some patients may need insulin
MODY 4	*IPF1*; also known as *PDX1*	Insulin promoter factor 1	Rare	Early adulthood; can present later	Autosomal dominant	No	Permanent	Oral sulfonylureas; some patients may need insulin
MODY 5	*TCF2*	Hepatic nuclear factor 1β (beta) or HNF1B	Rare	Adolescence or early adulthood	Autosomal dominant	No	Permanent	Insulin; patients also may need treatment for related conditions such as kidney failure or cysts
MODY 6	*NeuroD1*, or *BETA2*	Neurogenic differentiation factor 1	Rare	In the fourth decade of life	Autosomal dominant	No	Permanent	Insulin

The disease results from mutations of the gene encoding forkhead box P3 (FOXP3), which functions as a transcription factor and master switch for regulatory (suppressor) $CD4^+CD25^+CD127^{-/low}$ T lymphocytes.[101] Lack of such regulatory T cells leads to overwhelming autoimmunity. This is an important syndrome to recognize early, as bone marrow transplantation, affording restoration of functional T-regulatory cells (even with partial chimerism), has proved to be therapeutic.

The Genetic Risk Score

A recent advance in the ability to stratify patients with high risk for developing T1DM has occurred through construction of cumulative genetic risk models utilizing logistic regression algorithms that incorporate HLA and non-HLA alleles associated with T1DM.[68–70] Although HLA predominates risk, the inclusion of non-HLA loci increases model accuracy for classifying individuals as T1DM patients versus those at low risk. Although not currently used in standard clinical care, the GRS is being applied in the research space to improve subject selection and stratification. Genetic risk models enable prediction of T1DM progression,[102] differentiation of T1DM from T2DM and monogenic forms of diabetes,[68,69] and determination of T1DM onset prevalence in individuals older than 30 years. In the prospective TEDDY study, a GRS model improved the ability to identify infants with high risk of developing anti-islet autoantibodies by 6 years of age.[103] One caveat is due to inherent biases resulting from the predominance of GWAS and genotype/phenotype studies being conducted in majority Caucasian populations from Western Europe and the United States. The need for studies characterizing the T1DM genetic risk profile and the development of new GRS models in non-Caucasian populations represents a clear limitation to clinical introduction of current GRS modeling.[70]

Environmental Factors

Environmental factors have long been considered to influence the pathogenesis of T1DM. Regional differences in disease rates based on geography, seasonality in its diagnosis, rising incidence trends, and variance among twins are consistent with this conclusion. However, despite decades of research, no single environmental agent has been identified that would universally explain these observations. Identification of any such agent is especially difficult due to facets of the disorder's pathogenesis (e.g., the long prodromal/prediabetic phase that often precedes T1DM, that 85% of new cases arise from the general population rather than having a family history of T1DM, the limited disease frequency of 1 in 300).

The fivefold increase in T1DM rates over the past half-century has been considered strong evidence that environmental factors related to diabetes risk have changed since the 1960s. Factors that increase diabetes risk may be increasing, or just as likely, factors that suppress the development of T1DM may be decreasing. It is interesting that the rising incidence as a function of age at onset is occurring globally at different rates. The increase in Finland is highest for children in whom the disease develops before age 5 years, yet in the United States, the recent Search for Diabetes in Youth Study (SEARCH) showed the most rapid rise in teenagers.[56,104]

Models to Explain the Influence of Environment

Although the mechanisms by which environment influences T1DM are not known, numerous hypothetical models have been proposed. The accelerator and overload hypotheses suggest that childhood obesity increases insulin demand, overloading the islet cells and accelerating beta-cell autoimmune damage.[105,106] In the Copenhagen model, beta-cell destruction is proposed to result from interactions between the environment, immune system, and the beta cells themselves in genetically susceptible individuals.[107] The hygiene hypothesis attributes the rising incidence of autoimmune disorders to the decline in infections with reduced stimulation of the immune system.[108] Conversely, the fertile field hypothesis proposes that microbial infection induces a temporary state in which other antigens can more easily react to yield autoreactive T cells.[109] Also implicating the gut, the old friends hypothesis implicates dietary exposure as a possible direct regulator of the immune system and of self-tolerance by altering gut microbiota and intestinal permeability.[110] Finally, the threshold hypothesis suggests that the etiologic influences of genetics and environment, when evaluated as intersecting and reciprocal odds-ratio–based trend lines, result in a method to define the attributable risk for T1DM.[111]

A popular model of T1DM involves an environmental "trigger" presumed to initiate disease. Whereas it is true that the underlying genetic susceptibility to T1DM might allow for a triggering of anti–beta-cell immunity by an environmental event, it is increasingly accepted that environmental agents may contribute far beyond an initial triggering and rather act throughout the natural history of T1DM development, such as by modulating the ongoing autoimmune process (e.g., genes controlling immune regulation). There is no shortage of potential environmental candidates (e.g., breastfeeding, antibiotics, infant and childhood diet, viruses) that, either alone or in combination, may contribute to the disorder's pathogenesis. However, most fall into the groups involving infection (particularly viruses), vaccination, and diet.

Candidate Environmental Factors

Infection

The best evidence, if not the only strong evidence, for a specific environmental agent to contribute to T1DM pathogenesis involves congenital rubella infection, which, in contrast to noncongenital infection, greatly increases development of T1DM.[112] The mechanism(s) by which this congenital infection increases diabetes development is currently unknown, with hypotheses ranging from molecular mimicry to long-term alteration in T-cell function secondary to the congenital insult.

Enteroviruses are the most often discussed agents associated with T1DM. Enteroviruses are small RNA viruses that frequently infect young children. Coxsackie virus (a form of enterovirus) infections were implicated after initial anecdotal reports involving children who had severe infections and who died at T1DM onset. These case studies preceded the recognition that T1DM is not an acute disease but one involving a chronic prediabetic period of autoimmunity. At the time of presentation with diabetes, almost all children have a rising elevation in HbA_{1c}, reflecting what is likely months of hyperglycemia preceding diagnosis.[113] Thus, it is likely that viral infection at the onset of T1DM is most often incidental.

The potential importance of enteroviral infection was evaluated in studies from Scandinavia, with enteroviral infection (detected by changes in antiviral antibodies or detection of enteroviral RNA by molecular techniques) assessed during pregnancy and in infancy.[114] Some studies report an increased incidence of enteroviral infection during pregnancy among mothers whose children later developed T1DM, but others have not.[114,115] Considering that infants with a genetic risk for development of diabetes are

followed from birth, it becomes possible to prospectively analyze expression of enterovirus RNA. Enteroviral infections have been associated with appearance of anti-islet autoantibodies in some regions (Finland)[114] but not in others (Colorado),[115] a finding that may be related to differing frequency and/or timing of enteroviral infections in these two populations.

Rotavirus infection, another virus that commonly infects young children, has been associated with increased anti-islet autoantibodies in one study from Australia,[116] but an increase in rotavirus infection was not found in children with T1DM compared with controls. Other viruses are also being evaluated for association with the triggering of autoimmunity.

Vaccines

A series of studies have been performed addressing the concern that childhood vaccine timing might influence development of T1DM, yet none has provided any evidence that childhood vaccinations influence the development of diabetes.[117] This is an important health concern if parents alter their family's childhood vaccination schedule because of concern about development of T1DM.

Dietary Factors

Dietary factors as environmental T1DM triggers have also been extensively investigated. Early introduction of bovine milk in the form of infant formula has been hypothesized to increase T1DM, based primarily on retrospective studies associating early or increased bovine milk ingestion (or less breastfeeding) with an increased risk of disease. Several prospective studies in which infants were observed until the development of anti-islet autoantibodies failed to find any association or found only a weak association with either breastfeeding or bovine milk ingestion. Pilot studies of an infant formula lacking bovine milk proteins were initiated in Finland and suggest that weaning to insulin-free cow's milk formula might reduce the development of anti-islet autoantibodies in the first 3 years of life.[118] In contrast, the TEDDY study found no association between choice of first infant formula and risk of developing anti-islet autoantibodies.[119]

Studies from Germany and Denver, Colorado, in the United States provided evidence that early (<3 months) introduction of cereals may increase development of islet autoimmunity.[120] Both low vitamin D and ω-3 fatty acids, which can influence immune function, have also been associated with risk of T1DM.[121] Dietary interventions with these factors to date have not been shown to be therapeutic.

Natural History of T1DM—Emerging Concepts

Beta-Cell Mass Is Not Equal in All Individuals

Most historical models of T1DM have assumed a normal beta-cell mass at birth that declines once the autoimmune attack occurs (see Fig. 36.3). However, recent studies of cadaveric pancreata have shown beta-cell mass in normal, nondiabetic humans varies threefold to fivefold, independent of adult age or body mass index, with beta-cell mass likely mostly determined in the first two decades of life.[122] This has important implications when one considers the starting point for declining beta-cell mass during autoimmune beta-cell destruction. An individual's timeline to diabetes onset could be determined not by the severity of the autoimmune attack but the starting point for beta-cell mass (see Fig. 36.3). The reasons for variation in beta-cell mass are unknown but could include the in utero environment, events during the first decade of life, and yet unknown genetic or environmental determinants. Given the aforementioned observations of a smaller total pancreatic mass in individuals with new-onset T1DM,[40,41] both pancreatic mass and beta-cell mass might be impacted, because endocrine islet cells and exocrine cells share a common embryologic heritage. Together, these structural changes emphasize the need to better understand the timeline and determinants of human beta-cell mass.

Pancreatic Exocrine Abnormalities in T1DM

Autopsy studies first revealed reduced pancreas weights in deceased persons with T1DM. More recently, studies of higher-quality organ donor tissues reveal that this phenomenon affects not only subjects with long-standing T1DM but also recent-onset T1DM and nondiabetic persons with anti-islet autoantibodies.[40] Noninvasive imaging methods permit studies of pancreas volume in living subjects, which confirm that the pancreas in generally smaller in T1DM patients than in those without disease.[41] Islets constitute only 1% to 2% of the entire pancreatic volume; hence, beta-cell loss alone cannot account for the reduction in total pancreas size. Insulinopenic effects were initially thought to cause loss of pancreatic size or weight following T1DM diagnosis. However, detection of low pancreas weight at T1DM onset and in anti-islet autoantibody–positive organ donors without diabetes,[40,41] together with certain histopathologic findings in the T1DM exocrine pancreas (e.g., invasion of exocrine pancreatic tissue by $CD8^+$ T cells, $CD4^+$ T cells, $CD11c^+$ dendritic cells, and neutrophils[32,33] and C4d complement deposition[123]), suggest early loss of pancreas mass prior to diagnosis. Moreover, serum levels of trypsinogen, which are indicative of exocrine pancreas function, are reduced in patients with long-standing T1DM, in those with recent-onset T1DM, and in nondiabetic individuals with anti-islet autoantibodies[124] implying that disease-related effects within the exocrine pancreas may be present prior to T1DM diagnosis.

Metabolic Progression Before Hyperglycemia

The intravenous glucose tolerance test aids in evaluating the time to onset of diabetes among persons expressing anti-islet autoantibodies. Glucose is given at 0.5 g/kg over 5 minutes (maximum 35 g, 25 g/dL), and insulin levels are measured before and 1 and 3 minutes after the glucose infusion.[125] Most persons who are within 1 year of developing overt T1DM have no first-phase insulin secretion after intravenous glucose administration. A simpler measurement in someone progressing to T1DM is the HbA_{1c}, which in most prediabetic individuals increases progressively (although in the normal range) 1 or 2 years before overt hyperglycemia develops.

The diagnosis of T1DM usually relies on the presence of fasting hyperglycemia (≥126 mg/dL, 7.0 mmol/L), but with prospective evaluation, many persons have diabetes determined by OGTT according to the 120-minute criterion (≥200 mg/dL, 11.1 mmol/L), with a nondiagnostic fasting glucose level. Impaired fasting glucose (100–125 mg/dL, 5.6–6.9 mmol/L) or impaired glucose tolerance (glucose at 120 minutes on OGTT, 140–199 mg/dL, 7.8–11.0 mmol/L) is usually present within 6 months before the onset of overt T1DM.

C-Peptide Loss After Hyperglycemia

After diabetes is diagnosed, levels of C-peptide in serum can be used to assess remaining beta-cell function, because C-peptide is

released from the beta cell in equimolar concentrations with insulin but does not undergo hepatic extraction and has a longer half-life in circulation. C-peptide levels are usually measured in the fasting state, after intravenous glucagon, or with a standard liquid meal. Such measurements are primarily of importance for trials of therapies to alleviate loss of insulin secretion after diagnosis. Determination of the C-peptide concentration provides the best current measure for assessing the impact of new therapies. A small amount of remaining C-peptide is associated with impressive metabolic benefit, as shown in the Diabetes Control and Complications Trial (DCCT).

Transient Hyperglycemia

A significant number of children are evaluated by endocrinologists for transient hyperglycemia. The usual history is one of severe stress associated with hyperglycemia that resolves within days to 1 month. Such children may be in the honeymoon phase of T1DM, or they may truly have a transient episode of hyperglycemia. Rarely, diabetes in children is misdiagnosed. Children without severe stress who have transient hyperglycemia or who have a relative with T1DM are more likely to have early T1DM. Absence of anti-islet autoantibodies and a normal first-phase insulin secretion during an intravenous glucose tolerance test strongly indicate transient hyperglycemia and not T1DM. It is not known whether children with transient hyperglycemia are at increased risk for T2DM later in life.

Emerging Biomarkers of T1DM Natural History and Diagnosis

As mentioned throughout this chapter, seroconversion to anti-islet autoantibody positivity is the most reliable predictive biomarker of T1DM identified to date,[11] but as noted, as many as 10% of T1DM patients are negative for anti-islet autoantibodies at the time of diagnosis.[126] Early diagnosis and treatment with exogenous insulin has been associated with improved disease management and fewer complications. Moreover, there are notions that early intervention prior to the first signs of autoimmunity might offer the greatest chance to successfully prevent T1DM progression and onset in high-risk individuals. Hence, a substantial effort has been directed at the discovery and characterization of new biomarkers to improve our understanding of the disease pathogenesis, as well as our ability to predict and stage disease progression, facilitating early diagnosis.

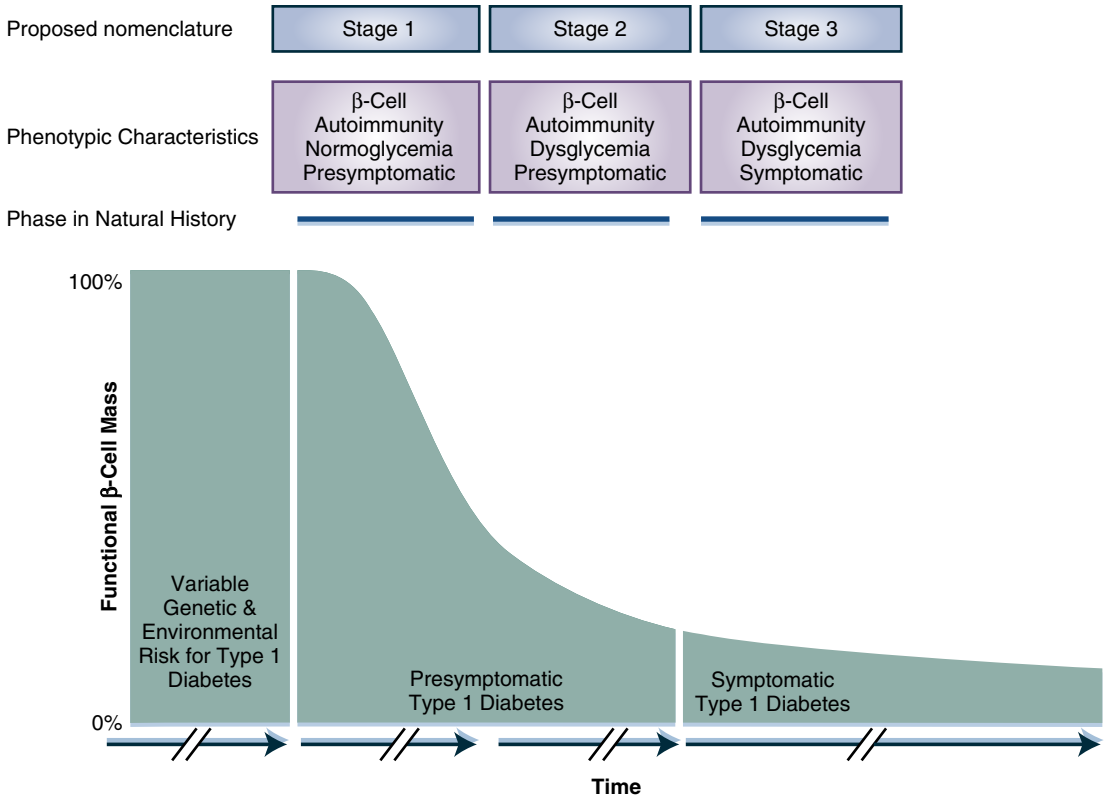
The majority of biomarkers identified signify metabolic defects across T1DM progression. Specifically, so-called beta-cell death assays, proinsulin/C-peptide ratio, and the T1D Diagnostic Index60 (Index60) monitor the decline in functional beta-cell mass related to autoimmune pathology and/or beta-cell stress in T1DM. Specifically, beta-cell death assays involve the measurement of cell-free unmethylated insulin (*INS*) DNA from blood or serum.[127] Cell-specific methylation patterns can be used to identify *INS* DNA released from necrotic beta cells as opposed to other cell types. The short half-life of cell-free DNA in circulation is a notable limitation, but as a single example, cell-free unmethylated *INS* DNA assays were able to detect a decline in beta-cell death consistent with preserved beta-cell function (measured by C-peptide) following treatment with teplizumab (anti-CD3) in a clinical trial setting.[128] Similarly, an elevated serum proinsulin/C-peptide ratio measured under fasting conditions implies impaired processing of proinsulin to insulin and C-peptide within the beta cell, suggesting duress related to metabolic, oxidative, or immunologic stress.[129] These assays are expected to have the greatest utility in the research setting, but the ability to evaluate changes in beta-cell death and dysfunction in real time represents a massive step forward. Meanwhile, the Index60 has been introduced as a clinical/diagnostic biomarker measuring functional beta-cell mass. The test requires an OGTT with calculations incorporating the log-adjusted fasting C-peptide level, 60-minute C-peptide level, and 60-minute blood glucose level.[130] An Index60 value greater than 2 in an individual with anti-islet autoantibodies is considered indicative of T1DM onset, but the need for standard clinical C-peptide assays has limited its widespread implementation.

Alterations to the microbiome have been suggested as yet another biomarker of T1DM. Although less conclusive than the metabolic markers discussed previously, prospective studies of infants and children with high genetic risk for T1DM have revealed the gut microbiome as an early indicator of disease progression, even prior to seroconversion. Extensive profiling of the gut microbial community is generally accomplished by sequencing the microbial 16S ribosomal RNA gene from fecal samples. Overall, a high Bacteroides to Firmicutes ratio has been associated with anti-islet autoimmunity, whereas the presence of butyrate-producing bacteria may protect against T1DM by promoting the synthesis of mucin and reducing intestinal leakiness.[131] In addition, low diversity and poor stability of the gut microbial community are associated with T1DM,[132] although a cause-and-effect relationship has not been determined. Yet these T1DM-related microbiome biomarkers from high-risk infants enrolled in the TEDDY study have also shown geographically distinct patterns with the relative abundance of the most prominent strains and overall microbial diversity differing across six study sites (the US states of Colorado, Georgia/Florida, and Washington, as well as Finland, Germany, and Sweden).[58] At present, it is unknown what factors contribute toward these shifts in the pre-T1DM gut microbiome, but they have been hypothesized to include diet, antibiotics, infections, and/or household environment (e.g., exposure to pets, allergens, pollutants).

Stages in the Natural History of T1DM

Whereas the etiology and precise mechanisms leading to T1DM remain somewhat elusive, studies over the past three to four decades have generated, without question, a wealth of information—be it immunologic, serologic, metabolic, or genetics based—regarding the natural history of pre-T1DM through the time of its formal diagnosis.[133] As but one example and as noted earlier, anti-islet autoimmunity in the form of anti-islet autoantibodies, in the context of genetic susceptibility and metabolic assessments, have clearly been identified as predictive tools for progression to overt disease.[11] Large international cohorts (e.g., TEDDY, Diabetes Prevention Trial Type 1 [DPT-1], DAISY, BabyDiab) have been studied in both relatives of patients with T1DM and in the general population who are at high genetic risk; this, to better understand the disease, reduced rates of DKA, decrease mortality, and set the stage for research-based efforts seeking T1DM prevention. Indeed, these natural history studies often led to the earlier diagnosis of T1DM and a reduced frequency of DKA at onset in those followed prospectively.[134] Several recent studies have also demonstrated long-term glycemic benefits seen for those patients who did not experience the severe metabolic derangement of DKA at formal diagnosis.[135]

Although the diagnosis of diabetes has traditionally been based on ADA criteria,[136] it is increasingly clear that the actual onset of



• **Fig. 36.5** Staging of pre–type 1 diabetes mellitus. (Redrawn from Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care*. 2015;38[10]:1964–1974.)

the disease, per se, often occurs months to years before the onset of symptoms. Thus, T1DM is a unique physiologic state where autoimmunity is present and progression to metabolic derangement and clinical onset can be predicted, especially in younger children and adolescents. As such, the ADA, JDRF, and Endocrine Society released a joint position statement calling for the staging of pre-T1DM[137] (Fig. 36.5). Stage 1 is defined by the presence of two or more anti-islet autoantibodies with normoglycemia (normal glucose tolerance on 2-hour OGTT). Stage 2 shows progression to dysglycemia (impaired glucose tolerance) in the setting of two or more anti-islet autoantibodies. Stage 3 occurs when a patient meets ADA criteria for the diagnosis of diabetes.

To be clear, there are no evidence-based recommendations for practitioners caring for anti-islet autoantibody–positive patients, or for performing formal "T1DM staging," other than to encourage enrollment in research studies. Nonetheless, close monitoring of high-risk patients in natural history studies markedly reduces DKA rates at diagnosis, and when combined with the notion that partaking in research is critical to finding a means of preventing T1DM, research participation in family members of those with T1DM should be encouraged. Yet only when an effective preventative strategy for T1DM is identified will the notion of universal risk screening and T1DM staging for all children be considered justifiable.

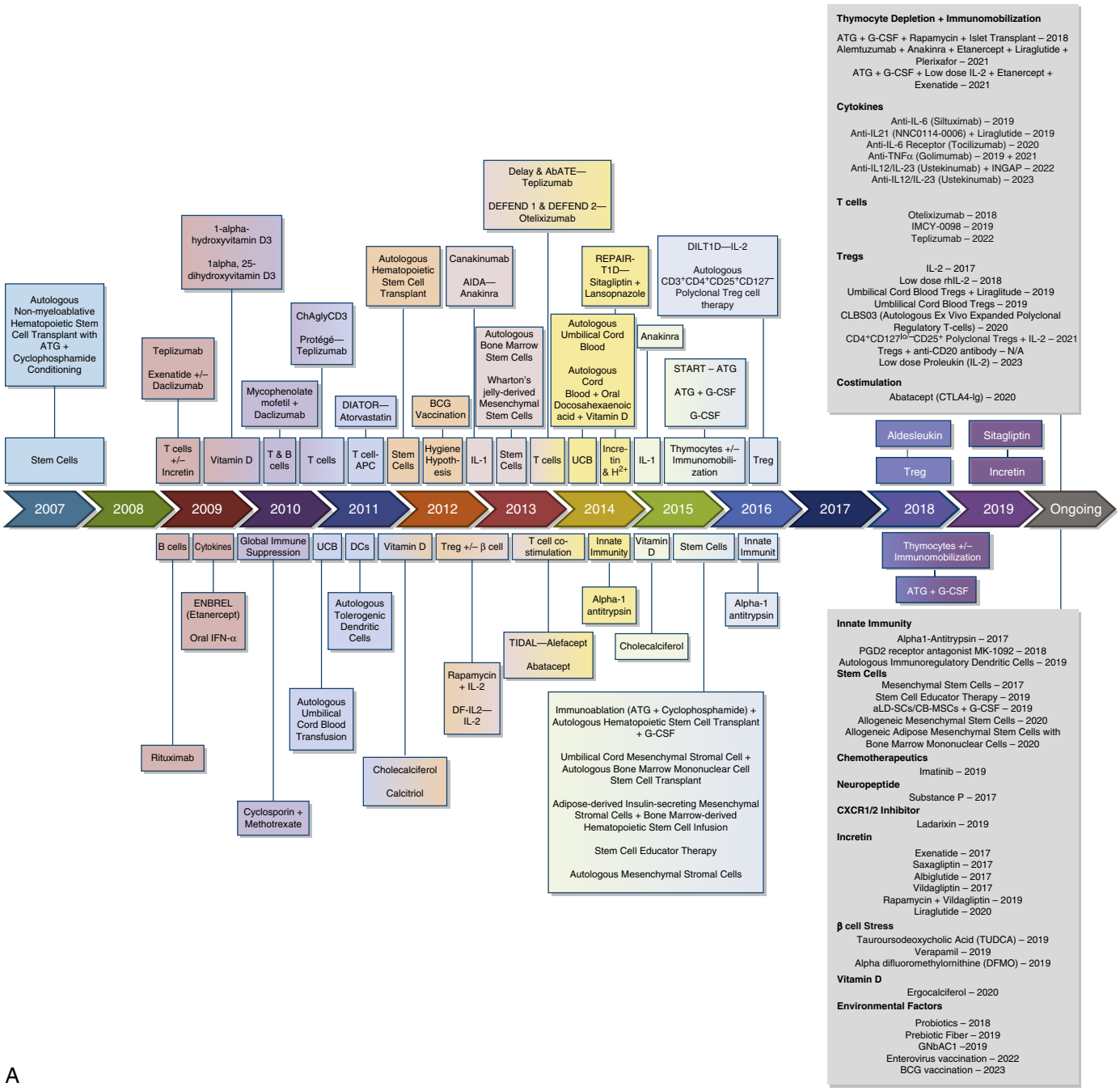
Immunotherapy for the Prevention and Reversal of T1DM

The intersection of two independent events in the 1970s—the generalized acknowledgment that T1DM represents an autoimmune disease, alongside the development of immunosuppressive agents—led to decades of efforts directed toward immunotherapy as a means to either prevent T1DM (i.e., avert symptomatic onset) or, once diagnosed, reverse it (commonly termed *intervention*). To be clear, studies attempting to prevent the development of T1DM involve treatment of persons at varying stages of disease risk based on a combination of genetic susceptibility, the number of T1DM-associated autoantibodies, and the degree to which glucose levels are elevated.[138] Although the populations subject to such procedures would differ, the common goal is to prevent further loss of beta-cell function.

At present, in a setting of either disease prevention or intervention, there is no proven safe and effective immunotherapy for use in a general public health care setting that prevents T1DM or reverses the disease, as judged by preservation of C-peptide. However, in research settings, potentially promising candidates do exist for disease prevention (e.g., oral insulin, GAD vaccine) or intervention (e.g., anti-CD3, antithymocyte globulin [ATG], ATG plus granulocyte colony stimulating factor, CTLA4-Ig).[139–143] Indeed, dozens of clinical trials, ranging from small to large, have been completed in both settings; a limited number are discussed later based on their proposed method of action (Fig. 36.6).

Immunosuppression

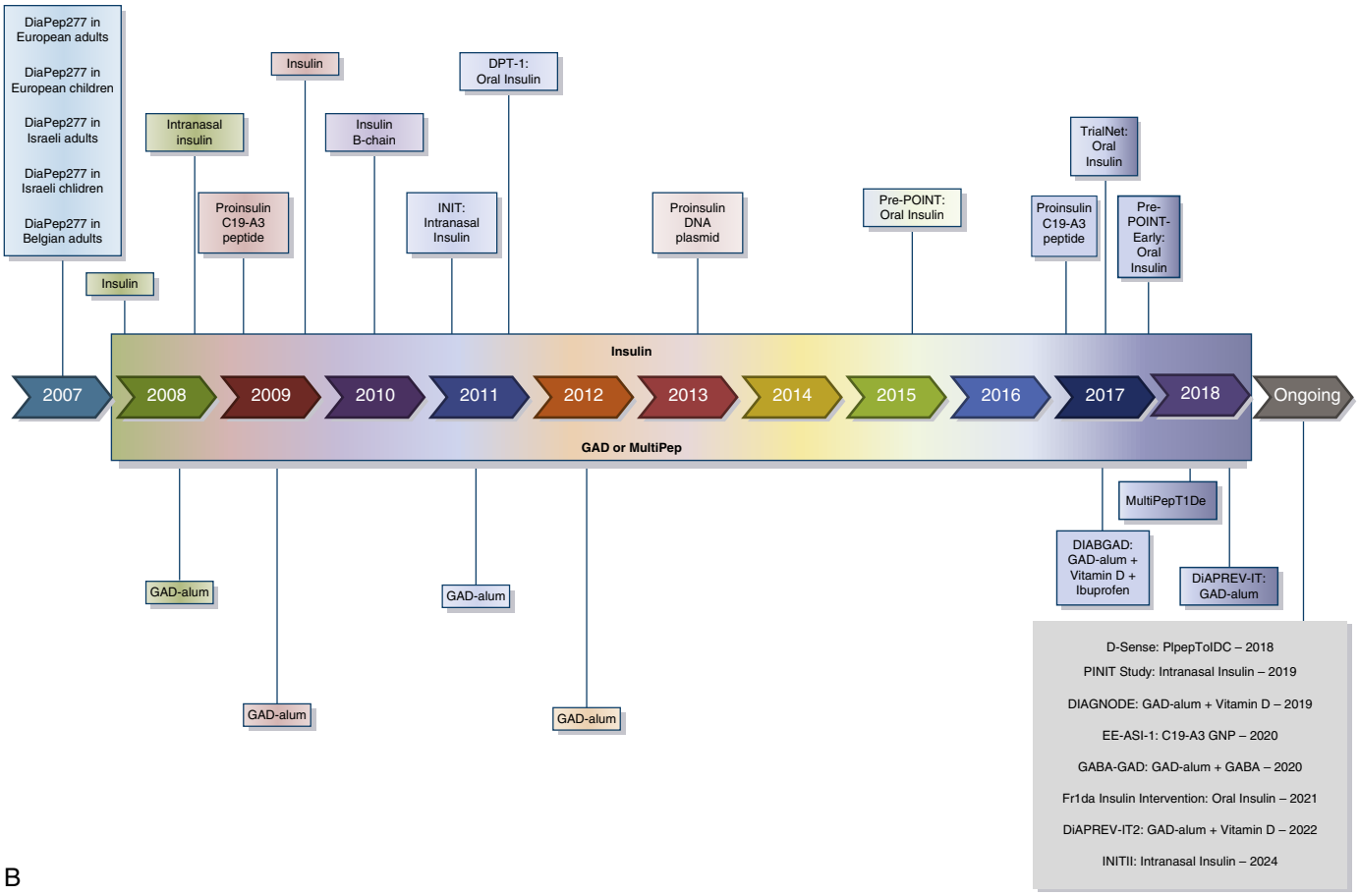
The earliest studies of therapies to prevent beta-cell destruction used immunosuppressive agents. Large intervention trials of cyclosporine indicated that this agent prevented further loss of C-peptide secretion and improved metabolic function when it was administered in recent-onset T1DM cases. Cyclosporine therapy



A

• **Fig. 36.6** (A) Shifts in T1DM clinical research emphasis over 10 years from 2007 to 2017. Therapeutic agents and target mechanisms/objectives are noted for T1DM clinical trials over the past decade, organized by their year of first publication. Current ongoing studies are noted with their expected year of completion. *AIDA,* Anti-Interleukin-1 in Diabetes Action trial; *APC,* antigen-presenting cell; *ATG,* antithymocyte globulin; *BCG,* bacillus Calmette-Guérin; *DCs,* dendritic cells; *DF,* dose finding; *G-CSF,* granulocyte colony-stimulating factor; *IL,* interleukin; *T1DM,* type 1 diabetes mellitus; *UCB,* umbilical cord blood. (A, modified from Atkinson MA, Posgai A, Wheeler DCS, Peakman M. The challenge of modulating β-cell autoimmunity in type 1 diabetes. *Lancet Diabetes Endocrinol.* 2019;7[1]:52–64.

did not, however, maintain a nondiabetic state when therapy was instituted after the onset of diabetes, and with discontinuation of the drug, patients rapidly lost C-peptide reserve. The combination of inability to cure diabetes and toxicities associated with cyclosporine (particularly nephrotoxicity and concern about increased risk of malignancy) ruled out its acceptance for use. Other immunosuppressive agents used in subsequent studies, such as prednisone and azathioprine, demonstrated relatively little effect in disease intervention. In the time since, a litany of agents (e.g., anti-CD20, ATG, mycophenolate plus anti-IL2 receptor α-monoclonal antibody) thought to act at least in part via immune depletion, a form of immune suppression, have demonstrated mixed results in T1DM intervention efforts (see Fig. 36.6A). Some provide no therapeutic benefit (i.e., preservation of C-peptide, reduced



B

Fig. 36.6, cont'd (B) Timeline of completed and ongoing antigen-specific trials for T1DM. B, modified from Roep BO, Wheeler DCS, Peakman M. Antigen-based immune modulation therapy for type 1 diabetes in the era of precision medicine. *Lancet Diabetes Endocrinol*. 2019;7[1]:65–74.)

insulin needs, reduction in hypoglycemic events), whereas others have achieved at least one of these beneficial parameters, usually C-peptide preservation. However, even when efficacious, for most subjects, the beneficial effect was not durable (i.e., 6 months to 1 year of benefit).[141,143] It is possible that the treatment effect may persist for a longer term with repeat dosing, although this approach has not been tested. Therefore, at present, although T1DM is an immune-mediated disorder, it is not treated with immunotherapeutic agents in clinical practice. This is not to say that hope is lost for such being the case in the future. Indeed, in recent times (e.g., year 2000 to the present), studies related to two forms of modified antibodies against the CD3 molecule have, without question, generated the most research interest and offer some degree of eventual optimism.

Throughout several phase I and phase II intervention studies, a single course of anti-CD3 therapy decreased the loss of C-peptide secretion over a 12- to 24-month period in new-onset T1DM patients.[141] However, phase III trials were not considered of therapeutic benefit to the extent that commercialization of these agents was to some degree disrupted. Concerns related to the activation of Epstein-Barr virus infection, the duration of C-peptide preservation, and the protocol by which the drugs are administered have further limited their development. Thus, limited efforts continue to move anti-CD3 back into the therapeutic setting. A recent study demonstrated that a short course of the anti-CD3 monoclonal antibody, teplizumab, delayed progression to clinical T1DM in high-risk patients.[144]

Immunologic Vaccination

In animal models (especially the NOD mouse), it is relatively easy to prevent T1DM. Indeed, although somewhat dated, a widely recognized effort notes more than 200 such means.[145] Yet animal models have permitted development of the most exciting potential prevention or intervention modality of immunologic vaccination. Excitement is derived from the specificity and the relatively low risk of these therapies compared with immunosuppression. The basic concept behind immunologic vaccination is the induction of regulatory T lymphocytes that target a given beta-cell antigen and, on encountering the target antigen (e.g., insulin, GAD) in the context of the vaccine formula or route of administration, produce cytokines and cell-mediated effects that suppress autoimmunity and tissue destruction.[146]

Induction of a protective immune response may depend on the route of administration of the given antigen (e.g., oral, nasal, intradermal) or on the use of an altered antigen (e.g., altered peptide ligands). For example, insulin given either orally or by subcutaneous injection prevents diabetes in NOD mice.[147,148] Intact insulin is not necessary, because the insulin B chain and an immunodominant B(9-23) peptide of insulin were also effective.[149] The latter molecules have no insulin-like metabolic effect but are able to activate regulatory T lymphocytes that target insulin.

In terms of translation, DPT-1 studied both oral insulin and parenteral injections of low doses of insulin (see Fig. 36.6B). The results of the parenteral trial did not demonstrate a reduction in

the risk of developing diabetes. The oral trial did not document an overall benefit of insulin, but in the subgroup with higher levels of IAA at entry, a statistically significant delay in progression to diabetes was observed.[150] Although the National Institutes of Health TrialNet conducted a follow-up study testing the efficacy of oral insulin to prevent T1DM in those having certain criteria related to age and IAA level, no benefit of oral insulin for delay or prevention of T1DM was seen.[151] Nevertheless, a pilot trial (Pre-POINT) led by investigators in Germany involving the administration of high-dose oral insulin in children at high genetic risk for T1DM with a family history of the disease reported that this form of therapy was capable of altering the anti-insulin immune response without inducing hypoglycemia.[152] Thus, potential value of oral insulin remains uncertain.

Therapeutic Targeting for Mechanisms of Beta-Cell Death

As noted, the most prominent cell found in human islets in settings of T1DM is the $CD8^+$ cytotoxic T cell, which likely participates in beta-cell killing because of its ability to recognize targets via antigen in the context of MHC class I, which is elevated in many islets in those with the disease.[39] Therapeutically, lymphocytes and memory lymphocytes of the adaptive immune response can be targeted by anti–T-cell drugs directed at CD3 (teplizumab), CD2 (LFA3-Ig), and certain co-stimulatory blockers.[141,153,154] Indeed, partial success of such compounds in recently diagnosed T1DM, defined by preservation of C-peptide production over several months to years, speaks toward an important role for such autoreactive lymphocytes in beta-cell destruction, at least late in the pathogenesis of this disease. Inflammatory cytokines are also known to harm islets, and therefore anti-inflammatory therapies targeting cytokines may hold promise, as observed in a trial blocking TNF.[155] These observations provide further support for the concept of combination therapies.

With the growing concept that multiple mechanisms likely contribute to the pathogenesis of T1DM, enthusiasm has increased for utilizing combination therapies to prevent or reverse the disease. Examples of combinations with respect to immune intervention would include an induction component using drugs targeting inflammation and T-/B-cell memory and a maintenance component that could involve antigens to induce tolerance to beta cells. At the same time, emerging data support an increasing role for beta-cell stress and loss of function as potential contributing factors for T1DM development; therefore, combination therapy involving the addition of drugs that stabilize and maintain beta-cell survival and function should be considered.[156]

Pancreas and Islet Cell Transplantation

Pancreatic transplantation for patients requiring a kidney transplant has become an accepted clinical procedure, but one that for a variety of reasons, particularly the shortage of organ donors, remains relatively rare. Patients with a kidney transplant receive immunosuppressive drugs that, given the nature of clinical monitoring for renal transplant rejection (e.g., creatinine, albumin), allow for careful (i.e., tailored) drug delivery for optimal care. Several studies suggest that improved results for pancreatic transplantation occur in the setting of simultaneous pancreas and kidney transplantation versus transplantation of pancreas alone.[157] Indeed, there is considerable debate concerning pancreas transplantation without transplantation of a kidney. With a successful pancreas transplant, hyperglycemia is immediately reversed for most, and there is growing evidence of improved long-term outcomes. Nevertheless, the surgery is extensive, and there are many potential complications associated with the transplant. Perhaps for these reasons, along with improvements in diabetes management (and thus reducing long-term complications, including kidney failure), it appears that the procedure peaked in the mid- to late 2000s, with declines in the number of procedures performed in the United States thereafter.[158]

Beyond therapeutic interest in pancreatic transplantation, additional research has focused on the mechanisms of pancreas rejection for what it might reveal regarding the immunologic mechanisms of T1DM. In that regard, in T1DM patients provided with a pancreatic transplant, diabetes (i.e., a repeated need for exogenous insulin replacement therapy) can recur as a result of mechanisms considered attributable to either recurrent autoimmunity (as typified by the recurrence of T1DM-associated autoantibodies once lost) or, more often, allograft rejection.[159] It is difficult to monitor and determine which of these two forms of islet destruction are occurring with the development of hyperglycemia, and currently there are no specific means in terms of treatment that would separate the two, although at least one study has provided evidence for recurrent autoimmunity manifested by the induction of anti-islet autoantibodies prior to graft rejection.[160]

With respect to islet (vs. pancreas) transplant, therapeutic hopes for this procedure have been high since the late 1960s when early pioneering approaches were developed for islet isolation from whole pancreata. Indeed, with islet autotransplantation in patients with pancreatitis, most patients become insulin independent and remain so.[161] Yet it was not until the early 2000s that a group from Edmonton, Canada, used meticulous islet isolation techniques, transplantation of islets from multiple pancreata, avoided the use of steroids, and utilized an immunosuppressive regimen involving the drug rapamycin, resulting in improved outcomes for patients with T1DM. This so-called Edmonton protocol has subsequently been tested in a series of specialized centers throughout North America and Europe. It is clear that in many centers, albeit with varying degrees of success, islet cell transplant can prove effective (i.e., ~60% insulin independent at 1 year), especially for patients with severe hypoglycemia when the goal is long-term prevention of severe hypoglycemic episodes. For most of the patients who achieve insulin independence, resumption of use of low doses of insulin is necessary within 2 years, and by 5 years the benefits of the form of therapy wane.[162] Even with these positive results, the number of islets available from cadaveric donors for transplantation is quite limited, and the toxicities of the drugs used probably exceed the level of benefit achieved, except for those most subject to disease-associated complications (e.g., severe, recurrent hypoglycemia). Further research to achieve tolerance without long-term immunosuppression is essential, as is continued development of systems allowing for xenogeneic transplantation.

Of immense promise for the future are therapies involving the use of surrogate beta-like cells (i.e., insulin producing, glucose responsive) produced from stem cells, either embryonic or induced from pluripotent cells originating from a variety of tissues. In addition, use of encapsulation or biosynthetic membranes to protect transplanted islets, combined with the increasing use of long-term continuous glucose monitoring (CGM), combined with improvements in modes of insulin delivery (e.g., analogues, pumps) or use of secondary agents in

patients with T1DM, will almost certainly raise the bar for consideration of the risks and benefits of both islet and pancreatic transplantation.

Stem Cell or Xenogeneic Islet Cell Transplantation

As noted, improved metabolic control averting the need for exogenous insulin replacement can be achieved with beta-cell replacement in those with T1DM, either through whole-pancreas transplantation or via pancreatic islet cell transplantation. Therefore, although whole-pancreas and islet cell transplantation have both become highly operationalized and often effective procedures, the quantitative paucity of suitable organ donor tissues, taken together with complications associated with immune suppression, have severely limited their widespread therapeutic application.[163] As a result, numerous research efforts have been extended over the past decade toward the development of sustainable sources of "surrogate" insulin-producing cells (e.g., xenogeneic islets, induced pluripotent stem cells, fetal stem cells) alongside additional methodological investigations, all designed to improve therapeutic outcomes utilizing such cells (e.g., genetic modification, encapsulation).[164,165] Importantly, engineering and/or genetic modification–based systems have been proposed to avert aberrant and deleterious immune responses associated with T1DM and/or the use of allogenic cells.[166]

Indeed, remarkable progress has been made over a relatively short period of time with respect to generating beta-cell equivalents (i.e., glucose-responsive insulin-producing cells) from human stem cell populations, be they derived from induced pluripotent stem cells or embryonic stem cells.[167] Xenogeneic efforts (e.g., porcine islets), for a variety of reasons, although still a potential therapeutic option, are currently less favored than stem cells due to a variety of technical challenges, as well as concerns over safety.[168]

Stem cell therapy–based efforts as a therapy for T1DM largely arose from a strategy designed in laboratory-based settings to recapitulate the islet cell developmental pathway while generating single hormone-positive beta cells capable of glucose-stimulated insulin secretion.[169] Progress has been remarkable, with clinical trials already planned involving the use of stem cell–derived pancreatic progenitors capable of developing into functional beta and islet cells.[170]

Although the future of stem cells as a clinical therapy is promising, a series of concerns/limitations remain. First, beta cells, the primary cell sought for stem cell development, in their natural setting do not exist in isolation (i.e., beta cells alone) but rather exist in a heterogenous three-dimensional structure comprised of the islets of Langerhans. Hence, the notion of generating a cell therapy in the absence of a full complement of islet cells (i.e., alpha, beta, delta, pancreatic polypeptide; see Chapter 33) must be considered moving forward, especially in the context of hormonal counterregulation. In addition strong consideration must continue to be given toward averting what likely would be an assault on the target tissue—both autoimmune and alloimmune, depending on the setting. To this end, effort for any so-called protective devices should encompass a need to (1) provide biocompatibility; (2) see ample blood supply to sustain cellular survival and functional beta-cell mass capable of imparting normoglycemia; (3) prevent sensitization and be immune protective; (4) trap potentially oncogenic cells; and (5) allow for rapid response of insulin, in terms of both activating and suppressing insulin secretion, to changing glucose concentrations.[166]

Disorders Associated With Immunity to Insulin/Insulin Receptor

Insulin Autoimmune Syndrome

The insulin autoimmune syndrome, also termed *Hirata syndrome*, is rare and is typically associated with hypoglycemia.[171] These patients have extremely high concentrations of autoantibodies reacting with human insulin in the absence of exogenous insulin therapy. It is thought that inappropriate (i.e., nonregulated by the overall blood glucose level) release of autoantibody-bound insulin produces the hypoglycemia. Interestingly, and for reasons unknown, the disease occurs most commonly in persons of Asian descent.[172] Among 50 Japanese patients with the syndrome and possessing the typical polyclonal anti-IAA, 96% had an HLA-DR4 allele, and 84% possessed a DRB1*04:06 allele. In contrast, patients with monoclonal anti-IAA do not possess such a profound and specific HLA association.[173] Most patients with this disorder develop the disease in association with treatment of sulfhydryl-containing medications, particularly methimazole, as well as alpha-lipoic acid.[174] Treatment usually consists of stopping these medications, and for more than 75% of the patients, the disease remits.

Insulin Allergy

Mild immune reactivity against exogenously administered insulin is not an uncommon feature in T1DM management. Indeed, essentially all patients treated with recombinant human insulin produce anti-insulin antibodies.[175] The levels of these antibodies are relatively low, and they do not appear to interfere with insulin therapy for the majority of individuals. Most studies show no relationship between the presence of anti-insulin antibodies and complications associated with T1DM (e.g., retinopathy, neuropathy).[175] However, there are reports correlating insulin antibodies with macrosomia.[176] With the introduction of recombinant human insulin replacing animal insulins, symptomatic immune responses to insulin such as immediate hypersensitivity, delayed hypersensitivity, lipoatrophy, and lipohypertrophy have decreased.

Allergic reactions can occur with insulin analogues, modifications of recombinant human insulin, although this is uncommon. More common features include allergies to affiliated lubricants; preservatives; and plastics in bottles, stoppers, syringes, and needles. The usual mode of therapy for such situations consists of substituting the type or formulation of insulin and administration of oral antihistamines or topical mast cell stabilizers for immunoglobulin E–mediated local reactions, followed by insulin desensitization or addition of small amounts of glucocorticoids to the insulin injected for local delayed hypersensitivity reactions.[177,178]

Anti-Insulin Receptor Autoantibodies

Anti-insulin receptor autoantibodies (i.e., type B insulin resistance) are associated with hypoglycemia, hypercatabolism, severe acanthosis nigricans, and insulin resistance. It appears that anti-insulin receptor autoantibodies can act as either antagonists or agonists for this disease. This syndrome is quite rare, is often associated with non–organ-specific autoimmunity, and treatments usually involve various forms of immunosuppression (e.g., rituximab (anti-CD20), steroids, intravenous immune globulin, cyclophosphamide), albeit with mixed success.[179]

Clinical Presentation

The peak age for presentation of T1DM is at or during puberty, with a smaller peak in children between 5 and 7 years of age. The symptoms and signs are related to the presence of hyperglycemia and the resulting effects on fluid and electrolyte balance, which include polyuria, polydipsia, polyphagia, weight loss, and blurred vision.[9] Because infection may have precipitated or preceded the initial presentation, symptoms of infection may also be present, such as fever, sore throat, cough, or dysuria. Particularly in children, the onset of symptoms can occur over a brief period, and families may be able to date the onset with considerable accuracy. In older persons with T1DM, the onset of symptoms may be insidious over months, and many are mistakenly diagnosed as having T2DM by screening during this asymptomatic period.

If onset of T1DM is associated with DKA, which is not uncommon, additional symptoms related to this acute metabolic complication of diabetes are also present. These symptoms can include abdominal pain, nausea, and vomiting. Variable effects on mental status may be seen, ranging from slight drowsiness to profound lethargy and even coma if the condition has been untreated for a significant period.

Laboratory Findings at Presentation

Plasma glucose concentrations at presentation of T1DM are elevated, usually in the range of 300 to 500 mg/dL (16.7–27.8 mmol/L). If the presentation is uncomplicated, the remainder of the fluid and electrolyte measurements may be completely normal. However, if DKA is present, the measurements will reflect the presence of an acidosis, as well as more severe dehydration. DKA is discussed more fully later in this chapter.

At presentation, the C-peptide level (a surrogate marker for insulin secretion) is generally in the low-normal range and declines over time. However, residual C-peptide may be detected throughout the natural history of diabetes. In reference laboratories, pancreatic autoantibodies are present in approximately 98% of individuals at diagnosis, but most commercial laboratories do not provide either the full spectrum of assays or equivalently sensitive or specific assays, resulting in both false-negative and -positive assays (discussed previously). Furthermore, antibody titers diminish over time and may be less prevalent in certain ethnicities.[180]

Treatment

Principles of T1DM Management

Despite advances in understanding the autoimmune nature of T1DM, this disease remains incurable yet manageable. Management requires orchestrated efforts on the part of the person with diabetes, family members, and the multidisciplinary health care team. Important stakeholders may vary, depending on the age of onset of the disease, comorbid conditions, stages of transition throughout the life span, from early childhood through adolescence and young adulthood to adulthood and the middle-age years, then finally culminating in the advancing years of the geriatric population. Although diabetes diagnosed in childhood is predominantly due to T1DM,[181] there is increasing recognition that T1DM is diagnosed in adulthood.[182] The likelihood for residual beta-cell function is greater with an older age of onset of disease.[183] Nonetheless, a diagnosis of T1DM at any age requires exogenous insulin therapy given by injection or continuous subcutaneous insulin infusion (CSII); careful attention to dietary intake, especially with regard to carbohydrates, the major contributor to glycemic excursions; frequent or continuous monitoring of glucose levels with a blood glucose meter or CGM, respectively; and an exercise program that is as much therapeutic as recreational in nature. These management activities require constant vigilance, with attention to treatment needs 24 hours per day, 7 days a week, every year.

Such a rigorous management program requires input of a multidisciplinary treatment team, composed of an endocrinologist or physician with specific training and interest in diabetes, a certified diabetes educator, a dietician, and a mental health professional (either a social worker or psychologist) with diabetes behavioral health expertise. Other team members can include an exercise physiologist, an ophthalmologist or optometrist with diabetes expertise, a podiatrist, a nephrologist, and other specialists as indicated. Most importantly, persons with T1DM of all ages require continuous access to a health care provider through an emergency contact number in case of emergence of acute complications, such as severe hypoglycemia or DKA.

Visit frequency for diabetes care depends on the age at diagnosis, duration of disease, and presence of comorbidities. At T1DM diagnosis, initiation of insulin therapy is amenable to outpatient management when electrolytes are stable and in the absence of acidosis. Initial visits usually entail 2 to 3 sequential days of multidisciplinary visits, followed by visits in 2 to 3 weeks, then 1 to 2 months later. Thereafter, diabetes follow-up care generally occurs quarterly, especially in childhood when there is need to monitor growth and development. Both weight and height, along with vital signs, should be obtained at every quarterly visit, and Tanner staging should be performed at least annually for pediatric patients. Similarly, weights and vital signs should be obtained at each visit along with timely screening for complications and cardiovascular risk factors.

In childhood, initial management education is directed to the parents/guardians, whereas a family-based approach to care continues throughout adolescence.[184,185] As children get older, diabetes management tasks and the delivery of medical care gradually shift onto the person with diabetes, although T1DM at any age is not a do-it-yourself condition, given the many daily treatment demands and nuances of advanced diabetes technologies. In adulthood, following the visit frequency required for all persons newly diagnosed with T1DM, follow-up visits may occur every 3 to 6 months, depending on the achievement of glycemic goals and the presence of comorbidities.[180,186,187]

Diabetes education for all stakeholders occurs in a developmentally appropriate manner, geared to ensure comprehension of all involved in care. Diabetes management is also tailored to the needs and desires of the person with diabetes, creating an individualized care approach. Thus, a collaborative care model allows for optimal selection of one of the various approaches to insulin delivery, from the simpler two-injection-per-day regimen to the more complex semiautomated approach to insulin delivery using CSII and CGM. It remains important to reassess management approaches during the various developmental stages, especially during puberty, when physiologic insulin resistance coupled with psychosocial turmoil can lead to increased insulin needs and deteriorating glycemic control.[188,189] Similarly, adult needs change and require equal attention by the provider to the individual's preferences.

Glycemic Targets

The fundamental approach of individualized care is based on the treatment goal of achieving glycemic targets. The DCCT was the randomized clinical trial that laid the foundation of intensive insulin therapy as the standard of care.[190] In the DCCT, intensive insulin was compared with conventional insulin therapy in 1441 persons, 13 to 39 years old, with established T1DM. Intensive insulin therapy consisted of physiologic insulin replacement with either basal bolus therapy or CSII; conventional therapy included one to two injections of insulin daily. Notably, at the time of that clinical trial, there were no rapid-acting or long-acting insulin analogues; only short- and intermediate-acting insulin preparations were available (see insulin regimens in the following).

Intensive insulin therapy yielded superior glycemic control to conventional insulin therapy, with HbA_{1c} levels that were approximately 2% lower in the intensively treated group than in the conventionally treated group, medians of 7% and 9%, respectively.[190] The intensively treated group experienced a 35% to 76% reduction in the occurrence of microvascular complications, including retinopathy, nephropathy, and neuropathy compared than in the conventionally treated group. During the subsequent 20 to 25 years of the DCCT observational follow-up study, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, the intensively treated group compared with the conventionally treated group continued to demonstrate a reduced risk of both micro- and macrovascular complications and overall mortality, mainly attributable to the difference in glycemic control during the DCCT.[191–196] These findings help derive glycemic targets in practice today.

Glycemic targets are based on HbA_{1c} levels, especially given that the explanation for complication risk in the DCCT and EDIC studies was attributable to glycemia.[197] Various national and international professional organizations have created HbA_{1c} goals. The ADA recommends HbA_{1c} levels of less than 7% for adults and less than 7.5% for younger children and adolescents; additionally, HbA_{1c} levels should be routinely monitored quarterly.[180,186,187]

Prior to 2014, the ADA's pediatric HbA_{1c} goals were higher for younger children at less than 8.5% for those younger than 6 years and less than 8% for those 6 to 12 years old, whereas the goal was less than 7.5% for those 13 to 19 years old, due to previous concerns regarding neurocognitive damage from severe, recurrent hypoglycemia in young children.[198,199] The earlier data suggesting neurocognitive damage from hypoglycemia occurred during the era prior to modern insulins and physiologic insulin replacement. Recent data demonstrate that intensive insulin therapy confers no additional risk to neurocognitive function in young children with T1DM.[200,201] Additional research suggests that hyperglycemia and glycemic variability may incur adverse short-term outcomes on neurocognitive function and risk on central nervous system development based on magnetic resonance imaging changes of white matter.[202–205]

In consideration of emerging data on risk of hyperglycemia for the central nervous system, along with available management tools to minimize risk of hypoglycemia in young persons with T1DM, HbA_{1c} targets have been lowered by some professional organizations, including the International Society for Pediatric and Adolescent Diabetes (ISPAD) and the National Institute for Health and Clinical Excellence Guidelines in the United Kingdom. ISPAD now recommends HbA_{1c} targets of less than 7%, and the National Institute for Health and Clinical Excellence recommends targets HbA_{1c} of 6.5% or less.[206] Similarly, for adults, the American Association of Clinical Endocrinologists recommends an HbA_{1c} target of 6.5% or less.[207]

Although HbA_{1c} target recommendations may have become more rigorous over recent years, the ADA and other organizations emphasize that glycemic targets need to be individualized. The target HbA_{1c} goal for any single person with diabetes needs to reflect his or her risk of severe hypoglycemia, as well as hyperglycemia, considering the presence of comorbidities that may interfere with survival. For example, now the ADA offers higher HbA_{1c} goals of less than 8% for persons with a history of severe hypoglycemia, limited life expectancy, or advanced complications, as well as more stringent HbA_{1c} goals of less than 6.5% for those without hypoglycemia risk.[187] ISPAD also considers less stringent goals for regions of the world where advanced diabetes therapies and technologies may be unavailable to reduce risk of severe hypoglycemia.[206] Thus, personalized glycemic goals are tailored to individual needs and consideration of risk of severe hypoglycemia (see Chapter 38).

Lifestyle Management: Nutrition and Exercise

Lifestyle management is a major component of the care of persons with T1DM. Attention to both nutrition and exercise is fundamental in glycemic management, as well for overall health and cardiovascular disease prevention. There is need to provide education on these lifestyle issues to the persons with diabetes and to the family members of young children and adolescents with diabetes, especially given the global epidemic of childhood and adult overweight/obesity.[208,209] Overall, all diet and exercise recommendations are individualized to meet the patient's and family's needs. These knowledge areas are addressed in diabetes self-management education and support and should begin at diagnosis and progress as disease duration increases and the needs of the person with diabetes change.

Nutrition Therapy

Medical nutrition therapy addresses diabetes-specific issues, such as those related to the carbohydrate content of foods, to limit postprandial glycemic excursions, as well as ways to maintain normal growth in the pediatric population and to avoid excessive weight gain across the life span. Nutrition education, generally provided by a registered dietitian annually following the initial education at diagnosis, includes both personal and family dietary choices, food preference changes over time, challenges regarding access to food, meal schedules, patterns of exercise, growth and development, weight status, cardiovascular risk factors, and consideration of disordered eating behaviors.

For the growing child with diabetes, provision of adequate calories to maintain normal growth and development is fundamental. Underweight is a rare occurrence today in youth with T1DM. In fact, one third of pediatric patients with T1DM are overweight or obese.[210–212] Diet prescriptions for adults with T1DM should also account for weight status, given the rise in overweight and obesity among those with T1DM, affecting up to 50%,[213] and the association of increased weight with cardiovascular risk in T1DM.[214]

Nutrition education addresses the way foods impact glucose excursions, how diet can help achieve glycemic goals, and dietary approaches to avoiding hypo- and hyperglycemia with exercise. Nutrition education addresses numeracy, literacy, and self-management abilities of those with diabetes and their family members. Overall, adherence to nutrition management is associated with better glycemic control in youth with T1DM.[215]

Principles of general nutrition include a focus on whole foods and the standard composition of macronutrients with approximately 50% of energy intake as carbohydrates, 20% as protein, and 30% as fat. Fat intake should include less than 10% of energy intake as saturated fat, less than 10% as polyunsaturated fat, and greater than 10% as monounsaturated fat. One should try to eat five portions of fruits and vegetables daily. Often, young persons with diabetes, with the support of their parents, may choose to eat less healthy food choices, like prepackaged foods that include the carbohydrate content on the label, instead of fruits or vegetables.[216] Nutrition education follows published guidelines by professional organizations, such as the ADA[187] and ISPAD.[217] Very low carbohydrates should be avoided, as such diets can lead to elevations in lipid levels and there can be risk for ketosis, especially with insulin dose reductions, disordered eating behaviors, or use of the sodium-glucose cotransporter 2 (SGLT2) class of oral agents, currently unapproved for use in persons with T1DM (see discussion of adjunctive therapies in the following).

The major focus of nutrition education is on carbohydrate counting, because carbohydrates have the largest impact on glycemic excursions. So-called carbohydrate counting can provide flexible diet management by allowing for a wide range of food choices. It can also offer a way to select insulin doses based on food intake. When numeracy skills are deficient, experienced estimation of carbohydrate intake suffices. In fact, previous studies have shown that exactitude in carbohydrate counting is unnecessary, because under- or overestimating the carbohydrate content by 5 to 7 g, or by +/–15%, does not produce significant hypo- or hyperglycemia, respectively.[218,219] Furthermore, it is more important to be consistent rather than exact with carbohydrate counting, because the former is associated with better glycemic control.[220]

Additional studies have addressed the need to consider protein and fat intake on glycemic excursions.[221–223] After one has mastered carbohydrate counting, additional advanced nutrition education can include issues related to meal composition, carbohydrate quality (the glycemic index), and the contributions of protein and fat to glycemic excursions. Considering that meals containing substantial protein and fat can delay gastric emptying and the subsequent postprandial glycemic excursion, nutrition education can address advanced approaches to insulin bolusing. For example, one can use a square (or extended) wave bolus or dual (or combination) wave bolus with CSII, or one can divide the injected bolus doses into two separate injections separated in time by 60 to 90 minutes.

Physical Activity and Exercise

Physical activity is important for persons with and without diabetes. Exercise offers physical fitness, strength building, weight management, socialization, enhancement of self-esteem, and cardiovascular risk factor management. Exercise is a key component of diabetes management. It is important to recognize how exercise affects glycemic excursions, as exercise can lead to both hypoglycemia and hyperglycemia. Recommendations for youth include 60 minutes or more of daily physical activity, which should include muscle- and bone-strengthening activities on 3 or more days each week.[224] Recommendations for adults include at least 150 minutes of moderate-intensity aerobic activity or 75 minutes of vigorous-intensity aerobic activity each week plus muscle-strengthening activities on 2 or more days each week.[225] Adults with diabetes, and occasionally pediatric patients with diabetes, should receive medical clearance before embarking on an exercise program to evaluate the presence of any comorbid conditions or diabetes complications that could limit exercise participation, such as previously undiagnosed coronary heart disease.

Exercise impacts fuel metabolism through the recruitment of various hormones (insulin, glucagon, catecholamines, and glucocorticoids), and the impact of exercise on metabolism varies by the type, intensity, and duration of the activity.[226,227] With intact islet cells, including fully functioning beta cells, the body maintains euglycemia during exercise by balancing glucose uptake with hepatic glucose production. In T1DM, it is not possible to reduce insulin production following administration of an insulin dose; therefore, the circulating insulin can lead to hypoglycemia by both inhibiting hepatic glucose production and promoting exercise-related glucose uptake. Alternatively, in T1DM, high-intensity activities, such as sprints or resistance training, can lead to hyperglycemia when there is not enough circulating insulin due to the intense exercise's stimulation of counterregulatory hormone release, which, in turn, will increase hepatic glucose production and inhibit glucose uptake into skeletal muscle.

In general, most persons with T1DM, as well as their family members, worry more about exercise-induced hypoglycemia. Further, they are often frustrated and puzzled by the appearance of hyperglycemia with exercise. Hypoglycemia can occur during and immediately following the activity, and again 7 to 11 hours after the exercise, corresponding to the so-called lag effect of exercise. The latter phenomenon was shown in insulin clamp studies[228] and likely results from enhanced insulin sensitivity, blunted counterregulatory hormone release, and increased glucose uptake by the liver and skeletal muscles to replenish glycogen stores after exercise. The late risk of hypoglycemia often occurs overnight when persons with diabetes exercise in the late afternoon or evening, causing particular concern for patients and family members. For some persons with T1DM, there can be additional impairment of counterregulatory hormone responses due to blunting during sleep, antecedent hypoglycemia, and autonomic failure.[229–231] Thus, exercise-related hypoglycemia, along with fear of hypoglycemia, can curtail exercise participation. Fortunately, there are ways to limit the risk of exercise-related hypoglycemia and hyperglycemia while managing glycemia with exercise. Management approaches follow; for more in-depth discussion, there are recently published exercise management guidelines.[226,227,232]

To prevent hypoglycemia, one should begin exercise with a glucose of 100 mg/dL (5.6 mmol/L) or higher and plan to ingest carbohydrates during and/or after the exercise according to its duration and intensity. One can consider providing 0.25 g to up to 1.00 g of carbohydrate per minute of exercise when the activity lasts 40 minutes or longer, individualized based on the person's size (i.e., toddler or child), his or her needs, and past experience. The additional carbohydrates can be provided by increasing food intake. Alternatively, one can reduce bolus insulin doses, by approximately 50% as a starting point, for any meal or snack within 2 hours of the planned activity, which may be preferable to increasing carbohydrate ingestion for weight management. For those receiving CSII, basal rates can also be lowered by approximately 50% or even suspended for periods of 1 to 2 hours for exercise based on the patient's needs.[233] To manage the lag effect or late hypoglycemia risk after exercise, basal rates can be reduced by approximately 20% for up to 6 hours at bedtime for those treated with CSII, or long-acting insulin doses can be reduced by approximately 20% at bedtime.[234] Despite these insulin-dosing strategies, it remains important to have carbohydrates available for ingestion and to ensure continuous or frequent glucose monitoring for safe exercise.

To prevent significant hyperglycemia with exercise and induction of ketosis, it is important not to exercise when one is in an insulin-deficient state. The person with diabetes should exercise only when feeling well and in the absence of significant ketosis, defined as more than small urinary ketones or a blood beta-hydroxybutyrate level of 1.5 mmol/L or more.[226,227,232] One should also postpone intense exercise with significant hyperglycemia, defined as a glucose level of 350 mg/dL (19.4 mmol/L) or more, until corrected with insulin.

Exercise education can help limit the hypo- and hyperglycemic effects of exercise. When available, an exercise physiologist can add training with respect to exercise management, which can be especially important for the competitive athlete.

Insulin Therapy

Background

Normal physiologic insulin secretion results in low levels of insulin in the fasting and overnight period, and rapid increases in insulin following food intake, stress, and certain medications. Low levels of basal insulin secretion are necessary for covering basal metabolic needs, suppressing lipolysis, and balancing glucose utilization by organs such as the brain with hepatic glucose production.[235] Increases in insulin in response to rising blood glucose levels facilitate cellular glucose uptake and suppress hepatic glucose production. Normally, this is achieved through a substantial initial first-phase release, followed by an extended second-phase secretion into the portal circulation. The goal of insulin therapy in persons with T1DM is to mirror pancreatic insulin patterns, and this biphasic insulin release is particularly difficult to replicate.[235] Fortunately, incremental improvements in the pharmacology and delivery of insulin have made it increasingly possible to achieve reasonable insulin levels in persons with diabetes. The discovery and initial isolation of animal insulin in 1921 first made it possible to treat persons with T1DM. In the late 1940s, regular animal insulin was combined with protamine (neutral protamine Hagedorn insulin, NPH) to delay absorption and increase duration of action. In 1977, recombinant DNA technology allowed purification of insulin and production of synthetic "human" insulin. This major breakthrough generated the production of large amounts of insulin and also resulted in a reduction of insulin antibodies and insulin allergies. This development was followed by the biochemical modification of the insulin molecule to vary insulin action, giving rise to the so-called insulin analogues.

In 1993, the DCCT established a reduced risk of the development and progression of both micro- and macrovascular complications and all-cause mortality among those treated with intensive insulin therapy with the goal of achieving near-normal blood glucose levels.[190,191] In the DCCT, intensive insulin therapy was associated with a high rate of severe hypoglycemia (62 episodes per 100 patient-years of intensive insulin therapy); this related, in part, to the utilization of older nonanalogue insulin formulations.[190] The treatment of diabetes was further improved by subsequent development of rapid-acting insulin analogues in the 1990s and long-acting (basal) insulin analogues in the early 2000s, which has led to lower rates of hypoglycemia, along with less weight gain and improved glycemic control, in persons with T1DM.[236–238]

Insulin Preparations

Examples of commercially available insulin preparations are shown in Table 36.6 and vary in time to onset, peak, and duration of action. The typical insulin concentration is U-100 (100 units of insulin per 1 mL); however, more concentrated preparations are commercially available, including U-200 insulin degludec (Novo Nordisk Tresiba pen), U-300 insulin glargine (Sanofi Toujeo pen), U-200 lispro (Humalog U-200 KwikPen), and U-500 regular human insulin (Lilly Humulin pen or vial). These concentrated insulin preparations are most commonly used in patients with T2DM, but they are beneficial for some individuals with T1DM who have significant insulin resistance and high insulin requirements.[239] In very young children requiring small doses of insulin (e.g., <0.5 unit bolus doses), rapid-acting insulin diluted to a tenth

TABLE 36.6 Insulin Types and Action Profiles

	Product	Onset of Action	Peak Action	Duration
Rapid acting	Aspart (*Novolog* [Novo Nordisk, Princeton, NJ]) Lispro (*Humalog U-100* [Eli Lilly, Indianapoils, IN], *Humalog U-200* [Eli Lilly, Indianapoils, IN], *Admelog* [Sanofi, Bridgewater, NJ]) Glulisine (*Apidra* [Sanofi, Bridgewater, NJ])	10–30 min	30–180 min	3–5 h
	Aspart (*Fiasp* [Novo Nordisk, Princeton, NJ]) Insulin human (*Afrezza* [MannKind, Westlake Village, CA]) inhalation powder	2.5 min 12 min	40–50 min 35–45 min	 1.5–3 h
Short acting	Regular U-100 (Humulin R U-100 [Eli Lilly, Indianapolis, IN]) Regular U-100 (Novolin R [Novo Nordisk, Princeton, NJ]) Regular U-500 (Humulin U-500)	30–60 min	2–4 h	U-100: Up to 10 h U-500: Up to 24 h
Intermediate Acting	NPH (Humulin N) NPH (Novolin N)	2–4 h	4–8 h	12–18 h
Long Acting	Detemir (*Levemir* [Novo Nordisk, Princeton, NJ]) Glargine (*Lantus* [Sanofi, Bridgewater, NJ], *Basaglar* [Eli Lilly, Indianapoils, IN], *Toujeo U-300* [Sanofi, Bridgewater, NJ]) Degludec (*Tresiba U-100* [Novo Nordisk, Princeton, NJ], *Tresiba U-200*)	2–4 h	Minimal	Detemir: 12–24 h Glargine: up to 24 h Degludec: up to 48 h
Premixed	70/30 (NPH/Aspart) (*Novolog 70/30*) 70/30 NPH/Regular (*Humulin 70/30*) 75/25 (NPH/Lispro) (*Humalog 75/25*) 50/50 (NPH/Lispro) (*Humalog 50/50*) [Other combinations may be available in Europe.]	5–60 min	Dual	12–18 h

the usual concentration (10 units/mL, often called *U-10*) or other concentrations (U-50, U-25) may be used, after education by the child's diabetes health care team; diluted insulin may be prepared by a compounding pharmacy or by the young child's family using a diluent provided by the insulin manufacturer.

Insulin can be dispensed in vials and administered via syringes or continuous infusion pumps or dispensed and administered in pen form. Rapid-acting inhaled insulin is also available, and it has been demonstrated to be noninferior when compared with insulin aspart for mean change in HbA_{1c}, although more patients treated with insulin aspart achieved an HbA_{1c} of less than 7% compared with patients treated with inhaled insulin (31% vs. 18%)[240]; availability of limited dosing increments has been one barrier to treatment with inhaled insulin.

In light of results from the DCCT, intensive insulin therapy is the standard of care for treatment of T1DM and can be achieved with multiple daily injections (MDI) basal-bolus therapy or insulin pump therapy. In both MDI and pump therapy, rapid-acting insulin is given as a bolus at meals and snacks and periodically to correct hyperglycemia (no more frequently than every 2–3 hours) and as basal insulin given once or twice a day with long-acting insulin (for MDI) or as a basal rate (for insulin pumps). A small proportion of patients and families require simplified insulin regimens rather than intensive insulin therapy, which will be discussed in the following.

Initial Management of Newly Diagnosed T1DM

In pediatric patients with newly diagnosed diabetes, initial management includes resuscitation with oral or intravenous fluids to rehydrate and correct electrolyte imbalances, administration of insulin to halt lipolysis and reverse hepatic gluconeogenesis and ketogenesis, and commencement of diabetes education. Depending on factors such as the age of the individual patient and severity of his or her clinical presentation, family factors, and availability of diabetes-related resources, initial management may occur in an intensive care unit, inpatient hospital setting, or outpatient unit. Initial presentation of T1DM is often DKA. Please refer to the discussion on DKA.

Initiation of Insulin Therapy

For those individuals who do not initially present in DKA, treatment with subcutaneous insulin should be started at diagnosis of diabetes. For those who present in DKA, subcutaneous insulin should begin with resolution of DKA (pH ≥7.3, CO_2 ≥18 mEq/L [18 mmol/L], and normalization of the anion gap) in conjunction with the first meal being eaten post-DKA. Determination of the appropriate starting insulin dose must consider the patient's weight, age, pubertal status, and history of DKA. For young children and postpubertal adults who are not obese and do not present in DKA, a typical starting dose is 0.3 to 0.5 units/kg per day. Higher doses will be needed for individuals who are obese, children in puberty, or following presentation with DKA, such that a pubertal child with obesity and resolved DKA may receive a starting dose of 1 unit/kg per day. This total daily dose (TDD) of insulin is typically given in a divided program of MDI with about 50% of the TDD delivered as a once-daily dose of basal insulin and 50% given as boluses.

Insulin Dose Determination

Treatment with basal-bolus therapy requires the calculation of the appropriate insulin bolus at each meal and snack based on the premeal blood glucose level and the planned carbohydrate intake. To calculate a bolus, the person will first calculate the proper carbohydrate coverage for the meal using his or her individualized insulin-to-carbohydrate ratio. The carbohydrate ratio is the number of grams of carbohydrate covered by 1 unit of insulin (roughly 450 divided by the total daily insulin dose, TDD). Next, the person will determine the amount of insulin required to correct for the premeal blood glucose level, using his or her individualized correction factor (also sometimes called *sensitivity factor* or *sensitivity index*). The correction factor is the expected decrease in blood glucose resulting from 1 unit of insulin (approximately 1650 divided by the total daily insulin dose, TDD). The bolus delivered with the meal or snack is the sum of the calculated carbohydrate coverage and the calculated blood glucose correction. Most patients do these calculations by hand, mental math, or calculators, or with the help of apps or pump pen bolus wizards, but some families require sliding scales or charts for determination of bolus dosing. Rapid-acting insulin boluses should be given 10 to 15 minutes before the start of a meal or snack because onset of action is about 15 minutes. In cases where food intake is less predictable (e.g., very young children, gastroenteritis), postprandial administration of a rapid-acting insulin bolus is safe and effective.[241] Since rapid-acting insulin has a 3-hour action time, a correction factor is usually not given any more frequently than every 3 hours to avoid hypoglycemia from "stacking" of insulin action. Anticipated activity (i.e., recent or planned exercise) may factor into insulin bolus calculations, with less insulin required before, during, or after certain types of exercise. Additionally, a blood glucose trend based on continuous glucose monitor arrows may also factor into the insulin bolus calculation at mealtime.[242,243]

Other Insulin Regimen Options

In individualizing each person's insulin regimen, clinicians must consider the frequency of blood glucose monitoring, number of daily injections, the need for flexibility in meal planning, and family schedule. In a minority of patients with T1DM, treatment with intensive insulin therapy and mealtime bolus calculations may not be feasible or appropriate. In these cases, intermediate-acting insulin (NPH) may be prescribed, often in conjunction with rapid- or short-acting insulin, and sometimes in premixed form (70/30, 75/25, 50/50). To avoid hypoglycemia in such regimens, meals are typically fixed with regard to timing and amount of carbohydrates consumed. Table 36.7 provides examples of insulin regimens.

Blood Glucose Monitoring

Self-management of diabetes requires the routine monitoring of glucose levels to select and adjust insulin doses, to understand the impact of various foods on glycemic excursions, to reduce the risk of exercise related hypo- or hyperglycemia, and to react to periods of illness or stress that can lead to increased glucose variability and hyperglycemia with ketosis along with risk for ketoacidosis. There are numerous glucose meters available that utilize a small volume of blood (<1 mL), provide results in less than 5 seconds, offer alternative site use beyond fingertips, and meet recent standards of accuracy. Accuracy standards include the need for at least 95% of blood glucose meter results to fall within ±15 mg/dL (0.8 mmol/L) for blood glucose <100 mg/dL (5.6 mmol/L) and within ±15% for blood glucose ≥100 mg/dL (5.6 mmol/L) performed with a reference method,[244] with recommendations allowing for ongoing assessment of meter performance.[245] Each year, the ADA provides an update of available meters, along with a cataloging of their various features.[246] Although there may be substantial choice in the selection of glucose meters, one must mainly consider insurance coverage and the need to link the meter with a pump for CSII.

TABLE 36.7 Examples of Insulin Regimens Used in Type 1 Diabetes

Intensive Insulin Regimens
Multiple Daily Injections
Long-acting insulin given once daily, and rapid-acting insulin given at meals, snacks, and periodically to correct high blood sugars
Continuous Subcutaneous Insulin Infusion via Insulin Pump
Rapid-acting insulin given at basal rate (rate can vary throughout the day) and as a bolus at meals, snacks, and as needed to correct high blood sugars
Rapid-acting insulin given via pump according to automated insulin delivery algorithm and based on continuous glucose monitoring
Simplified Insulin Regimens
Multiple Daily Injections
Long-acting insulin given once daily, NPH given at breakfast, and rapid-acting insulin given at breakfast and dinner (may be used when lunchtime insulin dosing is logistically difficult; lunch carbohydrate intake must be consistent)
NPH given twice daily (breakfast and bedtime), and rapid-acting insulin given at meals
NPH and rapid- or short-acting insulin given twice daily (breakfast and dinner)
Premixed insulin (70/30, 75/25, 50/50) given twice daily (breakfast and dinner)

Daily blood glucose monitoring frequency has been repeatedly linked to lower HbA_{1c} levels.[247–249] For persons with T1DM, blood glucose monitoring frequency is generally recommended at least four times daily and up to 10 to 12 times daily. Monitoring generally occurs before meals/snacks and at bedtime, with monitoring at other salient times, such as postprandially, overnight, before/during/after exercise, and during periods of illness or stress. More frequent monitoring is often recommended for persons with hypoglycemic unawareness (i.e., prior to driving) and for those unable to communicate symptoms, such as the very young or the elderly. It is important to download the glucose meter at routine appointments to review glycemic patterns with patients and families and to utilize the data to encourage self-management for insulin dose adjustments. Modern CGM technologies, especially those with nonadjunctive claims for insulin management, have supplanted the need for frequent blood glucose monitoring (see the next section).

Continuous Glucose Monitoring

Real-time CGM is increasingly utilized for routine diabetes care in adults, children, and adolescents with T1DM. CGM is a portable device that allows for measuring and visualizing the glucose concentration continuously in real time for several days to weeks.[250] CGM devices are composed of three main units: a sensor, a transmitter, and a receiver. The sensing electrode is placed in the subcutaneous space and measures glucose in the interstitial fluid, which is correlated to blood glucose via a calibration procedure. The sensing electrode is inserted by the user or a caregiver for most devices or implanted by a clinical team for the new long-term sensor. Each sensor can last between 5 and 14 days for the minimally invasive CGMs, and from 90 to 180 days for the implantable one. Information from the sensing electrode is transmitted to a dedicated receiver or to a smart device like a phone or an insulin pump. The CGM transmitter is attached on top of the sensor and can last 3 to 6 months of use or is rechargeable in some devices. The transmitter unit utilizes either a proprietary radio or Bluetooth Low Energy (BTLE) communication to connect to the receiver unit or mobile device. The dedicated receiver, an app on a smart phone or an insulin pump, receives the glucose readings, which then can be used to visualize glucose trajectories and provide hypoglycemia and hyperglycemia alerts and alarms to the user and followers. The CGM is calibrated using self-monitoring blood glucose (SMBG) devices. Calibration for some of the CGMs occurs as often as every 12 hours. Calibration values can be entered on the mobile device, CGM receiver, or insulin pump. Recent CGM technology enables a calibration-free option or factory calibration without loss of accuracy, which liberates users from capillary glucose measurements, although calibration is still optional.

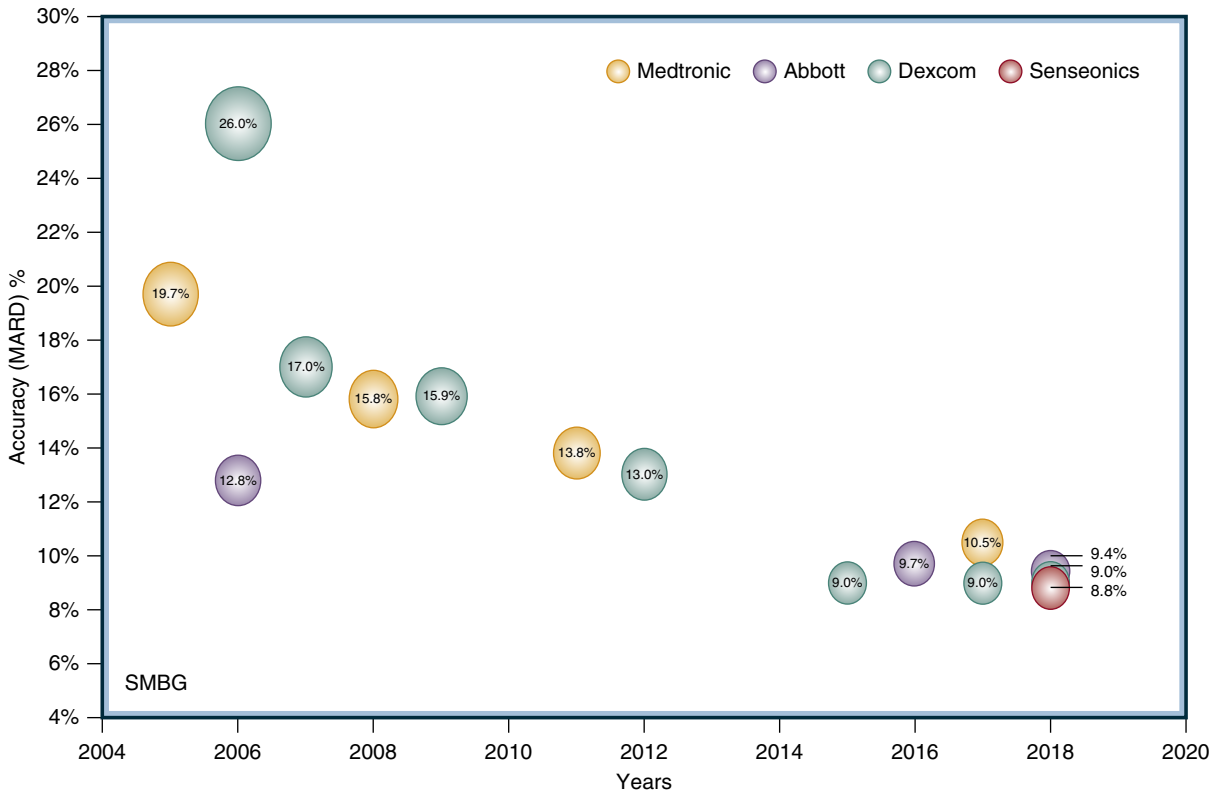
The first modern CGM devices were introduced by Medtronic Diabetes (Northridge, CA) as a diagnostic tool providing 72 hours of retrospective data when calibrated with blood glucose meter values; the latter were to be used for insulin dosing or other therapeutic adjustments. New devices added the use of trend arrows and predictions, and with each newer generation, the sensor use time increased and the accuracy measured as mean absolute relative difference.[251–257] CGM technology, based on electrochemical detection of glucose using enzymatic chemistry that employs a glucose oxidase reaction, has improved dramatically in the past decade, as depicted in Fig. 36.7.[258,259] Modern CGM technology has reached the accuracy of SMBG with the new Dexcom G6 (San Diego, CA), which is factory calibrated and approved for insulin dosing, as well as the new Abbott FreeStyle Libre (Alameda, CA), which has reduced the warm-up time to 1 hour and can run for 14 days (Fig. 36.8). The Senseonics (Germantown, MD) CGM is a new implantable device based on fluorescent chemistry that functions from 90 to 180 days using a removable rechargeable receiver and transmits glucose to a mobile app[260–264] (see Fig. 36.8).

Innovations are ongoing with smaller devices and longer use time between sensor insertions, integration of CGM devices with insulin pumps and mobile apps together enhancing the usability and have opened the door for automated insulin delivery (AID) or artificial pancreas.[259]

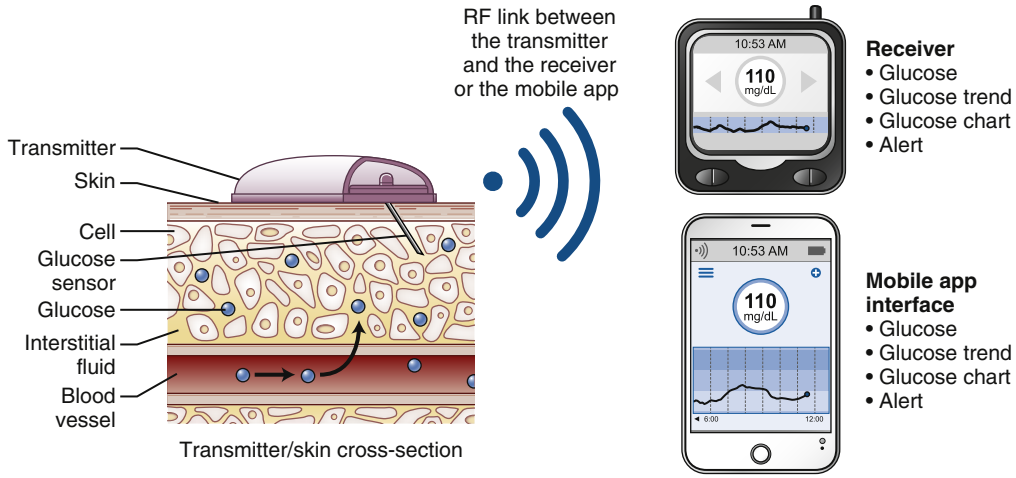
Benefits of CGM devices have accrued with improved performance of the devices over time.[265–267] Consistent CGM use of 6 or more days per week has been associated with improvements in glycemic control, reflected in lower HbA_{1c} levels, more patients achieving HbA_{1c} targets, and reduction in time in the hypoglycemic range of less than 70 mg/dL (<3.9 mmol/L) or less than 54 mg/dL (3 mmol/L).[268–271] Similarly, more frequent scanning of the Freestyle Libre has also been associated with improved glycemic control, noted by an increase of glucose time in range, with reductions of time in hypoglycemia and hyperglycemia; mean scanning frequency was about 16 times per day in one study.[272] Finally, recent publications from the Endocrine Society have advocated the use of CGM trend arrows to fine tune insulin bolus doses at mealtimes in pediatric and adult patients to avoid extreme postprandial glucose excursions, especially given the recognition that the glucose trajectory and the magnitude of the glucose level impact the response to the insulin bolus and ingested meal.[242,273]

Continuous Subcutaneous Insulin Infusion

CSII is a minimally invasive form of insulin delivery that enables intensive insulin therapy. One of the advantages of the use of



• **Fig. 36.7** Accuracy of current continuous glucose monitoring devices in the past decade. *MARD,* mean absolute relative difference; *SMBG,* self-monitoring blood glucose. (Modified from Facchinetti A. Continuous glucose monitoring sensors: past, present and future algorithmic challenges. *Sensors (Basel)*. 2016;16[12]:E2093.)



• **Fig. 36.8** The components of a continuous glucose monitoring system, which include the glucose sensor that resides in the subcutaneous tissue along with the transmitter that wirelessly transmits the glucose data to a separate receiver that can be a stand-alone device or an application on a mobile device. Wireless transmission occurs via radio frequency (RF) and Bluetooth. Both glucose concentrations and trends can be displayed. Alerts can notify the user when glucose levels are rising or falling.

CSII, as its name implies, is its ability to continuously deliver insulin and allow the users to respond more rapidly to any changes in their physiologic insulin requirements. Second, most modern CSII pumps enable the user to program and modify key parameters that effect insulin delivery, such as (1) the basal rate profile, in which several basal rates with multiple insulin segments can be entered to address changes in insulin requirements between workdays and weekends, active days or sick days, and more; (2) the storing of multiple sensitivity factors, as well as carbohydrate to insulin ratio; (3) alerts and alarms; (4) bolus calculators and insulin-on-board estimators, to prevent overstacking of insulin.

CSII therapy has been associated with improved glycemic control, decreased hypoglycemia, and better quality of life.[274–276] Still, there are several disadvantages of this delivery route. Using

TABLE 36.8 Modern Insulin Pumps in the United States

Product	Reservoir	Basal Range	Bolus Range	Main Features
Omnipod INSULET CORP. (Acton, MA)	200 U	0.05–30 U/h in 0.05-U increments	0.05–30 U; Increments of 0.05, 0.1, 0.5, or 1 U	• No tubing • Patch design • Pod is waterproof for up to 25 feet deep for 60 minutes • Personal Diabetes Manager (PDM) controls the pod • New system that uses BTLE communication and a smart PDM
MiniMed 530G System MEDTRONIC DIABETES	300 U	0.025–35 U/h in 0.025-U increments	0.025–25 U; Increments of 0.1 U	The MiniMed 530G combo pump–CGM uses SmartGuard technology to stop insulin delivery for up to 2 hours if the glucose level reaches a preset low limit and the user does not react to a low-glucose alarm.
MiniMed 670G System MEDTRONIC DIABETES	300 U	0.025–35 U/h in 0.025-U increments	0.025–25 U; Increments of 0.1 U	The MiniMed 670G, a hybrid closed-loop pump, uses SmartGuard technology to allow users to choose from increasing levels of automation that best fit their diabetes management needs.
T:slim X2 Pump TANDEM DIABETES CARE (San Diego, CA)	300 U	0.1–15 U/h in 0.001-U increments	0.05–25 U; Increments of 0.01 U	The T:slim X2 uses the Tandem Device Updater to remotely update software from a computer without requiring purchase of a new device. Integrated with Dexcom's G5 CGM. New Basal-IQ pump supports the Dexcom G6 and has a PLGS feature.

BTLE, Bluetooth low energy; *CGM*, continuous glucose monitoring; *PLGS*, predictive low-glucose suspend.
Modified from Diabetes Forecast: Consumer Guide. 2018. diabetesforecast.org. Available at http://main.diabetes.org/dforg/pdfs/2018/2018-cg-insulin-pumps.pdf.

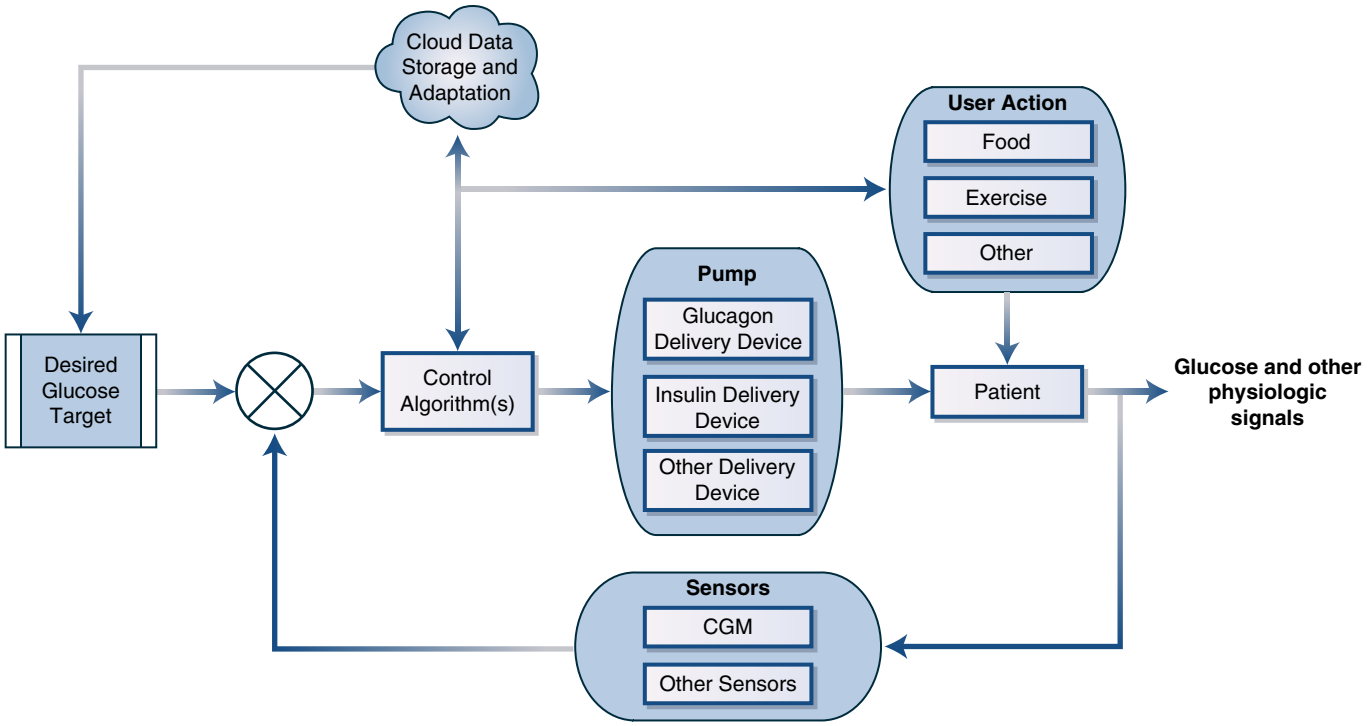
CSII means that the user is attached to an external device, which may be inconvenient in daily life. Additionally, it is recommended to change the infusion set every 3 days to reduce problems with site irritation and irregularity in insulin delivery caused by blocked catheters, insulin leakage, or cannula dislodgement.[277] A failure of the infusion site can lead to hyperglycemia, and potentially DKA, if the problem is not detected in time. Pump technology has progressed to support smarter devices that integrate CGM functionality, as well as algorithms that enable insulin suspension based on fix threshold or predictive design, to minimize hypoglycemia and improve glycemic outcomes. The latest leap forward in pump technology is the development of closed-loop insulin delivery devices that build on top of the current small and smart devices, and add a control algorithm that is tasked with calculating the safe dose of insulin, in most cases, or a combination of insulin and glucagon. As can be seen in Table 36.8, several pumps are available to people with T1DM, with different features, sizes, and technology to support their individual needs and requirements.

The first advantage of connected care between CGM and the insulin pump delivery system is the introduction of integrated or linked systems that enable automated insulin suspension. Suspending basal insulin delivery for low sensor glucose levels has been shown to distinctly reduce hypoglycemia without worsening glycemia.[278] Sensor-augmented pumps that preemptively suspend insulin delivery when sensor glucose levels are predicted to be low show promise in minimizing hypoglycemia.[279–282] The greatest potential for improved glycemic control is the dynamic regulation of insulin delivery for both high and low glucose levels. "Hybrid" closed-loop systems that modulate basal insulin delivery by sensor glucose levels increase time spent within target glucose ranges, reduce hyper- and hypoglycemia exposure, lower HbA_{1C} levels, and improve measures of quality of life in both adult and adolescent subjects.[283–287] Translation of AID from research to clinical care will require educating users and providers to optimize outcomes and understand the new technology and the best way to use it.[288,289] Users of current hybrid closed-loop systems must still count carbohydrates and bolus manually for meals. Systems that reduce reliance on carbohydrate counting, and systems that administer glucagon under automated control to mitigate the risk of hypoglycemia, remain in development.[290] A recent systematic review and meta-analysis of randomized controlled trials suggest that artificial pancreas systems uniformly improve glucose control in outpatient settings despite heterogenous technical and clinical factors.[291]

AID: "The Artificial Pancreas"

The combination of continuous glucose technology with insulin pumps has enabled the development of AID systems (closed-loop or artificial pancreas devices). A controller, an algorithm, adjusts insulin delivery based on CGM data to a glucose target or range that is preset and can be changed based on user lifestyle or need.[292–294] These feedback control algorithms achieve safe and effective glucose regulation for people with T1DM, maximize time spent within the euglycemic safe range of 70 to 180 mg/dL, minimize hypoglycemic events, and prevent postprandial hyperglycemia with little user intervention.

A closed-loop glucose control or AID system is composed of several basic elements, as depicted in Fig. 36.9. The source of information is the sensor module that is based on a CGM. Additional sensors that monitor other physiologic parameters (e.g., heart rate, accelerometry, skin temperature) are being integrated in new AID systems.[295] The delivery module is based on an insulin pump, while a combination of insulin and other hormones or medications are also being explored. The core module, the control algorithm, processes the sensor measurements with or without additional input provided by the user, such as meal or exercise information, to achieve the desired glucose set-point target or range. The control signal is sent to the user's pump to manipulate insulin delivery and other drugs, such as glucagon or pramlintide, in a variable rate or microdose. Current artificial pancreas systems are based on subcutaneous sensing and delivery; however, the intraperitoneal space has been investigated as a potential alternative site to insulin delivery and glucose sensing, one that will be closer to physiologic insulin secretion.[296,297] The core of AID



• **Fig. 36.9** Feedback control loop for automated glucose management. *CGM*, continuous glucose monitoring.

systems is the control algorithm. Many control techniques are being explored in academic studies, and some have translated to industrial development and implementation as part of new AID systems. The lead feedback control algorithms for automated glucose regulation are proportional integral derivative (PID) control, fuzzy logic control, and model predictive control (MPC).

Proportional Integral Derivative AID

The PID controller is the most common strategy of implementing feedback control.[298] PID control consists of the summation of three distinct terms or components: the proportional term that captures the current tracking error (the difference between the current control variable and the desired set-point), the integral term that captures historical accumulation of the error, and the derivative term that captures anticipated change of the error.[298] Steil and colleagues[299] have pioneered the development of PID-based AID systems as part of Medtronic Diabetes. PID control algorithms have been used in several automated closed-loop insulin delivery systems, including the first US Food and Drug Administration (FDA)-approved hybrid closed-loop system—the Medtronic 670G.[299–302]

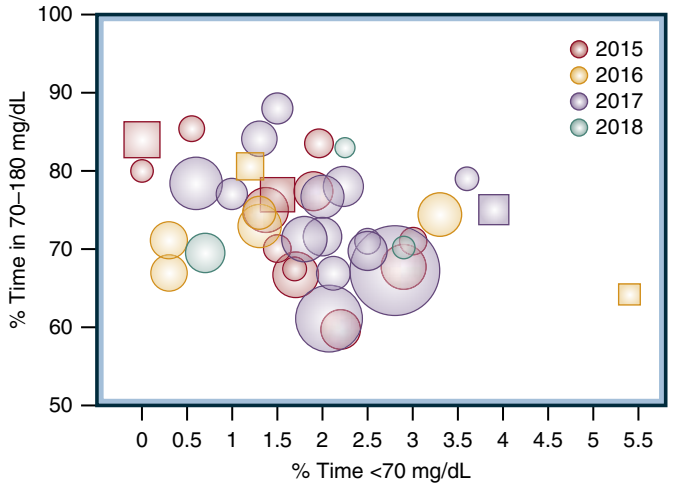
Fuzzy Logic AID

Closed-loop algorithms using fuzzy logic follow specially designed dosing rules to incorporate expert clinical knowledge in diabetes decision making.[303,304] A fuzzy logic–based controller has three main stages: (1) input fuzzification, mapping the glucose and/or its rate of change using membership functions; (2) fuzzy inference, combining the fuzzy inputs from the first stage and a set of fuzzy rules to determine the degree of activation of output membership functions; and (3) output defuzzification, where the final output—insulin dose—is calculated. One fuzzy logic–based artificial pancreas system is known as the MD-Logic Artificial Pancreas, which has been developed by researchers at Schneider Children's Medical Center of Israel. The system has been used in multiple clinical studies and is proven to regulate glucose levels across age groups.[305] The MD-Logic closed-loop algorithm consists of two control modules: a control to range module that aims to keep glucose in the 80 to 120 mg/dL (4.4–6.7 mmol/L) range and a control to target module.

Model Predictive Control AID

MPC is one of the most widely used approaches in advanced control system design of AID devices due to its ability to directly handle practical constraints and multiple inputs and outputs, formulate control objectives into a constrained optimization problem, and find the optimal control inputs by solving the optimization problem in a receding-horizon fashion. MPC is a model-based control strategy using models in two ways: (1) utilizing a reliable model to predict the effect of past control moves on future outputs, assuming no future moves, and (2) using the same model to compute optimal controller moves, implementing the first move and repeat procedure. So far, this approach has been successfully utilized in the development of AID systems by multiple research groups and industry translation of the technology.[284,285,287,290,306,307]

AID systems can be divided into two main classes: insulin-only and bihormonal systems that use insulin and glucagon or other drugs to improve care. In the past decade, multiple clinical studies have been launched to evaluate the safety and efficacy of AID systems in both groups. Different control algorithms, CGMs, and delivery devices were evaluated in these studies. AID systems have been tested with multiple challenges that people with T1DM are facing, such as nocturnal glucose control and both announced and



• **Fig. 36.10** Clinical performance of insulin-only (disks) and insulin and glucagon (squares) automated insulin delivery systems, where the size of the shape depicts the number of subjects in the study and the color depicts the publication year. (Modified from Dassau E. The path to a medical internet of things (IoT) and human-centric design of an artificial pancreas. Presented at the Symposium on Artificial Pancreas and Decision Support Approaches at the American Diabetes Association 78th Scientific Sessions, Orlando, FL, 2018.)

unannounced meals and physical activity. These studies started in a tight, supervised environment and gradually transitioned to hotel and at-home clinical studies that ranged from short visits to prolonged multiweek studies.[284,290,295,300,305,307–317] As depicted in Fig. 36.10, both insulin-only and bihormonal AID have demonstrated safe and effective glucose regulation with minimal time, less than 1.5% spent under 70 mg/dL (3.9 mmol/L) and approximately 75% in the 70 to 180 mg/dL (3.9–10.0 mmol/L) range. Additional work with new technology, as well as added wearable sensors, and/or drugs may improve these remarkable outcomes. Adjunctive therapies to treat T1DM such as pramlintide and glucagon-like peptide 1 (GLP1) receptor agonists (e.g., liraglutide, exenatide) or SGLT2 inhibitors that are used in clinical care are yet to be fully evaluated with AID systems, and only limited clinical studies have been done to evaluate how to integrate them with AID systems.[318,319]

Decision Support Systems

An emerging field in diabetes care is decision support that uses connected care devices such as CGM, smart insulin pens, SMBG, and insulin pumps to provide guidance and advice to people with T1DM via smart apps or cloud-based algorithms. This evolving field can improve care of both MDI and CSII users by providing tools to adjust basal boluses, as well as meal and correction boluses, based on glucose input and peoples' lifestyle. These tools can provide additional insight to the clinical team with enhanced visualization and advice on insulin management.[320–323]

Use of Adjunctive Drugs in T1DM

Advances in treatment for T1DM have primarily targeted the development of insulin analogues and devices that optimize insulin delivery and mimic normal physiologic serum insulin levels. Even with these advances, insulin therapy has been associated with hypoglycemia and weight gain. To offset some of these side effects of insulin therapy, several drugs that are currently used in T2DM have been investigated for adjunctive use in T1DM, to assess potential benefits related to improved glycemic control, less hypoglycemia, reduced glucose variability, and less weight gain. To date, pramlintide is the only drug that is approved by the FDA for adjunctive use in adults with T1DM; no adjunctive treatments have been FDA approved for use in children with T1DM.

Pramlintide

Pramlintide, an injectable amylin analogue, slows gastric emptying, suppresses postprandial glucagon secretion, and enhances satiety. Several large, randomized, placebo-controlled, double-blinded trials have shown that the addition of pramlintide to insulin at mealtime in adults with T1DM improved glycemic control, including modest reductions in HbA_{1c} by 0.3% to 0.6%, and decreased postprandial glucose excursions.[324,325] Pramlintide was also associated with modest weight loss (mean, 0.4–1.3 kg) and reduced insulin dose; nausea was the most frequent side effect. In a recent closed-loop insulin delivery study, the addition of mealtime pramlintide was associated with a reduction in postprandial hyperglycemia compared with closed loop alone.[326] Pramlintide is approved for adjunctive use in adults with T1DM but not in children.

Metformin

Several clinical trials have studied the addition of metformin to target insulin resistance, particularly in individuals who are obese and during puberty. Clinical trials have failed to demonstrate improvement in HbA_{1c} with adjunctive use of metformin, although some studies in youth have shown decreases in weight and insulin requirement.[327,328] In a recent randomized controlled trial of adjunctive metformin in adults with T1DM at risk for cardiovascular disease, the metformin group exhibited improvements in body weight, low-density lipoprotein cholesterol, and estimated glomerular filtration rate, but metformin did not significantly improve carotid artery intima-media thickness (the primary study outcome) or glycemic control.[329] Metformin is not FDA approved for treatment of T1DM.

Incretin-Based Therapies

Incretin-based therapies include GLP1 receptor agonists and dipeptidyl peptidase 4 (DPP4) inhibitors. GLP1 is an incretin hormone produced in the gut that has been shown to boost glucose-dependent insulin secretion, suppress glucagon secretion, delay gastric emptying, and promote satiety. Individuals with T1DM appear to have normal GLP1 production but dysregulation between the pancreas and the gut, leading to ineffective glucagon suppression.[330] A recent 24-week randomized, double-blind, placebo-controlled trial of overweight adults with T1DM and HbA_{1c} greater than 8% showed that the addition of the once-daily injectable GLP1 receptor agonist liraglutide was associated with weight loss and decreased insulin dose, but change in HbA_{1c} from baseline did not differ between groups.[331] In a recent closed-loop insulin delivery study, the addition of once-daily liraglutide was associated with fewer postprandial increases in blood glucose, weight loss, and decreased insulin compared with closed-loop control.[326] Considering that endogenous incretins such as GLP1 are rapidly degraded by the enzyme DPP4, treatment with DPP4 inhibitors has been shown to have many of the same glycemic benefits as GLP1 receptor agonists in the treatment of T2DM. Trials of DPP4 inhibitors in T1DM to date have not yet shown significant clinical benefit.[332] Incretin-based therapies are not FDA approved for use in T1DM.

SGTL1 and SGLT2 Inhibitors

Oral SGLTs reduce postprandial hyperglycemia, reducing glucose reabsorption in the proximal renal tubule (SGLT2) and in some agents decreasing and slowing intestinal glucose absorption (SGLT1).[333] In those with T2DM, they have been associated with improvement of glycemic control, weight loss, and blood pressure reduction,[334–337] lower rates of cardiovascular morbidity and mortality in those at high cardiovascular risk,[338] and decreased albuminuria.[339] Two recent large, double-blinded, phase III randomized control trials studied the efficacy and safety of SGLTs in adults with T1DM combined with optimized insulin therapy.[340,341] The inTandem1 trial (n = 793) was a 1-year study of the dual SGLT1/SGLT2 inhibitor sotagliflozin, 200 or 400 mg once daily before breakfast combined with optimized insulin therapy.[341] The DEPICT-2 trial (n = 813) was a 24-week study of the SGLT2 inhibitor dapagliflozin, 5 or 10 mg once daily.[340] Both studies showed that, compared with placebo, adjunctive use of an SGLT inhibitor was associated with modest but durable dose-dependent improvements in HbA_{1c}, reductions in insulin dose, and weight loss. Unfortunately, those treated with SGLT inhibitors also had higher rates of genital mycotic infections and DKA (in Tandem1 trial participants with ≥1 DKA event in the 1-year study: 0.4% in the placebo group, 3.4% in the 200-mg sotagliflozin group, 4.2% in the 400-mg sotagliflozin group; DEPICT-2 trial participants with a definite DKA event over the 24-week study: 0 in the placebo group, 2.6% in the 5-mg dapagliflozin group, 2.2% in the 10-mg dapagliflozin group). Similar large phase III studies of once-daily oral doses of empagliflozin adjunctive to insulin therapy over 52 weeks in patients with T1DM (EASE-2 and EASE-3) have presented preliminary data showing similar benefits. Risk of DKA with SGLT2 inhibitors might be higher in patients with a history of DKA, individuals on a low-carbohydrate diet, and in those who do not check ketones in the setting of potential symptoms. SGLT inhibitors are yet not FDA approved for use in T1DM.

In considering the addition of adjunctive drugs in a person with T1DM, any potential benefits must be carefully weighed against risks and costs of the medications.

Outcomes Beyond HbA_{1c}

CGM offers a new opportunity to assess glycemic control beyond HbA_{1c}, which assesses average glycemic control over the previous 2 to 3 months, although it is more heavily weighted toward the most recent month. Yet there are challenges with the use of HbA_{1c} as a measure of glycemia, as it can reflect a wide range of mean glucose levels and can vary in persons of different races, in those with different rates of glycation, and according to red cell survival time.[342–346]

CGM data reflect overall glycemia with the collection of just 2 weeks of data.[347] Recent consensus conferences, reflecting major national and international professional organizations, have published a series of CGM metrics important in the assessment of glycemia in persons with diabetes,[348,349] based on the recognition that there can be an improved understanding of a person's glycemia beyond the HbA_{1c}. At present, there is a series of glucose metrics reported on CGM data downloads, along with an effort to standardize the CGM metrics and to include glucose data collected over a 2-week period, with the goal that regulators will also accept these metrics as important glycemic outcomes.[350] The metrics include measures of overall glucose levels and glucose variability. The major measures include glucose mean, glucose standard deviation, coefficient of variation ([standard deviation/mean] × 100 as a percentage), glucose time in range (70–180 mg/dL [3.9–10.0 mmol/L]), time in hyperglycemia (>180 mg/dL [10.0 mmol/L] and >250 mg/dL [13.9 mmol/L]), and time in hypoglycemia (<70 mg/dL [3.9 mmol/L] and <54 mg/dL [3.0 mmol/L]). Glucose time in range, as well as time in hyperglycemia and hypoglycemia, should be reported per unit time, as in the percentage of the values in the specified range of 24 hours/day. A clinically meaningful episode of hypoglycemia has been defined as 15 minutes or more of consecutive CGM readings less than 54 mg/dL (3.0 mmol/L). There is no doubt that CGM has heralded in a new era to enhance our understanding of outcomes beyond HbA_{1c}.

In addition to glycemic metrics beyond HbA_{1c}, there are numerous patient-reported outcomes that warrant attention. These include quality of life, well-being, fear of hypoglycemia, diabetes distress or burden, treatment satisfaction, self-efficacy, disordered eating behaviors, anxiety, depression, diabetes family conflict, sleep deprivation, and others, many of which have been discussed in a recent position statement by the ADA.[351] Several validated surveys to assess such patient-reported outcomes are catalogued in this publication. With the continued development of adjunctive therapies and advanced diabetes technologies, there will be a continued need to consider the patient- and family-reported outcomes in addition to metrics of glycemic control.

Transition in Care From Pediatric to Adult Health Care Settings and Beyond

The delivery of pediatric diabetes care focuses on the young person with diabetes, as well as family members who assist with diabetes management tasks and provide support and guidance. Adult care generally tends to follow a medical model with a focus almost exclusively on the person with diabetes. This variation in approaches can produce a disconnect for the young person with T1DM who transitions between health care settings. Additionally, transition generally occurs between the ages of 18 and 22 years, although it can occur at younger ages (down to age 14–16 years) or older ages (up to 26 years), depending on the health care system. Nonetheless, transition often occurs during the fragile development stage of young adulthood, between the ages of 18 and 25 years, when the young person faces many competing challenges related myriad choices concerning education, occupation, socialization, intimacy, economic self-sufficiency, and potential geographic separation from the family unit, among other issues.[352]

Transition is considered as the process that begins during adolescence when the teen accepts more diabetes self-care responsibilities and attends part of the diabetes encounter without parents or guardians. During transition, the teen or young adult is expected to master self-care skills, as well as learn how to make follow-up appointments, seek on-call help, and obtain prescriptions, for example. At the end of transition, there is the actual transfer to adult care, the final step that entails the need for an introduction to a different health care delivery model.

There has been increased interest in the study of transition from pediatric to adult diabetes care due to the recognition that young adults experience many adverse outcomes, including achieving the highest mean HbA_{1c} level of 9.2% at the age of 19 years.[353] Many young adults with T1DM often have substantial gaps in care exceeding 6 to 12 months between visits, resulting in deteriorating glycemic control, the need for emergency room use, and the potential emergence of diabetes complications that go undetected and untreated in a timely manner.[354–356] The ADA, along with other major national and international societies, created a

joint consensus statement on transition, based mainly on expert consensus, which has helped lay the groundwork for continued research in this area.[185,356,357] There are few published controlled trials, although there are studies evaluating the use of care coordinator, often called a *patient navigator*[358] or care *ambassador*,[359–361] at time of transfer from the pediatric to adult team.[362,363]

In general, growing teens should begin to learn about the process of transition to adult heath care starting in early to mid-adolescence from the pediatric diabetes health care team. In the final year of pediatric care, the young adult should receive names and contact information for adult diabetes care providers. Then, both the pediatric and adult providers can help support the transfer and ensure the appropriate transmission of important diabetes medical information. Numerous online resources have been developed to assist in the transition process.[364,365] Finally, although the topic of transition from pediatric to adult care continues to generate substantial clinical and research interest, another transition period, specifically the one from adulthood to a more mature older adult group, also requires ongoing research. This is especially important given that there are substantial numbers of persons with T1DM living many decades with diabetes and surviving well into the geriatric years, indicating a need to reassess the cognitive abilities and hypoglycemia risk in the older person with diabetes.[366,367]

Acute Diabetic Emergencies

Diabetic Ketoacidosis

DKA is a significant, expensive, and possibly lethal and potentially avoidable acute complication of T1DM.

Pathophysiology of DKA

DKA is defined by hyperglycemia (blood glucose >200 mg/dL, ~11 mmol/L), ketonemia (blood beta-hydroxybutyrate ≥3 mmol/L or moderate/large urine ketones), and acidosis (venous pH <7.3 or serum bicarbonate <15 mmol/L).[368] It is the result of relative or absolute insulin deficiency causing an increase in glucagon concentrations and accompanying increases in epinephrine, norepinephrine, cortisol, and growth hormone levels.[369] These hormone changes lead to a catabolic state in the periphery, mobilizing substrates that the liver then uses to increase production of glucose and ketone bodies. When serum glucose concentrations exceed the renal threshold of approximately 180 mg/dL (10 mmol/L), osmotic diuresis leads to dehydration and loss of electrolytes. Vomiting, which commonly occurs with severe ketosis, can further contribute to dehydration and electrolyte abnormalities. The vicious cycle continues with additional stress hormones production, which leads to worsening insulin resistance, hyperglycemia, and ketosis.[368]

Epidemiology and Risk Factors for DKA

DKA is a common presentation in new-onset T1DM. The estimated proportion of children with new-onset T1DM who present with DKA ranges from 15% to 70% in different studies and in different countries.[368,370–372] Individuals who are young (<5 years), do not have a first-degree relative with T1DM, and with lower socioeconomic status are at a greater risk of DKA at diagnosis.[372] In a review of international DKA rates, higher DKA incidence at first presentation of T1DM was associated with lower gross domestic product, lower latitude, and lower background incidence of T1DM.[371] Most cases of DKA, in fact, occur in those with established diabetes, where the risk of DKA is between 1 and 10 per 100 person-years, and is higher in patients with poor metabolic control and/or history of insulin omission; a history of a prior episode of DKA; gastrointestinal illness; psychiatric disorders, including eating disorders; low socioeconomic status; and, for unclear reasons, females in adolescence.[353,368,373] Insulin pump use is actually associated with lower rates of DKA compared with MDI,[374,375] although insulin pump failure is an important cause of hyperglycemia, ketonemia, and DKA.

Prevention of DKA /Sick Day Management

Appropriate management of sick days in individuals with T1DM can usually be achieved in the outpatient setting with ongoing education and support. Guidelines are available for management of sick days in children and adolescents with diabetes.[376] The prevention of progression to DKA and avoidance of hypoglycemia rely on modifying doses of but never omitting insulin, frequent monitoring of blood glucose and ketone levels every 1 to 3 hours, preventing/treating dehydration and hypoglycemia, treating the underlying illness, and maintaining frequent contact with the diabetes team. Monitoring of ketones using blood ketone meters that measure beta-hydroxybutyrate is preferred over monitoring of urine ketone strips, due to easier sampling, lower rates of hospitalization/emergency department visits, and the potential for earlier and more accurate identification of clinical worsening or improvement.[376–378]

Insulin Dosing During Sick Days

The degree of hyperglycemia and ketosis, along with the rate of change of these parameters, helps guide insulin dose adjustments. Supplemental rapid-acting insulin boluses (correction doses or 5–20% of the total daily insulin dose, TDD) should be given every 2 to 3 hours while ketones persist; see Table 36.9 for bolus dosing suggestions. For ketones with hypoglycemia, consider reducing basal rates for a short period of time for patients on insulin pumps. For ketones, hypoglycemia, and decreased oral intake over a prolonged period, consider reducing the insulin glargine dose for patients on MDI and basal rates for those on insulin pumps.

Prevention/Treatment of Dehydration During Sick Days

Persons with T1DM and ketonemia require extra fluid due to risk of dehydration. A reasonable target oral fluid intake is 0.5 to 1.0 ounce per year of age per hour (maximum of 8 ounces/hour). The type of drink should be adjusted according to blood glucose levels such that when blood glucose is 250 mg/dL (~14 mmol/L) or less, rehydrate with sugar-containing clear liquids (e.g., sports drinks, soda, popsicles, oral electrolyte solution), and when blood glucose is greater than 250 mg/dL (14 mmol/L), give sugar-free clear liquids (e.g., water, diet soda, seltzer, diet juices).

Mini-Dose Glucagon

When blood sugars remain low and ketones remain elevated, small doses of glucagon (mini-dose glucagon) may be given. As long as there are adequate hepatic glycogen stores, mini-dose glucagon can help with resolution of hypoglycemia in the setting of prolonged vomiting or fasting.[379] Glucagon should be reconstituted with the diluent in the glucagon kit, then the desired mini-dose glucagon is drawn up and administered with an insulin syringe. Table 36.10 provides information on dosing.[380]

Education

The ADA recommends yearly education on sick day management and DKA prevention for persons with T1DM and their caregivers,

TABLE 36.9 Guidelines for Insulin Adjustment With Ketones

	BG <90 mg/dL (BG <5 mmol/L)	BG 90–179 mg/dL (BG 5–9.9 mmol/L)	BG 180–249 mg/dL (BG 10–13.9 mmol/L)	BG >250 mg/dL (BG >14 mmol/L)
Ketones <0.6 mmol/L (negative/trace)	Ordinary bolus for carbohydrates	Ordinary bolus	Ordinary bolus	Increase bolus by 5%
Ketones 0.6–1.4 mmol/L (small)	Ordinary bolus for carbohydrates	Ordinary bolus	Increase bolus by 5%	Increase bolus by 10%
Ketones 1.5–2.9 mmol/L (moderate)	Ordinary bolus for carbohydrates	Increase bolus by 5%	Increase bolus by 10%	Increase bolus by 15%
Ketones ≥3 mmol/L (large)	Ordinary bolus for carbohydrates	Increase bolus by 10%	Increase bolus by 15%	Increase bolus by 20%

BG, Blood glucose.

TABLE 36.10 Glucagon Mini-Dosing

	Glucagon Dose (μg)	Glucagon Dose (mg)	Unit Markings on Insulin Syringe
Age <2 years	20	0.02	2
2–15 years	10 per year of age (range, 20–150)	0.01 per year of age (range, 0.02–0.15)	1 per year of age (range, 1–15)
Age >15 years	150	0.15	15

including the importance of insulin administration, as well as glucose and ketone monitoring.[381] Patients and families should be aware that they should seek immediate medical attention for signs of dehydration, prolonged vomiting for more than a few hours, persistent hyperglycemia (>250–300 mg/dL, >14–16.7 mmol/L), ketones that do not improve after 12 hours, or symptoms of DKA (abdominal pain, nausea, vomiting, fruity-smelling breath, hyperventilation, or altered mental status).

Treatment of DKA

Detailed guidelines for the treatment of DKA are available.[368] Immediate initial treatment of DKA begins with fluid replacement, typically with one to two intravenous fluid boluses with normal saline, 10 mL/kg each. Rehydration should begin before starting insulin therapy, and initial fluid resuscitation is then followed by continued cautious rehydration with the goal of replacing the estimated fluid deficit over the first 24 to 48 hours. Initiation of an insulin drip at a rate of 0.05 to 0.1 units/kg per hour, without an insulin bolus, should begin at least 1 hour after fluid replacement is started. Patients must be monitored very closely for clinical and biochemical response to treatment. Fluid content and rate, and occasionally insulin rate, should be titrated so that blood glucose falls gradually at a rate of 50 to 75 mg/dL per hour (2.8–4.2 mmol/L per hour). Fluid should typically contain saline with potassium, phosphate, and acetate. Bicarbonate is not typically recommended in the treatment of DKA, except in unusual circumstances related to severe acidosis or hyperkalemia. Potassium replacement is usually required due to total-body potassium depletion, but potassium supplementation should be deferred until urine output is documented.

The Pediatric Emergency Care Applied Research Network (PECARN) Fluid Therapies Under Investigation in DKA (FLUID) study was a multisite prospective randomized factorial trial examining long- and short-term neurologic outcomes associated with the rate of intravenous fluid administration (rapid or slow) and the sodium chloride content of intravenous fluids (0.9% or 0.45%).[382] In the 1389 episodes of DKA reported in 1255 children, the primary outcome of a decline in mental status (two consecutive Glasgow Coma Scale scores <14; possible scores range from 3–15) was met in 48 episodes of DKA (3.5%) and was not associated with either the rate of fluid administration or the sodium chloride content of the fluids. Secondary neurologic outcomes including clinically apparent brain injury during treatment for DKA, short-term memory, and memory and IQ measured 2 to 6 months after recovery from DKA were also not associated with fluid administration rate or content.

One important cause of morbidity and mortality in DKA is cerebral edema. Risk factors for cerebral edema include young age (<5 years), new-onset T1DM, longer duration of symptoms, lower initial pH, higher initial blood urea nitrogen, treatment with bicarbonate, and serum sodium concentrations increasing during initial treatment of DKA. In patients at high risk of cerebral edema, or suspected of having cerebral edema, timely intervention with mannitol or hypertonic saline is important.

Other Complications

Hypoglycemia is the other major acute complication of T1DM and is discussed in Chapter 38. Chronic complications are discussed in Chapter 37.

Comorbidities

There is a higher prevalence of additional autoimmune conditions among persons with T1DM compared with the general population. In an analysis of more than 25,000 participants in the T1D Exchange Registry, 27% of children and adults with T1DM were diagnosed with one or more additional autoimmune diseases, and prevalence was higher in those of older age, female sex, and non-Hispanic white race.[383] The most common comorbidities are thyroid dysfunction and celiac disease, but autoimmune primary adrenal insufficiency (Addison disease), Graves disease, inflammatory bowel disease, collagen vascular disease, skin disease, and others can occur as well. Regular assessment for signs and symptoms

of other autoimmune diseases and periodic screening in asymptomatic individuals is recommended.

Thyroid Disease

Approximately 20% of individuals with T1DM have hypothyroidism or hyperthyroidism, making thyroid disease the most common autoimmune disorder associated with T1DM.[383] The ADA and ISPAD recommend measuring thyroid antibodies and thyroid-stimulating hormone (TSH) levels soon after diagnosis of T1DM.[381,384] One large study of children with T1DM found that about 25% of children had positive thyroid peroxidase autoantibodies at the time of T1DM diagnosis.[385] Thyroid function tests may be slightly abnormal at the time of T1DM diagnosis due to sick euthyroid syndrome; if this is the case, thyroid function tests should be reassessed once glycemic control improves. If antibodies are negative and TSH levels are normal soon after diagnosis, regular measurement of TSH concentrations is recommended every 1 to 2 years, and sooner if the patient develops any new symptoms or signs concerning for thyroid dysfunction, including thyromegaly, abnormal growth pattern, or unexplained fluctuations in blood glucose level. There is some evidence that subclinical hypothyroidism is associated with decreased linear growth velocity and increased risk of symptomatic hypoglycemia.[386]

Celiac Disease

Celiac disease is the second most common coexistent autoimmune disorder in patients with T1DM, is diagnosed in 2% to 8% of youth with T1DM and is more prevalent in those who were younger at T1DM diagnosis and those of white race.[383,387,388] Introduction of gluten-containing foods between 4 and 6 months of age (compared with introduction in the first 3 months of life or in the seventh month or later) in infants at increased risk for celiac disease and T1DM appears to mitigate the risk of celiac disease.[389] Individuals with T1DM and celiac disease may experience increased glycemic variability and unexplained hypoglycemia.[390] The ADA and ISPAD recommend screening for celiac disease in children with T1DM soon after diagnosis and at regular intervals after diagnosis (ADA: rescreen within 2 years and again after 5 years; ISPAD: screen every 1–2 years).[381,384] Screening labs include immunoglobulin A (IgA) tissue transglutaminase antibodies, with documentation of normal total serum IgA levels; if an individual is found to be IgA deficient, send immunoglobulin G–specific antibody tests. More frequent screening may be necessary if symptoms develop or in children who have a first-degree relative with celiac disease. If screening tests are positive, referral to gastroenterology is recommended before prescribing a gluten-free diet. Typically, a biopsy is done to confirm the diagnosis of celiac disease, especially in asymptomatic children.[391] In symptomatic individuals with high antibody titers, some providers may prescribe dietary changes without a confirmatory biopsy. Once the diagnosis is confirmed, children with celiac disease should be placed on a gluten-free diet and be referred to a dietitian experienced in managing both diabetes and celiac disease, because the nutritional management of a person with both T1DM and celiac disease can be complex and burdensome. A gluten-free diet may reduce gastrointestinal complaints, metabolic symptoms, and hypoglycemia.[392]

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The complete list of references is available online at ExpertConsult.com.

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Complications of Diabetes Mellitus

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CHAPTER OUTLINE

KEY POINTS

- Chronic hyperglycemia causes retinopathy and nephropathy. The effects of former high HbA_{1c} levels can persist for years of subsequent lower HbA_{1c} values. Both chronic hyperglycemia and the consequences of insulin resistance cause neuropathy, atherosclerosis, and cardiomyopathy. In some patients, chronic unresolved inflammation is as great a cardiovascular disease risk factor as high blood pressure and cholesterol. Mitochondrial dysfunction is a component of many mechanisms underlying complications.
- Proliferative diabetic retinopathy and diabetic macular edema can both cause severe visual loss. Treatment of diabetic macular edema with intraocular injection of vascular endothelial growth factor (VEGF) inhibitors—medications that inhibit vascular endothelial growth factor—reduces diabetes-induced abnormal retinal thickening and improves long-term visual acuity outcomes. Treatment with panretinal photocoagulation reduces blindness from proliferative diabetic retinopathy by over 90%. Intraocular anti-VEGF therapy can now also effectively treat proliferative diabetic retinopathy.
- Chronic diabetic kidney disease increases the risk of cardiovascular disease and death. Optimal management includes early control of blood pressure using renin-angiotensin-aldosterone system blockade and other agents, control of hyperglycemia (glucagon-like peptide-1 [GLP1]) analogs and sodium glucose cotransporter-2 (SGLT2) inhibitors that may be more renoprotective, and control of dyslipidemia.
- Diabetic neuropathies include distal symmetric polyneuropathy, mononeuropathies, and a variety of autonomic neuropathies. Loss of heart rate variability due to autonomic neuropathy increases the risk of cardiac events more than fourfold. The cornerstone of treatment is blood glucose control. Specific antidepressants, anticonvulsants, and the γ-aminobutyric acid (GABA) analogue pregabalin are used to treat painful neuropathy.
- Prediabetes and diabetes both substantially increase risk of coronary disease, early and late myocardial infarction fatality rates, and risk of congestive heart failure, even after adjustment for other cardiovascular risk factors. Hyperglycemia and insulin resistance interact synergistically with hypertension and dyslipidemia. Optimal treatment includes intensive lowering of low-density lipoprotein (LDL) cholesterol with early use of statins or other agents, blood pressure control, and use of glucose-lowering agents that have proven efficacy to reduce cardiovascular events, including metformin, GLP1 receptor agonists, and SGLT2 inhibitors. In general, coronary artery bypass grafting may provide better outcomes than coronary stenting in patients with diabetes. Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) reduce post–myocardial infarction mortality risks.
- Diabetic foot ulcers are the major cause of nontraumatic leg amputation. Risk factors are sensory neuropathic loss of proprioception, motor neuropathic foot deformity, and peripheral vascular disease. Simple clinical interventions reduce amputation by up to 80%. Management involves pressure off-loading, parenteral antibiotics for infection, and ensuring adequate arterial inflow.

Biochemistry and Molecular Cell Biology

Clinical Overview

All forms of diabetes mellitus are characterized by insufficient or absolute lack of insulin secretion, insulin resistance, altered fuel metabolism, and development of diabetes-specific complications in the eye, kidney, and peripheral nerve. Diabetes is also associated with accelerated atherosclerosis affecting arteries of the heart, brain, and lower extremities. In coronary arteries, diabetic atherosclerosis is more diffuse, with greater inflammatory infiltrate and larger necrotic core size.[1] Diabetes increases heart failure risk fourfold, and doubles both early and late post–myocardial infarction fatality rates.[2] As a consequence of these complications, diabetes is the leading cause of new blindness in people 20 to 74 years old,[3] the leading cause of end-stage renal disease (ESRD), and the

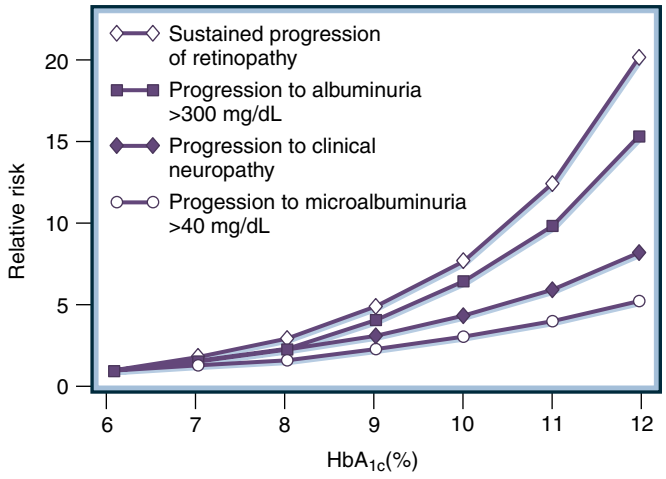
leading cause of both neuropathy and nontraumatic lower extremity amputations. Life expectancy for patients with diabetic ESRD is under 4 years. Neuropathy affects more than 60% of patients with diabetes. Diabetic neuropathy includes distal symmetric polyneuropathy, mononeuropathies, and a variety of autonomic neuropathies causing cardiac arrhythmias, hypotension, erectile dysfunction, urinary incontinence, gastroparesis, and nocturnal diarrhea.

Overall life expectancy is about 11 to 13 years shorter for people with type 1 diabetes mellitus (T1DM), and 7 to 10 years shorter for people with type 2 diabetes mellitus (T2DM) compared to people without diabetes.[4–6] Accelerated cardiovascular disease is the leading cause of death in people with both T1DM and T2DM diabetes. T1DM patients diagnosed before the age of 10 years have a 30-fold increased risk of coronary heart disease and acute myocardial infarction occurring in their early adult years.[7] While the incidence of diabetes-related ESRD, acute myocardial infarction, and amputation has declined over the past 25 years, the rates are still 10-fold higher than for the overall adult population.[8]

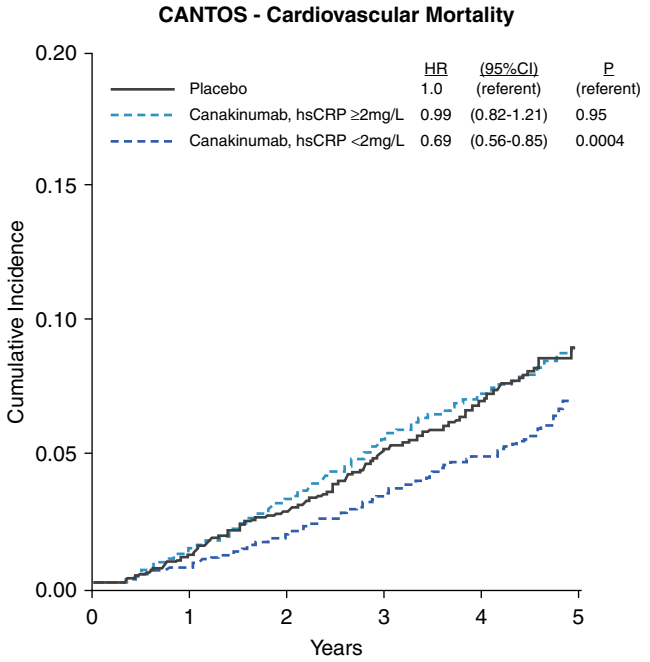
A strong relationship between hyperglycemia and diabetic retinopathy and nephropathy has been demonstrated in both T1DM and T2DM large prospective clinical studies—the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), respectively.[9,10] There is a continuous (although not linear) relationship between the level of glycemia and the risk of development and progression of these microvascular complications (Fig. 37.1). In T1DM, hyperglycemia is similarly associated with neuropathy, while in T2DM, it is more closely associated with the consequences of insulin resistance/hyperinsulinemia.[11,12] In contrast to the beneficial effects of glycemic reduction on microvascular complications, a meta-analysis of randomized controlled trials shows reduction in myocardial infarction rates but limited benefits of intensive glucose-lowering treatment on all-cause mortality and deaths from cardiovascular causes.[13] While consequences of insulin resistance/hyperinsulinemia and chronic hyperglycemia both play a role in the pathobiology of diabetic cardiovascular complications, the consequences of insulin resistance/hyperinsulinemia may play a larger role.[14,15] In the San Antonio Heart Study, for example, individuals with normal glucose tolerance and higher insulin resistance had twice the 8-year risk of adverse cardiovascular outcomes than those with lower insulin resistance, even after adjustment for 11 risk factors.[16,17]

Chronic, unresolved systemic inflammation is a cardiovascular risk factor independent of traditional lipid and nonlipid risk factors. In healthy men followed prospectively as part of the Physician's Health Study, those with the highest quartile concentrations of high sensitivity C-reactive protein (hsCRP) had a 2.9-fold greater risk of myocardial infarction than those in the lowest quartile, a magnitude of independent risk as great as hypertension and cholesterol.[18] Directly reducing inflammation with canakinumab, an interleukin 1β (IL1β) neutralizing monoclonal antibody, reduced cardiovascular event rates in a population receiving lipid-lowering therapy at baseline. The greatest reduction occurred among those achieving hsCRP concentrations below 2 mg/L[19] (Fig. 37.2). Individuals with and without diabetes had similar magnitude of benefit from canakinumab suggesting the IL1β pathway is not unique to diabetes. Chronic inflammation also contributes to chronic kidney disease, mediated in part by activation of the NLRP3 (nucleotide-binding oligomerization domain-like receptors [NLR] family pyrin domain containing three) inflammasome in renal cells. Inhibition of IL1β with canakinumab also reduced major cardiovascular event rates among high-risk patients with chronic kidney disease.[20] Recent studies suggest defective resolution of chronic inflammation is also a general feature of most diabetic complications.

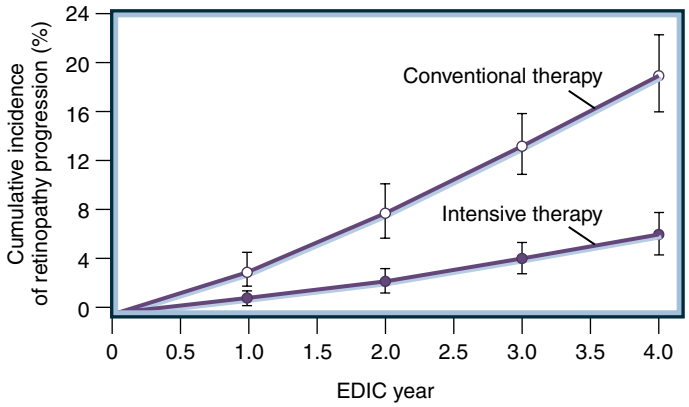
For each of the complications of diabetes, the effects of former high HbA_{1c} levels can persist for years after HbA_{1c} values



• **Fig. 37.1** Relative risks for the development of diabetic complications at different mean levels of glycosylated hemoglobin (HbA_{1c}) in patients with type 1 diabetes obtained from the Diabetes Control and Complications Trial. (Adapted from Skyler J. Diabetic complications: the importance of glucose control. *Endocrinol Metab Clin North Am*. 1996;25:243–254.)



• **Fig. 37.2** Cumulative incidence and hazard ratios of cardiovascular mortality among CANTOS participants allocated to either placebo or canakinumab according to whether postrandomization on-treatment hsCRP levels were above or below 2 mg/L. Hazard ratios are adjusted for age, sex, smoking status, hypertension, diabetes, body mass index, baseline concentration of hsCRP, and baseline concentration of LDL-C. *CI,* Confidence interval; *HR,* hazard ratio. (From Aday AW, Ridker PM. Anti-inflammatory therapy in clinical care: the CANTOS trial and beyond. *Front Cardiovasc Med*. 2018;5:62.)



• **Fig. 37.3** Cumulative incidence of further progression of retinopathy 4 years after the end of the Diabetes Control and Complications Trial. The median glycosylated hemoglobin level was 8.2% for the conventional therapy group and 7.9% for the intensive therapy group. *EDIC,* Epidemiology of Diabetes, Interventions, and Complications Research Group. (Modified from Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group, Lachin JM, Genuth S, et al. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. *N Engl J Med*. 2000;342:381–389.)

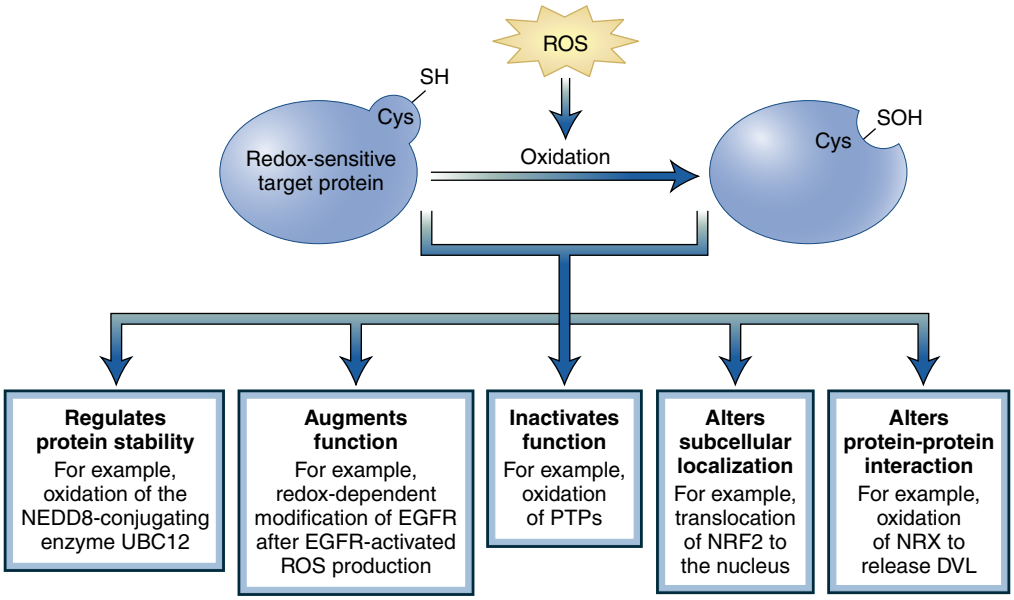
have been lowered (called "metabolic memory" by the DCCT and long-term observational follow-up Epidemiology of Diabetes Interventions and Complications [EDIC] study investigators and "a legacy effect" by the UKPDS investigators). Data from DCCT/EDIC studies proved effects of former intensive and conventional therapy persist for at least 18 years.[21,22] In the conventional therapy group, effects of previous high HbA_{1c} on poststudy retinopathy, nephropathy, and major adverse cardiovascular events (MACE; comprised of nonfatal myocardial infarction or stroke, or cardiovascular death) persisted as if there had been no improvement in HbA_{1c} at all (Fig. 37.3). Atherosclerotic changes not even present at the end of the DCCT appeared subsequently in the previously higher HbA_{1c} group, followed by a twofold increase in heart attacks, strokes, and cardiovascular death, even though the HbA_{1c} level in these patients since the end of the DCCT was identical to that of the formerly intensive-control group during the entire time that these arterial changes developed.[23] On the other hand, the beneficial effects of previous lower HbA_{1c} persisted in the intensive treatment group after their HbA_{1c} increased after the DCCT intervention ended, as if there had been no deterioration in their HbA_{1c}.

Thus T1DM patients in the DCCT with long-term exposure to a higher level of hyperglycemia during the study became more susceptible to damage despite subsequent lower levels of hyperglycemia than when they first started the trial. In contrast, lower levels of hyperglycemia during the trial made patients more resistant to damage from subsequent higher levels. Data from long-term follow-up studies from the UKPDS confirm a similar effect for T2DM patients associated with intensive glucose control, long after the cessation of randomized intervention.[24]

Much current knowledge about diabetic complication mechanisms comes from studies in murine models and of cultured cells. Murine models are valuable tools for defining the pathogenesis of diabetic complications. However, it is important to recognize the limits of murine models. No diabetic animal model, regardless of genetic background, develops the advanced stages of human diabetic complications, such as proliferative retinopathy, end-stage renal failure, significant nerve fiber loss, or complex atherosclerotic plaques and plaque rupture, nor do they develop the degree of fibrosis seen in human hearts with late-stage diabetes–associated heart failure. Also, since much of the mechanistic data currently available from studies of cell and mouse models reflect the earliest stages of each complication, mechanisms dominant in the pathogenesis of later stages of each complication are likely different from those dominant in the early stages. Recent proteomic, genomic, and molecular studies of tissue from patients with longer duration of diabetes have contributed to enormous recent progress in understanding the biochemical, molecular, and cellular mechanisms involved in the pathogenesis of diabetic complications. Many of the multiple mechanisms discussed in this section share a common element: prolonged increases in reactive oxygen species (ROS) production in cells critical for the development of diabetic complications in the retina, kidney, peripheral nerve, arteries, and heart. In diabetic mouse models, transgenic overexpression of mitochondrial superoxide dismutases or catalase prevents retinopathy,[25] nephropathy,[26,27] neuropathy,[28] atherosclerosis,[29] and cardiomyopathy.[30,31]

Physiologic Reactive Oxygen Species Production Is Essential for Normal Intracellular Signaling and Cellular Homeostasis

In normal physiology, ROS production is coupled to metabolic networks and circadian clocks, and ROS species (hydrogen peroxide, H_2O_2) function as signaling molecules essential for normal cellular homeostasis.[32–34] Physiologic ROS (H_2O_2) signaling is essential for normal intracellular communication, cell differentiation, autophagy, response to insulin and growth factor stimulation, and the generation of physiologic inflammatory responses. ROS regulate protein stability, augment protein function, inactivate function, alter subcellular localization, and alter protein-protein interactions (Fig. 37.4). In normal cardiovascular function, for example, enhanced production of H_2O_2 from a mitochondrial source of superoxide leads to increased flow rates in human coronary resistance vessels. H_2O_2 hyperpolarizes and dilates human coronary arterioles through opening of Ca^{2+}-activated K^+ channels. Catalase, a scavenger of H_2O_2, greatly inhibits this flow-induced dilation.[35] Similarly, H_2O_2 exerts a beneficial effect on vasodilator function and reduces blood pressure in transgenic mice with endothelium-targeted nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4 (Nox4) overexpression.[36] In the heart, low levels of hydrogen peroxide induce proliferation of mouse embryonic stem cells as well as neonatal cardiomyocytes, and ROS induce expression of cardiac-specific genes, transcription factors, and growth factors in embryonic stem cells. These effects are dampened by free radical scavengers. ROS also act as transducers of mechanical strain–induced cardiovascular differentiation of embryonic stem cells.[37] ROS-dependent activation of integrins and subsequent induction of PI3-K/Akt (phosphatidylinositol-4,5-bisphosphate 3-kinase/RACα/β serine/threonine-protein kinase) signaling are also involved in cyclic strain–mediated cardiomyogenesis.[38] Localized ROS production contributes to stretch-induced augmentation of cardiac contractile activity as well.[39] Physiologic excitation–contraction coupling in heart muscle may also involve ROS signaling as physiologic stretch rapidly activates NOX2 located on sarcolemma and T tubule membranes in cardiomyocytes.[39] The local ROS produced sensitizes ryanodine receptors in the sarcoplasmic reticulum. This triggers a burst of Ca^{2+} sparks, thereby increasing Ca^{2+} signaling sensitivity in healthy cardiomyocytes. Thus ROS



• **Fig. 37.4** The multiple ways in which oxidant signaling can affect cellular function. Oxidation of a reactive cysteine residue SH (thiol) to SOH (sulphenic acid) leads to an alteration in the reduction-oxidation (redox)–sensitive target that can then have myriad effects on protein stability, activity, localization, or protein-protein interaction. *Cys,* Cysteine; *DVL,* dishevelled; *EGFR,* epidermal growth factor receptor; *NEDD8,* neural precursor cell expressed developmentally down regulated-8; *NRF2,* nuclear related factor-2; *NRX,* nucleoredoxin; *PTP,* protein tyrosine phosphatase; *ROS,* reactive oxygen species. (From Holmström KM, Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signaling; UBC12, ubiquitin carrier protein 12. *Nat Rev Mol Cell Biol*. 2014;15:411–421.)

production at the right time, place, level, and duration plays a crucial role in physiologic homeostasis.[40] However, ROS production at too high a level, for too long, or at an inappropriate subcellular location, leads to impaired cellular function and diabetic tissue pathology.

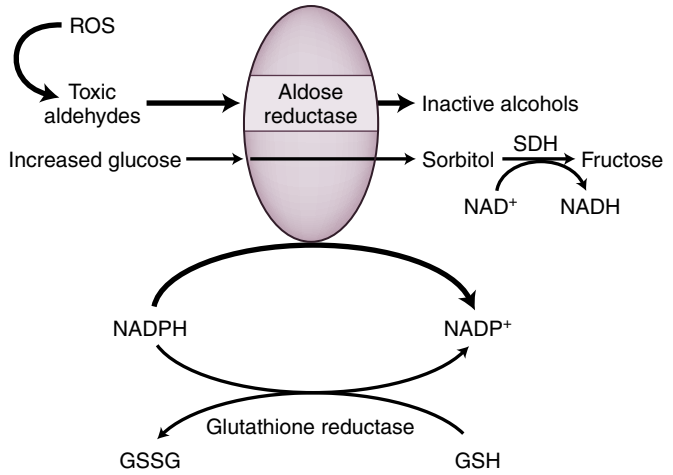
Recent data suggest the site at which ROS are generated is as important as the amount of ROS produced.[41] ROS generated by mitochondrial complex III are released into the mitochondrial matrix, intermembrane space, outer membrane, and cytosol. In contrast, ROS generated by mitochondrial complex I are released into the matrix. ROS from these two sites reacted with distinct subsets of proteins involved in different physiologic pathways.[42] Importantly, ROS from mitochondrial complex I caused a high percentage of irreversible overoxidations of cysteine thiols, consistent with persistent oxidative damage. They also cause nuclear deoxyribonucleic acid (DNA) double-strand breaks and oxidize mitochondrial DNA, both of which have adverse consequences.[43–45] In contrast, ROS from mitochondrial complex III caused reversible oxidation of cysteine thiols, consistent with their function as redox switches in critical signaling pathways. Use of indiscriminate antioxidants, which block both the damaging and the physiologic effects of mitochondrial ROS, likely explain the negative findings of clinical trials with selected antioxidant compounds.

Mechanisms of Hyperglycemia-Induced Damage

Increased Aldose Reductase Substrate Conversion

Aldose reductase (AKR1B1), a member of the large aldo-keto reductase superfamily, catalyzes reduction of a wide variety of hydrophobic and hydrophilic carbonyl-containing compounds—including glucose and several glycolytic intermediates—to their corresponding alcohols. This enzyme is cytosolic and requires NADPH as a cofactor. In some cell types, where glucose is converted to the sugar alcohol sorbitol, sorbitol is then converted to fructose by another enzyme, sorbitol dehydrogenase, using NAD^+ (nicotinamide adenine dinucleotide) as a cofactor. This series of reactions, termed the *polyol pathway,* has been implicated in the pathogenesis of several diabetic complications. However, the amount of substrate converted to product per second (K_{cat}) of human aldose reductase for glucose is 0.15 [s^{-1}]. K_{cat} values for most enzymes are between 1 and 10^4. Moreover, because the intracellular glucose concentration in capillary retinal endothelial cells incubated in 25 mM glucose is approximately 0.15 mM,[46] while the K_m of aldose reductase for glucose reported by Bohren and coworkers is 100 mM and the K_{cat}/K_m is 1.3 [s^{-1} M^{-1}], the predicted rate of aldose reductase reduction of glucose to sorbitol in microvascular endothelial cells would be expected to be rather low. However, aldose reductase has high affinity and enzyme activity for a variety of other substrates, including several glycolytic intermediates such as glyceraldehyde 3-phosphate, and its degradation product, methylglyoxal.[47,48] AKR1B1 also efficiently reduces oxidative stress-generated lipid aldehydes such as 4-hydroxynonenal with a K_m in the micromolar range (10–30 μM) as compared to a K_m of 50–100 mM for glucose[49] (Fig. 37.5).

In vivo studies of aldose reductase inhibition in a 5-year study in dogs showed that diabetic neuropathy was prevented, but aldose reductase inhibition failed to prevent retinopathy or capillary basement membrane thickening in the retina, kidney, or muscle.[50] In response to this preclinical result with neuropathy, 32 clinical trials were completed in the 1990s and early 2000s.[51] None of these trials showed clinical efficacy. In diabetic apolipoprotein E (ApoE) knockout mice, overexpression of human aldose reductase accelerated atherosclerosis, and pharmacologic inhibition of the enzyme

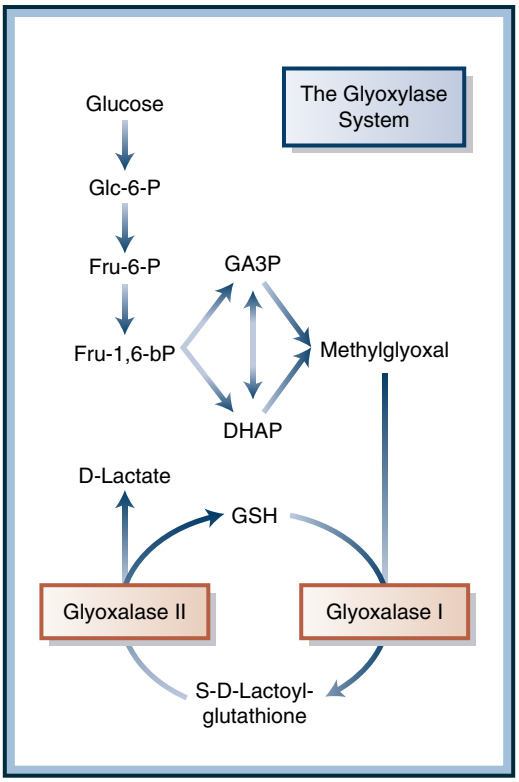


• **Fig. 37.5** Aldose reductase (AKR1B1) catalyzes reduction of the ROS-generated and the polyol pathway. Aldose reductase reduces reactive oxygen species (ROS)–generated toxic aldehydes such as lipid-derived 4-hydroxynonenal to inactive alcohols, and in some cell types, glucose to sorbitol, using triphosphopyridine nucleotide (NADPH), the reduced form of nicotinamide adenine dinucleotide phosphate ($NADP^+$), as a cofactor. It also reduces glyceraldehyde 3-phosphate and its reactive degradation product methylglyoxal. *GSH,* Reduced glutathione; *GSSG,* oxidized glutathione; *SDH,* sorbitol dehydrogenase.

prevented this.[52] In contrast, in mice expressing physiologic levels of aldose reductase, knockout or pharmacologic inhibition of the enzyme unexpectedly caused increased early atherosclerotic lesion size in both diabetic and nondiabetic mice.[53] Differences in total enzyme activity, cofactor levels, and levels of alternative intracellular substrates, coupled with known differences in enzyme kinetics for different substrates, likely explain these seemingly paradoxic observations.

Increased Intracellular Formation of the Major Advanced Glycation End Products—Precursor Methylglyoxal

The post-translational modifications of proteins called advanced glycation end products (AGEs) are formed by glucose-derived dicarbonyls reacting with amino groups of unprotonated lysine and arginine residues of proteins. Methylglyoxal, formed by the nonenzymatic fragmentation of the glycolytic intermediate triose phosphate, accounts for the majority of hyperglycemia-induced increase in AGE adducts in diabetic tissues.[54] Intracellular methylglyoxal is detoxified by the glyoxalase system.[55] The enzyme glyoxalase I, together with glyoxalase II and a catalytic amount of glutathione, reduces this highly reactive α-oxoaldehyde to D-lactate (Fig. 37.6). In cells, methylglyoxal reacts with unprotonated arginine residues to form the major methylglyoxal-derived epitope MG-H1 (methylglyoxal hydroimidazolone 1). Intracellular production of AGE precursors damages target cells by three general mechanisms (Fig. 37.7). First, AGE modification of intracellular proteins changes their function. Second, AGE modification of extracellular matrix components alters their interaction with other matrix components and with integrin matrix receptors. Third, intracellular methylglyoxal increases expression of both the pattern recognition receptor for AGEs (RAGEs) and its major endogenous ligands, the proinflammatory S100 calgranulins.[56] Ligation of these ligands with RAGE causes cooperative interaction with the innate immune system signaling molecule toll-like receptor 4 (TLR4).[57] Expression of RAGE, S100A8, S100A12, and high mobility group box 1 (HMGB1) are all increased by

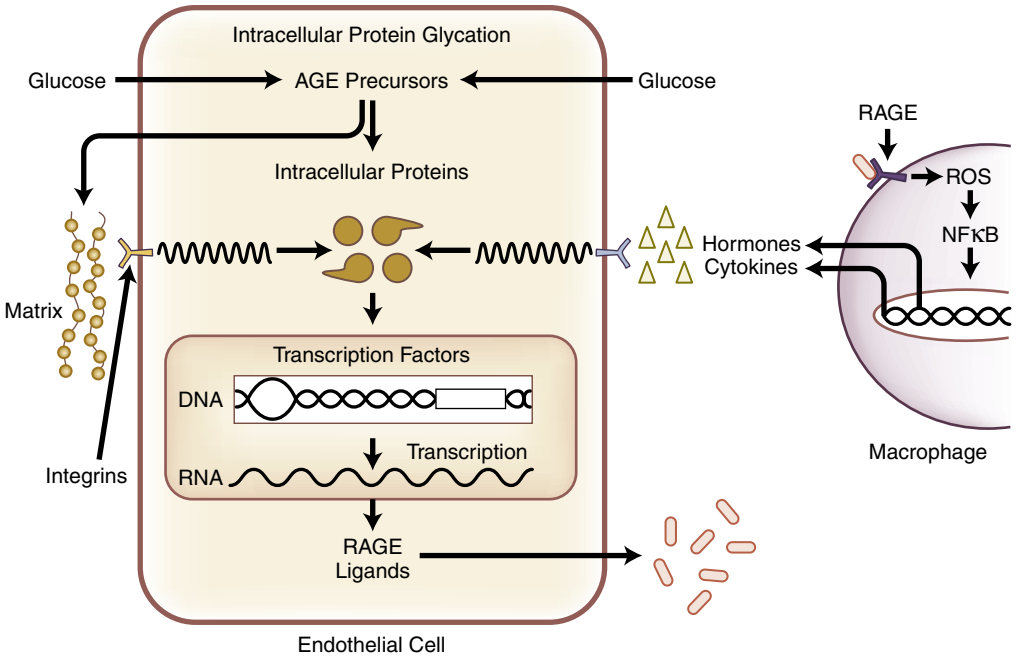


• **Fig. 37.6** Intracellular methylglyoxal, the major advanced glycation end product (AGE) precursor, is detoxified by the glyoxalase system. The enzyme glyoxalase I, together with glyoxalase II and a catalytic amount of glutathione, reduces this highly reactive α-oxoaldehyde to D-lactate. *DHAP,* Dihydroxyacetone phosphate; *GA3P,* glyceraldehyde 3-phosphate; *GSH,* glutathione; *Glc-6-P,* glucose-6-phosphate; *Fru-6-P,* fructose-6-phosphate; *Fru-1,6-bP,* fructose-1,6-bisphosphate. (From Schmoch T, Uhle F, Siegler BH, et al. The glyoxalase system and methylglyoxal-derived carbonyl stress in sepsis: glycotoxic aspects of sepsis pathophysiology. *Int J Mol Sci.* 2017;18.)

high glucose concentrations in cell culture and in diabetic animals. This hyperglycemia-induced overexpression is mediated by ROS-induced increases in methylglyoxal, which increase binding of the transcription factors nuclear factor-κB (NFκB) and activator protein 1 (AP1) to the promoters of RAGE and of RAGE ligands, respectively.[56]

Diabetes increases levels of the major methylglyoxal-derived adduct in retina, renal glomerulus, and sciatic nerve of rats.[58,59] In the retina, diabetic mice with knockouts of four transient receptor potential cation channels (Trpc1/4/5/6 mice) have increased levels of glyoxalase 1 protein and activity, which protects them from the initial phenotype of diabetic retinopathy, pericyte dropout, and acellular capillary formation.[60] In the kidneys of nondiabetic mice, knockdown of glyoxalase I (GLO1) increases to diabetic levels both methylglyoxal modification of glomerular proteins and oxidative stress, causing alterations in kidney morphology indistinguishable from those caused by diabetes, while in kidneys of diabetic mice, GLO1 overexpression completely prevents diabetes-induced increases in methylgloxal modification of glomerular proteins, increased oxidative stress, and the development of diabetic kidney pathology, despite unchanged levels of diabetic hyperglycemia.[61]

Increased levels of methylglyoxal may contribute to painful diabetic neuropathy by altering the function of the voltage-gated sodium channel Nav1.8. Nav1.8 is expressed exclusively



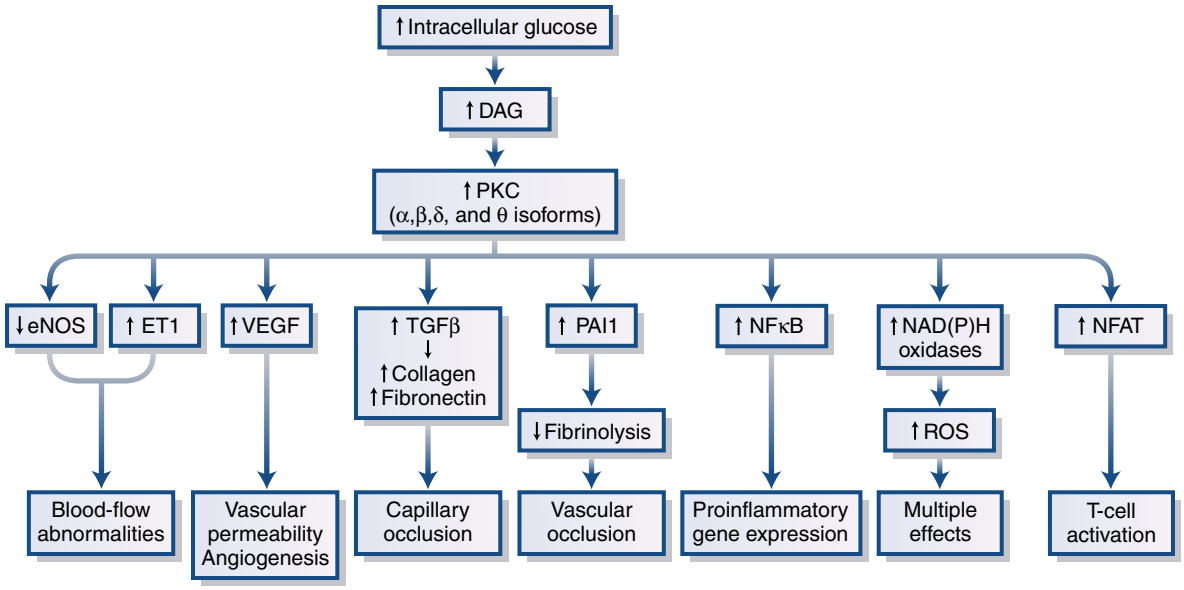
• **Fig. 37.7** Potential mechanisms by which intracellular production of advanced glycation end product (AGE) precursors damage vascular cells. First, intracellular protein modification alters protein function. Second, extracellular matrix modified by AGE precursors has abnormal functional properties. Third, intracellular methylglyoxal increases expression of both the receptor for AGEs (RAGE) and its major endogenous ligands. Ligand binding to RAGE induces receptor-mediated production of deleterious gene products such as cytokines. *NFκB,* Nuclear factor-κB; *ROS,* reactive oxygen species. (Adapted from Brownlee M. Lilly lecture 1993: glycation and diabetic complications. *Diabetes*. 1994;43:836–841.)

in unmyelinated, small-diameter sensory neurons called C fibers. Post-translational modification of Nav1.8 by methylglyoxal depolarizes sensory neurons, facilitating firing of these pain pathway neurons. In streptozotocin-induced and genetic mouse models of diabetes but not in Nav1.8 knockout ($Scn10^{-/-}$) diabetic mice,[62] post-translation modification of Nav1.8 by methylglyoxal increased electrical excitability and facilitated firing of pain pathway neurons, which in turn facilitated neurosecretion of calcitonin gene-related peptide, increasing cyclooxygenase-2 (COX2) expression. Methylglyoxal treatment also evoked thermal and mechanical hyperalgesia, reflected by increased blood flow in brain regions that are involved in pain processing. Plasma methylglyoxal concentrations above 600 nM discriminated between diabetes-affected individuals with painful neuropathy and those without pain.

Increased methylglyoxal is also an important element in the pathogenesis of both diabetic atherosclerosis and diabetic cardiomyopathy. In nondiabetic ApoE null mice, increasing plasma methylglyoxal levels to diabetic levels using a glyoxalase-1 inhibitor caused endothelial inflammation and atherogenesis similar to that induced by diabetes mellitus.[63] In human atherosclerotic plaques, MG-H1 (methylglyoxal hydroimidazolone 1) levels were associated with rupture-prone plaques having increased levels of the inflammatory mediators IL8 and monocyte chemotactic protein-1 (MCP1), and higher matrix metalloproteinase-9 (MMP9) activity. MG-H1 was primarily found in lesion macrophages surrounding the necrotic core, and co-localized with cleaved caspase-3.[64] In the diabetic heart, methylglyoxal preferentially reacts with both ryanodine receptor 2, the major myocardial intracellular mediator of calcium-induced calcium release, and with sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a), which is responsible for the synchronized reuptake of released intracellular calcium.[65] This coordinated process of calcium cycling is critical for efficient cardiac contractions, and diabetes-induced defects caused by increased methylglyoxal adduct formation likely contribute to impaired systolic function.

Increased methylglyoxal production also seems to be responsible for poor cardiac stem cell–mediated repair and angiogenic capacity.[66] Cardiac stem cells from biopsies of hearts from human diabetics were less able to repair postinfarction damage in immunodeficient mice than cardiac stem cells from nondiabetic patients, and conditioned medium from these cells had less angiogenic capacity. Culture of nondiabetic murine cardiac stem cells in high glucose induced the same cardiac repair and angiogenesis defects seen in human diabetic cells. In both human and mouse cells, overexpression of GLO1 restored the angiogenic defects.[66] In diabetic mice with defective postischemia hindlimb revascularization, overexpression of the methylglyoxal-metabolizing enzyme GLO1 exclusively in bone marrow cells was sufficient to restore bone marrow cell function and neovascularization of ischemic tissue in diabetes mellitus.[67]

Increased methylglyoxal also activates the unfolded protein response in cardiomyocytes.[68] Although transient activation of the unfolded protein response relieves endoplasmic reticulum (ER) stress, prolonged activation of the unfolded protein response in cardiovascular disease triggers apoptosis, mediated by the downstream effector C/EBP-homologous protein (CHOP). CHOP plays a critical role in macrophage apoptosis, a process involved in plaque necrosis in advanced atheromata. In $Chop^{-/-}Apoe^{-/-}$ mice, lesion area plaque necrosis was reduced by 50%. In high fat–fed $ApoE^{-/-}$ and low-density lipoprotein (LDL) $receptor^{-/-}$ mice, CHOP promoted plaque growth, apoptosis, and plaque necrosis.[69] In cardiomyocytes, methylglyoxal also induces apoptosis via CHOP.[68] Infusion of methylglyoxal in nondiabetic mice induced



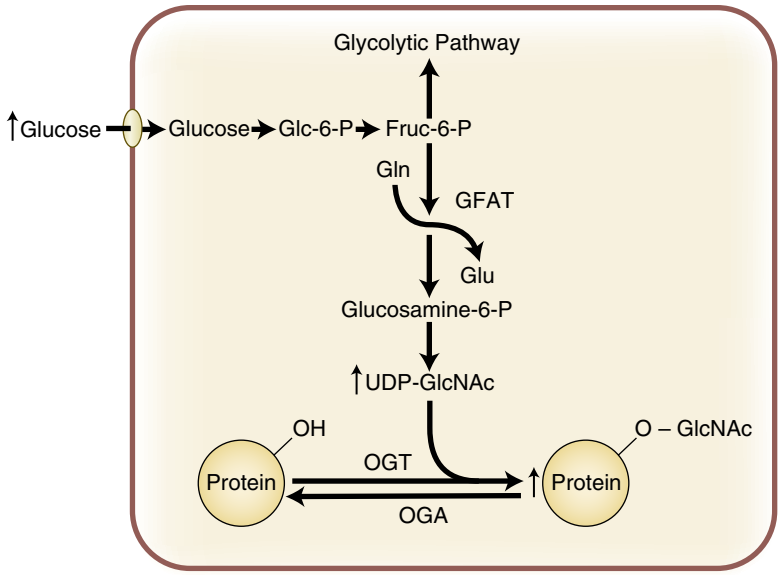
• **Fig. 37.8** Potential consequences of hyperglycemia-induced protein kinase C (PKC) activation. Hyperglycemia increases diacylglycerol (DAG) content, which activates several PKC isoforms. Activated PKC has a number of pathogenic consequences. *eNOS,* Endothelial nitric oxide synthase; *ET1,* endothelin 1; *NAD(P)H,* nicotinamide adenine dinucleotide phosphate; *NFAT,* nuclear factor of activated T cells; *NFκB,* nuclear factor-κB; *PAI,* plasminogen activator inhibitor; *ROS,* reactive oxygen species; *TGF,* transforming growth factor; *VEGF,* vascular endothelial growth factor. (Adapted from Koya D, Jirousek MR, Lin YW, et al. Characterization of protein kinase C beta isoform activation on the gene expression of transforming growth factor-beta, extracellular matrix components, and prostanoids in the glomeruli of diabetic rats. *J Clin Invest.* 1997;100:115–126.)

cardiomyocyte apoptosis, inflammation, and a significant reduction in left ventricular fractional shortening and left ventricular ejection fraction. Each of these adverse effects was prevented in *CHOP*$^{-/-}$ mice. In the hearts of diabetic mice, overexpression of GLO1 in the vasculature[70] prevented diabetes mellitus–induced reduction of myocardial capillary density, increased apoptosis, and loss of cardiac function. Neuregulin production, which transduces signals between the heart's microvasculature and cardiomyocytes,[71] endothelial nitric oxide synthase (eNOS) dimerization, and expression of the antiapoptotic protein BCL-2 were also maintained in the diabetic *GLO1* transgenic hearts.

Activation of Protein Kinase C β, δ, and θ

Protein kinase C (PKC) is a family of protein kinase enzymes with 15 isoforms that are involved in the regulation of protein function. Nine of these 15 PKC isoforms are activated by a lipid second messenger, diacylglycerol. Elevated intracellular glucose increases diacylglycerol levels in a variety of diabetic target tissues, including arterial smooth muscle cells and cardiomyocytes, by de novo synthesis.[72–74] Hyperglycemia primarily activates the β and δ isoforms of PKC, but increases in activity of several other isoforms have also been found (Fig. 37.8). These PKC isoforms can also be activated by intracellular ROS in the absence of diacylglycerol or Ca^{2+}. The regulatory domain of these PKC isoforms contains two pairs of zinc fingers with six cysteine residues and two zinc atoms, which can be oxidized by intracellular ROS. Oxidation alters zinc-finger conformation and activates PKC.[75] Intracellular hyperglycemia activates PKC in retina and glomeruli of diabetic animals.[72,76] In contrast to other complication-prone tissues, total PKC activity is reduced in the peripheral nerve of diabetic animals. Schwann cell PKCα activity is reduced, while vascular wall PKCβII activity is increased.[77]

In early experimental diabetes, activation of PKCβ isoforms mediates retinal and renal blood flow abnormalities,[78] perhaps by depressing nitric oxide (NO) production and increasing endothelin-1 activity. In the diabetic retina, hyperglycemia persistently activates PKC and p38a mitogen-activated protein kinase (MAPK) to increase expression of a previously unknown target of PKC signaling, Src homology-2 domain-containing phosphatase-1 (SHP1), a protein tyrosine phosphatase. This signaling cascade leads to platelet-derived growth factor (PDGF) receptor-β dephosphorylation and a reduction in downstream signaling from this receptor, resulting in pericyte apoptosis.[79] Abnormal activation of PKC also has been implicated in the decreased glomerular production of NO induced by experimental diabetes[80] and in the decreased smooth muscle cell NO production induced by hyperglycemia.[81] PKC activation also mediates glucose-enhanced extracellular matrix accumulation in rat glomerular mesangial cells.[82] Hyperglycemia increases endothelin 1–stimulated MAPK activity in glomerular mesangial cells by activating PKC isoforms.[83] The increased endothelial cell permeability induced by high glucose concentrations in cultured cells is mediated by activation of PKCα.[84] Activation of PKC by elevated glucose levels also induces expression of the permeability-enhancing vascular endothelial growth factor (VEGF) in vascular smooth muscle cells.[85] In addition to affecting hyperglycemia-induced abnormalities of blood flow and permeability, activation of PKC contributes to increased matrix protein accumulation, inducing expression of transforming growth factor beta-1 (TGFβ1), fibronectin, and α1 type IV collagen in glomeruli of diabetic rats.[80] Many cellular abnormalities involved in diabetic cardiovascular disease have also been linked to PKC activation. These include endothelial dysfunction, increased vascular permeability, impaired angiogenesis, and increased apoptosis. Molecular mechanisms affected by diabetes-induced PKC



• **Fig. 37.9** Schematic representation of the hexosamine biosynthetic pathway (HBP). The glycolytic intermediate fructose 6-phosphate (Fruc-6-P) is converted to glucosamine 6-phosphate (GlcN-6-P) by the enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT). Increased donation of *N*-acetylglucosamine moieties from UDP-GlcNAc to serine and threonine residues of proteins by *O*-GlcNAc transferase (OGT) and their removal by *O*-GlcNAcase (OGA) affect proteins that regulate gene expression, translation, protein degradation, signal transduction, protein localization, epigenetics, and mitochondrial bioenergetics. *Glc,* Glucose, *GlcNAc, N*-acetylglucosamine; *OGT, O*-linked *N*-acetylglucosamine (GlcNAc) transferase; *UDP,* uridine diphosphate. (Modified from Slawson C, Copeland RJ, Hart GW. O-GlcNAc signaling: a metabolic link between diabetes and cancer? *Trends Biochem Sci.* 2010;35:547–555.)

activation include alterations in functionally significant enzymatic activities, such as mitogen-activated protein kinase, cytosolic phospholipase A2, and Na^+-K^+-ATPase, and alterations in several transcription factors.[86]

Hyperglycemia-induced activation of PKCβ promotes vascular inflammation and acceleration of atherosclerosis in diabetic ApoE null mice by augmenting expression of inflammatory mediators. In addition, it increases macrophage expression of cluster of differentiation 11c (CD11c), chemokines (C-C motif ligand 2), and interleukin-1β, via increased extracellular signal-regulated kinase 1 and 2 (ERK1 and ERK2), and c-Jun-N terminus kinase (JNK)–mitogen-activated kinase.[87] In this same diabetic model, PKCβ activation increased transcription of the proinflammatory cytokine IL18 and inhibited transcription of IL18-binding protein in the aorta. Diabetic mice showed increased plaque formation, cholesteryl ester content, and macrophage infiltration. Treatment with a PKCβ inhibitor prevented these.[88] PKCβ2 in endothelial cells from transgenic ApoE null mice overexpressing PKCβ decreased insulin- stimulated Akt/eNOS (endothelial nitric oxide synthase) activation and increased basal and angiotensin-induced expression of the vasoconstrictor endothelin-1. These dual effects increased endothelial dysfunction and accelerated atherosclerosis in this model compared with $ApoE^{-/-}$ mice.[89]

PKC activity has been linked to myocardial dysfunction, causing cardiomyopathy and cardiac failure. Selective overexpression of PKCβ2 in the myocardium of diabetic mice increased expression of connective tissue growth factor and transforming growth factor-β1, cardiomyopathy, and cardiac fibrosis.[90] More recently, activation of PKCα/β in diabetic hearts has been shown to mediate reactivation of fetal splicing programs by phosphorylation and upregulation of the RNA-binding proteins CUGBP elav-like family member 1 (CELF1) and RNA binding fox-1 homolog 2 (Rbfox2).[91] Chronic activation of PKC isozymes α, β, and δ promotes diastolic and systolic dysfunction, fibrosis, cardiomyocyte hypertrophy, and apoptosis[92] Another PKC isoform, PKCθ, plays crucial roles in the proliferation, differentiation, and activation of mature T cells via activation of several transcription factors in the nuclei of T cells, including nuclear factor of activated T cells (NFAT), c-Jun, c-Fos, and AP1. Diabetes-induced cardiac interstitial fibrosis, reduced contractility, reduced expression of the tight junction maintaining protein ZO1, and T-cell infiltration were all improved by treatment with an isoform-specific PKCθ inhibitor.[93]

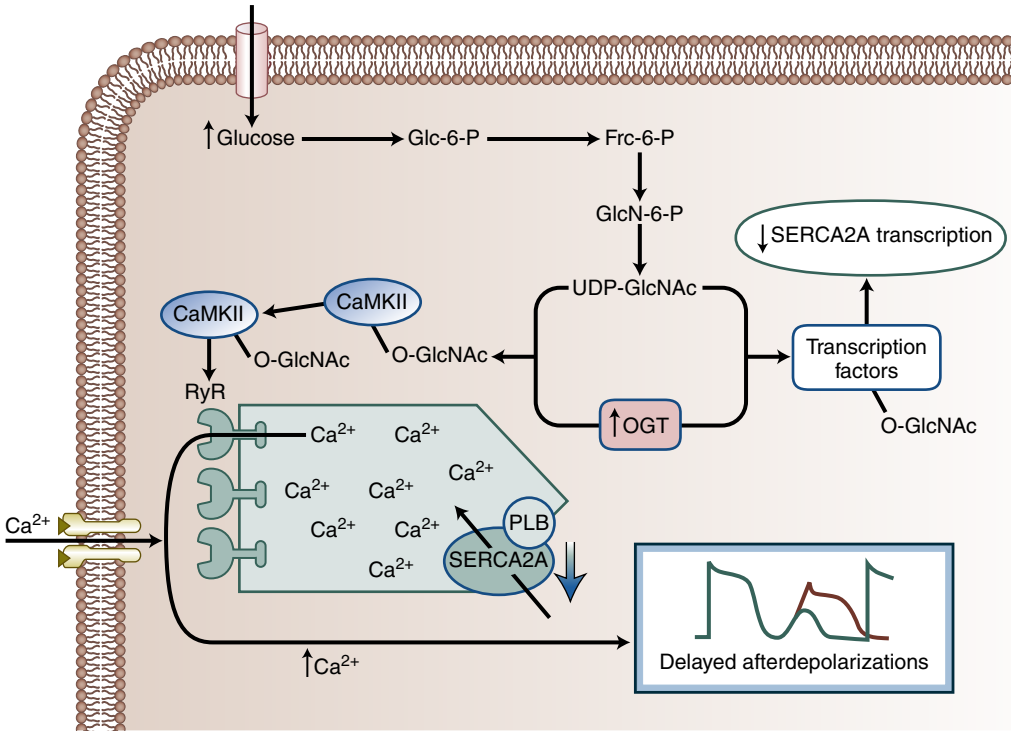
Increased Protein Modification by O-GlcNAc

The hexosamine pathway causes reversible post-translational modification of intracellular protein serine and threonine residues by *N*-acetylglucosamine. *O*-GlcNAc modifies proteins that regulate gene expression, translation, protein degradation, signal transduction, protein localization, epigenetics, and mitochondrial bioenergetics.[94] In cells damaged by hyperglycemia, excess intracellular glucose provides increased fructose-6-phosphate, which is converted to glucosamine-6-phosphate by the rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase. Glucosamine-6-phosphate is further converted to *N*-acetylglucosamine-6-phosphate (GlcNAc-6-P) and finally to UDP-*N*-acetylglucosamine (UDP-GlcNAc). The enzyme *O*-GlcNAc transferase (OGT) uses UDP-GlcNAc to transfer *N*-acetylglucosamine to a variety of proteins, resulting in increased protein modification by *N*-acetylglucosamine. Another enzyme, *N*-acetylglucosaminidase (*O*-GlcNAcase [OGA]), removes this protein modification (Fig. 37.9). Alternative splicing of the genes encoding the *O*-linked GlcNAc cycling enzymes OGT and OGA yields isoforms targeted to discrete sites in the nucleus,

cytoplasm, and mitochondria. *O*-GlcNAc serves as a nutrient/stress sensor regulating cellular homeostasis by altering signaling, transcription, metabolism, organelle biogenesis, cytoskeletal dynamics, and apoptosis.[95,96] Aberrant *O*-GlcNAc processing reduced mitochondrial protein expression and respiration, mitochondrial proteins involved in the respiratory chain and the tricarboxylic acid (TCA) cycle were decreased, and mitochondrial morphology was altered.[97]

In mouse coronary endothelial cells isolated from diabetic mice, *O*-GlcNAcase protein expression was significantly decreased compared with coronary endothelial cells from control mice. In contrast, OGT protein expression was markedly increased.[98] The resultant increased protein modification by *O*-GlcNAc was responsible for decreased endothelium-dependent relaxation of the coronary arteries and reduced capillary density in the left ventricle. Both of these defects were restored by overexpression of *O*-GlcNAcase. Decreased endothelium-dependent relaxation of the coronary arteries and reduced capillary density both reflect inhibition of eNOS, which is required for endothelium-dependent arterial relaxation and for mobilization of stem and progenitor cells from the bone marrow compartment.[99] In human arterial endothelial cells, activation of eNOS by phosphorylation at serine 1177 is inhibited directly by hyperglycemia-induced *O*-GlcNAcylation at this site.[100] eNOS activity is also affected by several other post-translational modifications, but the effect of diabetes on these has not yet been determined.[101] Carotid plaques from diabetic patients have a marked increase of *O*-GlcNAcylation in both cytoplasm and nuclear compartments of endothelial cells compared with nondiabetic subjects.[102] Increased *O*-GlcNAcylation may also contribute to diabetes-accelerated atherosclerosis by increasing ubiquitination and proteasomal degradation of the anti-inflammatory NFκB inhibitory protein A20 in coronary endothelial and smooth muscle cells.[103]

Chronically elevated *O*-GlcNAc levels also adversely affect myocardial function. Cardiac effects of increased *O*-GlcNAcylation include decreased mitochondrial function, decreased autophagic signaling, and decreased contractile function.[104] Ventricular contraction and relaxation are controlled mainly by release and uptake of Ca^{2+} by the SERCA2 pump. In hypertrophied and failing myocardium, SERCA2 protein level and its Ca^{2+} uptake function are depressed. Overexpression of OGT significantly reduced transcription of SERCA2, causing decreased calcium reuptake and impaired diastolic relaxation.[105] High glucose also increased *O*-GlcNAc modification of the calcium^{2+}/calmodulin-dependent protein kinase IIδ (CaMKIIδ), an enzyme critical for Ca^{2+} homeostasis and reuptake in cardiomyocytes. *O*-GlcNAc-modified CaMKII at serine 279 is increased in the heart of diabetic humans and rats,[106] causing autonomous activation of CaMKII. Thus CaMKII remains activated even after intracellular Ca^{2+} declines. This contributes to decreased cardiac contractility and potentially fatal arrhythmias, such as premature ventricular complexes and delayed afterdepolarizations (Fig. 37.10). Delayed afterdepolarizations are associated with the initiation of long QT-interval arrhythmias such as torsade de pointes. Overexpression of GlcNAcase or



• **Fig. 37.10** Hyperglycemia-induced myocardial protein modification by β-linked *N*-acetylglucosamine (*O*-GlcNAc) causes increased intracellular Ca^{2+} and delayed afterpolarizations. Increased *O*-GlcNAc modification of calcium/calmodulin-dependent protein kinase IIδ (CaMKII) causes autonomous CaMKII activation. CaMKII increases intracellular Ca^{2+} by phosphorylating ryanodine receptor 2 (RyR). *O*-linked *N*-acetylglucosamine (GlcNAc) transferase (OGT) also modifies transcription complex factors regulating expression of sarcoplasmic reticulum Ca^{2+}-ATPase *(SERCA2a),* reducing SERCA2A expression and contributing to increased intracellular Ca^{2+}. Increased *O*-GlcNAc modification of these proteins causes delayed afterdepolarizations in cardiomyocytes. *PLB,* Phospholamban. (Redrawn from Shah M, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circ Res.* 2016;118:1808–1829.)

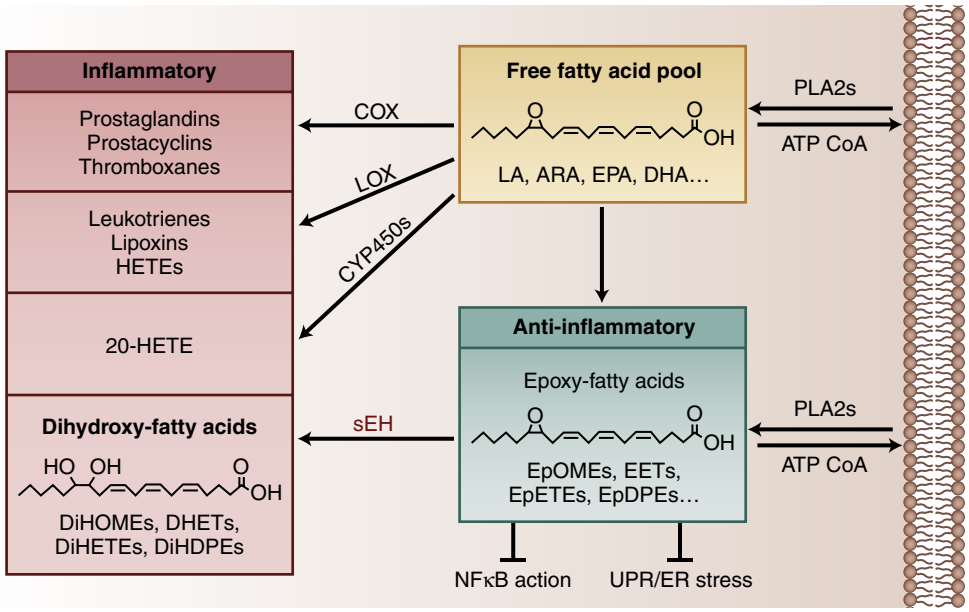
inhibition of GlcNAc modification increased expression of *SERCA2a*, ablated sarcoplasmic reticulum Ca^{2+} leak, improved cardiac contractility, and reduced arrhythmic events. Increased levels of ROS also cause autonomous activation of CaMKII by oxidation of adjacent methionine residues in its regulatory domain.[107] Activation of this mitochondrial ROS–oxidized CaMKII pathway increased mortality after myocardial infarction in diabetic mouse models.[108] Mitochondrial OGT is increased in diabetic cardiac mitochondria, whereas *O*-GlcNAcase (OGA) is reduced, causing increased *O*-GlcNAcylation of cardiac mitochondrial proteins. Inhibition of OGA and the resulting increased mitochondrial protein modification by *O*-GlcNAc increases oxygen consumption and reduces reserve capacity.[109] Reduced bioenergetic reserve capacity makes cells more sensitive to stress and cell death.

Increased Soluble Epoxide Hydrolase

Soluble epoxide hydrolase (sEH) is the dominant member of the epoxide hydrolase family in humans. It binds to specific epoxides such as epoxyeicosatrienoic acids (EETs) and rapidly converts them to less active or inactive dihydrodiols, the dihydroxy-eicosatrienoic acids (diHETrEs). EETs are signaling molecules formed from arachidonic acid by cytochrome P450 enzymes such as CYP2J2. In cell and animal models EETs have major anti-inflammatory activity.[110] They reduce activation of NFκB, causing transcriptional downregulation of the proinflammatory enzymes inducible nitric oxide synthase (iNOS), lipoxygenase 5 (LOX5), cyclooxygenase 2 (COX2), and of several pro-inflammatory cytokines (Fig. 37.11). EETs also prevent the signaling cascade activated by the three major unfolded protein response/ER stress pathway sensors: inositol requiring protein 1α (IRE1α), protein kinase RNA-like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6).

Expression of sEH was increased in diabetic mouse retinas and in retinas and vitreous humor of patients with diabetes.[111] As a consequence, levels of the sEH diol product 19,20-dihydroxydocosapentaenoic acid (19,20-DHDP) were increased. Nonproliferative diabetic retinopathy is characterized by pericyte loss and endothelial cell dysfunction. 19,20-DHDP prevented the association of presenilin 1 with N-cadherin and VE-cadherin in retinal vessels, compromising pericyte-endothelial cell interactions and interendothelial cell junctions.[111] Inhibition of sEH prevented the early nonproliferative stage of diabetic retinopathy in animal models. Similarly, sEH in glomerular podocytes is a significant contributor to hyperglycemia-induced renal injury. Podocyte sEH deficiency was associated with attenuated hyperglycemia-induced renal endoplasmic reticulum stress, inflammation and fibrosis, and enhanced autophagy. These effects were recapitulated in immortalized murine podocytes treated with a selective sEH pharmacologic inhibitor.[112] EETs and other epoxides also reduce pain caused by diabetic peripheral neuropathy.[113]

In the arterial wall, anti-inflammatory actions of EETs include attenuation of cytokine-induced endothelial activation and leukocyte adhesion, prevention of endothelium-dependent vascular remodeling, and improvement of vascular inflammation and endothelial dysfunction. EETs also inhibit platelet aggregation and promote fibrinolysis, and may inhibit vascular smooth muscle cell proliferation. In human studies, two single nucleotide polymorphisms in the sEH coding region of the *EPHX2* gene are associated with increased risk of developing coronary artery disease in different populations. Caucasians homozygous for the nonsynonymous K55R allele, with higher apparent soluble epoxide hydrolase activity in vivo, have a 3.5-fold increased risk of developing coronary heart disease.[114] Low levels of EET and high levels of soluble epoxide hydrolase are also associated with cardiac hypertrophy.



• **Fig. 37.11** Overview of the three proinflammatory branches of the arachidonic acid cascade generated by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450s). Opposing these are the anti-inflammatory epoxy fatty acids generated by P450 enzymes such as CYP2J2. These anti-inflammatory epoxides are reduced to their corresponding 1,2-diols by the enzyme soluble epoxide hydrolase. *ARA,* Arachidonic acid; *DHA,* docosahexaenoic acid; *EETs,* epoxyeicosatrienoic acids; *EPA,* eicosapentaenoic acid; *EpDPEs,* DHA epoxygenase metabolites; *EpETEs,* epoxyeicosatetraenoic acids; *EpOMEs,* cis epoxy fatty acids; *ER,* endoplasmic reticulum; *HETE,* hydroxyeicosatetraenoic acid; *LA,* linoleic acid; *NFκB,* nuclear factor kappa-light-chain-enhancer of activated B cells; *PLA2,* phospholipase A2; *sEH,* soluble epoxide hydrolase, *UPR,* unfolded protein response. (Modified from Morisseau C, Hammock BD. Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. *Annu Rev Pharmacol Toxicol.* 2013;53:37–58.)

Increasing EETs by inhibition of soluble epoxide hydrolase lowers fetal gene markers of cardiac hypertrophy, reduces heart size, and decreases cardiac fibrosis in animal models.[115–118] EETs appear to act in part by reducing inflammation in cardiomyocytes.

Upregulation of sEH decreases levels of the arachidonic acid-derived inflammation stop signal lipoxin A4, while inhibition of sEH increased lipoxin A4 by increasing its EET precursor.[119] Lipoxins are one of four classes of proresolving mediators of inflammation.[120,121] Lipoxin A4 is a polymorphonuclear cell (PMN) stop signal that limits further recruitment. It also stimulates macrophage efferocytosis, the phagocytosis of apoptotic PMNs and debris. A failure to resolve arterial inflammation, which normally involves the suppression of inflammatory cell influx, effective clearance of apoptotic cells, and promotion of inflammatory cell egress, promotes the progression of atherosclerotic lesions into unstable plaques, which can trigger atherothrombotic vascular events.[122,123]

Reduced Pyruvate Kinase M2 Activity

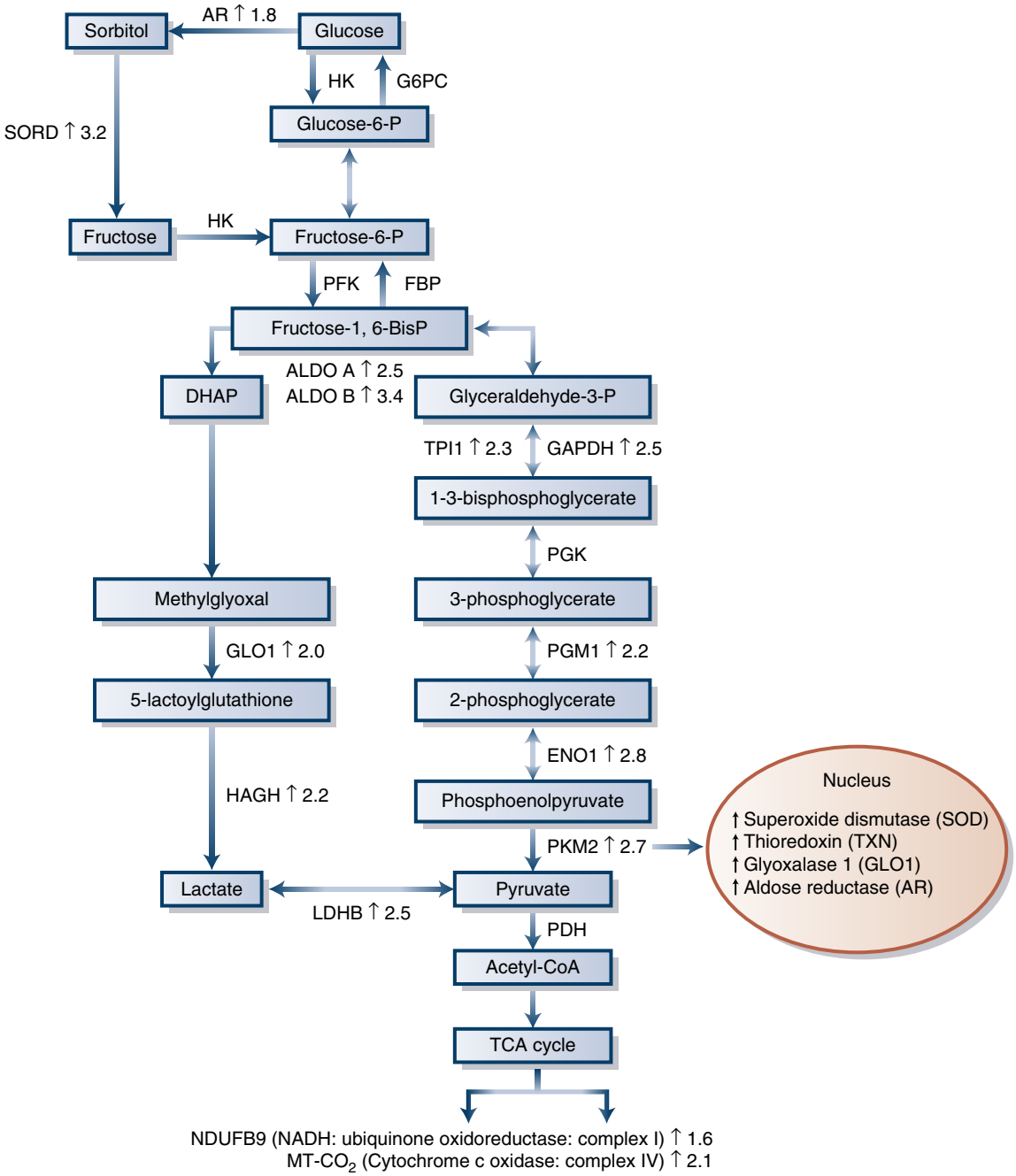
The less active isoform of the glycolytic enzyme, pyruvate kinase M2 (PKM2), is only active as a homotetramer. Its activity can be further inhibited by tyrosine kinases, acetylation, *O*-GlycNAcylation,[124] and oxidation of cysteine residue 358.[125] Although PKM2 is expressed in normal differentiated, nonproliferating cells, the increased expression of this less active isoform in most tumors has focused attention on the potential of PKM2 to divert glycolytic intermediates toward biosynthetic pathways in tumor cells. Independent of its enzymatic activity, PKM2 can also regulate aerobic glycolysis by acting as a dual-specificity protein kinase, increasing transcription of itself, the glucose transporter GLUT1, and lactate dehydrogenase A (LDLA).[126–128] In postmortem kidneys from people with T1DM for more than 50 years, an unbiased proteomic analysis identified expression of glomerular PKM2 as a critical determinant of susceptibility or resistance to diabetic nephropathy (Fig. 37.12).[129] Individuals with no or mild nephropathy showed significant upregulation of mitochondrial encoded cytochrome C oxidase II, a subunit of mitochondrial electron transport chain complex IV, the two methylglyoxal detoxifying enzymes glyoxalase 1 and AKRB1 (aldo/keto reductase family 1 member 1B), and the antioxidant proteins superoxide dismutase 1 (SOD1) and thioredoxin. Interestingly, PKM2 protein levels in the nephropathy susceptible group were identical to nondiabetics, while protein levels in the protected group were 40-fold higher than in nondiabetics. PK enzymatic activity in the protected group were not different from PK activity in nondiabetics, while PK activity in the nonprotected group was significantly lower. The median level of sulfenylated and oxidized PKM2 was fourfold higher in the nonprotected group, consistent with the observed relative differences in PKM2 protein level versus activity. Mitochondrial cytochrome C oxidase II protein levels in glomeruli from protected diabetic individuals were also nearly 30-fold higher than in both nonprotected individuals and nondiabetic controls, and protein levels of the methylglyoxal degrading enzymes glyoxalase 1 and AKR1B1 were 10-fold higher.

Different Hyperglycemia-Induced Pathogenic Mechanisms Reflect a Single Upstream Process: Mitochondrial Overproduction of ROS

A single upstream hyperglycemia–induced process—mitochondrial overproduction of superoxide—activates all the pathogenic mechanisms described in the previous sections.[130,131] Enhanced intracellular glucose transport and oxidation leads to mitochondrial overproduction of superoxide.[43,131] This can, in turn, activate other superoxide production pathways that may amplify the original damaging effect of hyperglycemia. Examples of amplification mechanisms include increased mitochondrial fission mediated by the *rho*-associated protein kinase 1 (ROCK1),[132,133] and ROS-mediated uncoupling of eNOS dimers to superoxide-producing eNOS monomers in endothelial cells.[134] Although NADPH oxidase 4 (Nox4) has been hypothesized to be a direct source of increased ROS in the diabetic kidney,[135,136] it appears to act indirectly by increasing mitochondrial ROS production. Nox4 is constitutively active and associates with mitochondria, inhibiting mitochondrial biogenesis and function.[137] The resultant decrease in maximal respiration suggests oxidative damage to enzymes of the tricarboxylic acid (TCA) cycle, the electron transport chain (ETC), or inner mitochondrial membrane lipids has occurred.[138,139] Knockout of Nox4 increases mitochondrial biogenesis and maximal respiratory capacity dramatically, which would prevent substrate-driven increased mitochondrial ROS production.[137] Mitochondrial ROS cause activation of other NOX isoforms.[140]

The initiating role of mitochondrial ROS is suggested by the observation that cells lacking mitochondrial electron transport chain function (ρ0 cells)[43] fail to increase ROS production in response to high glucose.

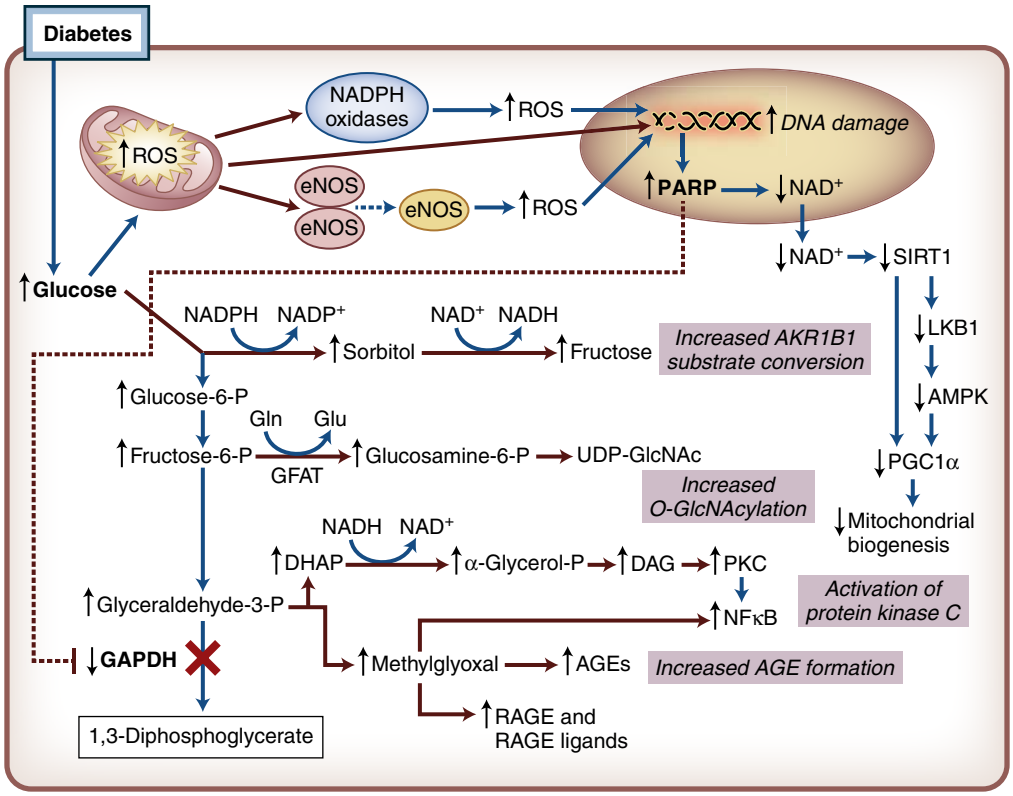
Dugan and associates[141] have suggested diabetic nephropathy is caused by reduced, rather than increased, mitochondrial ROS production, based on the observation that AMPK activity is decreased in the diabetic kidney. Protein levels of the master regulator of mitochondrial biogenesis and function, PGC1α, and mitochondrial density were also decreased. These authors proposed a model in which these observations reflect a feed-forward cycle initiated and maintained by decreased mitochondrial ROS. However, in human endothelial cells, silencing of AMPK causes increased, not decreased, ROS.[142] An alternate model of a feed-forward cycle involving decreased AMPK activity and decreased mitochondrial biogenesis caused by increased mitochondrial ROS is more consistent with these and other observations. In this model, increased mitochondrial superoxide causes the release of Fe^{++} from ferritin and iron sulfur cluster–containing proteins. Interaction of this released free iron in the nucleus with mitochondrial superoxide–derived hydrogen peroxide forms hydroxyl radicals, the only ROS species capable of cleaving bonds in macromolecules (Fig. 37.13).[143] This results in ROS-mediated DNA double-strand breaks in the nucleus. DNA double-strand breaks activate DNA repair mechanisms, including the enzyme poly-ADP-ribose polymerase 1 (PARP1). Activation of PARP1 inhibits the key glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) by poly-ADP-ribosylation, and depletes intracellular NAD^+ by degrading it to synthesize ADP-ribose. Inhibition of GAPDH activity causes upstream accumulation of early glycolytic intermediates, which are diverted into pathogenic signaling pathways.[43] Diversion of glucose increases polyol pathway flux, whereas diversion of fructose-6-phosphate increases hexosamine pathway activity. Diversion of glyceraldehyde-3-phosphate to α-glycerol phosphate activates PKC, and reduced activity of GAPDH diverts accumulated triose phosphates toward methylglyoxal formation. Methylglyoxal increases expression of the receptor for advance glycation end products (RAGE) and its activating ligand S100A8/9. Hyperglycemia-induced ROS reduce intracellular NAD^+ content by 50%.[144] Reduced content of NAD^+ inhibits the activity of the NAD^+-dependent protein deacetylase sirtuin 1 (SIRT1), which normally deacetylates and activates both the master regulator of mitochondrial biogenesis, PGC1α,



• **Fig. 37.12** Schema illustrating the significant alterations of glucose metabolism and glycolysis pathway proteins from individuals with T1DM 50 years or more protected versus nonprotected from diabetic nephropathy. *aldo A/B,* aldolase A and B; *AR,* aldose reductase; *DAG,* diglyceride; *DHAP,* dihydroxyacetone phosphate; *ENO1,* enolase 1; *FBP,* fructose-1,6-diphosphatase; *G6PC,* glucose-6-phosphatase, catalytic subunit; *GAPDH,* glyceraldehyde 3-phosphate dehydrogenase; *GLO1,* glyoxalase 1; *GPI,* glucose phosphate isomerase; *HAGH,* hydroxyacyl glutathione hydrolase; *HK,* hexokinase; *LDHB,* lactate dehydrogenase; *PDH,* pyruvate dehydrogenase; *PFK,* phosphofructokinase; *PGK,* phosphoglycerate kinase; *PGM1,* phosphoglucomutase-1; *PHGDH,* 3-phosphoglycerate dehydrogenase; *PKM,* pyruvate kinase isoenzyme type M2; *SORD,* sorbitol dehydrogenase; *TPI1,* triosephosphate isomerase 1. (Modified from Qi W, Keenan HA, Li Q, et al. Pyruvate kinase M2 activation may protect against the progression of diabetic glomerular pathology and mitochondrial dysfunction. *Nat Med.* 2017;23:753–765.)

and of liver kinase B1 (LKB1), the kinase that activates AMPK. Thus decreased SIRT1 activity would decrease activity of LKB1, PGC1α, and AMPK, as observed by Nishikawa and coworkers, but causing increased, not decreased, mitochondrial ROS production.[145]

SIRT1 also deacetylates and thereby inactivates the p65 subunit of NFκB, and skews macrophages to their reparative polarity.[146] Consistent with this model, diabetic *db/db* mice with a conditional deletion of SIRT1 in podocytes developed more proteinuria and kidney injury compared with *db/db* control mice,[147]

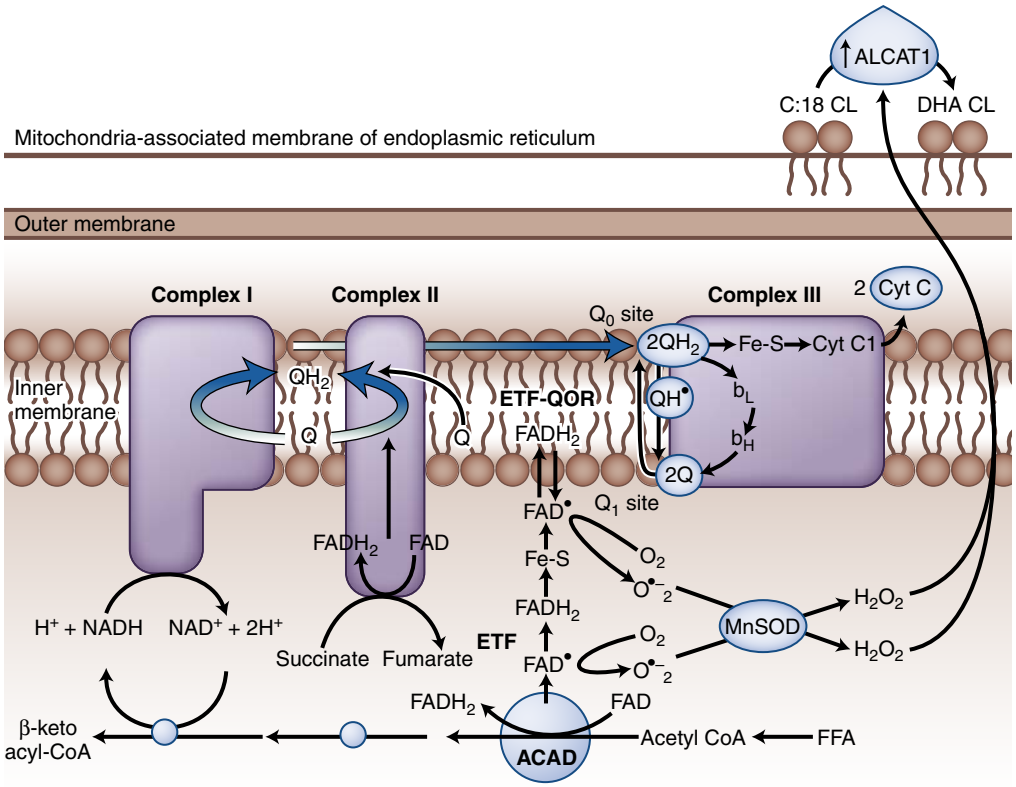


• **Fig. 37.13** Four hyperglycemia-induced pathogenic mechanisms are activated by overproduction of reactive oxygen species (ROS). Increased intracellular glucose flux causes mitochondrial overproduction of ROS, which can be further amplified by activating NADPH oxidases and uncoupling endothelial nitric oxide synthase (eNOS). Stable ROS species (hydrogen peroxide, H_2O_2) diffuse into the nucleus, where they cause DNA damage, which activates poly(ADPribose) polymerase (PARP). Poly-ADPribosylation of glyceraldehyde-3-dehydrogenase (GAPDH) by PARP reduces GAPDH activity, causing upstream accumulation of early glycolytic intermediates, which are diverted into four pathogenic signaling pathways. PARP activity depletes intracellular NAD^+ by degrading it to synthesize ADPribose. This reduces activity of the NAD^+-dependent deacetylase sirtuin 1 (SIRT1), which reduces activity of liver kinase B1 (LKB1), the enzyme that activates AMP-activated protein kinase (AMPK), which activates peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α) by phosphorylation, and of PGC1α itself (which requires deacetylation for activation). This results in decreased mitochondrial biogenesis. *AGEs,* Advanced glycation end products; *AKR1B1,* aldose reductase; *DAG,* diacylglycerol; *DHAP,* dihydroxyacetone phosphate; *eNOS,* endothelial nitric oxide synthase; *GAPDH,* glyceraldehyde-3-phosphate dehydrogenase; *GFAT,* glutamine fructose-6-phosphate amidotransferase; *Gln,* glucosamine; *Glu,* glucose; *NAD,* nicotinamide adenine dinucleotide; *NADPH,* nicotinamide adenine dinucleotide phosphate; *NFκB,* nuclear factor-κB; *PKC,* protein kinase C; *RAGE,* receptor for AGEs; *UDP-GlcNAc,* uridine diphosphate *N*-acetylglucosamine. (Modified from Shah M, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circ Res.* 2016;118:1808–1829.)

and PGC1α knockout diabetic mice developed a more severe neuropathy, with mitochondrial degeneration and an increase in oxidative modification of intracellular proteins.[148] Recently, real-time in vivo studies using a mitochondrial matrix localized GFP-based redox biosensor in *db*/*db* mice confirmed increased mitochondrial ROS production in diabetic nephropathy.[149]

Together, these diversions and pathway activations lead to cellular dysfunction, inflammation, apoptosis, and fibrosis in cells exposed to high intracellular glucose. The central importance of ROS in initiating each of these processes is illustrated by the fact that each can be prevented when hyperglycemia-mediated ROS generation is curtailed by transgenic expression of the enzymes superoxide dismutase or catalase.

Intracellular formation of ROS also upregulates soluble epoxide hydrolase mRNA and protein in mouse endothelial cells.[150] Essential mitochondrial genes are downregulated in glomeruli of the diabetic nephropathy–susceptible mouse strain DBA/2J, but not in the diabetic nephropathy–resistant strain C57BL/6J. Diabetic nephropathy–susceptible DBA/2J mice had increased tissue levels and urinary excretion of 8-oxo-deoxyguanosine, a major product of DNA oxidation. In humans with diabetic kidney disease, biopsies showed increased mitochondrial DNA damage, and increased urinary 8-oxo-deoxyguanosine.[151] Glomerular podocyte-specific knockout of PKM2 in diabetic nephropathy–susceptible mice had reduced expression of PGC1α, reduced mitochondrial mass, increased mitochondrial fission, and increased ROS. Increased ROS caused DNA strand breaks, increased activated PKCδ, and increased methylglyoxal levels. Activities of PARP and GAPDH were not reported. These changes were associated with accelerated podocyte apoptosis and glomerular pathology. Analysis of cellular respiration using Seahorse Extracellular Flux (XF) Analyzers showed that high glucose decreased mitochondrial maximal respiration by about 36%, suggesting that oxidative damage to enzymes of the tricarboxylic acid cycle, the electron transport chain, or the inner mitochondrial membrane lipids had occurred.[138,139] In cultured podocytes and in diabetic mice, activation of PKM2 with a small molecular weight



• **Fig. 37.14** Increased fatty acid oxidation, reactive oxygen species (ROS) formation, and cardiolipin (CL) remodeling. Insulin resistance–induced increased β oxidation of free fatty acids (FFAs) causes greater H_2O_2 production than does increased glucose oxidation because of increased electron leakage from the electron transfer flavoprotein (ETF) complex. These ROS activate acyl-CoA:lysocardiolipin acyltransferase 1 *(ALCAT1)* transcription. ALCAT1, located in the mitochondrial-associated membrane of the endoplasmic reticulum, causes pathologic remodeling of cardiolipin from tetra 18:2 cardiolipin to cardiolipin with highly unsaturated fatty acid side chains and cardiolipin deficiency because of oxidative damage. This reduces ETC (electron transport chain) electron flux, ATP synthesis, and further increases ROS. *ACAD,* acyl-CoA dehydrogenase. (Redrawn from Shah M, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circ Res.* 2016;118:1808–1829.)

activator increased mitochondrial biogenesis, normalized mitochondrial maximal respiration, prevented high glucose–induced elevated ROS, and restored levels of PKCδ and methylglyoxal to low glucose levels. These effects are consistent with increased shunting of glucose to lactate, thereby reducing nonproductive increased flux through the mitochondrial electron transport chain.

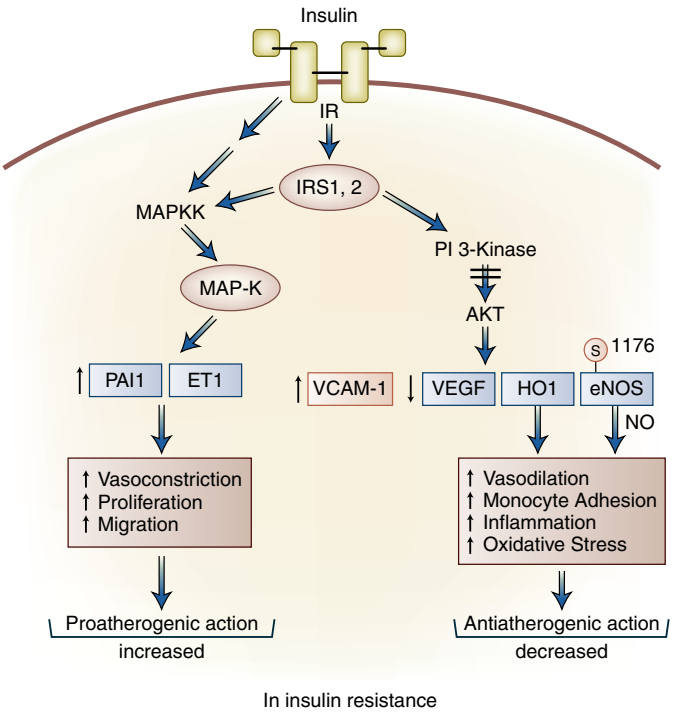
Insulin Resistance Increases Fatty Acid Oxidation, Causing Mitochondrial Overproduction of ROS

Insulin resistance occurs in most patients with T2DM. To isolate the effects of insulin resistance from those of hyperglycemia and diabetes, insulin resistance was evaluated in persons without diabetes or impaired glucose tolerance. Those in the highest quintile of insulin resistance had a 2.5-fold increase in cardiovascular disease risk compared with those in the lowest quintile, after correction for 11 known cardiovascular risk factors, including LDL, triglycerides, systolic blood pressure, and smoking.[17] This observation suggests that insulin resistance itself is a major cause of cardiovascular disease in T2DM. Adipocyte insulin resistance increases lipolysis and circulating free fatty acids (FFAs). Hydrolysis of triglyceride-rich lipoproteins by cell surface lipoprotein lipases floods the coronary arteries, heart, and liver with triglyceride-derived fatty acids. In the liver, this drives hepatic lipoprotein synthesis and secretion, and suppresses hepatic glucose production.[152–154] Patients with T1DM are also insulin resistant, with insulin sensitivity reduced by about 50%. T1DM patients have significant insulin resistance in adipose tissue, as well as in liver and skeletal muscle.[155,156] In T1DM patients, insulin resistance predicts the extent of coronary artery calcification and likely contributes to their increased risk of cardiovascular disease.[157] The role of insulin itself remains controversial. In mice fed a high-fat diet, genetically induced reduction of hyperinsulinemia decreased adipose tissue inflammation and increased insulin responsiveness.[153]

Insulin resistance/hyperinsulinemia increases mitochondrial fatty acid oxidation in arterial endothelial cells, macrophages, and cardiomyocytes, causing excessive ROS production. The major site of electron leakage from increased fatty acid oxidation is electron transfer flavoprotein, which receives electrons from the $FADH_2$ formed during the first oxidation step of β-oxidation[158] (Fig. 37.14). These fatty acid oxidation-derived ROS induce expression of the noncoding RNA *gadd7,* which amplifies oxidative stress and its induction of the endoplasmic reticulum stress response in a feed-forward loop.[159] In two insulin-resistant nondiabetic animal models, inhibition of either FFA release from adipocytes or FFA oxidation in arterial endothelium

prevented the increased production of ROS and its damaging effects.[160] In human arterial endothelial cells, this FFA-induced increase in ROS activates the same damaging pathways seen with high glucose. FFA-induced overproduction of superoxide also inactivates two important antiatherogenic enzymes: prostacyclin synthase and eNOS.[160]

In vitro studies suggest that at the level of the vessel wall, insulin has both antiatherogenic and proatherogenic effects (Fig. 37.15).[161] One major antiatherogenic effect is the stimulation of endothelial NO production. NO released from endothelial cells is a potent inhibitor of platelet aggregation and adhesion to the vascular wall. Endothelial NO also controls the expression of genes involved in atherogenesis. It decreases expression of monocyte chemoattractant protein 1 (MCP1) and of surface adhesion molecules such as CD11/CD18, P-selectin, vascular cell adhesion molecule 1 (VCAM1), and intercellular adhesion molecule 1 (ICAM1). Endothelial cell NO also reduces vascular permeability and decreases the rate of oxidation of LDL to its more proatherogenic form. Finally, endothelial cell NO inhibits proliferation of vascular smooth muscle cells (VSMCs).[162] However, in insulin resistance, overproduction of ROS leads to oxidation of tetrahydrobiopterin (BH_4), the essential cofactor of endothelial nitric oxide synthase. Decreased BH_4 uncouples oxygen reduction from NO synthesis, thereby converting eNOS to a superoxide-producing enzyme.[163] Although this important antiatherogenic effect of insulin is blocked by insulin resistance–induced ROS, two major proatherogenic effects of insulin are not. Insulin both potentiates platelet-derived growth factor (PDGF)–induced VSMC proliferation and stimulates endothelial and VSMC production of the thrombolysis-inhibitor plasminogen activator inhibitor 1 (PAI1).[164–166]



• **Fig. 37.15** Selective insulin resistance in vascular cells. Selective insulin resistance in vascular cells occurs when angiotensin II, elevated free fatty acids (FFAs) and glucose levels, and proinflammatory cytokines induced by diabetes and insulin resistance inhibit only the IRS/PI3K/Akt pathway. In contrast, insulin's stimulation of the SOS/Grb2/MAPK pathway is unaffected or even enhanced. The selective loss of insulin's actions via the IRS/PI3K/Akt pathway causes the reduction of insulin's antiatherosclerotic action and contributes to the acceleration of atherosclerosis and other cardiovascular pathologies in diabetes. *AKT,* protein kinase B; *eNOS,* endothelial nitric oxide synthase; *ET1,* endothelin-1; *HO1,* heme oxygenase-1; *ICAM,* intracellular adhesion molecule; *IR,* insulin receptor; *IRS1,2,* insulin receptor substrate 1,2; *MAP-K,* mitogen-activated protein kinase; *MAPKK,* MAPK kinase; *NO,* nitric oxide; *PAI,* plasminogen activator inhibitor; *PI,* phosphatidylinositol; *TNF,* tumor necrosis factor; *VCAM1,* vascular cell adhesion molecule-1; *VEGF,* vascular endothelial growth factor; *VSMC,* vascular smooth muscle cell. (Data from King G, Brownlee M. The cellular and molecular mechanisms of diabetic complications. *Endocrinol Metab Clin North Am*. 1996;25:255–270; King GL, Park K, Li Q. Selective insulin resistance and the development of cardiovascular diseases in diabetes: the 2015 Edwin Bierman Award Lecture. *Diabetes.* 2016;65:1462–1471.)

Macrophages are a central participant in atherogenesis. While most atherosclerotic lesions are stable, insulin resistance increases macrophage ROS, which drive chronic inflammation and accelerate their progression to unstable rupture-prone plaques. These plaques have a greater inflammatory infiltrate, a larger prothrombotic necrotic core, and increased production of matrix metaloproteinases that weaken the fibrous cap. Insulin resistance in macrophages induces mitochondrial ROS production by activating the CHOP branch of the stress unfolded protein response (UPR).[167,168] Plaque necrosis is caused by a combination of increased macrophage apoptosis and decreased phagocytic clearance of the apoptotic macrophages by a process called *efferocytosis,* which results in post–apoptotic necrosis of apoptotic cells and inflammation. Both of these processes are promoted by macrophage insulin resistance.[169] Apoptotic cells can inappropriately express the ROS-TNFα-NFκB-inducible "don't eat me" cell surface signal CD47,[170] and efferocyte receptors on phagocytes such as MER tyrosine kinase (MerTK) can be cleaved and inactivated by the mitochondrial ROS-activated protease ADAM17 (a disintegrin and metalloproteinase).[171] Blocking antibodies to CD47 prevent progression of established lesions, protect against plaque rupture, and induce regression of the necrotic core in several mouse models.[123,170,172,173]

Fatty acid oxidation appears to be the source of mitochondrial ROS production in macrophages, directly or indirectly via altered mitochondrial cardiolipin fatty acid composition.[174,175] When fatty acids are oxidized instead of glucose, the ratio of NADH to $FADH_2$ changes from 5:1 to 2:1. This causes an overreduction of the mitochondrial electron transport chain coenzyme Q (CoQ) pool, and an increase in reverse electron transport (RET) and ROS production by complex I. ROS from mitochondrial electron transport chain complex I cause a high percentage of irreversible overoxidation of cysteine thiols.[41,42] Reverse electron transport to complex I, driven by increased complex II oxidation of the TCA cycle intermediate succinate, drives the polarization of macrophages to a proinflammatory phenotype.[176]

In diabetic heart, increased myocardial ROS production occurs early, before accumulation of triglycerides and subsequent synthesis of C16:0 ceramides are evident.[177,178] This likely reflects increased fatty acid oxidation and resultant oxidative damage to mitochondrial cardiolipin,[179] the specific phospholipid of mitochondrial membranes. Cardiolipin is important for efficient electron flux, ATP synthesis, and reduced ROS formation. In addition, cardiolipin is involved in mitochondrial-mediated apoptosis, and it plays a critical role in regulating mitochondrial fission and fusion.[180–182] In nondiabetic heart, the major species of cardiolipin contains four linoleic acids (tetra 18:2 cardiolipin). This unique acyl composition is not derived from de novo synthesis of cardiolipin, but rather from a remodeling process that involves phospholipases and acyltransferase-transacylases. In diabetic myocardium from murine models with insulin resistance (*ob/ob, db/*

db, and high-fat diet) and in models of severe insulin deficient type 1 diabetes, the fatty acyl content of the more saturated 18:2 cardiolipin is dramatically reduced, whereas the content of longer chain, more unsaturated fatty acyl cardiolipin such as 20:4 is substantially increased.[183] Because of this increase in highly unsaturated side chains, diabetic heart cardiolipin is more vulnerable to oxidative damage. Cardiac overexpression of cardiolipin synthase increases tetra 18:2 cardiolipin in diabetic mice and prevents diabetes-induced changes in cardiolipin lipid remodeling. The cardiolipin deficiency and profound remodeling caused by diabetes and insulin resistance is caused by ROS-induced transcription of acyl-CoA:lysocardiolipin acyltransferase 1 *(ALCAT1)* (see Fig. 37.14). ALCAT1 catalyzes the transfer of linoleoyl-CoA onto monolysocardiolipin or dilysocardiolipin. Overexpression of ALCAT1 caused cardiolipin deficiency and fatty acid compositional changes similar to diabetes and obesity, with increased production of ROS, whereas ALCAT1 deficiency increased levels of tetra 18:2 cardiolipin in mouse heart and reduced ROS production.[184]

High levels of ROS are also a major proximal activator of the FOXO family of transcription factors. Increased ROS stimulate FOXO translocation from the cytosol into the nucleus by increasing FOXO GlcNAcylation, Jun-N-terminus kinase signaling, and CaMKII activation.[185,186] Cysteine oxidation also increases FOXO transcriptional output. In the hearts of diabetic mice and of mice with high-fat diet–induced insulin resistance, FOXO proteins were persistently activated.[187] This persistent activation was associated with downregulation of insulin receptor substrate 1 (IRS1), reduced activity of IRS1 and its downstream target Akt, and the development of cardiomyopathy. In cardiomyocyte-specific FOXO1 knockout mice fed a high-fat diet, neither insulin resistance nor cardiomyopathy occurred.

Diabetes Reduces Activity of Nuclear Erythroid-Related Factor 2, the Master Regulator of Antioxidant Gene Expression

The number of recognized ROS-regulating enzymes has increased substantially over the past 15 years.[40] Examples include superoxide dismutases, catalases, glutathione peroxidases, glutathione reductase, thioredoxins, thioredoxin reductases, methionine sulfoxide reductases, and peroxiredoxins. The activity of these enzymes is largely determined by ROS-induced changes in their transcription. Increased transcription of many of these antioxidant enzymes is mediated by the transcription factor nuclear erythroid–related factor 2 (Nrf2), a member of the cap 'n' collar subfamily of basic region leucine zipper transcription factors.[188] By regulating oxidant levels and oxidant signaling, Nrf2 participates in the control of the unfolded protein response, apoptosis, mitochondrial biogenesis, and stem cell regulation. Nrf2 also increases transcription of GLO1, the rate-limiting enzyme of the glyoxalase system, which prevents post-translational modification of proteins and histones by methylglyoxal, the major AGE precursor.[189,190] It also increases transcription of the rate-controlling enzyme in the nonoxidative branch of the pentose phosphate pathway, transketolase. Activation of transketolase inhibits three of the major hyperglycemia-driven pathways implicated in the pathogenesis of diabetic vascular damage (the diacylglycerol-PKC pathway, the methylglyoxal-AGE formation pathway, and the hexosamine pathway) and inhibits hyperglycemia-induced NFκB activation.[58] Preclinical studies using Nrf2 activators or Nrf2-deficient diabetic mice established that Nrf2 is a crucial endogenous modulator of ROS and protects from experimental diabetic nephropathy.[191–193] In the kidneys of *db/db* mice, treatment with the tetracycline antibiotic minocycline increased Nrf2 protein levels, reduced glomerular oxidative stress markers, and ameliorated diabetic nephropathy.[194]

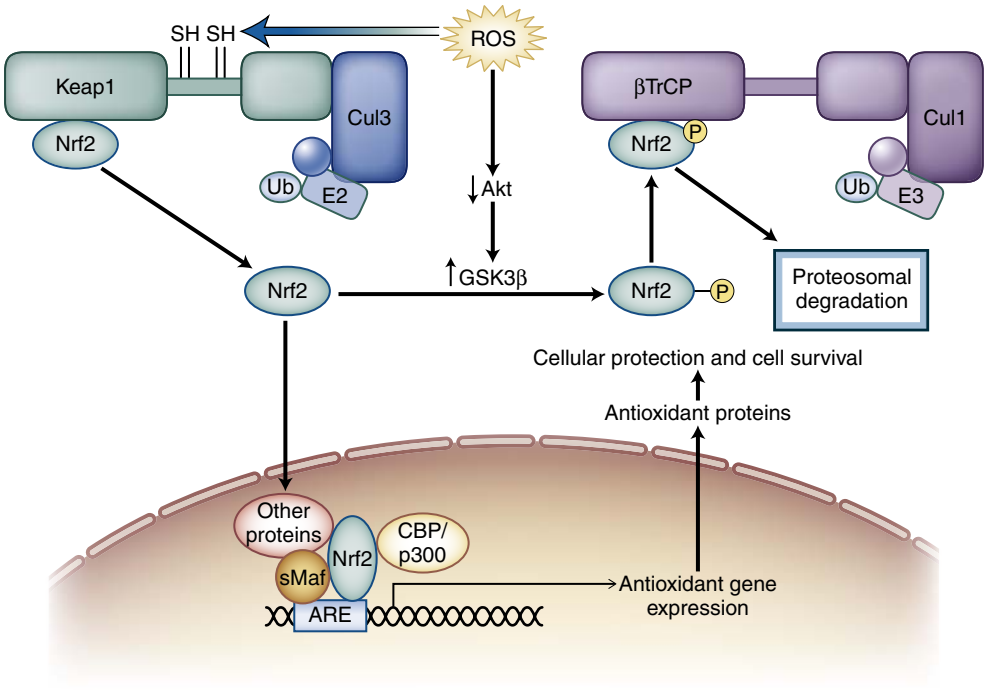
Nrf2 is expressed constitutively, and its intranuclear levels are controlled post-translationally. In the absence of inducers, Nrf2 associates with the redox-sensitive protein Kelch-like erythroid cell–derived protein with cap 'n' collar homology–associated protein 1 (Keap1), where it is rapidly polyubiquinated by Keap1-associated cullin-3–RING E2 ubiquitin ligase proteins and degraded by proteasomes. Nrf2 bound to Keap1 is released by ROS oxidation of critical cysteine thiols of Keap1, or by the reaction of these thiols with ROS-generated electrophiles such as glycolysis-derived methylgloxal and unsaturated fatty acid peroxidation-derived 4-hydroxynonenal. Phosphorylation of Nrf2 by protein kinases such as casein kinase 2 (CK2) may help target Nrf2 to the nucleus. After forming heterodimers with small Maf proteins, Nrf2 binds to the antioxidant response element (ARE) to induce transcription of its target genes. Export of Nrf2 from the nucleus is controlled by phosphorylation. Src family members such as Fyn phosphorylate Nrf2 at Tyr568, causing export from the nucleus and degradation.[195] Reduction of Nrf2 protein in the cytosolic compartment is mediated by β-transducin repeat–containing protein, a substrate adaptor for the S-phase kinase-associated protein 1–Cul1–F-box protein E3 ubiquitin ligase, which targets GSK3β-phosphorylated Nrf2 to the proteosome[196] (Fig. 37.16).

Modification of critical Keap1 cysteine thiols by sulforaphane, a dietary isothiocyanate found in cruciferous vegetables, also releases Nrf2.[199] In endothelial cells, sulforaphane prevented hyperglycemia-induced activation of the hexosamine and PKC pathways and prevented increased cellular accumulation and excretion of the major AGE precursor methylglyoxal.[197] In the aortae of diabetic mice, sulforaphane treatment restored aortic levels of Nrf2 and Nrf2-dependent antioxidant gene expression, preventing diabetes-induced increases in wall thickness, fibrosis, inflammation (tumor necrosis factor-α [TNFα] and vascular cell adhesion molecule-1 expression), apoptosis, and increased cell proliferation.[198] Diabetic cardiomyopathy was also prevented in mouse models by sulforaphane treatment.[199] Normalized Nrf2 activity also prevented diabetes-associated cardiac inflammation, fibrosis, lipid accumulation, and impaired autophagy.

In hearts from diabetic patients, Nrf2 protein is significantly reduced. In mice, cardiac Nrf2 protein was similarly reduced after 5 months of diabetes mellitus.[200] Hyperglycemia-induced mitochondrial overproduction of ROS has been shown to increase glycogen synthase kinase 3 beta (GSK3β) activity by inhibiting Akt1-dependent phosphorylation of GSK3β at serine 9.[143] Activated GSK3β targets cytoplasmic Nrf2 for increased proteasomal degradation mediated by the β transducin repeat–containing protein/S-phase kinase-associated protein 1–Cul1–F-box protein E3 ubiquitin ligase.[196]

Diabetes Activates the NLR Family Pyrin Domain Containing 3 Inflammasome

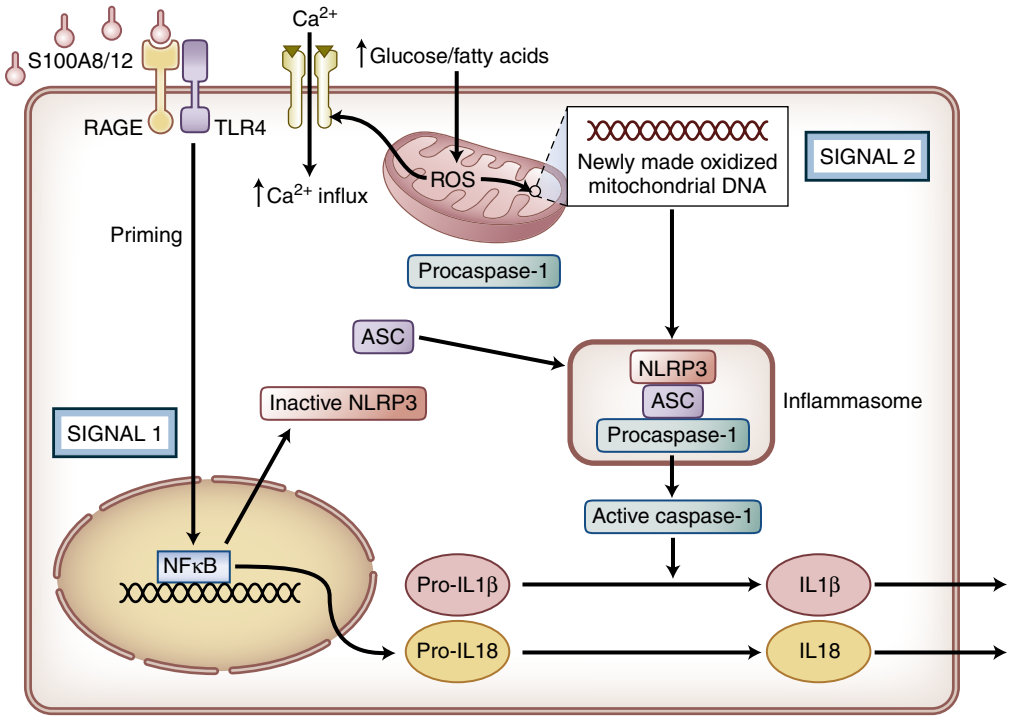
Dysregulated NLRP3 inflammasome activity underlies many chronic inflammatory states. The NLRP3 inflammasome is formed by oligomerization of inactive NLRP3, associated with apoptosis-associated speck–like protein (ASC), and procaspase-1. This complex, in turn, catalyzes the conversion of procaspase-1 to caspase-1, which contributes to the production and secretion of mature proinflammatory IL1β and IL18.[201] Activation requires two steps. The first, called *priming,* activates the transcription factor NFκB, which promotes transcription of NLRP3, pro-IL1β,



• **Fig. 37.16** Diabetes mellitus reduces nuclear erythroid–related factor 2 (Nrf2) protein in diabetic kidney and heart. Nrf2, the master regulator of antioxidant gene expression, associates with the redox-sensitive protein Kelch-like erythroid cell–derived protein with cap 'n' collar homology–associated protein 1 (Keap1), where it is rapidly polyubiquitinated by Keap1-associated cullin-3 (Cul3)–RING E2 ubiquitin ligase proteins and degraded by proteasomes. Reactive oxygen species (ROS) oxidation of critical cysteine thiols of Keap1 causes release of bound Nrf2 protein. Phosphorylation of Nrf2 by protein kinases such as CK2 help target Nrf2 to the nucleus. Nrf2 forms heterodimers with small Maf proteins, which bind to the antioxidant response element (ARE) in its target gene promoters. After export of Nrf2 from the nucleus, cytosolic Nrf2 is phosphorylated by GSK3β. This phosphorylated Nrf2 is recognized by β-transducin repeat–containing protein (βTrCP), a substrate adaptor for the S-phase kinase–associated protein-1–Cul1–F-box protein E3 ubiquitin ligase, which targets Nrf2 phosphorylated by GSK3β to the proteasome. *Akt,* protein kinase B; *CBP/p300,* CREB-binding protein and its homologue p300; *CK2,* casein kinase II; *E2*, ubiquitin-conjugating enzymes; *GSK3β,* glycogen synthase kinase-3β; *NRF2,* nuclear factor (erythroid-derived 2)–like 2; *sMaf,* small musculoaponeurotic fibrosarcoma proteins; *Ub,* ubiquitin. (Redrawn from Shah M, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circ Res.* 2016;118:1808–1829.)

and pro-IL18.[202] In mononuclear cells from diabetic patients and in vascular endothelial cells from diabetic rats, NFκB is chronically active.[203] This likely results from TLR4 or TLR4/RAGE heterodimer signaling stimulated by binding of ROS-induced S100A8/9 calgranulins. A second signal activates the NLRP3 inflammasome by facilitating the oligomerization of inactive NLRP3, apoptosis-associated speck–like protein, and procaspase-1. Although diverse molecular cues can trigger this step, several studies had suggested that these might all act through a mitochondrial pathway associated with high levels of mitochondrial ROS. Blocking ROS generation by mitochondria has been shown to abolish NLRP3 inflammasome activation, whereas artificial induction of mitochondrial ROS can spontaneously induce NLRP3-mediated IL1β secretion.[204,205] Mitochondrial ROS stimulate calcium influx via the TRPM2 channel (transient receptor potential cation channel subfamily M member 2), and macrophages deficient in TRPM2 have drastically impaired NLRP3 inflammasome activation and IL1β secretion.[206] Synthesis and oxidation of mitochondrial DNA also drive this activation step. TLR4 signaling increases levels of the human mitochondrial CMP kinase enzyme CMPK2, which increases nucleotide cytidine triphosphate, causing synthesis of mitochondrial DNA. Oxidized DNA fragments then exit the mitochondria, bind to the NLRP3 inflammasome, and activate it (Fig. 37.17).

Increased expression of the NLRP3 inflammasome components Nlrp3 and apoptosis-associated speck–like protein was found in monocytes from new, untreated patients with T2DM. Along with increased expression, there was increased inflammasome activation. Consistent with this, drug-naive T2DM patients had significantly higher serum levels of the proinflammatory cytokines IL1β and IL18 than did healthy individuals.[207] In kidneys from diabetic humans and in murine models of both T2DM and T1DM, inflammasome activation was detected in glomerular endothelial cells and podocytes.[208] Nlrp3 deficiency protected these mice from experimental diabetic nephropathy. Analysis of gene expression data in the Nephromine database revealed persistently elevated glomerular expression of inflammasome markers in patients with diabetic nephropathy and in murine models.[209] NLRP3 inflammasome activation also occurs in diabetic renal tubulointerstitial cells. Tubulointerstitial fibrosis is the final common pathway for loss of renal function in diabetic nephropathy, and tubuloepithelial to mesenchymal transdifferentiation is one



• **Fig. 37.17** NOD-like receptor family, pyrin domain-containing 3 (NRLP3) inflammasome activation in diabetic monocytes, kidney, coronary arteries, and heart. Hyperglycemia and increased fatty acids induce reactive oxygen species (ROS). These increase receptor for advanced glycation end products (RAGE) expression, which heterodimerizes with toll-like receptor 4 (TLR4). Signaling from this complex causes nuclear factor-κB (NFκB)–mediated transcription of inactive NRLP3, prointerleukin 1β (Pro-IL1β), and Pro-IL18. Increased intracellular Ca^{2+} and/or newly made oxidized mitochondrial DNA triggers oligomerization of inactive NLRP3, associated with apoptosis-associated speck–like protein (ASC), and procaspase-1. This activated inflammasome complex catalyzes the conversion of procaspase-1 to caspase-1, and of pro-IL1β and proIL-18 to mature IL1β and IL18. *S100A8/12,* Calgranulin A/B heterodimer ligand for RAGE. (Data from Shah M, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circ Res*. 2016;118:1808–1829; Murphy MP. Newly made mitochondrial DNA drives inflammation. *Nature.* 2018;560:176–177.)

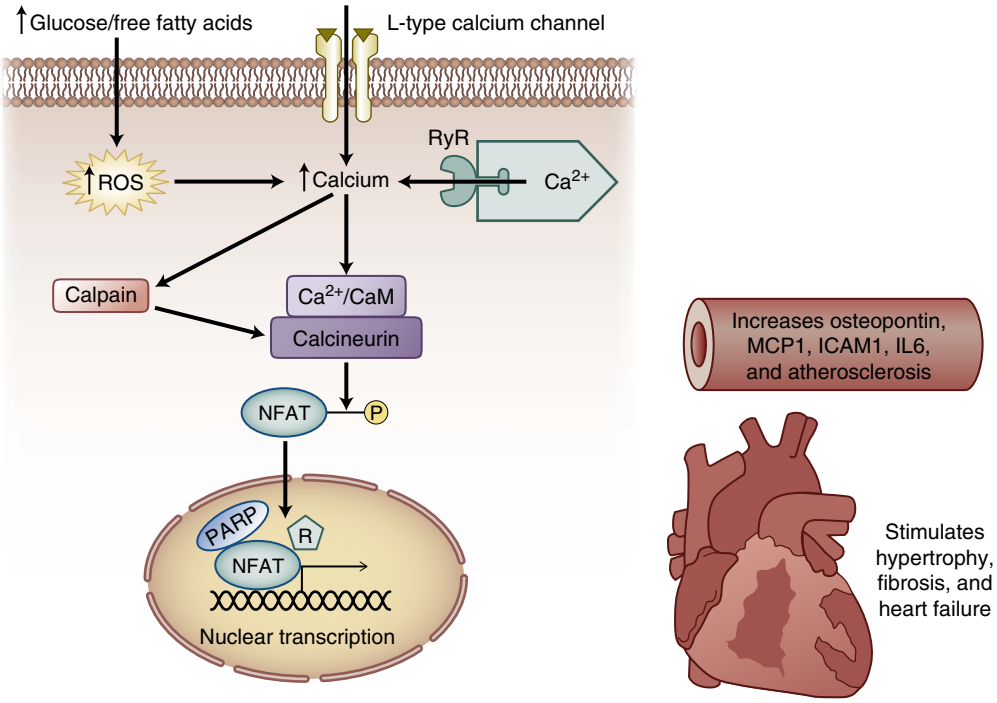
source of renal interstitial fibrogenic myofibroblasts. In diabetic kidney disease, the degree of tubulointerstitial fibrosis is probably a stronger predictor of GFR decline than glomerular changes.[210] A critical mediator of renal fibrosis is the ROS-activated redox-sensitive kinase apoptosis signal-regulating kinase 1 (ASK1), which activates p38 and JNK downstream signaling pathways. In renal biopsies from patients with diabetic kidney disease, ASK1 activation was associated with myofibroblasts, and in *db*/*db* eNOS$^{-/-}$ diabetic mice, inhibition of ASK1 arrested progressive GFR decline, glomerulosclerosis, and proteinuria.[211]

In a T2DM rat model, excessive activation of NLRP3 was associated with cardiac inflammation, cell death, disorganized ultrastructure, and fibrosis. NLRP3 gene silencing ameliorated cardiac inflammation, apoptosis, fibrosis, and left ventricular cardiac dysfunction.[212] In a well-established porcine model of diabetic atherosclerosis, which develops complex atherosclerotic plaques resembling those of humans, the cleavage processing of the sterol regulatory element-binding protein transcription factors (SREBP1 and SREBP2) and expression of their target genes, which increase fatty acid synthesis and cholesterol biosynthesis, were increased in endothelial cells and infiltrating macrophages of both fatty streaks and advanced lesions with fibrous caps, necrotic cores, and cholesterol cores. SIRT1 and AMPK activity were also reduced.[213] Increased SREBP1a in macrophages directly upregulates transcription of NLRP3,[214] and in porcine diabetic atherosclerosis, increased NLRP3 was found both in macrophages of advanced lesions and in endothelial cells and smooth muscle cells. The changes found in porcine diabetic atherosclerosis were also present in coronary atherosclerosis samples from diabetic humans.

Diabetes Activates the Transcription Factor NFAT

The transcription factor NFAT plays a role in the development of diabetic retinopathy, nephropathy, atherosclerosis, and cardiomyopathy. In resting cells, NFAT proteins are phosphorylated and are located in the cytoplasm. In diabetes, intracellular calcium is increased by increased ROS. Increased intracellular calcium then activates NFATc1-c4 by increasing NFAT dephosphorylation by the Ca^{2+}/calmodulin-dependent serine/threonine phosphatase calcineurin, which facilitates NFAT translocation into the nucleus. Once in the nucleus, NFAT interacts with coregulators to achieve optimal NFAT activation.[215] In the nucleus, ADP-ribosylation mediated by PARP1 acts as a molecular switch to positively regulate NFAT-dependent cytokine gene transcription[216] (Fig. 37.18). In diabetes, nuclear PARP1 is activated by intracellular ROS-induced DNA strand breaks (see Fig. 37.13).

In diabetic retinopathy, upregulation of proinflammatory cytokines such as TNFα and upregulation of retinal microvascular endothelial cell adhesion proteins are important components



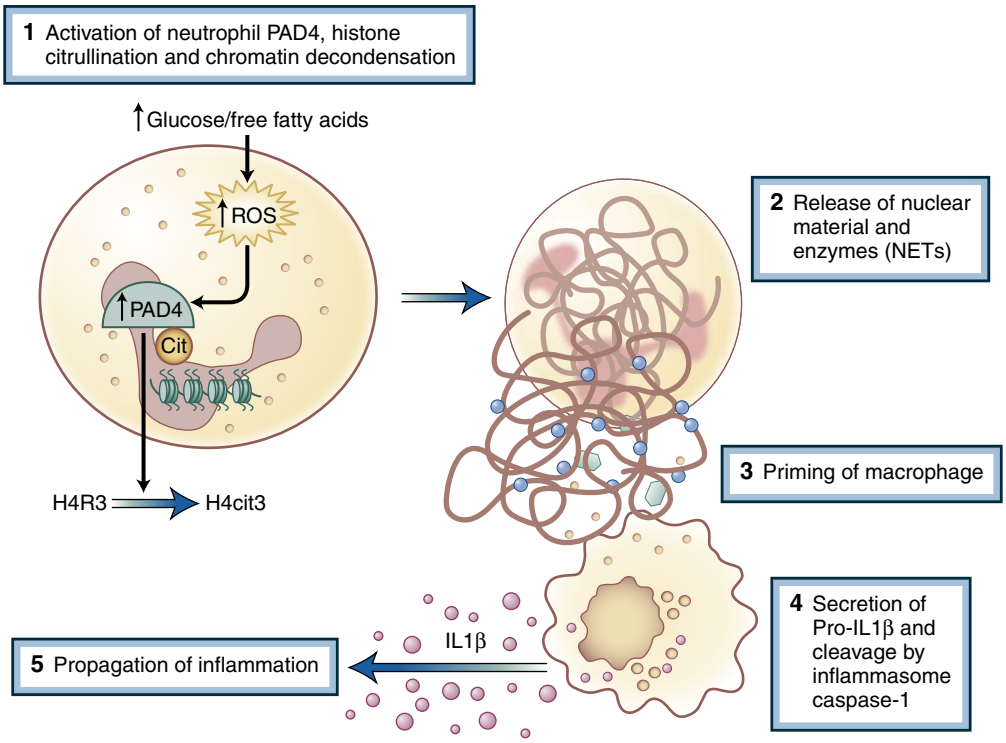
• **Fig. 37.18** Increased mitochondrial oxidation of glucose or fatty acids activates nuclear factor of activated T cells (NFAT)–mediated transcription of genes promoting diabetic retinopathy, nephropathy, atherosclerosis, and cardiomyopathy. Mitochondrial overproduction of reactive oxygen species (ROS) causes increased intracellular Ca^{2+}, which activates the calcium-activated neutral cysteine protease calpain. Calpain then activates the Ca^{2+}/calmodulin-dependent (CaM; Ca^{2+}) serine/threonine phosphatase calcineurin. Dephosphorylation facilitates nuclear translocation of the transcription factor NFAT. In the nucleus, NFAT interacts with poly-ADPribose polymerase (PARP), which increases NFAT transcriptional activity via NFAT poly-ADPribosylation. *ICAM1,* Intercellular adhesion molecule-1; *IL6,* interleukin-6; *MCP1,* monocyte chemoattractant protein-1; *RyR,* ryanodine receptor. (Modified from Shah M, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circ Res.* 2016;118:1808–1829.)

of chronic inflammation.[217] In human retinal microvascular endothelial cells, TNFα activates NFAT signaling. Activated NFAT specifically upregulated expression of the adhesion protein VCAM1, which increases adhesion of lymphocytes and macrophages to endothelium, the inflammatory cytokine CX3CL1 (C-X3-C motif chemokine ligand 1), which promotes leukocyte adhesion, the neutrophil chemotactic cytokine CXCL6, and the activated T-cell chemotactic cytokine CXC11.[218] In human renal glomerular podocytes exposed to sera from diabetic kidney disease patients, increased TNFα production caused NFATc1-mediated repression of the cholesterol efflux regulator ATP-binding cassette transporter A1 (ABCA1), and reduced activity of sterol-O-acyltransferase 1 (SOAT1), resulting in free cholesterol-mediated podocyte injury.[219]

In arteries of diabetic mice, activated NFATc3 induces arterial smooth muscle cell expression of the proinflammatory matrix protein osteopontin, a cytokine that promotes atherosclerosis and diabetic vascular disease. NFAT inhibition effectively reduced arterial wall osteopontin, IL6, MCP1, intercellular adhesion molecule-1, CD68, and tissue factor expression, and lowered plasma IL6 in diabetic mice.[220] In diabetic $ApoE^{-/-}$mice, inhibition of NFAT-signaling completely suppressed a 2.2-fold increase in atherosclerotic plaque area. Inhibition of NFAT also reduced lipid content in the plaque of diabetic mice independent of plasma glucose and lipid levels.[221] NFATc3 activated by increased mitochondrial ROS production also increases arterial vasoconstrictor reactivity to endothelin-1.[222] NFAT activation also appears to play a role in cardiac hypertrophy, fibrosis, and cardiomyocyte apoptosis.[223] In the diabetic heart, NFAT is activated by the calcium-activated neutral cysteine protease calpain, which in turn activates calcineurin. In cardiomyocytes, increased calpain activity activates NFAT-dependent cardiac hypertrophy and heart failure.[224] In two mouse models of diabetes, cardiac-specific deletion of calpain reduced myocardial hypertrophy and fibrosis, leading to improvement of myocardial function. Calpain activation correlated with increased activity of NFAT and NFκB, consistent with calpain's role in activation of calcineurin and degradation of the cytosolic NFκB inhibitor, NFκB inhibitor α.[225,226]

Diabetes Increases Neutrophil Extracellular Traps

In atherosclerotic plaques, neutrophils prime macrophages for proinflammatory responses.[227] This priming is mediated by extracellular webs of DNA bound to cytotoxic histones, which are released by activated neutrophils, called *neutrophil extracellular traps* (NETs).[228] This process follows a coordinated multistep process: histone citrullination, chromatin decondensation, migration of elastase and other granule enzymes into the nucleus, disintegration of the nuclear membrane, and release of DNA, histones, and granule proteins into the extracellular space.[229] The release of NETs primes macrophages to produce pro-IL1β, which is cleaved to mature proinflammatory IL1β by caspase-1. Caspase-1, in turn, is secreted by macrophages in response to activation of the



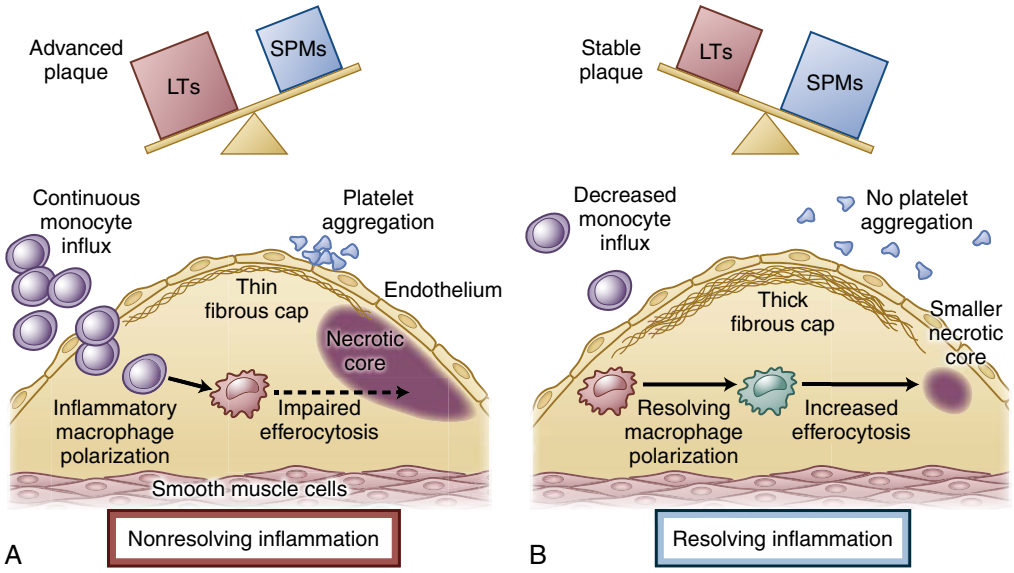
• **Fig. 37.19** Diabetes increases neutrophil extracellular traps (NETs), priming macrophages for inflammation. Increased reactive oxygen species (ROS) increase transcription and activation of peptidylarginine deiminase 4 (PAD4), the enzyme that initiates formation and release of NETs by citrullination (Cit) of histones. Released NETs prime macrophages to produce pro-interleukin 1β (Pro-IL1β), which is cleaved to mature proinflammatory IL1β by caspase-1 secreted by macrophages in response to NOD-like receptor family, pyrin domain-containing 3 inflammasome activation. *H4cit3,* Histone 4 with arginine residue 3 converted to citrulline; *H4R3,* histone 4 arginine 3; ROS, reactive oxygen species. (Modified from Shah M, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circ Res.* 2016;118:1808–1829.)

NLRP3 inflammasome.[227] Both ROS and increased intracellular Ca^{2+} activate NET formation (Fig. 37.19).[230] Atherosclerosis-prone $Apoe^{-/-}$ mice with deletions of two serine proteases that localize to NETs developed dramatically smaller atherosclerotic lesions compared with $Apoe^{-/-}$ control animals, despite similar lipid concentrations and leukocyte counts in blood. These triple mutant mice had no NETs, lower systemic IL1β concentration, and fewer lesional IL17-producing T cells.[231] Quenching mitochondrial ROS in a mouse model of atherosclerosis (aged myeloid cell mCAT→LDL receptor null [$Ldlr^{-/-}$] chimeric mice) decreased lesional NETs compared with age-matched $Ldlr^{-/-}$ mice, and also decreased total macrophage content in atherosclerotic lesions.[232] Oxidized LDL (oxLDL) and 7-keto-cholesterol, the most abundant oxysterol in human oxLDL, induced mitochondrial ROS production and highly proinflammatory oxidized mitochondrial DNA-bound NETs.[233] Neutrophils from both type 1 and type 2 diabetic humans have elevated expression of peptidyl arginine deiminase 4, the enzyme critical for histone citrullination-mediated chromatin decondensation and NET formation.[234]

Nonresolving Inflammation in Metabolic Syndrome, Diabetes, and Atherosclerosis

Chronic low-grade inflammation is associated with metabolic syndrome, diabetes, and atherosclerosis.[235–240] In normal physiology, inflammation has two temporal phases: an initiation phase of acute inflammation and a resolution phase. The initiation phase involves recruitment of neutrophils followed by monocytes, which differentiate into proinflammatory macrophages. These cells engulf the inflammatory stimulus and remove damaged tissue debris. Resolution of inflammation is an active process involving a switch from synthesis of arachidonic acid–derived proinflammatory prostaglandins and leukotrienes to synthesis of at least four families of specialized proresolving mediators (SPMs). These SPM families are arachidonic acid–derived lipoxins, and omega-3 polyunsaturated fatty acid–derived resolvins, protectins, and maresins.[121] SPMs decrease further recruitment of PMNs, stimulate macrophage uptake and clearance of apoptotic cells (efferocytosis), shift macrophage phenotype from proinflammatory to proresolution, and initiate tissue repair processes. Obesity and diabetes delay PMN apoptosis and impair macrophage efferocytosis. In human arteries, high levels of resolvin D1 and low levels of the proinflammatory leukotriene B4 were associated with plaques having less necrosis and thicker fibrous caps. In contrast, high levels of leukotriene B4 and low levels of resolvin D1 were associated with plaques having large necrotic cores and thin fibrous caps.[241]

Current evidence suggests diabetes causes defective SPM biosynthesis downstream of their fatty acid precursors.[238,239] The resultant nonresolving inflammation causes continuous leukocyte influx into atherosclerotic lesions, inflammatory macrophage polarization, and defective efferocytosis. These drive the progression of atherosclerosis to the development of clinically dangerous lesions with large necrotic, procoagulant cores and thin fibrous caps. SPMs limit plaque progression by suppressing inflammation, enhancing efferocytosis, and promoting an increase in thickness



• **Fig. 37.20** Nonresolving inflammation in atherosclerosis. Reduced specialized proresolving mediators *(SPMs)* and increased leukotrienes (LTs) promotes instability of atherosclerotic plaques. When the SPM:LT ratio is low (A), resolution of inflammation is impaired, leading to sustained inflammatory monocyte influx, platelet aggregation, proinflammatory macrophage polarization, impaired efferocytosis, large necrotic cores, and thin fibrous caps. Conversely, when the SPM:LT ratio is high (B), resolution of inflammation is characterized by decreased monocyte influx, proresolving macrophage polarization, enhanced efferocytosis, decreased necrotic core formation, and thicker fibrous caps. (Redrawn from Kasikara C, Doran A, Cai B, et al. The role of non-resolving inflammation in atherosclerosis. *J Clin Invest.* 2018;128:2713–2723.)

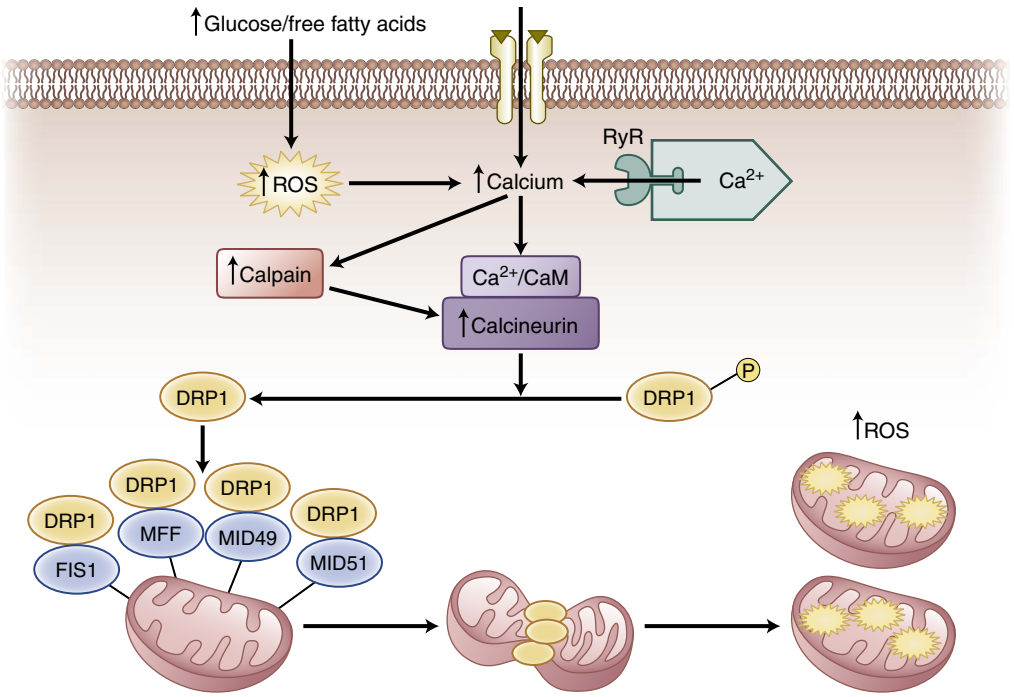
of the fibrous cap (Fig. 37.20). Enhanced efferocytosis promotes macrophage production of several SPMs, including lipoxin A4, resolvin E1, and protectin D1. Lipoxins also recruit monocytes as macrophages further enhance efferocytosis in the resolving lesions. Efferocytosis requires specific phagocytotic receptors, which bind to apoptotic cells. Deletion of one of these receptors, Mer tyrosine kinase (MerTK), caused impaired efferocytosis, accumulation of apoptotic cells, and large necrotic cores in murine atherosclerotic lesions. It also caused impaired SPM production by macrophages, because MerTK signaling decreases proinflammatory leukotriene synthesis and increases synthesis of proresolving lipoxin A4.[239]

MerTK can be inactivated by the metalloproteinase ADAM17, which, when activated by macrophage overproduction of mitochondrial ROS, cleaves the receptor.[171] High levels of soluble MerTK are found in advanced human atherosclerotic lesions, suggesting that this is one mechanism impairing efferocytosis. Increased levels of ROS cause autonomous activation of CaMKII by oxidation of adjacent methionine residues in its regulatory domain.[107] In arteries, activated CaMKII suppresses efferocytosis in macrophages and promotes atherosclerotic plaque necrosis.[242]

Diabetes Alters Mitochondrial Dynamics

Mitochondria dynamics are the continuous processes of mitochondrial fusion, fission, biogenesis, and mitophagy, which together maintain optimal cellular bioenergetics and ROS homeostasis.[243] In normal physiology, fission helps facilitate mitophagy, which degrades and recycles damaged mitochondria and mitochondrial fragments, although fission also increases ROS production due to incomplete transfer of electrons through the electron transport chain. Mitochondrial fission is a critical process that enables macrophages to clear multiple apoptotic cells. When fission is disabled, phagosome formation around secondarily encountered apoptotic cells is impaired.[244] The primary regulators of outer mitochondrial fusion are dynamin-related guanosine triphosphate hydrolases (GTPases), termed mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2), while inner mitochondrial fusion and cristae stabilization involve optic atrophy protein 1 (Opa1). The primary regulator of mammalian mitochondrial fission is the GTPase dynamin-related protein 1 (Drp1), which is recruited from the cytosol to the mitochondrial outer membrane where it binds to four Drp1 receptors: mitochondrial fission factor (MFF), mitochondrial dynamics protein of 49 kDa (miD49) and 51 kDa (miD51), and mitochondrial fission 1 protein (FIS1).[245] Oligomerization of Drp1 is thought to provide the mechanical force to constrict mitochondrial membranes and to fragment the organelle. Mitochondrial fission occurs at mitochondria-endoplasmic reticulum contact sites, with involvement of the mitochondrial phospholipid cardiolipin and calcium transfer.[246]

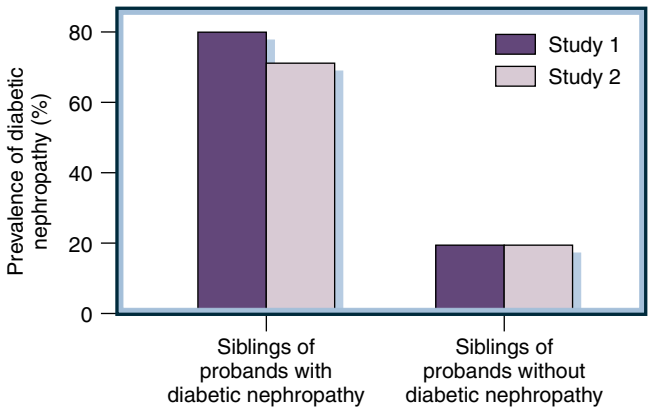
Increased mitochondrial fission caused by increased mitochondrial recruitment of Drp1 is associated with key features of diabetic nephropathy in diabetic mouse models[133] (Fig. 37.21). In diabetic mice lacking Drp1, decreased fission protected against progression of diabetic nephropathy.[247] Increased mitochondrial fission also occurs in mitochondria from mouse coronary endothelial cells, where the level of the fusion protein Opa1 is decreased and the level of Drp1 is increased.[248] This response may represent an important proresolving response in atherosclerotic lesions, since mice lacking myeloid Drp1 have defective efferocytosis.[235,244] Persistent increased fission is also associated with myocardial dysfunction in humans with type 2 diabetes, due in part to decreased expression of the fusion protein mitofusin 1.[249] Drp1 recruitment to mitochondria is regulated by its phosphorylation at serine 616 by either CaMKII or ROCK1. In the diabetic heart and kidney, ROS can activate both of these kinases.[108,133]



• **Fig. 37.21** Diabetes induces increased mitochondrial fission in kidney, coronary artery, and myocardium. Increased reactive oxygen species (ROS) from either excess glucose or fatty acids increases intracellular Ca^{2+}. Increased Ca^{2+} activates the calcium-activated neutral cysteine protease calpain, which activates the Ca^{2+}/calmodulin-dependent (CaM; Ca^{2+}) serine/threonine phosphatase calcineurin. Calcineurin dephosphorylates the GTPase dynamin–related protein 1 (DRP1), which is then recruited from the cytosol to the mitochondrial outer membrane where it binds to four DRP1 receptors—mitochondrial fission factor (MFF), mitochondrial dynamics protein of 49 kDa (MID49), and 51 kDa (MID51), and mitochondrial fission 1 protein (FIS1). Drp1 oligomerization provides the mechanical force to constrict mitochondrial membranes and fragment the organelle (mitochondrial fission). Increased fission causes further increases in ROS production and mitochondrial dysfunction. (Modified from Shah M, Brownlee M. Molecular and cellular mechanisms of cardiovascular disorders in diabetes. *Circ Res.* 2016;118:1808–1829.)

Genetic Determinants of Susceptibility to Microvascular Complications

Clinicians have long observed that different patients with similar duration and degree of hyperglycemia differ markedly in their susceptibility to microvascular complications. Such observations suggested that genetic differences exist that affect the pathways by which hyperglycemia damages microvascular cells. The leveling of risk of overt proteinuria after 30 years' duration of T1DM at 27% is evidence that only a subset of patients are susceptible to development of diabetic nephropathy.[250] A role for genetic determinants of susceptibility to diabetic nephropathy is more directly supported by familial clustering, with an estimated heritability of at least 40%. In two studies of families in which two or more siblings had T1DM, the risk of nephropathy in a diabetic sibling was 83% or 72% if the proband diabetic sibling had advanced diabetic nephropathy, but only 17% or 22% if the index patient did not have diabetic nephropathy (Fig. 37.22).[251,252] Familial clustering for the risk of severe diabetic retinopathy was also reported by the DCCT. Likewise, familial aggregation of coronary artery calcification occurs in families with T2DM.[253] Numerous associations have been made between various candidate gene polymorphisms and the risk of diabetic complications. However, in a meta-analysis of three large cohorts from the Genetics of Nephropathy—an International Effort (GENIE), no associations remained significant after correction for multiple testing or application of stringent significance thresholds.[254]



• **Fig. 37.22** Familial clustering of diabetic nephropathy. Prevalence of diabetic nephropathy in two studies of diabetic siblings of probands with or without diabetic nephropathy. (Adapted from Seaquist ER, Goetz FC, Rich S, et al. Familial clustering of diabetic kidney disease: evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med.* 1989;320:1161–1165; Quinn M, Angelico MC, Warram JH, et al. Familial factors determine the development of diabetic nephropathy in patients with IDDM. *Diabetologia*. 1996;39:940–945.)

While genome-wide association studies (GWAS) performed in large, well-characterized study populations have identified a few susceptibility loci for diabetic retinopathy, nephropathy, and cardiovascular disease,[255] the cumulative effects of disease-associated

single nucleotide polymorphisms (SNPs) have failed to account for the majority of complex-trait heritability for a large number of diseases, including diabetes and its complications (the "missing heritability" problem). Ultimately, the central unanswered question is: How is coordinated gene expression regulatory information conveyed—the information that determines when, where, at what level, in what combinations, and for how long, genes are expressed? With protein coding sequences representing approximately 2% of the genome, research in the post-GWAS era is currently focused on noncoding RNAs, genetic-epigenetic interactions, and long-range chromatin 3D looping architecture.[256,257]

Noncoding RNAs and Diabetic Complications

Although piwi-interacting RNAs (piRNAs), endogenous small interfering RNAs (siRNAs), intron-derived microRNAs (miRNAs), and a host of long noncoding RNAs all have regulatory roles, the best understood noncoding RNAs with respect to diabetic complications are the miRNAs, which regulate several key biologic pathways and cellular functions involved in diabetic complications. Most individual miRNAs target hundreds of specific mRNAs,[258] thereby coordinately regulating complex gene networks. In the retina, diabetes increased levels of several microRNA mediators of inflammation (miR146, miR155, and miR132),[259] while decreasing levels of the anti-inflammation mediator miR146a. Levels of miR146a are also reduced in sciatic nerve of *db/db* mice. Treatment with a miR146a mimetic reduced macrophage activation and improved nerve conduction velocity, intraepidermal nerve fiber density, and axonal degeneration. miR146a was also decreased in kidneys and hearts of type 1 and type 2 diabetic animals.[260] In diabetic mouse kidney cortex, levels of the profibrotic miR192 are also increased, and miR192 knockdown prevented diabetes-induced proteinuria and renal fibrosis.[261] Diabetes also increased miR29c in the kidney, which induces cell apoptosis and increases extracellular matrix protein accumulation. Knockdown of miR29c prevented high glucose–induced cell apoptosis and significantly reduced albuminuria and kidney mesangial matrix accumulation in *db/db* mice.[262]

miR33 regulates cellular functions associated with cardiovascular disease. It increases macrophage activation, inhibits mitochondrial biogenesis, and represses autophagy.[263] In mice placed on an atherogenic diet for 16 weeks and then made diabetic by streptozotocin injection, anti-miR33 treatment decreased atherosclerotic plaque macrophage content and inflammatory gene expression. The decreased macrophage content in anti-miR33–treated diabetic mice was associated with a blunting of hyperglycemia-induced monocytosis and reduced monocyte recruitment to the plaque.[264] Diabetes increases circulating numbers of neutrophils and monocytes through an ROS-mediated mechanism and impairs regression of early atherosclerosis by increasing macrophage entry into the lesion in mouse models.[265,266]

MicroRNAs may also play a role in the pathogenesis of diabetic cardiomyopathy. miR499, miR133a, and miR373 are downregulated in diabetic cardiomyocytes.[267] Downregulation of miR133a in a normal adult genetic background was sufficient to induce cardiac hypertrophy, and its downregulation is a prerequisite for the development of apoptosis, fibrosis, and prolongation of the QT interval in animal models.[268] The protective capacity of cardiac progenitor cells after myocardial infarction is also enhanced by miR133a.[269]

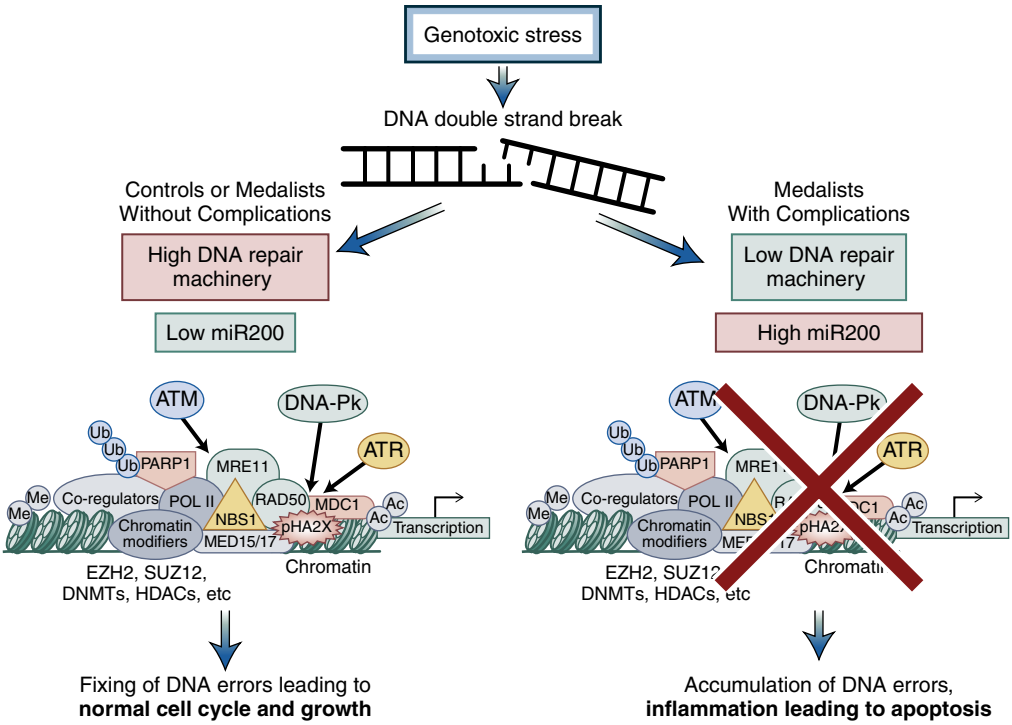
In patients with over 50 years of T1DM, expression levels of miR200 differentiated those with no complications from those with complications.[44] Induced pluripotent stem cells (iPSCs) from fibroblasts of patients with no complications had low miR200 levels, while iPSCs from fibroblasts of patients with complications (retinopathy, nephropathy, neuropathy, and cardiovascular disease) had high miR200 levels. miR200 was found to inhibit DNA damage checkpoint protein expression, causing accumulation of DNA double-strand breaks, inflammation, and apoptosis (Fig. 37.23). Knockdown of miR200 in fibroblasts from patients with complications rescued checkpoint protein expression and reduced DNA damage. In differentiated neurons derived from all clinical backgrounds, exogenous overexpression of miR200 produced the DNA damage mark γH2AX phosphorylation (pH2AX). These data are consistent with the model of ROS-mediated DNA damage activating multiple hyperglycemia/IR-induced pathogenic mechanisms discussed in previous sections.

Molecular Basis for Metabolic Memory

Stable cellular phenotypes are maintained by a multitude of interconnected multilevel positive and negative feedback loops,[270,271] interacting with combinatorial extracellular signals from adjacent cells, from receptor ligands secreted by cells in other tissues, and from changes in metabolite flux and concentration. Familiar examples of stable alterations in cellular phenotype are the induction of pluripotency in terminally differentiated cells, and the differentiation of these iPS cells into a variety of cell types.[272] In preclinical models of T1DM and T2DM, diabetes irreversibly depletes two bone marrow–derived mesenchymal progenitor subpopulations having provasculogenic expression profiles, and this deficit in vascular progenitor cells is not correctable by restoring glucose homeostasis in vitro or in vivo.[273] These data suggest that diabetes causes permanent modifications in subpopulations of mesenchymal progenitor cells that are passed to daughter cells with each division.

Similarly, when fibroblasts from patients with T1DM for more than 50 years with complications were used to make iPS cells, the miR200 family was upregulated in fibroblasts from patients with complications, in the reprogrammed iPS cells from these fibroblasts, and in neurons differentiated from iPS cells from the cohort with complications. These neurons had increased susceptibility to genotoxic stressors, including exposure to high glucose and various sources of increased ROS. The miR200 family target transcripts encoding DNA damage checkpoint proteins, and there was a concomitant increase in DNA damage and a loss of DNA damage checkpoint proteins.[44] These findings were confirmed in aortic tissue from patients with complications and were reversed in fibroblasts and iPS cells by knockdown of miR200. In fibroblasts from patients with complications, there was also a striking impairment in reprogramming efficiency, and the induced iPCs had impaired cellular growth and differentiation. In tissue from patients with complications, there was increased inflammation due to macrophage infiltration. There was also increased apoptosis due to elevated levels of DNA damage, which was not compensated by differentiation of progenitor cells. Taken together, the observations from both these studies could reflect underlying genetic or epigenetic causes. However, since the DCCT study patients were randomly assigned to one of two different treatment groups, treatment-related epigenetic causes are a more likely explanation for the long-term metabolic memory observed subsequently in the EDIC study.

Epigenetic causes are those that change DNA or RNA structure and function, but do not change DNA-RNA sequence. Along with the activities of microRNAs and other noncoding RNAs, a



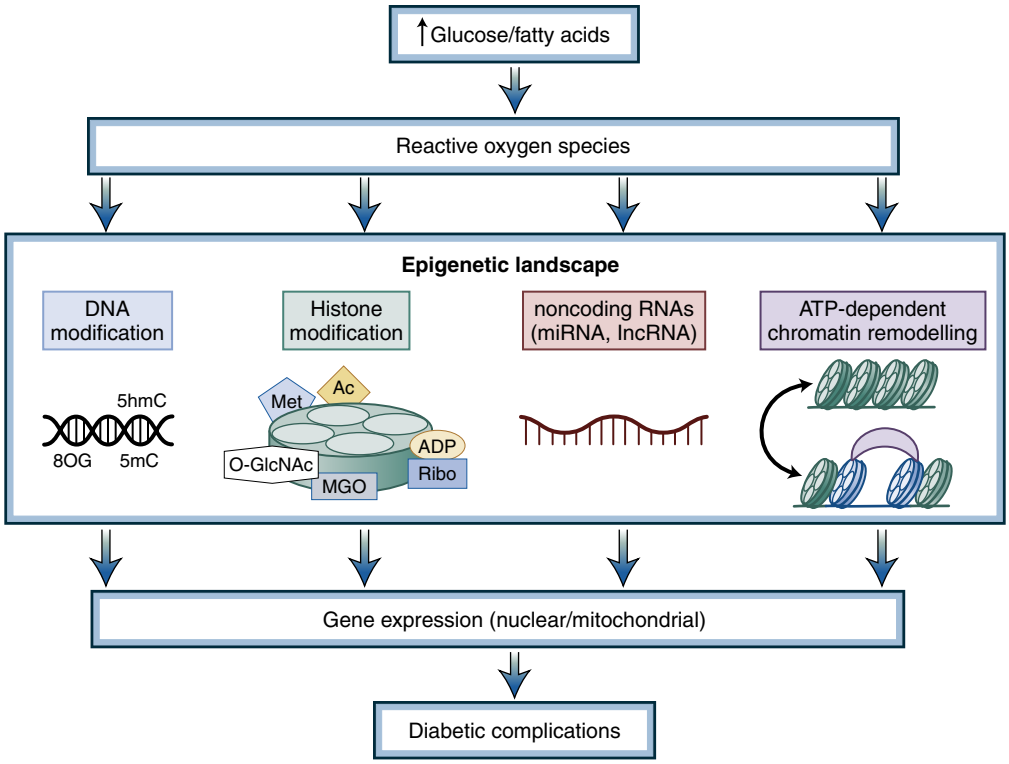
• **Fig. 37.23** Expression levels of microRNA (miR) miR200 from patients with over 50 years of T1DM differentiate those with no complications from those with complications. Induced pluripotent stem cells (iPSCs) from fibroblasts of patients with no complications had low miR200 levels, while iPSCs from fibroblasts of patients with complications (retinopathy, nephropathy, neuropathy, and cardiovascular disease) had high miR200 levels. miR200 was found to inhibit DNA damage checkpoint protein expression, causing accumulation of DNA double-strand breaks, inflammation, and apoptosis. *ATM,* Ataxia telangiectasia mutated; *ATR,* ataxia telangiectasia and Rad3–related protein; *DNA-PK,* DNA-dependent protein kinase; *DNMTs,* DNA methyltransferases; *EZH2,* enhancer of zeste homolog 2; *HDACs,* histone deacetylases; *MDC1,* mediator of DNA damage checkpoint 1; *Me,* methyl; *MED 15/17,* mediator of RNA polymerase II transcription subunits 15 and 17; *MRE11,* MRE11 homolog, double-strand break repair nuclease; *NBS1,* Nijmegen breakage syndrome protein 1; *PARP1,* poly-ADPribose polymerase 1; *pH2AX,* phosphorylated H2A histone family member X; *POL II,* RNA polymerase II; *RAD 50,* DNA repair protein RAD50; *SUZ12,* suppressor of zeste 12 protein homolog; *Ub,* ubiquitin. (Modified from Bhatt S, Gupta MK, Khamaisi M, et al. Preserved DNA damage checkpoint pathway protects against complications in long-standing type 1 diabetes. *Cell Metab.* 2015;22:239–252.)

variety of enzymes modify chromatin by adding and removing chemical groups to DNA (primarily cytosine methylation) and to the protruding N-terminal tails of nucleosome histone proteins (monomethylation, dimethylation, and trimethylation of lysine and arginine residues, acetylation of lysine residues, and modification of arginines by methylglyoxal and of serines and threonines by *O*-GlcNAc).[190,274] In conjunction with remodeling complexes, these modifications mediate the availability or nonavailability of DNA sequences for transcription (Fig. 37.24). Increased ROS modulate several epigenetic mechanisms in different cell types and contexts.[275–277]

DNA methylation can repress or activate transcription, depending on its location near promoters or near enhancers in intergenic regions.[275] Similarly, methylation of histone tails can repress or activate transcription, depending on the position of the modified residue in a specific histone tail, and on the degree of methylation. Acetylation of histone tails generally facilitates transcription.

In whole blood and isolated monocyte samples from DCCT/EDIC type 1 diabetic patients at the EDIC study baseline and in samples from the same patients obtained during EDIC years 16 to 17, DNA methylation profiling revealed strong connections with networks associated with diabetic complications, even though the post-DCCT HbA_{1c} values for both intensive and standard treatment groups became identical at the end of the DCCT (~8%), and remained identical for the duration of the EDIC study. The most significant persistent DNA methylation change was hypomethylation of thioredoxin-interacting protein (TXNIP).[278] TXNIP binds to and inhibits thioredoxin, increasing ROS production and inhibiting renal tubular autophagy.[279] The importance of this DNA methylation profiling study is that it shows, for the first time, that differential methylation at several loci persists for more than 16 to 17 years in circulating leukocytes from the same diabetic patient cohort. Every diabetic complication is a complex heterocellular process, however, and each different cell type involved has a distinct epigenome regulating gene expression and cell type–specific pathologies despite sharing a common genetic sequence.[275] A similar but technically more limited study of selected histone modifications in monocytes showed enrichment of activating histone 3 lysine 9 acetylation (H3K9Ac) in promoters of more than 15 genes related to the NFκB inflammatory pathway.[280]

Transient hyperglycemia, at a level sufficient to increase mitochondrial ROS production, induces long-lasting activating epigenetic changes (increased monomethylation of histone 3 lysine 4) in the proximal promoter of the NFκB subunit p65 in vitro in

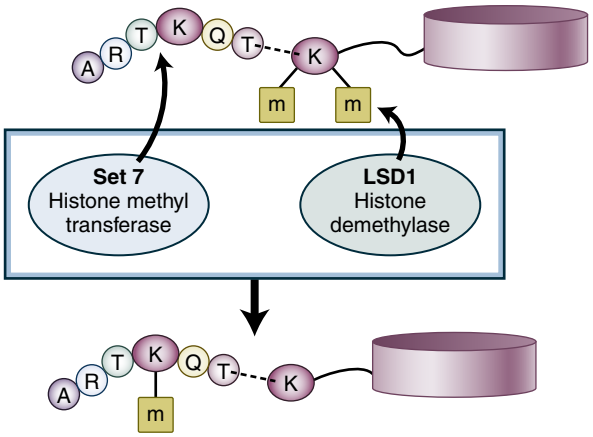


• **Fig. 37.24** Increased metabolite-generated reactive oxygen species (ROS) modulate the epigenetic landscape, altering histone modifications, DNA modifications, expression of noncoding RNAs, and ATP-dependent chromatin remodeling. These changes subsequently affect gene expression patterns implicated in the pathogenesis of diabetic complications. DNA modifications include cytosine methylation (5mC), hydroxymethylation (5hmC), or 8-oxo-2'-deoxyguanosine (8OG) formation. Histone modifications include methylation (Met), acetylation (Ac), ADP-ribosylation (ADP-Ribo), phosphorylation (P), glycation by methylglyoxal (MGO), and glycosylation by *O*-linked *N*-acetylglucosamine (O-GlcNAc). Noncoding RNAs include microRNAs (miRNA) and long noncoding RNAs (lncRNA). ATP-dependent chromatin remodeling includes moving and adding/removing nucleosomes by ATPase-containing complexes. (Modified from Kietzmann T, Petry A, Shvetsova A, et al. The epigenetic landscape related to reactive oxygen species formation in the cardiovascular system. *Br J Pharmacol.* 2017;174:1533–1554.)

human aortic endothelial cells (16-hour exposure), and in vivo in aortic cells in nondiabetic mice (6-hour exposure). These epigenetic changes caused sustained increases in p65 gene expression and in the expression of p65-dependent proinflammatory genes. Both the epigenetic changes and the gene expression changes persist for at least 6 days of subsequent normal glycemia in cultured cells and for months in previously diabetic mice whose beta-cell function had recovered.[281,282] Hyperglycemia-induced epigenetic changes and increased p65 expression are prevented by normalizing mitochondrial superoxide production or superoxide-induced methylglyoxal. Demethylation of another histone lysine residue, histone 3 lysine 9, is also induced by hyperglycemia-induced overproduction of ROS. This reduces inhibition of p65 gene expression, and therefore acts synergistically with the activating methylation of histone 3 lysine 4. The specific H3K4m1 enrichment is mediated by the lysine methyltransferase Set7, which is recruited to the nucleus by high glucose–induced mechanisms.[283] Genome-wide analysis of Set7 function in human vascular endothelial cells confirmed its role in regulating NFκB-dependent pathways, affecting a large number of genes associated with vascular function by both histone lysine methylation and lysine methylation on nonhistone substrates such as transcription factors.[284,285] The demethylation of histone 3 lysine 9 is mediated by the histone demethylase LSD1 (lysine-specific histone demethylase 1) (Fig. 37.25).

Another component of epigenetic regulation is the action of epigenetic reader proteins that bind to modified histone residues and facilitate the formation of transcriptional complexes. In endothelial cells, proinflammatory activation of NFκB led to rapid recruitment of NFκB to newly formed superenhancers, and rapid large-scale redistribution of the epigenetic reader bromodomain and extraterminal domain 4 (abbreviated BET4 or BRD4) from decommissioned preexisting basal enhancers to the newly formed superenhancers.[286] This recruitment caused increased levels of H3K27ac and immediate transcription of inflammatory genes. Blocking the association of BRD4 with acetylated lysines in LDL receptor–deficient hypercholesterolemic mice attenuated both early and late atherosclerosis development.

BRD4 and other members of the BET family are also critical effectors of pathologic cardiac remodeling and heart failure via their ability to coactivate multiple master transcription factors known to initiate and promote heart failure, including NFκB and NFAT.[287] BET inhibition suppresses pathologic cardiac gene expression programs and arrests pathologic hypertrophy and heart failure in vivo. BRD4 (most potently its splice isoform BRD4B) also functions as an endogenous inhibitor of DNA damage response signaling, which enhances radiation ROS-induced cell death. In contrast, knockdown of this isoform caused rapid cell-cycle checkpoint recovery and enhanced cell survival.[288] Much has been learned since the original clinical description of metabolic memory, but much more research remains to be done before the molecular basis for metabolic memory is clearly understood.



• **Fig. 37.25** Transient hyperglycemia induces long-lasting activating epigenetic changes in the proximal promoter of the NFκB subunit p65 in endothelial cells. Hyperglycemia-induced reactive oxygen species (ROS) and methylglyoxal cause activating modifications of histone 3 lysine 4 (monomethylation) and derepressing modifications of histone 3 lysine 9 (removal of two methyl groups) at the NFκB p65 proximal promoter. K is the symbol for the amino acid lysine. The chains of circled letters are the N-terminal tails of histone H3. *LSD1*, lysine-specific histone demethylase 1A; *Set 7*, SET domain-containing protein 7. (From Brasacchio D, Okabe J, Tikellis C, et al. Hyperglycemia induces a dynamic cooperativity of histone methylase and demethylase enzymes associated with gene-activating epigenetic marks that co-exist on the lysine tail. *Diabetes*. 2009;58:1229–1236; El-Osta A, Brasacchio D, Yao D, et al. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. *J Exp Med.* 2008;205:2409–2417.)

Diabetic Retinopathy and Other Ocular Complications of Diabetes[a]

Diabetic retinopathy is a well-characterized sight-threatening chronic microvascular complication that eventually afflicts virtually all patients with diabetes mellitus.[289] Diabetic retinopathy is characterized by gradually progressive alterations in the retinal microvasculature, leading to areas of retinal nonperfusion, increased vascular permeability, and pathologic intraocular proliferation of retinal vessels. The complications associated with retinal neovascularization, termed *proliferative diabetic retinopathy* (PDR), and increased vascular permeability, termed *diabetic macular edema* (DME), can result in severe and permanent vision loss.

Early-stage diabetic retinopathy eventually occurs in nearly all patients, although early intensive treatment of hyperglycemia delays its onset and progression in T1DM. In selected patients with T2DM, systemic treatment with fenofibrate, ACE inhibitors, and angiotensin II receptor blockers (ARBs) may affect the development and progression of diabetic retinopathy. Multiple phase 3 clinical trials over the past 5 years have now established intravitreously delivered anti-VEGF therapy as the new standard of care for most eyes with visual impairment from center-involved DME.[290,291] Currently, more than 90% of severe vision loss resulting from PDR can be prevented, and approximately 50% of eyes with visual loss from DME can resolve their retinal thickening and/or recover vision of 20/20 or better with appropriate medical and ophthalmologic care.[292,292a] Thus the primary clinical care emphasis for the prevention of vision loss in the diabetic patient is on early identification, accurate classification, and timely treatment of retinopathy through lifelong routine ophthalmologic follow-up appointments and optimized treatment of hyperglycemia, hypertension, and hyperlipidemia.

[a] Portions of this section draw on, among others, Aiello LM, Cavallerano JD, Aiello LP. Diagnosis, management, and treatment of nonproliferative diabetic retinopathy and diabetic macular edema. In: Albert DM, Jakobiec FA, eds. *Principles and Practice of Ophthalmology.* 2nd ed. Philadelphia: WB Saunders; 2000:1900–1914; Aiello LP, Cavallerano J, Klein R. Diabetic eye disease. In DeGroot LJ, James JL, eds. *Endocrinology*, 5th ed. Philadelphia: WB Saunders; 2005:1305–1317; Aiello LP, Gardner TW, King GL, et al. Diabetic retinopathy: technical review. American Diabetes Association. *Diabetes Care.* 1998;21:143–156; Aiello LP, Cavallerano J. Diabetic retinopathy. In Johnstone MT, Veves A, eds. *Contemporary Cardiology: Diabetes and Cardiovascular Disease*. Totowa, NJ: Humana Press; 2001:385–398.

Epidemiology and Impact of Proliferative Diabetic Retinopathy and Diabetic Macular Edema

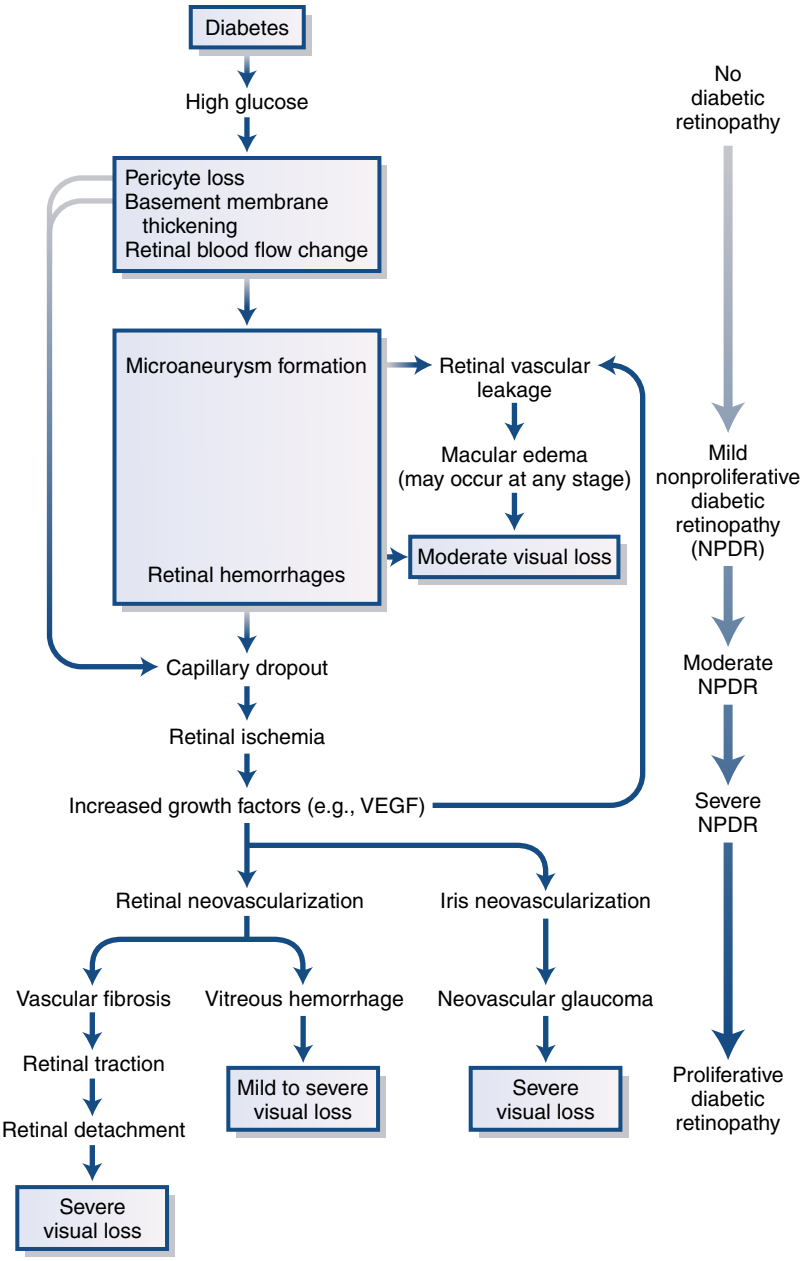
There is a higher risk of more frequent and severe ocular complications in T1DM than T2DM.[293] Approximately 25% of patients with T1DM have retinopathy after 5 years, and this figure increases to 60% and 80% after 10 and 15 years, respectively. The most visually threatening form of retinopathy (PDR) is present in about 67% of T1DM patients who have had diabetes for 35 years.[294] However, because T2DM accounts for 90% to 95% of the diabetic population in the United States, type 2 disease accounts for a higher fraction of patients with vision loss.

In the United States, an estimated 700,000 people have PDR, 130,000 of whom have high-risk PDR. Among people with diabetes, 500,000 have macular edema, of whom 325,000 have clinically significant macular edema (CSME).[295–299] An estimated 63,000 cases of PDR, 29,000 cases of high-risk PDR, 80,000 cases of macular edema, 56,000 cases of CSME, and 12,000 to 24,000 new cases of legal blindness occur each year as a result of diabetic retinopathy.[295,296,300] Blindness is estimated to be 25 times more common in persons with diabetes than in those without the disease.[301,302] Rates of progression to PDR or severe visual loss have declined over the last four decades in developed countries due to improvements in medical management, earlier identification, and treatment of diabetic retinopathy with laser photocoagulation.[303] However, given the dramatically increasing prevalence of diabetes worldwide, with 629 million individuals expected to be affected by diabetes by the year 2045,[304] a proportionally larger number of individuals will be at risk for vision loss from diabetic eye complications over the next few decades. Threefold increases in vision-threatening diabetic retinopathy will affect 3.4 million Americans aged 40 and older by 2050, as estimated by the Centers for Disease Control and Prevention (CDC).[306]

Many individuals with diabetes and vision-threatening diabetic retinopathy are not aware of the presence or the severity of their disease. The 2010 National Health and Nutrition Examination Survey revealed that only 45% of Americans with DME were aware that diabetes had affected their eyes and nearly 60% of these individuals had not had a dilated eye examination within the past year. These findings reflect both a lack of awareness among patients at risk for vision loss from diabetic eye complications and insufficient evaluation for many patients with vision-threatening retinopathy.[306]

Pathophysiology of Diabetic Retinopathy

A detailed discussion of the pathophysiologic mechanisms underlying diabetic retinopathy and other diabetes-related complications has been presented earlier in this chapter. The earliest

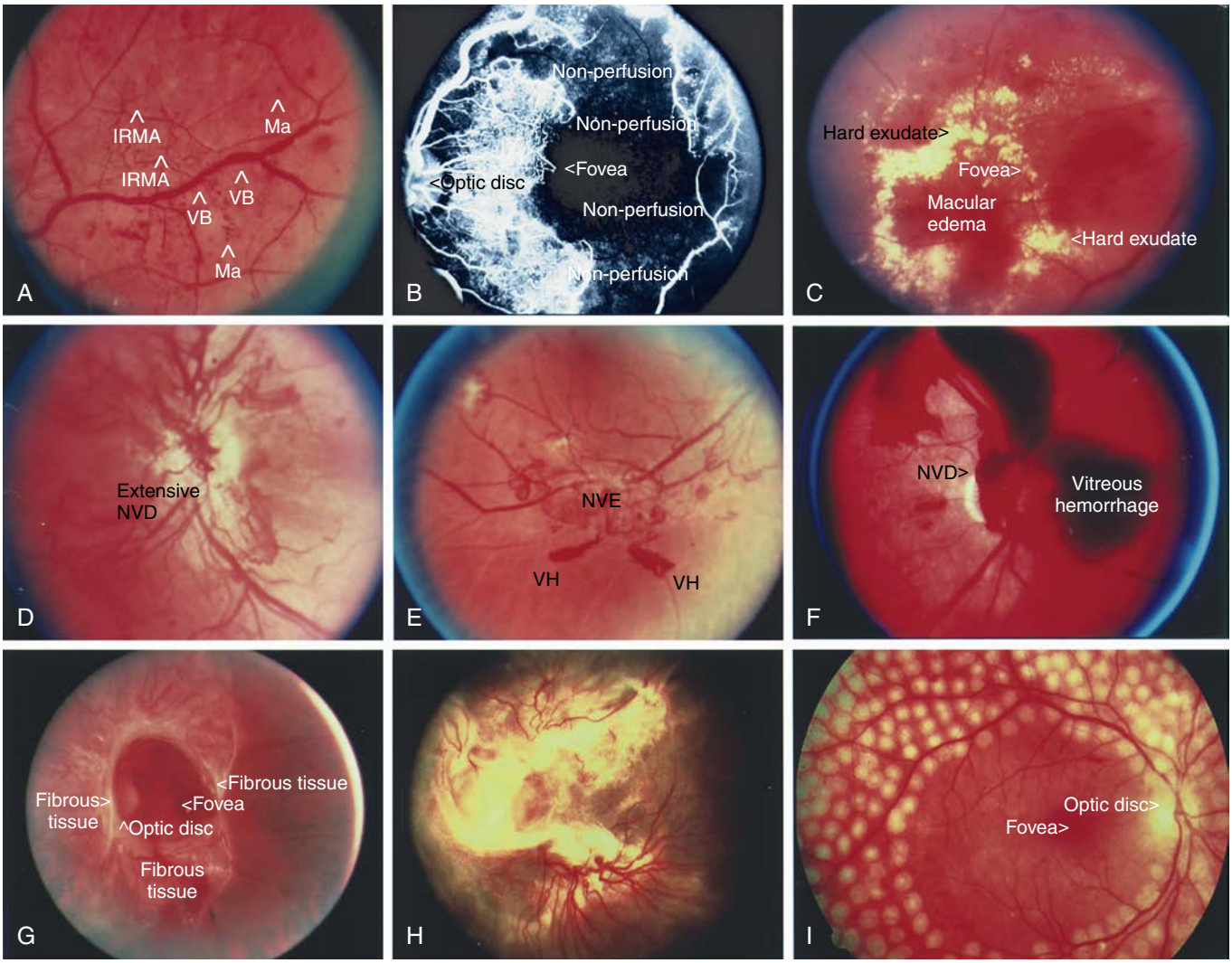


• **Fig. 37.26** Pathogenesis of diabetic retinopathy. This schematic flow chart represents the major preclinical and clinical findings associated with the full spectrum of diabetic retinopathy and macular edema. *VEGF,* Vascular endothelial growth factor.

histologic effects of diabetes mellitus in the eye include loss of retinal vascular pericytes (supporting cells for retinal endothelial cells), thickening of vascular endothelium basement membrane, and alterations in retinal blood flow (Fig. 37.26).[307–310] With increasing loss of retinal pericytes, the retinal vessel wall develops outpouchings (microaneurysms) and becomes fragile.

Clinically, microaneurysms and small retinal hemorrhages might not always be readily distinguishable from each other and are usually evaluated together as "hemorrhages and microaneurysms" (Fig. 37.27A). Rheologic changes occur in diabetic retinopathy and result from increased platelet aggregation, integrin-mediated leukocyte adhesion, and endothelial damage.[311–313] Disruption of the blood-retina barrier can ensue, characterized by increased vascular permeability.[314,315] Subsequent leakage of blood and serum from the retinal vessels results in retinal hemorrhages, retinal edema, and hard exudates (see Fig. 37.27A and C). Vision loss can follow if the fovea is affected by the leakage.[316]

With time, increasing sclerosis and endothelial cell loss lead to narrowing of the retinal vessels, which decreases vascular perfusion and can ultimately lead to obliteration of the capillaries and small vessels (see Fig. 37.27B). The resulting retinal ischemia is a potent inducer of angiogenic growth factors. Several angiogenic growth factors have been isolated from eyes with diabetic retinopathy, including insulin-like growth factors, basic fibroblast growth factor (bFGF), and VEGF.[317,318] These factors promote the development of new vessel growth and retinal vascular permeability.[319,320] Indeed, inhibition of angiogenic molecules such as VEGF and their signaling pathways can suppress the development of retinal



• **Fig. 37.27** Clinical features of diabetic retinopathy: Some typical findings in human diabetic retinopathy. (A) Findings in severe nonproliferative diabetic retinopathy, including microaneurysms (Ma), venous beading *(VB),* and intraretinal microvascular abnormalities (IRMA). (B) Fluorescein angiogram showing marked capillary nonperfusion. (C) Clinically significant macular edema with retinal thickening and hard exudates involving the fovea. (D) Extensive neovascularization of the optic disc (NVD), illustrating high-risk proliferative diabetic retinopathy. (E) Neovascularization elsewhere (NVE) and two small vitreous hemorrhages (VH), also illustrating high-risk proliferative diabetic retinopathy. (F) Extensive vitreous hemorrhage arising from severe neovascularization of the disc (NVD). (G) Severe fibrovascular proliferation surrounding the fovea. (H) Traction retinal detachment from extensive fibrovascular proliferation. (I) Scars from scatter (panretinal) laser photocoagulation. The macula, fovea, and optic disc are not treated to preserve central vision. Laser burns are evident as white retinal lesions. (Adapted from Aiello LP. Eye complications of diabetes. In Korenman SG, Kahn CR, eds. *Atlas of Clinical Endocrinology.* Vol 2: *Diabetes.* Philadelphia, PA: Blackwell Scientific; 1999.)

neovascularization and retinal vascular permeability.[321,322] VEGF-independent endogenous inhibitors of angiogenesis and vascular permeability such as pigment epithelial-derived factor (PEDF), plasma kallikrein, and erythropoietin have also been found in the eye, and these have therapeutic potential.[323,324]

New vessels tend to grow in regions of strong vitreous adhesion to the retina, such as at the optic disc and major vascular arcades (see Fig. 37.27D and E). The posterior vitreous face also serves as a scaffold for pathologic neovascularization, and the new vessels commonly arise at the junctions between perfused and nonperfused retina. When the retina is severely ischemic, the concentration of angiogenic growth factors can reach sufficient concentration in the anterior chamber to cause abnormal new vessel proliferation on the iris and the anterior chamber angle.[318,325] Uncontrolled anterior segment neovascularization can result in neovascular glaucoma because the fibrovascular proliferation in the angle of the eye causes blockage of aqueous outflow through the trabecular meshwork.[305]

Proliferating new vessels in diabetic retinopathy are fragile and have a tendency to bleed, which results in preretinal and vitreous hemorrhages (see Fig. 37.27E and F). Although the presence of a large amount of blood in the preretinal space or vitreous cavity is not damaging to the retina, these intraocular hemorrhages often cause vision loss by blocking the visual axis. Vitreous hemorrhage

can clear spontaneously without intervention, but eyes in which hemorrhage is nonclearing may need vitrectomy surgery to restore vision. Vitreous hemorrhage can also decrease the ability to visualize the retina and thereby limit the ability to adequately diagnose and treat other retinal disease. Membranes on the retinal surface can be induced by blood and result in wrinkling and traction on the retina. Although all retinal neovascularization, given sufficient time, eventually becomes quiescent, as with most scarring processes there is progressive fibrosis of the new vessel complexes that is associated with contraction. In the eye, such forces can exert traction on the retina, leading to tractional retinal detachment and retinal tears that can cause severe and permanent vision loss if left untreated (see Fig. 37.27G and H).

In short, causes of vision loss from complications of diabetes mellitus include retinal ischemia involving the fovea, macular edema at or near the fovea, preretinal or vitreous hemorrhage, retinal detachment, and neovascular glaucoma. Vision loss can also result from more indirect effects of vasculopathy in diabetic patients, such as retinal vessel occlusion, accelerated atherosclerotic disease, and embolic phenomena.

Clinical Features of Diabetic Retinopathy

Risk Factors

Duration of diabetes is closely associated with the onset and severity of diabetic retinopathy. Nearly all patients with T1DM develop some degree of retinopathy after 20 years.[293,296,326] In the United States approximately 20% of patients with newly diagnosed T2DM have retinopathy at the time of diagnosis,[326] and more than 60% develop some degree of retinopathy over subsequent decades. In the UKPDS study of T2DM, 35% of female subjects and 39% of male subjects had some level of diabetic retinopathy at the time of diabetes diagnosis.[327]

Age of onset of diabetes is another risk factor. Diabetic retinopathy is rare in prepubescent patients with T1DM.[87] In the Wisconsin Epidemiologic Study of Diabetic Retinopathy, approximately 4% of patients younger than 30 years of age at diagnosis and nearly 2% of patients older than 30 years of age at diagnosis were legally blind. In the younger-onset group, 86% of blindness was attributable to diabetic retinopathy. In the older-onset group, in which other eye diseases were also common, 33% of the cases of legal blindness were due to diabetic retinopathy.[294,326,328]

Lack of appropriate glycemic control is the most significant known risk factor for the onset and progression of diabetic retinopathy. The DCCT demonstrated a clear relationship between hyperglycemia and higher risk of diabetic microvascular complications, including retinopathy, in 1441 patients with T1DM.[9,329–332] Intensive insulin therapy reduced or prevented the development of retinopathy by 27% as compared with conventional therapy in patients monitored for 4 to 9 years in the DCCT. Additionally, intensive insulin therapy reduced the progression of diabetic retinopathy by 34%, to 76%, and had a substantial beneficial effect over the entire range of retinopathy severity. These results underscore the fact that although intensive therapy might not prevent retinopathy completely, it reduces the risk of retinopathy onset and progression.

Interestingly, the effect of reducing the HbA_{1c} in this group, from 9.1% for conventional treatment to the 7.3% for intensive treatment, resulted in a benefit maintained through 18 years of subsequent follow-up in the EDIC study, even though the difference in mean HbA_{1c} levels of the two former randomized treatment groups was only 0.4% at 1 year ($p < 0.001$), continued to narrow, and became statistically nonsignificant from 5 years onward (8.1% vs. 8.2%, $p = 0.09$). The rate of further progression of complications from their levels at the end of the DCCT remains less in the former intensive treatment group. Thus the benefits of 6.5 years of intensive treatment continue to accrue well beyond the period of initial HbA_{1c} difference.[9,22,330,333–335] Beyond retinopathy progression itself, the risk for diabetes-related ocular surgery was reduced 48% in the intensive therapy group[336] over a median follow-up of 23 years. Applying DCCT intensive insulin therapy to all persons in the United States with T1DM would result in a gain of 920,000 person-years of sight,[331] although the costs of intensive therapy are three times that of conventional therapy.[337]

Presence of renal disease, as manifested by microalbuminuria and proteinuria, is yet another significant risk factor for onset and progression of diabetic retinopathy.[338,339] Hypertension is associated with PDR and is an established risk factor for the development of macular edema.[340] Independent of renal disease, elevated serum lipid levels are associated with extravasated lipid in the retina (hard exudates) and vision loss.[341]

Clinical Findings

Clinical findings associated with early and progressing diabetic retinopathy include microaneurysms, hemorrhages, cotton-wool spots, hard exudates, intraretinal microvascular abnormalities, and venous abnormalities such as venous loops, venous tortuosity, and venous beading (see Fig. 37.27A and C). Microaneurysms are outpouchings of the capillary walls that can leak fluid and result in intraretinal edema and hemorrhages. The intraretinal hemorrhages can be flame shaped or dot-blot–like in appearance, reflecting the architecture of the retinal layer in which they occur. Flame-shaped hemorrhages occur in inner retina closer to the vitreous, and dot-blot hemorrhages occur deeper in the retina. Intraretinal microvascular abnormalities are either new vessel growth within the retinal tissue itself or shunt vessels through areas of poor vascular perfusion. It is common for intraretinal microvascular abnormalities to be located adjacent to cotton-wool spots. Cotton-wool spots are caused by microinfarcts in the nerve fiber layer of the retina. Venous caliber abnormalities, also known as venous beading, are generally a sign of severe retinal hypoxia. In some cases of extensive vascular loss, however, the retina may actually appear free of nonproliferative lesions on clinical examination. Such areas are termed *featureless retina* and are a sign of severe retinal hypoxia.

Vision loss from diabetic retinopathy generally results from persistent nonclearing vitreous hemorrhage, traction retinal detachment, retinal nonperfusion, or DME (see Figs. 37.26 and 37.27). Neovascularization with fibrous tissue contraction can distort the retina and lead to traction retinal detachment. The new vessels can bleed, causing preretinal or vitreous hemorrhage. The most common cause of vision loss from diabetes, however, is macular disease and macular edema. Macular edema is more likely to occur in patients with T2DM. In diabetic macular disease, macular edema involving the fovea or nonperfusion of the capillaries in the central macula is responsible for the loss of vision.

Classification Systems

Classification of Diabetic Retinopathy

Diabetic retinopathy is broadly classified into *nonproliferative diabetic retinopathy* (NPDR) and *proliferative diabetic retinopathy* categories.[342,343] Macular edema can be present in eyes with nonproliferative or proliferative disease and is not used in the classification of level of retinopathy. The historic terms *background retinopathy* and *preproliferative diabetic retinopathy* have been

replaced to reflect the specific characteristics and risk stratification of the prognostically important subgroups in NPDR (Table 37.1).

Generally, diabetic retinopathy progresses from no retinopathy through mild, moderate, severe, and very severe NPDR and eventually to PDR. The level of NPDR is determined by the extent and location of clinical manifestations of retinopathy. Mild NPDR is characterized by limited microvascular abnormalities such as hemorrhages or microaneurysms, cotton-wool spots, and increased vascular permeability. Moderate and severe NPDR is characterized by increasing severity of hemorrhages or microaneurysms, venous caliber abnormalities, intraretinal microvascular abnormalities, and vascular closure. The level of NPDR establishes the risk of progression to sight-threatening retinopathy and dictates appropriate clinical management and follow-up.

TABLE 37.1 Glossary and Abbreviations Pertinent to Diabetic Eye Disease

Term	Definition
Antivascular endothelial growth factor (anti-VEGF) therapies; VEGF inhibitors	For the purposes of this chapter, these refer to VEGF inhibitors (including aflibercept, bevacizumab, and ranibizumab) that are given intravitreously for the treatment of diabetic macular edema and proliferative diabetic retinopathy.
Background diabetic retinopathy (BDR)	An outdated term referring to some stages of NPDR; not closely associated with disease progression; replaced by the various levels of NPDR.
Center-involved (or central-involved) diabetic macular edema (ciDME)	Abnormal thickening of the central retina (usually 1 mm in diameter central retinal subfield) due to increased vascular permeability. Because central involvement is more likely to cause visual impairment, it is commonly used as a threshold for treatment.
Clinically significant macular edema (CSME)	Thickening of the retina in the macular region of sufficient extent and location to threaten central visual function.
Cotton-wool spot	A gray or white area lesion in the nerve fiber layer of the retina resulting from stasis of axoplasmic flow caused by microinfarcts of the retinal nerve fiber layer.
Diabetes Control and Complications Trial (DCCT)	A multicenter, randomized clinical trial designed to address whether intensive insulin therapy could prevent or slow the progression of systemic complications of diabetes mellitus.
Diabetic retinopathy (DR)	Retinal damage related to the underlying systemic disease of diabetes mellitus.
Diabetic Retinopathy Study (DRS)	The first multicenter, randomized clinical trial to demonstrate the value of scatter (panretinal) photocoagulation in reducing the risk of vision loss among patients with all levels of diabetic retinopathy.
Diabetic Retinopathy Vitrectomy Study (DRVS)	A multicenter clinical trial evaluating early vitrectomy for patients with very advanced diabetic retinopathy or nonresolving vitreous hemorrhage.
Early Treatment Diabetic Retinopathy Study (ETDRS)	A multicenter, randomized clinical trial that addressed at what stage of retinopathy scatter (panretinal) photocoagulation was indicated, whether focal photocoagulation was effective for preventing moderate vision loss due to clinically significant macular edema, and whether aspirin therapy altered the progression of diabetic retinopathy.
Focal or grid laser photocoagulation	A type of laser treatment whose main goal is to reduce vascular leakage, either by focal treatment of leaking retinal microaneurysms or by application of therapy in a gridlike pattern for patients with clinically significant macular edema.
Hard exudate	Lipid accumulation within the retina as a result of increased vasopermeability.
High-risk characteristic proliferative diabetic retinopathy (HRC-PDR)	Proliferative diabetic retinopathy of defined extent, location, or clinical findings that is particularly associated with severe vision loss.
Microaneurysm	An early vascular abnormality consisting of an outpouching of the retinal microvasculature.
Neovascular glaucoma (NVG)	Elevation of intraocular pressure caused by the development of neovascularization in the anterior segment of the eye.
Neovascularization at the disc (NVD)	Retinal neovascularization occurring at or within 1500 μm of the optic disc.
Neovascularization elsewhere (NVE)	Retinal neovascularization that is located more than 1500 μm away from the optic disc.
Neovascularization of the iris (NVI)	Neovascularization occurring on the iris (rubeosis iridis), usually as a result of extensive retinal ischemia.
No light perception (NLP)	The inability to perceive light.
Nonproliferative diabetic retinopathy (NPDR)	Severities (mild, moderate, severe) of clinically evident diabetic retinopathy that precede the development of PDR.
Preproliferative diabetic retinopathy (PPDR)	An outdated term referring to more advanced levels of NPDR; not closely associated with disease progression; replaced by the various levels of NPDR.
Proliferative diabetic retinopathy (PDR)	An advanced level of diabetic retinopathy in which proliferation of new vessels or fibrous tissue occurs on or within the retina.
Rubeosis iridis	Neovascularization of the iris.

PDR is characterized by vasoproliferation of the retina and its complications, including new vessels on the optic disc (NVD), new vessels elsewhere on the retina (NVE), preretinal hemorrhage (PRH), vitreous hemorrhage, and fibrous tissue proliferation (FP). On the basis of the extent and location of these lesions, PDR is classified as *early PDR* or *high-risk PDR*. Larger areas of these complications as well as new vessels that are near the optic disc are associated with greater risks of vision loss.

Classification of Diabetic Macular Edema

DME can be present at any severity of diabetic retinopathy. Historically, eyes with DME were categorized as having nonclinically significant or clinically significant macular edema. The term CSME was first introduced in the Early Treatment Diabetic Retinopathy Study (ETDRS) to indicate an increased risk for moderate visual loss and was used as a threshold to determine need for laser treatment. CSME exists if there is retinal thickening at or within 500 μm of the fovea, hard exudates at or within 500 μm of the fovea with adjacent retinal thickening, or an area or areas of retinal thickening one disc area or more in size, any part of which is within 1500 μm of the fovea.[342,344,345] CSME is a clinical diagnosis that is not dependent on visual acuity or results of ancillary testing such as fluorescein angiography and can be present even when vision is 20/20 or better.

In the recent era, with the advent of ocular coherence tomography (OCT) and its objective quantitative measurement of retinal thickness, clinical care and clinical trial endpoints have shifted to evaluating whether the center of the macula is involved or not. Data from the ETDRS evaluating eyes with macular edema have shown the presence or absence of thickening involving the center of the macula, now termed *center-involved DME,* which is highly associated with short-term and long-term visual acuity outcomes. In the ETDRS, eyes with center-involved DME had nearly a 10-fold greater risk for developing moderate visual loss compared to eyes without center involvement. The identification of center-involved DME is critical because it will generally indicate how likely the DME is to cause visual impairment and is a key criteria for determining the need for treatment. Noncenter-involved DME does not typically cause visual loss or symptoms, and frequently does not progress to center-involved DME. Thus if the center of the macula is not involved there is often not a compelling reason to treat. As a result, recent clinical studies and guidelines for clinical care have generally used the presence or absence of thickening of the central 1-mm diameter of retina, along with visual status, as the defining criteria to consider initiation of treatment for eyes with DME.

International Classification of Diabetic Retinopathy and Diabetic Macular Edema

The American Academy of Ophthalmology initiated a project to establish a consensus International Classification of Diabetic Retinopathy and Diabetic Macular Edema in an effort to simplify classification and standardize communication among diabetes health care providers.[346,347] This international classification describes five clinical levels of diabetic retinopathy: no apparent retinopathy (no abnormalities), mild NPDR (microaneurysms only), moderate NPDR (more than microaneurysms only but less than severe NPDR), severe NPDR (any of the following: >20 intraretinal hemorrhages in each of four retinal quadrants, definite venous beading in two or more retinal quadrants, prominent intraretinal microvascular abnormalities in one or more retinal quadrants, and no PDR), and PDR (one or more of retinal neovascularization, vitreous hemorrhage, or preretinal hemorrhage). Table 37.2 compares levels of retinopathy in the international classification to those defined by the landmark ETDRS.

In regard to DME, the international classification identifies two broad categories: macular edema apparently absent (no apparent retinal thickening or hard exudates in the posterior pole) and macular edema apparently present (some apparent retinal thickening or hard exudates in the posterior pole). Macular edema is subclassified as mild (some retinal thickening or hard exudates in the posterior pole but distant from the center of the macula), moderate (retinal thickening or hard exudates approaching the center of the macula but not involving the center), or severe (retinal thickening or hard exudates involving the center of the macula). Table 37.3 compares levels of DME in the international classification to ETDRS levels of DME.

As compared with ETDRS retinopathy grading, the International Classification of Diabetic Retinopathy and Diabetic Macular Edema reduces the number of levels of diabetic retinopathy, simplifies descriptions of the categories, and describes the levels

TABLE 37.2 Levels of Diabetic Retinopathy

International Classification Level	ETDRS Level
No apparent retinopathy	Level 10: DR absent
Mild NPDR	Level 20: very mild NPDR
Moderate NPDR	Levels 35, 43, and 47: moderate NPDR
Severe NPDR	Levels 53A–E: severe to very severe NPDR
PDR	Levels 61, 65, 71, 75, 81, 85: PDR, high-risk PDR, very severe or advanced PDR

DR, Diabetic retinopathy; *ETDRS,* Early Treatment Diabetic Retinopathy Study; *NPDR,* nonproliferative diabetic retinopathy; *PDR,* proliferative diabetic retinopathy.

From Grading diabetic retinopathy from stereoscopic color fundus photographs—an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. *Ophthalmology.* 1991;98:S786–S806.

TABLE 37.3 International Classification of Diabetic Macular Edema (DME)

Disease Severity Level	Ophthalmic Findings (Retinal Thickening or Hard Exudates in Posterior Pole)	ETDRS Scale Equivalent
DME apparently absent	None apparent	
DME apparently present	Some apparent	
Mild DME	Some findings present but distant from center of the macula	DME but not CSME
Moderate DME	Findings approaching the center but not involving the center	CSME
Severe DME	Findings involving the center of the macula	CSME

CSME, Clinically significant macular edema; *ETDRS,* Early Treatment Diabetic Retinopathy Study.

without relying on reference to the standard photographs of the Airlie House Classification of diabetic retinopathy. This approach makes clinical use easier and more uniform among practitioners not versed in the complexities of the ETDRS grading system. However, because of this simplification, the International Classification of Diabetic Retinopathy and Diabetic Macular Edema is not a replacement for ETDRS levels of diabetic retinopathy in large-scale clinical trials or studies for which precise retinopathy classification is required.

Other Ocular Manifestations of Diabetes

All structures of the eye are susceptible to complications of diabetes. The consequence of these changes can range from being unnoticed by both patient and physician, to symptomatic but not sight threatening, to requiring evaluation to rule out potentially life-threatening underlying causes other than diabetes.

Mononeuropathies of the third, fourth, or sixth cranial nerves can arise in association with diabetes; mononeuropathy of the fourth cranial nerve is least likely associated with diabetes and warrants workup for other causes.[348–350] Nerve palsies present a significant diagnostic challenge because misdiagnosis can result in a life-threatening lesion remaining untreated. In one review of cranial nerve palsies treated in a diabetic patient population in 1967, 42% of mononeuropathies were not diabetic in origin.[349] This finding underscores the danger of routinely attributing mononeuropathies to the diabetic condition itself without carefully ruling out other potential causes. The percentage of all extraocular muscle palsies attributable to diabetes mellitus is estimated at 4.5% to 6%.[350] Mononeuropathies may be the initial presenting sign of new-onset diabetes, and diabetes should therefore be considered in the differential diagnosis of any mononeuropathy affecting the extraocular muscles, even in patients who do not claim a history of diabetes. Diabetes-induced third-nerve, fourth-nerve, and sixth-nerve palsies are usually self-limited and should resolve spontaneously in 2 to 6 months. Palsies can recur or subsequently develop in the contralateral eye.

The optic disc can be affected by diabetes in a variety of ways other than vasoproliferation. Diabetic papillopathy is a diagnosis of exclusion and must be distinguished from other causes of disc swelling such as true papilledema from increased intracranial pressure, pseudopapilledema such as optic nerve head drusen, toxic optic neuropathies, neoplasms of the optic nerve, and hypertension.[351] Optic disc pallor can occur following spontaneous remission of proliferative retinopathy or remission following scatter (panretinal) laser photocoagulation (see Fig. 37.27I). Because diabetes poses an increased risk for developing open-angle glaucoma, the disc pallor following remission of retinopathy or laser photocoagulation must be considered when evaluating the optic nerve head for glaucoma.

A diabetic ocular complication with potentially serious consequences is neovascularization of the iris. Neovascularization of the iris occurs in 4% to 7% of diabetic eyes and may be present in 40% to 60% of eyes with proliferative retinopathy. Usually new iris vessels are first observed at the pupillary border, followed by a fine network of vessels over the iris tissue progressing into the filtration angle of the eye. Closure of the angle by the fibrovascular network results in neovascular glaucoma, which can lead to irreversible optic nerve damage and vision loss due to the rise in intraocular pressure.[354] Neovascular glaucoma is difficult to manage and requires aggressive treatment. Diabetes is the second leading cause of neovascular glaucoma, accounting for 32% of cases.[355] When possible, scatter (panretinal) laser photocoagulation is the primary therapy for neovascular glaucoma and provides durable regression of neovascularization of the iris and angle for most eyes. Other approaches such as goniophotocoagulation, topical or systemic antiglaucoma medications, and antiglaucomatous filtration surgery are available when needed.[356–358] Intravitreal administration of VEGF inhibitors is now frequently utilized in eyes with acute neovascular glaucoma and results in remarkably rapid regression of the neovascularization with resulting normalization of the intraocular pressure.[359] However, the effects of anti-VEGF agents are often transient, and there is frequently recurrence of neovascularization unless injections are given on a monthly basis. Thus a more durable therapy such as scatter laser photocoagulation is often performed.

The cornea of the diabetic person is more susceptible to injury and slower to heal after injury than is the nondiabetic cornea.[359a,360] The diabetic cornea is also more prone to infectious corneal ulcers, which can lead to rapid loss of vision, need for corneal transplant, or loss of the eye if it is not treated aggressively. Consequently, diabetic patients using contact lenses should exercise caution to avoid contact lens overwear and to maintain careful monitoring.

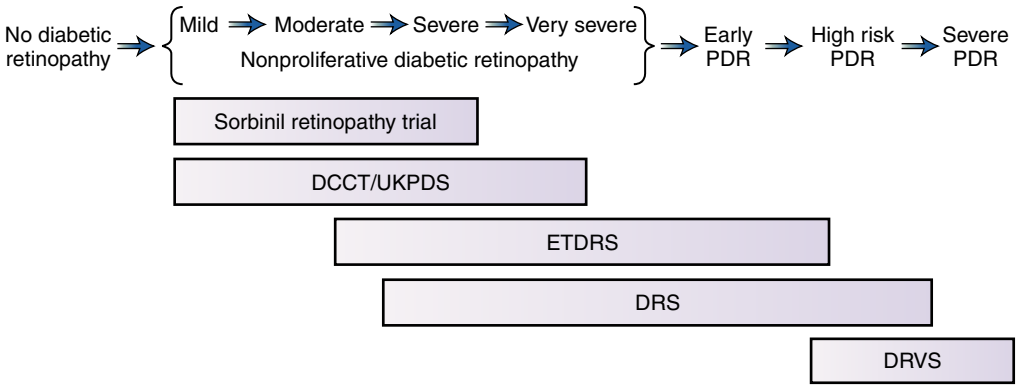
Open-angle glaucoma is 1.4 times more common in the diabetic population than in the nondiabetic population.[361] The prevalence of glaucoma increases with age and duration of diabetes, but medical therapy for open-angle glaucoma is generally effective. In a study of 76,318 women enrolled in the Nurses' Health Study, Pasquale and coworkers found that T2DM is associated with an increased risk of primary open-angle glaucoma in women.[362]

Diabetes effects on the crystalline lens can result in transient refractive changes, alterations in accommodative ability,[363] and cataracts. Refractive change can be significant and is related to fluctuation of blood glucose levels with osmotic lens swelling.[365] Cataracts can occur earlier in life and progress more rapidly in the presence of diabetes.[364,365] Cataracts are 1.6 times more common in people with diabetes than in those without diabetes.[364,365] In patients with earlier onset diabetes, duration of diabetes, retinopathy status, diuretic use, and HbA_{1c} levels are risk factors.[366] In patients with later onset diabetes, age of the patient, lower intraocular pressure, smoking, and lower diastolic BP may be additional risk factors.[367,368] Diabetic patients undergoing simultaneous kidney and pancreas transplantation are at an increased risk of developing all types of cataracts, independent of the use of corticosteroids after transplantation.[369] Both phacoemulsification and extracapsular cataract extraction with intraocular lens implantation are appropriate surgical therapies. The principal determinant of postoperative vision and progression of retinopathy is related to the preoperative presence of DME and the severity of diabetic retinopathy.[370,371]

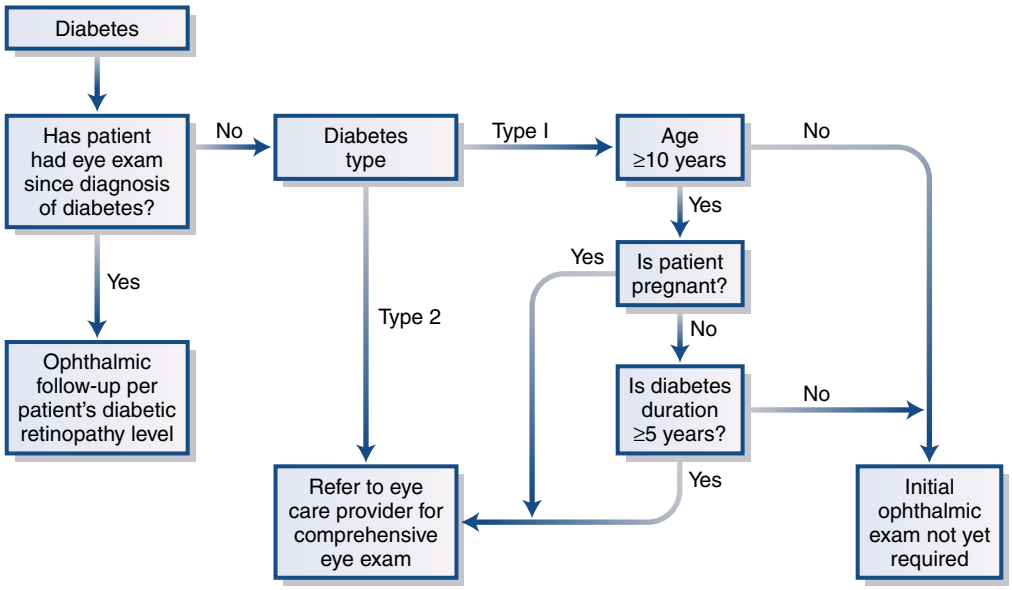
Other findings with higher incidence among patients with diabetes include xanthelasma,[348] microaneurysms of the bulbar conjunctiva,[372] posterior vitreous detachment,[373] and the rare but often fatal orbital fungal infection Mucorales phycomycosis.[352,353] Prompt diagnosis and treatment of phycomycosis caused by *Mucor* species are crucial, although the survival rate remains at only 57%.[353,374]

Monitoring and Treatment of Diabetic Retinopathy

Appropriate clinical management of diabetic retinopathy has been defined by results of major randomized, multicenter clinical trials (Fig. 37.28): the Diabetic Retinopathy Clinical Research Network



• **Fig. 37.28** Schematic representation of the major multicenter clinical trials of diabetic retinopathy and the severity levels of diabetic retinopathy that they primarily addressed. *DCCT,* Diabetes Control and Complications Trial; *DRS,* Diabetic Retinopathy Study; *DRVS,* Diabetic Retinopathy Vitrectomy Study; *ETDRS,* Early Treatment Diabetic Retinopathy Study; *PDR,* proliferative diabetic retinopathy; *UKPDS,* United Kingdom Prospective Diabetes Study.



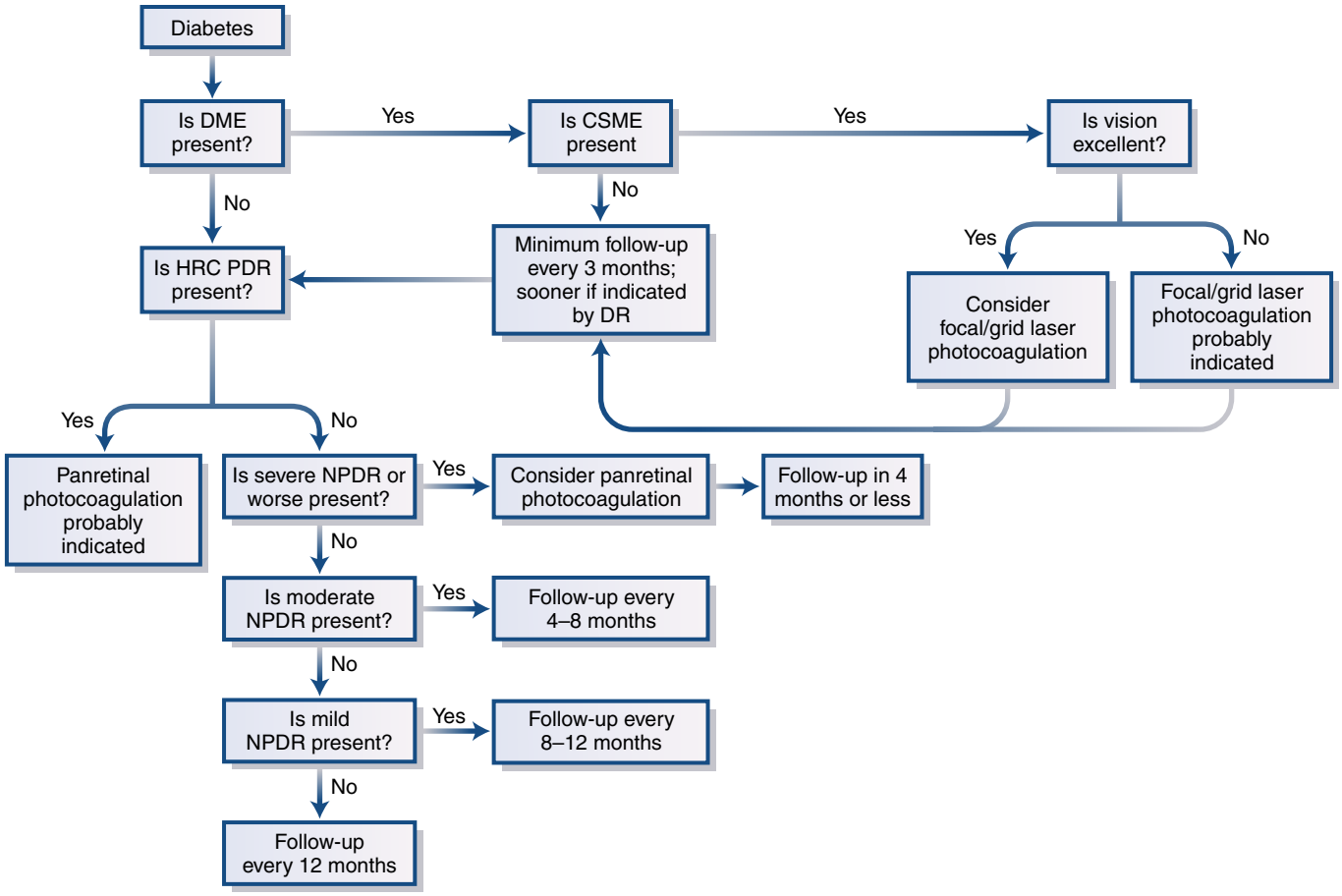
• **Fig. 37.29** Schematic flow chart of major principles involved in determining the timing of initial ophthalmic examination after a diagnosis of diabetes mellitus. These are minimal recommended times. Ocular symptoms, complaints, or other associated medical issues can necessitate earlier evaluation. Guidelines are regularly reevaluated based on new study results.

Protocols I, S, and T,[290,291,375] Diabetic Retinopathy Study (DRS),[376] ETDRS,[316] Diabetic Retinopathy Vitrectomy Study (DRVS),[377] DCCT,[378] and UKPDS.[379] These studies have elucidated the progression rates of each level of diabetic retinopathy, guided follow-up intervals, and demonstrated the proper delivery, timing, and resulting effectiveness of glycemic control, laser photocoagulation, and intravitreous anti-VEGF therapy (Figs. 37.29, 37.30, 37.31, and 37.32). They have also established recommendations for vitrectomy surgery for diabetic eye complications.

Comprehensive Eye Examination

An accurate ocular examination detailing the extent and location of retinopathy-associated findings is critical for determining monitoring and treatment decisions in patients with diabetic retinopathy. As detailed later, most of the blindness associated with advanced stages of retinopathy can be averted with appropriate and timely diagnosis and therapy. Unfortunately, many diabetic patients do not receive adequate eye care at an appropriate stage of their disease.[380,381] In fact, 11% of T1DM and 7% of T2DM patients with high-risk PDR necessitating prompt treatment had not been examined by an ophthalmologist within the past 2 years.[381] In one study, 55% of patients with high-risk PDR or CSME had never had laser photocoagulation.[380]

The comprehensive eye examination is the mainstay of such evaluation and is necessary on a repetitive, lifelong basis for patients with diabetes.[342,382] Such an evaluation has four major components: history, examination, diagnosis, and treatment as needed. Annual retinal evaluation to assess the presence and level of diabetic retinopathy and DME is essential to guide patient care. The fundamentals of a comprehensive eye examination for the nondiabetic patient have been detailed by the American Academy of Ophthalmology[382] and the American Optometric Association.[383]



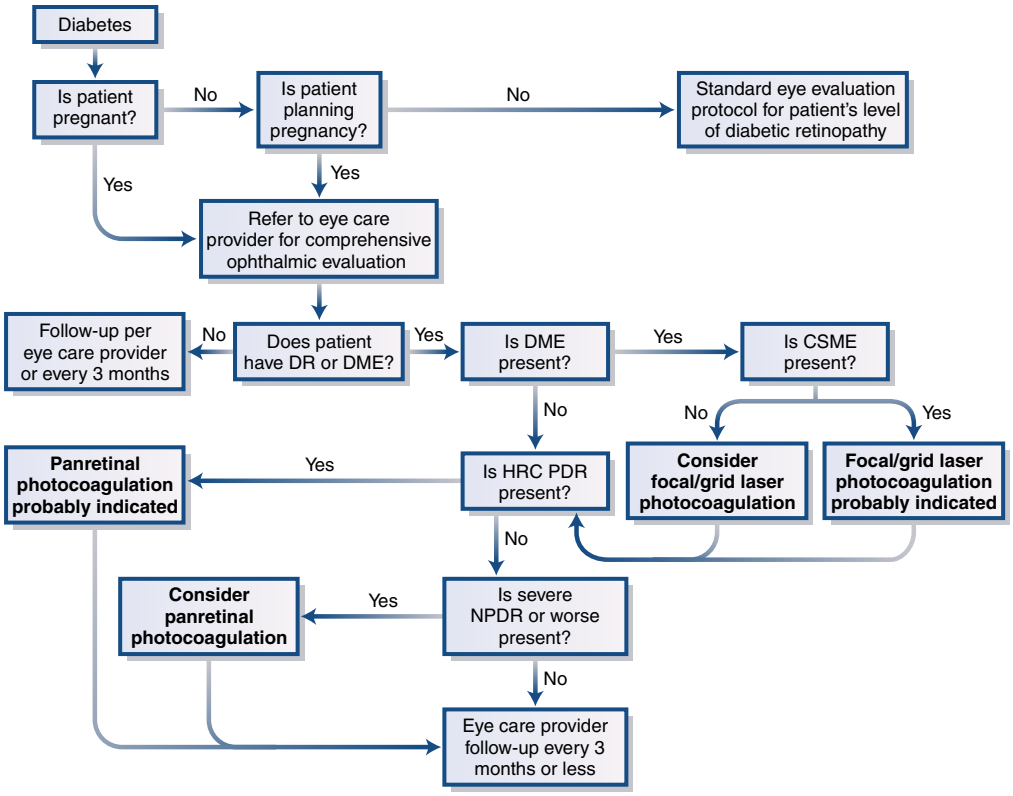
• **Fig. 37.30** Diabetic retinopathy and macular edema examination and treatment flow chart: nonpregnant patients. The schematic flow chart presents major principles involved in determining routine ophthalmic follow-up and indications for treatment in nonpregnant patients with diabetes. These intervals are only general, minimal recommended frequencies. Ocular symptoms, complaints, or other associated ophthalmic or medical issues can necessitate earlier evaluation or an altered approach. Guidelines are regularly reevaluated based on new study results. *CSME,* Clinically significant macular edema; *DME,* diabetic macular edema; *DR,* diabetic retinopathy; *HRC PDR,* high-risk characteristic proliferative diabetic retinopathy; *NPDR,* nonproliferative diabetic retinopathy; *PDR,* proliferative diabetic retinopathy.

The examination of the patient with diabetes should be similar, with additional emphasis on portions of the examination that relate to problems particularly relevant to diabetes.

Dilated ophthalmic examination is superior to undilated evaluation because only 50% of eyes are correctly classified as to presence and severity of retinopathy through undilated pupils.[384,385] Appropriate ophthalmic evaluation entails pupillary dilation, slit-lamp biomicroscopy, examination of the retinal periphery with indirect ophthalmoscopy or mirrored contact lens, and sometimes gonioscopy.[382,383] Because of the complexities of the diagnosis and treatment of PDR and CSME, ophthalmologists with specialized knowledge and experience in the management of diabetic retinopathy are required to determine and provide appropriate surgical intervention.[386] Thus it is recommended that all patients with diabetes should have dilated ocular examinations by an experienced eye care provider (ophthalmologist or optometrist), and diabetic patients should be under the direct or consulting care of an ophthalmologist experienced in the management of diabetic retinopathy at least by the time moderate diabetic retinopathy or DME is present. Retinal imaging that has demonstrated equivalency to dilated retinal fundus examination or the accepted standard of ETDRS seven-standard-field stereoscopic retinal imaging when interpreted by a trained eye care provider can also be appropriate for retinal evaluation.[387,388] Furthermore, ocular telehealth programs for diabetic retinopathy that utilize validated means of retinal imaging have the potential to expand access to highly effective evidence-based diabetes eye care and provide cost-effective alternative methods of care.[389]

Initial Ophthalmic Evaluation

The recommendation for initial ocular examination in persons with diabetes is based on the prevalence rates of retinopathy and the incidence of subsequent vision threatening diabetic eye complications (see Fig. 37.29). Approximately 80% of T1DM patients have retinopathy after 15 years of disease, but only about 25% have any retinopathy after 5 years of diabetes.[385] The prevalence of PDR is less than 2% at 5 years and 25% by 15 years. For T2DM, the onset date of diabetes is usually unknown, and severe retinal disease can be observed even at the time of diabetes diagnosis. Up to 3% of patients whose diabetes is first diagnosed after age 30 years (T2DM) have CSME or high-risk PDR at the time of initial diagnosis of diabetes.[390] Thus in patients older than 10 years, initial ophthalmic examination is recommended beginning 5 years after the diagnosis of T1DM and immediately after diagnosis of T2DM (see Fig. 37.29).[342,391]



• **Fig. 37.31** Diabetic retinopathy and macular edema examination and treatment flow chart: pregnant patients. The schematic flow chart shows major principles involved in determining routine ophthalmic follow-up and indications for treatment in pregnant patients with diabetes. These intervals are only general, minimal recommended frequencies. Ocular symptoms, complaints, or other associated ophthalmic or medical issues can necessitate earlier evaluation or an altered approach. Because retinopathy can progress rapidly in pregnant patients with diabetes, careful and more frequent evaluation is often indicated. Guidelines are regularly reevaluated based on new study results. *CSME,* Clinically significant macular edema; *DME,* diabetic macular edema; *DR,* diabetic retinopathy; *HRC PDR,* high-risk characteristic proliferative diabetic retinopathy; *NPDR,* nonproliferative diabetic retinopathy.

Puberty and pregnancy can accelerate retinopathy progression. The onset of vision-threatening retinopathy is rare in children prior to puberty, regardless of the duration of diabetes[391,392]; however, significant retinopathy can arise within 6 years of disease if diabetes is diagnosed between the ages of 10 and 30 years.[295] Diabetic retinopathy can become particularly aggressive during pregnancy in patients with diabetes.[393,394] In the past, the prognosis for pregnancy in the diabetic patient with microvascular complications was so poor that pregnant diabetic patients were commonly advised to avoid or terminate pregnancies.[395] With recognition of the importance of glycemic control, diabetic patients in the childbearing age now can experience safe pregnancy and childbirth with minimal risk to both the mother and the baby.[396]

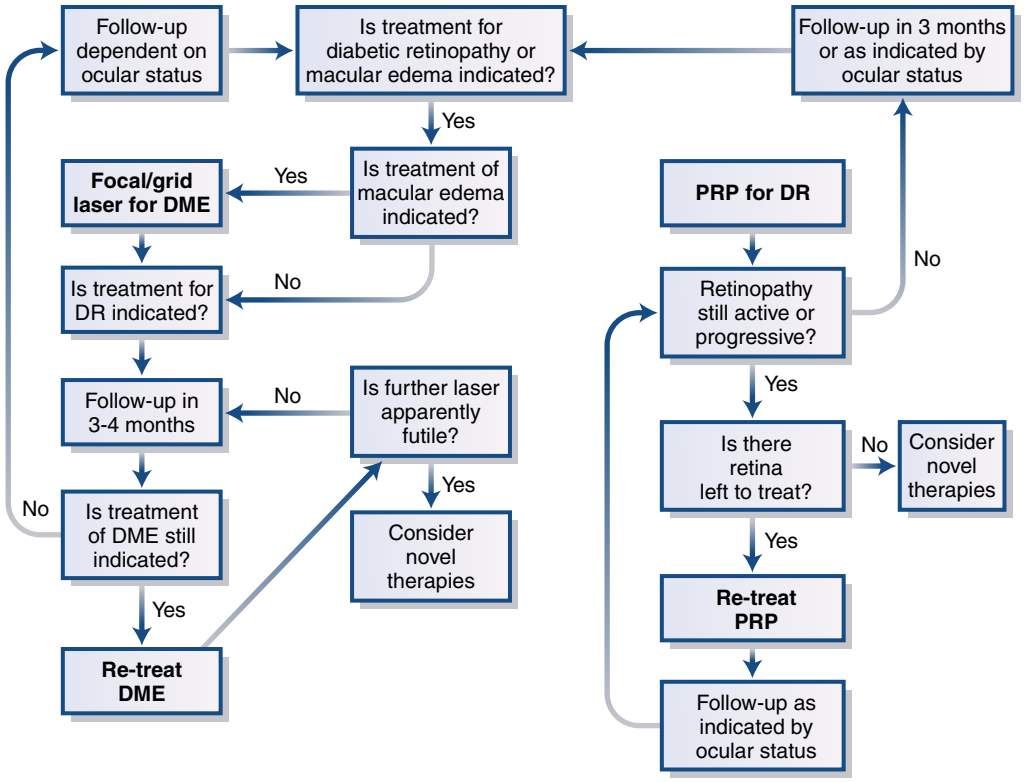
Ideally, patients with diabetes who are planning pregnancy should have a comprehensive eye examination within 1 year prior to conception (see Fig. 37.31). Treatment may be indicated prior to conception for patients who are at risk for vision loss if their diabetic retinopathy were to worsen during pregnancy. Patients who become pregnant should have a comprehensive eye examination in the first trimester of pregnancy. Close follow-up throughout pregnancy is indicated, with subsequent examinations determined by the findings present at the first-trimester examination.[342] This recommendation does not apply to women who develop gestational diabetes because they are not at increased risk of developing diabetic retinopathy.

Follow-Up Ophthalmic Examination

Follow-up ocular examination is determined from the risk of disease progression at any particular retinopathy severity (see Fig. 37.30). NPDR is clinically categorized into four levels of severity based on clinical findings compared to stereo fundus photographic standards: mild, moderate, severe, and very severe.[397] Progression of nonproliferative retinopathy to the visually threatening level of high-risk PDR is closely correlated with baseline NPDR severity (Table 37.4). Progression rates from each individual NPDR level to any other retinopathy level are also known. These are used to define standard minimal follow-up intervals as detailed in Fig. 37.30 and Table 37.5. Because significant sight-threatening retinopathy can initially occur with no or minimal visual symptoms, patients with no clinically evident diabetic retinopathy and no known ocular problems still require annual comprehensive ophthalmic examinations even if they are totally asymptomatic.

Evaluation and Treatment of Proliferative Diabetic Retinopathy

The extent and location of neovascularization determine the level of PDR.[398,399] PDR is best evaluated by dilated fundus examination using slit-lamp biomicroscopy combined with indirect ophthalmoscopy or stereo fundus photography. Fluorescein angiography and optical coherence tomography angiography can also



• **Fig. 37.32** Photocoagulation flow chart. This schematic flow chart details general photocoagulation treatment approaches in patients with diabetic retinopathy or diabetic macular edema. These are only general guidelines, and actual treatment choices can be affected by numerous other factors, including findings in the same eye or in the contralateral eye and systemic issues. *DME,* Diabetic macular edema; *DR,* diabetic retinopathy; *PRP,* scatter (panretinal) photocoagulation.

TABLE 37.4 Progression to Proliferative Diabetic Retinopathy by Level of Nonproliferative Diabetic Retinopathy

	CHANCE OF HIGH-RISK PDR (%)	
Retinopathy Level	1 Year	5 Years
Mild NPDR	1	16
Moderate NPDR	3–8	27–39
Severe NPDR	15	56
Very severe NPDR	45	71
PDR with fewer high-risk characteristics	22–46	64–75

NPDR, Nonproliferative diabetic retinopathy; *PDR,* proliferative diabetic retinopathy.

From Aiello LP, Gardner TW, King GL, et al. Diabetic retinopathy. *Diabetes Care.* 1998;21:143–156.

aid in identifying small, subtle patches of retinal neovascularization. However, these imaging modalities are usually not required for the diagnosis of PDR because these findings are clinically evident in most cases (Fig. 37.33).

The presence of PDR in an eye substantially increases the risk for severe vision loss. Severe visual loss is defined as best corrected acuity of 5/200 or worse on two consecutive visits 4 months apart. This represents vision loss substantially worse than the 20/200 or worse limit defined as legal blindness. Without treatment, eyes with high-risk PDR have a 28% risk of severe vision loss within 2 years. This risk compares with a 7% risk of severe vision loss after 2 years for eyes with PDR but without high-risk characteristics.[398]

Until this decade, scatter (panretinal) laser photocoagulation was the sole therapy shown to be effective against PDR. The DRS demonstrated that panretinal laser photocoagulation was effective in reducing the risk of severe vision loss from PDR by 50% or more. The ETDRS further demonstrated that panretinal laser photocoagulation applied when an eye approaches or just reaches high-risk PDR reduces the risk of severe vision loss to less than 4%. Based on these results, prompt scatter photocoagulation is considered appropriate for all patients with high-risk PDR, usually appropriate for patients with PDR less than high risk, and may be advisable for patients with severe or very severe NPDR, especially in the setting of T2DM (see Fig. 37.30).[316,398-401]

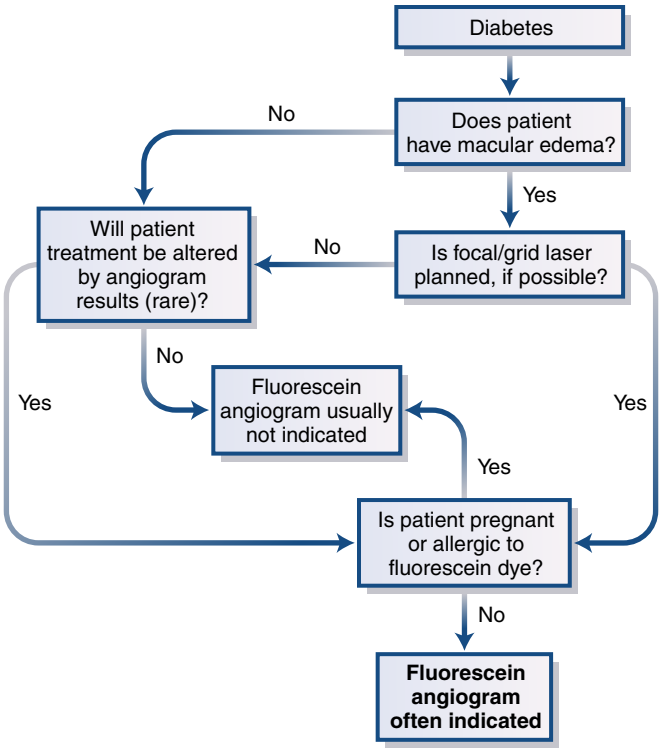
Recent progression of eye disease, status of the fellow eye, compliance with follow-up, concurrent health concerns such as hypertension or kidney disease, and other factors must be considered in determining if laser surgery should be performed in these patients. In particular, patients with T2DM should be considered for scatter photocoagulation before high-risk PDR develops because the risk of severe vision loss and the need for pars plana vitrectomy (PPV) can be reduced by 50% in these patients, especially when macular edema is present.[401]

In scatter photocoagulation, 1200 to 1800 laser burns are applied to the peripheral retinal tissue, actually focally destroying the outer photoreceptor and retinal pigment epithelium of the retina (see Fig. 37.27I). Large vessels are avoided, as are areas of

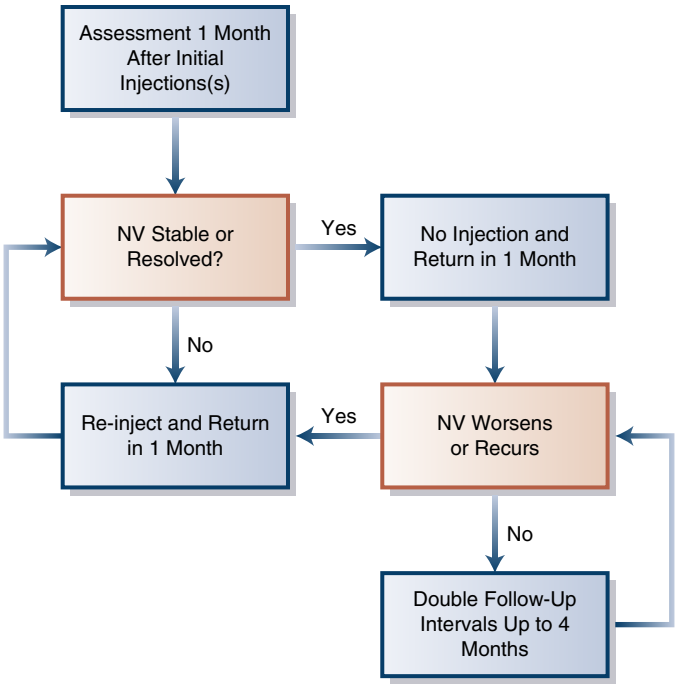
TABLE 37.5 Recommended General Management of Diabetic Retinopathy

	RISK OF PROGRESSION (%)		EVALUATION			TREATMENT			
Level of DR	To PDR (1 yr)	To High-Risk PDR (5 yr)	Color Photo	OCT	FA	Scatter Laser (PRP)	Focal Laser	Intravitreal VEGF Inhibitors	Follow-Up (mo)
Mild NPDR									
All	5	15							
No DME			No	No	No	No	No	No	12
Non-ciDME			Yes	Yes	Occ	No	Occ	Occ	4–6
ciDME			Yes	Yes	Yes	No	Occ	Yes	1–4
Moderate NPDR									
All	12–27	33							
No DME			Yes	No	No	No	No	No	6–8
Non-ciDME			Yes	Yes	Occ	No	Occ	Occ	4–6
ciDME			Yes	Yes	Yes	No	Yes	Yes	1–4
Severe NPDR									
All	52	60							
No DME			Yes	No	No	Rarely	No	No	3–4
Non-ciDME			Yes	Yes	Occ	Occ after DME treatment	Occ	Occ	2–3
ciDME			Yes	Yes	Yes	Occ after DME treatment	Yes	Yes	1–3
Very Severe NPDR									
All	75	75							
No DME			Yes	No	No	Occ	No	No	2–3
Non-ciDME			Yes	Yes	Occ	Occ after DME treatment	Occ	Occ	2–3
ciDME			Yes	Yes	Yes	Occ after DME treatment	Yes	Yes	1–3
PDR < High Risk									
All	—	75							
No DME			Yes	No	No	Occ	No	Occ	2–3
Non-ciDME			Yes	Yes	Occ	Occ after DME treatment	Occ	Occ	2–3
ciDME			Yes	Yes	Yes	Occ after DME treatment	Yes	Yes	1–3
PDR With High-Risk Characteristics									
All	—	—							
No DME			Yes	No	No	Yes	No	Occ	2–3
Non-ciDME			Yes	Yes	Occ	Yes	Usually	Occ	1–2
ciDME			Yes	Yes	Yes	Yes	Yes	Yes	1–2

ciDME, Center-involved diabetic macular edema; *DME*, diabetic macular edema; *FA*, fluorescein angiography; *ME*, macular edema; *NPDR*, nonproliferative diabetic retinopathy; *Occ*, occasionally; *OCT*, optical coherence tomography; *PDR*, proliferative diabetic retinopathy; *VEGF*, vascular endothelial growth factor.



• **Fig. 37.33** Fluorescein angiogram flow chart. The schematic flow chart details a general algorithm for appropriate use of fluorescein angiography in the ocular evaluation of patients with diabetes mellitus. In unusual cases, confounding factors can alter the appropriate approach.



• **Fig. 37.34** Principles of the Diabetic Retinopathy Clinical Research Network (DRCR.net) antivascular endothelial growth factor treatment algorithm for proliferative diabetic retinopathy. *NV,* Neovascularization. (Redrawn from Sun JK, Glassman AR, Beaulieu WT, et al. Rationale and application of the protocol S anti-vascular endothelial growth factor algorithm for proliferative diabetic retinopathy. *Ophthalmol*. 2018;125.)

preretinal hemorrhage. The treatment is thought to exert its effect by increasing oxygen delivery to the inner retina, decreasing viable hypoxic growth factor–producing cells, and increasing the relative perfusion per area of viable retina. The total treatment is usually applied over one to three sessions, spaced 1 to 2 weeks apart. Follow-up evaluation usually occurs at 3 months.

The response to scatter photocoagulation varies. The most desirable effect is to see a regression of the new vessels, although stabilization of the neovascularization with no further growth can result. This latter situation requires careful clinical monitoring. In some cases, new vessels continue to proliferate, requiring additional scatter photocoagulation or adjuvant treatment with intravitreous anti-VEGF agents (see Fig. 37.32).

Recent studies have shown that anti-VEGF treatment with aflibercept or ranibizumab is an effective first-line alternative to scatter photocoagulation in carefully selected patients with PDR[375,402,403] Neovascular processes are exquisitely sensitive to anti-VEGF agents, and eyes with severe neovascularization of the retina or anterior segment have demonstrated dramatic and rapid improvement with anti-VEGF therapy.[322,404] The Diabetic Retinopathy Clinical Research (DRCR) Network Protocol S demonstrated that visual acuity outcomes at 2 and 5 years were noninferior in eyes treated with intravitreal ranibizumab to those obtained with scatter photocoagulation. Anti-VEGF treatment with ranibizumab was associated with several advantages over laser therapy. There was greater average visual gain over the course of the first 2 years, reduced rates of visual impairment from DME onset, less loss of peripheral visual field, and decreased need for vitrectomy surgery at both 2 and 5 years in the ranibizumab group.

When delivered by a standardized retreatment algorithm such as that developed for Protocol S (Fig. 37.34), anti-VEGF therapy is highly effective at reducing retinal neovascularization. In Protocol S, 38% of the eyes treated with ranibizumab were eligible to defer monthly injections due to sustained stability of neovascularization at least once over the first 2 years of follow-up, and retinal neovascularization was completely resolved at 44% of visits through 2 years.[405] Nonetheless, eyes randomized to ranibizumab treatment in Protocol S continued to need a median of three injections per year through 2 to 5 years. It should also be realized that patients with PDR often have systemic comorbidities that may contribute to missed or rescheduled office visits. Thus patient compliance with follow-up and treatment recommendations is a critical aspect of ensuring successful outcomes with anti-VEGF therapy for PDR. If compliance is expected to be a problem for a specific patient, then treatment with laser panretinal photocoagulation alone, or in combination with a VEGF inhibitor, is the preferred therapeutic approach.

Surgical intervention with PPV is usually reserved for eyes with nonclearing vitreous hemorrhage or traction detachment from PDR. The DRVS, completed in 1989, demonstrated that early PPV in persons with severe fibrovascular proliferation was more likely to result in better vision and less likely to result in poor vision, particularly in patients with T1DM.[377] PPV aims primarily to remove abnormal fibrovascular tissue, alleviate retinal traction, allow the retina to obtain a more anatomically normal position, and remove vitreous opacities such as vitreous hemorrhage. The actual outcome data from this study is not entirely applicable today due to dramatic advances in surgical techniques and the advent of laser endophotocoagulation that have occurred in the intervening years. Nevertheless, it is clear that PPV can save and restore vision in many cases of severe retinal disease that are not amenable, or not responsive, to laser photocoagulation.

Treatment of Nonproliferative Diabetic Retinopathy

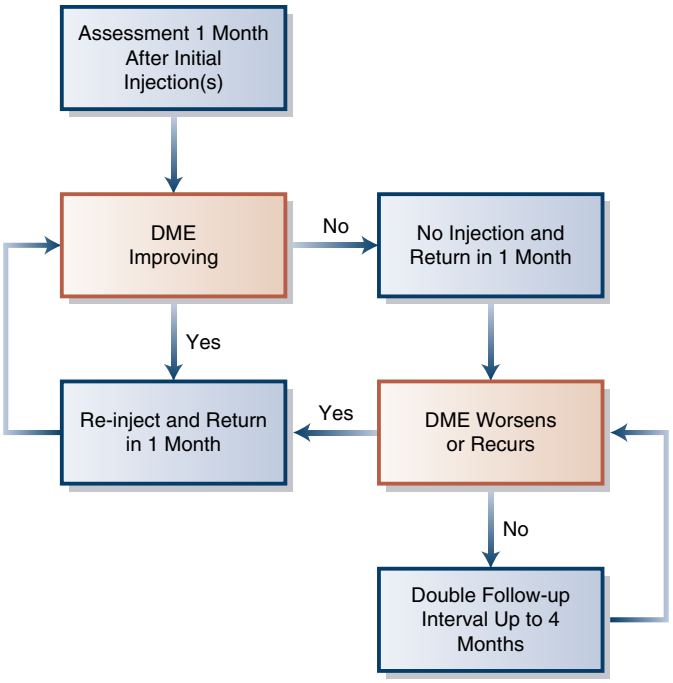
Studies of intravitreous therapy with VEGF inhibitors and steroids have demonstrated beneficial effects of these medications on DR severity and rates of progression to PDR-related complications such as vitreous hemorrhage and need for vitrectomy. Nearly 40% of eyes achieve two or more step regression of diabetic retinopathy severity after 3 years of monthly continuous anti-VEGF therapy for DME.[406–408] Decreased rates of PDR onset and need for PDR treatment are also observed after ranibizumab therapy as compared to sham treatment.[407] After an average of 4.4 injections of aflibercept over 6 months, 58% of eyes experienced a two or more step improvement in diabetic retinopathy severity as compared to 6% of sham-treated eyes.[408] Steroid therapy, whether delivered by the peribulbar or intravitreal route, also resulted in decreased risk of diabetic retinopathy worsening. Over 2 years, intravitreal steroid administration resulted in a 32% relative risk reduction for retinopathy progression as compared to sham treatment.[407] Although these results are promising, there is no treatment mandate at this time for eyes with nonproliferative diabetic retinopathy since long-term visual outcomes have not been definitively shown to be superior after treatment with any of these intraocular therapeutic approaches versus medical care alone.

Treatment of Diabetic Macular Edema

Untreated CSME is associated with an approximately 25% chance of moderate vision loss after 3 years (defined as at least doubling the visual angle; e.g., 20/40 reduced to 20/80).[316] Macular edema is best evaluated clinically by dilated examination using slit-lamp biomicroscopy or stereo fundus photography. The newer diagnostic ophthalmic imaging technique of OCT has provided a means to objectively quantify retinal thickening and is currently the objective method of choice. When used in conjunction with visual acuity measurement, OCT has been used to monitor response to treatment and help determine timing of intervention.[409,410] As discussed earlier, the current standard for macular edema evaluation focuses on determining the presence or absence of edema involving the center of the macula.

Current first-line therapy for most eyes with center-involved DME and DME-related visual impairment of 20/32 or worse is intravitreous injections of VEGF inhibitors following a defined protocol such as that described by the DRCR Network[291] (Fig. 37.35). Typically, injections are performed monthly with a loading dose of at least four to six injections. On average, eight to nine injections are performed in the first year of treatment. The average number of injections required to maintain the beneficial visual acuity gains declines substantially to three or four in the second year, one or two in the third year, and zero to one in years 4 and 5.

Results from an initial multicenter randomized controlled clinical trial evaluating intravitreal administration of ranibizumab either with immediate laser or with deferred laser show a marked benefit of the anti-VEGF agent compared to laser alone. After 1 year, ranibizumab as applied in the trial resulted in a nine-letter mean gain ($p < 0.001$) when combined either with prompt laser or deferral of laser for at least 24 weeks. This was more effective than prompt laser alone (three-letter gain) for the treatment of center-involving DME in eyes with central thickening and vision reduced to 20/32 to 20/320. The number of eyes gaining two or more lines of vision almost doubled in the anti-VEGF groups as compared with laser alone. Conversely, eyes losing two or more lines of vision were about one-third as many in the anti-VEGF groups as compared to laser alone. Results were sustained over 5 years despite a substantially decreased number of injections



• **Fig. 37.35** Diagram showing the Diabetic Retinopathy Clinical Research Network (DRCR.net) rationale for treatment and follow-up of center-involved diabetic macular edema (DME) with antivascular endothelial growth factor therapy. (Modified from Aiello LP, Beck RW, Bressler NM, et al. Rationale for the Diabetic Retinopathy Clinical Research Network intravitreal anti-VEGF treatment and follow-up protocol for center-involved diabetic macular edema. *Ophthalmol.* 2011;118:e5–e14.)

throughout years 2 to 5.[411,412] No increased systemic events or serious ocular adverse events were attributed to the treatment other than the known small risk of endophthalmitis (1 per 1000 injections) associated with intravitreal injections themselves.

A head-to-head comparative effectiveness trial demonstrated overall efficacy of all three currently commercially available intraocular VEGF inhibitors (aflibercept, bevacizumab, and ranibizumab) in improving visual acuity and reducing retinal thickening in eyes with center-involved DME.[291] Eyes with only mild visual impairment from DME and baseline vision of 20/32 to 20/40 had similar visual acuity outcomes on average regardless of therapeutic group assignment. However, in eyes with baseline vision of 20/50 or worse, aflibercept provided visual gains superior to those achieved with bevacizumab at 1 and 2 years, and vision outcomes were superior to ranibizumab at 1 but not 2 years.

Although VEGF inhibitors are highly successful in many eyes, approximately 40% to 50% of eyes still have persistent DME or visual impairment despite chronic monthly therapy. Thus there is a continued unmet need for novel therapies that address VEGF-independent mechanisms for DME onset, persistence, and worsening.

Focal laser photocoagulation may still be indicated for some patients with DME (see Figs. 37.27C and 37.30) without center involvement or in eyes with good vision or in patients who cannot tolerate a regimen of intravitreal injections. The ETDRS demonstrated focal laser photocoagulation for CSME reduced the 5-year risk of moderate vision loss by 50%, but 15% of patients continue to experience vision loss.[345] In focal laser photocoagulation, lesions from 500 to 3000 μm from the center of the macula that are contributing to thickening of the macular area are generally

directly photocoagulated. These lesions are identified clinically or by fluorescein angiography and consist primarily of leaking microaneurysms. When leakage is diffuse or microaneurysms are extensive, photocoagulation may be applied to the macula in a grid configuration, avoiding the fovea region.

If macular laser is planned, fluorescein angiography can be a useful test for guiding the focal treatment of microaneurysm that are leaking fluid into the retina and identifying areas of macular capillary nonperfusion that might benefit from grid laser treatment (see Fig. 37.33). Because there are risks associated with fluorescein angiography, including nausea, urticaria, hives, and rarely death (1 in 222,000 patients) or severe medical sequelae (1 in 2000 patients),[413–415] fluorescein angiography is not otherwise part of the routine examination of a diabetic patient without diabetic retinopathy, and the procedure is usually contraindicated in patients with known allergy to fluorescein dye or during pregnancy.

Follow-up evaluation after focal laser treatment generally occurs after 3 months (see Fig. 37.30). In cases in which macular edema persists, further treatment may be necessary. In the presence of macular edema, patients with PDR or severe NPDR should be considered for anti-VEGF or laser treatment of macular edema whether or not the macular edema is center involved because they are likely to require scatter laser photocoagulation in the near future and because scatter photocoagulation, while beneficial for PDR, can exacerbate existing macular edema.

The ophthalmic use of corticosteroids administered either through the periocular or intravitreal routes for the treatment of DME gained widespread use due to early case reports and uncontrolled clinical trials documenting its rapid and often dramatic effect on retinal thickening. Two multicenter randomized prospective clinical trials were undertaken to address both the effectiveness and safety of both routes of steroid administration. In the first, peribulbar steroid injections were found to have no significant benefit for the treatment of DME.[416] The 3-year results of a second multicenter randomized controlled trial comparing intravitreal steroids to focal laser therapy have shown that despite an initial rapid reduction in retinal thickness and improvement in vision with the intravitreal steroid injection, by 1 year the results were no better than laser photocoagulation, and at 2 years through 3 years, steroid was inferior to the laser treatment in both visual outcome and retinal thickness improvement.[417,418] Intravitreal steroid injections were associated with an approximately fourfold increase in the rate of intraocular pressure complications and fourfold increase in need for cataract surgery compared to laser treatment. The development of cataract was likely a large contributor to declining visual acuity in the steroid-treated group after the first 6 months. This hypothesis is supported by the fact that patients who had already undergone cataract surgery prior to initiating intravitreal steroid treatment demonstrated vision improvement comparable to that seen in the anti-VEGF-treated group.[375]

Intravitreous steroid therapy has also been investigated as a potential additive therapy to intravitreous VEGF inhibitors in patients who have persistent retinal thickening and visual impairment despite at least six injections of anti-VEGF. Although retinal thickening is significantly reduced in eyes after combined steroid and anti-VEGF therapy compared to those receiving anti-VEGF treatment alone, visual acuity outcomes do not appear to be improved.[419] Thus, currently, intravitreal steroid alone is not the preferred primary therapy for DME or a recommended adjuvant therapy in eyes that have not responded successfully to anti-VEGF therapy. Intraocular steroid treatment may have a role, however, for treatment of patients with DME who cannot receive anti-VEGF agents or who are pseudophakic before treatment.

The pathogenesis of DME is highly complex, and a variable response to treatment modalities has been observed in many patients. Investigations of varying drug dosages to identify the optimal treatment concentration and development of sustained drug delivery devices to limit risks, costs, and inconvenience associated with repeated intraocular injections are underway. Multiple potential therapeutics are also being tested in clinical trials for the treatment of DME, including a bispecific antibody against VEGF and angiotensin II, plasma kallikrein inhibition, photobiomodulation therapy, and other novel approaches.

Control of Systemic Disorders and Effect of Systemic Medications

In addition to the importance of intensive glycemic control in reducing the onset and progression of diabetic retinopathy as discussed earlier, it is critical for the optimal ocular health of diabetic patients that several other systemic considerations be optimized.

Elevated blood pressure exacerbates the development and progression of diabetic retinopathy. Concomitant hypertension is common in diabetes. Patients with T1DM have a 17% prevalence of hypertension at baseline and a 25% incidence after 10 years.[420] There is a 38% to 68% prevalence in T2DM.[421–423] In most studies, hypertension correlates with other retinopathy risk factors, including duration of diabetes, higher HbA_{1c} level, presence of proteinuria, and male gender. The risk of PDR is associated with the presence of hypertension at the baseline visit, higher HbA_{1c} levels, and presence of more severe levels of retinopathy at the initial visit.[424] Patients with hypertension are more likely to develop retinopathy, diffuse macular edema, and more severe levels of retinopathy[425–427] and have more rapid progression of retinopathy when compared with diabetic patients who do not have hypertension.[427–429]

The large randomized, prospective UKPDS in 1148 patients with T2DM demonstrated a 34% ($p = 0.0004$) and 47% ($p < 0.004$) reduction in risk of diabetic retinopathy progression and moderate visual acuity loss, respectively, in patients assigned to intensive blood control.[430] Effects were independent of glycemic control, and risk reductions were similar regardless of whether the hypertension was controlled with an angiotensin-converting enzyme (ACE) inhibitor (captopril) or a beta blocker (atenolol). Overall, hypertension appears to be a significant risk factor in the development and progression of diabetic retinopathy and should be rigorously controlled. Until the results of specific trials investigating the blood pressure levels required to minimize end-organ damage in patients with diabetes are known,[431] target blood pressure should most likely be maintained as low as safely possible.

Associations between renal and retinal angiopathy are numerous. Both microalbuminuria and proteinuria are associated with retinopathy.[432–435] The presence and severity of diabetic retinopathy are indicators of the risk of gross proteinuria,[436,437] and conversely, proteinuria predicts PDR.[433,438,439] Half of all T1DM patients with PDR and 10 or more years of diabetes have concomitant proteinuria.[432] In T1DM, the prevalence of PDR increases from 7% at onset of microalbuminuria to 29% 4 years after onset of albuminuria, compared with 3% and 8%, respectively, in patients without persistent microalbuminuria.[440] The Appropriate Blood Pressure Control in Diabetes (ABCD) trial found both the severity and progression of retinopathy were associated with overt albuminuria.[441–443] The presence of gross proteinuria at baseline is associated with 95% increased risk of developing macular edema among patients with T1DM,[424] and dialysis can improve macular edema in diabetic patients with renal failure.

Despite these associations, a causal relationship between diabetic kidney disease and diabetic retinopathy has not been established. The frequent coexistence of retinal and renal microangiopathies with factors that affect both complications, such as associated hypertension and disease duration, make it difficult to establish.[444] Overall, it is important to carefully consider the renal status of any patient with diabetes and to ensure that the patient is receiving optimal care in this regard. In addition, rapidly progressive retinopathy, especially in a patient with a long history of diabetes where retinopathy previously has been stable, should suggest the need for renal evaluation.

Low hematocrit was an independent risk factor in the ETDRS analysis of baseline risk factors for development of high-risk PDR and severe vision loss.[445] A cross-sectional study involving 1691 patients revealed a twofold increased risk of any retinopathy in patients with a hemoglobin level less than 12 g/dL compared to those with a higher hemoglobin concentration, using multivariate analyses controlling for serum creatinine, proteinuria, and other factors.[446] In patients with retinopathy, those with low hemoglobin levels have a fivefold increased risk of severe retinopathy compared with those with higher hemoglobin levels. There have been limited reports of resolution of macular edema and hard exudate with improvement or stabilization of visual acuity in erythropoietin-treated patients after an increase in mean hematocrit.[447] In view of the potential association of low hematocrit and diabetic retinopathy, it is important to ensure that patients with diabetic retinopathy and anemia are receiving appropriate management.

In summary, diabetes is clearly a multisystem disease requiring a comprehensive medical team approach. Even with regard to ocular health, this necessitates the involvement of multiple health care specialists for optimal patient care.

Diabetic Nephropathy

Diabetic nephropathy remains a major cause of morbidity and death for persons with either T1DM or T2DM. In Western countries, diabetes is the leading single cause of end-stage renal disease.[210] Indeed, in many countries such as the United States, more than 50% of patients in renal replacement therapy programs have diabetes as the major cause of their renal failure. However, the full impact of diabetic nephropathy is far greater.[448] Globally most patients with diabetes are in developing countries that do not have the resources or health infrastructure to provide universal renal replacement therapy.[449] Even in developed countries, for every 20 patients with diabetes and chronic kidney disease, less than one will survive to end-stage renal disease, succumbing instead to atherosclerotic cardiovascular disease, heart failure, or infection. Furthermore, the presence and severity of diabetic renal disease significantly contributes to the risk of these conditions. For example, almost all of the excess in cardiovascular deaths in persons with diabetes younger than 50 years can be attributed to nephropathy.[450] Indeed, in T1DM subjects without nephropathy there may be a lower risk of premature death,[451] although a recent review of the FinnDiane cohort indicates that in nonalbuminuric T1DM subjects there is still evidence of increased mortality.[452] In patients with T2DM, microalbuminuria is associated with a twofold to fourfold increase in the risk of death. In patients with overt proteinuria and hypertension, the risk is even higher.[453] Consequently, the goal to reduce ESRD in patients with diabetes is only one component as part of the overall benefit in preventing diabetic kidney disease.

It is estimated that 25% to 40% of patients with T1DM and 5% to 40% of patients with T2DM ultimately develop some features of diabetic kidney disease.[454,455] Over 20% of patients with T2DM already have diabetic kidney disease when they are diagnosed with diabetes,[456] and a further 20% to 40% develop diabetic nephropathy, mostly within 10 years of diagnosis. Although nephropathy appears be more common in T1DM, because of the large and increasing number of persons with T2DM,[449,457] more than 80% of diabetic patients in renal replacement programs have T2DM.

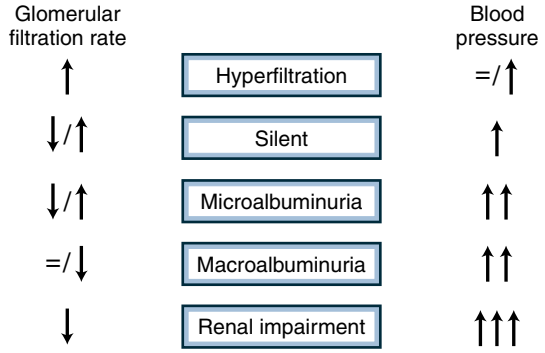
Natural History of Nephropathy in Type 1 Diabetes

Nephropathy and specifically proteinuria in the setting of diabetes have been known for more than 100 years, and the classic structural features of glomerulosclerosis were described more than 70 years ago.[458] However, it is only since the 1980s that the natural history of this condition has been extensively delineated. This is partly because significantly more patients are surviving to see the full presentation of this condition. For example, in 1971, the median survival time of patients with T1DM and overt nephropathy was 5 years, with less than 10% surviving more than 10 years. Consequently, few patients were able to survive the course of their renal disease. By comparison, in 1996, the median survival in an equivalent population was more than 17 years. Not surprisingly, nearly 10 times more patients with T1DM are now entering ESRD programs.

Diabetic nephropathy is characterized clinically as a triad of hypertension, proteinuria, and ultimately renal impairment.[459] The classic five stages of nephropathy as described by Mogensen and colleagues,[460] although not totally accurate, remain the best way of describing this condition (Fig. 37.36). This description relies on functional evaluation of the renal disease and is based on serial measurements of glomerular filtration rate (GFR) and albuminuria.

Stage 1: Hyperfiltration

The initial phase has been termed the *hyperfiltration* phase. It is associated with an elevation of glomerular filtration rate[460] and presumably an increase in capillary glomerular pressure. Although invariably present in animal models of T1DM,[461] an elevation of GFR occurs in up to 40% of type 1 diabetic patients. Hyperfiltration is considered to occur as a result of concomitant renal hypertrophy[462] and partly due to a range of intrarenal hemodynamic abnormalities that occur in the diabetic milieu that contribute to glomerular hypertension.[461] The pathophysiology of



• **Fig. 37.36** The phases (natural history) of diabetic nephropathy.

renal hypertrophy associated with diabetes remains unexplained, although specific growth factors such as the growth hormone (GH)/insulin-like growth hormone (IGF1) system and transforming growth factor β (TGFβ) have been implicated.[463,464] Notably, there is not only glomerular but also tubular hypertrophy. Indeed, the tubular hypertrophy explains the increased kidney weight in diabetes because tubules make up more than 90% of the kidney weight.[465] In addition, increased salt reabsorption associated with proximal tubular hypertrophy can also contribute to glomerular hyperfiltration via tubuloglomerular feedback.[463]

The second explanation for the increase in GFR associated with diabetes relates to hemodynamic changes within the kidney. Although not directly tested in humans, micropuncture studies in rodents, particularly by Brenner's group in the 1980s, revealed that experimental diabetes was associated with a range of intrarenal hemodynamic changes.[461] Alongside hyperfiltration, there is an increase in effective renal plasma flow, and thus some investigators call this the *hyperperfusion-hyperfiltration* phase of diabetic nephropathy. At the same time, intraglomerular capillary pressure is increased, reflecting relative efferent versus afferent arteriolar vasoconstriction[461] with activation of the intrarenal renin-angiotensin system (RAS) and reduced synthesis of the vasodilator nitric oxide.

The importance of this hyperfiltration phase in predicting and leading to diabetic nephropathy remains controversial. Several groups have confirmed the initial relationship between elevated GFR and later development of proteinuria as described by Mogensen and Christensen.[466] However, this has not been a universal finding. Nevertheless, subsequent studies with antihypertensive agents, and in particular agents that interrupt the renin-angiotensin system, have shown attenuation of some of these glomerular hemodynamic abnormalities.[467] This provides justification to consider that at least some of these intrarenal hemodynamic changes in diabetes play a role in the development and progression of nephropathy. However, in insulin-dependent diabetic patients the ACE inhibitor captopril had the smallest effect (17%) in reducing the risk of doubling serum creatinine in patients with serum creatinine of 1 mg/dL and the largest effect (76%) in patients whose serum creatinine was 2 mg/dL. These data do not seem to support a primary role for early hyperfiltration in the development of early diabetic nephropathy. Results suggest that in patients with type 2 diabetes with advanced renal disease, a link to hyperfiltration was not apparent.[468,469]

This field has recently been reactivated with the advent of sodium glucose cotransporter-2 (SGLT2) inhibitors, which influence tubuloglomerular feedback and have been shown to reduce intraglomerular pressure via effects on afferent arteriolar dilation.[470] Indeed, these agents have impressive effects in attenuating diabetes-associated hyperfiltration, as reported initially in T1DM but subsequently in T2DM.[471,472] Whether these hemodynamic benefits explain, at least in part, the renoprotection seen with these agents in large clinical studies, including Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients–Removing Excess Glucose (EMPA-REG OUTCOME) and Canagliflozin Cardiovascular Assessment Study (CANVAS), remains to be determined.[473,474]

Stage 2: The Silent Stage

The next stage is known as the *silent stage,* where, from a clinical point of view, there is no overt evidence of any form of renal dysfunction. Patients usually have normal GFR with no evidence of albuminuria. However, this phase is associated with significant structural changes, including basement membrane thickening and mesangial expansion. More recently, loss of the glomerular cells known as podocytes has also been described as a relatively early structural change in the diabetic kidney. Indeed, by performing detailed quantitative studies of renal morphology it is often possible to detect those who will develop renal damage.[475] This is a very important phase clinically because it is hoped that investigators will be able to develop new tests, such as biomarkers in plasma or urine or sophisticated assessments from renal biopsy material, to identify which patients will progress to more advanced renal disease. Because overall less than 40% of T1DM subjects will progress, it is critical that we detect those who are the potential progressors and could be candidates for early prevention and treatment strategies to avoid end-stage renal disease. As yet, no reliable clinically translatable surrogate markers or predictors have been identified as worth pursuing in clinical practice during this silent phase of the disease.

Extensive studies have tested various plasma markers, such as prorenin,[476] tumor necrosis factor α (TNFα) receptor,[477] advanced glycation end products,[478] and precursors such as methylglyoxal,[479] but the predictive value has not been conclusive. Although diabetic kidney disease probably has at least in part a genetic basis, few potentially relevant gene polymorphisms such as the angiotensin-converting enzyme gene have been identified.[480] Recent genome-wide association studies have not generally been very helpful.[481,482] The measurement of albumin fragments (ghost albumin) in the urine of patients with diabetes may be another, albeit unproven, approach.[483] Serial prospective ambulatory blood pressure monitoring studies have also demonstrated modest rises in blood pressure in patients in this silent phase up to 5 years before urinary albumin excretion begins to increase.[484] However, none of these markers has been proved to be sensitive or specific enough on further clinical evaluation for widespread clinical application.

Stage 3: Microalbuminuria

The third phase is known as *microalbuminuria* or the stage of *incipient nephropathy.* At this stage, often 5 to 15 years after the initial diagnosis of T1DM, the urinary albumin excretion rate has increased into the microalbuminuric range of 20 to 200 μg per minute or 30 to 300 mg per 24 hours.[485] In the past, microalbuminuria was considered to be a predictor rather than a manifestation of diabetic kidney disease. Increasingly it has been appreciated, particularly when based on interpretation of renal morphologic studies, that in the microalbuminuric phase there is often but not always widespread evidence of advanced glomerular structural changes.[486,487] Concomitant with these changes, systolic and diastolic blood pressures are increased. Furthermore, the nocturnal dip in blood pressure seen in normal persons is often lost with the development of microalbuminuria.[488] Renal function during this phase may be increased, normal, or reduced.

The best approach to screen for microalbuminuria remains controversial. The original studies used 24-hour or overnight urine sampling methods. However, a spot urine albumin:creatinine ratio in an early-morning urine specimen has been validated and appears to be a practical option for routine clinical practice.[210,485] Because the onset of persistent microalbuminuria, if left untreated, is often a reliable harbinger of overt nephropathy,[466] it is incumbent on clinicians to perform serial measurements of this parameter and to repeat the measurement if there is an isolated elevation in urinary albumin excretion.

Studies suggest that in many patients with T1DM, microalbuminuria can be transient and can reverse to normoalbuminuria.[489]

Thus the onset of microalbuminuria does not irrevocably seal the fate of the patient. A study of 386 patients with persistent microalbuminuria at study entry showed that regression of microalbuminuria occurred in 58% of patients,[489] although other groups have reported much lower rates of this phenomenon.[490] Notably, in that study, microalbuminuria of short duration, optimal levels of HbA_{1c} (<8%), low systolic blood pressure (<115 mm Hg), and low levels of both cholesterol and triglycerides were independently associated with the regression of microalbuminuria. Therefore screening of diabetic patients for nephropathy is now recommended to include at least twice-annual measurements of urinary albumin concentrations in T1DM patients.

Stage 4: Macroalbuminuria

The next stage is the *macroalbuminuria* phase or *overt nephropathy*. This stage represents the phase that has been previously described as diabetic nephropathy and is highly predictive of subsequent renal failure if left untreated. It is characterized by a urinary albumin excretion rate greater than 300 mg per 24 hours (200 μg/minute). This phase usually occurs after 10 to 15 years of diabetes, but the risk of overt renal disease never truly disappears and can appear after 40 or 50 years of T1DM.

There are at least two peaks of incidence of overt nephropathy, and this has been termed by some investigators as representing slow and fast trackers.[491] The key contributors to this marked variation in the timing of onset of proteinuria, independent of glycemic or blood pressure control, remain elusive, although a range of genetic, molecular, and environmental factors have been proposed. In association with this increase in proteinuria, more than two-thirds of patients have overt systemic hypertension.[492] During this phase, if left untreated, blood pressure continues to rise, accelerating the decline in GFR, which promotes a further rise in blood pressure, creating a vicious cycle of progressive renal impairment that ultimately leads to end-stage renal disease.

Stage 5: Uremia

The final *uremic* phase, which historically occurred in up to 40% of T1DM subjects,[454] requires the institution of renal replacement therapy. As recently as the 1970s, patients with diabetes were not considered candidates for renal replacement therapy because of their abysmal prognosis. However, improvements in the management of cardiovascular disease and renal replacement options have seen the survival on dialysis approach that of patients with renal disease from other causes. Many patients with diabetes and end-stage renal disease are also now considered candidates for renal transplantation, which is associated with better outcomes than remaining on dialysis. However, there is evidence that the renal lesions of diabetes often recur in the transplanted kidney, though the lead time to develop ESRD means that few kidneys are lost through recurrent disease.

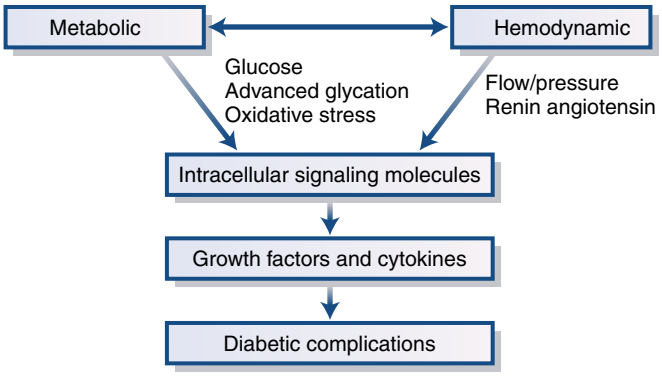
Increasingly, single pancreas-kidney (SPK) and pancreas-after-kidney (PAK) transplantation have become therapeutic options for patients with T1DM and end-stage renal disease, and these newer options appear to offer advantages over kidney-alone transplantation. In particular there is some evidence that maintaining euglycemia following pancreas transplantation can lead to resolution of many diabetes-related renal lesions such as mesangial expansion.[493] This reversal is often not apparent until after 10 years of euglycemia, emphasizing the slow turnover of matrix and the potential long-term effects of hyperglycemic memory on the kidney.

Natural History of Nephropathy in Type 2 Diabetes

The natural history of diabetic nephropathy in patients with T2DM is less well understood than in patients with T1DM. This partly reflects the fact that T2DM is largely a disease of an older population, with associated obesity, hypertension, dyslipidemia, and high rates of cardiovascular disease, which are all factors associated with increased risk of renal disease. In addition, approximately 7% of patients with T2DM already have microalbuminuria at the time of diagnosis. This may be partly related to the fact that most of these patients have had untreated diabetes for 5 to 10 years (on average) before diagnosis. Within 5 years of a diagnosis of T2DM, up to 18% of patients have microalbuminuria, especially those with poor metabolic control and high blood pressure. This has led some investigators to suggest that nephropathy in T2DM is different from that seen in patients with T1DM.

However, the natural history of nephropathy in T2DM has more similarities than differences from that seen in T1DM. Hyperfiltration does occur in T2DM,[494] although it has been reported to be less commonly observed than in T1DM. This observation must be interpreted with caution because GFR normally declines with age, and hyperfiltration can still exist, although the GFR remains in the normal adult range. Microalbuminuria also occurs in T2DM. However, the finding of microalbuminuria in T2DM might not be as specific for diabetic renal disease as described in the seminal studies in T1DM. In the context of a very high prevalence of cardiovascular disease, microalbuminuria may be more closely associated with nonrenal events such as stroke and myocardial infarction.[439] Furthermore, incipient or overt cardiac failure, urinary tract infection, and urinary obstruction (e.g., enlarged prostate) can also lead to microalbuminuria.

Many patients with T2DM and microalbuminuria also progress to overt proteinuria. However, it is increasingly appreciated that the situation has become more complex, and many groups have now described subjects with T1DM[495] as well as T2DM[496] who develop renal impairment with progressive decline in GFR in the absence of significant proteinuria. The exact explanation for this phenomenon is unknown, and ongoing studies are exploring if these patients have different renal morphologic changes from those with the more classic syndrome of diabetic nephropathy: overt proteinuria and declining GFR. Preliminary studies suggest a prominent vascular component for this form of nonproteinuric renal dysfunction. Nonetheless, it appears that the risk of end-stage renal disease in patients with T2DM and renal impairment is similar in the presence or absence of microalbuminuria, underlining the importance of an estimated GFR in the management of patients with T2DM. This has led to many of the national and international guidelines now recommending the inclusion of regular measurements of serum creatinine and determination of estimated GFR using a variety of different formulas. The frequency of these measurements differs among the various guidelines, but at a minimum these measurements should be performed on a yearly basis. Importantly, in T2DM, many subjects will develop a progressive decline in GFR without the development of albuminuria. Indeed, recent renal biopsy studies although not conclusive suggest that there may be a difference in renal morphologic lesions between albuminuric and nonalbuminuric forms of diabetic kidney disease.[497]



• **Fig. 37.37** Interactions between metabolic and hemodynamic factors in promoting diabetic complications, including nephropathy.

Pathogenesis of Diabetic Nephropathy

It is likely that many of the mechanisms implicated in diabetic microvascular complications, in general, play a central role in the development and progression of diabetic nephropathy (Fig. 37.37).[43] It is evident that hyperglycemia is necessary for the initiation of renal injury, because patients without diabetes do not develop this type of nephropathy. Moreover, intensive therapy designed to achieve improved glycemic control is able to attenuate the development of nephropathy, as assessed by urinary albumin excretion, although it is not fully prevented.[334] However, it is now clear that other factors must also be involved because continuous florid hyperglycemia is not necessarily required for diabetic hyperfiltration and kidney growth to occur. Indeed, glomerular hyperfiltration and tubular hypertrophy can persist in patients with T1DM even after euglycemia is achieved through aggressive insulin therapy.[498]

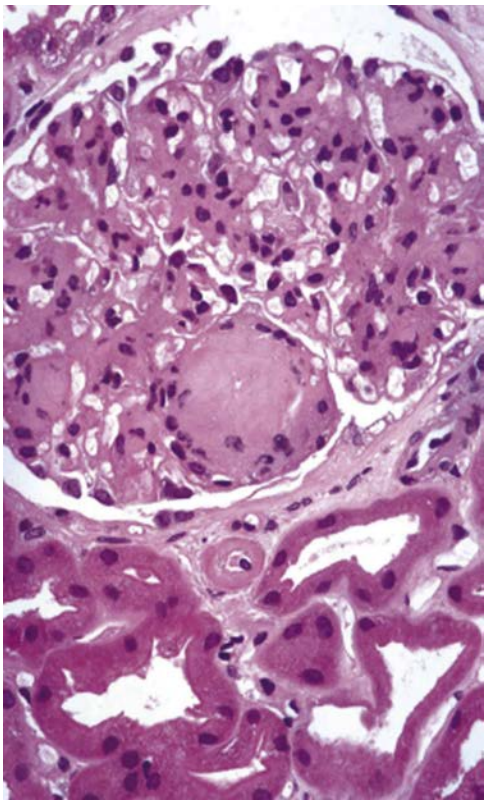
Other pathways that may be involved in diabetic nephropathy include generation of mitochondrial ROS, accumulation of AGEs, and activation of intracellular signaling molecules such as PKC.[131,499] Many of the seminal studies performed in endothelial cells demonstrating a central role for mitochondrial ROS in activating pathways implicated in diabetic vascular complications have been reproduced in mesangial cells.[500] Advanced glycation, which occurs at an accelerated rate in diabetic patients, is a prominent phenomenon in the kidney. Not only is the kidney the major site for excretion of AGEs, but also many of the proteins with a long life, such as collagen, are extensively glycated in patients with diabetes.[501] Furthermore, various AGE receptors (e.g., RAGE) have been described in the kidney, which appear to play a role in mediating some of the deleterious effects of AGEs, such as stimulation of growth factor expression and induction of important phenotypic changes within certain renal cell populations to promote scarring.[56] Preliminary studies have used various approaches to inhibit renal AGE accumulation and action, including a form of soluble RAGE (sRAGE). A range of pharmacologic agents has shown promising results, but clinical translation of these findings remains to be fully defined.[501] Selective PKC isoform inhibitors have been evaluated in small clinical trials, but their role in renal disease remains controversial.[502] Some exciting pilot studies evaluating a number of cytosolic sources of oxidative stress, such as nicotinamide adenine dinucleotide phosphate oxidase, suggest that certain NADPH oxidase isoforms such as Nox4 may be excellent targets for new renoprotective therapies.[503] This is strengthened by the advent of orally bioavailable NADPH oxidase inhibitors,[136] which are now under clinical investigation.

In addition to the mechanisms described here, the diabetic kidney appears to be readily modulated by a range of vasoactive hormones. Indeed, it is increasingly appreciated that there may be important interactions between metabolic pathways and various hemodynamic factors, including vasoactive hormones such as angiotensin II in mediating renal injury in diabetes (see Fig. 37.37).[504,505] Although many drugs that modulate hormone levels or action might not be specific for diabetic kidney disease, interruption of the renin-angiotensin system appears to be an excellent approach not only for reducing blood pressure but also for correcting many of the cellular, biochemical, hemodynamic, and structural abnormalities seen in the diabetic kidney. These agents appear to be very powerful antiproteinuric agents at all stages of renal disease, including microalbuminuria and macroalbuminuria, although the exact mechanism of action remains to be fully defined. Furthermore, these agents influence renal structure although these findings are primarily deduced from preclinical studies. In addition, as seen in the Reduction in Endpoints in Noninsulin-Dependent Diabetes Mellitus with the Angiotensin II Antagonist Losartan (RENAAL) study,[506] they also attenuate decline in renal function in type 2 subjects with advanced renal disease. Based on the discovery in the late 1990s that proteinuria in a range of nephropathies could occur as a result of molecular and structural abnormalities in a highly specialized structure known as the slit diaphragm within the glomerular epithelial cell (podocyte), a number of experimental studies, subsequently confirmed in humans, showed that depletion of one of these slit pore proteins, nephrin, could be attenuated or prevented by agents that interrupt the RAS.[507]

In addition to promoting glomerular nephrin depletion, angiotensin II also appears to have other actions that promote the development of proteinuria, including trophic effects on the kidney and increasing glomerular membrane pore size.[508] Although many investigators have focused on the RAS and, in particular, the vasoconstrictor angiotensin II, it is increasingly appreciated that other vasoconstrictors may be important. These include endothelin and a number of vasodilators, such as nitric oxide, bradykinin, atrial natriuretic peptide, and vasodilatory angiotensins such as angiotensin 1-7.[509] This exploration of the role of vasoactive hormones and their respective receptors in the diabetic kidney is critical for designing new treatments for this condition, because these pathways are ideal targets for drug development. This point has already been demonstrated for agents that interrupt the renin-angiotensin system, including ACE inhibitors and ARBs. However, the use of dual RAS inhibition, specifically the combination of an ACE inhibitor and an ARB, despite positive effects on albuminuria,[510] is not recommended due to increased risks of hyperkalemia and acute kidney injury, as was also seen with RAS blockade with a combination of a renin inhibitor and an ARB.[511]

Pathology of Diabetic Renal Disease

Diabetic renal disease was originally described as a glomerulopathy associated with diffuse or nodular glomerulosclerosis.[458] Subsequent studies using electron microscopy have revealed that glomerular basement membrane thickening and mesangial expansion are prominent glomerular abnormalities in diabetes[475] (Fig. 37.38). Indeed, prospective studies have shown that these changes predict to a certain degree the development of overt renal disease in patients with T1DM. However, fewer than one-third of diabetic patients with microalbuminuria have the



• **Fig. 37.38** Glomerular and tubular manifestations of diabetic nephropathy. *GBM,* Glomerular basement membrane; *TBM,* tubular basement membrane.

typical glomerulopathy described by Kimmelsteil and Wilson in 1936.[458,512] Although initial studies emphasized the mesangial cell changes in the glomerulus, glomerular epithelial cell abnormalities represent new areas of active research.[507] Podocyte dysfunction and subsequent apoptosis, ultimately leading to depletion of podocytes within the glomerulus appears to play a pivotal role in the development of proteinuria in diabetes.[513]

Although most of the focus has been on glomerular changes in the diabetic kidney, more recent studies have identified important changes in the other sites within the kidney, including the tubules, interstitium, medulla, and papilla.[465] *Diabetic tubulopathy* is characterized by a variety of structural and functional changes, including tubuloepithelial cell hypertrophy, tubular basement membrane thickening, epithelial-mesenchymal transition,[514] and the accumulation of glycogen (see Fig. 37.38). There is also an expansion of the interstitial space with infiltration of various cell types, including myofibroblasts and macrophages.

These tubular changes represent more than just the aftermath of diabetic nephropathy. The dysregulation of tubular functions in diabetes can precede or at least accompany the changes in the renal glomerulus and the onset of albuminuria.[512] Indeed, the functional and structural changes in the proximal tubule may be key to contributing to the development and progression of diabetic nephropathy.[465] For example, it has been suggested that tubuloglomerular feedback mechanisms can drive hyperfiltration associated with diabetes[462] and that tubular dysfunction can contribute to albuminuria due to defective uptake and lysosomal processing.[483] Indeed, renal function and prognosis correlate better with structural lesions in the tubules and cortical interstitium than with classic glomerular changes of diabetic nephropathy.

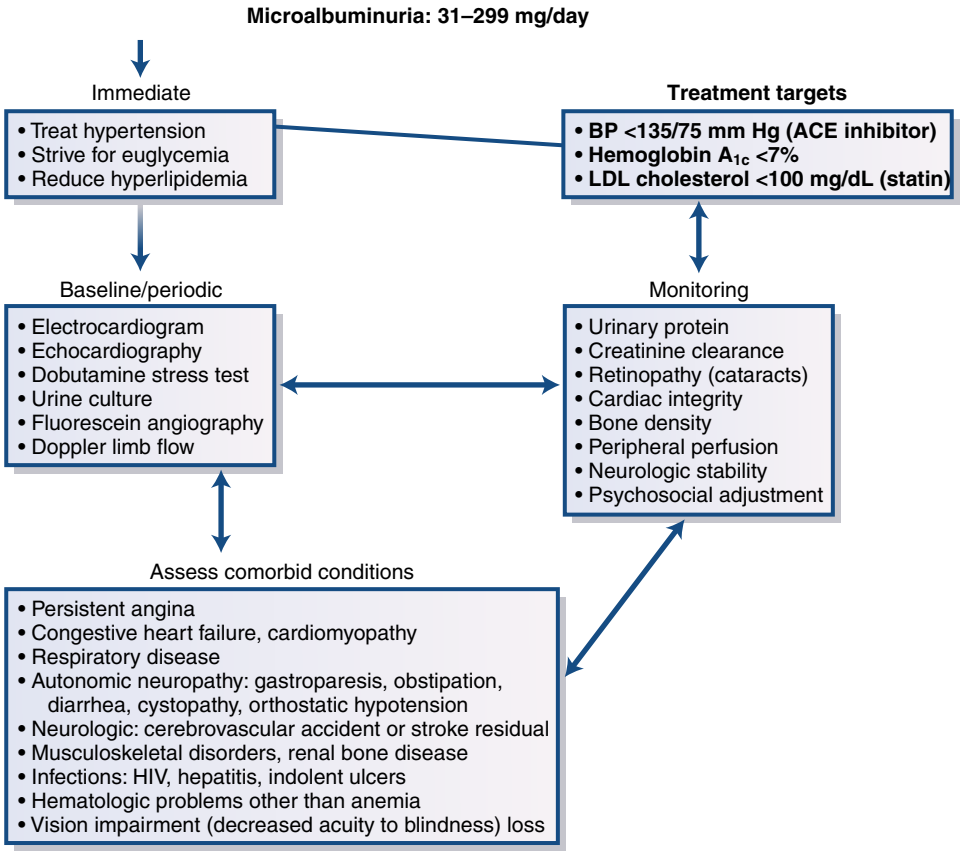
Other Renal Manifestations of Diabetes

Renal Artery Stenosis

Because diabetic patients have, in general, an increased burden of atherosclerosis, they appear to have a higher risk of renal artery stenosis. However, although angiographic studies have demonstrated a high prevalence of renal artery stenosis in diabetic patients, these lesions are often of no hemodynamic significance. Nevertheless, a small subgroup will have a hemodynamically significant stenosis, enhancing hypertension, increasing the risk of acute pulmonary edema, and inducing progressive renal impairment.[515] In such subjects, specific interventions such as surgery or angioplasty need to be considered.[516] Furthermore, some patients have bilateral renal artery stenosis that, on commencement of an agent such as an ACE inhibitor, can lead to acute renal failure.[517] Fortunately, in most patients, if the renal failure is diagnosed early, cessation of the ACE inhibitor leads to rapid restoration of renal function in this situation.

Renal Papillary Necrosis

Renal papillary necrosis involves a severe destructive process, presumably as a result of ischemia to the medulla and papilla.[518] Beethoven's final illness might have been papillary necrosis in the context of diabetes.[519] The papilla is very sensitive to these ischemic changes because even in the normal setting it is exposed to a relatively hypoxic environment. Concomitant exacerbating factors include urinary tract infection and analgesic abuse. The importance of ischemia and possibly angiotensin II in this disorder has been suggested in experimental studies in transgenic rats that overexpress renin and angiotensin II in their kidney after induction of diabetes.[520] In these rats,



• **Fig. 37.39** Flow chart illustrating the management of diabetic nephropathy before the onset of renal failure. *ACE,* Angiotensin-converting enzyme; *BP,* blood pressure; *HIV,* human immunodeficiency virus; *LDL,* low-density lipoprotein.

diabetes was associated with development of papillary necrosis, which was prevented by blockade of the renin-angiotensin system. Clinically, papillary necrosis often manifests as flank pain, hematuria, and fever. Urinalysis reveals red and white blood cells, bacteria, and papillary fragments. Ureteric obstruction can occur as a result of these fragments and must be addressed as an emergency.

Renal Tubular Acidosis

A well-known functional abnormality associated with diabetic tubulopathy is *renal tubular acidosis (type 4)*, manifesting as hyperkalemia and hyperchloremic metabolic acidosis.[521] This is thought to be a manifestation of hyporeninemic hypoaldosteronism associated with diabetes, resulting in proximal tubule ammonia production being reduced to levels inadequate to buffer acid in the distal nephron. The precise cause of this abnormality remains to be established. In some patients, there appears to be a defect in the conversion of prorenin to active renin.[522] It has also been suggested that damage to the tubular cells of the juxtaglomerular apparatus associated with diabetes can contribute to impaired renin release, possibly due to reduced renal prostaglandin production and elevated vasopressin levels.[523]

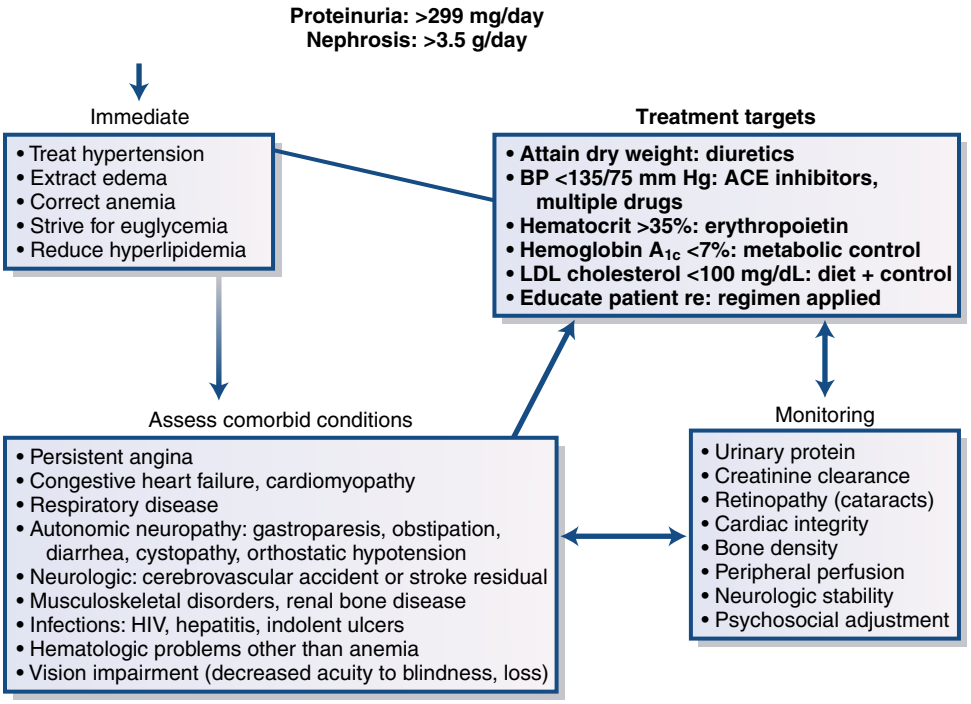
A major risk associated with hyporeninemic hypoaldosteronism is the development of life-threatening hyperkalemia. This is an increasingly important issue with the widespread use of ACE inhibitors and ARBs, often in combination, in this population. This is further exacerbated by the use of potassium-sparing diuretics (such as spironolactone) and beta blockers.

Contrast-Induced Nephropathy

Because many diabetic patients have impaired renal function, they are at high risk of increased renal impairment from certain nephrotoxic agents. One of the most important risks relates to radiocontrast dyes.[524] Where possible, patients with diabetes and renal impairment should avoid imaging studies that involve contrast and, in particular, multiple studies performed in rapid succession. Where intravenous contrast forms an indispensable tool to management, low-osmolality, nonionic, or gadolinium-based contrast media may be less nephrotoxic in patients with renal failure.[525,526] It has been suggested that patients who require such procedures should be well hydrated before, during, and after the procedure. However, a recent report suggests that this approach may not be as effective as previously presumed.[527] The role of *N*-acetylcysteine, a thiol-containing antioxidant, shows promise to protect against contrast-induced nephropathy.[528] The oral hypoglycemic drug metformin should also be discontinued before contrast procedures to prevent life-threatening lactic acidosis.

Management of Diabetic Kidney Disease

Blood pressure and glycemic control represent the major cornerstones for preventing and treating diabetic nephropathy (Figs. 37.39 and 37.40). In the early 1980s, a number of Scandinavian researchers found that aggressive blood pressure reduction reduces the rate of progression of diabetic nephropathy,[529,530] and in the 1990s other researchers found that intensified glycemic control has a similar benefit in both T1DM (DCCT)[9] and T2DM (UKPDS)



• **Fig. 37.40** Flow chart illustrating the management of diabetic nephropathy after the onset of clinical proteinuria. *ACE,* Angiotensin-converting enzyme; *BP,* blood pressure; *HIV,* human immunodeficiency virus; *LDL,* low-density lipoprotein.

diabetic subjects. These findings have led to the view that optimization of blood pressure and plasma glucose levels should be the mainstay of therapy for diabetic nephropathy.

Glycemic Control in Diabetic Kidney Disease

The importance of glucose as a factor in the progression of diabetic kidney disease, as initially suggested from epidemiologic and preclinical studies, was clearly demonstrated in the DCCT study in patients with T1DM.[9] In both the primary and secondary prevention arm of the study, any decrease in HbA_{1c} was strongly associated with a reduction in the risk of development of microalbuminuria as well as a decrease in the risk of progression to overt nephropathy. The follow-up EDIC study has confirmed long-lasting benefits of this therapeutic approach.[531] The UKPDS clearly demonstrated a role for intensified glycemic control in newly diagnosed T2DM subjects when treatment led to a reduction in HbA_{1c} from 7.9% to 7%. The Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) study has demonstrated that a further reduction of HbA_{1c} to an average of 6.5% was associated with a further reduction in renal events, as assessed by the development and progression of microalbuminuria.[532] A subsequent evaluation of this study[533] demonstrated intensified glycemic control that reduced the development of end-stage renal disease, emphasizing the fact that tighter glycemic control continues to confer renal benefits even in the setting of more advanced renal disease. This renal benefit persisted, as seen in the follow-up study known as ADVANCE-ON.[534] Thus despite the ongoing controversy as to the appropriate HbA_{1c} target to reduce macrovascular disease as a result of the findings from the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study,[535] no such controversy as to a possible deleterious effect of intensified glycemic control has been reported with respect to nephropathy.

It remains to be determined how useful the intensification of glycemic control is in the setting of overt nephropathy as a last-ditch strategy to delay the onset of end-stage renal disease. Aggressive management of hypertension and lipid lowering are clearly more important than glycemic control in reducing cardiovascular events and slowing renal disease progression at this stage of relatively advanced disease, although some studies suggest that poor glycemic control can accelerate the loss of renal function in diabetic nephropathy.[536] However, a number of large studies have failed to show any evidence that strict glycemic control per se retards renal progression once overt nephropathy is present.[537] In fact, in the Veterans Administration Diabetes Trial (VADT) tight glycemic control reduced albuminuria but failed to demonstrate any effect on GFR.[538] In addition, as renal function fails, tight glycemic control becomes more hazardous, with an increased risk of hypoglycemia. Nonetheless, because there is sufficient evidence that glycemic control can reduce both macrovascular events and microvascular complications of diabetes at other sites, it is reasonable to suggest that optimization of metabolic control in patients with overt nephropathy remains worthwhile.

The choice of agent, however, remains controversial. Certainly, several types of drugs are able to improve glycemic control in patients with T2DM[539]; however, the particular advantages of one class over another for preventing and treating diabetic nephropathy remain to be established. Nevertheless, this issue appears to have become more clinically relevant with the widespread use of newer classes of glucose-lowering drugs, including dipeptidyl peptidase 4 (DDP4) inhibitors, GLP1 analogs, and SGLT2 inhibitors.[539] With respect to DPP4 inhibitors, modest renal benefits have been suggested, particularly with saxagliptin in the Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus (SAVOR) study[540] and in pooled analyses with linagliptin.[541] Pending results from studies such as the

Cardiovascular and Renal Microvascular Outcome Study With Linagliptin in Patients With Type 2 Diabetes Mellitus (CARMELINA)[542] will further clarify the potential renoprotective role of these agents. Recent studies have been performed with GLP1 analogues, including liraglutide and semaglutide.[543–545] Both agents, in addition to conferring cardiovascular protection, afforded renal benefits. In the Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER) trial, liraglutide-treated patients had a 22% lower incidence of new onset of persistent macroalbuminuria and a slower decline in the eGFR over time, particularly in the subgroup of patients who had evidence of kidney damage at baseline.[545] In the Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes (SUSTAIN 6), semaglutide-treated patients had a similar reduction in renal outcomes. However, semaglutide-treated patients also had increased risk of serious diabetic retinopathy, including 1.91-fold increased risk of retinal photocoagulation and a 2.29-fold increased risk of vitreous hemorrhage,[543,544] which may be due to more severe retinal disease at baseline and rapid glycemic improvements. Finally, SGLT2 inhibitors have been shown to have strong renoprotective effects. For example, the EMPA-REG OUTCOME study demonstrated an approximately 50% decrease in composite renal outcomes, including benefits on reducing hard renal endpoints such as end-stage renal failure.[474] Similar benefits have also been reported in the CANVAS study using the SGLT2 inhibitor canagliflozin.[473] Of major interest is the finding that renoprotection is observed even in the absence of glucose lowering with these agents as seen in subjects with eGFR below 45 mL per minute.[546] This suggests that these agents confer renoprotection at least in part in a glucose-independent manner, but the mechanism(s) are unknown. Changes in sodium balance, intrarenal hemodynamics, plasma volume, decreased insulin resistance, and triglyceride-rich lipoprotein production and modest actions in reducing blood pressure, body weight, and uric acid have all been suggested as possible mechanisms.

A number of differences in the side effect profiles should influence prescribing habits. In patients with renal impairment, particular care must be exercised in selecting and dosing oral diabetic therapy because an accumulation of either the drug or active metabolites can lead to hypoglycemia (e.g., with glyburide) and other serious adverse effects such as lactic acidosis (with metformin). Thiazolidinediones (TZDs), such as pioglitazone and rosiglitazone, should be used with caution in patients with advanced nephropathy who have or are at risk of heart failure, although renal impairment per se is not a contraindication for this class of drugs.

Blood Pressure Control in Diabetic Kidney Disease

A sustained reduction in blood pressure appears to be one of the most important interventions to prevent progressive nephropathy in T1DM and T2DM. For example, in the UKPDS, a reduction in blood pressure from 154 to 144 mm Hg was associated with a 30% reduction in microalbuminuria. All national and international guidelines now emphasize the importance of blood pressure reduction in the diabetic patient. Although many guidelines suggest that specific targets should be achieved, no such threshold appears to exist for any renal endpoint in patients with diabetes. In particular, the risk of progressive diabetic nephropathy continues to decrease, with blood pressure reductions into the normal range and below, meaning that the lowest achievable blood pressure is associated with the best clinical outcomes. This is particularly important in those with the greatest risk of renal damage, patients with overt nephropathy. In these patients, it has been suggested that optimal blood pressure control is less than 125/75 mm Hg.[547] Indeed, in a subanalysis from the blood pressure arm of the ADVANCE study, no blood pressure threshold was detected. Specifically, in those subjects in whom achieved blood pressure was reduced to levels even lower than currently recommended in national and international guidelines, there was a further decrease in renal events.[548] Thus it is possible that if individuals can tolerate lower blood pressures without major side effects such as dizziness and syncope it may be worth considering treating some T2DM subjects to blood pressure levels below those currently recommended. However, syncope is often a side effect limiting the options to intensify blood pressure–lowering therapy, particularly in those diabetic subjects with systolic hypertension as a result of diabetes-associated vascular stiffness.

There is good evidence that tight blood pressure control, no matter how it was achieved, is associated with a significant reduction in the risk of microalbuminuria (primary prevention). Although blood pressure reduction appears paramount, there is also evidence that ACE inhibitors[549] have renoprotective actions beyond their antihypertensive effects for primary prevention.[550,551] However, if treatment should commence in the normoalbuminuric stage, such a strategy would involve treating the majority of patients who are not at risk of nephropathy. Ideally, it would be useful to be able to identify patients, still normoalbuminuric, whose likelihood of progression is increased. As of yet, no such markers of predisposition to renal disease are available, although serum prorenin[476] and modest elevations in urinary albumin excretion albeit still within the normal range (borderline microalbuminuria)[489] might ultimately be examples of such markers.

The issue of primary prevention has been readdressed in two studies in which subjects with normoalbuminuria were treated with agents that interrupt the RAS. In the first smaller trial, which included the performance of sequential renal biopsies, no benefit of early institution of either the ACE enalapril or the angiotensin II receptor antagonist losartan was observed, as defined not only as a lack of effect on albuminuria but also no significant retardation in progression of renal morphologic injury despite benefits on retinopathy.[552] In the second, much larger trial known as the Diabetic Retinopathy Candesartan Trials (DIRECT) study, the angiotensin II antagonist candesartan, despite some modest retinoprotective effects, had no major impact on reducing the new onset of microalbuminuria.[553]

In secondary prevention studies, the additional benefits achieved from blocking the renin-angiotensin system are clearer. A meta-analysis incorporating the findings of more than 10 studies in patients with microalbuminuria has demonstrated the ability of ACE inhibitors not only to retard the development of overt proteinuria but also to decrease urinary albumin excretion by more than 30%. In some patients with microalbuminuria, ACE inhibition can reduce urinary albumin excretion into the normoalbuminuric range.[550] In patients with T1DM and overt proteinuria, aggressive blood pressure reduction reduced proteinuria by up to 50% and retarded the rate of decline in renal function.[529,530]

Similar studies have been performed in T2DM patients. Two landmark trials, RENAAL and Irbesartan in Diabetic Nephropathy Trial (IDNT), examined the renoprotective effects of the ARBs losartan and irbesartan, respectively.[506,469] In both studies, when compared to various alternative antihypertensive agents such as calcium antagonists (but not ACE inhibitors), ARB treatment was associated with a reduction in end-stage renal failure, a greater than 30% decrease in proteinuria, and a major reduction

in hospitalization for heart failure. As a result of these studies, ARBs are recommended as first-line treatment for blood pressure reduction in T2DM patients with overt proteinuria.[554]

Although ACE inhibitors have not been as extensively studied in this population, the Diabetics Exposed to Telmisartan and Enalapril (DETAIL) trial suggested similar renoprotective actions for both drug classes.[555] Similar findings comparing the angiotensin II antagonist telmisartan to the ACE inhibitor, ramipril, are also reported in the much larger ONTARGET study, albeit that trial was not performed exclusively in diabetic subjects.[556] Thus from a clinical perspective no clear difference between these two drug classes has been identified. The one exception is cough, which occurs in 5% to 30% of patients taking ACE inhibitors, depending on ethnicity, which is higher in Asian subjects. In microalbuminuric T2DM subjects, ARBs have also been demonstrated to have a role. For example, in the Irbesartan Microalbuminuria Type 2 (IRMA2) trial, irbesartan dose-dependently reduced the risk of development of macroproteinuria,[557] confirming the findings seen predominantly with ACE inhibitors in microalbuminuric T1DM subjects.[550]

Another approach to inhibit the renin-angiotensin system has involved the use of the recently introduced renin inhibitors such as aliskiren. For example, in the Safety and Efficacy of Aliskiren When Added to Standardized Losartan and Optimal Antihypertensive Therapy in Patients With Hypertension, Type 2 Diabetes and Proteinuria (AVOID) study in T2DM subjects,[558] this agent appeared to have an additional effect on albuminuria when administered with the angiotensin II antagonist losartan. Unfortunately, as reported in the larger subsequent Aliskiren Trial in Type 2 Diabetes Using Cardiorenal Endpoints (ALTITUDE) study,[511] aliskiren has failed to demonstrate superior cardiovascular or renal protection, and thus this approach of add-on renin inhibition is not recommended. Other approaches focusing on blood pressure reduction continue to be examined in these populations. Pilot studies with various mineralocorticoid (MC) antagonists, albeit not studied extensively, have revealed that spironolactone and eplerenone reduce albuminuria.[559,560] Unfortunately, these agents are associated with hyperkalemia, particularly in subjects with renal impairment and/or type 4 renal tubular acidosis. However, the advent of a newer MC antagonist, finerenone, which has a lower risk of hyperkalemia, has led to renewed interest in this treatment approach. Indeed, in the mineralocorticoid Receptor Antagonist Tolerability Study—Diabetic Nephropathy (ARTS-DN) trial, a dose-dependent effect of finerenone was associated with a progressive decrease in albuminuria.[561] These positive findings have led to larger trials with this drug that are currently in progress.

Evaluation of Additional Approaches to the Management of Diabetic Kidney Disease

Low-protein diets (0.75 g/kg/day) have been shown to retard the progression of renal disease, although the data are not totally convincing for diabetic nephropathy, per se. A meta-analysis of five studies in T1DM subjects supported a minor renoprotective role for these diets,[562] but this has not been a universal finding.[563] There are even fewer data in T2DM subjects with overt nephropathy.[564] However, the expected benefits that can be achieved through protein restriction in patients with diabetic nephropathy are at best modest in comparison with adequate blood pressure control and blockade of the renin-angiotensin system. Moreover, the nutritional impact of such interventions must be carefully considered, particularly in patients with brittle glycemic control.

The role of lipid-lowering agents as renoprotective drugs remains controversial. Although in rodents a large body of evidence suggests that lipids promote renal injury and that various lipid-lowering drugs reduce nephropathy, even in the setting of no or minimal effect on lipids,[565] the data in humans are variable.[566] However, in a study of fenofibrate in T2DM, known as the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, there was an impressive reduction in albuminuria.[567,568] Furthermore, in the Heart Protection Study, simvastatin appeared to retard the decline in renal function, although this analysis was not confined to the diabetic subgroup.[569] Another group has also reported a potential renoprotective effect of a statin,[570] although this effect has not been observed in all studies. Indeed, several meta-analyses have generally reported at best modest benefits of statins on renal disease.[571–573] Nevertheless, because cardiovascular disease is so prominent in diabetic patients, particularly those with incipient or overt renal disease, lipid-lowering treatment should be considered in most patients independent of its putative renoprotective actions.[574]

Other approaches to consider include correction of anemia with agents such as erythropoietin.[575] The role of these agents as renoprotective drugs remains to be clarified,[576] but the potential benefits on general patient well-being and in reducing left ventricular hypertrophy[577] provide a rationale for using such agents judiciously in diabetic patients. However, the Trial to Reduce Cardiovascular Events With Aranesp Therapy (TREAT) study, although focusing on cardiovascular events and mortality using the erythropoietin analogue darbepoetin, did not demonstrate that darbepoetin is renoprotective. Furthermore, the drug was unfortunately associated with a twofold increase in cerebrovascular events.[578] With the advent of new hemoglobin-raising drugs such as the hypoxia-inducible factor prolyl hydroxylase inhibitors,[579] there is likely to be a new approach for correcting anemia in diabetes-associated chronic kidney disease.

Over the past decade, several clinical trials targeting diabetic nephropathy with novel agents have yielded disappointing results. For example, PKCβ inhibition with ruboxistaurin, which had renal benefits in experimental diabetes, failed to show any major benefits on albuminuria in T2DM subjects.[580] Another promising agent, sulodexide, postulated to restore the glomerular charge by repleting the loss of glycosaminoglycans[581] and thereby act as an antiproteinuric and ultimately renoprotective drug, also failed to demonstrate any evidence of renoprotection in several large trials. An endothelin antagonist, avosentan, was assessed in the Assess the Effect of the Endothelin Receptor Antagonist Avosentan on Time to Doubling of Serum Creatinine, End Stage Renal Disease or Death in Patients With Type 2 Diabetes Mellitus and Diabetic Nephropathy (ASCEND) trial.[582] Although this drug was associated with impressive reductions in albuminuria, the associated side effect of fluid retention reduced the enthusiasm for this agent. However, another endothelin antagonist, atrasentan, with fewer side effects and being antiproteinuric,[583] was developed. However, this endothelin antagonist also unfortunately failed at a relatively advanced stage of clinical development. Finally, bardoxolone, an agonist of the transcription factor Nrf2, which appears to act as an antioxidant, has been investigated in T2DM subjects with impaired renal function. In the initial Bardoxolone Methyl Treatment: Renal Function in CKD/Type 2 Diabetes (BEAM) trial[584] an improvement in renal function was reported. However, a subsequent larger study in T2DM subjects with stage 4 chronic kidney disease known as the Bardoxolone Methyl Evaluation in Patients with Chronic Kidney Disease and Type 2 Diabetes Mellitus: the

Occurrence of Renal Events (BEACON) study was prematurely terminated because of increased cardiovascular events.[585] Nevertheless, this drug continues to be evaluated,[586] including in new studies, albeit with a lower dose, such as in the Phase II Study of Bardoxolone Methyl in Patients With Chronic Kidney Disease and Type 2 Diabetes (TSUBAKI) study where positive findings have been reported.

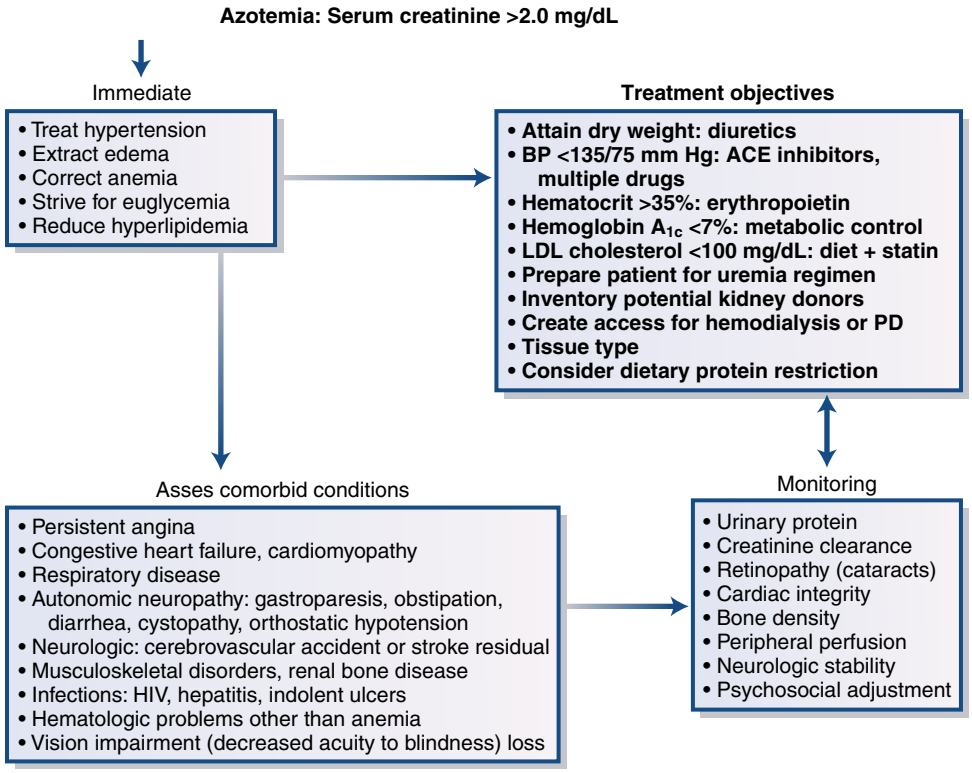
Treatment of the Diabetic Uremic Patient

Renal impairment in a patient with diabetes necessitates changes in therapy. Often, blood glucose control becomes more brittle because the half-life of insulin is prolonged and the renal response to hypoglycemia is impaired. High swinging blood glucose levels in a patient with nephropathy can often mistakenly lead to an increase in oral therapy. However, in patients with renal impairment, particular care must be exercised in the selection and dosing of oral hypoglycemic therapy. Fortunately, DPP4 inhibitors can be used safely in such individuals, albeit often the dose needs to be reduced in subjects with low GFR. Importantly, one DPP4 inhibitor, linagliptin, which is not excreted by the kidney, can be given without dose reduction, even in subjects on dialysis.[587] Nonsteroidal anti-inflammatory drugs (NSAIDs) and COX2 inhibitors should be avoided when possible because their use is associated with inadequate blood pressure control, often as a result of reduced efficacy of antihypertensive drug therapy. Patients at high risk for progressive deterioration in their renal function should be considered for an early referral to a nephrology service for management of renal failure (Fig. 37.41). This facilitates access to erythropoietin, control of calcium phosphate balance, and planning for renal replacement therapy with the preemptive placement of access catheters and lines. Delay in referral can result in a more precipitous start to renal replacement and usually a bad prognostic outcome.[588]

Many options are now available for the diabetic patient requiring renal replacement therapy.[589] These include home or facility hemodialysis, including overnight dialysis, peritoneal dialysis, renal transplantation (cadaveric or living related), or combined pancreas-kidney transplantation. Most patients choose hemodialysis rather than peritoneal dialysis, although data are conflicting regarding which approach leads to better survival (Table 37.6). Some patients opt for withdrawal of treatment because their quality of life, with advanced cardiovascular disease, visual impairment, and amputations, is poor.

The Burden of Nephropathy

One must never consider renal disease in a diabetic patient in isolation. Proteinuria, per se, is strongly associated with other complications such as macrovascular disease, heart failure, and retinopathy. Furthermore, treatments directed toward one complication may be useful for the other complications. Indeed, intensified glycemic control has been shown to be particularly useful for other microvascular complications.[9] In addition, the various antihypertensive regimens, particularly those using agents that interrupt the renin-angiotensin system as well as glucose and blood pressure lowering with SGLT2 inhibitors, also confer important cardiovascular benefits such as reducing heart failure.[469,506,590] Recent data also support use of angiotensin receptor neprilysin inhibitors (ARNi). A post hoc analysis of the Prospective Comparison of ARNI With ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial using



• **Fig. 37.41** Flow chart illustrating the management of diabetic nephropathy after onset of renal failure. *ACE,* Angiotensin-converting enzyme; *BP,* blood pressure; *HIV,* human immunodeficiency virus; *LDL,* low-density lipoprotein; *PD,* peritoneal dialysis.

TABLE 37.6 Options in Therapy for End-Stage Renal Disease in Diabetic Patients

Variable	Peritoneal Dialysis	Hemodialysis	Kidney Transplantation
Extensive extrarenal disease	No limitation	No limitation except for hypotension	Excluded in cardiovascular insufficiency
Geriatric patients	Limited by frailty, cognitive dysfunction; patient treatment satisfaction greater with assisted peritoneal dialysis	No limitation	Arbitrary exclusion as determined by program
Complete rehabilitation	Rare, if ever	Very few patients	Common as long as graft functions
Death rate	Much higher than for nondiabetic patients	Much higher than for nondiabetic patients	About the same as for nondiabetic patients
First-year survival rate	~75%	~75%	>90%
Morbidity during first year	~15 days in hospital	~12 days in hospital	Weeks to months hospitalized
Survival to second decade	Almost never	<5%	~1 in 5
Progression of complications	Usual and unremitting; hyperglycemia and hyperlipidemia	Usual and unremitting; might benefit from metabolic control	Interdicted by functioning pancreas plus kidney; partially ameliorated by correction of azotemia
Special advantage	Can be self-performed; avoids swings in solute and level of intravascular volume	Can be self-performed; efficient extraction of solute and water in hours	Cures uremia; freedom to travel
Disadvantages	Peritonitis; hyperinsulinemia; hyperglycemia, hyperlipidemia; long hours of treatment; more days hospitalized than with either hemodialysis or transplantation	Blood access a hazard for clotting, hemorrhage, and infection; cyclic hypotension, weakness, aluminum toxicity, amyloidosis	Cosmetic disfigurement, hypertension, personal expense for cytotoxic drugs; induced malignancy; HIV (human immunodeficiency virus) transmission
Patient acceptance	Variable, usual compliance with passive tolerance for regimen	Variable; often noncompliant with dietary, metabolic, or antihypertensive components of regimen	Enthusiastic during periods of good renal allograft function; exalted when pancreas proffers euglycemia
Relative cost	Most expensive over long run	Less expensive than kidney transplantation in the first year; subsequent years more expensive	Pancreas plus kidney engraftment most expensive uremia therapy for diabetics; after first year, kidney transplantation alone is lowest cost option

sacubitril/valsartan, a combination of neprilysin inhibition and angiotensin II receptor blockade, was reported to slow the rate of reduction of eGFR in patients with stage 2 to 4 heart failure with reduced ejection fraction, a finding which was more prominent in the participants with T2DM at study entry.[591] Interestingly this agent is also associated with modest glucose-lowering effects.[592]

Thus as clearly expounded in the Intensified Multifactorial Intervention in Patients With Type 2 Diabetes and Microalbuminuria (Steno-2) study, the multifactorial approach in microalbuminuric subjects will not only lead to renal benefits as confirmed in a follow-up study,[593] but will also confer other advantages to the diabetic patient, including reducing all-cause mortality.[594] Because those with renal disease have the greatest risk for nonrenal complications, it stands to reason that they also are likely to have the greatest absolute benefit from risk-reduction strategies.

Diabetic Neuropathies

Diabetic neuropathies are a group of clinical syndromes with distinct presentations secondary to different underlying pathogenetic mechanisms. These syndromes are most commonly classified into two broad groups: diffuse versus focal neuropathies. Among the diffuse diabetic neuropathies, distal symmetric polyneuropathy (DSPN) is the most common, followed by the family of autonomic neuropathies, including cardiac autonomic neuropathy (CAN), and autonomic neuropathy of the gastrointenstinal, urogenital, and sudomotor systems. Focal neuropathies are much less common than diffuse neuropathies and include isolated mononeuropathies of one or more peripheral nerves; even rarer are focal neuropathies of one or more nerve roots, classified as radiculopathy or polyradiculopathy, respectively (Tables 37.7 and 37.8).

Epidemiology and Impact of Diabetic Neuropathies

Diabetic neuropathies are the most common complications of T1DM and T2DM. Distal symmetric polyneuropathy represents the most commonly diagnosed and highly morbid of the diabetic neuropathies. While prevalence estimates vary depending on how the diagnosis is made, DSPN occurs in at least half if not more of all diabetic patients over time.[595] Prevalence as determined by clinical examination was approximately 45% after 25 years of diabetes in the classic study by Pirat following a cohort of 4400 diabetic patients between 1947 and 1973.[596] However, when more contemporary quantitative sensory testing or nerve conduction studies are used as part of the diagnostic criteria, the prevalence of DSPN increases from 60%[597] to greater than 75%.[598] Multiple studies now confirm that DPSN also occurs in

TABLE 37.7 Classification for Diabetic Neuropathies

Diabetic Neuropathies

A. Diffuse neuropathy
DSPN
- Primarily small fiber neuropathy
- Primarily large fiber neuropathy
- Mixed small and large fiber neuropathy (most common)

Autonomic
Cardiovascular
- Reduced HRV
- Resting tachycardia
- Orthostatic hypotension
- Sudden death (malignant arrhythmia)

Gastrointestinal
- Diabetic gastroparesis (gastropathy)
- Diabetic enteropathy (diarrhea)
- Colonic hypomotility (constipation)

Urogenital
- Diabetic cystopathy (neurogenic bladder)
- Erectile dysfunction
- Female sexual dysfunction

Sudomotor dysfunction
- Distal hypohydrosis/anhidrosis
- Gustatory sweating

Hyoglycemia unawareness

Abnormal pupillary function

B. Mononeuropathy (mononeuritis multiplex) (atypical forms)
Isolated cranial or peripheral nerve (e.g., CN III, ulnar, median, femoral, peroneal)
Mononeuritis multiplex (if confluent may resemble polyneuropathy)

C. Radiculopathy or polyradiculopathy (atypical forms)
Radiculoplexus neuropathy (a.k.a. lumbosacral polyradiculopathy, proximal motor amyotrophy)
Thoracic radiculopathy
Nondiabetic neuropathies common in diabetes
Pressure palsies
Chronic inflammatory demyelinating polyneuropathy
Radiculoplexus neuropathy
Acute painful small-fiber neuropathies (treatment induced)

DSPN, Distal symmetric polyneuropathy.
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TABLE 37.8 Mononeuritis and Entrapment Syndromes

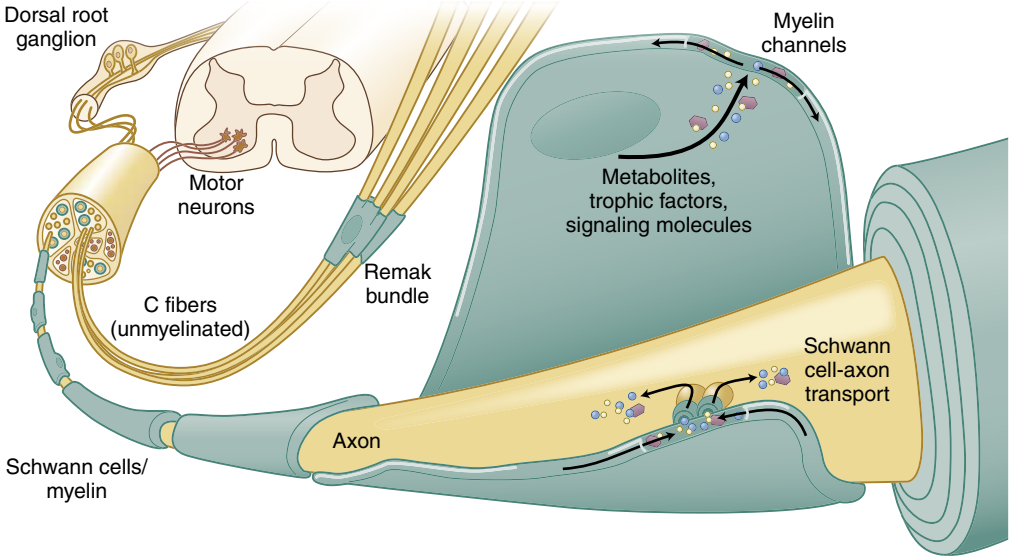
Feature	Mononeuritis	Entrapment
Onset	Sudden	Gradual
Nerves	Usually single but may be multiple	Single nerves exposed to trauma
Common nerves	C3, C6, C7, ulnar, median, peroneal	Median, ulnar, peroneal, medial and lateral plantar
Progression	Not progressive; resolves spontaneously	Progressive
Treatment	Symptomatic	Rest, splints, diuretics, steroid injections, surgery for paralysis

C3, C6, C7, Cervical spinal nerves 3, 6, and 7.
Modified from Vinik AI, Mehrabyan A. Diabetic neuropathies. *Med Clin North Am.* 2004;88:947–999.

patients with prediabetes and the metabolic syndrome[599–601] and while higher in those metabolic syndrome patients with hyperglycemia, ranging from prediabetic to T2DM levels, DSPN can also occur independent of glycemic status.[601] With the alarming rise of prediabetes, T2DM, and the metabolic syndrome, estimates range from 15 million to 30 million individuals in the United States are living with DSPN.[602]

DSPN leads to a poor quality of life, limiting a patient's function, especially DSPN associated with pain. Up to 70% of DSPN patients experience some form of disrupted sleep, depression, anxiety, and poor work productivity.[603] Impairment of physical functioning in DSPN is associated with a 15-fold increase in the likelihood of falling and fractures, particularly in older diabetics.[604] Foot ulceration leading to amputation is one of the most serious and adverse effects of DSPN. Foot ulcers are a strong predictor of early mortality in diabetic patients[605] and precede nearly all amputations.[606] Of patients with new foot ulcers, 5% die within 1 year of their first ulcer and 42.2% of people with existing ulcers die within 5 years, as shown in a recent study of 414,523 patients with diabetes.[607] A 2017 systematic review reported that the global foot ulcer prevalence is 6.3%, with the highest prevalence, 13%, occurring in North America.[608] Worldwide DSPN accounts for more hospitalizations than all the other diabetic complications combined and is the root cause of 50% to 75% of nontraumatic amputations in North America.[609,610] Though rates of lower extremity amputation among Medicare-enrolled diabetics have decreased by 28.8% between 2000 and 2010,[611] the cost of caring for DSPN complications has not decreased because of delay in diagnosis.[612]

Like DSPN, autonomic neuropathies adversely affect function in patients with T1DM and T2DM, affecting a wide variety of organ systems, including cardiovascular, gastrointestinal, urogenital, and sudomotor/thermoregulatory systems. Of these, cardiac autonomic neuropathy is the best studied. Multiple studies confirm early mortality and sudden death in diabetic CAN patients, with mortality estimates up to 56% over a 10-year period.[613] One-third of patients with autonomic neuropathy have impaired quality of life,[614] with erectile dysfunction predicting not only poor quality of life in the affected patient[615] but also in the patient's sexual partner.[616]



• **Fig. 37.42** The peripheral nervous system. The peripheral nervous system (PNS) is comprised of both neurons and Schwann cells (SCs), and the structure, location, and interaction of these components have important implications for PNS function. Efferent axons of motor neurons, whose cell bodies are located in the ventral horn of the spinal cord, carry signals from the central nervous system (CNS) to muscles and glands, whereas afferent axons of sensory neurons, whose cell bodies are located in the dorsal root ganglia, relay information from peripheral sensory receptors to the CNS. Thin and unmyelinated sensory axons, also known as C fibers or small fibers, are associated with nonmyelinating Schwann cells and are grouped as Remak bundles. These represent a large portion of the PNS neurons. Myelinated sensory axons, on the other hand, are surrounded by myelin sheaths made by Schwann cells that form distinct nodal domains important for saltatory conduction. They also form a tubular network of myelinic channels that connect the SC cytoplasm with the periaxonal space, providing substrate for energy generation to the axonal compartment. (Redrawn from Feldman EL, Nave KA, Jensen TS, et al. New horizons in diabetic neuropathy: mechanisms, bioenergetics, and pain. *Neuron.* 2017;93:1296–1313.)

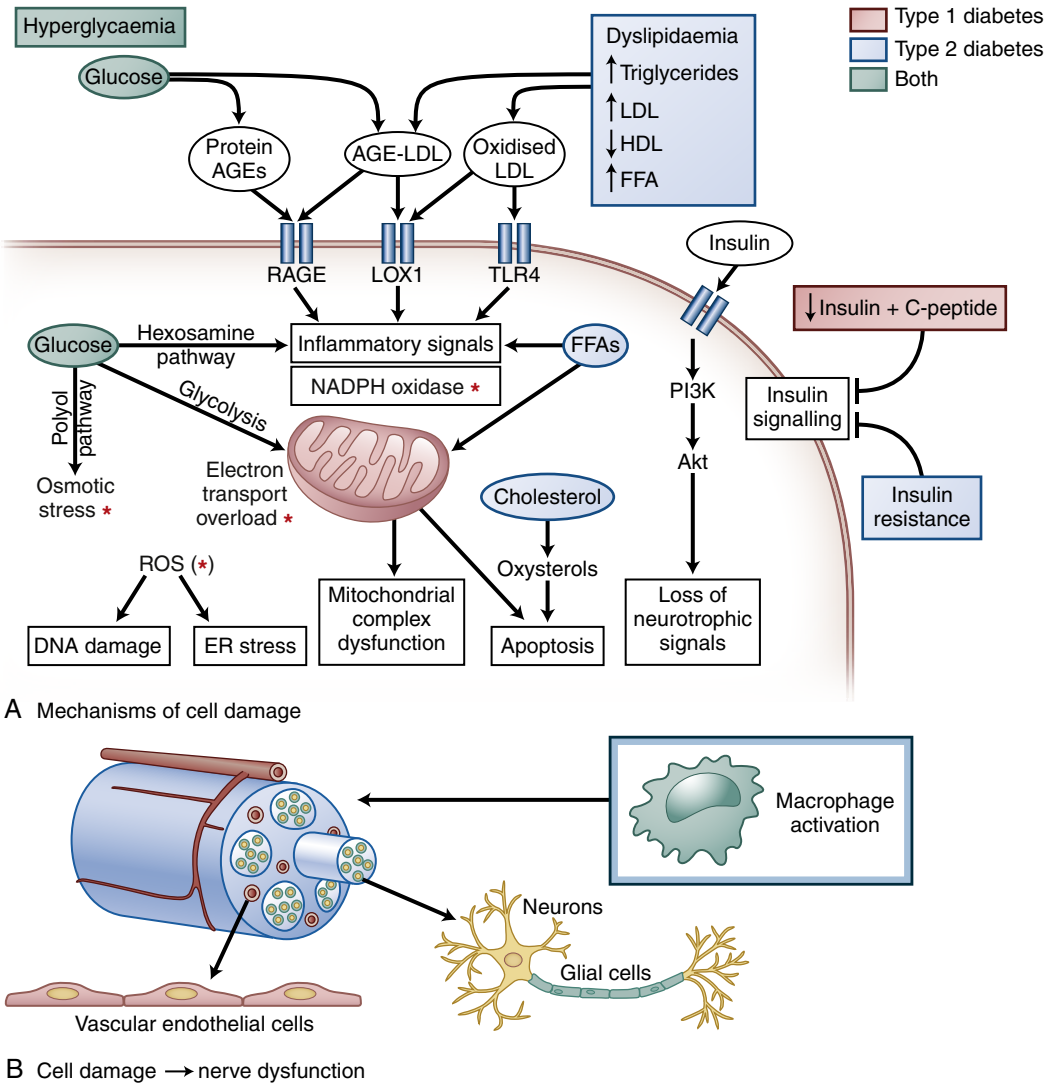
While cost estimates vary, the total annual cost of DSPN is estimated to be greater than $25 billion,[617] with total annual medical costs for a diabetic patient with painless DSPN estimated at $12,492 compared to $6632 for a diabetic patient without DSPN. Costs are even greater with painful DSPN, from $27,931 up to $30,755 per patient, depending on the severity of the pain[617] and the use of generic versus nongeneric medications.[618] Importantly, little money is spent in the United States on patient education or preventative foot care, an investment that has been proven to be effective in decreasing the incidence of DSPN complications.[619]

Pathophysiology of Diabetic Neuropathies

While the pathophysiology of diabetic neuropathy has been discussed earlier in this chapter, an understanding of nerve structure (Fig. 37.42) provides additional insight into neuropathy pathogenesis and clinical presentation. In the peripheral nervous system, afferent axons from sensory receptors send information to the dorsal root ganglia of the central nervous system (CNS), while efferent axons relay CNS signals to muscles and joints. Schwann cells envelope and myelinate large and midsize sensory axons, knowns as *large fibers*. In contrast, the cytoplasm from one Schwann cell can envelope 30 or more unmyelinated small axons, known as *small fibers*. These large and small fibers carry specific information: Large fibers relay vibratory, proprioception, and tactile sensation; small fibers relay thermal sensation and pain, and they also regulate microvascular blood flow. Small fiber damage usually precedes large fiber damage in diabetes[620] and frequently presents as burning pain, although patients also report tingling and prickly sensations.[621] With time, large fiber involvement occurs, with corresponding numbness and poor position sense.

How diabetes changes the interaction between Schwann cells and axons is not yet understood, and it remains unclear if the primary insult in DSPN lies in the sensory neuron, axon, or Schwann cell; logically, it is likely that all three components are adversely affected, in both distinct and overlapping ways. In the diabetic microenvironment, glycolytic intermediates are depleted in Schwann cells[622] while fatty acid oxidation is increased,[623] producing a state of cellular oxidative stress and excess cytosolic bioactive lipids.[624] Emerging evidence reveals that axon-Schwann cell communication, via myelinic channels between the two tissues, allows for the two-way transport of these glycolytic intermediates and bioactive lipids.[602,625] The concept of *crosstalk* between Schwann cells and axons is further supported by a recent finding that delivery of exosomes from Schwann cells cultured in high glucose to sciatic nerves of mice resulted in murine DSPN.[626] Finally, the idea of axon-Schwann cell crosstalk and reciprocal supply of critical intermediary metabolites is supported by Schwann cell–specific knockout experiments of a serine-threonine kinase required for Schwann cell energy regulation. This knockout caused degeneration of sensory greater than motor axons, especially in the small unmyelinated fibers.[627]

Clinical trials have yielded insights into potential differences underlying the pathogenesis of DSPN in T1DM and T2DM. Glucose control has a significant effect on DSPN onset and progression in T1DM but no effect or a modest 3% to 5% effect at



• **Fig. 37.43** Mechanisms of diabetic neuropathy. Factors linked to type 1 diabetes *(red),* type 2 diabetes *(blue),* and both *(green)* cause DNA damage, endoplasmic reticulum stress, mitochondrial complex dysfunction, apoptosis, and loss of neurotrophic signaling (A). These mechanisms can damage neurons, glial cells, and vascular endothelial cells and can trigger macrophage activation. Damage to each of these cell types can lead to nerve dysfunction and neuropathy (B). The relative importance of the pathways in this network will vary with cell type, disease profile, and time. *AGE,* Advanced glycation end products; *ER,* endoplasmic reticulum; *FFA,* free fatty acids; *HDL,* high-density lipoprotein; *LDL,* low-density lipoprotein; *LOX1,* oxidized LDL receptor 1; *PI3K,* phosphatidylinositol-3-kinase; *RAGE,* receptor for advanced glycation end products; *ROS,* reactive oxygen species (red star); *TLR4,* toll-like receptor 4. (Redrawn from Callaghan BC, Cheng HT, Stables CL, et al. Diabetic neuropathy: clinical manifestations and current treatments. *Lancet Neurol.* 2012;11:521–534.)

best on DSPN in T2DM.[628] The idea that DSPN is actually two distinct disorders, DSPN T1DM and DSPN T2DM, is a source of active investigation,[11] with the emerging concept that neurons can develop insulin resistance[629] that contributes to DSPN in T2DM (Fig. 37.43).[630]

Clinical Features of Diabetic Distal Symmetrical Polyneuropathy

Clinical Symptoms

DSPN is the most common and widely recognized form of diabetic neuropathy. An international consensus meeting defined DSPN as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes."[631,632] Alternate causes of DSPN include vitamin B_{12} deficiency, multiple myeloma, and hereditary neuropathies. The onset of DSPN is usually insidious but can be acute, as discussed later. DSPN is primarily a sensory neuropathy and can involve small fibers, large fibers, or both.[633] Motor involvement is only seen late in the disease, if at all.

Symptoms of DSPN secondary to diabetes depend on the affected fiber types. Early in the course of the disease, small fibers are preferentially involved, leading to symptoms of pain, hyperalgesia and allodynia in the lower limbs, followed over time by a loss of thermal sensitivity and pain perception.[621] These same early symptoms of DSPN are also the main constellation of presenting

symptoms in prediabetic neuropathy.[600] Symptoms often are exacerbated at night and are manifested in the feet more than the hands. Spontaneous episodes of pain can be severely disabling. The pain varies in intensity and character and has been variably described by patients as lancinating, stabbing, or sharp. Paresthesias or episodes of distorted sensation, such as pins and needles, tingling, coldness, numbness, or burning, often accompany the pain.[633] As a whole, these multiple symptoms are classified as *positive* symptoms, as the affected diabetic patient actively experiences discomfort.

In contrast, the symptoms of large fiber neuropathy are most commonly denoted as *negative* symptoms, with the patient presenting with a numb, insensate foot. Diabetic patients with large fiber predominant DSPN are frequently unaware of their neuropathy, and are surprised when a physical examination reveals absent sensation in their feet. As large fibers mediate position sense, poor balance with frank falling is a common presenting symptom in diabetic patients with a large fiber predominant DSPN. While less common, these patients can also experience a deep-seated, dull, toothache-like sensation in their feet and, in very severe cases, will experience symptoms of distal weakness of the toes and ankles, secondary to late-onset large motor fiber involvement.

While the distinction is classically made between small and large fiber predominant DSPN, a common presentation of DSPN in diabetic patients is a combination of symptoms from both fiber types. A few clinical "pearls" can assist the clinician in identifying symptoms that are atypical for DSPN secondary to diabetes. These include motor greater than sensory symptoms and a reportable asymmetry. If the physical examination confirms these symptoms, an alternative diagnosis to DSPN must be pursued.

Clinical Signs of Diabetic Neuropathy

A simple examination can distinguish between small and large fiber impairment. Inspection of the skin is an essential first part of the clinical examination because small fibers also subserve sudomotor function. The foot is inspected for dry skin, fissures, and callouses. Small fiber function is then assessed using a simple disposable safety pin, a cotton wisp, or by assessing the ability of a patient to distinguish temperatures between a proximal location (the face) and a distal location (the dorsum of the great toe). A simple bedside approach is to determine if the patient can differentiate between the sharp and dull end of the safety pin on the dorsum of the toe, and if he or she can perceive a cotton wisp in the same anatomic location. To assess temperature in the most simple manner, a cool tuning fork is placed on the patient's cheek, then the same instrument is placed on the great toe, and the patient is asked to report on perceiving the same cool temperature in both anatomic locations or not. Large fiber function is initially assessed by measuring the time in seconds that a patient can perceive vibration sensation on the great toe after a health care provider places a 128-Hz vibrating tuning fork on the dorsum of the patient's great toe. Proprioception is assessed by minor movements of the first metatarsophalangeal joint of the great toe. Ankle reflexes are an essential part of assessing large fiber function and are best produced with the examiner mildly dorsiflexing the patient's foot and striking the Achilles tendon with a Tromner or Queens Square reflex hammer.

Given that the glove and stocking distribution of the DSPN reflects nerve fiber length, when sensory loss reaches the midcalves, patients begin to perceive symmetric sensory loss of the fingertips. Examination of the upper extremity is most commonly performed as outlined earlier for the lower extremity, except the dorsum of the index finger is used by the examiner.

The Semmes-Weinstein 10-g filament as an assessment of touch pressure, a large fiber function, can be used as a screening tool for DSPN.[634] The filament is bent to a C-shape applying pressure to the plantar surface of the great toe. Because of the variability in the sensitivity and specificity of the 10-g filament,[635] it is recommended that a three-site test be done involving the plantar aspects of the great toe, the third metatarsal, and the fifth metatarsal. The ability to sense the 10-gram filament on the soles of the feet is also frequently used by podiatrists as a screening tool for DSPN, although it is generally held that this is not sufficiently sensitive to diagnose DSPN.[634]

In advanced cases of DSPN, examination will reveal wasting of the small muscles of the feet, with hammertoes and weakness of toe extension; in extreme cases, there is weakness of the anterior tibialis and the intrinsic hand muscles.

Differential Diagnosis of Diabetic Neuropathy

The American Academy of Neurology suggests checking a serum protein electrophoresis with immunofixation, a B_{12} level, and thyroid function tests in all patients suspected of having DSPN. If these are normal, and there are no atypical signs, the most likely diagnosis is prediabetic neuropathy or DSPN.[631] The importance of performing these screening tests was confirmed in the Rochester Diabetic Neuropathy Study, in which up to 10% of peripheral neuropathy in diabetic patients was determined to not be secondary to diabetes.[636]

Importantly, the symptoms and signs of DSPN are sensory and symmetrical, and any convincing asymmetry evidence of motor greater than sensory involvement, or a rapid and aggressive clinical course, should lead the clinician to suspect an alternative etiology for the patient's clinical presentation. In these atypical cases, it is essential to exclude other causes of neuropathy in patients with diabetes (Table 37.9).[637]

Classification of Diabetic DSPN

The Toronto Consensus panel convened in 2009 and redefined the minimal criteria for diagnosis of typical diabetic neuropathy[638]:

1. Possible DSPN. The presence of symptoms or signs of DSPN may include the following: symptoms—decreased sensation, positive neuropathic sensory symptoms (e.g., "asleep numbness," prickling or stabbing, burning or aching pain) predominantly in the toes, feet, or legs; or signs—symmetric decrease of distal sensation or unequivocally decreased or absent ankle reflexes.
2. Probable DSPN. The presence of a combination of symptoms and signs of neuropathy, including any two or more of the following: neuropathic symptoms, decreased distal sensation, or unequivocally decreased or absent ankle reflexes.
3. Confirmed DSPN: The presence of an abnormality of nerve conduction and a symptom(s) or sign(s) of neuropathy confirm DSPN. If nerve conduction is normal, a validated measure of small fiber neuropathy (with Class 1 evidence) may be used. To assess for the severity of DSPN, several graded clinical scores can be used that include various continuous measures of sum scores of neurologic signs, symptoms, or composite clinical and electrophysiologic scores; scores of function of activities of daily living; or scores of predetermined tasks or of disability.
4. Subclinical DSPN. The presence of no signs or symptoms of neuropathy are confirmed with abnormal nerve conduction or a validated measure of small fiber neuropathy (with Class 1 evidence). Definitions 1, 2, or 3 can be used for clinical practice, and definitions 3 or 4 can be used for research studies.

TABLE 37.9 Common Causes of Rare Subtypes of Peripheral Neuropathy

Localization	Condition
Diffuse, nonlength-dependent neuropathy; demyelinating sensory motor	AIDP; CIDP; CIDP variants: POEMS syndrome, IgM anti-MAG neuropathy, Waldenstrom acroglobulinemia, and MGUS; diphtheria; and toxic exposures (hexane, arsenic, and amiodarone)
Demyelinating sensory	Sensory CIDP or AIDP; and DADS (IgM anti-MAG neuropathy)
Demyelinating motor	MMN
Axonal sensory motor	Toxic exposures; ASMAN; and AIP
Axonal sensory	Paraneoplastic (Hu, CRMP5, and amphyphisin); Sjögren syndrome; chemotherapy (platinum based, bortezomib); vitamin B_6 toxicity; idiopathic; HIV, HTLV; autoimmune hepatitis; celiac disease; HSAN; Friedrich ataxia; CANVAS; SANDO; and CANOMAD
Axonal motor	ALS; PMA; postpolio syndrome; HIV, HTLV, WNV, enterovirus D68; MMN without conduction block; radiation; monomelic amyotrophy; HMN; SMA (including Kennedy syndrome); and complicated HSP
Multiple mononeuropathies	Systemic vasculitic neuropathy: microscopic polyangiitis, Wegener granulomatosis, polyarteritis nodosa, Churg-Strauss syndrome, cryoglobulinemia, Sjögren syndrome, rheumatoid arthritis, and SLE; nonsystemic vasculitic neuropathy; neoplasm (malignant and benign); HNPP; sarcoidosis; amyloidosis; MMN; and MADSAM
Polyradiculopathy	Compressive: disc herniation/spondylosis, osteomyelitis, and neoplasm; noncompressive: infection (CMV, VZV, Lyme, and tuberculosis), inflammatory (sarcoidosis), neoplastic (leukemia and lymphoma), and radiation
Plexopathy	Compressive: neoplasm and hemorrhage; noncompressive: infection (VZV, HSV, CMV, and Lyme), inflammatory (sarcoidosis), neoplastic (leukemia, lymphoma), and radiation
Radiculoplexus neuropathy	Diabetic lumbar (diabetic amyotrophy); diabetic cervical; postsurgical inflammatory; nondiabetic lumbar or cervical; infection (VZV, HSV, CMV, and Lyme); inflammatory (sarcoidosis); neoplastic (leukemia and lymphoma); and radiation

AIDP, Acute inflammatory demyelinating polyneuropathy; *AIP,* acute intermittent porphyria; *ALS,* amyotrophic lateral sclerosis; *ASMAN,* acute sensory motor axonal neuropathy; *CANOMAD,* chronic ataxic neuropathy, ophthalmoplegia, monoclonal IgM protein, cold agglutinins, disialosyl antibodies; *CANVAS,* cerebellar ataxia neuropathy vestibular areflexia syndrome; *CIDP,* chronic inflammatory demyelinating polyneuropathy; *CMV,* cytomegalovirus; *CRMP5,* collapsing response mediator protein 5; *DADS,* distal-acquired demyelinating syndrome; *HIV,* human immunodeficiency virus; *HMN,* hereditary motor neuropathy; *HNPP,* hereditary neuropathy with liability to pressure palsies; *HSAN,* hereditary sensory autonomic neuropathy; *HSP,* hereditary spastic paraplegia; *HSV,* herpes simplex virus; *HTLV,* human T-lymphotrophic virus; *MADSAM,* multifocal acquired demyelinating sensory and motor neuropathy; *MGUS,* monoclonal gammopathy of unclear significance; *MMN,* multifocal motor neuropathy; *PMA,* progressive muscular atrophy; *POEMS,* polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes; *SANDO,* sensory ataxia neuropathy dysarthria ophthalmoplegia; *SLE,* systemic lupus erythematosus; *SMA,* spinal muscular atrophy; *VZV,* varicella zoster virus; *WNV,* West Nile virus.

5. Small fiber neuropathy (SFN). SFN should be graded as follows: (1) possible: the presence of length-dependent symptoms and/or clinical signs of small fiber damage; (2) probable: the presence of length-dependent symptoms, clinical signs of small fiber damage, and normal sural nerve conduction; and (3) definite: the presence of length-dependent symptoms, clinical signs of small fiber damage, normal sural nerve conduction, and altered intraepidermal nerve fiber (IENF) density at the ankle and/or abnormal thermal thresholds at the foot (both of the later provide Class 1 evidence).

Graded Scores for the Classification of DSPN

While DSPN is diagnosed at the bedside, as outlined earlier, there are several scores that provide structure to the bedside examination and range from simple assessments of large and small fibers to more complex assessments requiring quantitative thermal testing, electrophysiology, or skin biopsies. These scores include the Neuropathy Impairment Score of the lower limb (NIS-LL),[639] Michigan Diabetic Neuropathy Score (MDNS),[640] Modified Toronto Clinical Neuropathy Score (mTCNS),[641] Total Neuropathy Score–Clinical (TNS-C),[642] the Utah Early Neuropathy Score (UENS),[643] and the Neuropathy Disability Score (NDS).[644] The sensitivities and specificities are listed in Table 37.10. Electrophysiologic testing or nerve conduction studies along with a referral to a neurologist is normally not needed, unless the patient has either atypical symptoms or signs.

The importance of the skin biopsy as a diagnostic tool for DSPN is increasingly recognized when no clear objective evidence of small or large fiber dysfunction is present on examination.[621] This technique quantitates small IENFs through antibody staining of the neuronal antigen protein gene product (PGP) 9.5. It is minimally invasive (3-mm-diameter punch biopsies) but enables direct study of small fibers that cannot be evaluated by standard nerve conduction studies. Prediabetic neuropathy and DSPN are accompanied by loss of these small fibers in a distal-to-proximal gradient.

Treatment of DSPN

Treatment of DSPN is commonly divided into either direct disease-modifying therapies or treatment of pain, a common occurrence in patients with DSPN. Accumulating evidence supports the idea that disease-modifying therapies are distinct for DSPN, depending if the patient has T1DM or T2DM.[645] In contrast, treatment of pain is not personalized to the type of diabetes,

TABLE 37.10 Sensitivity and Specificity for Tested Neuropathy Scales

	Cutoff	Sensitivity	Specificity	PPV	NPV	LRP	LRN
Modified Toronto Clinical Neuropathy Score (mTCNS)	3.00	98.00	97.00	0.99	0.94	31.20	0.03
Total Neuropathy Score (Clinical) (TNSc)	5.00	81.00	97.00	0.99	0.66	25.90	0.20
Utah Early Neuropathy Scale (UENS)	3.00	85.00	97.00	0.99	0.72	27.20	0.15
Early Neuropathy Score (ENS)	5.00	83.00	97.00	0.99	0.67	26.67	0.17
Michigan Diabetic Neuropathy Score (MDNS)	5.00	80.00	100.00	1.00	0.65	—	0.20
Neuropathy Impairment Score of the Lower Limb (NISLL)	3.00	83.00	97.00	0.98	0.69	26.47	0.18
Neuropathy Disability Score (NDS)	4.00	89.00	100.00	1.00	0.78	—	0.11

LRN, Likelihood ratio negative; *LRP,* likelihood ratio positive; *NPV,* negative predictive value; *PPV,* positive predictive value.

Modified from Zilliox LA, Ruby SK, Singh S, et al. Clinical neuropathy scales in neuropathy associated with impaired glucose tolerance. *J Diabetes Complications.* 2015;29:372–377.

although emerging research indicates there may be differences in the pain phenotypes among the different types of diabetes.[602]

Disease-Modifying Therapy in DSPN

There are no US Food and Drug Administration (FDA)–approved therapies that directly address DSPN as a disease entity. In the absence of pharmaceuticals, dietary and lifestyle interventions are recommended to modify the course of the illness. A 2012 Cochrane review reported that glucose control has only modest effects, if any at all, on DSPN in T2DM, while it is an important factor in determining the onset and progression of DSPN in T1DM.[645] In both prediabetes and T2DM, the components of the metabolic syndrome are collectively drivers of neuropathy. Multiple studies confirm that dyslipidemia, hypertension, obesity, and low hip:waist ratios are present in patients with prediabetic neuropathy and T2DM patients with DSPN. Modification of these factors, beyond glucose control alone, is required to effect a clinically meaningful change in neuropathy in both patient groups. Of interest, obese patients with the metabolic syndrome but normoglycemia also have neuropathy, further confirming that glucose control alone is insufficient to address neuropathy pathogenesis in the prediabetic or T2DM patient.[600]

The 2017 American Diabetes Association (ADA) recommendations are outlined in Table 37.11.[646]

Treatment of Painful Neuropathy

Pain is a common presentation of prediabetic neuropathy and present in approximately 20% of patients with DSPN.[647] There are no compelling studies that show glucose control is helpful in the treatment of pain. In contrast, aggressive glucose control over a short period of time can result in debilitating pain syndromes in both T1DM and T2DM patients.[648] These pain syndromes are known as treatment-induced neuropathies. It is now well established that the degree of a patient's impairment correlates with the rate of change of the HbA_{1c} (Fig. 37.44),[649] cautioning the clinician to achieve glycemic control at a rate of less than 1% per month HbA_{1c}.[649]

There are three FDA-approved drugs for the treatment of painful DSPN, with better evidence for duloxetine and pregabalin. The opioid tapentadol also is FDA approved, but with weaker evidence. In light of the current opioid epidemic in both North America and worldwide, opioid use is discouraged in the treatment of painful DSPN.

TABLE 37.11 American Diabetes Association Recommendations for Distal Symmetric Polyneuropathy (DSPN)

- Tight glucose control targeting near-normal glycemia in patients with type 1 diabetes dramatically reduces the incidence of distal symmetric polyneuropathy and is recommended for distal symmetric polyneuropathy prevention in type 1 diabetes. **A**
- In patients with type 2 diabetes with more advanced disease and multiple risk factors and comorbidities, intensive glucose control alone is modestly effective in preventing distal symmetric polyneuropathy, and patient-centered goals should be targeted. **B**
- Lifestyle interventions are recommended for distal symmetric polyneuropathy prevention in patients with prediabetes/metabolic syndrome and type 2 diabetes. **B**

ADA recommendations are assigned ratings of A, B, or C depending on the quality of evidence.

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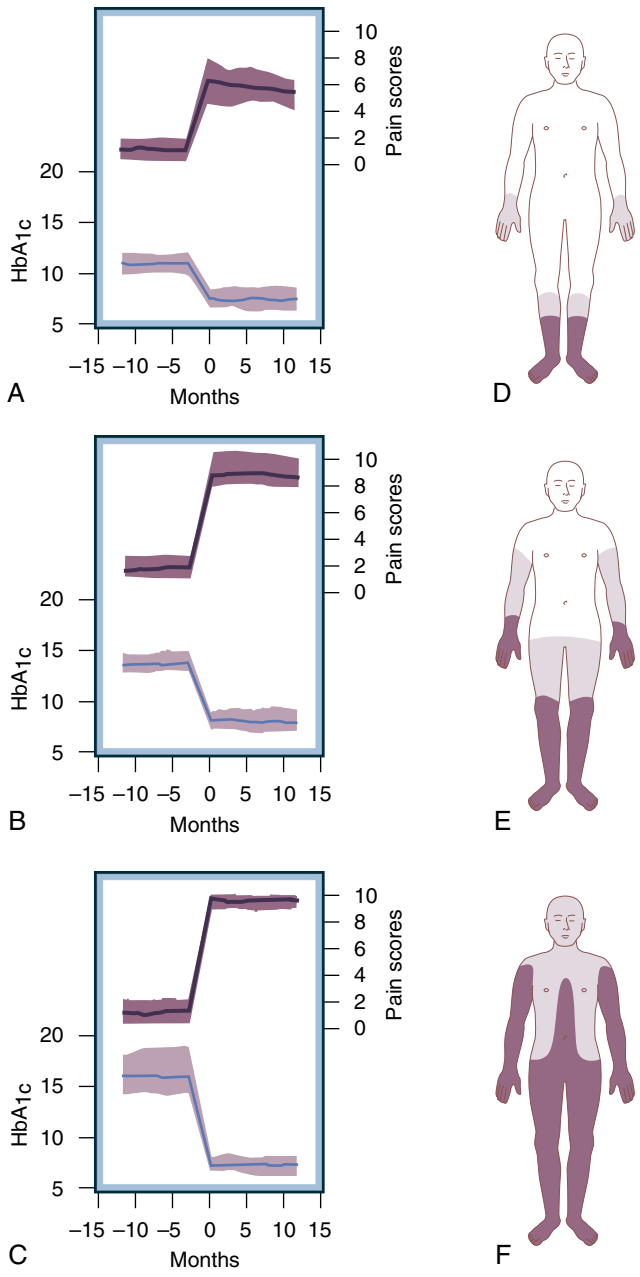
Both the American Academy of Neurology (AAN) and the European Federation of Neurological Societies (EFNS) have reviewed the Class 1 and 2 studies for the treatment of painful DSPN (Table 37.12). In general, three classes of therapies appear effective in the treatment of DSPN: the voltage-gated α2δ ligands (pregabalin, gabapentin), the serotonin-norepinephrine reuptake inhibitors (duloxetine, venlafaxine), and the secondary amine tricyclic antidepressants (amytriptyline, nortriptyline, desipramine). Fig. 37.45 provides a management algorithm for the patient with painful DSPN; Table 37.13 outlines the commonly used doses and range of side effects. A simple rule is to begin with one drug from one of the three classes, and maximize the dose of this drug to either side effects or known therapeutic dosage; if there is some clinical benefit, although not complete, then a second agent from a different drug class should be added for more symptom relief. This agent is again titrated slowly to either side effects or maximal known therapeutic dose. While figures vary, this approach generally provides relief for at least two-thirds, if not more, of patients[618]; there is also an emerging literature that coupling pain treatment with an exercise regimen provides even greater symptom



• **Fig. 37.44** Complications and risks associated with treatment-induced neuropathy of diabetes (TIND). (A–F) The 104 individuals with treatment-induced neuropathy of diabetes are grouped by change in glycosylated HbA_{1c} scores. (A) The 27 individuals with a decrease in HbA_{1c} of 2% to 3.9% are shown. (B) The 52 individuals with a decrease in HbA_{1c} of 4% to 7% are shown. (C) The 25 individuals with a decrease in HbA_{1c} of more than 7% are shown. (A–C) The lower portion of the graph *(left y-axis)* reveals the glycosylated haemoglobin (HbA_{1c}) scores over time. The mean value is shown in blue and the standard deviation in light purple. The upper portion of the graph *(right y-axis)* reveals neuropathic pain scores during the same time frame. The mean value is shown in black and the standard deviation in dark purple. The representative distribution of neuropathic is shown in (D–F), with the area in dark purple representing pain common to all individuals, and the area in light purple common to many individuals. (D) The least widespread pain distribution in the individuals with the smallest change in HbA_{1c} (corresponding to A). (E) The pain distribution in the individuals with moderate decreases in HbA_{1c} (B). (F) The group with the largest decrease in HbA_{1c}. (C) has widespread neuropathic pain. (Redrawn from Gibbons CH, Freeman R. Treatment-induced neuropathy of diabetes: an acute, iatrogenic complication of diabetes (part 1). *Brain.* 2015;138:43–52.)

relief.[650] This approach to pain is recommended in the 2011 AAN guidelines and the 2017 ADA position statement.

While not part of the ADA position statement, the AAN guidelines discuss the addition of selected therapies, which are briefly described later, for patients who do not achieve pain control with metabolic control, combination therapy, and exercise.[651] Alpha-lipoic acid is an antioxidant that is used in Europe at a dose of 600 mg per day as an adjunct to the treatment of painful DSPN.[652,653] Capsaicin is a natural substance that locally depletes substance P, resulting in a loss of small fibers. Capsaicin cream applied topically (0.075% four times/day) to the feet in patients with painful DSPN was reported to provide a modest improvement in pain.[654] Similarly local lidocaine applied to painful areas has been reported to improve pain in selected small studies,[655] although a Cochrane systematic review found no role for lidocaine in the treatment of painful neuropathy.[656] Studies on the use of transelectric stimulation (TENS) units are limited for painful DSPN, but do support its use as an adjuvant therapy in treatment-resistant patients.[657–659]

There is no role for opioids in the treatment of chronic painful DSPN, and limited use, if at all, in acute painful DSPN, possibly for the pain experienced with treatment-induced neuropathy. If pain management is not achieved without opioids, then referral to a pain clinic for the patient is the next suggested step.

Other Diabetic Somatic Neuropathies: Clinical Features and Treatment Paradigms

As presented in Table 37.7, there are other diabetic neuropathies. Cranial mononeuropathies occur primarily in the older population. Onset is usually acute and associated with pain, and the course is self-limited, resolving within 6 to 8 weeks. It is generally believed these neuropathies are secondary to vascular insufficiency.[660] The most common cranial mononeuropathy secondary to diabetes is a mononeuropathy of the third cranial nerve, presenting as a complete third nerve palsy with pupillary sparing. Less common are mononeuropathies of cranial nerves 4, 6, and 7. Because it is essential to distinguish these mononeuropathies from a more serious central etiology, including cranial aneurysms and stroke, neuroimaging is frequently required to confirm that the symptoms and signs are due soley to diabetes.

Mononeuropathies must be distinguished from entrapment syndromes, which start slowly, progress, and persist without intervention (see Table 37.8). The most common entrapment site in diabetic patients is the median nerve, although patients also experience ulnar, lateral cutaneous femoral, and peroneal nerve entrapment. Median nerve entrapment, commonly known as the carpal tunnel syndrome, occurs three times as often in persons with diabetes than in a normal, healthy population,[661,662] and its increased prevalence in diabetes may be related to diabetic cheiroarthropathy,[663] repeated undetected trauma (due to repetitious use of the hands), metabolic changes, or accumulation of fluid or edema within the confined space of the carpal tunnel.[664] Diagnosis can be confirmed by nerve conduction studies or ultrasound.[665] The mainstays of nonsurgical treatment are resting of the wrist, aided by the placement of a wrist splint in a neutral position for day and night use, and the addition of anti-inflammatory medications. Surgical treatment consists of sectioning the volar carpal ligament.[666] The decision to proceed with surgery should be based on several considerations,

TABLE 37.12 Class I and Class II Randomized Controlled Trials From the American Academy of Neurology and European Federation of Neurologic Societies Guidelines on the Treatment of Painful Diabetic Distal Symmetric Polyneuropathy

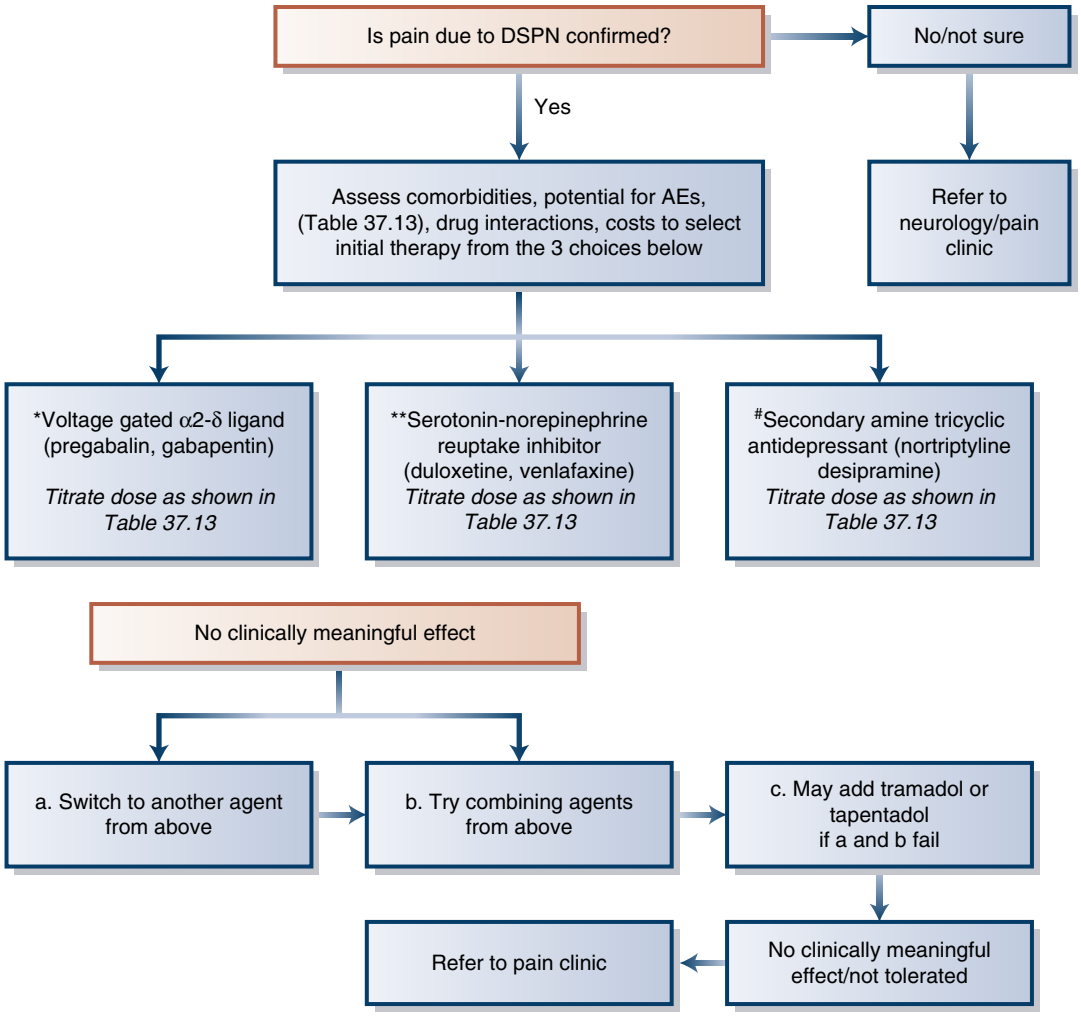
Source[c]	Treatment per Day	Evidence Class[a]	Study Duration (wk)	No. Receiving Treatment/ Total Sample[b]	Mean Pain Reduction on 0-10 Rating Scale vs. Placebo (95% CI)	PATIENTS WITH >50% PAIN REDUCTION		Common Adverse Effects
						Treatment Effect (%)	Placebo Effect (%)	
Lesser et al., 2004	Pregabalin, 300 mg		5	81/337	–1.26 (–1.86 to –0.65)	46	18	Dizziness, somnolence, peripheral edema, confusion, blurry vision
Rosenstock et al., 2004	Pregabalin, 300 mg	I	8	76/146	–1.47 (–2.19 to –0.75)	40	14.5	
Lesser et al., 2004	Pregabalin, 600 mg	I	5	82/337	–1.45 (–2.06 to –0.85)	48	18	
Richter et al., 2005	Pregabalin, 600 mg	I	6	72/223	–1.26 (–1.89 to –0.64)	39	15	
Freynhagen et al., 2005	Pregabalin, 300–600 mg	II	12	82/209	Approximately –1.4 to 1.6 ($p = 0.002$)	48-52	24	
Backonja et al., 1998	Gabapentin, 900–3600 mg	I	8	70/135	–1.2 (–1.9 to –0.6)	Not reported; 60% treated with gabapentin had at least moderate improvement (>30%) vs 33% treated with placebo		Dizziness, somnolence, confusion
Gorson et al., 1999	Gabapentin, 900 mg	II	6	19/30	No difference	Not reported; 42.5% treated with gabapentin reported moderate or excellent pain relief vs 22.5% treated with placebo		
Simpson, 2001	Gabapentin, 900-3600 mg	II	8	27/54	–1.9 (Not reported; $p < 0.01$)	Not reported; 55.5% treated with gabapentin reported much to moderate improvement vs 25.9% treated with placebo		
Vrethem et al., 1997	Amitriptyline, 75 mg	I	4	33/99	–1.8 (Not reported; $p < 0.001$)	Not reported; 63% of patients treated with amitriptyline had at least 20% improvement vs 22% treated with placebo		Dry mouth, sedation, vertigo
Max et al., 1987	Amitriptyline, 25–150 mg	II	6	29 (Crossover)	Not reported	Not reported; 65.5% treated with amitriptyline reported moderate to complete improvement vs 3.5% treated with placebo		
Raskin et al., 2005	Duloxetine, 60 mg	I	12	116/348	–0.9 (–1.39 to –0.42)	50	30	Nausea, somnolence, hyperhidrosis, anorexia

Goldstein et al., 2005	Duloxetine, 60 mg	II	12	86/344	–1.17 (–1.84 to –0.5)	49	26	
Wernicke et al., 2006	Duloxetine, 60 mg	II	12	85/248	–1.32 (–1.95 to –0.69)	43	27	
Raskin et al., 2005	Duloxetine, 120 mg	I	12	116/348	–0.87 (–1.36 to –0.39)	39	30	
Goldstein et al., 2005	Duloxetine, 120 mg	II	12	80/344	–1.45 (–2.13 to –0.78)	52	26	
Wernicke et al., 2006	Duloxetine, 120 mg	II	12	78/248	–1.44 (–2.08 to –0.81)	53	27	
Rowbotham et al., 2004	Venlafaxine, 150–225 mg	I	6	82/242	–0.7 (Not reported; $p < .001$)	56	34	Nausea, dyspepsia, sweating, somnolence, insomnia, blood pressure and cardia rhythm changes

[a]Class I randomized controlled trials must have allocation concealment, clearly defined primary outcomes, and inclusion and exclusion criteria with greater than 80% of patients completing the study. Class II randomized controlled trials lack one or more of the requirements listed for class I studies.

[b]Number of participants receiving the dosage in column 2 out of the total number of participants in the trial. Many trials had multiple intervention groups.

[c]Refer to source article for full reference listings.



• **Fig. 37.45** Algorithm for management of the patient with pain because of distal symmetric polyneuropathy (DSPN). *AE,* Adverse events. *Pregabalin is FDA approved for painful DSPN, whereas gabapentin is not. Pharmacokinetic profile, spectrum of AEs, drug interactions, comorbidities, and costs to be considered in selecting the agent of choice. **Duloxetine is FDA approved for painful DSPN, whereas venlafaxine is not. Pharmacokinetic profile, spectrum of AEs, drug interactions, comorbidities, and costs should be considered in selecting the agent of choice. #None is FDA approved for painful DSPN. Spectrum of AEs, drug interactions, and comorbidities need be considered if selecting these agents. (Modified from Pop-Busui R, Boulton AJ, Feldman EL, et al. Diabetic neuropathy: a position statement by the American Diabetes Association. *Diabetes Care.* 2017;40:136–154.)

including severity of symptoms, appearance of motor weakness, and failure of nonsurgical treatment.[667]

Another distinct class of diabetic neuropathies includes the diabetic polyradiculoneuropathies. This disorder is also known by many other names, including proximal motor neuropathy, diabetic amyotrophy, diabetic femoral neuropathy, and diabetic radiculoplexus neuropathy. It primarily affects older T2DM male patients. The onset of the disease can be gradual but is more commonly abrupt, presenting with excruciating pain in the thighs and hips or buttocks, followed by significant weakness of the proximal muscles of the lower limbs. The neuropathy begins unilaterally, but frequently spreads bilaterally, and coexists with DSPN. Electrophysiologic evaluation reveals lumbosacral plexopathy most commonly superimposed on DSPN.[668] Patients usually clinically resolve with time, and the general course of treatment is supportive. Use of intravenous gammaglobulin is reported to accelerate the resolution of symptoms,[669] but must be used with caution in diabetic patients because of the greater risk of inducing renal failure.[670]

Clinical Features of Diabetic Autonomic Neuropathy

Diabetic autonomic neuropathy is secondary to dysfunction in the sympathetic and parasympathetic nervous systems. The pathogenesis underlying diabetic autonomic neuropathy is generally considered to be similar to DSPN, with a distal-to-proximal dying back of axons due to multiple metabolic insults.[671] Because of the widespread innervation pattern of the autonomic nervous system to critical organs, the symptoms and signs of autonomic dysfunction are varied, dependent on the organ system. These are summarized in Table 37.14.[646]

Cardiac autonomic neuropathy is clinically the most important of the autonomic neuropathies because of its association with early cardiac arrhythmia, silent myocardial infarctions, and early mortality.[672] CAN may present as lightheadedness upon standing, palpitations, frank syncope, and a generalized sense of weakness. In very early stages, CAN may be asymptomatic and only

identified by abnormal heart rate variability, either with deep breathing or upon standing. Later signs include resting tachycardia with a heart rate above 100 beats per minute and orthostatic hypotension defined as a reduction of more than 20 mm Hg in systolic or 10 mm Hg in diastolic pressure when going from a lying to a standing position.

The 2017 ADA guidelines suggest screening for CAN in all diabetic patients with DSPN or other microvascular complications of diabetes (Table 37.15).[646] As with DSPN, there are no disease-modifying therapies, and T1DM patients are instructed to monitor glucose control, while T2DM patients are instructed to focus more on general lifestyle parameters and controlling all aspects of the metabolic syndrome. These recommendations are presented in Table 37.16 and parallel those for DSPN (see Table 37.11).

Diabetic autonomic neuropathy can involve the gastrointestinal tract, resulting in esophageal dysmotility, delayed gastric emptying, and both constipation and diarrhea with frank fecal incontinence. Among these disorders, the best studied is diabetic-mediated delayed gastric emptying, also known as gastroparesis. While estimates vary, the disorder is more prevalent in T1DM than T2DM and has a prevalence of approximately 5% in T2DM patients.[673] The 2017 ADA recommendations for the screening and diagnosis of gastroparesis is presented in Table 37.17.[646]

TABLE 37.13 Treatment for Pain Associated With Distal Symmetric Polyneuropathy

Drug Class	Agent[a]	DOSE		NNT Range 30–50% Improvement[c]	Common Adverse Events	Major Adverse Events
		Initial	Effective			
Anticonvulsants	Pregabalin[b]	25–75 mg, 1–3×/day	300–600 mg/day	3.3–8.3	Somnolence Dizziness Peripheral edema Headache Ataxia Fatigue Xerostomia Weight gain	Angioedema Hepatotoxicity Rhabdomyolysis Suicidal thoughts and behavior Seizures after rapid discontinuation Thrombocytopenia
	Gabapentin	100–300 mg, 1–3×/day	900–3600 mg/day	3.3–7.2	Somnolence Dizziness Ataxia Fatigue	Stevens-Johnson syndrome Suicidal thoughts and behavior Seizures after rapid discontinuation
Antidepressants						
Serotonin-norepinephrine reuptake inhibitors	Duloxetine[b]	20–30 mg/day	60–120 mg/day	3.8–11	Nausea Somnolence Dizziness Constipation Dyspepsia Diarrhea Xerostomia Anorexia Headache Diaphoresis Insomnia Fatigue Decreased libido	Stevens-Johnson syndrome Hepatotoxicity Hypertensive crisis Gastrointestinal hemorrhage Delirium Myocardial infarction Cardiac arrhythmias Glaucoma Suicidal thoughts and behavior Shift to mania in patients with bipolar disorder Seizures Severe hyponatremia Fragility bone fractures Serotonin syndrome Neuroleptic malignant syndrome
	Venlafaxine	37.5 mg/day	75–225 mg/day	5.2–8.4	Nausea Somnolence Dizziness Constipation Dyspepsia Diarrhea Xerostomia Anorexia Headache Diaphoresis Insomnia Fatigue Decreased libido	Same as duloxetine

Continued

TABLE 37.13 Treatment for Pain Associated With Distal Symmetric Polyneuropathy—cont'd

Drug Class	Agent[a]	DOSE		NNT Range 30–50% Improvement[c]	Common Adverse Events	Major Adverse Events
		Initial	Effective			
Tricyclic antidepressants	Amitriptyline	10–25 mg/day	25–100 mg/day	2.1–4.2	Xerostomia Somnolence Fatigue Headache Dizziness Insomnia Orthostatic hypotension Anorexia Nausea Urinary retention Constipation Blurred vision Accommodation Disturbance Mydriasis Weight gain	Delirium Cardiac arrhythmias Conduction abnormalities Myocardial infarction Heart failure exacerbation Stroke Seizures Hepatotoxicity Bone marrow suppression Suicidal thoughts and behavior Shift to mania in bipolar disorder Neuroleptic malignant syndrome Serotonin syndrome Severe hyponatremia Fragility bone fractures
	Desipramine				Same as above	Same as above
	Nortriptyline				Same as above	Same as above
Opioids	Tramadol	50 mg 1–2×/day	210 mg/day	3.1–6.4	Somnolence Nausea Vomiting Constipation Lightheadedness Dizziness Headache	Confusion Seizures Cardiac arrhythmias Hypertension Hypersensitivity reactions Stevens-Johnson syndrome
	Tapentadol[b]	Immediate release: 50–100 mg 4–6×/day	Immediate release: Day 1: 700 mg; after day 1, 60 mg/day	N/A	Somnolence Nausea	Respiratory depression Serotonin syndrome
		Extended release: 50 mg 2×/day	Extended release: 50 mg 2×/day		Vomiting Constipation Dizziness	Seizures Hypertension Neonatal opioid withdrawal syndrome

[a]Refer to source article for specific studies referenced for each agent.

[b]FDA-approved.

[c]FDA considers 30% to 50% improvement to be significant.

NNT, Number needed to treat.

Modified from Pop-Busui R, Boulton AJM, Feldman EL, et al. Diabetic neuropathies: a position statement by the American Diabetes Association. *Diabetes Care.* 2017;40:136–154.

Treatment of gastroparesis is multifaceted. Patients are instructed to eat multiple small meals with lower fat and fiber content and to discontinue any drugs that decrease gastric motility, such as tricyclic antidepressants or opioids. If these interventions do not provide sufficient symptomatic relief, patients can begin metoclopramide, the only FDA-approved drug for the treatment of gastroparesis. Patients should not take the drug for more than 5 consecutive days, secondary to the risk of developing extrapyramidal symptoms, and even this short regimen should only be undertaken when all other interventions have failed.[646]

Sexual and bladder function are also under the control of the autonomic nervous system, and dysfunction occurs commonly in both with long-term diabetes. Erectile dysfunction is common in men and may occur in up to 50% of men over the age of 40. Men are more likely to experience erectile dysfunction if they have T1DM or T2DM, with an increase in dysfunction with a longer duration of diabetes. Erectile dysfunction is further exacerbated by obesity, smoking, and hypertension, along with excessive alcohol use and selected medications.[674] While there is likely a component of autonomic dysfunction in most affected individuals, it is essential to address the modifiable risks factors that are outlined earlier. The phosphodiesterase type 5 inhibitors provide a first line of therapy for erectile dysfunction; these drugs include sildenafil, tadalafil, and vardenafil.[675] Additional therapeutic approaches

TABLE 37.14 Symptoms and Signs Associated With Diabetic Autonomic Neuropathy

Cardiac Autonomic Neuropathy	Gastrointestinal	Urogenital	Sudomotor
Resting tachycardia Abnormal blood pressure regulation • Nondipping • Reverse dipping	Gastroparesis (Gastropathy) • Nausea • Bloating • Loss of appetite • Early satiety • Postprandial vomiting • Brittle diabetes	Bladder dysfunction • Frequency • Urgency • Nocturia • Hesitancy • Weak stream • Dribbling • Urinary incontinence • Urinary retention	Dry skin • Anhidrosis • Gustatory sweating
Orthostatic hypotension (all with standing) • Lightheadedness • Weakness • Faintness • Visual impairment • Syncope	Esophageal dysfunction • Heartburn • Dysphagia for solids	Male sexual dysfunction • Erectile dysfunction • Decreased libido • Abnormal ejaculation	
Orthostatic tachycardia or bradycardia and chronotropic incompetence (all with standing) • Lightheadedness • Weakness • Faintness • Dizziness • Visual impairment • Syncope	Diabetic diarrhea • Profuse and watery diarrhea • Fecal incontinence • May alternate with constipation	Female sexual dysfunction • Decreased sexual desire • Increased pain during intercourse • Decreased sexual arousal • Inadequate lubrication	
Exercise intolerance	Constipation • May alternate with explosive diarrhea		

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TABLE 37.15 American Diabetes Association Recommendations for Cardiac Autonomic Neuropathy Screening and Diagnosis

- Symptoms and signs of autonomic neuropathy should be assessed in patients with microvascular and neuropathic complications. **E**
- In the presence of symptoms or signs of cardiovascular autonomic neuropathy, tests excluding other comorbidities or drug effects/interactions that could mimic cardiovascular autonomic neuropathy should be performed. **E**
- Consider assessing symptoms and signs of cardiovascular autonomic neuropathy in patients with hypoglycemia unawareness. **C**

ADA recommendations are assigned ratings of A, B, or C depending on the quality of evidence. Expert opinion E is a separate category for recommendations in which there is no evidence from clinical trials, in which clinical trials may be impractical, or in which there is conflicting evidence.

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TABLE 37.16 American Diabetes Association Recommendations for Cardiac Autonomic Neuropathy

- Optimize glucose control as early as possible to prevent or delay the development of cardiovascular autonomic neuropathy in people with type 1 diabetes. **A**
- Consider a multifactorial approach targeting glycemia among other risk factors to prevent cardiovascular autonomic neuropathy in people with type 2 diabetes. **C**
- Consider lifestyle modifications to improve cardiovascular autonomic neuropathy in patients with prediabetes. **C**

ADA recommendations are assigned ratings of A, B, or C depending on the quality of evidence.

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include intracavernosal injections, vacuum devices, psychosexual counseling, and cognitive behavioral therapy.[675] Generally, a combination of therapies provides the best therapeutic responses.

Bladder and lower urinary tract dysfunction can occur in men and women with diabetes. Symptoms include frequent urination with urinary urgency, nocturia, and a weak urinary stream. In older men, these symptoms can be attributed to benign prostatic hyperplasia (BPH), but are frequently exacerbated by diabetes. While estimates vary, studies suggest up to a twofold increase in lower urinary tract symptoms in men with diabetes.[676] Urinary incontinence occurs in 50% of middle-aged women with diabetes; in several studies, the presence of diabetes is reported to significantly increase the risk of female urinary incontinence.[676] This can be multifactorial, due to multiple pregnancies, obesity, and medications, in addition to diabetes. The studies that directly

TABLE 37.17 American Diabetes Association Recommendations for Gastrointestinal Neuropathies

- Evaluate for gastroparesis in people with diabetic neuropathy, retinopathy, and/or nephropathy by assessing for symptoms of unexpected glycemic variability, early satiety, bloating, nausea, and vomiting. **C**
- Exclusion of other causes documented to alter gastric emptying, such as use of opioids or glucagon-like peptide 1 receptor agonists and organic gastric outlet obstruction, is needed before performing specialized testing for gastroparesis. **C**
- To test for gastroparesis, either measure gastric emptying with scintigraphy of digestible solids at 15-min intervals for 4 h after food intake or use a ^{13}C-octanoic acid breath test. **B**

ADA recommendations are assigned ratings of A, B, or C depending on the quality of evidence.

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link bladder and lower urinary tract dysfunction to autonomic neuropathy are listed in Table 37.18[676] and suggest an association with bladder and lower urinary tract dysfunction to both CAN and DSPN. In both men and women, bladder and lower urinary tract dysfunction increase the risk of urinary tract infections.[677] As with all forms of diabetic autonomic neuropathy, patients are counseled to achieve glucose control and address other comorbidities, including obesity, hypertension, and hyperlipidemia, that may contribute to the disorder.[678]

Hypoglycemic unawareness is another form of diabetic autonomic neuropathy with significant patient morbidity. Hypoglycemia is defined as a plasma glucose level of less than 70 mg/dL (3.9 mmol/L) without autonomic warning signs.[679,680] In most patients, two categories of symptoms occur with hypoglycemia: neuroglycopenic and autonomic symptoms. Neuroglycopenic symptoms are directly correlated with lack of available glucose for brain function, including hunger, altered mental status, confusion, perioral paresthesias, difficulty speaking, diffuse weakness, and syncope. In more severe cases, patients develop seizures, become comatose, and (in extreme cases) die. Autonomic symptoms

TABLE 37.18 Studies of Autonomic Neuropathy and Bladder Dysfunction in Diabetes

Authors (Year)[b]	Overall Population (Diabetes Type)	Definition of Autonomic Neuropathy	Definition of Bladder Dysfunction	Findings
Male + Female				
Ueda et al., 1997	63 diabetes[a]	Sympathetic skin response	Volume at first desire to void Max bladder capacity Bladder pressure Residual urine	Mean vol. at first desire to void, max bladder capacity lower for sympathetic skin response absent. Mean bladder pressure and residual urine greater for sympathetic skin response absent
Low et al., 2004	231 T1DM/T2DM	Autonomic Symptom Profile (ASP) Composite Autonomic Severity Score (CASS)	ASP urinary domain: bladder dysfunction, sexual dysfunction (males only)	Significant correlations between ASP urinary domain and overall CASS and domain scores
Kebapci et al., 2007	54 T2DM 27 males 27 females	CAN: deep breathing, Valsalva, stand test	LUTS: IPSS, urinary incontinence, urodynamic studies	QT prolongation associated with increased postvoid residual urine OR 2.33 (0.16–34.89)
Pavy-Le Traon et al., 2010	684 T1DM	CAN severity Ewing Score (0–5): deep breathing, Valsalva, stand test, HRV, SBS	Bladder dysfunction symptoms	Bladder dysfunction independently associated with CAN
Males				
Pop-Busui et al., 2015	635 T1DM DCCT/EDIC study	CAN: R-R variation <15, or R-R variation 15–19.9 plus Valsalva ratio ≤1.5, 10 mmHg drop in DBP	LUTS: AUASI 8–35	LUTS prevalence: 158 (25 %) Odds of ED + LUTS: 2.65 (1.47–4.79)
Bansal et al., 2011	52 diabetes[a]	Sympathetic skin response: Medtronic electromyographic system	LUTS: IPSS 8–35 urodynamic studies	Diabetic cystopathy correlated with abnormal motor and sensory nerve conduction velocity studies and abnormal sympathetic skin responses
Females				
Hotaling et al., 2016	571 T1DM DCCT/EDIC study	CAN: R-R variation <15, or R-R variation 15–19.9 plus Valsalva ratio ≤1.5, 10 mm Hg drop in DBP	UI: Sandvik Severity Index 3–12	UI prevalence: 172 (30 %)

[a]Type of diabetes not indicated.

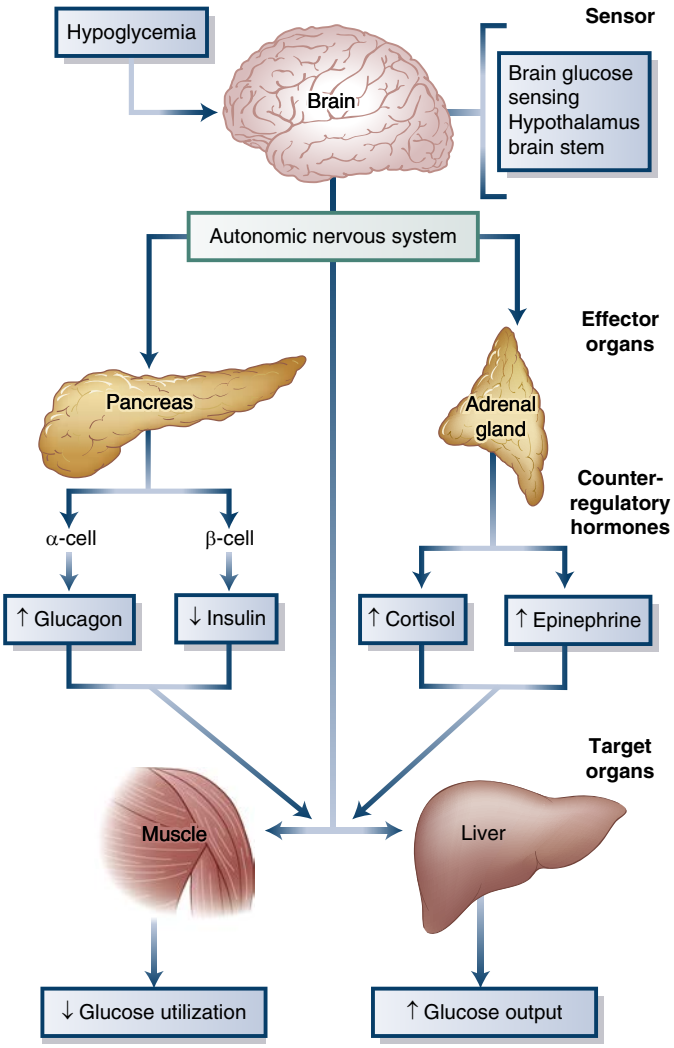
[b]Refer to source article for full reference listing of each study.

AUASI, American Urological Association Symptom Index; *CAN,* cardiovascular autonomic neuropathy; *DBP,* diastolic blood pressure; *DCCT/EDIC,* Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications; *ED,* erectile dysfunction; *HRV,* heart rate variability; *IIEF,* International Index of Erectile Dysfunction; *IPPS,* International Prostate Symptom Score; *LUTS,* lower urinary tract symptoms; *SBS,* spontaneous baroreflex slope; *T1DM,* type 1 diabetes mellitus; *T2DM,* type 2 diabetes mellitus; *UI,* urinary incontinence.

Modified from Braffett BH, Wessells H, Sarma AV. Urogenital autonomic dysfunction in diabetes. *Curr Diabetes Rep.* 2016;16:119.

include diaphoresis, tremulousness, pallor, palpitations, and generalized anxiety. Hypoglycemic unawareness is defined as the presence of neuroglycopenic symptoms prior to or in the absence of autonomic warning symptoms.[681]

Counterregulatory responses under the control of the autonomic nervous system are required to restore normal glycemia in response to hypoglycemia. These essential responses include stimulation of catecholamines (both norepinephrine and epinephrine), glucagon, cortisol, and growth hormone, while inhibiting insulin secretion.[682] These counterregulatory responses stimulate hepatic glucose production while decreasing glucose utilization in the periphery, and collectively restore euglycemia (Fig. 37.46). Recurrent hypoglycemic episodes decrease the plasma glucose level that serves as the setpoint for activating the counterregulatory response in both T1DM and T2DM patients.[682] Severe hypoglycemia with life-threatening neuroglycopenic symptoms is more common in T1DM patients with hypoglycemic unawareness and correlates with longer duration of diabetes, intensive control, and advancing age.[683,684] While a commonly cited statistic is that 40% of T1DM patients experience one or more episodes of hypoglycemic unawareness,[682] a recent multinational study using a patient self-reported assessment tool, the global Hypoglycemia Assessment Tool (HAT), reported the global incidence of hypoglycemic unawareness in T1DM and T2DM patients on insulin therapy as 97.4% and 95.3%, respectively, with approximately 6.9 events per month in T1DM patients and 2.4 events in T2DM patients.[685]



• **Fig. 37.46** Counterregulatory response to hypoglycemia in type 2 diabetes. (From Martín-Timón I, del Cañizo-Gómez FJ. Mechanisms of hypoglycemia unawareness and implications in diabetic patients. *World J Diabetes.* 2015;6:912–926.)

Regardless of the exact percentages, it is clear that hypoglycemic unawareness is common and a source of significant patient morbidity. There is a strong association between recurrent hypoglycemia and an increase in fatal cardiac arrhythmias.[686] An analysis of unrecognized hypoglycemia in 10,096 participants from the ACCORD trial revealed a relationship between the number of episodes of hypoglycemic unawareness and death, leading to early discontinuation of the trial.[687] While hypoglycemic unawareness does not result in significant permanent cognitive impairment in adults,[688,689] it represents a major and potentially disabling event in children and adolescents.[690] Recurrent episodes in young children can result in behavioral problems and neurocognitive dysfunction[691,692] associated with changes in brain structure.[693] In adults who drive, it can result in potentially fatal automobile accidents.

Management of hypoglycemic unawareness begins with prevention, with careful blood glucose monitoring and reasonable blood glucose targets personalized to each patient,[694] in parallel with patient education.[695] This approach can be augmented with β_2-adrenergic agents, caffeine, and serotonin reuptake inhibitors, although the effects are small and use of these agents is controversial.[682] Most therapeutic efforts focus on glycemic control. Continuous glucose monitoring (CGM) to identify hypoglycemia can decrease the number and severity of adverse hypoglycemic events. In a group of T1DM patients with hypoglycemic unawareness, CGM reduced severe hypoglycemic events from 8.1 to 0.6 events per year.[696] Pharmacologic treatment options begin with optimizing insulin regimens; for example, rapid-acting insulin analogs afford more flexibility for patients with exercise regimens and varying mealtimes,[697] while long-acting insulin analogs provide a flatter, less variable glycemic profile that decreases the rate of overall and nocturnal hypoglycemia.[698] The use of continuous subcutaneous insulin infusion (insulin pump) is very effective in decreasing the number of hypoglycemic events,[699] with reported improvements in patient-reported quality of life assessments.[700] Regardless of the insulin regimen chosen by the patient, personalized insulin treatment with appropriate glycemic targets is the cornerstone of treatment.[682,701]

Diabetic Heart Disease

Coronary Artery Disease

In recent decades, cardiovascular disease mortality rates have declined substantially in the general population in the United States. However, improvement in cardiovascular mortality rates has been significantly less in people with diabetes.[8] Diabetes remains associated with excess morbidity and mortality rates even after adjustment for traditional risk factors. Most studies of cardiovascular risk in diabetes have been in the T2DM population, who represent more than 90% of all patients with diabetes.

Effect of Diabetes on Risk of Coronary Heart Disease (CHD)

The Framingham Heart Study showed a twofold to threefold elevation in risk of clinically evident atherosclerotic disease in patients with T2DM compared to those without diabetes.[702] Diabetic

men in the Multiple Risk Factor Intervention Trial (MRFIT) had an absolute risk of cardiovascular death more than three times higher than that of the nondiabetic cohort, even after adjustment for established risk factors.[703] Seminal work from Finland showed that patients with T2DM without a previous myocardial infarction have a risk of myocardial infarction over 7 years as high as that of nondiabetic patients with a history of a myocardial infarct.[704] In this study, the case fatality rate after myocardial infarction was also substantially higher in patients with diabetes. In women, diabetes eliminates the cardioprotective effects of the premenopausal period, and women with diabetes have a cardiovascular mortality rate as high as that of diabetic men. The follow-up of the Diabetes UK cohort, a group of 23,751 subjects with insulin-treated diabetes diagnosed before the age of 30 years, also showed similar mortality rates for men and women, and the size of this cohort permitted robust gender-specific estimates of standardized mortality ratios.[705] For example, among those aged 30 to 39 years, the standardized mortality ratios for ischemic heart disease mortality were 8 and 41.6, respectively. Other forms of cardiovascular diseases, such as hypertension, valvular disease, cardiomyopathy, heart failure, and stroke, are also increased.

The risks of cardiovascular events and death conferred by T2DM have been examined in several prospective and observational trials with various populations of patients. The adjusted relative risk is generally increased by 1.3 to 1.6 for total mortality, mortality attributed to cardiovascular events, nonfatal myocardial infarction or stroke, and congestive heart failure.[706–709] Many epidemiologic studies observe the hazard ratio for cardiovascular mortality risk in patients with diabetes is similar to that in nondiabetic patients who had experienced a previous myocardial infarction. Whether or not the diagnosis of diabetes itself is a risk equivalent to having a history of prior myocardial infarction remains controversial.[709,710] However, all studies demonstrate diabetes results in a significantly increased risk of cardiovascular disease. Thus the strategy of considering diabetes as a CHD risk equivalent is appropriate for purposes of assessing risk and defining an aggressive multirisk treatment regimen. With more intensive management of cholesterol, blood pressure, and decreased smoking, rates of myocardial infarction, stroke, amputation, and mortality are decreasing among people with diabetes, especially in high-income countries. Yet the global burden of cardiovascular disease attributable to diabetes is not declining due to major increases in the number of people developing diabetes.[711]

Coronary heart disease risk has also been evaluated in smaller numbers of patients with T1DM. In the Framingham study, the cumulative cardiovascular mortality rate in patients with T1DM was approximately four times that of nondiabetic patients by age 55 years.[710] Patients with T1DM and a HbA_{1c} level of 6.9% or lower, considered at goal by most management guidelines, had a risk of death from any cause or from cardiovascular causes that was twice as high as the risk for matched controls.[712] As in patients with T2DM, the early deaths related to coronary heart disease in patients with T1DM may occur by the fourth decade of life, and the cumulative mortality rate increases at a similar rate in both T1DM and T2DM in the subsequent decades. The increased cardiovascular mortality rate with age in patients with T1DM is substantially even higher in those patients who also have nephropathy, with gradations of cardiovascular risk evident across the spectrum from microalbuminuria to macroalbumuria to end-stage renal disease, with a 2.8-fold, 9.2-fold, and 18.3-fold increase in mortality compared to the general population across these groups.[451] Therefore persistent proteinuria is a strong predictor of the development of coronary heart disease in this population. Studies suggest that proteinuria is a marker of generalized vascular damage that predisposes to atherosclerosis and coronary events.

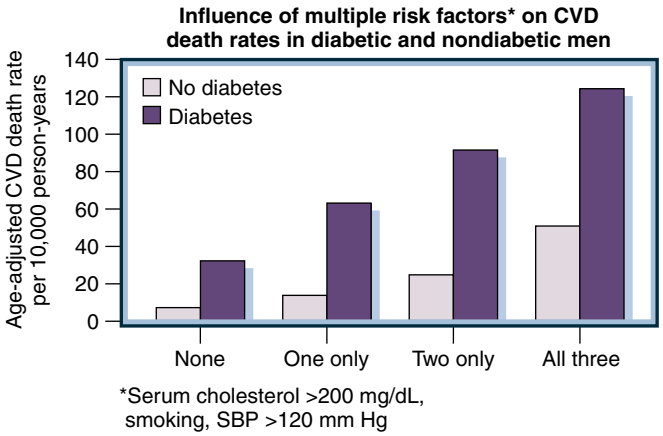
It is unclear whether there has been any recent decline in mortality or morbidity rates from coronary artery disease associated with T1DM. The Pittsburgh Epidemiology of Diabetes Complications (EDC) study reported no difference in the cumulative incidence of cardiovascular disease with 20, 25, or 30 years of disease duration according to year of diagnosis (1950 through 1980).[713] The benefits of improved treatment of hyperglycemia and concomitant hypertension and other comorbid conditions with standard care therefore do not appear to have reduced cardiovascular mortality rates associated with T1DM, in contrast to dramatic reductions realized for diabetic retinopathy and nephropathy.

Aggregation of Traditional CHD Risk Factors in Diabetes

Multiple traditional cardiovascular risk factors (e.g., hypertension, dyslipidemia, obesity, insulin resistance) occur together in patients with diabetes.[714] Approximately 50% of patients with T2DM have hypertension, and more than 30% have hypercholesterolemia at the time of diagnosis. As in nondiabetic patients, these risk factors independently predict cardiovascular mortality.[703] However, even in the presence of one or more concomitant risk factors, diabetes further increases the cardiovascular death rate (Fig. 37.47). It also appears that diabetes interacts synergistically with other risk factors to more sharply increase risk as the number of total risk factors increases.

The UKPDS further confirmed the importance of risk factor aggregation in T2DM as well as the relationship between specific risk factors and future cardiovascular events. In this large population of patients with newly diagnosed T2DM, higher LDL cholesterol levels, lower HDL cholesterol concentrations, higher HbA_{1c} levels and systolic blood pressure measurements, and a history of smoking measured at baseline were all associated with a risk of future cardiovascular disease.[10]

Cardiovascular risk associated with T2DM may be a consequence of insulin resistance and its associated abnormalities that are often present in the prediabetic state.[715] In patients spanning



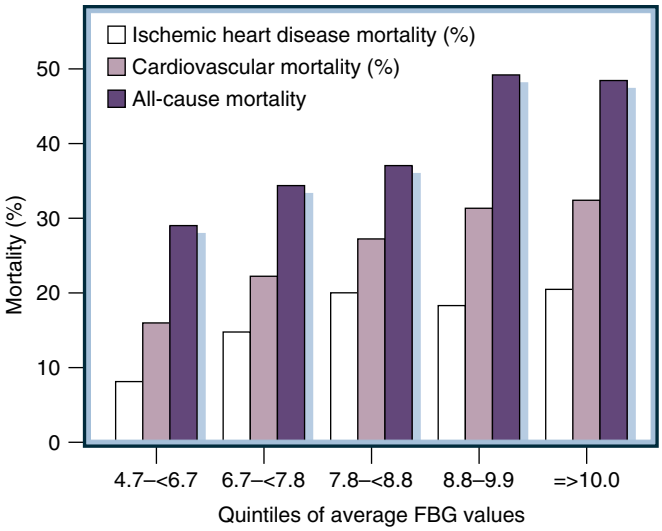
• **Fig. 37.47** Age-adjusted cardiovascular disease *(CVD)* death rates by number of risk factors for men with and without diabetes at baseline screened for the Multiple Risk Factor Intervention Trial. In the presence of diabetes, the cardiovascular death rate steeply rises at any level of concomitant risk factors. *SBP,* Systolic blood pressure. (From Stamler J, Vaccaro O, Neaton JD, et al. Diabetes, other risk factors, and 12-year cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care.* 1993;16:434–444.)

the spectrum of glucose tolerance, from normal to impaired glucose tolerance to frank diabetes, insulin resistance positively correlates with atherosclerosis as assessed by carotid intimal medial thickening.[716] The San Antonio Heart Study, a population-based study of diabetes and cardiovascular disease in Mexican Americans and non–Latin American whites, showed those who converted to diabetes from a prediabetic state and who were more insulin resistant had higher blood pressure, higher triglyceride levels, and lower HDL cholesterol levels.[17] Strikingly, individuals with normal glucose tolerance and high insulin resistance had twice the 8-year risk of adverse cardiovascular outcomes compared to those with low insulin resistance, even after adjustment for 11 risk factors. Although it is not possible to fully distinguish hyperinsulinemia from hyperglycemia, hyperinsulinemia has been hypothesized to contribute mechanistically to the relationship between hyperglycemia and coronary heart disease in patients with T2DM,[717] and a number of studies have shown hyperinsulinemia to independently predict cardiovascular risk. These results suggest treatment strategies that increase insulin sensitivity in these patients can reduce cardiovascular risk. However, no significant difference in the rates of death and major cardiovascular events were observed using insulin provisional compared to insulin sparing therapeutic strategies in patients with both T2DM and heart disease in the Bypass Angioplasty Revascularization Investigation 2 Diabetes Trial (BARI 2D).[718] Furthermore, when insulin therapies were used prospectively to lower HbA_{1C} in either patients with T2DM or prediabetes, they have not been shown to worsen cardiovascular outcomes. This suggests that hyperinsulinemia is a marker of insulin resistance but not a cause of the adverse cardiovascular effects of insulin resistance.

Given the multifactorial nature of atherogenic risk in patients with T2DM, it is reasonable to conclude that an aggressive multifactorial intervention could significantly reduce cardiovascular risk. The value of such a treatment regimen was tested in the Steno-2 study,[595] in which 160 patients with T2DM and microalbuminuria were randomized to receive either conventional treatment in accordance with national guidelines or intensive therapy that included behavior modification and targeted pharmacologic therapy for hyperglycemia, hypertension, dyslipidemia, and microalbuminuria along with secondary prevention of cardiovascular disease with aspirin. Over a mean follow-up period of 7.8 years, patients who received intensive treatment had greater improvements in HbA_{1c}, blood pressure, fasting serum cholesterol and triglyceride values, and urinary albumin excretion than did patients receiving conventional therapy. The greater degree of improvement in risk factors with intensive therapy was also reflected in outcomes. Patients receiving intensive therapy had an approximately 50% reduction in events comprising a multiple cardiovascular endpoint, and in retinopathy, nephropathy, and autonomic neuropathy.[594]

Insulin Resistance, Plasma Glucose, and Excess CHD Risk in Diabetes

Insulin resistance and its consequences increase cardiovascular risk in people with normal glucose tolerance. Yet diabetes confers increased risk that is not explained by the known risk factors associated with insulin resistance. Newer proatherogenic consequences of pathway-specific insulin resistance are discussed in the first section of this chapter. Although a meta-analysis of randomized controlled trials showed limited benefits of intensive glucose-lowering treatment on all-cause mortality and deaths from cardiovascular causes, using HbA_{1c} as an indicator,[13] effects of hyperglycemia not captured by HbA_{1c} may also explain excess cardiovascular risk in prediabetes as well as in diabetes. Hyperglycemia increases insulin resistance, and circadian variation in insulin resistance causes fasting hyperglycemia. One study showed that mortality rates from all causes, cardiovascular mortality, and ischemic heart disease increased progressively across quintiles of fasting blood glucose levels in patients with T2DM (Fig. 37.48).[719] Other data support a dose-response relationship between fasting hyperglycemia and cardiovascular mortality rate in diabetes, with patients with the highest levels of fasting blood glucose having a cardiovascular mortality rate almost five times higher than patients with the two lowest levels combined.[720]



• **Fig. 37.48** All-cause mortality, cardiovascular mortality, and ischemic heart disease mortality rates in patients with type 2 diabetes mellitus by quintiles of average fasting blood glucose (FBG) values. Cardiovascular mortality and all-cause mortality rates increase throughout the range of fasting plasma glucose in a graded fashion. (From Andersson DK, Svärdsudd K. Long-term glycemic control relates to mortality in type II diabetes. *Diabetes Care.* 1995;18:1534–1543.)

Prediabetes refers to the presence of *impaired fasting glucose* (≥100 mg/dL [≥5.5 mmol/L] and <120 mg/dL [<6.7 mmol/L]), and *impaired glucose tolerance,* defined as a 2-hour post–75-g oral glucose load value of 140 to 199 mg/dL (7.8–11 mmol/L) with the fasting level below 100 mg/dL (<5.5 mmol/L) and/or HbA_{1c} between 5.7% and 6.5%. Impaired glucose tolerance (IGT)—a prediabetic state associated with insulin resistance—is also a risk factor for mortality. In a large study of the relationship between 2-hour postload blood glucose and cardiovascular fatality, 17,869 male civil servants enrolled in the Whitehall Study from 1967 to 1969 were monitored, and outcomes were correlated with baseline measurements of the 2-hour postload blood glucose (2hBG) level after a 50-g oral glucose load.[721] The hazard ratio for cardiovascular mortality increased as a linear function of 2hBG for all values of 2hBG greater than 83 mg/dL (>4.6 mmol/L). With 2hBG values between 83 and 200 mg/dL (4.6 and 11.1 mmol/L), the age-adjusted hazard ratio for cardiovascular disease was 3.62 (95% CI, 2.3–5.6).

Tominaga and colleagues examined survival rates in a cohort of participants in a diabetes prevalence trial in Japan[722] and concluded the risk of cardiovascular fatality is associated with impaired glucose tolerance rather than impaired fasting glucose. Further substantiation of the role of impaired glucose tolerance in cardiovascular mortality risk was provided by an analysis of

data from the Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study.[723] In this study, more than 25,000 men and women were monitored for a mean of 7.3 years, and outcomes were correlated with measurements of fasting glucose and 2hBG after a 75-mg glucose load at baseline. The results indicated that the oral glucose tolerance test provides the best index of risk of mortality associated with impaired glucose tolerance.

The Nurses' Health Study also implicated the prediabetic state as a cardiovascular risk factor.[724] In this large cohort of women, 5894 developed diabetes over a 20-year follow-up. The age-adjusted relative rate for myocardial infarction was 3.75 (95% CI, 3.10–4.53) in the period before diagnosis of diabetes and 4.57 (95% CI, 3.87–5.39) after diagnosis, compared with women who did not develop diabetes, even after adjustment for other cardiovascular risk factors. The risk of stroke was also increased before onset of diabetes.

The continuum of cardiovascular risk with rising glucose levels has also been identified in patients with T1DM[725] and in people without clinically overt diabetes but with varying levels of glucose intolerance.

Cardiovascular Disease in Patients With Metabolic Syndrome

Definitions and Diagnosis

Metabolic syndrome is a term referring to the frequent clustering of the cardiovascular risk factors of hypertension, obesity, insulin resistance, dyslipidemia, and dysglycemia in a single patient. Separate diagnostic criteria for different risk components have been put forth by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III),[726] the World Health Organization (WHO),[727] and the International Diabetes Federation (IDF),[728] with clinical overlap among these definitions and the designation of prediabetes.[729]

According to prior NCEP guidelines,[729a] the metabolic syndrome is based on the presence of three of the following five risk factors[726]:

- Abdominal obesity (waist circumference >40 inches (>101.6 cm) in men and >35 inches (>88.9 cm) in women)
- Plasma triglycerides 150 mg/dL (1.7 mmol/L) or higher
- Plasma HDL cholesterol less than 40 mg/dL (<1.04 mmol/L) in men, less than 50 mg/dL (<1.30 mmol/L) in women
- Blood pressure 130/85 mm Hg or higher
- Fasting plasma glucose 110 mg/dL (6.1 mmol/L) or higher

The NCEP criteria give precedence to obesity as a contributor to the metabolic syndrome and apply cutoff points for triglycerides and HDL that are probably less stringent than would be used to identify a categoric risk factor, reflecting the fact that many marginal risk factors can result in a significant risk for CHD. The NCEP criteria do not require explicit demonstration of insulin resistance for diagnosis of the metabolic syndrome, and patients with diabetes are not excluded from the diagnosis.[727] There are modest differences between the NCEP criteria and those developed by the WHO[727] and the IDF.[728] One intriguing aspect of the IDF criteria is the recognition that individuals of Asian origin may have increased visceral adiposity with lower waist circumference than what is observed in Western countries and hence the difference in waist circumference criteria for such individuals.

Studies support the concept that insulin resistance during the prediabetic state contributes to atherogenic risk.[730] Prediabetic subjects who were insulin resistant had higher levels of inflammatory markers (C-reactive protein, PAI1, and fibrinogen) than converters to diabetes with predominantly low insulin secretion or nonconverters. Thus insulin resistance is associated with a proinflammatory state that can contribute to the atherogenic risk profile in prediabetic patients. Many aspects of insulin resistance and the proatherogenic and proinflammatory state of prediabetes are associated with increased visceral fat.[731]

By 2012, more than one-third of all US adults met the definition and criteria for metabolic syndrome agreed to jointly by several international organizations.[732] Although there continues to be debate about the validity and significance of identifying the metabolic syndrome or prediabetes in patients, clinicians, including primary care physicians, often find the construct of value in understanding the relevant underlying issues and explaining these to patients. Behind some of the reluctance to identify metabolic syndrome/prediabetic states in patients is the clinical question of what to do after such a diagnosis is made. Perhaps new data showing cardiovascular risk reduction with newer glucose-lowering therapies that do not cause hypoglycemia (discussed later) may shift this perspective. These new drug options, along with effective medications for reducing cholesterol and hypertension, support the clinical utility of continuing to identify prediabetes and the metabolic syndrome.

Cardiovascular Consequences of Metabolic Syndrome

Metabolic syndrome, prediabetes, and other overlapping definitions from the NCEP, WHO, and others have been evaluated in multiple studies regarding the incidence and mortality risk of CHD.[733–735] In general, age-adjusted cardiovascular mortality and all-cause mortality increase twofold to threefold in patients with metabolic syndrome, regardless of the diagnostic criteria used to define the syndrome. Increased risk is not seen in every study. Differences among these studies exist and involve when and how analyses were done and the metabolic syndrome definitions used, including key issues such as whether frank diabetes is included or excluded from metabolic syndrome definition and the hyperglycemia threshold used.

In a definitive meta-analysis of 87 studies involving 951,083 patients, the metabolic syndrome was found to be associated with an increased risk of cardiovascular disease (relative risk [RR]: 2.35; 95% CI: 2.02–2.73), cardiovascular mortality (RR: 2.40; 95% CI: 1.87–3.08), all-cause mortality (RR: 1.58; 95% CI: 1.39–1.78), myocardial infarction (RR: 1.99; 95% CI: 1.61–2.46), and stroke (RR: 2.27; 95% CI: 1.80–2.85); metabolic syndrome in the absence of diabetes was still associated with increased cardiovascular risk,[732] although it is based on fewer studies.

Another approach to assessing cardiovascular risk of metabolic syndrome involves consideration of this cohort in clinical trials. For example, in the West of Scotland Coronary Prevention Study,[735] those with the metabolic syndrome had an increased cardiovascular risk, along with elevated C-reactive protein levels versus the rest of the cohort, which added prognostic value for both cardiovascular disease and diabetes.

Controversies over metabolic syndrome and other prediabetic states exist.[736] One issue has been whether the cardiovascular risk associated with the metabolic syndrome differs from the additive risk conferred by the presence of each metabolic syndrome component. Another has been what therapeutic intervention should ensue once a diagnosis of metabolic syndrome is made. These and other issues have limited the extent to which this issue has been studied in trials or further considered by guidelines.[737] Evidence continues to emerge regarding the pathologic issues associated

with prediabetes, including intimal medial thickening and plaque in carotid arteries, and adverse functional cardiac parameters such as reduced diastolic filling (now referred to as *heart failure with preserved ejection fraction*).[738] For now, specific relatively subtle differences among diagnostic criteria of prediabetic states may be less relevant for the clinician than the recognition of the increased risk of cardiovascular disease and diabetes associated with metabolic syndrome, the commonly encountered clustering of these risk factors, and the impact lifestyle and modest weight reduction can have of many component risk factors. New data for cardiovascular event reduction in patients with diabetes with agents that do not cause hypoglycemia as well as weight loss agents may stimulate reexamination of these issues.

The Role of Glycemic Control in Improving Cardiovascular Outcomes

The UKPDS confirmed the positive association between plasma glucose levels and CHD risk for HbA_{1c} levels greater than 6.2% in patients with diabetes.[717] Each percentage point elevation in HbA_{1c} increased cardiovascular risk by 11%. Multiple other lines of evidence, as noted, confirm the relationship between hyperglycemia, diabetes, and increased cardiovascular risk. Despite this, it has been difficult to establish that glucose control in diabetes could decrease future macrovascular events, including trials investigating intensive versus standard diabetes treatment and different strategies of achieving glycemic control. Although meta-analyses of randomized controlled trials of diabetes treatments revealed improved glucose control was associated with reductions in nonfatal myocardial infarction rates, there has been no evidence for decreases in either cardiovascular death or all-cause mortality.[13] Given the clear increased cardiovascular risk with diabetes, the inability to reduce cardiovascular events in response to treating diabetes represented a clinical paradox, a contrast to contemporaneous studies showing the increased cardiovascular risk of hypercholesterolemia could be reversed by lowering LDL with statin therapy, including in patients with diabetes. Various hypotheses have been raised regarding this inability of diabetes treatment to show improved cardiovascular outcomes, including trial issues such as study design, sample size, duration, and poorly selected primary endpoints, and disease issues such as disease duration or treatment-specific factors (e.g., drugs used).

In 2008, the FDA mandated that novel glucose-lowering therapies seeking approval must demonstrate cardiovascular safety. The resulting data, involving over nine cardiovascular outcome trials reported to date involving 200,000 patients, have led to a striking pivot for the fields of both diabetes and cardiology with data demonstrating not only cardiovascular safety but also cardiovascular benefit for glucagon-like peptide 1 receptor agonists (GLP1RA) and sodium glucose transporter 2 inhibitors (SGLT2i). Other studies established cardiovascular safety but also identified potential concerns over an increased risk for inducing heart failure, as seen with some dipeptidyl-peptidase 4 inhibitors (DPP4i). Given the landmark nature of these trials, their impact on clinical management of diabetes, and the new questions posed by these findings, a review of the prior studies with older antidiabetic agents warrants review and provides context before the results with these newer agents are considered.

Earlier studies, such as the DCCT in T1DM and the smaller Veterans Affairs Diabetes Feasibility (VA) trial in T2DM, did not show a reduction in cardiovascular endpoints with intensive metabolic control. These well-conducted studies had inherent limitations. Although relatively large (N = 1441), the DCCT followed a relatively young (mean age 27 years) population of patients with T1DM for approximately 6 years. At the end of follow-up, few events had occurred.[9] Intensive therapy reduced the risk of coronary artery and peripheral vascular disease by 41% compared with conventional therapy, but the difference was not statistically significant. Similarly, in the VA trial, intensive blood glucose control in patients with T2DM trended to but did not significantly reduce cardiovascular endpoints.[739] Both studies lacked adequate power to detect a difference in macrovascular events between treatment groups because of the small number of events in each group, small patient populations, and relatively short follow-up.

In contrast, the 17-year follow-up of the 1441 patients from the DCCT trial (EDIC) demonstrated the benefit of early intensive glycemic control in T1DM on cardiovascular risk in the subsequent post-DCCT, EDIC observational setting.[740] This study and its findings are discussed in the sections on biochemistry and molecular cell biology and retinopathy earlier in the chapter. During follow-up, those who had previously been randomized to intensive treatment had an emergent reduction in cardiovascular risk of 42% (p = 0.02) and a reduction in the composite risk of nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death of 57% (p = 0.02). Of note, this separation was evident even though after 11 years of follow-up, HbA_{1c}, as well as blood pressure and lipid risk factors, were essentially identical between the two arms. Patients in the conventional treatment group had more albuminuria and microalbuminuria than intensively treated patients, but the differences in risk remained significant after adjustment for these factors. These findings indicate early intensive glycemic control reduced the long-term risk of cardiovascular disease in patients with T1DM while also raising intriguing mechanistic questions regarding metabolic memory (discussed in the first section of this chapter).

For T2DM, the UKPDS was larger and adequately powered to detect differences between groups in terms of macrovascular events in regular versus intensive glycemic control.[379] Those receiving intensive glycemic control showed a trend toward lower myocardial infarction rates compared to conventional treatment, which was not statistically significant (p = 0.052).[379] Despite the lack of overall efficacy of intensive treatment for management of macrovascular complications of diabetes in the UKPDS, there were indications that metformin as a specific therapy may be effective in reducing cardiovascular events.[379,741] In a retrospective analysis of an overweight subset (n = 342) of the UKPDS cohort who were randomized to metformin, significant reductions in the occurrence of any diabetes-related endpoint (32%), diabetes-related death (42%), and all-cause mortality (36%) were seen compared with conventionally treated patients.[741] Although this is subset data in a limited number of patients, these results, along with registry data suggesting decreased mortality with metformin use,[742] promoted acceptance of metformin having potential cardiovascular benefit.

Subsequent trials examined whether lowering the target for glycemic control (to HbA_{1c} <7%) would reduce cardiovascular events. The ACCORD trial randomized patients with T2DM who had established cardiovascular disease or multiple risk factors to a treatment-directed HbA_{1c} value lower than 7% or to standard therapy with an HbA_{1c} target value between 7% and 7.9%. Over 3.5 years of follow-up, total mortality rate increased in the intensively treated group without any significant reduction in cardiovascular events.[535] The VADT, which randomized patients to either intensive treatment (median HbA_{1c} 6.9%) or standard therapy (median HbA_{1c} 8.4%) also failed to demonstrate cardiovascular benefit through tighter HbA_{1c} control.[538] The ADVANCE trial,

which randomly assigned patients to an intensively treated group, who attained HbA_{1c} of 6.5% versus a standard control group who had HbA_{1c} values of 7.3%, demonstrated a relative reduction of 10% in the combined outcome of major macrovascular and microvascular events that was not statistically significant.[532] Of note, this statistically insignificant 10% relative decrease in cardiovascular events was driven largely by a 21% reduction in nephropathy. Taken together, these three studies do not support HbA_{1c} reduction beyond 7%, improving cardiovascular outcomes in patients with established coronary heart disease and treated with these agents.

An additional perspective on the prior data showing limited if any cardiovascular benefit through older glucose-lowering treatments is that HbA_{1c} represents a flawed surrogate marker for identifying risk of future macrovascular outcomes responsive to therapy, contrasting with the ability of HbA_{1c} to track efficacy in predicting microvascular responses to glucose-lowering treatment. Given that HbA_{1c} is used to define the disease of diabetes, enrolling patients based on HbA_{1c} may not consistently enroll those more likely to benefit in terms of macrovascular outcomes in response to the treatment under study.

Overall, many studies provide strong epidemiologic data showing that diabetes is associated with a large increase in cardiovascular risk. In contrast to studies of microvascular disease, where the associated risk is high per unit change in HbA_{1c}, the associated cardiovascular risk per unit change in HbA_{1c} is lower, once corrected for triglycerides, LDL cholesterol, hypertension, and smoking.

While the benefit of glucose lowering with metformin to reduce myocardial infarction was shown in the substudy of the UKPDS,[741] as previously mentioned, little data was available to support other therapeutic approaches or specific agents.

Studies Using Insulin-Sensitizer Medications

The BARI 2D trial randomized 2368 patients with T2DM and coronary heart disease to either prompt revascularization or intensive medical therapy alone and to either insulin sensitization or insulin provision diabetes therapy. Randomization was stratified by the proposed revascularization method. The 5-year survival and major cardiovascular event rates were similar in all study subgroups except for those patients undergoing coronary artery bypass grafting who had fewer major cardiovascular events after revascularization. There was less hypoglycemia and weight gain and greater apparent benefit from coronary artery bypass grafting in the insulin sensitization group.

To evaluate insulin versus other therapies for lowering glucose as a strategy to decrease cardiovascular events, in the Outcome Reduction With Initial Glargine Intervention (ORIGIN) trial, insulin or noninsulin therapies were used in patients with either prediabetes or early T2DM to achieve a fasting plasma glucose of 95 mg/dL versus 123 mg/dL (5.3 mmol/L vs 6.8 mmol/L) with other therapies.[743] No difference in cardiovascular outcomes was evident in the more aggressively treated arm.

Thiazolidinediones are direct insulin sensitizers, improving insulin sensitivity rather than increasing insulin levels. TZDs bind to and activate the nuclear receptors known as peroxisomal proliferator-activated receptor gamma (PPARγ) and to mitoNEET, a mitochondrial protein-regulating energy metabolism.[744] TZD effects in vascular and inflammatory cells limit atherosclerosis and inflammatory responses. In the PROactive study (i.e., Prospective Pioglitazone Clinical Trial in Macrovascular Events), 5238 patients with T2DM and evidence of macrovascular disease were randomized to either pioglitazone or usual care with the objective of similar glucose control in both arms.[745] After a mean of 34.5 months, there was no significant difference in the two treatment groups in the study's primary endpoint—a seven-component composite of all-cause mortality, nonfatal myocardial infarction, stroke, acute coronary syndrome, surgical intervention in the leg or coronary arteries, and amputation above the ankle. This broad primary endpoint, which extended beyond the more standard objective cardiovascular primary endpoint used in cardiovascular trials, was used to increase the prospect of demonstrating cardiovascular benefit, under the assumption that peripheral arterial disease behaves like coronary disease. However, improvements in peripheral arterial disease endpoints have been difficult to demonstrate, even with potent cardiovascular risk-reducing agents such as statins. Patients treated with pioglitazone had significantly lower risk for the prespecified secondary endpoint in this trial, which was more consistent with the standard primary cardiovascular trial endpoint of combined all-cause mortality, nonfatal myocardial infarction, and nonfatal stroke (HR: 0.84; 95% CI: 0.80–1.02; $p = 0.027$).

Two issues have complicated the use of TZDs. A meta-analysis of rosiglitazone effects on cardiovascular outcomes examining short-term trials of glycemic control suggested rosiglitazone was associated with an increased risk of myocardial infarction. Total event rates were low in the included studies and they did not examine cardiovascular events as primary endpoints nor were the cardiovascular events adjudicated.[746] In contrast, other meta-analyses, including ones using only randomized clinical trials with prespecified cardiovascular endpoints and/or adjudication, found no increase in cardiovascular events or cardiovascular death with either rosiglitazone or pioglitazone.[747] This conclusion was also supported by other subsequent studies and an FDA consensus review panel.[748] Differences do exist between pioglitazone and rosiglitazone. A second factor limiting use of these agents has been their known side effects, including fluid retention that may increase heart failure (although without myocardial changes or increased mortality), increased bone fractures, significant increases in weight among a subset of patients, and increases in bladder cancer.[749] Whether these untoward effects are offset by clinical cardiovascular benefit may require further consideration, especially given more recent positive clinical trial data discussed later in the chapter.

Prior inability to conclusively demonstrate cardiovascular event reduction with specific diabetes approaches or therapies could have been due to not having the right agent or targeting the most impactful mechanism rather than other explanations such as patient characteristics or study design.[750,751]

As a result of the 2008 FDA-instituted guidance that new antidiabetic drugs must provide cardiovascular safety data in their approval process, a large amount of new data regarding cardiovascular effects of newer antidiabetic agents has emerged and continues to do so, including new data showing cardiovascular risk reduction for patients treated with GLP1RA or SGLT2 inhibitors. These results have focused thinking on specific agents and potential mechanisms that are likely more complex than simply lowering blood glucose concentration.

Studies Using Newer Antidiabetic Medications: A New Era?

Dipeptidyl Peptidase 4 Inhibitors and Cardiovascular Disease

DPP4i lower glucose by inhibiting degradation of the incretin GLP1. The SAVOR–Thrombolysis in Myocardial Infarction (TIMI) 53 (SAVOR-TIMI 53) trial established safety for this

agent with no difference in major adverse cardiovascular events (MACE) (the composite of nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death); however, a 27% increase in hospital admissions for heart failure was seen.[752] The Examination of Cardiovascular Outcomes With Alogliptin Versus Standard of Care (EXAMINE) similarly established cardiovascular safety for the MACE outcome with this DPP4i.[753] Although EXAMINE investigators reported that alogliptin had no impact on heart failure,[754] differences in the design and endpoint measurements in this trial as compared to SAVOR-TIMI 53 may limit this conclusion.[755] In the Trial to Evaluate Cardiovascular Outcomes After Treatment With Sitagliptin (TECOS), sitagliptin showed no cardiovascular benefit but also no adverse effects, including no increased admissions for heart failure, all as compared to usual care. Other approaches to the issue of DPP4i and heart failure, like observational studies and meta-analyses, have varied in conclusion about whether specific agents differ in heart failure risk. The FDA has included heart failure warnings in DPP4i labeling. While none of these agents increase mortality, it is prudent to examine DPP4i-treated patients with diabetes for signs and symptoms of heart failure. Notably, prior to these outcome studies, less rigorous forms of data had suggested cardiovascular benefits with DPP4i, underscoring the primacy of randomized, prospective, blinded clinical trial data.

In the CARMELINA study,[756,756a] the effect of linagliptin (5 mg once daily) was compared to placebo (both added to standard of care) on cardiovascular outcomes in 6979 adults with T2DM and high cardiovascular risk, the majority of whom also had kidney disease. Linagliptin demonstrated a similar cardiovascular safety profile compared to placebo when added to standard of care. There was a similar kidney safety profile and no increase in heart failure compared to placebo.[756,756a] The companion Cardiovascular Outcome Study of Linagliptin Versus Glimepiride (CAROLINA)[757] is comparing linagliptin to the sulfonylurea glimepiride in patients with T2DM and higher cardiovascular risk (known cardiovascular disease or T2DM-associated end-organ damage or older age or two or more cardiovascular risk factors).

GLP1RAs and Cardiovascular Disease

In contrast to the DPP4 inhibitor data, studies with both GLP1RAs and SGLT2 inhibitors provided landmark data showing decreased cardiovascular events with glucose-lowering therapy. Not surprisingly, these positive findings have prompted intense scrutiny and further questioning regarding mechanisms explaining these results. Nevertheless, the findings stand. The fact that two different agents within each drug class have shown a positive cardiovascular primary endpoint reinforces the general validity of the data. Furthermore, both GLP1RAs and SGLT2 inhibitors improved renal outcomes (see "Glycemic Control in Diabetic Kidney Disease").

GLP1RAs have GLP1 receptor-dependent and receptor-independent effects on the cardiovascular system.[758,759] GLP1RAs can induce satiety, promote weight loss, lower blood pressure, and have other effects in preclinical and smaller translational studies that might promote cardiovascular benefit.[760] In the LEADER trial[543] (Table 37.19), 9340 patients with T2DM and increased cardiovascular risk received random assignment to either liraglutide (titrated to 1.8 mg once daily) or placebo, with median follow-up of 3.8 years. The primary time-to-event analysis, which was the combined results of first occurrence of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke, was significantly decreased among liraglutide (overall incidence 13%) versus placebo group (14.9%, $p < 0.001$ for noninferiority; $p = 0.01$ for superiority).[543]

Semaglutide, a GLP1RA administered once a week, was studied in the SUSTAIN 6 trial, which enrolled 3297 patients with T2D, of whom the majority had established cardiovascular disease, chronic kidney disease, or both. Participants received either semaglutide (0.5 or 1 mg) or placebo once weekly for 2 years. The primary composite outcome, which was the first occurrence of the same 3-point major adverse cardiovascular event, was significantly reduced in those on semaglutide ($p < 0.001$ for noninferiority), with nonsignificantly decreased nonfatal myocardial infarction and significantly decreased nonfatal stroke (see Table 37.19).[544] In further support of GLP1RAs having cardiovascular benefit, the Harmony Outcomes trial demonstrated that 9463 patients with T2DM and cardiovascular disease receiving albiglutide 30 to 50 mg based on glycemia and tolerability had a 22% reduction in cardiovascular events compared to those receiving placebo in conjunction with usual care[761] over a median follow-up of 1.5 years.

In contrast, other GLP1RAs have shown cardiovascular safety but no benefit in outcome trials. In the Evaluation of Lixisenatide in Acute Coronary Syndrome (ELIXA) trial, 6068 patients with T2DM with recent (<180 days) myocardial infarction or unstable angina hospitalization received lixisenatide 10 to 20 μg once daily or placebo[762] with usual care with median follow-up of 25 months. The primary composite endpoint of cardiovascular death,

TABLE 37.19 Key Findings From GLP1 Receptor Agonist Cardiovascular Outcome Trials

Trial	GLP1RA	Sample Size	Follow-Up Years	Primary CV Composite Outcome[a]	Myocardial Infarction	Nonfatal Stroke	CV Mortality	All-Cause Mortality	Hospitalization for Heart Failure
LEADER	Liraglutide	9340	3.5	0.87 (0.78-0.97); $p = 0.01$	↓14% $p = 0.046$	↓11% $p = 0.30$ NS	↓22% $p = 0.007$	↓15% $p = 0.02$	↓13% $p = 0.14$ NS
SUSTAIN-6	Semaglutide	3297	2.0	0.74 (0.58-0.95); $p = 0.02$	↓15% $p = 0.38$ NS	↓39% $p = 0.04$	→ $p = 0.92$ NS	→ $p = 0.79$ NS	→ $p = 0.57$ NS

Results are shown as hazard ratio or odds ratio with (95% confidence intervals), or as percent change.

Full trial names and references are given in the text.

[a]CV death, myocardial infarction, or stroke.

CV, Cardiovascular; *GLP1RAs,* glucagon-like peptide-1 receptor agonists.

myocardial infarction, stroke, or unstable angina hospitalization showed no superiority, while also establishing safety given noninferiority versus placebo. The Exenatide Study of Cardiovascular Event Lowering (EXSCEL) trial, with extended-release exenatide also showed safety but no benefit when studied in 14,752 patients with T2DM treated with either extended-release exenatide 2 mg weekly or placebo over 3.2 years; no difference was seen versus placebo on the primary composite outcome of first occurrence of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke (HR: 0.91; 95% CI: 0.83–1.00; $p < 0.001$ for noninferiority; $p = 0.06$ for superiority).[763]

The basis for positive cardiovascular outcomes with liraglutide, semaglutide, and albiglutide but not lixisenatide or extended-release exenatide remains obscure. Differences between clinical trials such as enrollment criteria, subtle differences in patient populations, study locations, trial duration, and concomitant medication use may be underlying factors. It remains possible some members of the same drug class may have more potent effects on discrete cardiovascular risk parameters, such as blood pressure or weight loss. Given the prior literature, differences in glucose control itself seem unlikely to explain variable outcome benefits within drug classes or between older and newer agents. Pharmacologic differences among the agents is difficult to exclude (e.g., short-acting vs long-acting activity with long-acting exenatide nearly reaching statistical significance).

Initial concerns about increased risk of pancreatitis, pancreatic cancer, and thyroid cancer with GLP1RAs have not been borne out in clinical trials, although a prior history of such issues is viewed as a potential contraindication to use. Given that GLP1 provides a gut-derived satiety signal to the brain, the gastrointestinal side effects seen with these drugs, mainly nausea, are not surprising, may contribute to beneficial weight loss, and are not a barrier for most patients. GLP1RAs do not pose concerns for patients with chronic kidney disease, within the limits of the populations enrolled. A decrease in progression of chronic kidney disease has been noted. For some patients, the injectable nature of GLP1RAs may be an issue. These agents on their own do not cause hypoglycemia, although caution may be needed when combined with insulin provisional diabetes treatments (e.g., sulfonylureas or insulin).

SGLT2 Inhibitors and Cardiovascular Disease

By blocking glucose absorption in the proximal tubule, SGLT2 inhibitors lower glucose levels, while also wasting an energy substrate and having a diuretic effect, with resulting weight loss and blood pressure lowering.[764]

In the EMPA-REG OUTCOME trial[765] (Table 37.20), 7020 patients with T2DM and established cardiovascular disease received empagliflozin, 10 mg or 25 mg, or placebo once daily in addition to usual care. After a median duration of 3.1 years, the primary major cardiovascular event composite outcome among those receiving empagliflozin was significantly decreased. Especially impressive was that this positive outcome was driven primarily by a major decrease in death, which was seen in both cardiovascular (38%) and all-cause mortality (32%). Hospitalization for heart failure was significantly reduced in those on empagliflozin (–35%, $p = 0.002$), as evident in both those with and without a history of heart failure, as possibly expected for an agent with diuretic effects. An antihypertensive effect was also evident. However, a similar magnitude of reduction in hospitalization for heart failure has not been seen in clinical trials of diuretics or other antihypertensive drugs, making this explanation less likely. Given that most participants did not have a heart failure history, this effect highlights the extent to which subclinical heart failure as well as risk for heart failure is a major issue in T2DM while also suggesting new directions for SGLT2 inhibitor use now being investigated. The rapid separation of curves in terms of mortality and heart failure, both occurring after approximately 6 months, was also noteworthy.

Canagliflozin, another SGLT2 inhibitor, was studied in the CANVAS[473] program (see Table 37.20), which involved two cardiovascular trials, including CANVAS-R that focused on patients with renal disease. These studies involved 10,142 subjects with T2DM and either a history of cardiovascular disease (approximately two-thirds) or just an additional cardiovascular risk factor (one-third). After a mean of 3.6 years (median 2.4 years), the primary major adverse cardiovascular endpoint (myocardial infarction, stroke, cardiovascular mortality) was significantly reduced. Although each of these components trended toward benefit, none of them were significant on their own, including cardiovascular mortality or total mortality, in contrast to the EMPA-REG OUTCOME trial. A signal for an increased risk of amputations was seen in CANVAS, without clear evidence for similar issues in the EMPA-REG OUTCOME trial. Other analyses have also raised concerns regarding possible increased risk for amputations with canagliflozin. The extent to which this finding is valid involves other SGLT2 inhibitors, and the potential underlying mechanism for this putative issue remains unresolved and awaiting further data, although the significant reduction in cardiovascular events is evident. In Dapagliflozin Effect on Cardiovascular Events (DECLARE-TIMI 58), the effects of dapagliflozin in 17,150 patients with inadequately controlled diabetes and either known cardiovascular disease (secondary prevention cohort) or at least two risk factors for cardiovascular disease (primary prevention cohort) was studied. In announced but not yet published topline results, dapagliflozin demonstrated cardiovascular safety and a reduction in a composite of cardiovascular death or hospitalization for heart failure but without a significant difference in the major adverse composite outcome of combined cardiovascular death, nonfatal myocardial infarction, or ischemic stroke.

Other large cardiovascular outcome trials are being done with other SGLT2 inhibitors. Ertugliflozin is also being investigated in a large outcome cardiovascular trial. Future studies in other specific populations will provide additional insight into SGLT2 inhibitor effects. Other real-world data analyses, performed outside of a randomized clinical trial setting, have supported cardiovascular benefits with SGLT2 inhibitors. For example, CVD-REAL[766] (see Table 37.20), which reviewed medical claims, hospital records, and registries from 309,056 patients in six countries taking SGLT2 inhibitors, found SGLT2 inhibitor use was associated with a 39% lower risk of heart failure hospitalization ($p < 0.001$) and a 51% reduction in total death ($p < 0.001$) independent of country.

The mechanism for cardiovascular benefits with SGLT2 inhibitors remains to be defined. The multitude of prior negative trials with glucose-lowering argues against the modest improvement in HbA_{1c} as underlying the observed benefit. Reductions in blood pressure, weight and volume, with concomitant increases in hematocrit as well as unique aspects to tissue-based volume shifts, have been hypothesized to contribute to the decrease in cardiovascular risk. However, the relative small changes in these parameters and the lack of similar effects in trials with diuretics or other antihypertensives suggest the involvement of other factors. Serum uric acid levels, previously linked to cardiovascular risk, are decreased with SGLT2 inhibition, but the rapidity with which the curves

TABLE 37.20 Key Findings From SGLT2 Inhibitor Cardiovascular Outcome and Observational Studies

Studies	SGLT2 Inhibitor Daily Dose vs Comparator	SGLT2i vs Placebo or Comparator, *N*	History of CVD Patients, %	Median Follow-Up Years	Primary CV Composite Outcome[a]	Myocardial Infarction (Fatal or Nonfatal)	Stroke (Fatal or Nonfatal)	CV Mortality	All-Cause Mortality	Hospitalization for Heart Failure
Randomized Controlled Trials vs Placebo										
EMPA-REG OUTCOME[1]	Empagliflozin 10 or 25 mg vs placebo	4687 vs 2333	99	3.1	0.86 (0.74-0.99); $p = 0.04$	0.87 (0.70-1.09); $p = 0.23$	1.18 (0.89-1.56); $p = 0.26$	0.62 (0.49-0.77); $p < 0.001$	0.68 (0.57-0.82); $p < 0.001$	0.65 (0.50-0.85); $p = 0.002$
CANVAS[2]	Canagliflozin 100–300 mg vs placebo	5795 vs 4347	65	2.4	0.86 (0.75-0.97); $p = 0.02$	0.85 (0.69-1.05); NS	0.90 (0.71-1.15); NS	0.87 (0.72-1.06); NS	0.87 (0.74-1.01); NS	0.67 (0.52-0.87)[b]
Observational Studies vs Active Comparator										
CVD-REAL[3]	SGLT2i (dapagliflozin 42%, canagliflozin 53%)	154528 vs 154528 (propensity matching)	13	0.6–0.7	NA	0.85 (0.72-1.00); $p = 0.05$	0.83 (0.71-0.97); $p = 0.02$	NA	0.49 (0.41-0.57); $p < 0.001$	0.61 (0.51-0.73); $p < 0.001$

Results are shown as hazard ratio or odds ratio (95% confidence intervals). *p* values are provided as available.

[a]Cardiovascular death, nonfatal myocardial infarction, or nonfatal strokes.

[b]Hospitalization for heart failure was prespecified exploratory cardiovascular outcome.

NA, Not available; *NS,* nonsignificant; *SGLT2,* sodium glucose cotransporter type 2.

Data from [1]Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med.* 2015;373:2117–2128; [2]Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med.* 2017;377:644–657; [3]Kosiborod M, Cavender MA, Fu AZ, et al. Lower risk of heart failure and death in patients initiated on sodium-glucose cotransporter-2 inhibitors versus other glucose-lowering drugs: the CVD-REAL study (comparative effectiveness of cardiovascular outcomes in new users of sodium-glucose cotransporter-2 inhibitors). *Circulation.* 2017;136:249–259.

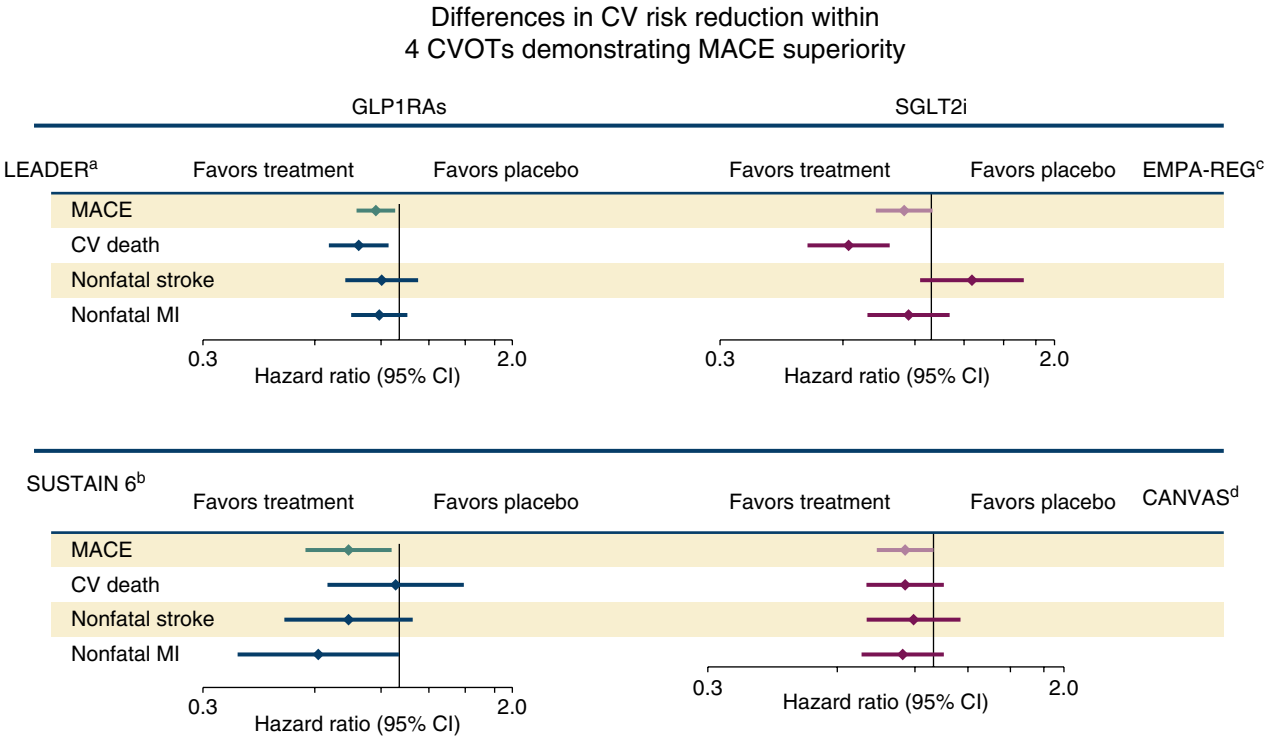
separate in the SGLT2 trials make this explanation less likely. Other proposed mechanisms include more efficient fuel switching in patients with T2DM and ischemia, through increased plasma β-hydroxybutyrate as a better energy substrate, changes in dysregulated neurohormonal signaling, and alterations in sodium-hydrogen exchange, among others.

Untoward effects with SGLT2 inhibition align in part with its known mechanism of action. Induced glucosuria can promote myocotic genital infections, which can be offset by good hygienic practices, with consideration of avoiding these agents in patients with a history of these issues. Increased diabetic ketoacidosis and euglycemic ketoacidosis have been reported with SGLT2 inhibitors although not seen in meta-analyses or the randomized trials discussed earlier. The question of increased amputation risk with canagliflozin was noted previously. Hypoglycemia is not an issue with this class of drugs, unless added to a regimen that includes insulin provisional therapies.

The positive cardiovascular outcome trials with both GLP1RAs and SGLT2 inhibitors (Fig. 37.49) raise fundamental questions regarding optimal T2DM management: How should these data be incorporated into guidelines, including the choice between GLP1RAs and SGLT2 inhibitors? Most guidelines have adopted an approach of recommending these agents in ways that align with the trials, for use mainly in patients with inadequately controlled HbA_{1c} and established or high risk for cardiovascular disease. This leaves unresolved the question of changing therapies for patients who have reasonable glycemic control but use antidiabetic drugs with no proven cardiovascular benefit. Separately, should cardiologists prescribe glucose-lowering agents, and how should previously encountered barriers to cardiologist engagement with diabetes treatment be overcome? Greater insight into the mechanisms underlying cardiovascular benefit from these newer agents may yield further refinement in their use, while also advancing continued drug development. Finally, the positive clinical trial data in T2DM patients with established cardiovascular disease or high cardiovascular risk have prompted further consideration of how the benefits seen with these therapies might be extended to other indications and patient groups, particularly T2DM patients without current cardiovascular disease. Clinical trials of these agents in people with T2DM and hypertension or heart failure are already under study. These agents, which do not cause hypoglycemia, may be especially appealing for reducing cardiovascular risk in those with prediabetes.

Characteristic Features and Treatment of Dyslipidemia in Diabetic Patients

Dyslipidemia has been well characterized as a risk factor that increases atherosclerosis in patients with T2DM. Various patterns of dyslipidemia are often encountered in diabetes and may



• **Fig. 37.49** Components of positive primary endpoint responses in landmark glucagon-like peptide 1 receptor agonist (GLP1RA) and sodium-glucose cotransporter-2 (SGLT2) inhibitor trials. Data shown for GLP1RAs (LEADER/liraglutide, SUSTAIN 6/semaglutide) and SGLT2 inhibitor (EMPA-REG/empagliflozin, CANVAS/canafliglozin) cardiovascular outcome trials (CVOT). *CI,* Confidence interval; *MACE,* major adverse cardiovascular events; *MI,* myocardial infarction. Full study names are noted in text. [a]Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2016;375:311–322; [b]Marso SP, Bain SC, Consoli A, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med*. 2016;375:1834–1844; [c]Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. 2015;373:2117–2128; [d]Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med*. 2017;377:644–657.

increase atherogenic risk (also see Chapter 41). Although patients with diabetes tend not to have marked elevations in plasma LDL cholesterol levels, their LDL cholesterol levels remain predictive of cardiovascular risk. LDL particles in diabetes are smaller and more dense than normal LDL. These small, dense triglyceride-rich LDL particles are especially pathologic. Small dense LDL particles are more susceptible to oxidation, particularly in the setting of poor glucose control. Other evidence suggests that glycation of LDL may be enhanced in diabetes, impairing recognition of the lipoprotein by its hepatic receptor and extending its half-life. Conversely, HDL cholesterol levels are decreased in patients with T2D.[767]

A key feature of diabetic dyslipidemia is an increase in hepatic very low-density lipoprotein (VLDL) production in response to elevations in FFA flux often seen in diabetes with insulin-resistant adipocytes. Although insulin and lipases such as lipoprotein lipase mediate the uptake of triglyceride-derived FFAs by striated muscle, reducing the levels presented to the liver, insulin resistance results in the opposite effect, increasing the levels of FFAs presented to the liver. The metabolic syndrome, with its characteristic abdominal obesity and insulin resistance, also increases FFA delivery to the liver. In addition, reduced lipoprotein lipase activity in T2DM leads to an accumulation of triglyceride-rich lipoproteins in the plasma of these patients and may also result in decreased physiologic delivery of lipolytically derived, biologically active molecules to cells. Such models align with genetic evidence in the general population. Gain-of-function variants with increased lipoprotein lipase activity, as well as those with genetic loss of function of inhibitors of the lipoprotein lipase pathway, appear to have less cardiovascular risk. Triglyceride-rich lipoproteins also play a role in the reduced levels of HDL cholesterol by increasing the transfer of cholesterol from these particles.

A number of landmark trials have established that lowering LDL cholesterol levels produces major clinical benefits in terms of reducing cardiovascular events in patients with and without a history of cardiovascular disease at baseline. These findings have now been extended to the population of subjects with T2DM and dyslipidemia. Although LDL levels are often within the average range in patients with diabetes, treatment with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) has consistently shown improved outcomes among both those with and without diabetes, including those with and without a prior history of heart disease. Statins are now incorporated into current diabetes management guidelines, discussed further later in the chapter.

In the Cholesterol and Recurrent Events (CARE) trial, T2DM patients with a prior cardiovascular event receiving pravastatin had a 25% reduction in the incidence of cardiovascular death, nonfatal myocardial infarction, coronary artery bypass graft, and revascularization procedures compared to placebo.[768] In the Long-Term Intervention With Pravastatin in Ischemic Disease (LIPID) study, patients with diabetes had a 19% reduction in major coronary heart disease events (fatal cardiovascular disease and nonfatal myocardial infarction).[769] In a post hoc subgroup analysis of secondary prevention in a large cohort of patients with diabetes, impaired glucose tolerance, or normal glucose tolerance, simvastatin normalized associated elevations in total cholesterol and triglycerides across the range of glucose values.[770] Treatment also significantly reduced major coronary events and revascularizations in patients with diabetes and reduced major coronary events, revascularizations, and total and coronary mortality in patients with impaired glucose tolerance.

Several studies have further validated the use of statins in patients with diabetes and related conditions.[567,771–774] In the Heart Protection Study, a large (N = 20,536), randomized, placebo-controlled trial of the use of simvastatin 40 mg in high-risk patients, roughly 29% of the study participants had T2DM.[775] Over the 5-year course of the study, treatment with simvastatin resulted in a significant reduction in the occurrence of major vascular events in patients with T2DM who had established coronary heart disease (33.4% vs 37.8% in simvastatin-treated and placebo-treated patients, respectively), in T2DM with no prior coronary heart disease (13.8% vs 18.6%), and in both categories combined (20.2% vs 25.1%). Overall, the study also demonstrated a highly significant 12% relative risk reduction in all-cause mortality and an 18% reduction in coronary mortality among all subjects treated with simvastatin.

The Collaborative Atorvastatin Diabetes Study (CARDS) had a fundamental impact on the management of diabetes because of the magnitude of beneficial effects seen in the statin-treated group. CARDS was a randomized, placebo-controlled trial that tested the effect of starting low dose atorvastatin (10 mg/day) in T2DM patients with no prior history of heart disease, with plasma LDL levels lower than 160 mg/dL (<4.1 mmol/L) (N = 2838) on preventing acute coronary heart events, coronary revascularization, or stroke.[574] The trial was terminated 2 years early because the prespecified efficacy criteria were met. After a median of 3.9 years follow-up, patients treated with atorvastatin had a relative risk reduction for first cardiovascular event of 37% (95% CI: 52–17% reduction; p = 0.001), compared with placebo-treated patients. Assessed separately, acute coronary heart disease events, coronary revascularizations, and stroke were significantly reduced, by 36%, 31%, and 48%, respectively. The CARDS trial showed that in patients with T2DM, a threshold LDL cholesterol level should not be the sole determinant of whether a statin is prescribed. Of note, stroke was reduced in CARDS, addressing prior questions about whether LDL lowering with statins might or might not reduce stroke to the same extent as they lower coronary heart disease. Currently, it is generally accepted that statins are effective in reducing cerebrovascular disease.[776] As a result of CARDS and other studies discussed, most if not all guidelines have endorsed statin use in most appropriately aged individuals with diabetes.

The Treating to New Targets (TNT) study compared the effects of atorvastatin 10 mg or 80 mg daily for a median follow-up period of 4.9 years in patients with clinically evident coronary heart disease who also met the NCEP criteria for diagnosis of the metabolic syndrome.[772] The study included 778 patients with T2DM, who constituted 22% of the study population. Treatment with atorvastatin 80 mg reduced major cardiovascular events 29% more than atorvastatin 10 mg, presumably due to the greater reduction in LDL cholesterol seen with higher dosing of atorvastatin.

Pooling of data from the multiple statin trials further supports statin benefits in those with diabetes. In the Cholesterol Treatment Trialists' (CTT) Collaboration,[777] analysis of 18,646 patients with diabetes in 14 different studies revealed a 9% proportional reduction in all-cause mortality per mmol/L reduction in LDL cholesterol (RR: 0.91, 99% CI: 0.82–1.01; p = 0.02) as compared to a similar 13% reduction in those without diabetes (RR: 0.87, 99% CI: 0.82–0.92; p < 0.0001).[778] A clinically and statistically significant 21% proportional reduction in major vascular events per mmol/L reduction in LDL cholesterol was observed in people with diabetes (RR: 0.79, 99% CI: 0.72–0.86; p < 0.0001).

In 2013, revised cholesterol treatment guidelines were provided by the American College of Cardiology (ACC) and the American Heart Association (AHA).[779] These recommendations depart from prior guidelines by focusing initiation of lipid-lowering therapy on four clinically defined patient groups who warranted either high-intensity, moderate-intensity, or low-intensity statin intervention while also abandoning use of LDL targets as a means of guiding therapy. Patients with diabetes were one of these four treatment groups, as supported by the data from primary and secondary prevention trials noted previously. A controversial aspect of these guidelines was their abandonment of specific LDL targets. Both TNT and the subsequent Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), which showed that adding the nonstatin LDL-lowering agent ezetimibe to stable statin therapy further decreased cardiovascular events in patients with coronary heart disease,[780,781] support the clinical utility of using specific LDL targets. The 2013 guidelines might be best viewed as a scientific statement given their precise focus on what had been proven in clinical trials, which explains the specific age cutoff points for statin treatment of patients with diabetes.

Clinical trials have also revealed statins are associated with an increase in new-onset diabetes among a small subset of patients at high risk for development of T2DM. In the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) study, which investigated rosuvastatin 20 mg versus placebo in 17,603 patients with no history of atherosclerotic events, baseline mean LDL levels of 108 mg/dL, and an elevated C-reactive protein of 4.2 mg/L, physician-reported new-onset diabetes was found in 270 patients on rosuvastatin versus 216 on placebo.[782] In the JUPITER population 41% of participants had metabolic syndrome at study entry. New-onset diabetes was found in 28% in patients with one or more risk factors for developing diabetes, who at the same time benefited by a 39% reduction in cardiovascular events and a 17% reduction in total mortality. Among those with no risk factors for diabetes, there was no increase in new diagnoses of diabetes.[783] Thus the modest increase in diabetes in response to statin treatment is markedly offset by major decreases in cardiovascular events. For patients in whom statin treatment is appropriate, concerns over increased conversion to diabetes should not be a factor in the decision to use statins. While increases in new diabetes has been seen with multiple statins, the mechanism underlying this statin effect remains obscure.

Additional data are now available regarding LDL cholesterol levels and cardiovascular risk associated with using additional new LDL-lowering therapies. In population studies, individuals with lifelong low LDL cholesterol levels were found to have genetic loss of function in the Proprotein Convertase Subtilisin/Kexin Type 9 *(PCSK9)* gene, which was associated with decreased atherosclerosis and cardiovascular events but no other untoward effects. Further supporting PCSK9 as a drug target, genetic variants with increased PCSK9 function resulted in a lipid and clinical picture that replicated familial hypercholesterolemia (FH). PCSK9 action targets the LDL receptor for proteosomal degradation rather than allowing it to return to the cell surface for another cycle of LDL clearance. In an impressive glimpse into future drug development prospects, in less than 10 years, PCSK9 inhibitors in the form of monoclonal antibodies were being tested for their clinical efficacy in cardiovascular event reduction. In FOURIER, adding the PCSK9 inhibitor evolocumab (Repatha) to statin therapy versus statin alone in 27,546 patients with established coronary disease reduced LDL levels by 59% (92 mg/dL to 30 mg/dL [2.4 mmol/L to 0.78 mmol/L]) and was associated with a statistically significant reduction in cardiovascular events of 15%.[784] The ODYSSEY trial with alirocumab (Praluent) in patients with recent acute coronary syndrome also showed a statistically significant reduction in cardiovascular events, as presented but not yet published.[785] Patients with diabetes are receiving attention in terms of PCSK9 inhibitor use as a function of their increased risk for cardiovascular events. Post hoc studies on outcomes in those with diabetes was a prespecified analysis in FOURIER and demonstrated similar benefit and no adverse issues among these patients (40%) compared to those who did not have diabetes at baseline (60%). In ODYSSEY, 29% of subjects had diabetes, indicating that there should be forthcoming data about this group. Other studies indicate no issues with alirocumab use in those with diabetes, with presentations suggesting an augmented impact on event reduction in acute coronary syndrome patients. An ongoing scientific question is evidence suggesting PCSK9 loss of function may be associated with increased risk of hyperglycemia and perhaps diabetes,[786] although no increased signal for new diabetes has been seen in the clinical trials published to date.

Outcomes of treatment with other nonstatin therapies have been variable. With niacin, data have been disappointing, with no additional benefit seen when added to statins in several trials.[773,787,788] Although the lower HDL levels found in patients with diabetes might make niacin an appealing treatment option, niacin's lack of proven efficacy combined with its side effects of increasing insulin resistance and relatively poor tolerability have limited its use. Recent genetic studies using Mendelian randomization have demonstrated that variants associated with higher HDL levels often do not protect against CV events.[789] Current attention is focused on the concept that the key element in the inverse relationship between HDL and cardiovascular disease is HDL functionality, which may not be reflected in total HDL levels.[790]

Fibric acid derivatives, which lower high triglycerides and raise HDL, have also been proposed as a treatment in people with diabetes given the usual nature of diabetic dyslipidemia. In the VA High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT), men given gemfibrozil had a significant decrease in coronary events and strokes in the absence of statin use.[774] Post hoc analyses suggesting these benefits were driven by the diabetes subgroup focused further attention on fibrates. The FIELD study assessed the effect of long-term fenofibrate therapy on cardiovascular events in patients with T2DM.[567] Patients were randomized to receive either micronized fenofibrate 200 mg per day (n = 4895) or placebo (n = 4900). During 5-year follow-up, 5.9% of the placebo-treated patients versus 5.2% of the fenofibrate-treated patients had a major coronary event (coronary heart disease death or nonfatal myocardial infarction), the primary endpoint of the trial, a difference that was not statistically significant. Fenofibrate therapy significantly reduced some secondary endpoints, including total cardiovascular events (HR: 0.89, 95% CI: 0.75–1.05, p = 0.035), coronary revascularization, progression of albuminuria, and the need for laser treatment of retinopathy. Other secondary endpoints such as cardiovascular mortality were higher, although not statistically significant, with fenofibrate. Statistical significance for the primary endpoint of the study might have been missed because a greater percentage of patients in the placebo group began statin therapy during the study period compared to the fenofibrate group, thus modulating their risk and potentially masking the treatment effect.

The ACCORD trial addressed the use of fibrates in combination with statins in patients with T2DM. This trial investigated whether combination therapy with simvastatin plus fenofibrate, compared with simvastatin alone, would reduce cardiovascular events in diabetic patients at high risk for cardiovascular disease. In this randomized clinical trial comparing fenofibrate to placebo in statin-treated patients, combination therapy did not reduce the rate of cardiovascular mortality, nonfatal myocardial infarction, or nonfatal stroke, although other secondary parameters such as first myocardial infarction were improved. In a prespecified subgroup with a high triglyceride ratio (triglycerides ≥204 mg/dL [≥5.3 mmol/L] and HDL ≤34 mg/dL [≤0.88 mmol/L]), fenofibrate did reduce the primary outcome as compared to the placebo group. A pattern has emerged in fibrate trials of greater benefit in those with significantly elevated triglycerides and lower HDL—the population in which fibrates would be commonly used. At this time, no formal recommendation exists that patients with significant dyslipidemia receive combination statin-fibrate therapy for further risk reduction on the basis of T2DM alone.[791] In those with a significant history of CVD, elevated triglycerides, and low HDL, expert opinion might endorse fibrates as an adjunct to statin therapy once LDL levels are appropriately controlled as a reasonable if not definitively established option. Importantly, improved glycemic control and decreased insulin resistance, whether through drug therapy or improved lifestyle interventions and modest weight loss, can improve diabetic dyslipidemia. Topline results from the Reduction of Cardiovascular Events With EPA—Intervention Trial (REDUCE-IT),[792] using a specific eicosapentaenoic acid only from fish oil in patients with established cardiovascular disease or diabetes mellitus and at least one other cardiovascular risk factor indicate a 25% reduction in the primary endpoint composite of the first occurrence of major adverse cardiovascular events, including cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina requiring hospitalization.

Genetic variants linked to low triglyceride levels, like apolipoprotein CIII (apoCIII) loss of function, have been reported to predict future cardiovascular events.[793] ApoCIII loss of function reduces plasma triglyceride, and the atherogenicity of chylomicrons, VLDL, LDL, and HDL particles. These findings provide a rationale for pursuing ApoCIII inhibition and genetic knockdown as a therapeutic strategy, which continues to be pursued despite initial FDA rejection of volanesorsen, an apoCIII antisense therapy, out of concerns for thrombocytopenia.[794]

Sources for cholesterol guidelines have transitioned from the prior NCEP Adult Treatment Panel recommendations, the last being the NCEP ATPIII, to joint guidance from the American College of Cardiology and the American Heart Association. Considering both the ATPIII recommendations and the most recent ACC/AHA approaches is valuable and provides additional perspective. The importance of dyslipidemia as a contributor to cardiovascular risk in patients with diabetes was incorporated in the NCEP ATPIII guidelines, which identified diabetes as being equal in cardiovascular risk to having had prior coronary heart events.[726] According to the NCEP ATPIII guidelines, patients with T2DM would receive cholesterol-lowering therapy if the LDL cholesterol level were higher than 130 mg/dL (>3.36 mmol/L), with the goal of reducing LDL cholesterol to less than 100 mg/dL (<2.57 mmol/L).[726] In practice, more aggressive approaches were often used, instituting drug treatment if LDL cholesterol level were higher than 100 mg/dL (>2.57 mmol/L). Subsequent guideline updates endorsed an LDL cholesterol goal of less than 70 mg/dL (<1.81 mg/dL) in higher risk patients even when the high-risk patient had a baseline LDL cholesterol level lower than 100 mg/dL (<2.57 mmol/L).[795] The most recent ACC/AHA cholesterol guidelines took a completely different approach. The guidelines abandon specific LDL targets, instead identifying four specific patient groups in whom clinical trial data supported statin use. Since clinical trials had not specifically targeted more-intensive versus less-intensive LDL goals with a single statin, the recommendations also focused on the appropriate statin dose intensity for given patient groups. The presence of diabetes was one of these four patient groups, with the recommendation that designated patients with diabetes (age 40–75 years), as defined by subjects enrolled in trials, receive a high-intensity statin (atorvastatin 40 or 80 mg, rosuvastatin 20 or 40 mg) if their 10-year calculated risk was greater than or equal to 7.5%, and a moderate-intensity statin (these same statins at their other lower doses and remaining other statins at their mid to highest doses) if calculated 10-year risk was less than 7.5%. This approach has been controversial, especially the abandonment of target LDL levels. However, data from multiple clinical trials, including those with ezetimibe, supported benefits from lower LDL levels. The subsequent PCSK9 inhibitor data has further strengthened evidence for additional cardiovascular benefit from achieving lower LDL levels as mentioned in further guideline updates and will likely be incorporated in an upcoming AHA/ACC guideline release.

Characteristic Features and Treatment of Hypertension in Diabetic Patients

Approximately 50% of patients with newly diagnosed diabetes also have hypertension. As with dyslipidemia, hypertension interacts with diabetes to amplify the risk of cardiac mortality (see Fig. 37.47). Although the cause of hypertension is multifactorial, the insulin-resistant state is one factor postulated to predispose patients to develop hypertension. In addition to its negative effects on the cardiovascular system, high blood pressure is a key contributor to the development of microvascular disease in diabetes. Based on the guidelines of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7), blood pressure should be reduced to less than 130/85 mm Hg in patients with diabetes.[547] A longer duration of diabetes has been linked to increased arterial stiffness, decreased vasomotor function, and changes in pulse pressure, which can contribute to patient symptoms in response to blood pressure treatment. Patients with diabetes may also lose the usual nocturnal blood pressure dip.

Results of the most recent clinical trials underscore the benefits of aggressive treatment of hypertension in patients with diabetes, although achieving mean blood pressure reductions to currently recommended targets is challenging both in clinical trials and in real-world settings. This may also help explain disconcordant trial results. Data have continued to support achieving a systolic blood pressure between 120 and 140 mm Hg in those with diabetes, while additional benefit is less clear with systolic blood pressure less than 120 mm Hg.[796]

Use of a long-acting dihydropyridine calcium channel blocker in the Systolic Hypertension in Europe (Syst-Eur) study resulted in substantial reductions in rates of total mortality (55%), cardiovascular mortality (76%), and cardiovascular events (69%) in the diabetic subgroup, greater benefits than were seen in the subgroup without diabetes. In the Heart Outcomes Prevention Evaluation (HOPE) study, in which almost 40% of patients had diabetes and one other cardiovascular risk factor, ramipril reduced the primary

outcome by 24% and total mortality risk by 25%.[797] Even in normotensive patients with diabetes, some benefit was seen with a drop in blood pressure (2–4 mm Hg) with ACE inhibitor therapy, raising questions about what mechanisms were responsible for the benefit seen with even modest blood pressure changes. Other rigorously designed studies, such as the UKPDS[798] and the Hypertension Optimal Treatment (HOT) study,[799] suggested that tight blood pressure control in patients with diabetes caused cardiovascular benefits of even greater magnitude. In the ADVANCE trial, over 11,000 type 2 diabetes patients received either a fixed-dose combination of perindopril and indapamide or placebo along with usual care.[800] Those on perindopril/indapamide had on average 5.6–mm Hg lower systolic blood pressures and 2.2–mm Hg diastolic blood pressures, with a 9% decrease in the relative risk of a major macrovascular or microvascular event as compared to 16.8% for those on placebo, with a hazard ratio 0.91. The macrovascular and microvascular events responded similarly but not independently, highlighting the pathogenic role of hypertension in both large and small vessel disease.

In the Losartan Intervention For Endpoint Reduction in Hypertension (LIFE) study, patients with diabetes, hypertension, and signs of left ventricular hypertrophy were randomly assigned to treatment with losartan-based (n = 586) or atenolol-based (n = 609) treatment for hypertension.[800a] Despite similar blood pressure reductions, losartan was more effective than atenolol for reducing rates of cardiovascular morbidity and mortality while also demonstrating fewer conversions to new diabetes. The ability of losartan to reduce events more effectively than atenolol may be related to the ability of angiotensin receptor blockers to reverse left ventricular hypertrophy more effectively than beta blockers.

Although beta blockers are thought to worsen glycemic control in patients with diabetes, it is not clear whether this is a property of all members of this drug class or whether this property persists if beta blockers are given in combination with renin-angiotensin system inhibitors that are known to increase insulin sensitivity. In the Glycemic Effects in Diabetes Mellitus: Carvedilol-Metoprolol Comparison in Hypertensives (GENINI) trial, patients with documented T2DM and hypertension who were taking a stable dose of either an ARB or an ACE inhibitor were randomized to receive either carvedilol, a nonselective beta blocker, which also blocks α_1-adrenergic receptors, or metoprolol, a β_1-selective adrenergic blocker. Although the degree of blood pressure control was similar with both beta blockers, HbA_{1c} and insulin resistance increased significantly with metoprolol but not with carvedilol. Therefore carvedilol appears not to cause the adverse effects that metoprolol does on glucose levels when used in combination with RAS inhibitors, although this conclusion needs to be confirmed in a longer term outcome trial.

The investigators of the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) compared outcomes during first-step treatment of hypertension in 31,512 patients with T2DM, impaired fasting glucose (IFG) levels, or normoglycemia with a calcium channel blocker (amlodipine 2.5–10 mg/day) or ACE inhibitor (lisinopril 10–40 mg/day) compared with a thiazide-type diuretic (chlorthalidone 12.5–25 mg/day).[801] There was no significant difference in the occurrence of the primary outcome (fatal cardiovascular disease or nonfatal myocardial infarction) in patients with T2DM treated with a calcium channel blocker or an ACE inhibitor compared with chlorthalidone. Patients with impaired fasting glucose treated with a calcium channel blocker had a significantly higher relative risk for the primary outcome than patients receiving chlorthalidone.

A major unresolved question has been whether treatment of hypertension to lower targets than currently recommended would further reduce cardiovascular risk in patients with type 2 diabetes. Most trials establishing the remarkable benefit of blood pressure lowering, regardless of the medications used, have studied people whose systolic blood pressure was higher than 140 mm Hg. In the ACCORD trial, the effect of lowering systolic blood pressure to two different levels on cardiovascular risk was assessed.[802] One group was randomized to a systolic blood pressure lower than 120 mm Hg (intensive therapy); the other group was treated to a systolic pressure lower than 140 mm Hg (standard therapy). Targeting the lower blood pressure level did not reduce the rate of fatal and nonfatal cardiovascular events. Based on this result, there is no recommendation to treat patients with T2DM and hypertension to a systolic blood pressure lower than the currently recommended target of 130 mm Hg to decrease cardiovascular events. However, further blood pressure reduction may reduce the incidence of diabetic nephropathy.[548,802] In contrast, in the Systolic Blood Pressure Intervention Trial (SPRINT), which did not enroll patients with diabetes, more aggressive blood pressure lowering was beneficial,[803] prompting subsequent discussions about potential discrepancies between SPRINT and these other studies, including differences in hypertension management in the presence or absence of diabetes.[804]

The HOT trial also studied intensive versus standard control in 18,790 participants, including 1501 subjects who had diabetes, with a focus on enrollment and targeted blood pressure based on diastolic measurements. While more intensive blood pressure control did not make a difference in the primary cardiovascular outcome in the study as a whole, the subgroup with diabetes experienced a 51% decrease in events, with optimal cardiovascular risk reduction found in those achieving a diastolic blood pressure of 82.6 mm Hg and systolic blood pressure of 138.5 mm Hg.[799]

Acute Coronary Syndromes in Diabetes Mellitus

The case fatality rate from myocardial infarction is almost twice as high in patients with diabetes as in nondiabetic patients. This excess risk is seen both during the acute phase and in the early and late postinfarction periods. A number of mechanisms have been hypothesized to be responsible for worse outcomes in patients with diabetes, including the following:

- Increased risk of heart failure due to maladaptive remodeling of the left ventricle[805–807]
- Increased risk of sudden death due to sympathovagal imbalance as a consequence of autonomic neuropathy[808–810]
- Increased likelihood of early reinfarction due to impaired fibrinolysis[811–813]
- Extensive underlying atherosclerosis[814,815]
- Changes in myocardial cell metabolism, including a shift from glucose oxidation to FFA oxidation, with less generation of ATP at any level of oxygen consumption[816,817]
- Associated cardiomyopathy[818]

Collective data provide strong evidence that a variety of treatment modalities can improve outcomes from myocardial infarction in patients with diabetes. In terms of interventions, patients with diabetes experiencing an acute infarct respond as favorably to fibrinolytic therapy as do nondiabetic patients.[814,815] Glycemic control is an essential component of overall management. Glucose levels at hospital admission have been independently correlated with early and late fatality after myocardial infarct in patients with and without diabetes mellitus.[820–823]

Studies such as the Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) study have assessed the impact of intensive glycemic control in patients with diabetes during the acute phase of myocardial infarction. Patients in this study were randomized to either intensive insulin therapy (insulin-glucose infusion for 24 hours, followed by subcutaneous insulin injection for 3 months) or standard glycemic control.[824] The intensive insulin regimen lowered blood glucose level during the first hour after admission and at discharge compared with conventional therapy. The 1-year mortality rate was reduced with the insulin infusion group compared with the control group, a difference that was maintained after 3.4 years of follow-up.

Although the mechanisms responsible for the potential benefit shown in the original DIGAMI study are not entirely clear, experimental data suggest that strict glycemic control can improve myocardial cell metabolism by increasing the availability of glucose as a substrate for ATP generation and reducing the formation of FFAs, thereby shifting cardiac metabolism from FFA oxidation to glycolysis and glucose oxidation. Intensive glycemic control can also reverse the impaired fibrinolysis that is typically seen in patients with diabetes.

However, DIGAMI 2, a prospective, randomized, open-label trial that followed up the DIGAMI trial, comparing outcomes in patients with either T1DM or T2DM, failed to corroborate the earlier reported improvement in outcomes with intensive insulin treatment.[825] The lack of effect of long-term insulin treatment on outcomes may have been perhaps partially influenced by protocol violations, including the patient group assigned to standard metabolic management, without insulin or glucose infusions, having such interventions, with 14% receiving insulin-glucose infusions and as many as 41% receiving extra insulin injections. As a result, the blood glucose levels in all three groups were not significantly different after treatment.

Results from the CREATE-ECLA (Clinical Trial of Reviparin and Metabolic Modulation in Acute Myocardial Infarction Treatment Evaluation—Estudios Cardiologicos Latin America), a randomized, controlled trial of 20,201 patients who presented with ST-segment elevation myocardial infarction within 12 hours after onset of symptoms, are consistent with DIGAMI 2 findings. Patients were randomized to either high-dose glucose-insulin-potassium (GIK) infusion (i.e., 25% glucose, 50 U/L regular insulin, and 80 mEq KCl) administered over 24 hours or to usual care.[826] Roughly 18% of the patients in both treatment arms had T2DM. After 30 days, there were no differences in the rate of occurrence of mortality, cardiac arrest, cardiogenic shock, or reinfarction in the two treatment groups.

Sulfonylureas have been implicated as increasing cardiovascular mortality rate, particularly in patients undergoing revascularization for acute myocardial infarction.[827] The UKPDS did not show a deleterious effect of these agents on the incidence of sudden death or myocardial infarction[379] or over 10 years of follow-up.[24] The sulfonylureas act through the sulfonylurea receptor component of ATP-sensitive potassium channels in the pancreatic beta cell. In the heart, ATP-sensitive potassium channels are involved in ischemic preconditioning and coronary vasodilation.[828–830] It is not clear whether the sulfonylureas modulate these channels in the heart or vascular system or whether they significantly increase risk in patients with diabetes who are experiencing an acute myocardial infarct.

ACE inhibitors dramatically reduce the mortality rate after a myocardial infarction in patients with diabetes, ostensibly through their effects to reduce infarct size and limit ventricular remodeling. In addition to these hemodynamic benefits, ACE inhibitors can also improve outcomes in diabetes by improving endothelial function,[831] improving fibrinolysis,[832] and decreasing insulin resistance.[833]

In a retrospective analysis of the GISSI-3 (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico) study,[834] lisinopril administration within 24 hours after hospital admission for acute myocardial infarction reduced both 6-week and 6-month mortality rates more in patients with diabetes than in patients without diabetes.

Similarly, in the Trandolapril Cardiac Evaluation Study (TRACE), which investigated trandolapril use in patients with left ventricular dysfunction after prior anterior myocardial infarct, a subgroup analysis of diabetes patients revealed trandolapril treatment greatly improved outcomes, with a 36% decrease in mortality from any cause and a 62% reduction in progression to severe heart failure as compared to placebo in those with diabetes, benefits that were more pronounced than the effects seen in TRACE subjects without diabetes (18% reduction in mortality, no effect on progression to severe CHF).[834a]

Beta blockers are now widely accepted for the treatment of acute coronary syndrome in patients with diabetes. Older, noncardioselective beta blockers might have adversely affected the lipid profile and inhibited the metabolic response to hypoglycemia, but more recent data with cardioselective beta blockers suggest that these agents have less negative effect on metabolic indices, perhaps because they increase peripheral blood flow and improve glucose delivery.[835,836] Clinical trial data confirm that beta blockers reduce the rates of mortality and reinfarction in patients with myocardial infarction in the presence of diabetes. In fact, the magnitude of their effects in patients with diabetes appear to exceed those seen in nondiabetic patients. A large review of data from more than 45,000 patients, 26% of whom had diabetes, showed beta blocker therapy was associated with a lower 1-year mortality rate in patients with diabetes than in those without diabetes, with no evidence of an increase in diabetes-related complications.[837]

Postulated mechanisms for the benefit of beta blockers in patients with diabetes include dampening of the sympathetic nervous system overactivity that arises as a consequence of autonomic neuropathy. Beta blockers can also reduce FFA levels and thereby reduce myocardial oxygen requirements. Carvedilol, although not a cardioselective beta blocker, has unique α_1-adrenergic receptor blocking properties along with purported antioxidant effects. These actions may underlie the decreased insulin resistance and greater blood pressure effects of this agent, which may be of particular benefit in patients with type 2 diabetes.[838]

Aspirin has been a cornerstone of therapy for the primary or secondary prevention of acute coronary syndrome in patients with T1DM and T2DM who do not have contraindications to its use. Aspirin significantly lowers the risk of myocardial infarction without increasing the risk of vitreous or retinal bleeding, even in patients with retinopathy.[839] Use of aspirin therapy (75–162 mg/day) as a secondary prevention strategy in those with diabetes and a history of atherosclerotic cardiovascular is currently recommended by the ADA,[840] with presumed benefits through modulation of the enhanced platelet aggregation seen with both T1DM and T2DM.[841] Despite this, controversy has arisen about the ideal dose of aspirin, if enteric coating might interfere with aspirin's effects,[842] and more recently, if aspirin benefits in primary prevention are offset by the risk of bleeding. The Aspirin in Reducing Events in the Elderly (ASPREE) trial studied aspirin 100 mg versus placebo in 19,114 subjects 70 years of age or older

with no known cardiovascular disease, including about 10% of participants who had diabetes, and found no benefit on cardiovascular outcomes, including the diabetes subgroup, but a significant increase in bleeding.[843] In contrast, in ASCEND, in which 15,480 participants with diabetes received either aspirin 100 mg or placebo for a mean of 7.4 years, the aspirin group had fewer serious vascular events (658 participants [8.5%] vs 743 [9.6%]; RR: 0.88, 95% CI: 0.79–0.97, p = 0.01) than placebo, but at the expense of more major gastrointestinal or other extracranial bleeding (4.1% on aspirin group vs 3.2% on placebo group, RR: 1.29, p = 0.003).[844] Aspirin use in patients with diabetes must be tailored to their particular overall cardiovascular and bleeding risk.

Antiplatelet therapy with the antagonist of the $P2Y_{12}$ subclass of ADP receptor clopidogrel also benefits patients with diabetes. The Clopidogrel Versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) trial compared outcomes in patients with non–ST-segment elevation myocardial infarction treated with aspirin or with clopidogrel and included 3866 patients with diabetes.[845] Although the event rate was higher among the diabetic patients than in the overall study population, the response to treatment was also better. The event rate for the primary endpoint (vascular death, ischemic stroke, myocardial infarction, or rehospitalization for ischemia or bleeding) was 17.7% for diabetic patients treated with aspirin and 15.6% for those randomized to clopidogrel, a significant RR of 12.5%. Newer antiplatelet therapies are also now available, including $P2Y_{12}$ receptor antagonists with greater potency. In the Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition With Prasugrel-Thrombolysis In Myocardial Infarction 38 (TRITON-TIMI 38) study, participants with diabetes had a 30% relative risk reduction in the primary endpoint (cardiovascular death, nonfatal myocardial infarct, or nonfatal stroke) without evidence for increases in major bleeding.[846] In the Platelet Inhibition and Patient Outcomes (PLATO) trial, ticagrelor equally reduced the rate of ischemic events in acute coronary syndrome patients both with and without diabetes; in the diabetes subgroup, reductions were seen in the primary composite endpoint (18%), all-cause mortality (18%), and stent thrombosis (35%) with no increase in major bleeding. Patients with diabetes are felt to have an increased risk for complications with percutaneous interventions like stent thrombosis, and may warrant more intensive oral antiplatelet therapy in appropriate patients.

Newer adjunct therapies, such as the platelet glycoprotein IIb/IIIa receptor antagonists that antagonize platelet action, have also been assessed in diabetic patients who present with unstable angina or non–Q-wave infarction. Overall, these agents appear to work equally well, or perhaps slightly better, in patients with diabetes compared with nondiabetic patients. In the Platelet Receptor Inhibition in Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms (PRISM-PLUS) study, the addition of tirofiban to heparin therapy reduced the 7-day composite endpoint, compared with heparin alone. This effect was greater in patients with diabetes than in patients without diabetes.[847]

In one study of patients undergoing percutaneous transluminal coronary angioplasty, glycoprotein IIb/IIIa antagonist therapy was associated with fewer acute events but a higher rate of long-term target-vessel revascularization in the diabetic cohort compared with the nondiabetic cohort.[848] However, in another trial, in which stents were used, the rate of target vessel revascularization at 6 months was significantly decreased with the addition of a glycoprotein IIb/IIIa antagonist compared with placebo.[849]

Results of the BARI study showed that coronary artery bypass grafting provides better outcomes than percutaneous transluminal coronary angioplasty in patients with diabetes, possibly because it addresses the extensive coronary vascular disease in these patients.[850] This study did not employ stents or glycoprotein IIb/IIIa inhibitors, two modalities that, when used together, appear to improve outcomes after percutaneous transluminal coronary angioplasty in patients with diabetes.

In terms of revascularization strategies, the Future Revascularization Evaluation in Patients With Diabetes Mellitus: Optimal Management of Multivessel Disease (FREEDOM) trial had a major impact on management decisions. This trial was an appropriately powered, randomized comparison of percutaneous coronary interventions (with drug-eluting stents) and coronary artery bypass graft (using arterial grafting) in patients with diabetes and multivessel coronary artery disease on concomitant optimal medical therapy. It provided strong evidence that that coronary bypass resulted in fewer major cardiovascular events, and reduced 5-year mortality compared to percutaneous coronary interventions.[851] Continued progress in stent technology and adjunctive therapies, as noted, continues to make this a challenging decision. Patients with diabetes with less extensive coronary disease for whom invasive intervention is warranted can certainly be managed with percutaneous interventions.

Cardiomyopathy in Patients With Diabetes Mellitus

Diabetes is associated with a fourfold increase in the risk of congestive heart failure, even after adjustment for other cardiovascular risk factors such as age, blood pressure, cholesterol level, obesity, and history of coronary heart disease.[852] Contributing factors include diabetes-induced cardiomyocyte dysfunction, impaired microvascular perfusion because of defective endothelial function, increased collagen deposition with fibrosis, and maladaptive remodeling after myocardial infarction, leading to both diastolic and systolic heart failure (also discussed in "Biochemistry and Molecular Cell Biology"). Current nomenclature[853] organizes heart failure into two broad categories: with preserved ejection fraction (HFpEF) and with reduction ejection fraction (HFrEF).[854] Patients with diabetes, who commonly present with either form, also experience higher rates of heart failure than do nondiabetic patients after an acute myocardial infarction, regardless of the size of the infarct zone.[806,855] These findings suggest that diabetes itself causes deleterious effects on the myocardium, leading to poorer outcomes.

A number of key structural, functional, and metabolic factors in diabetes have been implicated in the increased risk of maladaptive remodeling that leads to heart failure. For example, evidence of silent myocardial infarction is found in up to 40% of patients with diabetes who present with a clinically apparent infarct and can lead to unrecognized regional and global ventricular dysfunction.[834,856] As many as 50% of patients with diabetes and coronary heart disease have cardiac autonomic neuropathy, which is known to contribute to both systolic and diastolic dysfunction.[857] Like hypertension, diabetes can cause fibrosis of the myocardium and increased collagen deposition.[858,859] These effects are even more pronounced in patients with coexisting hypertension and diabetes and may contribute to the finding of diastolic dysfunction common in patients with diabetes.[860] Enhanced myocardial endothelial dysfunction in diabetes has also been described as a pathophysiologic pathway to impaired microvascular perfusion and ischemia.[861,862]

On a cellular level, both hyperglycemia and insulin resistance have direct negative effects on myocardial metabolism. Depression

of myocardial GLUT4 levels in the setting of diabetes inhibits glucose entry and glycolysis in the heart. As a result, intracellular metabolism shifts from glycolysis to FFA oxidation, thereby reducing glycolytic ATP generation, a major source of energy under anaerobic (i.e., ischemic) conditions.[816] The production of oxygen free radicals can also be enhanced in this situation, further depressing myocardial contractile function.[862] Mitochondrial dysfunction is a feature of ischemia-reperfusion injury, leading to generation of reactive oxygen species. In experimental models of ischemia-reperfusion, mitochondrial reactive oxygen species generation is mediated by reversal of succinate dehydrogenase, leading to succinate accumulation. On reperfusion, restoration of succinate dehydrogenase activity leads to rapid succinate oxidation, causing reactive oxygen species generation by reverse electron transport at mitochondrial complex I.[863] Increased myocardial production of succinate is a feature of acute ST-segment–elevation myocardial infarction in humans.[864]

A major advance in the heart failure field in general was the finding in PARADIGM-HF that the combination of an angiotensin receptor blocker (valsartan) and a neprilysin inhibitor (sacubitril) proved superior to the ACE inhibitor enalapril in patients with reduced ejection fraction (≤40%), significantly reducing cardiovascular death or hospitalization for heart failure, and doing so against a medicine previously shown to have a mortality benefit.[865] Progression of heart failure and renal dysfunction were also decreased. Of note, among the nearly 45% of patients in this study who had diabetes, there was evidence of greater decreases in HbA_{1c} and less progression to insulin use, raising intriguing mechanistic questions, while also potentially supporting the use of sacubitril/valsartan in patients with diabetes and reduced ejection fractions.[592]

Knowledge about the pathophysiology underlying increased coronary artery disease in people with prediabetes and type 2 diabetes has increased enormously in recent years. That knowledge has directed seminal clinical studies, which in turn have enlarged our understanding of pathophysiology. These seminal studies provide the rationale for an optimized multifactorial approach to the prevention and treatment of diabetic cardiovascular disease, including choice of drugs to manage hyperglycemia, dyslipidemia, and hypertension, and postinfarction sequelae. Data from ongoing clinical trials and new pharmaceutical developments will further improve the cardiovascular prognosis for people with diabetes.

The Diabetic Foot

Foot complications of ulceration and amputation are associated with high mortality, yet diabetic foot ulcers are the most preventable late adverse outcome of diabetes.[866–869] Elliott Joslin correctly noted in 1934 "diabetic gangrene is not heaven-sent, but earth-born." The neuropathic foot does not spontaneously ulcerate; insensitivity, deformity, and unperceived trauma (e.g., inappropriate footwear) promote skin breakdown. Most amputations are preceded by foot ulcers. Increased appreciation of the complex pathogenesis of foot ulcers permits design of screening programs for risk identification, preventive education, and design of multidisciplinary foot care programs. Much progress has been made, but suboptimal adherence to current professional guidelines has prevented translation of this into a universal decline in amputation rates.

Epidemiology of Diabetic Foot Ulceration

Foot ulceration is common and occurs in both T1DM and T2DM. The annual incidence of foot ulceration is about 2% to 2.5% in Western countries: Higher incident rates are found in select diabetic populations, including US Medicare recipients (6%), US veterans (5%), and the global diabetes population (6.3%).[870] Approximately 5% to 10% of diabetic patients have had past or present foot ulceration, and 1% have undergone amputation.[866] Although between 1990 and 2010, a significant reduction in certain diabetes-related complications was reported from the United States,[8] unfortunately there was no reduction in rates of amputations reported in this time frame. A large community-based study in the United Kingdom showed an annual incidence of ulceration of approximately 2%; this rose to 7% with known diabetic neuropathy and to as high as 50% with a past history of ulceration.[871] The lifetime risk for development of a foot ulcer in a diabetic patient is estimated to be as high as 25%.[868,872] Multiple factors likely contribute to different rates of foot ulceration, including diabetes duration, smoking, and other neurologic and cardiovascular risk factors. Foot ulceration is most common in patients with a history of similar problems. In experienced diabetic foot clinics, more than 50% of patients with new foot ulcers give a past ulcer history. Patients with other complications of diabetes, including retinopathy and renal dysfunction, are at increased risk for foot ulceration. Patients on dialysis are among those at the very highest risk for ulceration.[872a]

More than 80% of amputations are preceded by foot ulcers. Diabetes is the most frequent cause of nontraumatic lower limb amputation in the United States, and rates are 15 times greater than those in the nondiabetic population. Reduced amputation rates occur following implementation of foot screening and education programs.[873,874] Implementation of strategies to reduce ulceration and amputation are necessary and may benefit from additional education on behavioral aspects of diabetic foot care, including awareness of ulceration risk, compliance with daily foot inspection, and the importance of nonweightbearing with early foot infections.[875] Treatments to reduce cardiovascular and renal risk likely further reduce amputation rates, but this has been less clearly established.

Pathogenesis of Foot Ulceration

Foot ulceration results from multiple interacting component causes, none of which alone is sufficient to cause ulceration but when combined may lead to skin breakdown. A combination of two or more risk factors promotes diabetic foot ulceration, and the triad of component causes—neuropathy, deformity, and trauma—is present in 63% of incident ulcers. Edema and ischemia are also common component causes. These risk factors were demonstrated in a North American/United Kingdom collaborative study of more than 150 consecutive foot ulcer cases.[876]

Diabetic Neuropathy and the Foot Ulcer

All three components of neuropathy—sensory, motor, and autonomic—can contribute to foot ulceration. The risk of foot ulceration in patients with neuropathy is sevenfold higher than in those without this complication of diabetes.[871] Chronic sensorimotor neuropathy is common, affecting at least one-third of older diabetic patients in Western countries. Its onset is gradual and insidious, and symptoms may be so minimal that they go unnoticed. Although uncomfortable, painful, and paresthetic symptoms predominate in most patients, some never experience symptoms. Clinical examination usually reveals a sensory deficit

in a glove-and-stocking distribution, with signs of motor dysfunction such as small muscle wasting in the feet, and absent ankle reflexes. Although a history of typical symptoms strongly suggests a diagnosis of neuropathy, absence of symptoms does not exclude the diagnosis and must never be equated with a lack of foot ulcer risk. Therefore assessment of foot ulcer risk must always include a careful foot examination, including assessment of proprioception (i.e., pressure perception using a 10-g monofilament) regardless of history. Loss of sensation and proprioception reduce capillary perfusion and increase ischemia at sites of increased pressure load.

Sympathetic autonomic neuropathy affecting the lower limbs results in reduced sweating, dry skin, and development of cracks and fissures. In the absence of large-vessel arterial disease, there may be increased blood flow to the foot, with arteriovenous shunting leading to the warm but at-risk foot.

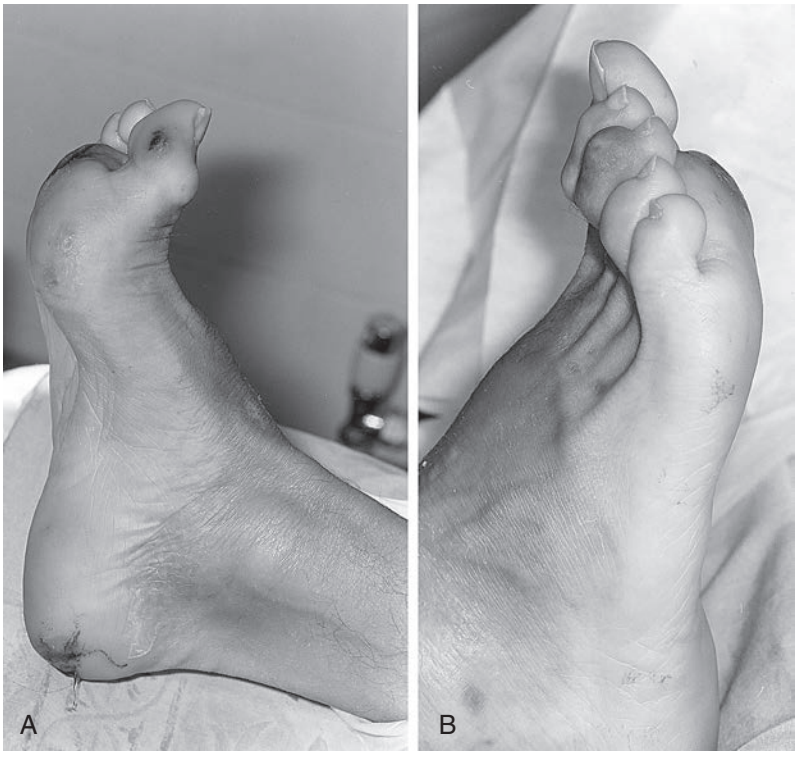
Callus, Deformity, and High Foot Pressures

Motor neuropathy, with imbalance of the flexor and extensor muscles in the foot, commonly results in foot deformity, with prominent metatarsal heads and clawing of the toes (Fig. 37.50). The combination of proprioceptive loss due to sensory neuropathy and the prominence of metatarsal heads lead to increased pressures and loads under the diabetic foot. Neuropathy is only one cause of high foot pressures. High pressures, together with dry skin, often result in the formation of callus under weightbearing areas of the metatarsal heads. Presence of plantar callus is a highly significant marker for foot ulcer risk. Conversely, removal of plantar callus is associated with reduced foot pressures and thereby a reduced foot ulcer risk.[877]

Peripheral Vascular Disease, Diabetic Foot Ulcers, and Amputation Risk

Peripheral vascular disease in isolation rarely causes ulceration. However, the common combination of vascular disease with minor trauma can lead to ulceration. Minor injury and subsequent infection increase the demand for blood supply beyond the circulatory capacity, and ischemic ulceration and risk of amputation develop. Early identification of patients at risk for peripheral vascular disease is essential, and appropriate investigation involving noninvasive Doppler studies, together with arteriography, often leads to revascularization procedures to improve lower extremity blood flow. Presence or absence of a dorsalis pedis or posterior tibial pulse may be the simplest and most reliable indicator of significant ischemia that can be elicited at the bedside.[878] However, Doppler-derived ankle pressure can be misleadingly high in patients with long-standing diabetes, so the use of the toe-brachial index together with Doppler waveforms of distal arteries can be useful in deciding which patients need further diagnostic intervention.[879]

Distal bypass surgery or endovascular interventions have good short-term but mixed long-term results in terms of limb salvage.[878,880] Advances in techniques and devices for both distal arterial bypass surgery and endovascular interventions have been made, and it is unclear which approach is preferred. A recent systematic review was unable to conclude any particular method was superior to any other.[881] However, a recent study in all patients with diabetic foot ulcers demonstrates that prompt vascular evaluation and revascularization within 2 weeks reduces the rate of amputations to the rate of nondiabetics with peripheral artery disease, supporting a change in clinical disease management.[882]



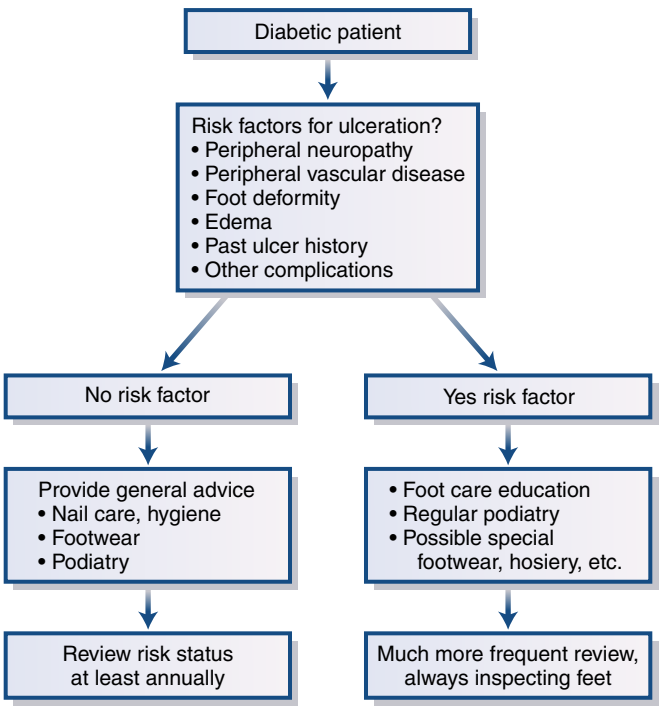
• **Fig. 37.50** The high-risk neuropathic foot. (A, B) Two lateral views of a patient with typical signs of a high-risk neuropathic foot. Notice the small-muscle wasting, clawing of the toes, and marked prominence of the metatarsal heads. At presentation with type 2 diabetes mellitus, this patient had severe neuropathy with foot ulceration on both the right foot *(shown here)* and the left foot. (From Andersson DK, Svärdsudd K. Long-term glycemic control relates to mortality in type II diabetes. *Diabetes Care.* 1995;18:1534–1543.)

Prevention of Foot Ulceration and Amputation

Relatively simple interventions can reduce amputations by up to 80%.[873,874] Therefore strategies for early identification of patients at potential risk for ulceration are required, and education programs that can be adapted for widespread application need to be developed. Because foot ulcers precede most amputations, are among the most common causes of hospital admission for patients with diabetes, and account for much morbidity and even fatality, the widespread application of preventive foot care strategies is urgently required.

Patients with any type of diabetes require regular foot examination for evidence of risk factors for ulceration, irrespective of disease duration. Neuropathy, vascular disease, and even foot ulceration may be the presenting feature of T2DM, so there can be no exception to the rule of screening (Fig. 37.51). At a minimum, such screening should be carried out annually. Of all the long-term complications of diabetes, foot problems and their risk factors are probably the easiest to detect. No expensive equipment is required, and feet can be examined for evidence of neuropathic and vascular deficits in the office setting using simple equipment.[871]

In 2008, a task force of the ADA published a report on the examination components that should be included in the annual Comprehensive Diabetic Foot Examination (CDFE) (Table 37.21).[884] The most important message is for practitioners to have patients remove shoes and socks to examine feet for the presence of callus, deformity, muscle wasting, and dry skin, all of which are clearly visible on inspection. A simple neurologic examination includes assessment of pressure perception using a 10-g monofilament. Sensory neuropathy with clinical evidence of peripheral arterial disease are the strongest predictors of future foot ulceration. Absence of the ability to perceive pressure from a 10-g monofilament, inability to perceive a vibrating 128-Hz tuning fork over the hallux, and absent ankle reflexes all have been shown to be predictors of foot ulceration.[868,869]



• **Fig. 37.51** Simple algorithm for risk screening in the diabetic foot.

The Diabetic Foot Care Team

Patients identified as being at high risk for foot ulceration should be managed by a team of specialists with expertise in the diabetic foot. The podiatrist usually takes responsibility for care of the skin and nails and, together with the specialist nurse or diabetes educator, provides foot care education. The orthotist, or shoe fitter, is invaluable to advise about and sometimes design footwear to protect high-risk feet. Team members should work closely with the diabetologist and the vascular and orthopedic surgeons. Patients with risk factors for ulceration require preventive foot care education, including self-foot care at home, frequent review by a foot surgeon or podiatrist, good foot wear with orthotics when indicated, and avoidance of potentially dangerous over-the-counter foot products.[874,877]

Classification of Foot Ulcers

Many different classification systems for grading diabetic foot ulcers have been reported.[868,877] One developed by Wagner[885] (Table 37.22) is widely used and accepted. More recently, the University of Texas (UT) group developed an alternative classification system that, in addition to ulcer depth (as in the Wagner system), accounts for presence or absence of infection and ischemia (Table 37.23). A prospective study from 2001 compared these two wound classification systems and concluded that the UT scheme is a better predictor of outcome than the older Wagner system.[885]

Management of Diabetic Foot Ulcers

Basic principles of wound healing apply equally to diabetic foot ulcers as to wounds in any other site or condition. In general, a

TABLE 37.21 Key Components of the Comprehensive Diabetic Foot Examination

Dermatologic

Skin status: color, thickness, dryness, cracking
Sweating
Infection: check between toes for fungal infection
Ulceration
Calluses/blistering: hemorrhage into callus?

Musculoskeletal

Deformity (e.g., claw toes, prominent metatarsal heads, Charcot joint)
Muscle wasting (guttering between metatarsals)
Assess whether shoes are appropriate for the feet (e.g., size, width)

Neurologic

Ability to perceive pressure from a 10-g monofilament plus one of the following:
- Vibration using 128-Hz tuning fork
- Pinprick sensation
- Ankle reflexes
- Vibratory perception threshold

Vascular

Foot pulses
Ankle-brachial index, if indicated

Adapted from Boulton AJ, Armstrong DG, Albert SF, et al. Comprehensive foot examination and risk assessment: a report of the Task Force of the Foot Care Interest Group of the American Diabetes Association, with endorsement by the American Association of Clinical Endocrinologists. *Diabetes Care.* 2008;31:1679–1685.

diabetic foot ulcer has high likelihood of healing if the following three conditions are satisfied:

- Arterial inflow is adequate.
- Infection is treated appropriately.
- Pressure is removed from the wound and the immediate surrounding area.

Although this approach appears simplistic, failure of diabetic foot ulcers to heal is usually a result of failure to pay sufficient attention to one or more contributing conditions, including pressure on the wound, infection, ischemia, and inadequate debridement.

The most common cause of nonhealing neuropathic foot ulcers is failure to remove pressure from the wound and immediate surrounding area. Patients who are advised not to put pressure over an ulcer find it especially difficult to adhere to such advice when peripheral sensation is lost or reduced. Pain results in protection of an injured area; the lack of pain permits pressure to be put directly onto the ulcer and results in nonhealing. A patient with normal sensation and a foot wound will limp to avoid putting pressure on the wound because doing so is painful. A patient who walks on a plantar wound without limping must have neuropathy.

The effect of pressure relief on the histopathologic features of neuropathic ulcers was assessed in a randomized study[886] with biopsy either at presentation or after 20 days of off-loading in a total-contact cast (TCC). Histologic features of chronic inflammation, with mononuclear infiltration, cellular debris, and scarce evidence of angiogenesis or granulation, were seen in patients who underwent biopsy at presentation; whereas granulation, neoangiogenesis, and a predominance of fibroblasts were seen in patients treated with TCC before biopsy. These important observations support that repetitive pressure on a neuropathic wound contributes to the chronicity of the wound, whereas pressure relief results in a reparative phase.

The next most common error is inappropriate management of infection. Topical applications are usually unhelpful, and if clinical infection is present, then it must be treated urgently with antibiotics. Most infections are polymicrobial, with gram-positive cocci, gram-negative rods, and anaerobes, with or without multidrug-resistant organisms (see later discussion).

Another common management error is failure to appreciate ischemic symptoms that are atypical due to altered pain sensation as a result of neuropathy. The most difficult ulcer to heal is the neuroischemic ulcer. Symptoms and even signs of ischemia may be altered in the diabetic state. Therefore appropriate noninvasive investigation and arteriography are indicated for patients with a nonhealing diabetic foot ulcer if there is any question about the vascular status.

Inappropriate wound debridement contributes to slow healing or nonhealing of a diabetic foot ulcer. Appropriate debridement and removal of all callus, dead, and macerated tissue is essential local treatment of a diabetic foot ulcer, which results in more rapid healing compared with inadequately debrided wounds.

The principles of management of neuropathic and neuroischemic foot ulcers are considered in the following sections referencing both the UT and the Wagner grading systems.

Neuropathic Foot Ulcer Without Osteomyelitis (Wagner Grades 1, 2; University of Texas Grades 1a, 1b, 2a, 2b)

Provision of adequate pressure relief is the most important feature in the management of neuropathic foot ulcers that occur under weightbearing areas such as the metatarsal heads and great toe. This is usually achieved by a TCC or a removable Scotch cast boot.[868,877]

The TCC has long been recognized as the gold standard for off-loading a foot wound. The TCC was associated with the shortest healing time in a randomized, controlled trial conducted by Anderson and colleagues comparing three off-loading techniques demonstrated.[887] When any cast device is used, regular removal of the cast is essential, because regular debridement of the wound by a specialist is essential, and any casting device could injure insensitive skin, especially over bony prominences. As the TCC requires a specially trained casting technician to apply it, and frequent removal is required for wound assessment, recent research has focused on alternative reusable devices that can only be removed in the specialist's office.

The removable cast walker (RCW) resulted in slower healing than the TCC, in the aforementioned trial by Armstrong

TABLE 37.22 Wagner Diabetic Foot Ulcer Classification System

Grade	Description
0	No ulcer, but high-risk foot (e.g., deformity, callus, insensitivity)
1	Superficial full-thickness ulcer
2	Deeper ulcer, penetrating tendons, no bone involvement
3	Deeper ulcer with bone involvement, osteitis
4	Partial gangrene (e.g., toes, forefoot)
5	Gangrene of whole foot

Modified from Oyibo S, Jude EB, Tarawneh I, et al. A comparison of two diabetic foot ulcer classification systems: the Wagner and the University of Texas wound classification systems. *Diabetes Care.* 2001;24:84–88.

TABLE 37.23 University of Texas Wound Classification System

Stage	Grade 0	Grade 1	Grade 2	Grade 3
A	Preulcer or postulcer lesion; no skin break	Superficial ulcer	Deep ulcer to tendon or capsule	Wound penetrating bone or joint
B	+ Infection	+ Infection	+ Infection	+ Infection
C	+ Ischemia	+ Ischemia	+ Ischemia	+ Ischemia
D	+ Infection and ischemia	+ Infection and ischemia	+ Infection and ischemia	+ Infection and ischemia

Modified from Armstrong DG, Lavery LA, Harkless LB. Validation of a diabetic wound classification system. The contribution of depth, infection, and ischemia to risk of amputation *Diabetes Care.* 1998;12:855–859.

et al,[887] even though prior gait laboratory studies suggested they are equally efficacious at off-loading. The reason for this disparity may be explained by the observation that although patients were instructed to wear the RCW at all times, patients used these devices for only 28% of all footsteps during a 24-hour period. To overcome this limitation the RCW, which can be applied by any clinic personnel and does not require specialist training, can be rendered irremovable by wrapping it in casting material. A controlled trial showed the irremovable RCW was as effective at healing neuropathic foot wounds as the TCC.[888]

Theoretically, complete healing of all superficial and neuropathic ulcers should be possible without the need for amputation. In the treatment of neuropathic ulcers with a good peripheral circulation, antibiotics are not indicated unless there are clear clinical signs of infection, including prominent discharge, local erythema, and cellulitis. The presence of any infective features in Wagner grade 1 or 2 ulcers would warrant reclassification in the UT system from 1a or 2a to 1b or 2b. In such cases, deep wound specimens should be taken and broad-spectrum oral antibiotic treatment should be started with, for example, either an amoxicillin–clavulanic acid combination (Augmentin) or clindamycin. The antibiotic may need to be altered after sensitivity results become available.[889]

Neuroischemic Ulcers (Wagner Grades 1, 2; University of Texas Grades 1c, 1d)

The principles of management of neuroischemic Wagner grade 1 and 2 ulcers are similar to those for neuropathic ulcers, with the following important differences. TCCs are not usually recommended for management of neuroischemic ulcers, although removable casts and pneumatic cast boots (Aircast) may be used in cases without infection. Antibiotic therapy is required for most neuroischemic ulcers. Investigation of the circulation is indicated, including noninvasive assessment and, if required, arteriography with appropriate subsequent surgical management or angioplasty.[878]

Osteomyelitis (Wagner Grade 3; University of Texas Grades 3b, 3d)

Wagner or UT grade 3 ulcers are deeper and involve underlying bone, often with abscess formation. Osteomyelitis is a serious complication of foot ulceration and may be present in as many as 50% of diabetic patients with moderate to severe foot infections.[868] If the physician can probe down to bone in a deep ulcer, the presence of osteomyelitis is strongly suggested. Plain radiographs are indicated for any nonhealing foot ulcer and are useful in the diagnosis of osteomyelitis in more than two-thirds of patients, although radiologic changes may be delayed. In difficult cases, further investigation, such as magnetic resonance imaging (MRI), bone scans, or an indium-111 (^{111}In)–labeled white blood cell scan can be useful in diagnosing bone infection.[890]

Although osteomyelitis treatment is traditionally surgical and involves resection of the infected bone, successful long-term treatment can occasionally be effective with antibiotics. *Staphylococcus aureus* is the most common infective bacteria. Therefore agents such as clindamycin (which penetrates bone well) or flucloxacillin are often used. Antibiotic therapy for 90 days was equally efficacious when compared to local surgery for diabetic foot osteomyelitis in a recent randomized trial.[891]

Gangrene (Wagner Grades 4, 5)

The presence of gangrene is always a serious sign in the diabetic foot. However, localized areas of gangrene, especially in the toes, that are without cellulitis, spreading infection, or discharge can occasionally be left to spontaneously auto-amputate. The presence of more extensive gangrene requires urgent hospital admission; treatment of infection, often with multiple antibiotics; glycemic control, usually with intravenous insulin; and detailed vascular assessment. It is in this situation that the team approach is most important, with close collaboration among the diabetes specialist, the vascular surgeon, and the radiologist.

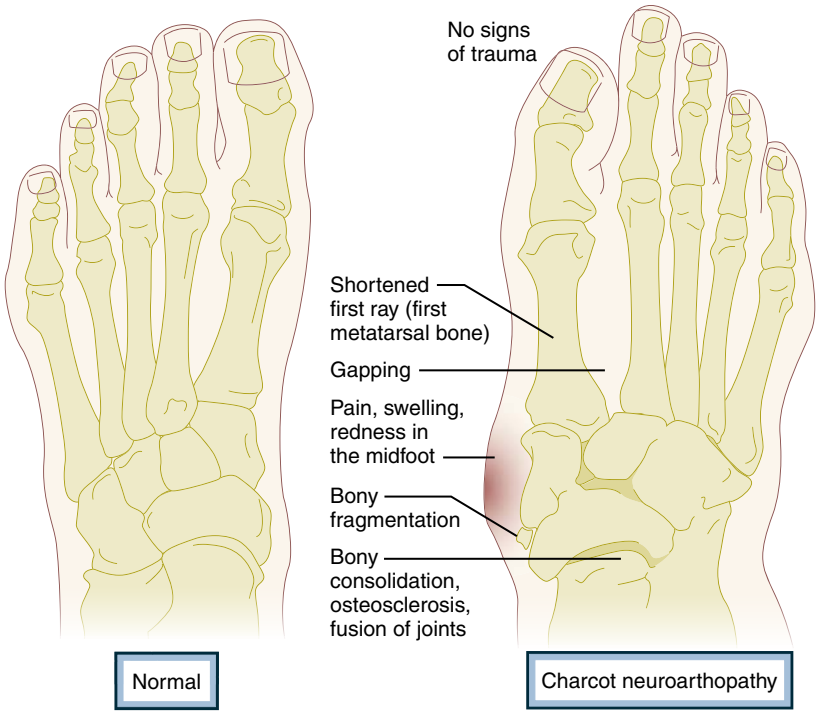
Charcot Neuroarthropathy

Charcot neuroarthropathy is a disabling condition affecting the joints and bones of the foot in the general diabetic population. It is much more frequent (10–13%) in high-risk diabetic patients. Permissive features for development of this condition include the presence of severe peripheral neuropathy and autonomic dysfunction with increased blood flow to the foot. The peripheral circulation is usually intact. In the Western world, diabetes is the most common cause of a Charcot foot, and increased awareness of this condition can enable earlier diagnosis and treatment to prevent severe deformity and disability.

The pathogenesis of the Charcot process remains poorly understood; however, the patient with peripheral insensitivity and autonomic dysfunction, with increased blood flow reflecting the autonomic dysfunction, to the foot is vulnerable to trauma, which the patient may not recall. Repetitive trauma results in increased blood flow through the bone, increased osteoclastic activity, and bone remodeling. In some cases, patients walk on a fracture, which leads to continuing bone and joint destruction in that area. Recent evidence suggests acute Charcot neuropathy may be triggered in the susceptible (i.e., neuropathic) individual by any event that leads to localized inflammation in the affected foot, triggering a vicious cycle in which there is increasing inflammation, increasing expression of receptor activator of nuclear factor-κB ligand (RANKL), a member of the tumor necrosis factor superfamily, and increasing bone breakdown.[892] Targeting activation of the RANKL/osteoprotegerin pathway might lead to novel, future treatments.

Charcot neuropathy can be difficult to distinguish from osteomyelitis or an inflammatory arthropathy.[890] A unilateral swollen, hot foot without an ulcer in a patient with neuropathy must be considered to be a Charcot foot until proved otherwise. Charcot arthropathy can be diagnosed in most patients by plain radiography and a high index of suspicion (Fig. 37.52). Radiographs reveal bone and joint destruction, fragmentation, and remodeling, although radiographic findings may be normal early in disease. In such cases, three-phase bisphosphonate bone scans show increased bone uptake, while ^{111}In-labeled bone scans will be negative in the absence of infection. In patients where osteomyelitis is suspected, ^{18}F-fluorodeoxyglucose–positron emission tomography–computed tomography (FDG-PET-CT) has the highest sensitivity for distinguishing Charcot arthropathy from osteomyelitis.

After diagnosis, management of the acute phase involves immobilization, usually in a TCC.[893] There is little evidence to support use of any pharmacologic treatment in the management of this condition. Although Charcot neuroarthropathy is rare, it should be suspected in any patient with unexplained swelling and heat in a neuropathic foot. Early intervention with immobilization may halt progression that, in the untreated state, can lead to marked foot deformity and require local or major amputations.



• **Fig. 37.52** Some of the key features of Charcot neuroarthropathy, an often overlooked complication of diabetes. (Redrawn from Botek G, Anderson MA, Taylor R. Charcot neuroarthropathy: an often overlooked complication of diabetes. *Cleve Clin J Med.* 2010;77:593–599.)

Adjunct Treatments for Foot Ulcers

Tissue-Engineered Skin and Platelet-Derived Growth Factors

Genetically derived growth factors and novel bioengineered skin substitutes have been developed as adjunctive treatments for diabetic foot ulcers, including Apligraf, a bilayered living human skin equivalent; Dermagraft, a human fibroblast-derived dermal substitute; and human platelet-derived growth factors. Although there are some benefits for each of these agents, they are costly[894] and should be reserved for ulcers that fail to respond to standard treatments. Any newer treatments should be considered an addition to good wound care, which must always include adequate off-loading and regular debridement. There is little objective evidence to date in support of routine use of these therapies, as well as others under study, including stem cell therapy and hyperbaric oxygen.[895]

Negative-Pressure Wound Therapy (NPWT)

NPWT, also known as vacuum-assisted closure, is increasingly used to treat large and complex diabetic foot wounds. The treatment may stimulate development of granulation tissue in previously nonhealing wounds and can also be helpful in the postoperative management of diabetic foot wounds, as supported by randomized, controlled trials in complex nonhealing diabetic foot ulcers[896] and postoperative cases.[895] Use of NPWT remains controversial, as discussed in a systematic review. The strongest evidence for efficacy is with postoperative diabetic foot wounds.[895]

Achilles Tendon Lengthening Procedures

Although some centers advocate and perform tendo-Achilles lengthening as an adjunct treatment to prevent recurrent diabetic foot ulcers posthealing, there are no adequate data supporting use of this procedure in the diabetic foot. Given the high risk of any foot surgery in the diabetic foot, the nonsurgical approach of prescribing footwear designed to reduce pressure on the metatarsal heads is preferable unless and until adequately powered prospective randomized clinical trials of Achilles tendon lengthening procedures demonstrate superior outcomes.

SGLT2 Inhibitors and Diabetic Foot Disease

An increased incidence of distal lower extremity amputations, mainly at the toe and metatarsal levels, was observed in the CANVAS trial[473] designed to evaluate the cardiovascular safety of canagliflozin. Numerous potential mechanisms have been suggested, but the cause of this finding remains enigmatic. However, a recent pharmacovigilance analysis confirmed that use of canagliflozin, but not dapagliflozin or empagliflozin, might be associated with an increased risk of amputations. The use of canagliflozin should probably be avoided in patients with distal neuropathy or peripheral arterial disease or with a history of foot disease. Although this does not appear to be a class effect,[897] use of other SGLT2 inhibitors should be used with caution in the abovementioned groups.

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The complete list of references is available online at ExpertConsult.com.

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Hypoglycemia

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CHAPTER OUTLINE

KEY POINTS

- Hypoglycemia—a plasma glucose concentration low enough to cause symptoms or signs—is a rare occurrence in individuals without diabetes but is common in sulfonylurea-, glinide-, or insulin-treated diabetes.
- The plasma glucose concentration is normally maintained in a relatively narrow range, 72 to 144 mg/dL (4.0–8.0 mmol/L), owing to a fine balance between glucose influx (exogenous glucose delivery and endogenous glucose production) and glucose efflux (glucose utilization by insulin-sensitive tissues, such as the skeletal muscle, and insulin-insensitive tissues, particularly the brain).
- Hypoglycemia results from an imbalance between glucose influx and glucose efflux due to either excessive glucose removal from the circulation, deficient glucose delivery into the circulation, or both.
- Hypoglycemia in diabetes is typically the result of the interplay of therapeutic hyperinsulinemia and compromised defenses against falling glucose levels resulting in hypoglycemia-associated autonomic failure (HAAF), including defective glucose counterregulation and impaired awareness of hypoglycemia.
- An attenuated sympathoadrenal response to falling glucose levels, the key feature of HAAF, is induced by recent antecedent hypoglycemia, sleep, or prior exercise, and reversible by short-term scrupulous avoidance of hypoglycemia.
- Iatrogenic hypoglycemia is associated with both morbidity and fatality in type 1 and type 2 diabetes mellitus.
- The practice of hypoglycemic risk reduction in persons with diabetes at risk of hypoglycemia includes addressing the issue, applying the principles of aggressive glycemic therapy, and considering the conventional risk factors and those indicative of HAAF.
- In the absence of diabetes, hypoglycemia in ill or medicated individuals can be caused by many drugs, critical illnesses, endocrine deficiencies, or non–islet cell tumors; in seemingly well individuals, it may be caused by endogenous hyperinsulinism or by various accidental, surreptitious, or malicious mechanisms. In children, it may also be the result of abnormalities in enzyme deficiencies that are key for the metabolism of fuel substrates that will contribute to abnormalities in glucose production and utilization.
- The decision to evaluate a given nondiabetic person systematically—seeking evidence of a metabolic disorder or an endogenous insulin excess during hypoglycemia—is recommended only for persons in whom Whipple triad (symptoms, signs, or both consistent with hypoglycemia; a low reliably measured plasma glucose; and resolution of those symptoms or signs after the glucose concentrations is raised) can be documented.
- Short-term treatment of hypoglycemia includes oral carbohydrates, or parenteral glucagon or glucose; long-term treatment requires correction of the hypoglycemic mechanism.

Iatrogenic hypoglycemia is the limiting factor in glycemic management of diabetes mellitus with insulin provisional therapies: insulin, a sulfonylurea, or a glinide.[1] Hypoglycemia is a distinctly uncommon clinical disorder in persons without diabetes.[2]

Glucose is an obligate metabolic fuel for the brain under physiologic conditions.[3] Because the brain cannot synthesize glucose, store more than a few minutes supply as glycogen, or utilize physiologic concentrations of other circulating fuels effectively, survival of the brain, and therefore the individual, requires a virtually continuous supply of glucose from the circulation. Blood-to-brain glucose transport is a direct function of the arterial plasma glucose concentration and requires the plasma glucose concentration be maintained within, or above, the physiologic range. Hypoglycemia causes functional brain failure, which is typically corrected after the plasma glucose concentration is raised (Table 38.1). Rarely, it causes a fatal cardiac arrhythmia or, if it is profound and prolonged, brain damage and death.

TABLE 38.1 Whipple Triad

Whipple triad is:
symptoms, signs, or both consistent with hypoglycemia, a low reliably measured plasma glucose concentration, and resolution of those symptoms or signs after the plasma glucose concentration is raised.[a]

Clinically, neuroglycopenic symptoms are more compelling than neurogenic symptoms.

The definition of "low" is judgmental, but a plasma glucose concentration of less than 55 mg/dL (3.0 mmol/L) is unequivocally low.

Documentation of Whipple triad is particularly important for the diagnosis of hypoglycemia in patients who do not have insulin-, sulfonylurea-, or glinide-treated diabetes because hypoglycemic disorders are uncommon in such individuals.

[a]Modified from Whipple AO. The surgical therapy of hyperinsulinism. *J Int Chir* 1938; 3:237–276.

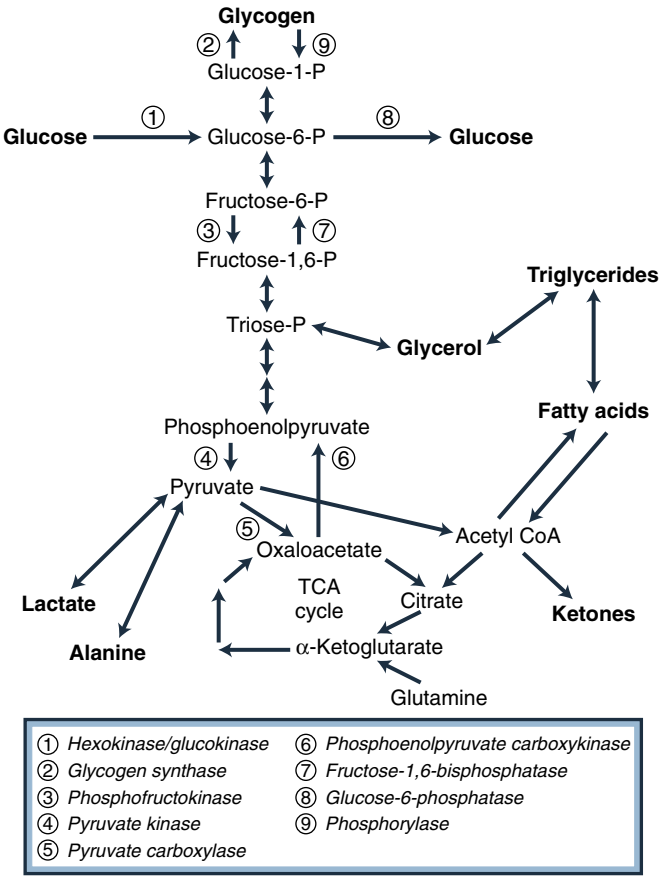
Given the survival requirement to maintain the plasma glucose concentration, it is not surprising that physiologic and behavioral mechanisms that prevent or rapidly correct hypoglycemia have evolved.[3] These mechanisms are so effective that hypoglycemia is an uncommon clinical event, except in persons who use drugs that lower the plasma glucose concentration, such as insulin, sulfonylureas, or alcohol.

Glucoregulatory failure resulting in hyperglycemia (diabetes mellitus) is discussed in Chapters 33 through 37; that resulting in hypoglycemia is discussed in the paragraphs that follow.

Physiology of Defense Against Hypoglycemia

Glucose Metabolism

Glucose is derived from three sources: intestinal absorption following digestion of dietary carbohydrates; *glycogenolysis*, which is the breakdown of glycogen, the polymerized storage form of glucose; and *gluconeogenesis*, which is the formation of glucose from precursors including lactate (and pyruvate), amino acids (especially alanine and glutamine), and, to a lesser extent, glycerol. There are multiple fates of glucose transported into cells (external losses are normally negligible) (Fig. 38.1). Glucose may be stored as glycogen, or may undergo glycolysis to pyruvate, which can be reduced to lactate, transaminated to form alanine, or converted to acetyl coenzyme A (CoA). Acetyl CoA, in turn, may be oxidized to carbon dioxide and water through the tricarboxylic acid cycle, converted to fatty acids that can be incorporated into triglycerides, oxidized, or utilized for synthesis of ketone bodies (acetoacetate, β-hydroxybutyrate) or cholesterol. Finally, glucose may be released into the circulation. Only liver and kidneys express glucose-6-phosphatase, the enzyme necessary for release of glucose into the circulation, at levels sufficient to permit substantial contributions to the systemic glucose pool. Many tissues express the enzymes required to synthesize and hydrolyze glycogen (glycogen synthase and phosphorylase, respectively). The liver and kidneys also express the enzymes necessary for gluconeogenesis, including the critical gluconeogenic enzymes pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and fructose-1,6-bisphosphatase.



• **Fig. 38.1** Schematic representation of glucose metabolism. *CoA*, coenzyme A; *P*, phosphate; *TCA*, tricarboxylic acid.

The liver is the major source of net endogenous glucose production (through glycogenolysis and gluconeogenesis). Conversely, the liver can be an organ of net glucose uptake and glycogen synthesis. The kidneys also produce glucose (through gluconeogenesis) and utilize glucose.

Muscle can take up and store glucose as glycogen, or metabolize glucose (through glycolysis) to pyruvate, which, among other fates, can be reduced to lactate or transaminated to form alanine. Lactate (and pyruvate) released from muscle can be transported to the liver and the kidneys, to serve as a gluconeogenic precursor (the Cori or glucose-lactate cycle). Alanine, glutamine, and other amino acids can also flow from muscle to liver and kidneys, where they also serve as gluconeogenic precursors. These constitute the glucose-alanine and glucose-glutamine cycles, in which new glucose formation is from precursors (e.g., amino acids) where the carbons are not derived from glucose. Although quantitatively less important than muscle, fat can also take up and metabolize glucose.

Glucose is essentially the sole metabolic fuel for the brain under physiologic conditions.[3] Glucose largely undergoes terminal oxidation in the brain. Although the adult human brain constitutes only about 2.5% of body weight, its oxidative metabolism accounts for approximately 25% of the basal metabolic rate and more than 50% of whole-body glucose utilization. The brain can utilize alternative fuels if their circulating levels rise high enough for them to enter the brain in quantity. For example, during extended fasting, markedly elevated circulating ketone levels can support the majority of the energy needs of the brain and reduce its utilization of glucose. Notably, ketogenesis is suppressed during episodes of insulin-mediated hypoglycemia such that again, the

TABLE 38.2 Systemic Glucose Balance[a] and Effects of Circulating Hormones on Endogenous Production and Use of Glucose

Source of glucose influx or efflux	HORMONAL EFFECTS		
	Insulin	Glucagon	Epinephrine
Glucose Influx Into the Circulation			
Exogenous glucose delivery			
Endogenous glucose delivery			
In liver: glycogenolysis and gluconeogenesis	↓	↑	↑
In kidneys: gluconeogenesis	↓		↑
Glucose Efflux Out of the Circulation			
Ongoing brain glucose utilization			
Variable glucose utilization by other tissues (e.g., muscle fat, liver, kidneys)	↑		↓

[a]Total glucose influx = total glucose efflux.

brain is critically dependent on a virtually continuous supply of glucose from the circulation.[3]

Systemic Glucose Balance

Normally, rates of endogenous glucose influx into the circulation and those of glucose efflux out of the circulation into tissues other than the brain are coordinately regulated—largely by the plasma glucose–lowering (regulatory) hormone insulin and the plasma glucose–raising (counterregulatory) hormones glucagon and epinephrine—such that systemic glucose balance is maintained, hypoglycemia (as well as hyperglycemia) is prevented, and a continuous supply of glucose to the brain is ensured (Table 38.2). This is accomplished despite wide variations in exogenous glucose influx (e.g., after meals vs. during fasting) and in glucose efflux (e.g., during exercise vs. rest). Hypoglycemia occurs when rates of glucose appearance in the circulation (the sum of endogenous glucose production and exogenous glucose delivery from ingested carbohydrates) fail to keep pace with rates of glucose disappearance from the circulation (the sum of ongoing glucose metabolism largely by the brain and of variable glucose utilization by tissues including muscle, fat, liver, and kidneys).

In healthy adults, the physiologic postabsorptive (fasting) plasma glucose concentration ranges from approximately 70 mg/dL (3.9 mmol/L) to 110 mg/dL (6.1 mmol/L), with a mean of about 90 mg/dL (5.0 mmol/L).[3] In the postabsorptive steady state, rates of glucose production and utilization average approximately 2.2 mg/kg per minute (12 μmol/kg per minute), with a range of 1.8 to 2.6 mg/kg per minute (10–14 μmol/kg per minute). These rates are as much as threefold higher in infants, at least in part because of their greater brain mass relative to body weight.

The liver is the predominant source of endogenous glucose production in the postabsorptive state; the kidneys, which both utilize and produce glucose, contribute little to net glucose production. However, as in the liver, glucose production in the kidney is regulated; it is suppressed by insulin and stimulated by epinephrine but not by glucagon. As a result, net renal glucose production occurs under some conditions, including hypoglycemia.[4] Therefore, endogenous glucose production cannot be equated solely with hepatic glucose production.

Gluconeogenesis and glycogenolysis are important for maintenance of the plasma glucose concentration.[5] The glucose pool—namely, free glucose in the extracellular fluids and in the cells of certain tissues (primarily the liver)—is only about 15 to 20 g (83–111 mmol). Glycogen that can be mobilized to provide circulating glucose (e.g., hepatic glycogen) contains approximately 70 g (390 mmol) of glucose, with a range of about 25 to 130 g (135–722 mmol). Therefore, in an adult of average size, preformed glucose can provide less than an 8-hour supply of energy, even at the diminished rate of glucose utilization that occurs in the postabsorptive state.

If fasting is prolonged to 24 to 48 hours, the plasma glucose concentration declines and then stabilizes; hepatic glycogen content falls to less than 10 g (55 mmol), and gluconeogenesis becomes the sole source of glucose production. Because amino acids are the main gluconeogenic precursors that result in net glucose formation, muscle protein is degraded. Glucose utilization by muscle and fat virtually ceases. As lipolysis and ketogenesis accelerate and circulating ketone levels rise, ketones become a major fuel for the brain. Glucose utilization by the brain declines by about half, which reduces the rate of gluconeogenesis required to maintain the plasma glucose concentration and hence decreases protein wasting.

After a meal, glucose absorption into the circulation increases to more than twice the rate of postabsorptive endogenous glucose production, depending on the carbohydrate content of the meal, the rate of gastric transit, and the rate of digestion and absorption. As glucose is absorbed, endogenous glucose production is suppressed, and glucose utilization by muscle, fat, and liver accelerates. The exogenous glucose is assimilated and, after a small rise, the plasma glucose concentration returns to the postabsorptive level.

Exercise increases glucose utilization (by muscle) to rates that can be several times greater than those of the postabsorptive state. Endogenous glucose production normally accelerates to match use so that the plasma glucose concentration is maintained.

In summary, the plasma glucose concentration is normally maintained within a relatively narrow range despite wide variations in glucose flux and thus maintains the systemic glucose balance. This remarkable homeostatic feat is accomplished by an array of hormonal, neural, and substrate glucoregulatory factors.

Responses to Hypoglycemia

Falling plasma glucose concentrations cause a sequence of responses, with defined glycemic thresholds, in healthy individuals[1,6–9] (Table 38.3). The first response is a decrease in insulin secretion. This decrease occurs as glucose levels decline within the physiologic range. Increased secretion of glucose counterregulatory hormones, including glucagon and epinephrine, occurs as glucose levels fall just below the physiologic range. Lower plasma glucose concentrations cause a more intense sympathoadrenal (sympathetic neural as well as adrenomedullary) response and symptoms. Even lower glucose levels cause cognitive dysfunction and additional manifestations of functional brain failure including seizure or coma.

Clinical Manifestations of Hypoglycemia

The symptoms and signs of hypoglycemia are nonspecific.[9] Clinical hypoglycemia—that sufficient to cause symptoms and signs[2]—is most convincingly documented by Whipple triad (see Table 38.1).

TABLE 38.3 Physiologic Responses to Decreasing Plasma Glucose Concentrations

Response	Glycemic Threshold[a] (mmol/L [mg/dL])	Physiologic Effects	Role in Prevention or Correction of Hypoglycemia (Glucose Counterregulation)
↓ Insulin	4.4–4.7 (80–85)	↑ R_a (↓ R_d)	Primary glucose regulatory factor, first defense against hypoglycemia
↑ Glucagon	3.6–3.9 (65–70)	↑ R_a	Primary glucose counterregulatory factor, second defense against hypoglycemia
↑ Epinephrine	3.6–3.9 (65–70)	↑ R_a, ↓ R_c	Involved, critical when glucagon is deficient, third defense against hypoglycemia
↑ Cortisol and growth hormone	3.6–3.9 (65–70)	↑ R_a, ↓ R_c	Involved, not critical
Symptoms	2.8–3.1 (50–55)	↑ Exogenous glucose	Prompt behavioral defense (food ingestion)
↓ Cognition	<2.8 (50)	—	(Compromises behavioral defense)
↓ Brain glucose metabolism	<2.8 (50)	—	—

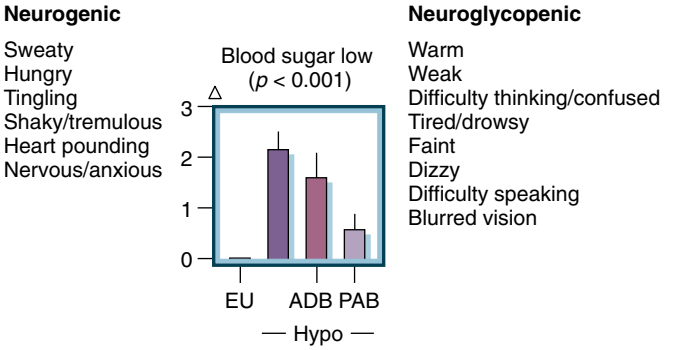
[a]Arterialized venous, not venous, plasma glucose concentrations.

R_a, Rate of glucose appearance, glucose production by the liver and kidneys; R_c, rate of glucose clearance by insulin-sensitive tissues; R_d, rate of glucose disappearance, glucose utilization by insulin-sensitive tissues such as skeletal muscle (no direct effect on central nervous system glucose utilization).

Neuroglycopenic symptoms are a direct result of brain glucose deprivation. They include cognitive impairments; behavioral changes; psychomotor abnormalities; and, at lower glucose levels, seizure and coma.[1,9] Neurogenic (or autonomic) symptoms are largely the result of the perception of physiologic changes caused by the sympathoadrenal (particularly the sympathetic neural[10]) discharge triggered by hypoglycemia. They include adrenergic (catecholamine-mediated) symptoms such as palpitations and tremor, and anxiety/arousal and cholinergic (acetylcholine-mediated) symptoms such as sweating, hunger, and paresthesias. Central mechanisms may also be involved in the generation of some of these symptoms (e.g., hunger).[11] Subjective awareness of hypoglycemia is largely the result of the perception of neurogenic symptoms[9] (Fig. 38.2).

The mechanism(s) of the signaling of the glucose regulatory and counterregulatory hormone and sympathetic responses to hypoglycemia are not known. However, those responses are not mediated by a decrease in brain glucose metabolism because the glycemic threshold for a decrease in the cerebral metabolic role of glucose, measured with [^{11}C]-glucose positron emission tomography, is at a lower plasma glucose concentration than the glycemic thresholds for the hormonal and sympathetic responses[12] (see Table 38.3).

Signs of hypoglycemia include pallor and diaphoresis, which result from adrenergic cutaneous vasoconstriction and cholinergic activation of sweat glands, respectively.[2,3] Heart rates and systolic blood pressures are raised, but often not greatly. Neuroglycopenic manifestations are often observable.



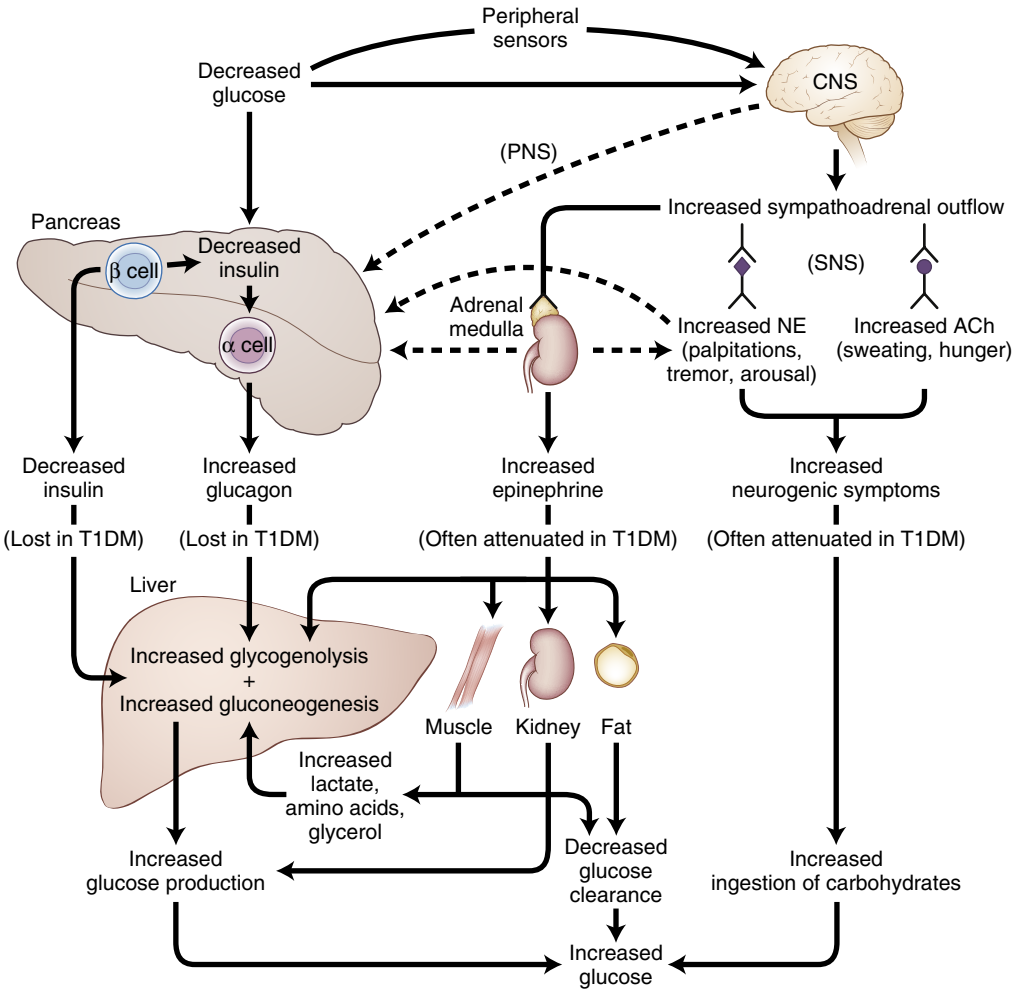
• **Fig. 38.2** Neurogenic (autonomic) and neuroglycopenic symptoms of hypoglycemia in healthy humans. Among the neurogenic symptoms, "sweaty," "hungry," and "tingling" are cholinergic, and "shaky/tremulous," "heart pounding," and "nervous/anxious" are adrenergic. See the text for discussion. Mean subject scores (± standard error) for awareness of hypoglycemia (low blood sugar) are shown during clamped euglycemia (EU) and during three conditions of hypoglycemia (Hypo): alone, with combined α- and β-adrenergic blockade by infused phentolamine and propranolol (ADB), and with combined α- and β-adrenergic blockade plus muscarinic cholinergic blockade by atropine (panautonomic blockade, PAB). (From Towler DA, Havlin CE, Craft S, Cryer P. Mechanism of awareness of hypoglycemia: perception of neurogenic [predominantly cholinergic] rather than neuroglycopenic symptoms. *Diabetes Care*. 1993;42:1791–1798, used with permission of the American Diabetes Association.)

Maintenance of Systemic Glucose Balance

Although obligatory glucose utilization, largely by the brain, is continuous, the delivery of exogenous glucose from dietary carbohydrates is intermittent. Systemic glucose balance (see Table 38.2) is normally maintained, and hypoglycemia and hyperglycemia are prevented, by dynamic, minute-to-minute regulation of endogenous glucose production from the liver and kidneys and of glucose utilization by tissues outside the central nervous system (CNS), such as muscle.[1,3] This regulation is exerted primarily by insulin, glucagon, and epinephrine[1–3] (Fig. 38.3; see also Table 38.3), although an array of hormones, neurotransmitters, and substrates is involved.[13]

The key physiologic defenses against falling plasma glucose concentrations are (1) a decrease in insulin, (2) an increase in glucagon, and, in the absence of the latter, (3) an increase in epinephrine.[3] The behavioral defense is carbohydrate ingestion prompted by symptoms that are largely sympathetic neural in origin[1,3,10] (see Table 38.3 and Fig. 38.3).

The first physiologic defense against hypoglycemia is a decrease in insulin secretion by the pancreatic islet beta cells. Signaled primarily by declining glucose levels at the beta cells, this response



• **Fig. 38.3** Physiologic and behavioral defenses against hypoglycemia in humans. *ACh,* acetylcholine; *α cell,* pancreatic islet alpha cell; *β cell,* pancreatic islet beta cell; *CNS,* central nervous system; *NE,* norepinephrine; *PNS,* parasympathetic nervous system; *SNS,* sympathetic nervous system; *T1DM,* type 1 diabetes mellitus. (From Cryer PE. Mechanisms of sympathoadrenal failure and hypoglycemia in diabetes. *J Clin Invest*. 2006;116:1470–1473, used with permission of the American Society for Clinical Investigation.)

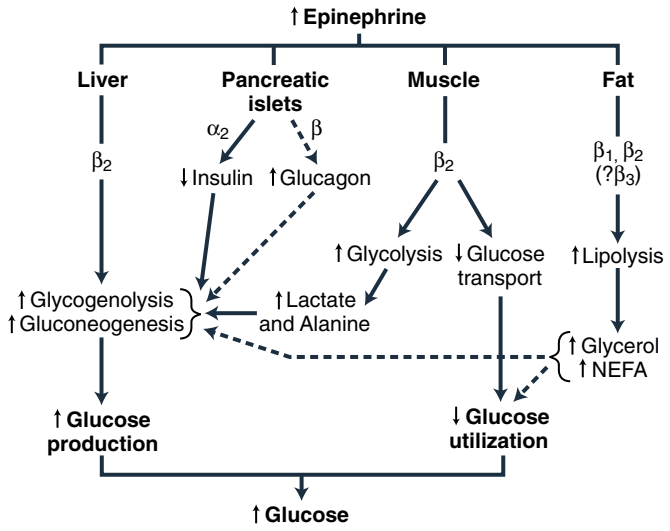
occurs as plasma glucose concentrations decline within the physiologic range[3] (see Table 38.3) and favors increased hepatic and renal glucose production with virtual cessation of glucose utilization by insulin-sensitive tissues such as muscle (see Fig. 38.3).

The second physiologic defense against hypoglycemia is an increase in glucagon secretion by pancreatic islet alpha cells. This increase occurs as plasma glucose concentrations fall just below the physiologic range (see Table 38.3) and stimulates hepatic glucose production, largely by stimulating glycogenolysis (see Fig. 38.3). This response is signaled primarily by a decrease in intraislet insulin, perhaps among other beta-cell secretory products, in the setting of low alpha-cell glucose concentrations[14–18] (see Fig. 38.3) and other signals that may include a decrease in somatostatin among others as yet to be determined mechanisms[14–18] and only secondarily by increased autonomic nervous system (sympathetic, parasympathetic, adrenomedullary) inputs.[19]

The third physiologic defense against hypoglycemia, which becomes critical when glucagon is deficient, is an increase in adrenomedullary epinephrine secretion. Signaled via the CNS, it also occurs as plasma glucose concentrations fall just below the physiologic range[1–3] (see Table 38.3) and raises plasma glucose concentrations largely by β_2-adrenergic stimulation of hepatic and renal glucose production (see Fig. 38.3). However, the plasma glucose–raising actions of epinephrine also involve limitation of glucose clearance by insulin-sensitive tissues, mobilization of gluconeogenic precursors such as lactate and amino acids from muscle and glycerol from fat, and α_2-adrenergic limitation of insulin secretion[20] (Fig. 38.4). Indeed, the adrenergic actions on beta-cell insulin secretion normally play an important role in the glycemic actions of epinephrine. α_2-Adrenergic limitation of insulin secretion permits the glycemic response; β_2-adrenergic stimulation alone has little effect because it also stimulates insulin secretion. However, some increase in insulin secretion—due to the rising glucose level, β_2-adrenergic stimulation, or both—limits the magnitude of the glycemic response to epinephrine. These physiologic interactions explain why glycemic sensitivity to epinephrine is increased in patients who cannot increase insulin secretion (e.g., those with type 1 diabetes mellitus [T1DM]).[20] Circulating epinephrine is derived almost exclusively from the adrenal medulla in adults.[10] Whereas circulating norepinephrine is derived largely from sympathetic nerve terminals under resting conditions and in many stimulated states (e.g., exercise), the plasma norepinephrine response to hypoglycemia is derived largely from the adrenal medulla.[10]

These physiologic defenses against hypoglycemia typically abort episodes of declining plasma glucose concentrations and prevent clinical (i.e., symptomatic) hypoglycemia. If they do not, the lower plasma glucose concentration causes a more intense sympathoadrenal response, resulting in symptoms[1,3] (see Table 38.3). These symptoms, particularly the neurogenic symptoms, cause awareness of hypoglycemia that prompts the behavioral defense against hypoglycemia—ingestion of carbohydrates[3] (see Fig. 38.3).

The integrated physiology of glucose counterregulation[1–3] is further illustrated in Fig. 38.5. Falling glucose levels within the pancreatic islet cells signal a decrease in insulin secretion and an increase in glucagon secretion. Falling glucose levels sensed in the periphery and in the CNS, acting through the hypothalamus, signal an increase in sympathoadrenal activity that results in an increase in adrenomedullary epinephrine secretion and in neurogenic symptoms, the latter largely resulting from increased sympathetic neural activity. Fig. 38.5 includes a putative cerebral network that may modulate the hypothalamic response.



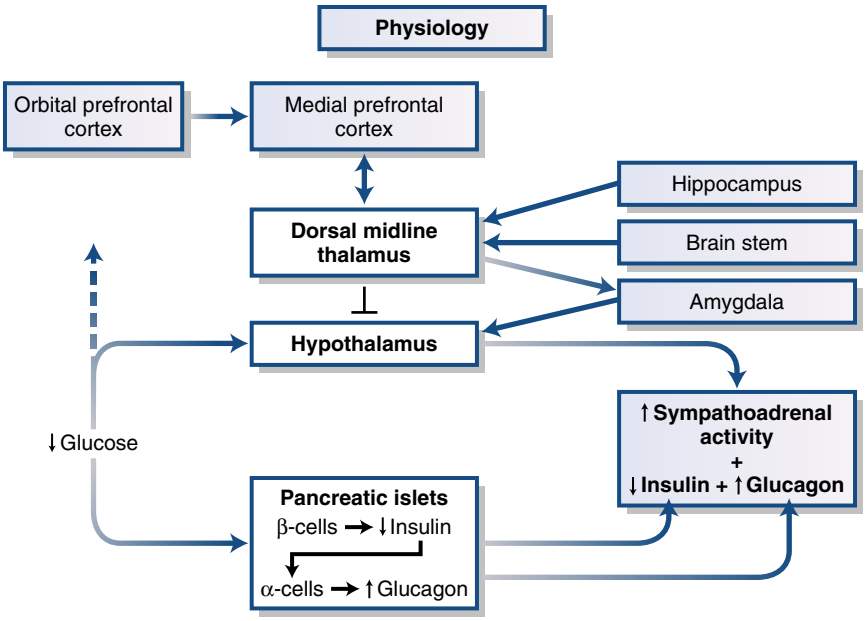
• **Fig. 38.4** Schematic representation of the mechanisms of the hyperglycemic effect of epinephrine, mediated by α- and β-adrenergic stimulation. *NEFA*, nonesterified fatty acid. (From Cryer PE. Catecholamines, pheochromocytoma and diabetes. *Diabetes Rev*. 1993;1:309–317, used with permission of the American Diabetes Association.)

Clinical Hypoglycemia

Definition and Diagnosis

Clinical hypoglycemia is, by definition, a plasma glucose concentration low enough to cause symptoms or signs, including impairment of brain function. The glycemic thresholds for symptoms and signs of hypoglycemia are dynamic; for example, they shift to lower plasma glucose concentrations in patients with recurrent hypoglycemia[21–23] and to higher concentrations in those with poorly controlled diabetes. Therefore, it is not possible to state a single plasma glucose concentration that categorically defines hypoglycemia. Furthermore, the symptoms and signs of hypoglycemia are nonspecific, and a low measured plasma glucose concentration can be artifactual. For all of these reasons, hypoglycemia is most convincingly documented by Whipple triad[1,2] (see Table 38.1).

Documentation of Whipple triad is particularly important when hypoglycemia is suspected in a person who does not have diabetes mellitus, because hypoglycemic disorders are rare. In the absence of diabetes, a thorough diagnostic evaluation is recommended only for patients in whom Whipple triad can be documented.[2] Ideally, patients with diabetes being treated with an insulin secretagogue or insulin should monitor their plasma glucose concentration whenever they suspect hypoglycemia. Nonetheless, the likelihood that a symptomatic episode is the result



• **Fig. 38.5** Schematic representation of the integrated mechanisms of the physiologic responses to hypoglycemia in humans: decrements in insulin secretion, increments in glucagon secretion, and increments in sympathoadrenal (adrenomedullary and sympathetic neural) activity. *α-cells*, pancreatic islet alpha cells; *β-cells*, pancreatic islet beta cells. (From Cryer PE. *Hypoglycemia in Diabetes: Pathophysiology, Prevalence and Prevention*. Alexandria, VA: American Diabetes Association; 2009, used with permission of the American Diabetes Association.)

of hypoglycemia is high, because hypoglycemia is common in patients treated with insulin, a sulfonylurea, or a glinide. It is also important to recognize that glucose concentrations measured in whole blood are approximately 15% lower than those in plasma and may be further reduced if the hematocrit is high.

Clinical Classification of Hypoglycemia

Causes of hypoglycemia[2] are outlined in Table 38.4. Drugs are, by far, the most common cause of hypoglycemia. Such drugs include insulin secretagogues and insulin used to treat diabetes. Although persons with diabetes can suffer from the same hypoglycemic disorders as those without diabetes, their hypoglycemic episodes are usually the result of treatment of their diabetes. Furthermore, the pathophysiology of hypoglycemia in diabetes is unique, and the diagnostic and management approaches are different from those for individuals without diabetes. Therefore, hypoglycemia in persons with diabetes and hypoglycemia in those without diabetes are discussed separately in this chapter.

Hypoglycemia in Persons With Diabetes

The Clinical Problem of Hypoglycemia in Diabetes

Iatrogenic hypoglycemia is the limiting factor in the glycemic management of diabetes with insulin, a sulfonylurea, or a glinide.[1]

TABLE 38.4 Causes of Hypoglycemia in Adults

Ill or Medicated Individual
Drugs
Insulin or insulin secretagogue
Alcohol
Others (see Table 38.8)
Critical Illnesses
Hepatic, renal, or cardiac failure
Sepsis
Inanition
Hormonal Deficiency
Cortisol
Glucagon and epinephrine (in insulin-deficient diabetes mellitus)
Non–Islet Cell Tumor
Seemingly Well Individual
Endogenous Hyperinsulinism
Insulinoma
Functional beta-cell disorders (nesidioblastosis)
Noninsulinoma pancreatogenous hypoglycemia
Post–gastric bypass hypoglycemia
Autoimmune hypoglycemia
Antibody to insulin
Antibody to insulin receptor
Insulin secretagogue
Other
Accidental, Surreptitious, or Malicious Hypoglycemia

From Cryer PE, Axelrod L, Grossman AB, et al. Evaluation and management of adult hypoglycemic disorders: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2009;94:709–728, used with permission of The Endocrine Society.

It causes recurrent morbidity in most persons with T1DM and in many of those with advanced type 2 diabetes mellitus (T2DM), and it is sometimes fatal. The barrier of hypoglycemia generally precludes maintenance of euglycemia over a lifetime of diabetes and thus full realization of the vascular benefits of glycemic control. Furthermore, hypoglycemia compromises physiologic and behavioral defenses against subsequent falling plasma glucose concentrations, resulting in a vicious cycle of recurrent hypoglycemia.

Hypoglycemia in diabetes is caused by pharmacokinetically imperfect treatment with insulin or an insulin secretagogue (e.g., a sulfonylurea or a glinide) that results in episodes of therapeutic hyperinsulinemia. Thus, it is fundamentally iatrogenic. Episodes of substantial absolute therapeutic hyperinsulinemia can cause isolated episodes of hypoglycemia. However, as developed later in this chapter, recurrent hypoglycemia in diabetes is typically the result of the interplay of relative or mild to moderate absolute therapeutic hyperinsulinemia and compromised physiologic and behavioral defenses against falling plasma glucose concentrations.[1,2]

Frequency of Hypoglycemia in Diabetes

Hypoglycemia is unfortunately a fact of life for people with T1DM.[24–26] The average patient suffers untold numbers of episodes of asymptomatic hypoglycemia (which are not benign, because they impair defenses against subsequent hypoglycemia), approximately two episodes of symptomatic hypoglycemia per week (thousands of such episodes over a lifetime of diabetes), and roughly one episode per year of severe, at least temporarily disabling hypoglycemia, often with seizure or coma. This problem has not abated since it was highlighted by the report of the Diabetes Control and Complications Trial (DCCT) in 1993.[24] In 2007, the United Kingdom Hypoglycaemia Study Group reported an incidence of severe hypoglycemia twice that reported in the DCCT among patients who had had T1DM for less than 5 years and five times the DCCT incidence among those with T1DM for more than 15 years in their population-based study.[27]

The overall incidence of hypoglycemia during treatment of T2DM with an insulin secretagogue, or even with insulin, is lower than that in patients with T1DM.[26–34] However, for pathophysiologic reasons discussed later, the incidence of hypoglycemia increases progressively over time as patients approach the absolute endogenous insulin-deficient end of the spectrum of T2DM.[27,29,30] Indeed, the incidence of hypoglycemia has been reported to be similar in patients with T2DM and in those with T1DM matched for duration of insulin therapy.[30] The United Kingdom Hypoglycaemia Study Group found a prevalence of severe hypoglycemia of 7% and an incidence of 10 episodes per 100 patient-years among patients with T2DM treated with insulin for less than 2 years; among those treated for longer than 5 years, these figures rose to 25% prevalence and 70 episodes per 100 patient-years.[27] The patterns for self-treated hypoglycemia were similar. Therefore, at least with current, less than euglycemic treatment goals, the frequency of iatrogenic hypoglycemia is relatively low during the first few years of treatment of T2DM with insulin but increases substantially in advanced T2DM, approaching the frequency among patients with T1DM. As discussed later in this chapter, new technologies, particularly continuous glucose monitoring (CGM) and drugs for the treatment of T2DM that do not cause hypoglycemia, will reduce the frequency of hypoglycemia for those who are able to use them.

Estimates of the incidence and prevalence of hypoglycemia in diabetes are generally underestimates because of the challenge of ascertainment.[1,2] Asymptomatic episodes will be missed unless

they are incidentally detected by self-monitoring of blood glucose or by CGM. Symptomatic episodes may not be recognized to be the result of hypoglycemia, because the symptoms of hypoglycemia are nonspecific. Even if they are recognized, they may not be remembered[35,36] and reported at periodic patient contacts. Because they are dramatic events that are more likely to be reported (by the patient or an associate), estimates of the frequency of severe hypoglycemia (that requiring the assistance of another person) are more reliable, although they represent only a small fraction of the total hypoglycemic burden. Prospective, population-based studies with a focus on hypoglycemia should provide the most reliable data.

The prospective, population-based data of Donnelly and colleagues[26] indicate that the overall incidences of any episode of hypoglycemia and of severe hypoglycemia in insulin-treated T2DM are about one-third of those in T1DM. Two other population-based studies reported the incidence of severe hypoglycemia requiring emergency treatment in insulin-treated T2DM to be 40%[31] and 100%[32] of that in T1DM. Taken together, and considering that the prevalence of T2DM is approximately 20-fold greater than that of T1DM and that most patients with T2DM ultimately require treatment with insulin, these data suggest that most episodes of iatrogenic hypoglycemia, including severe hypoglycemia, occur in persons with T2DM.

Impact of Hypoglycemia in Diabetes

Iatrogenic hypoglycemia causes recurrent physical and psychosocial morbidity and impairs glycemic defenses against subsequent hypoglycemia in many patients with diabetes.[1] The barrier of hypoglycemia generally precludes maintenance of euglycemia over a lifetime of diabetes. Hypoglycemia often causes functional brain failure that is typically reversed after the plasma glucose concentration is raised. Rarely, it causes sudden death, presumably the result of cardiac arrhythmia, or, if it is profound and prolonged, permanent brain dysfunction and death.

At a minimum, an episode of symptomatic hypoglycemia is a nuisance and a distraction. It can impair judgment, behavior, and performance of physical tasks such as driving. It can cause a seizure or loss of consciousness. Transient neurologic deficits sometimes occur, but permanent neurologic damage is rare. Systematic long-term follow-up of the DCCT patients suggests that recurrent iatrogenic hypoglycemia does not cause chronic cognitive impairments in young adults,[37] but the possibility that it does so in young children[38,39] and the elderly[40] remains. Among other psychological disorders, fear of hypoglycemia can be a barrier to glycemic control.[41]

Iatrogenic hypoglycemia can be fatal.[42–53] That hypoglycemia can kill has been known since the discovery of insulin.[42] There are epidemiologic associations of hypoglycemia with death,[43] reports of excessive mortality rates during intensive glycemic therapy in randomized controlled trials of patients with T2DM[43,44] and intensive care unit patients,[45,46] and reports of hypoglycemia mortality rates in series of patients with diabetes.[47–51] Early reports indicated that 2% to 4% of patients with T1DM died of hypoglycemia.[47–49] More recent reports indicate that 4%,[49,50] 7%,[51] 8%,[52] and 10% or greater[53] of deaths of persons with T1DM were caused by hypoglycemia. Indeed, hypoglycemia at the time of death has been documented by CGM.[54] Death rates of up to 10% of patients who suffer severe sulfonylurea-induced hypoglycemia have been reported.[55]

The finding of Currie and colleagues[56] that lower glycosylated hemoglobin (HbA_{1c}) levels are associated with increased mortality in patients with T2DM at higher risk of hypoglycemia is entirely consistent with this body of evidence that iatrogenic hypoglycemia is a risk factor for death in diabetes. Thus, there are iatrogenic mortality rates in both T1DM and T2DM.

Although prolonged, profound hypoglycemia can cause brain death, most instances of sudden hypoglycemic death are thought to be the result of cardiac arrhythmias triggered by an intense sympathoadrenal response to hypoglycemia.[44,57,58] These are mediated through β-adrenergic receptors.[58] Sudden death has been associated with QT interval prolongation and reduced baroreflex sensitivity in patients with classic diabetic autonomic neuropathy.[59–61] Adler and colleagues[62] demonstrated, in studies of nondiabetic individuals, that recent antecedent hypoglycemia causes reduced baroreflex sensitivity the following day. The induction of functional sympathetic failure by recent antecedent hypoglycemia as an additional, potentially fatal, feature of the concept of hypoglycemia-associated autonomic failure (HAAF) in diabetes[1] is discussed later in this chapter.

Clinical Definition and Classification of Hypoglycemia in Diabetes

The American Diabetes Association/Endocrine Society Workgroup on Hypoglycemia[63] (ADA/ES Workgroup) defined hypoglycemia in diabetes as "all episodes of abnormally low plasma glucose concentration that expose the individual to potential harm." That is broader than the recommended definition of clinical hypoglycemia in persons without diabetes (i.e., a plasma glucose concentration low enough to cause symptoms or signs[1,2]), because it includes asymptomatic episodes. The latter are not benign in persons with diabetes; they compromise defenses against subsequent hypoglycemia,[1,2] and they identify increased risk of imminent severe iatrogenic hypoglycemia.[64] Again, it is not possible to define a plasma glucose concentration that categorically defines hypoglycemia, because the glycemic thresholds for responses to hypoglycemia are dynamic. They shift to lower plasma glucose concentrations in patients with recurrent hypoglycemia[21,22] and to higher plasma glucose concentrations in those with poorly controlled diabetes.[20,21]

The ADA/ES Workgroup recommends that people with diabetes (implicitly those treated with an insulin secretagogue or insulin) become concerned about the possibility of developing hypoglycemia at a self-monitored plasma glucose concentration of 70 mg/dL (3.9 mmol/L) or less.[63] Within the error of self-monitoring (or CGM), that conservative alert value approximates the lower limit of the nondiabetic postabsorptive plasma glucose range[1,2] and the normal glycemic thresholds for activation of physiologic glucose counterregulatory systems[1,2] (see Table 38.3); it is low enough to reduce glycemic defenses against subsequent hypoglycemia in nondiabetic persons. Therefore, the recommended glucose alert level of 70 mg/dL (3.9 mmol/L) or less is data driven; it usually gives the patient time to take action to prevent a clinical hypoglycemic episode, and it provides some margin for the limited accuracy of glucose monitoring devices at low plasma glucose concentrations. It has been implicitly endorsed by the US Food and Drug Administration and the European Medicines Agency.

The recommended alert value does not, of course, mean that persons with diabetes should always self-treat for hypoglycemia at an estimated plasma glucose concentration of 70 mg/dL (3.9 mmol/L) or less. Rather, they should consider actions ranging from repeating the measurement in the near term, through behavioral actions such as avoiding exercise or driving until the glucose level is raised, to carbohydrate ingestion and subsequent adjustments of the therapeutic regimen.

The ADA/ES Workgroup[63] also recommended a clinical classification of hypoglycemia in diabetes (Table 38.5).

TABLE 38.5 Classification of Hypoglycemia in Diabetes

Clinical Classification	Definition
Severe hypoglycemia	An event requiring the assistance of another person to actively administer carbohydrate, glucagon, or other resuscitative actions. Plasma glucose measurements may not be available during such an event, but neurologic recovery attributable to the restoration of plasma glucose to a normal level is considered sufficient evidence that the event was induced by a low plasma glucose concentration.
Documented symptomatic hypoglycemia	An event during which typical symptoms of hypoglycemia are accompanied by a measured plasma glucose concentration of ≤70 mg/dL (3.9 mmol/L).
Asymptomatic hypoglycemia	An event not accompanied by typical symptoms of hypoglycemia but with a measured plasma glucose concentration of ≤70 mg/dL (3.9 mmol/L).
Probable symptomatic hypoglycemia	An event during which symptoms typical of hypoglycemia are not accompanied by a plasma glucose determination but were presumably caused by a plasma glucose concentration of ≤70 mg/dL (3.9 mmol/L).
Pseudohypoglycemia	An event during which the person with diabetes reports any of the typical symptoms of hypoglycemia and interprets those as indicative of hypoglycemia, with a measured plasma glucose concentration that is >70 mg/dL (3.9 mmol/L) but is approaching that level.

The International Hypoglycaemia Study Group[65] proposed three levels of iatrogenic hypoglycemia in diabetes:

- *Level 1*: A glucose alert value of 70 mg/dL (3.9 mmol/L) or less
- *Level 2*: A glucose level less than 54 mg/dL (3.0 mmol/L), which is sufficiently low to indicate serious clinically important hypoglycemia
- *Level 3*: Severe hypoglycemia as defined by the American Diabetes Association.

These levels were endorsed by the American Diabetes Association and the European Association for the Study of Diabetes.[65]

Based on the fact that the HbA_{1c} level provides useful information about long-term glycemic control but no information about short-term glycemic control, it has been suggested[66] that the classification of severe hypoglycemia (see Table 38.5) should be expanded to include a distinctly low plasma glucose concentration, perhaps less than 50 mg/dL (2.8 mmol/L) or less than 54 mg/dL (3.0 mmol/L),[65] detected by self-monitoring of plasma glucose, CGM, or a laboratory determination. This would be particularly useful if there were no symptoms at the time of the detection of the distinctly low glucose concentration, because it would document impaired awareness of hypoglycemia, a treatable condition.

Pathophysiology of Glucose Counterregulation in Diabetes

The pathophysiology of glucose counterregulation and its relationship to clinical hypoglycemia in diabetes[1,2] are summarized in the paragraphs that follow (see earlier discussion and Figs. 38.3 and 38.5). Again, the key physiologic defenses against falling plasma glucose concentrations are a decrease in insulin, an increase in glucagon, and, in the absence of the latter, an increase in epinephrine. The behavioral defense is carbohydrate ingestion prompted by symptoms[9] that are largely sympathetic neural in origin.[10]

Insulin Excess

Episodes of therapeutic hyperinsulinemia, produced by treatment with an insulin secretagogue (a sulfonylurea or a glinide) or with insulin, are a prerequisite for the development of iatrogenic hypoglycemia. Marked absolute insulin excess can cause isolated episodes of hypoglycemia. However, iatrogenic hypoglycemia is typically the result of the interplay of relative or mild to moderate absolute therapeutic hyperinsulinemia and compromised physiologic and behavioral defenses against falling plasma glucose concentrations[1,2] (Fig. 38.6).

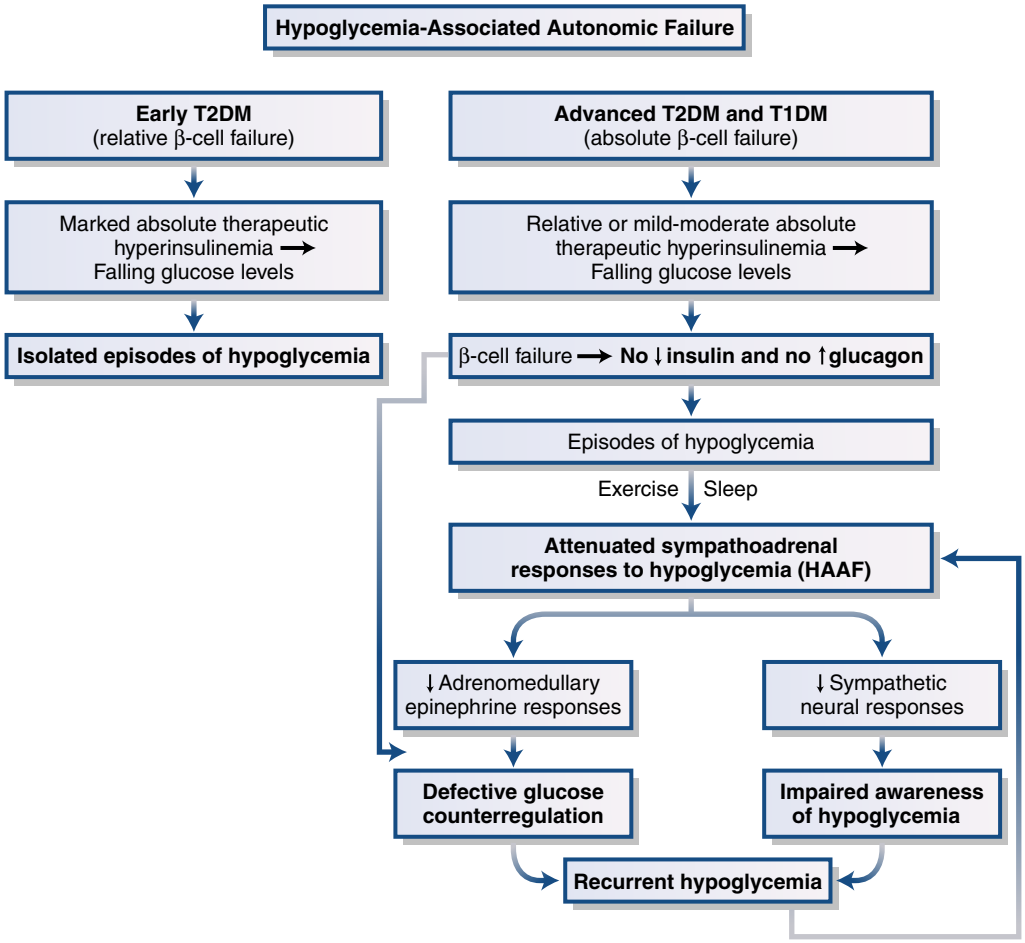
Defective Glucose Counterregulation and Hypoglycemia Unawareness

In established (absolute deficiency of endogenous insulin) T1DM, circulating (exogenous) insulin concentrations do not decrease as plasma glucose concentrations fall in response to therapeutic hyperinsulinemia. That is the result of absolute beta-cell failure with no regulated endogenous insulin secretion. Therefore, the first physiologic defense against hypoglycemia[3] is lost. Furthermore, despite the presence of functional alpha cells, there is no increase in glucagon secretion[1,2,18] (Fig. 38.7). That also is largely the result of beta-cell failure,[14–18] which causes loss of the decrement in insulin that normally signals an increase in alpha-cell glucagon secretion during hypoglycemia[1,18] (see Figs. 38.3 and 38.5). Therefore, the second physiologic defense against hypoglycemia[3] is lost as well.

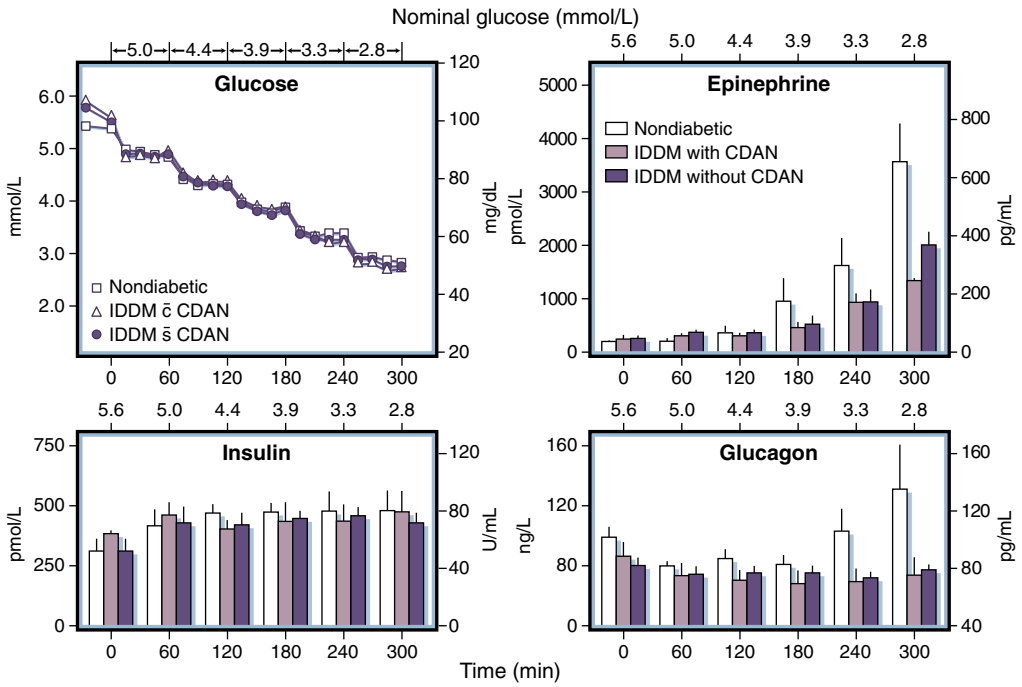
In addition, the increase in epinephrine secretion, the third physiologic defense against hypoglycemia,[3] is typically attenuated[1,2,67] (see Fig. 38.7). In the setting of absent insulin and glucagon responses, the attenuated epinephrine response causes the clinical syndrome of defective glucose counterregulation[67–69] (see Fig. 38.6), which is associated with a 25-fold[69] or greater[70] increased risk of severe hypoglycemia in T1DM. The attenuated epinephrine response is a marker of attenuation of the sympathoadrenal response (including the sympathetic neural response) that normally causes neurogenic symptoms that largely prompt the behavioral response leading to carbohydrate ingestion (see Figs. 38.3 and 38.5). This attenuated response (largely the attenuated sympathetic neural response) causes the clinical syndrome of hypoglycemia unawareness[1,2] (see Fig. 38.6).

Although the term *hypoglycemia unawareness* is used widely, "impaired awareness of hypoglycemia"[71] is more precise because there is a spectrum ranging from partial to complete loss of symptoms. Attenuated sympathoadrenal responses to falling plasma glucose concentrations can be caused by recent antecedent hypoglycemia[72] (Figs. 38.8 and 38.9), prior exercise,[73] or sleep,[74,75] but the precise mechanism is not known. (See later discussion on HAAF.)

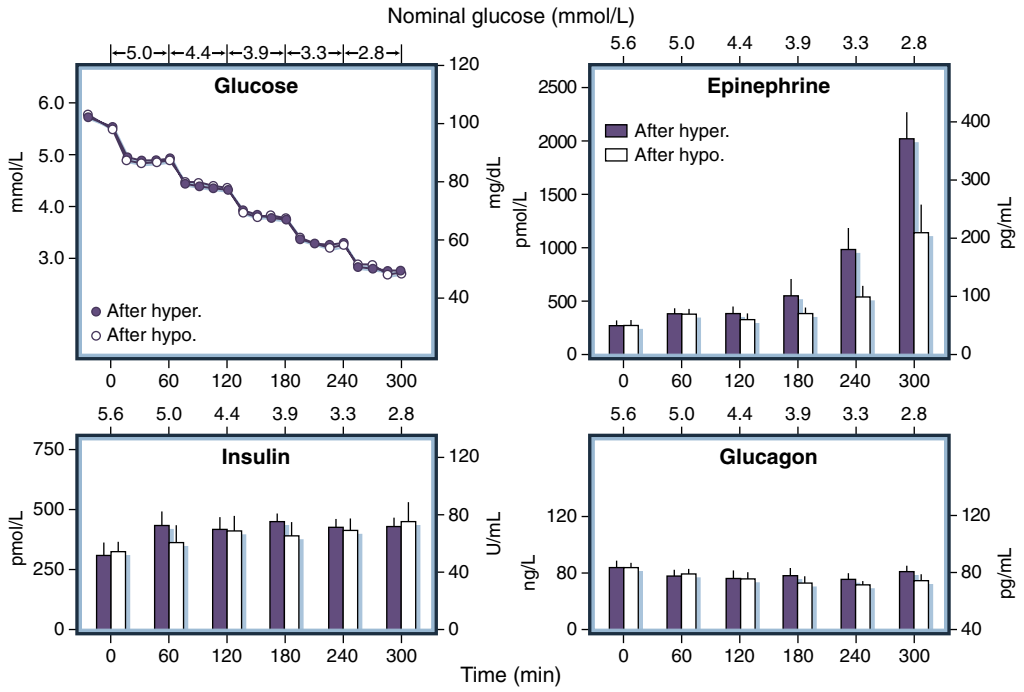
Impaired awareness of hypoglycemia is largely the result of reduced release of the sympathetic neurotransmitters norepinephrine and acetylcholine.[10] There is evidence of decreased β-adrenergic sensitivity, specifically reduced cardiac chronotropic sensitivity to isoproterenol, in affected patients.[76,77] However, vascular sensitivity to a β-adrenergic agonist was not found to be reduced in unaware patients.[78] Reduced sensitivity to β-adrenergic



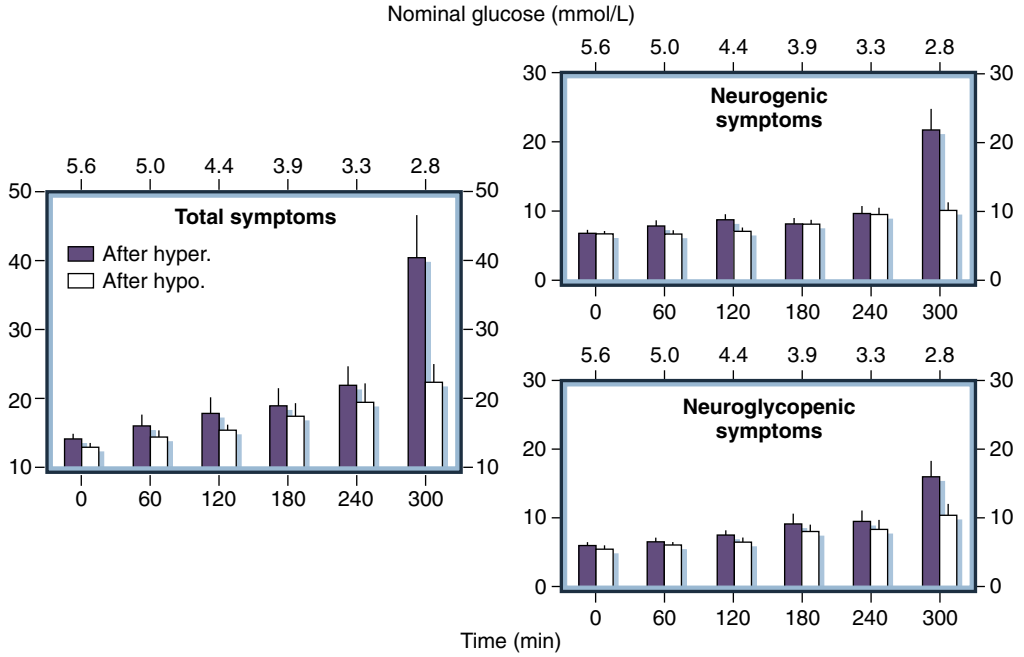
• **Fig. 38.6** Schematic representation of the concept of hypoglycemia-associated autonomic failure (HAAF) in diabetes and the pathogenesis of iatrogenic hypoglycemia including the clinical syndromes of defective glucose counterregulation and impaired awareness of hypoglycemia. *β-cell,* pancreatic islet beta cell; *T1DM,* type 1 diabetes mellitus; *T2DM,* type 2 diabetes mellitus.



• **Fig. 38.7** Mean (± standard error) plasma glucose, insulin, epinephrine, and glucagon concentrations during hyperinsulinemic stepped hypoglycemic glucose clamps in three groups of subjects: nondiabetic subjects (*open squares* and *columns*), people with type 1 diabetes (IDDM) who have classic diabetic autonomic neuropathy (CDAN; *open triangles* and *crosshatched columns*), and people with IDDM without CDAN (*closed circles* and *columns*). *IDDM,* insulin-dependent diabetes mellitus. (From Dagogo-Jack SE, Craft S, Cryer PE. Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. *J Clin Invest*. 1993;91:819–828, used with permission of the American Society for Clinical Investigation.)



• **Fig. 38.8** Mean (± standard error) plasma glucose, insulin, epinephrine, and glucagon concentrations during hyperinsulinemic stepped hypoglycemic glucose clamps in patients with type 1 (insulin-dependent) diabetes mellitus without classical diabetic autonomic neuropathy on mornings after afternoon hyperglycemia (After hyper.; *closed circles* and *columns*) and on mornings after afternoon hypoglycemia (After hypo.; *open circles* and *columns*). (From Dagogo-Jack SE, Craft S, Cryer PE. Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. *J Clin Invest*. 1993;91:819–828, used with permission of the American Society for Clinical Investigation.)



• **Fig. 38.9** Mean (± standard error) total, neurogenic, and neuroglycopenic symptom scores during hyperinsulinemic stepped hypoglycemic glucose clamps in patients with type 1 (insulin-dependent) diabetes mellitus without classical diabetic autonomic neuropathy on mornings after afternoon hyperglycemia (After hyper., *closed columns*) and on mornings after afternoon hypoglycemia (After hypo., *open columns*). (From Dagogo-Jack SE, Craft S, Cryer PE. Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. *J Clin Invest*. 1993;91:819–828, used with permission of the American Society for Clinical Investigation.)

signaling of neurogenic symptoms remains to be demonstrated in patients with unawareness, and it would be necessary to also postulate decreased cholinergic sensitivity to explain reduced cholinergic symptoms such as sweating.

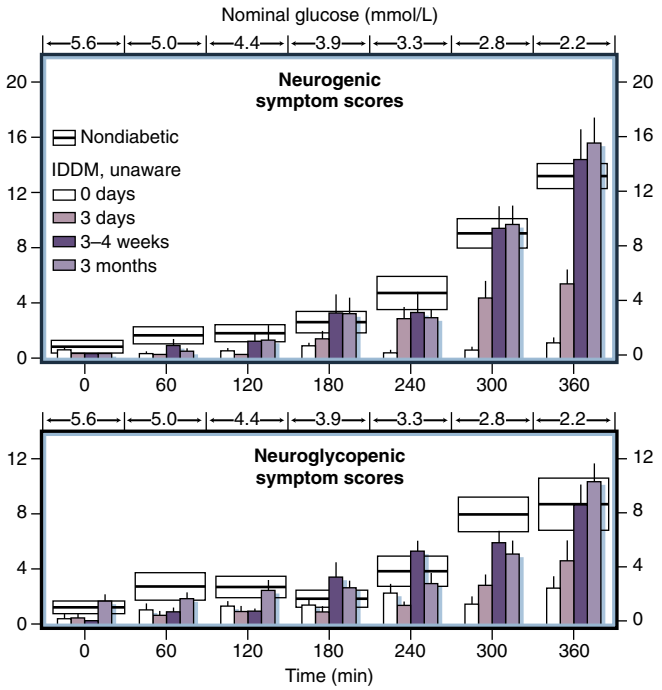
In contrast to these compromised defenses in T1DM, defenses against hypoglycemia are intact early in the course of T2DM. However, they become compromised over time.[1,79] In advanced T2DM (i.e., with absolutely deficient endogenous insulin), insulin and glucagon responses to falling plasma glucose concentrations are lost and sympathoadrenal responses to hypoglycemia are reduced by recent antecedent hypoglycemia, as is observed in T1DM.[79]

In summary, the pathophysiology of glucose counterregulation is the same in T1DM and T2DM but with different time courses.[67,79,80] The pathogenesis of an episode of iatrogenic hypoglycemia involves therapeutic hyperinsulinemia resulting in falling plasma glucose concentrations. With the absence of appropriate decrements in insulin and of increments in glucagon, hypoglycemia occurs. That, in turn, causes attenuated sympathoadrenal responses to subsequent falling glucose levels and recurrent episodes of hypoglycemia (see Fig. 38.6). Because beta-cell failure, which causes loss of both insulin and glucagon responses, occurs rapidly in T1DM but slowly in T2DM, the syndromes of defective glucose counterregulation and impaired awareness of hypoglycemia develop early in T1DM but later in T2DM. That temporal pattern of compromised glycemic defenses explains why iatrogenic hypoglycemia becomes progressively more frequent as patients approach the insulin-deficient end of the spectrum of T2DM.[27]

HAAF in Diabetes

The concept of HAAF[1,2,67,68,81] in diabetes developed in humans posits that in patients with absolute endogenous insulin deficient diabetes (T1DM or advanced T2DM), necessarily imperfect insulin replacement results in falling plasma glucose concentrations, but no decrease in insulin secretion and no increase in glucagon secretion, and thus recurrent episodes of hypoglycemia. Those episodes[72] (as well as sleep[74,75] or prior exercise[73]) attenuate adrenomedullary epinephrine secretion and sympathetic neural activation in response to subsequent hypoglycemia. In the setting of absent insulin and glucagon responses, the attenuated epinephrine responses cause the clinical syndrome of defective glucose counterregulation, which is associated with a 25-fold[69] or greater[70] increased risk of severe iatrogenic hypoglycemia during intensive glycemic therapy. The attenuated sympathetic neural responses cause the clinical syndrome of impaired awareness of hypoglycemia, which is associated with at least a sixfold increased risk of severe iatrogenic hypoglycemia during intensive glycemic therapy.[71] The resulting recurrent hypoglycemia further attenuates the sympathoadrenal response to falling plasma glucose concentrations (see Fig. 38.6). HAAF is a functional form of autonomic failure, distinct from classic diabetic autonomic neuropathy.[82] HAAF has cardiovascular implications because, like autonomic neuropathy, it reduces baroreflex sensitivity[62] and may predispose patients to cardiac arrhythmias.

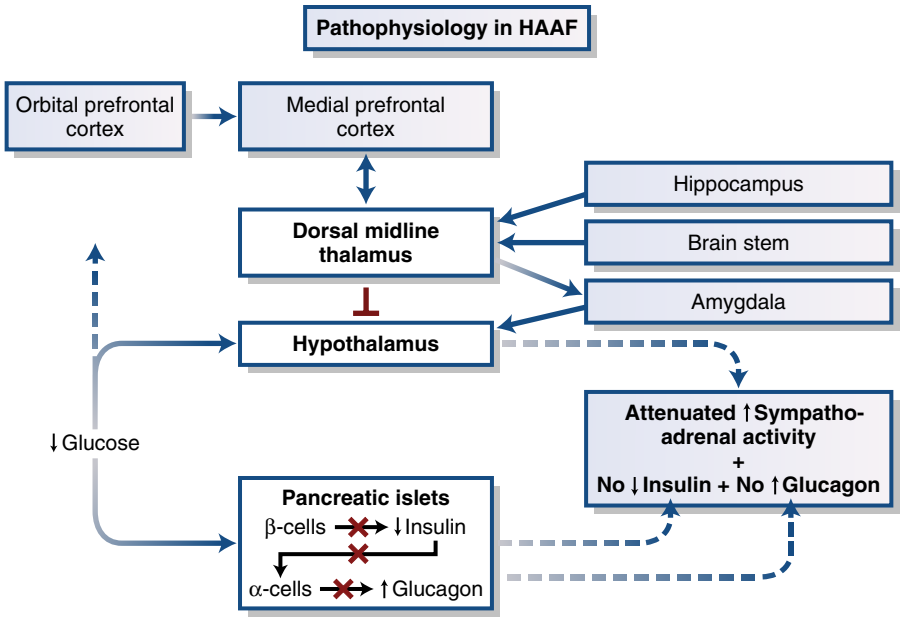
Recent antecedent hypoglycemia, even asymptomatic nocturnal hypoglycemia, reduces epinephrine and symptomatic responses to a given level of subsequent hypoglycemia,[83] reduces detection of hypoglycemia in the clinical setting,[84] and reduces glycemic defense against hyperinsulinemia[80] in T1DM. Perhaps the most compelling support for the clinical relevance of HAAF is the finding, originally from three independent laboratories,[85–88] that as little as 2 to 3 weeks of scrupulous avoidance of hypoglycemia reverses impaired awareness of hypoglycemia (Fig. 38.10) and improves the attenuated epinephrine component of defective glucose counterregulation in most affected patients. HAAF also occurs in advanced T2DM.[79]



• **Fig. 38.10** Mean (± standard error) neurogenic and neuroglycopenic symptom scores during hyperinsulinemic stepped hypoglycemic glucose clamps in nondiabetic subjects (*rectangles*) and in people with type 1 diabetes mellitus (IDDM) selected for clinical impaired awareness of hypoglycemia studied at various time points during scrupulous avoidance of iatrogenic hypoglycemia: at baseline (0 days), after 3 days, after 3 to 4 weeks, and after 3 months (see the key in the figure). *IDDM,* insulin-dependent diabetes mellitus. (From Dagogo-Jack S, Rattarasarn C, Cryer PE. Reversal of hypoglycemia unawareness, but not defective glucose counterregulation, in IDDM. *Diabetes*. 1994;43:1426–1434, used with permission of the American Diabetes Association.)

There are three recognized causes of a reversibly attenuated sympathoadrenal response to hypoglycemia and, therefore, three forms of HAAF.[1,2] Antecedent hypoglycemia–related HAAF led to the concept.[72,80] Exercise-related HAAF[73] is exemplified by late postexercise hypoglycemia, which typically occurs 6 to 15 hours after strenuous exercise and is often nocturnal.[89,90] Sleep-related HAAF[74,75] is the result of further attenuation of the sympathoadrenal response to hypoglycemia during sleep. Sleeping patients with T1DM are much less likely to be awakened by hypoglycemia than nondiabetic individuals,[75] probably because of their attenuated sympathoadrenal responses. There may well be additional, unrecognized causes of HAAF, and there may also be a structural (neuropathic) component.[1,2]

The integrated pathophysiologic mechanisms of HAAF are illustrated in Fig. 38.11. Loss of insulin and glucagon responses to falling plasma glucose concentrations caused by therapeutic hyperinsulinemia is the result of beta-cell failure in T1DM and advanced T2DM.[1,2,67] Neither can be attributed to loss of islet autonomic innervation, because low glucose concentrations decrease insulin secretion and increase glucagon secretion in patients with a transplanted (i.e., denervated) pancreas,[91] in dogs with a denervated pancreas,[92] and in isolated perfused pancreas and perifused islets. The mechanism of the attenuated sympathoadrenal response is



• **Fig. 38.11** Schematic representation of the integrated mechanisms of hypoglycemia-associated autonomic failure (HAAF) in diabetes. Compare with Fig. 38.5; see the text for discussion. *α-cells,* pancreatic islet alpha cells; *β-cells,* pancreatic islet beta cells. (From Cryer PE. The barrier of hypoglycemia in diabetes. Diabetes. 2008;57:3169–3176 and Cryer PE. *Hypoglycemia in Diabetes: Pathophysiology, Prevalence and Prevention*. Alexandria, VA: American Diabetes Association; 2009, used with permission of the American Diabetes Association.)

not known, but it must involve the brain or the afferent or efferent components of the sympathoadrenal system.[67] The proposed mechanisms include the systemic mediator, brain fuel transport, brain metabolism, and cerebral network hypotheses.[93–97] There is considerable evidence against the first two of these proposed mechanisms.[67] Among many potential mechanisms, an effect of lactate on brain metabolism has been studied[98–100] and includes evidence of reduced glutamate release within the ventromedial nucleus of the rat hypothalamus.[101]

Much of the fundamental research into the mechanism of HAAF has focused on the hypothalamus, the central integrator of the sympathoadrenal response to hypoglycemia. It is conceivable, however, that the changes in the hypothalamic/sympathoadrenal response reflect modulation by higher brain centers.[67,95–97] For example, measurements of regional cerebral blood flow using oxygen-15–labeled water and positron emission tomography indicate that hypoglycemia increases synaptic activity in widespread but interconnected brain regions in humans[95] and that recent antecedent hypoglycemia both reduces sympathoadrenal and symptomatic responses (a model of HAAF) and increases synaptic activation in the dorsal midline thalamus (and only in that brain region) during subsequent hypoglycemia.[96,97] Therefore, it has been suggested that there may be a cerebral network that results in thalamic inhibition of hypothalamic activity in HAAF.[96,97] Such a putative cerebral network is included in Fig. 38.11, although it remains theoretical.

Risk Factors for Hypoglycemia in Diabetes

The risk factors for iatrogenic hypoglycemia[102] (Table 38.6) follow directly from the pathophysiology of glucose counterregulation in diabetes and are based on the tenet that iatrogenic hypoglycemia is typically the result of the interplay of relative or absolute therapeutic insulin excess and compromised physiologic and behavioral defenses against falling plasma glucose concentrations (i.e., HAAF) in T1DM and advanced T2DM, as discussed earlier.

TABLE 38.6 Risk Factors for Hypoglycemia in Diabetes

Conventional Risk Factors: Absolute or Relative Insulin Excess

- Insulin or insulin secretagogue doses are excessive, ill timed, or of the wrong type
- Exogenous glucose delivery is decreased (e.g., after missed meals, during the overnight fast)
- Glucose utilization is increased (e.g., during exercise)
- Endogenous glucose production is decreased (e.g., after alcohol ingestion)
- Sensitivity to insulin is increased (e.g., after weight loss, with improved fitness or improved glycemic control, in the middle of the night)
- Insulin clearance is decreased (e.g., with renal failure)

Risk Factors for Hypoglycemia-Associated Autonomic Failure

- Absolute endogenous insulin deficiency
- A history of severe hypoglycemia, impaired awareness of hypoglycemia, or both, and recent antecedent hypoglycemia, prior exercise, or sleep
- Aggressive glycemic therapy per se (lower HbA_{1c} levels, lower glycemic goals, or both)

HbA_{1c}, Glycosylated hemoglobin.

Absolute or Relative Insulin Excess

The conventional risk factors for hypoglycemia in diabetes[1,102] (see Table 38.6) are based on the premise that absolute or relative insulin excess is the sole determinant of risk. Absolute therapeutic insulin excess occurs when doses of insulin or insulin secretagogue are excessive, ill timed, or of the wrong type, or when the clearance

of insulin is decreased (as in renal failure). Relative insulin excess occurs when exogenous glucose delivery is reduced, glucose utilization is increased, endogenous glucose production is decreased, or sensitivity to insulin is increased. Persons with diabetes and their caregivers must consider these risk factors, including each of the examples listed in Table 38.6, when iatrogenic hypoglycemia is recognized to be a problem. However, these factors explain only a minority of episodes.[103]

Clinical risk factors for hypoglycemia include the presence of HAAF (including both defective glucose counterregulation and impaired awareness of hypoglycemia), a history of severe hypoglycemia, chronic kidney disease, a long duration of diabetes, and malnutrition.[1]

Risk Factors for HAAF

The risk factors for HAAF[102,104–109] (see Table 38-6) include the following:

1. Absolute endogenous insulin deficiency[1,102,104–108]
2. A history of severe iatrogenic hypoglycemia, impaired awareness of hypoglycemia, or both, and recent antecedent hypoglycemia, prior exercise, or sleep[102,105–107]
3. Aggressive glycemic therapy per se (i.e., lower HbA_{1c} levels, lower glycemic goals, or both).[102,105–109]

As discussed earlier, the degree of beta-cell failure determines the extent to which insulin levels will not decrease and glucagon levels will not increase as glucose levels fall in response to therapeutic hyperinsulinemia. The importance of loss of a decrease in insulin as glucose levels decline in response to therapeutic hyperinsulinemia is underscored by the fact that patients with T1DM and some degree of preserved insulin secretion have a lower incidence of hypoglycemia.[110] A history of severe hypoglycemia indicates (and that of impaired awareness of hypoglycemia implies) recent antecedent hypoglycemia. Like prior exercise or sleep, recent antecedent hypoglycemia causes attenuated sympathoadrenal and symptomatic responses to subsequent hypoglycemia, the key feature of HAAF. Aggressive glycemic therapy is a risk factor for hypoglycemia. That does not mean, however, that one cannot both improve glycemic control and minimize the risk of hypoglycemia in many patients[102] (see later discussion).

Prevention of Hypoglycemia in Diabetes

Hypoglycemia risk factor reduction[102,111] (Table 38.7) is an empiric approach to minimizing the risk of iatrogenic hypoglycemia while maintaining or improving glycemic control in persons with diabetes. It involves four steps: acknowledge the problem, apply the principles of aggressive glycemic therapy,[102] consider the conventional risk factors, and consider the risk factors for HAAF (see Table 38.6).

TABLE 38.7 Hypoglycemia Risk Factor Reduction[a]

1. Acknowledge the problem.
2. Apply the principles of aggressive glycemic therapy:
   - Diabetes self-management (patient education and empowerment)
   - Frequent self-monitoring of blood glucose (and in some instances, continuous glucose sensing)
   - Flexible and appropriate insulin (and other drug) regimens
   - Individualized glycemic goals
   - Ongoing professional guidance and support
3. Consider the conventional risk factors for hypoglycemia.[a]
4. Consider the risk factors for HAAF.[a]

[a]See Table 38.6.

HAAF, Hypoglycemia-associated autonomic failure in diabetes.

Acknowledge the Problem

The problem of hypoglycemia should be addressed, at least in persons treated with an insulin secretagogue or with insulin, in every patient contact. Acknowledging the issue allows the caregiver to move on if hypoglycemia is not a problem, or to deal with it and keep it in perspective if hypoglycemia is a problem. Some patients are reluctant to raise the issue, but their concerns about the reality, or even the possibility, of hypoglycemia can be a barrier to glycemic control.[112–114] If possible, close associates of the patient should be asked whether they have observed clues to episodes not recognized by the patient. Even if no concerns are expressed, review of the record of self-monitoring of blood glucose (or CGM data) may disclose that hypoglycemia is a problem.

Apply the Principles of Aggressive Glycemic Therapy

These principles[111] include diabetes self-management by a well-informed patient, frequent self-monitoring of blood glucose levels (and in some instances CGM), flexible and appropriate insulin and other drug regimens, individualized glycemic goals, and ongoing professional guidance and support (see Table 38.7).

As the therapeutic regimen becomes progressively more complex—early in T1DM and later in T2DM—successful glycemic management becomes progressively more dependent on the many decisions and the skills of a well-informed person with diabetes. Therefore, patient education and empowerment are fundamentally important. Patients treated with insulin secretagogues or insulin need to know the common symptoms of hypoglycemia, their individual most meaningful symptoms, and how to treat (and not overtreat) an episode. They need to know the relevant conventional risk factors for hypoglycemia, including the temporal patterns of the glucose-lowering actions of their individual secretagogue or insulin preparations and the effects of missed meals, the overnight fast, exercise, and alcohol ingestion. They also need to know that increasing episodes of hypoglycemia signal an increased likelihood of future, often more severe, hypoglycemia.[105–107] Close associates also need to know how to recognize hypoglycemia, to understand why a neuroglycopenic patient may become uncooperative, and to know when and how to administer glucagon. Finally, patients need to learn to apply the data from their self-monitoring of blood glucose concentrations (or from CGM) toward the goal of minimizing hypoglycemia (as well as hyperglycemia). Structured education focused on avoidance of hypoglycemia can restore awareness of hypoglycemia and reduce the incidence of severe hypoglycemia without deterioration of glycemic control.[115–117]

Frequent self-monitoring also becomes vital to diabetes self-management as the therapeutic regimen becomes more complex, early in T1DM and later in T2DM. Ideally, patients should monitor their glucose level whenever they suspect hypoglycemia. That would confirm or refute an episode and help the patients learn their individual key symptoms and might lead to regimen adjustments. It is important for patients with impaired awareness of hypoglycemia to monitor glucose levels before performing critical tasks such as driving. Unfortunately, conventional blood glucose monitoring, in which a measurement is obtained at one point in time, does not indicate whether the glucose level is falling, stable, or rising. That shortcoming is being addressed by evolving technologies for CGM.[118,119]

Despite considerable enthusiasm for continuous subcutaneous insulin infusion (CSII) over multiple daily injections (MDI) of insulin,[120] one meta-analysis[121] disclosed no significant difference in minor or severe hypoglycemia events with CSII or MDI, and a randomized trial[122] that specifically included structured patient education in both groups revealed no difference in severe hypoglycemia with CSII or MDI.

There is convincing evidence that insulin analogues reduce the incidence of severe hypoglycemia in patients with diabetes who are at high risk for hypoglycemia[123] that includes both rapid-acting prandial insulins (lispro, aspart) and the long-acting basal insulins degludec[124] and glargine U-300,[125] both compared with glargine U-100 once daily.

There is increasing evidence that hybrid closed-loop insulin replacement systems, specifically those that temporarily suspend insulin infusion when a low glucose concentration is predicted, reduce hypoglycemia in T1DM.[126] Pancreatic islet transplantation prevents hypoglycemia, as long as the transplant is functional, and improves glucose counterregulation.[127]

Therapeutic hyperinsulinemia, a prerequisite for iatrogenic hypoglycemia, can occur during treatment with an insulin secretagogue (e.g., a sulfonylurea or a glinide) or with insulin. Early in its course, T2DM may respond to drugs that do not raise insulin levels at normal or low plasma glucose concentrations and therefore should not, and probably do not,[1,128] cause hypoglycemia. Such drugs include the biguanide metformin, sodium-glucose cotransporter 2 inhibitors, thiazolidinediones, α-glucosidase inhibitors, glucagon-like peptide 1 (GLP1) receptor agonists, and dipeptidyl peptidase 4 inhibitors. All of these drugs require endogenous insulin secretion to lower plasma glucose concentrations, and insulin secretion declines appropriately as glucose levels fall into the normal range. That is true even for the GLP1 receptor agonists and dipeptidyl peptidase 4 inhibitors, which enhance glucose-stimulated insulin secretion (among other actions). Nonetheless, all six categories of drugs increase the risk of hypoglycemia if used with an insulin secretagogue or insulin.

Among the commonly prescribed sulfonylureas, hypoglycemia is more often associated with the longer-acting glyburide (glibenclamide) than with the shorter-acting glimepiride.[129] Despite some advantages,[123–125] insulin analogues can cause hypoglycemia.[130,131]

As reviewed in detail elsewhere,[132] there is compelling evidence from randomized controlled trials that tight glycemic control partially prevents or delays microvascular complications (retinopathy, nephropathy, neuropathy) in T1DM and T2DM. As reviewed there and developed earlier in this chapter, there is also compelling evidence that tight glycemic control increases hypoglycemic morbidity and mortality risks. Thus, it follows that the selection of a glycemic goal should be linked to the risk of hypoglycemia.[132]

Given the premise that glycemic goals in diabetes are a trade-off between glycemic control and iatrogenic hypoglycemia,[132] it has been suggested that a reasonable individualized glycemic goal is the lowest HbA_{1c} that does not cause severe hypoglycemia and preserves awareness of hypoglycemia, preferably with little or no symptomatic or even asymptomatic hypoglycemia, at a given stage in the evolution of the individual's diabetes. Parenthetically, the substantial relationship between a lower HbA_{1c} level and a higher incidence of severe hypoglycemia has been consistently documented in randomized control clinical trials in both T1DM[28,133] and T2DM.[13,43,52,134,135] In these trials, when patients with diabetes were randomly assigned to intensive glycemic therapy, largely with insulin, and shown to have lower HbA_{1c} levels or to more conventional glycemic goals and shown to have higher HbA_{1c} levels, the incidence of severe hypoglycemia was two- to threefold higher in each of the groups with the lower HbA_{1c} levels. The frequency of hypoglycemia was inversely related to the HbA_{1c} level in both the original DCCT and in the follow-up Epidemiology of Diabetes Intervention and Complications (EDIC) phase,[28,133] although the slope was less steep in the EDIC phase. The extent to which the latter is the result of insulin analogues, improved insulin delivery, glucose monitoring, patient education, patient or caregiver skill, or some other fact is not known.

There is increasing evidence that hypoglycemia is proarrhythmogenic.[136–138] Holter monitoring during CGM detected episodes of iatrogenic hypoglycemia has detected runs of cardiac arrhythmias ranging from ventricular tachycardia[136] to brachycardia,[137] and repolarization abnormalities have been identified in persons with diabetes.[138] Thus, fatal cardiac arrhythmias almost assuredly underlie hypoglycemic cardiovascular mortality in diabetes.

In an extensive review, with the exception of a 15% reduction of nonfatal myocardial infarction, Rodriguez-Gutierrez and Montori[139] found no significant impact of tight glycemic control of T2DM on outcomes important to patients—end stage renal disease/dialysis, renal death, blindness, clinical neuropathy, cardiovascular or all-cause mortality, stroke, amputation, or peripheral vascular disease. They did find a two- to threefold increase in severe hypoglycemia during intensive therapy. The authors concluded that the overwhelming consensus in favor of tight glycemic control to prevent complications needs to be recalibrated. There is evidence of an association between mortality and substantially elevated HbA_{1c} levels in T1DM.[140,141] In a 27-year follow-up of DCCT patients, a rise in mortality above that of the US general population began only with a HbA_{1c} level greater than 9% (75 mmol/mol).[140] Analysis of a much larger dataset[141] disclosed the same finding. Notably, 8% of all of the deaths of DCCT/EDIC patients attributed to hypoglycemia and hypoglycemia with seizure, coma, or both were associated with mortality.[52] At 30 years of follow-up, previous intensive glycemic therapy (i.e., that during the DCCT) did not reduce major cardiovascular events (nonfatal myocardial infarctions, stroke, or cardiovascular death) significantly, although the trend was in that direction.[142] Given these data,[52,139–142] it would seem that the overwhelming consensus in favor of tight glycemic control for the prevention of macrovascular complications,[143] like that for neurovascular complications,[139] is stronger than the evidence to support it. However, these trials antedated T2DM drugs that do not cause hypoglycemia, such as GLP1 receptor agonists and sodium-glucose cotransporter 2 inhibitors, and newer technologies, including CGM, which undoubtedly will reduce the risk of intensive glycemic therapy in patients who can use them.

Clearly, one should strive to balance benefits and harms in the selection of a glycemic goal for a person with diabetes. Many persons with diabetes—those with HAAF, a history of severe hypoglycemia, chronic kidney disease, a long duration of diabetes, or malnutrition—are at risk of harm from iatrogenic hypoglycemia. Those with chronic vascular complications, serious comorbidities, or a short life expectancy have little likelihood of benefit. In both of these groups, a less stringent glycemic goal is indicated,[144] perhaps an HbA_{1c} less than 8.5% (69 mmol/mol).[145]

Finally, because the glycemic management of diabetes is empiric, caregivers should work with each patient to find the most effective and safest regimen at a given point in the course of that patient's diabetes.

Consider the Conventional Risk Factors

The conventional risk factors are those that result in relative, as well as absolute, insulin excess (see Table 38.6). In addition to insulin secretagogue doses, timing, and type, they include conditions in which exogenous glucose delivery or endogenous glucose production is decreased, glucose utilization or sensitivity to insulin is increased, or insulin clearance is decreased.

Consider the Risk Factors for HAAF

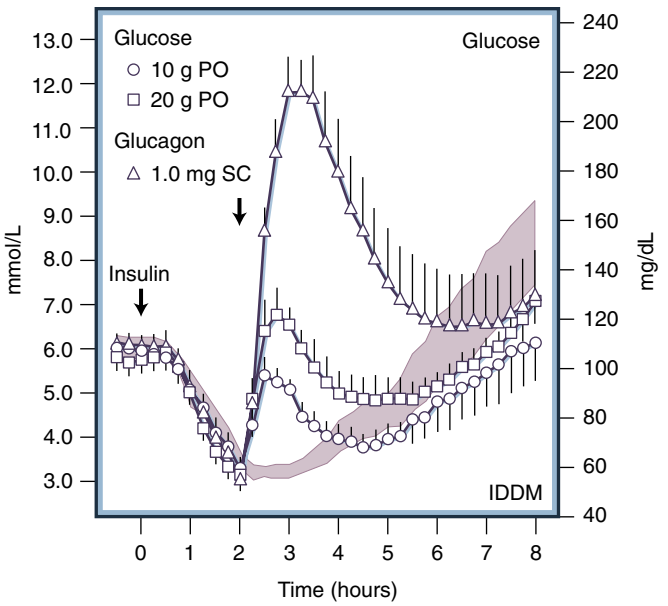
The risk factors indicative of HAAF (see Table 38.6) include the degree of endogenous insulin deficiency; a history of severe hypoglycemia, impaired awareness of hypoglycemia, or both, and recent antecedent hypoglycemia, prior exercise, or sleep; and aggressive glycemic therapy per se. An episode of severe hypoglycemia is a clinical red flag. Unless the cause is easily explainable, it should prompt consideration of a fundamental change in the therapeutic regimen. Without such a change, the risk of a subsequent episode of severe hypoglycemia is high.[105–107] In a patient with impaired awareness of hypoglycemia, patient reeducation[115–117] and a 2- to 3-week period of scrupulous avoidance of hypoglycemia is advisable and can be expected to restore awareness.[85–88] This approach usually requires somewhat higher glycemic goals in the short term. A history of late postexercise hypoglycemia, nocturnal hypoglycemia, or both should prompt appropriately timed regimen adjustments to provide more carbohydrate intake, less insulin action, or both.

Treatment of Hypoglycemia in Diabetes

Most episodes of symptomatic hypoglycemia or of asymptomatic hypoglycemia detected by self-monitoring or CGM are effectively self-treated by ingestion of glucose tablets or carbohydrates.[1] A reasonable dose is 20 g of glucose.[1] A meta-analysis indicated that glucose tablets result in a higher rate of relief of symptomatic hypoglycemia than dietary sugar.[146] Clinical improvement should occur in 15 to 20 minutes. The temptation to overtreat is understandable but should be avoided. With ongoing hyperinsulinemia, the glycemic response to oral glucose is transient, typically lasting less than 2 hours (Fig. 38.12). Therefore, ingestion of a snack or meal shortly after the glucose level is raised is usually advisable.

In a patient who is unable or unwilling (because of neuroglycopenia) to take carbohydrate orally, parenteral therapy is necessary. Glucagon, in a dose of 1.0 mg in adults, can be injected subcutaneously or intramuscularly by an associate of the patient. Administration of glucagon can be lifesaving, but it often causes substantial, albeit transient, hyperglycemia (see Fig. 38.12), and it can cause nausea and even vomiting. Smaller doses of glucagon (e.g., 150 µg), repeated if necessary, have been found to be effective without side effects.[147] For the prompt treatment of episodes of severe iatrogenic hypoglycemia and particularly for infusion, with insulin, in a bihormonal artificial pancreas, a glucagon analogue that is stable in solution and that retains the rapid plasma glucose–raising action has been developed. That preparation[147] and a nasal glucagon preparation[148] represent major improvement for the treatment of hypoglycemia with glucagon because they simplify treatment.

Because it acts by stimulating glycogenolysis, glucagon is ineffective in glycogen-depleted individuals (e.g., after a binge of alcohol ingestion). Although it is not an issue in T1DM or advanced T2DM, glucagon stimulates insulin secretion. Indeed, glucagon has been reported to cause hypoglycemia in nondiabetic individuals (see later discussion). Glucagon can be administered intravenously by medical personnel; however, in that setting, intravenous



• **Fig. 38.12** Mean (± standard error) plasma glucose concentrations during hypoglycemia produced by subcutaneous insulin injection in people with type 1 diabetes mellitus in response to 10 g (*circles*) or 20 g (*squares*) of oral (PO) glucose or 1.0 mg of subcutaneous (SC) glucagon (*triangles*) compared with placebo (*shaded area*). *IDDM,* insulin-dependent diabetes mellitus. (From Wiethop BV, Cryer PE. Alanine and terbutaline in treatment of hypoglycemia in IDDM. *Diabetes Care*. 1993;16:1131–1136, used with permission of the American Diabetes Association.)

(IV) glucose (dextrose) is the standard parenteral therapy. A common initial dose is 25 g, but lower doses are generally advisable to avoid posthypoglycemic hyperglycemia.[13]

The glycemic response to IV glucose is, of course, transient in the setting of ongoing hyperinsulinemia. Therefore, IV glucose administration often needs to be followed by glucose infusion and, once it is practical, by carbohydrate feeding.

The duration of an episode of severe hypoglycemia is a function of its cause. An episode caused by a rapid-acting insulin secretagogue or insulin analogue will be relatively brief. That caused by a long-acting insulin secretagogue or insulin analogue can result in prolonged hypoglycemia requiring hospitalization.

Finally, research on the development of glucose-responsive insulin formulations that would not cause hypoglycemia continues.[149]

Hypoglycemia in Persons Without Diabetes

The Decision to Evaluate for Hypoglycemia

Although hypoglycemia is common in persons with diabetes,[1] it is a distinctly uncommon clinical event in persons who do not have diabetes[2,150] because of the effectiveness of the normal physiologic and behavioral defenses against falling plasma glucose concentrations. Therefore, in the absence of diabetes, a thorough evaluation for hypoglycemia is recommended only for patients in whom Whipple triad (see Table 38.1) can be documented. In the absence of such documentation, evaluation for hypoglycemia may expose the patient to unnecessary evaluation, costs, and potential harm without expectation of benefit.[2]

Plasma glucose concentrations used to document Whipple triad must be measured with a reliable laboratory method and not with a blood glucose self-monitor. A reliably measured low plasma glucose concentration obtained in the absence of recognized

symptoms or signs should not be ignored. However, such a finding raises the possibility of "pseudohypoglycemia," an artifact of continued glucose metabolism by the formed elements of the blood after the blood sample is drawn.[2] This may occur if the sample is collected in a container that does not include an inhibitor of glycolysis, such as sodium fluoride or ethylenediaminetetraacetic acid-citrate (EDTA), and separation of the plasma or serum from the formed elements is delayed, particularly in the setting of erythrocytosis, leukocytosis, or thrombocytosis.

Venous sampling is standard in the clinical setting, but it is the arterial plasma glucose concentration that fuels the brain. Arteriovenous plasma glucose concentration differences are negligible in the postabsorptive state, but antecubital venous glucose levels are as much as one third lower than arterial glucose levels when insulin secretion is increased (e.g., after an oral glucose load) stimulating glucose extraction across the forearm.[151] Because of the provision of alternative fuels (specifically ketones) to the brain, plasma glucose concentrations lower than the overnight fasted physiologic range occur in healthy individuals, especially women and children, during extended fasting.[3] Finally, the glycemic thresholds of responses to hypoglycemia shift to lower plasma glucose concentrations in patients with recurrent hypoglycemia.[21,22] For all of these reasons, it is important to document Whipple triad before concluding that a hypoglycemic disorder exists in a person without diabetes.[2] However, a reliably measured, unequivocally normal plasma glucose concentration (e.g., >70 mg/dL [3.9 mmol/L]) during a symptomatic episode provides strong evidence that the symptoms were not the result of hypoglycemia.[2]

Clinical Classification of Hypoglycemic Disorders

The traditional classification of hypoglycemic disorders in nondiabetic persons, as either postabsorptive (fasting) or postprandial (reactive) hypoglycemias, has been supplanted by a clinical categorization. This distinguishes a patient who has a relevant disease or treatment from a patient who is otherwise seemingly well[2,152] (see Table 38.4). The presence of postprandial symptoms without Whipple triad (see Table 38.1), previously called *reactive hypoglycemia*, is now considered a functional disorder in which symptoms are not due to hypoglycemia and for which an oral glucose tolerance test is not indicated.[2]

Ill or Medicated Individual

Drugs are the most common cause of hypoglycemia.[153–159] In addition to insulin secretagogues and insulin (discussed earlier), offending drugs include alcohol, among many others[154–158] (Table 38.8). Drugs, often in the setting of critical illnesses including renal failure, are the most common cause of hypoglycemia in hospitals.[153] Again, insulin or insulin secretagogues are common offending drugs,[153,160] particularly if they are administered when enteral or parenteral nutrition is interrupted.

Ethanol inhibits gluconeogenesis. Clinical alcohol-induced hypoglycemia typically follows a binge of alcohol consumption during which the person eats little food (i.e., in the setting of glycogen depletion).[155] Alcohol-induced hypoglycemia can be fatal, but with restoration of euglycemia and supportive care, recovery is the rule. Ethanol is usually measurable in blood at the time of presentation.

Hypoglycemia sometimes occurs in patients with critical illnesses[2] (see Table 38.4). Hepatogenous hypoglycemia occurs most commonly when destruction of the liver is rapid and massive (e.g., in toxic hepatitis). It is unusual in common forms of cirrhosis or hepatitis, although glucose metabolism is measurably altered in uncomplicated viral hepatitis.[161] Hypoglycemia is also unusual in metastatic liver disease despite extensive hepatic replacement.[162] The pathogenesis of hypoglycemia in some patients with renal failure is unknown and likely multifactorial; it has been attributed to drugs, sepsis, or inanition.[153,163–165] Reduced renal clearance of insulin and reduced renal glucose production might be relevant factors. However, renal transplantation did not correct hypoglycemia in patients with glucose-6-phosphatase deficiency.[166]

The pathogenesis of the hypoglycemia occasionally seen in patients with severe cardiac failure is also not understood. The finding of elevated blood lactate levels associated with hypoglycemia[167] raises the possibility of inhibited gluconeogenesis. Sepsis is a relatively common cause of hypoglycemia.[153,154,168] Increased glucose utilization (by skeletal muscle and by macrophage-rich tissues, e.g., liver, spleen, and lung), which is thought to be cytokine mediated and is matched initially by increased glucose production, characterizes experimental sepsis.[169–171] The later decline in glucose production, which in the setting of persistently high glucose utilization results in hypoglycemia, is not the result of glucose counterregulatory failure; rather, it is caused by decreased responsiveness to appropriate glucose counterregulatory signals (i.e., low insulin and high glucagon and epinephrine levels).[172]

TABLE 38.8 Drugs, Other Than Antihyperglycemic Agents and Alcohol, Reported to Cause Hypoglycemia

Moderate Quality of Evidence

Cibenzoline
Gatifloxacin
Pentamidine
Quinine
Indomethacin
Glucagon (during endoscopy)

Low Quality of Evidence

Chloroquinoxaline sulfonamide
Artesunate/artemisinin/artemether
Insulin-like growth factor type 1
Lithium
Propoxyphene/dextropropoxyphene

Very Low Quality of Evidence

>25 Cases Identified

Angiotensin-converting enzyme inhibitors
Angiotensin receptor antagonists
β-Adrenergic receptor antagonists
Levofloxacin
Mifepristone
Disopyramide
Trimethoprim-sulfamethoxazole
Heparin
6-Mercaptopurine

<25 Cases Identified

See Murad et al.,[157] 2009

From Cryer PE, Axelrod L, Grossman AB, et al. Evaluation and management of adult hypoglycemic disorders: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2009;94:709–728; based on Murad MH, Coto-Yglesias F, Wang AT, et al. Drug-induced hypoglycemia: a systematic review. *J Clin Endocrinol Metab.* 2009;94:741–745, with permission of the Endocrine Society.

Hypoglycemia can be caused by inanition.[173] A plausible suggestion is that glucose becomes the sole oxidative fuel in the setting of total body fat depletion, and the resulting high rates of glucose utilization exceed the capacity to produce glucose because of a limited supply of gluconeogenic precursors (e.g., amino acids). Postabsorptive hypoglycemia (with low circulating alanine concentrations) has been reported in patients with profound muscle atrophy[174,175]; hypoglycemia is presumably the result of substrate limitation of gluconeogenesis in such patients.

With the notable exception of HAAF in patients with T1DM and advanced T2DM (discussed earlier), hormone deficiencies resulting in hypoglycemia are not common. Postabsorptive hypoglycemia, typically after a period of caloric deprivation caused by an intercurrent illness, can occur in patients with deficient secretion of cortisol, growth hormone, or both, particularly infants and young children.[176–178] Glycemic intolerance of fasting is largely corrected by glucocorticoid replacement; growth hormone replacement has a lesser effect.[177,178] Because cortisol normally supports gluconeogenesis by increasing gluconeogenic enzyme activities and mobilizing gluconeogenic precursors,[177,179] the hypoglycemic mechanism is thought to be reduced glucose production in the setting of glycogen deficiency. Nonetheless, most adults with deficiencies of these hormones do not experience hypoglycemia. Indeed, plasma glucose concentrations and rates of endogenous glucose production after overnight fasting have been reported to be indistinguishable from normal values in short-term glucocorticoid-withdrawn patients with hypopituitarism never treated with growth hormone.[180] Hypoglycemia has been reported in adrenocorticotropic hormone–deficient adults when glucose utilization or loss was increased (as during exercise or pregnancy, respectively)[181] or when glucose production is impaired (as after alcohol ingestion).[182]

Non–islet cell tumor hypoglycemia (NICTH) is rare. The tumors are usually, but not invariably, large, clinically apparent, and mesenchymal in origin. NICTH is often the result of overproduction of incompletely processed pro–insulin-like growth factor 2 (pro-IGF2),[183–186] but hypoglycemia attributed to overproduction of insulin-like growth factor 1 (IGF1) has also been reported.[187] The pro-IGF2 binds poorly to its binding proteins and therefore more freely enters tissue spaces, where its insulin-like actions cause hypoglycemia. Concentrations of plasma free IGF2 (or IGF1[187]) are elevated.[184] Because of suppression of growth hormone secretion and the resulting low IGF1 levels, the ratio of plasma IGF2 to IGF1 is elevated in pro-IGF2–mediated hypoglycemia. Plasma total IGF2 levels may be within the normal range, but the ratio of pro-IGF2 to IGF2 may be elevated.[185] Endogenous insulin secretion is suppressed appropriately during hypoglycemia in NICTH. Treatment of the tumor is seldom curative but may alleviate hypoglycemia. Treatment with a glucocorticoid, growth hormone, or both is sometimes effective.

Seemingly Well Individual

In seemingly well individuals with no evidence of drug, critical illness, hormone deficiency, or non–islet cell tumor as a cause of their hypoglycemia, the differential diagnosis narrows to two categories: (1) accidental, surreptitious, or even malicious hypoglycemia and (2) endogenous hyperinsulinism[2,150,152,188–190] (see Table 38.4). Consideration of the former possibility should precede a systematic assessment of the latter. Medical, pharmacy, and hospital errors can result in hypoglycemia and do occur. Surreptitious hypoglycemia[188–192] is more common in people with knowledge of, and access to, glucose-lowering medications. Malicious hypoglycemia[188,189] can be accomplished by administration of an insulin secretagogue or insulin.

Insulinomas (insulin-secreting pancreatic beta-cell tumors) are the prototypical, but not the only, cause of endogenous hyperinsulinemic hypoglycemia.[2,150,193–197] Patients with an insulinoma typically present with a history of episodes of neuroglycopenia occurring in the postabsorptive (fasting) state. However, an appreciable subset of patients (6% in one series[193]) report symptoms exclusively in the postprandial state. Insulinomas are rare; an incidence of 1 in 250,000 patient-years has been reported. Less than 10% of the patients have malignant insulinomas, multiple insulinomas, or multiple endocrine neoplasia type 1 (MEN1) syndrome.[197] Long-term survival is the rule after successful surgical removal of an insulinoma.[197]

Some patients (4% of one series[197]) with fasting endogenous hyperinsulinemic hypoglycemia do not have an insulinoma but have diffuse islet involvement with islet hypertrophy, sometimes with hyperplasia, and enlarged and hyperchromatic beta-cell nuclei. This condition is often termed *nesidioblastosis*, although the histologic finding of islets budding from pancreatic ducts is not invariably present. Such patients are clinically indistinguishable from those with an insulinoma.[198–202] Other patients have noninsulinoma pancreatogenous hypoglycemia syndrome (NIPHS)[202–205] or post–gastric bypass hypoglycemia.[206–216]

NIPHS[202–205] is characterized by spells of neuroglycopenia caused by endogenous hyperinsulinemic hypoglycemia occurring typically, but not invariably, after a meal. NIPHS is less common than insulinoma.[2,150] Because the syndrome is diffuse, anatomic tumor imaging studies are uniformly negative. Given documented postprandial hyperinsulinemic hypoglycemia, documentation of diffuse beta-cell hyperfunction depends on a positive selective arterial calcium stimulation test. The findings from that test can be used to guide partial pancreatectomy if empiric medical therapy (e.g., diet, an α-glucosidase inhibitor, diazoxide, octreotide) fails. In one relatively large surgical series, most patients improved; however, recurrence of symptoms was the rule, and some patients were not helped after partial pancreatectomy.[204]

Some patients who have undergone Roux-en-Y gastric bypass develop postprandial endogenous hyperinsulinemic hypoglycemia several months to years after the surgery.[206–214,217,218] Affected patients have accelerated absorption of ingested glucose that triggers a large insulin secretory response that is mediated, at least in part, by a robust increase in GLP1.[209–213] Post–gastric bypass hypoglycemia is rare, reported to occur in 0.2% of operated patients.[214] GLP1 receptor blockade has reported to eliminate hypoglycemia,[215] although this remains experimental. Suggested treatments include a low-carbohydrate diet, acarbose, somatostatin analogues, diazoxide, and possibly feeding into the bypassed stomach.[208]

Autoimmune hypoglycemia caused by an antibody to insulin is rare.[216,219] Affected individuals often have a history of other autoimmune disorders. Hypoglycemia occurs in the late postprandial period as insulin, which is secreted in response to the meal and then bound to the circulating antibody, dissociates from the antibody in an unregulated fashion. A clue to the diagnosis is the finding of very high measured plasma insulin levels during hypoglycemia. The diagnosis is made by the finding of high-titer serum insulin antibodies. There is no consistently effective therapy. A similar disorder has been reported in patients with a high-capacity insulin-binding monoclonal paraprotein.[220]

Accidental or surreptitious ingestion of an insulin secretagogue causes endogenous hyperinsulinemic hypoglycemia indistinguishable from that caused by an insulinoma aside from the presence

of a measurable oral hypoglycemic agent in the circulation at the time of hypoglycemia.[2,150]

Very rare causes of insulin-related hypoglycemia have been linked to a mutation of the insulin receptor,[221] to exercise-induced hyperinsulinemia,[222] or to an agonist antibody to the insulin receptor.[223] In the latter case, endogenous insulin secretion is suppressed appropriately, and inappropriately high insulin levels are thought to result from blockade of receptor-mediated insulin clearance by the antibody. Finally, although seemingly convincing cases of ectopic insulin secretion have been reported (e.g., by Seckl and colleagues[224]), the condition must be extraordinarily rare.

Diagnostic Approach

Patients with a hypoglycemic disorder may present in several different ways, including with a history of symptomatic episodes compatible with hypoglycemia, a serendipitously measured low plasma glucose concentration, or a familial syndrome that includes a hypoglycemic disorder (e.g., MEN1).[2,152,188] A careful history of any spells—including the specific symptoms, their timing in relation to meals, their duration, and any factors that aggravate or alleviate them—is important for the formulation of a diagnostic plan. A history that includes neuroglycopenia is particularly compelling.[2] Again, documentation of Whipple triad establishes that a hypoglycemic disorder exists.[2] The diagnostic strategy recommended in the Endocrine Society clinical practice guideline[2] is described here.

First, review the history, physical findings, and all available laboratory data, seeking clues to specific disorders such as drugs, critical illnesses, hormone deficiencies, or non–islet cell tumors (see Table 38.4), then pursue those.[2] This approach will identify the cause of hypoglycemia in most instances. Again, drugs[2] are, by far, the most common cause of hypoglycemia (see Table 38.8).

If the cause of a hypoglycemic disorder is not evident (i.e., in a seemingly healthy individual), measure plasma glucose, insulin, C-peptide, proinsulin, and β-hydroxybutyrate concentrations and screen for oral hypoglycemic agents during an episode of spontaneous hypoglycemia, and observe the plasma glucose response to IV injection of 1.0 mg of glucagon.[2] In addition, measure insulin antibodies.[2]

Failure of insulin secretion to fall to very low rates as plasma glucose concentrations fall to hypoglycemic levels is the key pathophysiologic feature of endogenous hyperinsulinism. Hypoglycemia is the result of low rates of glucose production rather than high rates of glucose utilization.[225] Plasma insulin, C-peptide, and proinsulin concentrations may not always be high relative to normal values obtained under euglycemic conditions, but they are essentially always inappropriately high relative to the low plasma glucose concentrations.[2,152,188] The traditional critical diagnostic criteria (assuming Whipple triad is documented) are plasma insulin concentrations of 3 μU/mL (18 pmol/L) or higher, plasma C-peptide concentrations of 0.6 ng/mL (0.2 nmol/L) or higher, and plasma proinsulin concentrations of 5.0 pmol/L or higher when plasma glucose concentrations are less than 55 mg/dL (3.0 mmol/L) (Table 38.9). These data, first published in 1995,[152] reassessed in 2009,[193,196] and incorporated into the Endocrine Society clinical practice guideline on adult hypoglycemic disorders,[2] were independently reassessed in 2013.[196] Notably, however, the extent to which the unaffected subjects in the latter study had low plasma glucose concentrations at the time of insulin, C-peptide, and proinsulin sampling is not made clear.[196] The insulin criterion of 18 pmol/L (3 μU/mL) or higher was generally supported: 93% sensitivity with 95% to 100% specificity[193] and 98% sensitivity with 60% specificity.[196] The C-peptide criterion of 0.6 ng/mL (0.2 nmol/L) or higher was not supported: sensitivity at 100% or lower with specificity of 60% to 78%[193] versus sensitivity at

TABLE 38.9 Patterns of Findings During Fasting or After a Mixed Meal in Normal Individuals[a] and in Individuals With Hyperinsulinemic (or IGF-Mediated) Hypoglycemia or Hypoglycemia Caused by Other Mechanisms

Symptoms, Signs, or Both	Glucose (mg/dL)	Insulin (μU/mL)	C-Peptide (nmol/L)	Proinsulin (pmol/L)	β-Hydroxybutyrate (mmol/L)	Glucose Increase After Glucagon (mg/dL)	Circulating Oral Hypoglycemic Agent	Antibody to Insulin	Diagnostic Interpretation
No	<55	<3	<0.2	<5	>2.7	<25	No	No	Normal
Yes	<55	≫3	<0.2	<5	≤2.7	>25	No	Neg (Pos)	Exogenous insulin
Yes	<55	≥3	≥0.2	≥5	≤2.7	>25	No	Neg	Insulinoma, NIPHS, PGBH
Yes	<55	≥3	≥0.2	≥5	≤2.7	>25	Yes	Neg	Oral hypoglycemic agent
Yes	<55	≫3	≫0.2[b]	≫5[b]	≤2.7	>25	No	Pos	Insulin autoimmune
Yes	<55	<3	<0.2	<5	≤2.7	>25	No	Neg	IGF[c]
Yes	<55	<3	<0.2	<5	>2.7	<25	No	Neg	Not insulin- or IGF mediated

[a]Normal individuals are those with no symptoms or signs despite relatively low plasma glucose concentrations (i.e., those in whom Whipple triad is not documented).

[b]Concentrations of free C-peptide and proinsulin are low.

[c]Increased pro-IGF2, free IGF2, and IGF2/IGF1 ratio.

IGF, Insulin-like growth factor; *NIPHS,* noninsulinoma pancreatogenous hypoglycemia; *PGBH,* post–gastric bypass hypoglycemia.

From Cryer PE, Axelrod L, Grossman AB, et al. Evaluation and management of adult hypoglycemic disorders: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2009;94:709–728, used with permission of the Endocrine Society. Data from Service[152] and Placzkowski and associates.[193] See discussion of Guettier and associates[196] for independent data.

100% but specificity only 10%[196]; raising the criterion to 2.3 ng/mL (0.8 nmol/L) or higher lowered sensitivity to 84% but raised specificity to 76%.[196] The proinsulin criterion of 5 pmol/L or higher provided 100% sensitivity in both series but with specificity of 68% to 78%[193] and 41%[196]; raising the criterion to 27 pmol/L or higher still provided 100% sensitivity but with 100% specificity.[196] Plasma β-hydroxybutyrate concentrations of 2.7 mmol/L or lower and an increase in the plasma glucose concentration of more than 25 mg/dL (1.4 mmol/L) over the low value over 30 minutes after glucagon injection provide evidence of biologic actions of inappropriately high insulin (or IGF) levels, with suppression of lipolysis and ketogenesis and preservation of hepatic glycogen stores, respectively. The patterns of findings in patients with a hypoglycemic disorder and in those with hyperinsulinemic (or IGF-mediated) hypoglycemia are summarized in Table 38.9. Occasionally, a patient with an insulinoma may not fulfill these criteria even during a 72-hour fast,[2,226] and a few have plasma insulin levels lower than 3 μU/mL (18 pmol/L) during hypoglycemia; however, at least in some series,[227] plasma C-peptide and proinsulin levels are elevated in such patients.

If Whipple triad has not been documented and the measurements described have not been obtained during an episode of spontaneous hypoglycemia, an attempt should be made to re-create the circumstances in which symptomatic hypoglycemia is likely to occur.[2] This can be accomplished by withholding food in a patient with a history suggestive of fasting hypoglycemia or by providing a mixed meal likely to cause a symptomatic episode in a patient with a history suggestive of postprandial hypoglycemia. Failing these relatively informal procedures, a patient with a history suggestive of fasting hypoglycemia should undergo a prolonged supervised fast.[2,226] The fast should be continued until Whipple triad is documented (or until a plasma glucose concentration of less than 55 mg/dL [<3.0 mmol/L] is reached if Whipple triad was unequivocally documented previously[226]) unless a progressive increase in plasma β-hydroxybutyrate levels signals a negative fast. Serial plasma glucose concentrations should be measured with a precise method, not with a point-of-care glucose monitor. About two thirds of patients with an insulinoma meet the diagnostic criteria during a fast of less than 24 hours; most, but not all, do so in less than 48 hours.[226] Therefore, the diagnostic fast can be initiated, and often completed, in the outpatient setting and continued in the inpatient setting if necessary. However, a patient with a history suggestive of postprandial hypoglycemia should undergo a mixed meal test conducted over 5 hours. Standards for interpretation of the findings of the mixed meal test have not been established; current usage[2] is to apply the criteria developed under fasting conditions.[152] Detailed suggestions for performance of a prolonged supervised fast and of a mixed meal test have been published.[2]

An insulinoma may well be present in a patient with documented Whipple triad; inappropriately high levels of insulin, C-peptide, and proinsulin along with no detectable circulating oral hypoglycemic agent; suppressed β-hydroxybutyrate levels; a brisk glycemic response to IV glucagon during fasting (or even postprandial[193]) hypoglycemia; and no circulating insulin antibody. However, as noted earlier, there are other causes of hyperinsulinemic hypoglycemia (see Tables 38.4 and 38.9). Therefore, the next step is to attempt to localize an insulinoma.[2]

Computed tomography, magnetic resonance imaging, and transabdominal ultrasonography detect approximately 75% of insulinomas.[193,228,229] They also detect metastases in the minority of patients who have a malignant insulinoma. Older somatostatin receptor scintigraphy is somewhat less sensitive.[230] Endoscopic pancreatic ultrasonography, with the option of fine-needle aspiration of a detected tumor, has a sensitivity greater than 90%.[230,231] With the combination of noninvasive imaging and, if necessary, endoscopic pancreatic ultrasonography, preoperative localization of insulinomas has become the rule.[193] Given the promise of positron emission tomography with radiotracers such as ^{68}Gallium DOTA-(Tyr3)-octreotate,[232] noninvasive localization of insulinomas may become the preferred approach. If anatomic localization of insulinoma is negative or equivocal, selective pancreatic arterial calcium injections, with an end point of at least a twofold[232,233] increase (or perhaps a greater than fivefold increase with contemporary assays[234]) in hepatic venous insulin levels over baseline, regionalize insulinomas with high sensitivity.[233,235] Although this invasive procedure is seldom necessary in patients with an insulinoma, it is the procedure of choice for confirming NIPHS[216,219] and hypoglycemia occurring after Roux-en-Y gastric bypass[206–214] (Table 38.10). Finally, intraoperative pancreatic ultrasonography almost invariably localizes tumors that are not apparent even to the experienced pancreatic surgeon.

TABLE 38.10 Causes of Hypoglycemia Unique to, or Typically With Onset in, Infancy and Childhood

Intolerance of Fasting

Preterm or small-for-gestational-age infants
Hypopituitarism, adrenal hypoplasia, congenital adrenal hyperplasia
Ketotic hypoglycemia of childhood

Hyperinsulinism

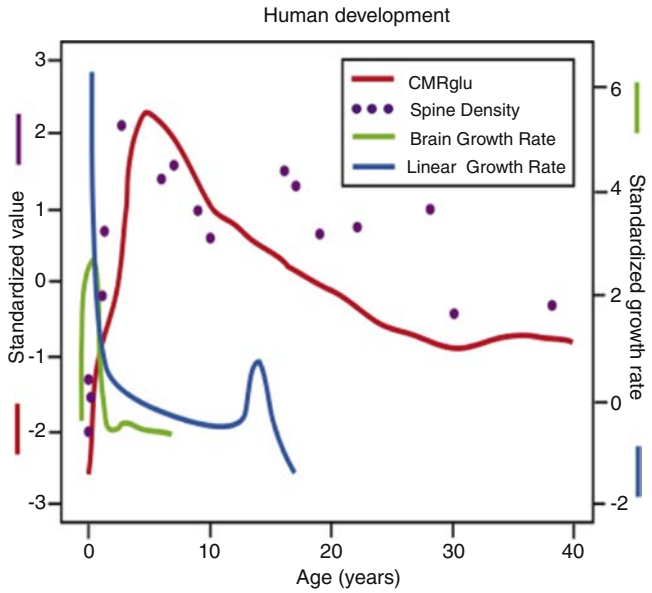
Infant of a diabetic mother
Maternal drugs (sulfonylurea, β_2-adrenergic agonist)
Congenital hyperinsulinism
Perinatal stress, small for gestational age
Others: Rh incompatibility, Beckwith-Wiedemann syndrome, exchange transfusions, perinatal stress, insulin-secreting tumors of pancreatic islet cells (insulinomas)

Inborn Errors of Metabolism (Enzyme Defects)

Carbohydrate metabolism: Glycogen storage disease types I, III, and VI; glycogen synthase deficiency; fructose-1,6-bisphosphatase deficiency; fructose-1-phosphate aldolase deficiency; galactose-1-phosphate uridyltransferase deficiency
Protein metabolism: Branched-chain α-keto acid dehydrogenase complex deficiency
Fat metabolism: Fatty acid oxidation defects including deficiencies in the carnitine cycle, the β-oxidation spiral, the electron transport system, and the ketogenesis sequence

Treatment of Hypoglycemia Disorders

Prevention of ongoing or recurrent hypoglycemia requires treatment that corrects or circumvents the hypoglycemic mechanism.[2] Obviously, treatment should be tailored to the specific hypoglycemic disorder identified. Offending drugs can be discontinued or their dosage reduced. Critical illnesses can often be treated. Deficient hormones, such as cortisol, can be replaced. Reduction of non–islet cell tumor mass with surgery, irradiation, or chemotherapy may alleviate hypoglycemia even if the tumor cannot be cured. Treatment with a glucocorticoid, growth hormone, or even octreotide may alleviate hypoglycemia in such patients. Surgical resection of a benign insulinoma typically is curative.



• **Fig. 38.13** Brain (*green*) and linear (*blue*) growth rates, dendritic spine density (*purple*), and cerebral metabolic rate of glucose (*red*) values across the life span during human development. *CMRglu*, cerebral metabolic rate of glucose. (Data from Goyal MS, Venkatesh S, Milbrandt J, et al. Feeding the brain and nurturing the mind: linking nutrition and the gut microbiota to brain development. *Proc Natl Acad Sci U S A.* 2015;112[46]:14105–14112.)

In unresectable disease, empirical treatments (diet, diazoxide, octreotide) can be tried; there has been progress with chemotherapy (e.g., everolimus).[236] Diet, including frequent feedings, an α-glucosidase inhibitor, diazoxide, or octreotide, can be tried in patients with NIPHS or post–gastric bypass hypoglycemia. Treatment of autoimmune hypoglycemia (with a glucocorticoid or another immunosuppressive medication) is problematic, but the disorder is sometimes self-limited. Failing these treatments, frequent feedings during the day and bedtime administration of large doses of uncooked cornstarch or even overnight intragastric glucose infusion may be necessary. Interestingly, a missense MAFA mutation leading to familial insulinomas or diabetes mellitus has been identified in two families.[237]

Hypoglycemia in Infancy and Childhood

The fetus receives a continuous supply of glucose from the maternal circulation across the placenta to meet a substantial proportion of its energy needs, and its glucose levels are mostly reflective of the maternal glycemic level. Hepatic glucose production and gluconeogenesis are absent during fetal life, due to the absence or very low activity of pyruvate carboxylase, phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and fructose 1,6 diphosphatase.[238] These rate-limiting enzymes of gluconeogenesis rapidly increase within the first few hours of life. Even though the enzymes needed for glycogen synthesis and glycogenolysis are present in the fetal liver, hepatic glycogen stores do not increase much until the third trimester, accounting for about 5% of the total liver weight at birth.[239]

Plasma glucose concentrations decline after birth as placental blood flow is interrupted, and they reach their nadir usually in the first 2 hours of life.[240] This is accompanied by a decrease in insulin and activation of glucose counterregulatory systems that favors not only mobilization of glycogen stores and gluconeogenesis but also lipolysis and eventually ketogenesis in the neonate. Because mobilizable glycogen stores are limited and quickly depleted, and feeding is intermittent, the newborn is forced to increase its endogenous glucose production and thus become largely dependent on gluconeogenesis initially. In most infants, the plasma glucose concentration is usually steady or is increasing by 4 to 6 hours of life. As feedings are spaced and the fasting period is prolonged, lipolysis and ketogenesis increase and ketones become a significant fuel source, particularly for the brain.

The brain relies almost exclusively on a constant supply of glucose to meet its metabolic demands and fuel brain growth and key maturational changes. The brain cannot do gluconeogenesis or use free fatty acids, because they are not transported across the blood-brain barrier. However, under special circumstances, it can use ketone bodies (beta-hydroxybutyric acid and acetoacetic acid) or lactate as transient surrogate fuels if levels rise significantly enough. In the first years of life, the human brain accounts for nearly 50% of the body's glucose consumption due to the proportionally larger brain to body mass ratio,[238,241] with the peak of cerebral glucose consumption occurring during middle childhood (~5–10 years of age).[242] Likewise, this metabolic peak is mirrored by increases in synaptic density and synaptic pruning, which occurs well beyond the periods of greatest linear brain growth and differs across brain regions[243] (Fig. 38.13). The rate of total-body and brain glucose metabolism then falls to normal adult rates. Overall, the rates of glucose flux (production and utilization) are nearly three times higher in infants and children than in adults. Throughout the lifespan, this glucose flux is finely regulated to maintain euglycemia by insulin, counterregulatory hormones (glucagon, catecholamines, cortisol, and growth hormone), incretins, other gut hormones (ghrelin and leptin), and neuronal inputs.[244] However, transient or persistent hypoglycemia may occur when glucose supplies are low and ketone body production is small or impaired, as in children with hyperinsulinism

or inborn errors of metabolism, or when there is impairment of the glucoregulatory mechanisms.[245,246] Hypoglycemia, if severe, may cause neurologic compromise or death. The extent of brain injury seems to be influenced by multiple factors, such as duration and degree of hypoglycemia.

It is key to recognize that transient low blood glucose concentrations are common in healthy infants in the first 24 to 48 hours of life as the glucose supply changes from a continuous transplacental supply to an intermittent supply from feeds, resulting in a quick depletion of the glycogen stores. Furthermore, newborns in these first hours of life are relatively hyperinsulinemic compared with older children due to a lower glycemic threshold for insulin secretion shortly after birth—55 to 65 mg/dL (3.0–3.6 mmol/L) compared with 80 to 85 mg/dL (4.4–4.7 mmol/L) in older infants, children, and adults.[247] Therefore, if the first feedings after birth are delayed, a considerable amount of normal term newborns cannot maintain a plasma glucose concentration above 40 mg/dL (2.2 mmol/L).[240] But as feeds are established, maintenance of plasma glucose concentrations is no longer primarily dependent on gluconeogenesis, and hypoglycemia becomes less common. Thus, it is important to distinguish this normal physiologic transitional response from disorders that result in persistent or recurrent hypoglycemia beyond the first 3 days of life. Because of potential difficulty in distinguishing a persistent hypoglycemia disorder from transitional neonatal glucose concentrations during the first 48 hours of life, the Pediatric Endocrine Society suggests delaying diagnostic evaluations for hypoglycemia until 2 to 3 days after birth. However, in infants who are at risk for hypoglycemia, glucose screening should be performed after the first feed, which should occur within 1 hour after birth. Plasma glucose concentrations should continue to be measured every 3 to 6 hours before feeds for the first 24 to 48 hours of life, because many at-risk newborns present with their first documented low glucose concentrations during this period.[247]

As in adults,[2] the diagnosis of clinical hypoglycemia should be considered in pediatric patients that have Whipple triad (see Table 38.1). Signs and symptoms of hypoglycemia are quite nonspecific in a neonate or children and may be difficult to recognize. Some of these may include jitteriness/tremors, hypotonia, changes in the level of consciousness, apnea/bradycardia, cyanosis, tachypnea, poor suck or feeding, hypothermia, and/or seizures. In addition, as in adults, it is not possible to state a single low plasma glucose concentration that categorically defines hypoglycemia in infants and children; its precise definition continues to be controversial. This is because (1) normal distributions of glucose values will vary depending on age; (2) glucose values will vary depending on conditions of feeding and fasting; (3) there are potential artifacts that may lead to inaccuracies in glucose determination; (4) the thresholds for specific responses to hypoglycemia can be altered by recent antecedent hypoglycemia; and (5) there is lack of outcome data, as there is not a single glucose value that has been associated with brain injury.[245–247] However, most clinical guidelines postulate arbitrary glucose thresholds to provide guidance on when to initiate a workup to identify children with an underlying disorder and when to initiate an intervention to reduce potential harm from hypoglycemia and minimize possible overtreatment.

The American Academy of Pediatrics in 2011, followed by the Pediatric Endocrine Society in 2015, wrote clinical reports and guidelines on screening and subsequent management of neonatal hypoglycemia.[247,248] Although these guidelines do not coincide on the glycemic thresholds for the diagnosis of hypoglycemia, they both endorse that hypoglycemia persisting beyond 48 hours is not likely to be transitional, and they emphasize the need for early identification of the infant at risk for severe hypoglycemia during the first 48 hours after delivery and for persistent hypoglycemia beyond 48 hours of life to determine the need for screening. These include not only the infant of a diabetic mother or the child with a family history of a genetic form of hypoglycemia or a congenital syndrome associated with hypoglycemia, such as congenital hyperinsulinism, Beckwith-Wiedemann syndrome, or hypopituitarism, but also those with relatively more common perinatal stress hyperinsulinism: birth asphyxia, intrauterine growth restriction, or toxemia, or infants receiving total parenteral nutrition.

The Pediatric Endocrine Society suggests a plasma glucose concentration of 50 mg/dL (2.8 mmol/L) or less as an appropriate threshold to trigger further diagnostic testing in a child less than 48 hours old and 60 mg/dL (3.3 mmol/L) or less after 48 hours of age.[247] Nevertheless, many will criticize that it is a relatively conservative threshold, whereas others will say that it may lead to overtreatment of many neonates, but the neonatal threshold is intended to avoid discharging newborns who may be at risk for recurrent and severe hypoglycemia.[247,249] The diagnostic evaluation for neonatal hypoglycemia should include a plasma confirmation of a low blood glucose value and simultaneous measure of carbon dioxide to determine if there is an associated acidosis, insulin level and ketone bodies to determine if there is evidence of hyperinsulinism and measure of the counterregulatory hormones (cortisol for cortisol deficiency and growth hormone for growth hormone deficiency), free fatty acids to see if there is a defect on fatty acid oxidation, and a lactate level. If outside of the newborn period, or if there are concerns for exogenous hyperinsulinism, C-peptide, which is a by-product of the metabolism of insulin, should also be also measured. In the case of a child with hypoglycemia of unknown cause, a glucagon stimulation test at the time of hypoglycemia can provide very useful diagnostic information about glycogen stores and possible hyperinsulinism. In such a test, 0.03 mg/kg of glucagon is given intravenously or by intramuscular injection when the child is hypoglycemic. Plasma glucose levels are measured prior to the injection and 10, 20, and 30 minutes after. If plasma glucose rises by more than 30 mg/dL (1.7 mmol/L) within the first 30 minutes after glucagon administration, it suggests that the child has hyperinsulinemia, because during hyperinsulinism there is inappropriate buildup of hepatic glycogen stores that are released in response to glucagon administration.

If the patient persists in being hypoglycemic or becomes hypoglycemic after obtaining the critical laboratory samples and doing the glucagon stimulation test, it is key to treat the low plasma glucose to prevent adverse outcomes. If the patient is conscious and able to drink and swallow safely, then he or she can be treated with administration of 10 to 20 g of rapidly absorbed carbohydrates by mouth or by a gastric tube, if available. This process may be repeated after 15 minutes, but if the hypoglycemia does not improve within 30 minutes, parenteral glucose is recommended. In infants and children with altered consciousness, IV dextrose should be given. A slow bolus of 2 mL/kg of 10% dextrose solution can be given, followed by a continuous infusion of dextrose at 6 to 9 mg/kg per minute. If IV access is not readily available and the patient has documented hyperinsulinism, then intramuscular glucagon (0.03 mg/kg up to a maximum of 1 mg) should be given.

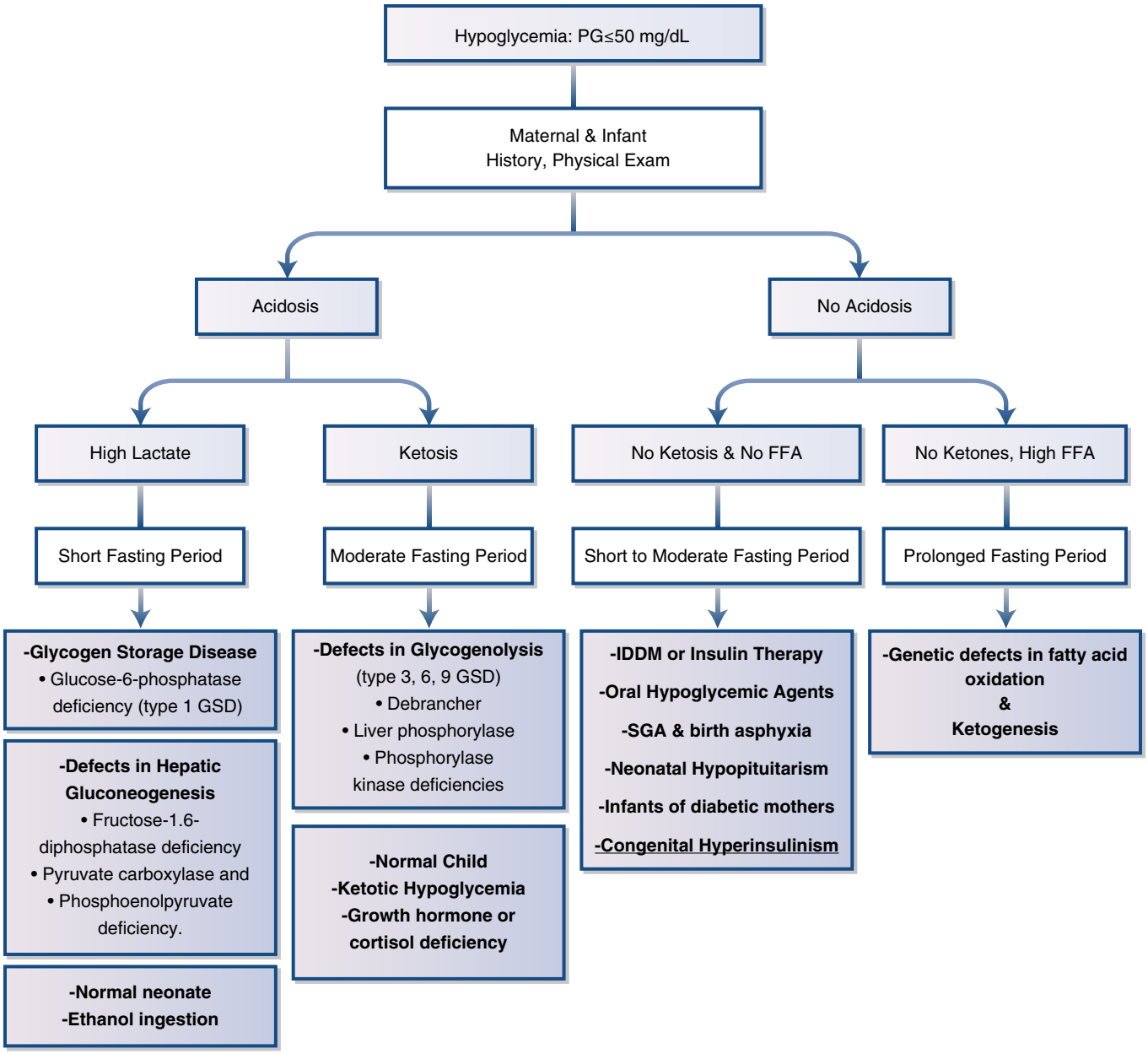
Close monitoring for further evaluation and treatment is needed in children found to have low plasma glucose. In neonates, monitoring should continue until plasma glucose concentrations

can be maintained with regular feedings at above 50 mg/dL (2.8 mmol/L) in newborns less than 48 hours old and above 60 mg/dL (3.3 mmol/L) in newborns more than 48 hours old. However, the mean plasma glucose concentration in the postabsorptive state of children and neonates after 48 hours of age does not differ from that in adults (70–100 mg/dL [3.9–5.5 mmol/L]). Thus, in children with the diagnosis of a disorder causing persistent hypoglycemia or a known risk of a persistent hypoglycemic disorder, the goal of treatment is to maintain a plasma glucose concentration above 70 mg/dL (3.89 mmol/L). Therefore, a safety fast (6-hour fast), in which a term neonate over 3 days of life is asked to skip a single feed, should always be done before discharge to ensure that plasma glucose concentrations can be maintained above that range.

Even though hypoglycemia in children can be caused by the same mechanisms as in adults[2] (see Table 38.4), including drugs and critical illnesses, there are several hypoglycemic disorders unique to infancy or childhood that can be classified as intolerance of fasting, hyperinsulinism, or are due to inborn errors of metabolism[245,246] (see Table 38.10). The presentation of these different conditions may vary, as well as the age of onset and the tolerance of the fasting period (Fig. 38.14). Thus, a good clinical history, physical examination, and critical laboratory samples are key to determine the diagnosis.

Intolerance of Fasting

Infants and toddlers may have a plasma glucose concentration less than 70 mg/dL (3.9 mmol/L) and hyperketonemia after overnight fasting because of limited fasting tolerance.[250–252] It is particularly common in preterm or small-for-gestational-age infants



• **Fig. 38.14** Diagnostic algorithm for determining the etiology of hypoglycemia in children. *FFA,* free fatty acid; *GSD,* glycogen storage disease; *PG,* plasma glucose. (Modified from Sprague JE, Arbeláez AM. Glucose counterregulatory responses to hypoglycemia. *Pediatr Endocrinol Rev.* 2011;9[1]:463–475.)

and is thought to result partially from incomplete development of gluconeogenic mechanisms.[2] When hypoglycemia occurs, glycogenolysis is the first mechanism that occurs, followed by gluconeogenesis, to try to raise systemic blood sugars. However, if fasting is prolonged or feeding is interrupted, as during an intercurrent illness in children who are least tolerant of fasting, there is lack of increased renal gluconeogenesis, and the endogenous substrate availability from muscles or liver is low[253,254] and ketotic hypoglycemia occurs. The syndrome of ketotic hypoglycemia of childhood typically occurs in 2- to 5-year-old children and remits spontaneously before age 10 years, when the ratio of brain to body weight changes. This should be a diagnosis of exclusion after other conditions that can present with ketosis, such as growth hormone deficiency, hypopituitarism, adrenocorticotropic hormone unresponsiveness, and glycogen synthase deficiency, are ruled out. Cortisol and growth hormone deficiency reduce gluconeogenesis and hepatic glucose production. Moreover, there are low plasma concentrations of gluconeogenic substrate, and there is a blunted glucagon response.[255]

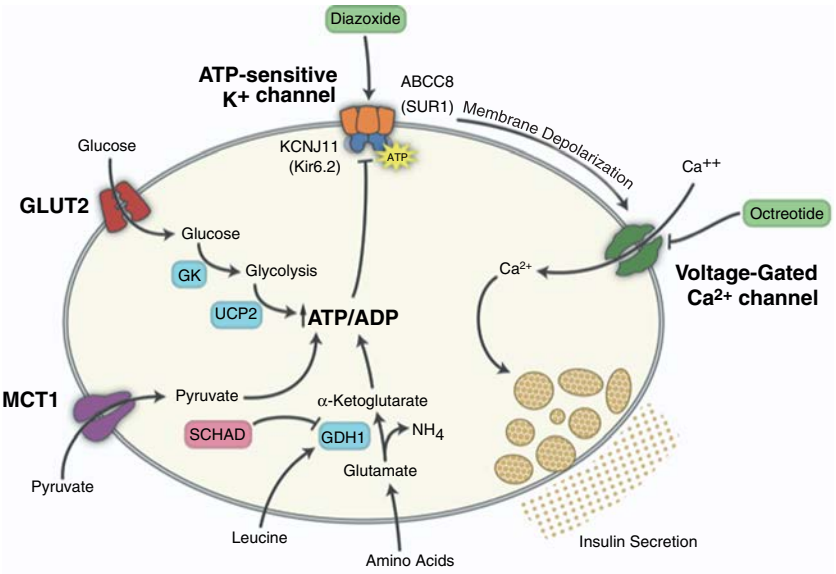
Hyperinsulinism

Hyperinsulinism can be suspected when the plasma insulin concentration is inappropriately normal or elevated for the level of hypoglycemia, and plasma or urine ketones levels, as well as free fatty acids, are low. In addition, this condition should also be suspected when there is a glycemic response to glucagon at the time of hypoglycemia. In the neonatal period, these children are typically large for gestational age, and hypoglycemia occurs after a short to moderate fasting period.

Maternal diabetes is a common cause of neonatal hypoglycemia due to transient hyperinsulinism in the offspring.[246] Infants of diabetic mothers have been hyperglycemic in utero due to the mother's hyperglycemia, which causes chronic stimulation of fetal insulin secretion in utero. Therefore, shortly after birth, there is failure of insulin to fall normally as glucose levels decline and transient neonatal hypoglycemia develops. Transient hyperinsulinemia also causes transient hypoglycemia and can occur due to other conditions listed in Table 38.10. Infants with perinatal stress, prematurity, or small for gestational age can develop transient hypoglycemia secondary to hyperinsulinism, which is usually responsive to diazoxide and resolves by 6 months of age. In children, postprandial hypoglycemia analogous to that occurring after gastric bypass can follow Nissen fundoplication. Insulin-secreting tumors of pancreatic islet cells are rare in children but have been reported in association with MEN1 and occur outside of the neonatal period. As in adults, hyperinsulinemic hypoglycemia can be accidental, surreptitious, or even malicious. When suspecting those cases, a C-peptide test can be very informative.

Congenital hyperinsulinism[256–258] is the most common cause of nontransient neonatal hypoglycemia, although it occurs in only 1 of every 30,000 to 50,000 live births. However, it can be as common as 1 in 2,500 live births in highly consanguineous communities.[259] Hypoglycemia may persist from the neonatal period or become apparent during the first year of life. These conditions usually have a significant associated risk of hypoglycemic seizures and developmental delays. The need for very high glucose infusion rates is a diagnostic clue.

Glucose-stimulated insulin secretion normally involves increased glucose transport into beta cells, glucokinase-mediated phosphorylation of glucose, and glucose metabolism via the glycolytic pathway, resulting in an increase in the ratio of adenosine triphosphate (ATP) to adenosine diphosphate. This leads to closure of membrane ATP-gated potassium (K_{ATP}) channels and their sulfonylurea receptor 1 (SUR1) and potassium inward rectifying channel (Kir6.2) subunits, membrane depolarization, calcium influx, and exocytosis of insulin. Insulin secretion is usually stimulated by glucose oxidation via glucokinase, but it can also occur due to leucine stimulation of glutamate oxidation via glutamate dehydrogenase. Several inherited abnormalities of these different cellular mechanisms (Fig. 38.15) are now known to cause congenital hyperinsulinism and hypoglycemia in neonates and infants.[256–258,260,261]



• **Fig. 38.15** Cellular mechanisms affecting beta-cell insulin secretion in congenital hyperinsulinism and therapeutic targets. Medications used to treat congenital hyperinsulinism are in *green*. *ADP,* adenosine diphosphate; *ATP,* adenosine triphosphate; *GDH1,* glutamate dehydrogenase 1; *GK,* glucokinase; *GLUT2,* glucose transporter 2; *MCT1,* monocarboxylate transporter 1; *SUR1;* sulfonylurea receptor 1; *UCP2,* uncoupling protein 2. (Courtesy Dr. Stephen Stone.)

Congenital hyperinsulinism has been associated with mutations of an increasing array of genes,[256–258,260,261] including SUR1 (encoded by *ABCC8*), Kir6.2 (encoded by *KCNJ11*), glucokinase (GK, encoded by *GCK*), glutamate dehydrogenase (GDH, encoded by *GLUD1*), short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD encoded by *HADH*), ectopic expression on beta-cell plasma membranes of *SLD16A1* (which encodes monocarboxylate transporter 1 [MCT1]), and mutations of *HNF4A* and *HNF1A*. Many patients with hyperinsulinemic hypoglycemia respond to the K_{ATP} channel opener diazoxide[262] and others to octreotide,[263] whereas some do not respond to medical management.

Identification of the genetic mutation in patients with congenital hyperinsulinism is key, as the management and prognosis will vary depending on the gene that is affected. Inactivating SUR1 or Kir6.2 mutations are the most common and most severe causes of congenital hyperinsulinism. They result in reduced K_{ATP} channel activity and, consequently, increased constitutive insulin secretion. Therefore, most affected patients do not respond to treatment with the K_{ATP} channel opener diazoxide, which normally suppresses insulin secretion. In contrast, patients with dominantly inherited mutations do retain responsiveness to diazoxide. Homozygous or compound heterozygous recessive mutations in either the *ABCC8* or *KCNJ11* gene typically result in diffuse congenital hyperinsulinism. However, patients with paternally recessively inherited mutations of the *ABCC8* or *KCNJ11* gene and a specific loss of maternal alleles of the imprinted chromosome region 11p15 develop focal congenital hyperinsulinism, in which only a small portion of the pancreas is affected with somatic loss of heterozygosity and paternal isodisomy of the chromosome 11p15 region that contains the *ABCC8* and *KCNJ11* genes in addition to several imprinted genes that increase beta-cell proliferation.[264] For those who do not have a sustained response to medical therapy (e.g., frequent feedings, diazoxide, octreotide), near-total pancreatectomy is required. Those with a focal lesion do not respond to diazoxide and are usually cured by surgical resection of the lesion. Focal lesions can be detected noninvasively with [^{18}F]-dihydroxyphenylalanine positron emission tomography[265] with a sensitivity of 85%, a specificity of 96%, and a 96% positive predictive value for diagnosis of focal hyperinsulinism.[266] These children should be evaluated by clinicians experienced with congenital hyperinsulinism and the multiple subspecialty services that are necessary for the management of the disorder.[267]

Activating, dominantly inherited mutations of the glutamate dehydrogenase gene cause hyperinsulinemia and hyperammonemia syndrome, the second most common form of congenital hyperinsulinism. Hypoglycemia typically develops after several months of life and is responsive to diazoxide. Activating, dominantly inherited mutations of the glucokinase gene cause varying degrees of hypoglycemia that may respond to diazoxide but may require pancreatectomy. Recessively inherited mutations of the SCHAD gene result in hypoglycemia that is generally responsive to diazoxide. In addition to hyperinsulinemic hypoglycemia, biochemical markers include increased levels of plasma 3-hydroxybutyrylcarnitine and increased urinary 3-hydroxyglutarate. Dominantly inherited mutations of the monocarboxylate transporter 1 gene (*SLC16A1*), resulting in increased pyruvate transport into beta cells and hyperinsulinism, have been associated with exercise-induced hypoglycemia.

Clinical manifestations, including low plasma glucose concentrations and inappropriately high levels of plasma insulin and C-peptide coupled with low plasma β-hydroxybutyrate levels and a brisk glycemic response to administered glucagon—all analogous to hyperinsulinemic hypoglycemia in adults[2]—characterize congenital hyperinsulinism in neonates and infants.[256–258,260,261] As in adults with endogenous hyperinsulinism,[2] plasma insulin concentrations are not invariably 3 μU/mL (18 pmol/L) or higher during hypoglycemia in patients with congenital hyperinsulinism,[257] although hyperinsulinism is often documented by serial insulin measurements and by inclusion of C-peptide measurements during hypoglycemia. Genetic testing for many mutations is available commercially. Finally, an association of hypertrophic cardiomyopathy and congenital hyperinsulinism has been recognized.[268]

Activating mutations in the postreceptor insulin signaling pathway (e.g., in the RAC-beta serine/threonine-protein kinase [*AKT2*] gene[269,270]) results in hypoinsulinemic hypoglycemia that resembles hyperinsulinemic hypoglycemia aside from evidence of reduced insulin secretion.

Given the array of potential causes of hypoglycemia in neonates and infants, the differential diagnosis is facilitated by an array of measurements during hypoglycemia when the precise hypoglycemic mechanism is obscure.[271] In addition to glucose, insulin, C-peptide, and β-hydroxybutyrate levels (and the glycemic response to glucagon), such measurements should include plasma bicarbonate, lactate, nonesterified fatty acid (NEFA), growth hormone, and cortisol levels. When laboratory findings are not consistent with hyperinsulinism, other causes of hypoglycemia should then be explored. In those instances, a plasma acylcarnitine profile and measurement of ammonia and urine organic acids are also needed, but they do not have to be obtained during hypoglycemia.

Inborn Errors of Metabolism (Enzyme Deficiencies)

Hypoglycemia that develops in infancy and persists into adult life with effective therapy can be caused by enzymatic defects in carbohydrate, protein, or fat metabolism[245] (see Table 38.10). Hypoglycemia usually becomes apparent later in infancy as the intervals between feedings become longer.

Abnormalities in the metabolism of carbohydrates are usually due to enzymatic deficiencies in the synthesis or metabolism of glycogen, gluconeogenesis, or metabolism of galactose or fructose. Glycogen storage diseases (GSDs) are due to different enzyme deficiencies that present early in childhood and are usually characterized by hypoglycemia after short fasting periods, that may have mild to moderate ketosis, and may present with or without hepatomegaly and have no response to glucagon stimulation. GSD type 0 is caused by glycogen synthase deficiency, which results from mutations in *GYS2* and does not cause hepatomegaly, but is characterized by preprandial ketotic hypoglycemia and postprandial hyperglycemia and lactic acidemia. GSD type Ia (von Gierke disease) is caused by mutations in *G6PC*, the gene that encodes glucose-6-phosphatase hydrolase activity. It occurs in approximately 1 of every 100,000 live births[272] and accounts for 80% of GSD type I cases. Given that glucose-6-phosphatase is the final enzyme in the hepatic release of glucose from gluconeogenic and glycogenolytic pathways, absence of its activity results in low rates of endogenous glucose production and severe fasting hypoglycemia[272] with no glycemic response to administered glucagon. Clinical findings include failure to thrive, hepatomegaly (due to both glycogen and fat accumulation), hypertriglyceridemia, accelerated lipolysis and ketogenesis, hyperuricemia, platelet dysfunction, and

marked lactic acidosis (from the metabolism of glucose-6-phosphate). With the exception of hepatomegaly, these abnormalities can be reversed by effective prevention of hypoglycemia with frequent feedings during waking hours and continuous intragastric glucose infusion during sleep or bedtime administration of large doses of uncooked cornstarch. Liver transplantation corrects hypoglycemia and the associated metabolic abnormalities. Late complications include progressive renal disease due to glycogen accumulation in the kidneys and hepatic adenomas. GSD type Ib is caused by mutations in *G6PT1*, the glucose-6-phosphate microsomal transporter. Their clinical presentation and biochemical findings are identical to GSD type Ia, but these patients also have chronic or intermittent neutropenia and neutrophil dysfunction, and are susceptible to recurrent infections. The diagnosis of GSD type Ia and type Ib is confirmed by mutation analysis of *G6PC* and *G6PT1*. Hypoglycemia is less prominent in GSD type III (hepatic amylo-1,6-glucosidase deficiency due to mutations in *AGL*), GSD type VI (hepatic glycogen phosphorylase deficiency due to mutations in *PYGL*), and GSD type IX (hepatic phosphorylase kinase deficiency due to mutations in *PHKA2*) because hepatic gluconeogenesis is preserved and these defects in glycogenolysis are rarely complete. These forms of GSD are rare and are treated with avoidance of hypoglycemia by frequent administration of frequent high-carbohydrate feedings and uncooked cornstarch, particularly at bedtime; in GSD type III, a high-protein diet can be of benefit.

Hypoglycemia can also be caused by enzymatic defects in gluconeogenesis including fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxylase deficiencies.[245] Glucose-6-phosphatase deficiency is considered by some as a gluconeogenesis defect; however, it was discussed earlier because it is also involved in glycogenolysis. The disorders of gluconeogenesis are characterized by hypoglycemia after moderate fasting periods that occur when hepatic glycogen stores are depleted. Thus, they present with lactic acidemia, ketosis, hyperlipidemia, and no response to glucagon stimulation, except in fructose-1,6-bisphosphatase deficiency, which may have a response in the fed state. Fructose-1,6, bisphosphatase deficiency presents similar to GSD type I, except hepatic glycogen accumulation does not occur, and hepatomegaly, which is the result of lipid accumulation instead of glycogen, is usually mild and liver function is normal. This condition is usually treated with a high-carbohydrate diet and frequent feeding. PEPCK deficiency and pyruvate carboxylase are quite rare. In patients with a mutation in the glucose transporter 1 (GLUT1) gene, plasma glucose concentrations are normal but brain glucose levels are low, causing neuroglycopenia. Hypoglycemia has been attributed to GLUT2 deficiency in Fanconi-Bickel syndrome. Postprandial, rather than postabsorptive, hypoglycemia occurs in galactosemia and in hereditary fructose intolerance (fructose-1-phosphate aldolase deficiency). Other disorders of gluconeogenesis may be due to lack of some gluconeogenic substrates such as galactose, fructose, or amino acids. In galactosemia, there is a deficiency of galactose-1-phosphate uridyl transferase (GALT), in which infants are unable to metabolize galactose to glucose, causing hepatic accumulation of galactose-1-phosphate, which inhibits enzymes of glycogenolysis. This can be a big issue in very young children considering that galactose is a by-product of lactose hydrolyzation and lactose is the major dietary carbohydrate for infants. These children may present with hypoglycemia, vomiting after ingestion of lactose or galactose, failure to thrive, or sepsis. Hypoglycemia in hereditary fructose intolerance, caused by a deficiency of the enzyme aldolase B, occurs after ingestion of fructose or sucrose (a disaccharide that is hydrolyzed to glucose and fructose). The main goal of long-term treatment of classic galactosemia is to minimize dietary galactose intake, and in patients with hereditary fructose intolerance, complete elimination of fructose and sucrose from the diet is an effective treatment for most people, although this can be challenging. Impaired gluconeogenesis may also occur due to alcohol intoxication and salicylate poisoning.

Deficiencies of enzymes involved in protein metabolism (see Table 38.10) that can cause fasting hypoglycemia include branched-chain ketoaciduria (maple syrup urine disease and tyrosinemia). They present with profound acidosis and failure to thrive. The pathogenesis of hypoglycemia is unclear but includes defective gluconeogenesis due to liver disease.

Several defects that ultimately impair fatty acid oxidation result in hypoglycemia with hypoketonemia during extended fasting.[269] Mitochondrial fatty acid oxidation and ketogenesis require transport of fatty acids across the plasma membrane, formation of fatty acyl-CoA derivatives, and transport of those derivatives into mitochondria. Because the inner mitochondrial membranes are not permeable to long-chain (as opposed to medium-chain and short-chain) fatty acyl-CoA esters, the long-chain fatty acyl-CoA esters are transesterified to fatty acylcarnitines at the outer surface of the membranes (by carnitine palmitoyltransferase 1, CPT1), transported across the membranes (by a translocase), and reconverted to the fatty acyl-CoA esters (by carnitine palmitoyltransferase 2, CPT2) at the inner surface of the membranes. Then, they can be oxidized or converted to ketones. Insulin decreases fat oxidation and ketogenesis by decreasing lipolysis and by increasing lipogenesis and the formation of malonyl CoA, which inhibits CPT1. Conversely, low insulin levels favor fatty acid oxidation and ketogenesis. High glucagon levels do so by decreasing malonyl CoA; catecholamines do so largely by stimulating lipolysis. Any defect in this complex sequence (see Table 38.10) decreases fatty acid oxidation (and ketogenesis) and reciprocally increases glucose oxidation, resulting in hypoketonemic postabsorptive hypoglycemia. Reduced plasma carnitine levels (20–50% of normal) are the rule in these disorders, but extremely low carnitine levels characterize the carnitine transport defect—a true carnitine deficiency state that is responsive to carnitine supplementation.[270] The diagnosis of specific fatty acid oxidation defects is typically accomplished by blood acylcarnitine profiling,[271] although molecular diagnosis is increasingly possible.

There are many fatty acid oxidation disorders that result in hypoketonemic hypoglycemia.[a] The most common is medium-chain acyl-CoA dehydrogenase deficiency. Because affected patients can become symptomatic—fatigue, vomiting, seizure, and coma—before becoming hypoglycemic, a normal acylcarnitine profile should be documented before a diagnostic fast is performed in such infants. Other fatty oxidation disorders include very long-, long-, and short-chain acyl-CoA dehydrogenase defects, as well as defects in electron transfer (glutaric acidemia type 2), 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) synthase deficiency, and HMG-CoA lyase deficiency. Defects in carnitine transport and in the carnitine cycle that cause hypoketonemic hypoglycemia include primary carnitine deficiency due to autosomal recessive mutations in the carnitine transporter (*OCTN2*). Others are CPT1 deficiency, carnitine acylcarnitine translocase (*CACT*) deficiency, and CPT2 deficiency. Treatment includes frequent feedings and a low-fat diet rich in medium-chain triglycerides in CPT1 deficiency[276] and carnitine supplementation in primary carnitine deficiency.

In summary, neonatal hypoglycemia may present with or without acidosis. If acidosis is present, this may be associated with lactic

[a]References 245, 246, 253, 256, 257, 260–263, 265, 268, 273–275.

acidemia or ketosis. If the patient has no acidosis and suppressed levels of NEFAs and β-hydroxybutyrate, it suggests hyperinsulinism; if the patient has high NEFA but low β-hydroxybutyrate, it suggests a defect in fatty acid oxidation or ketogenesis. Hypoglycemia with acidosis and high lactate levels suggests a defect in gluconeogenesis or glucose release. If the patient has high concentrations of both NEFA and β-hydroxybutyrate, it suggests a defect in glucose production or release including deficiency of cortisol, although NEFA and ketone levels need not be elevated in patients with hypopituitarism (see Fig. 38.14).

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This chapter was written after the senior author chaired a panel that developed *Evaluation and Management of Adult Hypoglycemic Disorders*, an Endocrine Society clinical practice guideline,[2] and shortly after publication of the third edition of the author's book, *Hypoglycemia in Diabetes: Pathophysiology, Prevalence and Prevention.*[1] Therefore, much of the conceptual and interpretive content here is the same, as is no small part of the phraseology.

Disclosures

The senior author has served as a consultant to Novo Nordisk A/S in recent years.

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The complete list of references is available online at ExpertConsult.com.

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39

Neuroendocrine Control of Energy Stores

MARTIN G. MYERS, JR., AND DAVID P. OLSON

CHAPTER OUTLINE

KEY POINTS

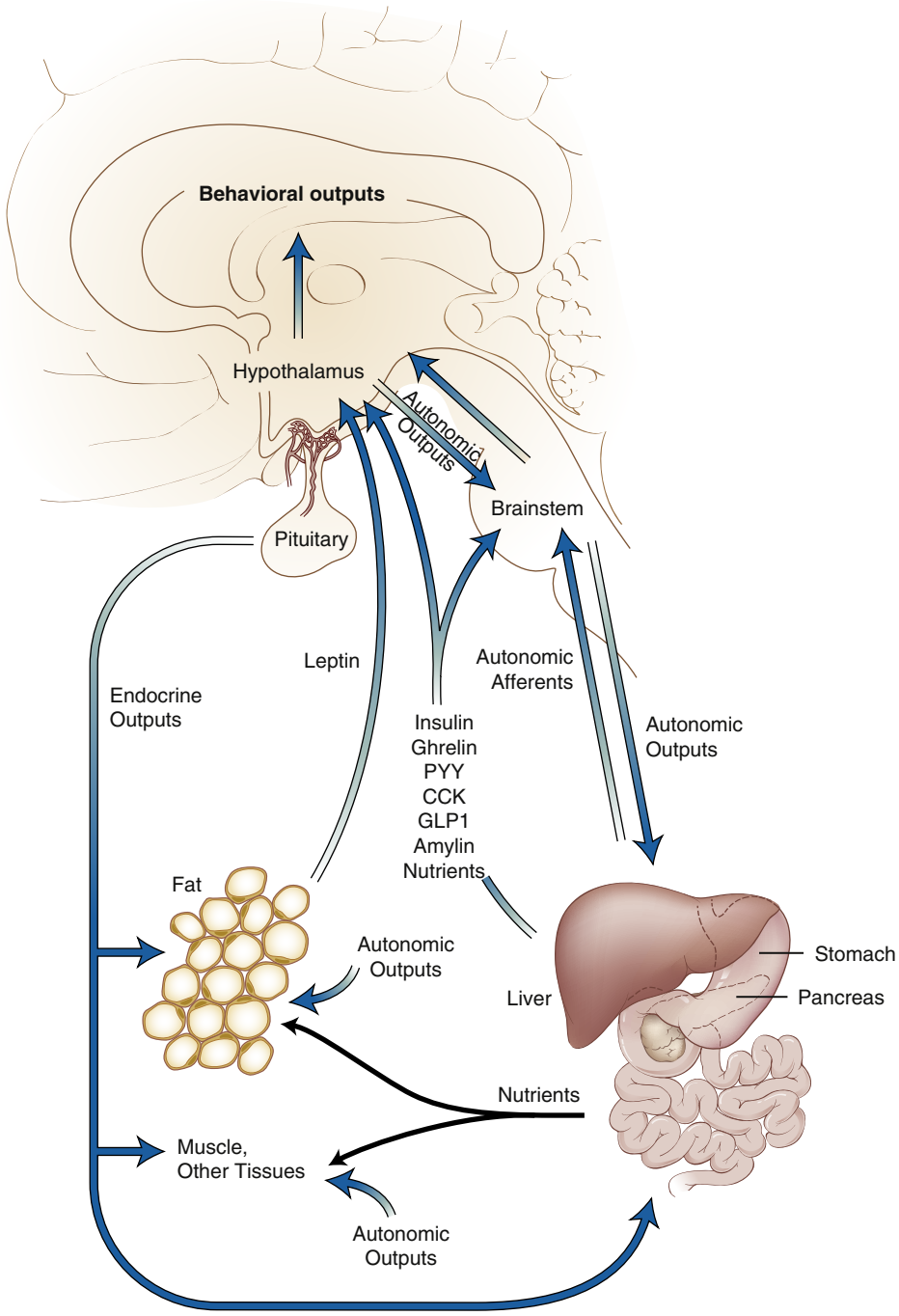
- The body's physiologic systems maintain stable energy stores and body weight by matching long-term food intake to energy expenditure.
- The study of monogenetic obesity syndromes in rodents and humans and the identification of human genetic variants that predispose to obesity have revealed key gene products and brain systems (such as the adipose-derived hormone, leptin, and the hypothalamic melanocortin system) that participate in the regulation of energy balance.
- Hypothalamic circuits mediate the long-term balance between energy intake and expenditure; hypothalamic nuclei that play important roles in energy homeostasis include the arcuate nucleus, the ventromedial nucleus, the dorsomedial nucleus, the paraventricular nucleus, and the lateral hypothalamic area.
- Brain regions such as the ventromedial nucleus, along with melanocortin circuits, also play a role in the central control of glucose homeostasis.
- Food and drug rewards share some common neural substrates, and the signals and systems that control food intake and body weight modulate the brain circuits that control reward. Understanding the molecular and neural mechanisms that modulate rewarding aspects of feeding may reveal sites for therapeutic intervention in obesity.
- The brainstem has traditionally been considered to control short-term feeding by mediating the effects of gut-derived satiety signals, but the brainstem represents the likely site of action for several antiobesity medications that decrease food intake and body weight over the long term. Brainstem nuclei that contribute to the control of food intake include the area postrema, the nucleus of the solitary tract, the dorsal motor nucleus of the vagus, and the lateral parabrachial nucleus.

The Biologic Control of Energy Balance

The law of conservation of energy dictates that body energy stores reflect the difference between energy taken in and energy expended. Consuming more calories than one expends leads to the storage of excess energy (generally, in adipose tissue); chronically, such positive energy balance causes obesity. Conversely, when expenditure exceeds intake, energy/fat stores decline.

While often thought of as a cosmetic problem, obesity represents a major health concern that contributes to the development of diabetes, cardiovascular disease, and cancer (among other illnesses).[1] In addition to their human toll, obesity and its complications cost $147 billion each year in the United States alone.[1] Furthermore, while common wisdom holds that voluntary behaviors (i.e., choosing to eat too much) dictate energy balance and underlie the development of obesity, several lines of evidence demonstrate the biologic (involuntary) basis for the regulation of body weight and adiposity.[2]

Humans and other animals exhibit a remarkable degree of weight stability over the long term because the depletion of body energy stores activates compensatory physiologic systems to counteract weight deviations and return energy stores to their previous levels or set-point.[2] Energy deficit causes hyperphagia and decreased energy expenditure, while forced overfeeding causes anorexia with increased energy expenditure, such that body weight ultimately returns to previous levels upon ad libitum feeding. Such observations prompted Gordon Kennedy[3] to propose a model of body weight regulation in which a signal proportional to energy



• **Fig. 39.1** Circuits and hormones that contribute to the control of food intake and energy homeostasis by the brain-gut-adipose axis. *CCK,* cholecystokinin; *GLP1,* glucagon-like peptide 1; *PYY,* peptide YY.

stores elicited compensatory changes in food intake and energy expenditure to maintain adipose mass at a presumed set-point.

It has long been understood that organic lesions within specific regions of the brain can cause obesity. For example, at the end of the 19th century clinicians, including Alfred Fröhlich, described a condition characterized by pituitary tumors associated with obesity and hypogonadism.[4,5] While several groups (including Harvey Cushing and his colleagues) argued that the syndrome was due to disruption of the pituitary gland,[6–8] removing the pituitary gland without damage to the overlying hypothalamus did not result in obesity in dogs.[9] Indeed, it was subsequently shown that destruction of the medial basal hypothalamus (which contains the arcuate and ventromedial nuclei) without damage to the pituitary gland caused morbid obesity and neuroendocrine derangements similar to those described by Frölich.[10] Conversely, lesions in other brain regions (such as the lateral hypothalamic area) suppressed feeding, resulting in leanness.[11] Thus the hypothalamus plays crucial roles in the control of energy balance and neuroendocrine function with early formulations suggesting that the lateral hypothalamic area contains a putative feeding center, while the medial basal hypothalamus contains a presumptive satiety center.[11]

The past several decades have expanded our understanding of the systems that contribute to the control of energy balance.[2] In addition to the originally described hypothalamic centers, we have

learned a great deal about brainstem systems that control feeding, the circuits that modulate the incentive value of food, and the peripheral signals that modulate them (Fig. 39.1).

Leptin Signals the Repletion of Adipose Stores

Parabiosis (joining the circulatory systems of two animals to permit the exchange of hormones) between obese rats with medial basal hypothalamus lesions and nonlesioned rats led to starvation and weight loss in the latter, while parabiosis between two medial basal hypothalamus-lesioned rats did not alter energy balance in either animal.[12–14] Thus medial basal hypothalamus-lesioned obese rats must produce a circulating factor that inhibits feeding in normal animals and acts via a medial basal hypothalamus satiety center.[11] An important series of experiments by Douglas Coleman almost 50 years ago provided the first insights into a potential mediator of this effect. Coleman used mice homozygous for *ob*, a recessive allele that causes hyperphagia, decreased energy expenditure, endocrine dysfunction, and obesity, and for *db*, which lies at a different locus but produces a phenotype similar to that of *ob*. Parabiosis of lean (wild-type) mice with *ob/ob* mice suppressed weight gain in the *ob/ob* mice, whereas parabiosis of wild-type and *db/db* mice caused profound hypophagia and weight loss in the wild-type mice.[15–17] Based upon these results, Coleman predicted that the *ob* locus produces a circulating satiety factor, while the *db* locus encodes a component required for the response to the presumptive *ob* hormone.

Cloning of the causative gene mutations in *ob* and *db* strains confirmed the predictions of these parabiosis studies: The gene mutated in *ob* encodes a hormone of the type 1 cytokine family (subsequently named leptin [from the Greek *leptos*, meaning "thin"]), while *db* affects the gene that encodes the leptin receptor (LepR), a member of the type 1 cytokine receptor family.[18–20] Treatment with leptin decreases feeding, adipose mass, and body weight in leptin-deficient *ob/ob* ($Lep^{ob/ob}$) mice and in lean normal mice, but fails to alter *db/db* ($Lepr^{db/db}$) mice.[21–23]

Adipose tissue produces leptin in approximate proportion to triglyceride stores, serving as a signal of the repletion of adipose energy stores to the central nervous system to control energy balance. Decreased leptin following caloric restriction initiates the neuroendocrine starvation response, increasing food-seeking and appetite and suppressing the expenditure of energy by neuroendocrine systems (resulting in infertility, decreased sympathetic nervous system tone, thyroid function, etc.).[24,25] Exogenous leptin reverses the neuroendocrine manifestations of starvation as well as the neuroendocrine dysfunction of $Lep^{ob/ob}$ mice. Leptin also reverses the hyperphagia, obesity, and neuroendocrine dysfunction of rare human patients with congenital leptin deficiency.[26–28]

Similarly, human patients and transgenic animals that lack adipose tissue (lipodystrophy) exhibit hyperphagia along with a predisposition to insulin resistance and other endocrine and metabolic abnormalities that are not corrected with caloric restriction.[29,30] Due to the dearth of adipose tissue in lipodystrophy, this syndrome causes low leptin concentrations, and leptin treatment improves hunger indices and endocrine/metabolic abnormalities. Indeed, leptin was recently approved for the treatment of lipodystrophy syndromes in humans.[31]

In contrast to the dramatic ability of leptin to reverse the hyperphagia and neuroendocrine abnormalities associated with congenital leptin deficiency and lipodystrophy, exogenous leptin modestly and briefly blunts food intake and body weight in normal-weight animals and minimally alters energy balance in obese humans or animals that have elevated endogenous leptin due to increased fat mass.[32] Indeed, serum leptin levels in humans are generally proportional to adipose mass,[33,34] thus the vast majority of obese humans are not leptin deficient, but rather fail to respond to elevated leptin concentrations with weight loss. Hence the absence of leptin sends a more powerful physiologic signal than does its excess. Indeed, while low leptin clearly represents the crucial signal of energy deficit, leptin does not mediate the anorexia that follows forced overfeeding,[35] suggesting the existence of a separate signal important for the suppression of food intake during nutritional surfeit.

The Hypothalamic Melanocortin System

In 1902, French geneticist Lucien Cuenot described the obese agouti mouse (A^y/a; also termed *lethal yellow* because homozygotes for the allele die prenatally) that had been bred for decades by European mouse fanciers.[36] The A^y/a strain is notable for the dominant inheritance of an obesity phenotype proportional to the intensity of its yellow coat color.[36] The increased body weight of A^y/a mice results mainly from hyperphagia and reflects both increased fat mass and lean body mass (with increased body length).[37]

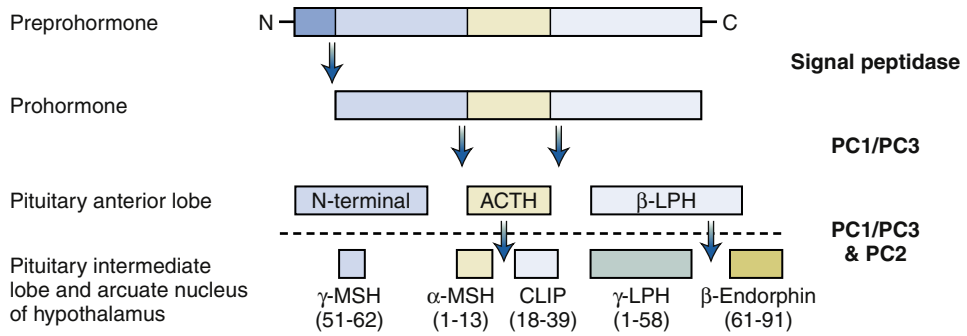
The A^y allele results from a genetic rearrangement that deletes the *Raly* gene and fuses the *Asip* gene to the *Raly* promoter, resulting in continuous expression of agouti signaling protein (ASIP) throughout the body.[38,39] ASIP is a secreted peptide that binds to and inhibits melanocortin receptors. The yellow coat color of the A^y/a mouse results from overexpression of ASIP in the skin, which blocks α-melanocyte stimulating hormone (αMSH) signaling at MC1 receptors (MC1R) in the hair follicle.[38] Since MC1R promotes the synthesis of eumelanin (black pigment) instead of pheomelanin (yellow pigment) in melanocytes, ASIP-mediated antagonism of hair follicle MC1R results in yellow coat color.

The brain contains two predominant melanocortin receptor isoforms: MC3R and MC4R,[40] both of which are activated by αMSH and inhibited by ASIP. Intracerebroventricular administration of αMSH or other melanocortin receptor agonists decreases food intake and body weight; overexpression of ASIP in the brain antagonizes the anorectic action of αMSH signaling and blunts endogenous melanocortin receptor activity resulting in hyperphagia. Thus the hypothalamic melanocortin system is crucial for the control of food intake and energy balance.

At its core, the hypothalamic melanocortin system includes arcuate nucleus neurons that express pro-opiomelanocortin (POMC), the precursor peptide for melanocortin receptor agonists. POMC in the arcuate nucleus is processed to produce αMSH (and at times, β-endorphin), although other POMC-expressing cells in the pituitary and elsewhere produce other POMC-derived peptides[41] (Fig. 39.2). Mice null for *Pomc* throughout the body or specifically in the arcuate nucleus are hyperphagic and weigh approximately twice as much as control animals in adulthood.[42] These animals also exhibit adrenal insufficiency due to the lack of POMC-derived adrenocorticotropic hormone (ACTH) in the anterior pituitary.

In addition to POMC neurons, the arcuate nucleus contains distinct neurons that express the ASIP homolog, agouti-related peptide (AgRP).[38] AgRP (like ASIP) binds and inhibits melanocortin receptors (especially MC3 and MC4 receptors, which are mainly expressed in the brain), and intracerebroventricular administration of AgRP elicits a long-lasting hyperphagic response in rodents.[38] These neurons also contain the inhibitory transmitters neuropeptide Y (NPY, which, like AgRP, promotes feeding when administered intracerebroventricularly) and gamma amino butyric acid (GABA). Negative energy balance (e.g., caloric restriction) activates these arcuate nucleus NPY/AgRP–containing neurons and increases the expression of *Agrp* and *Npy*.[43]

Mice null for *Mc4r* display substantial hyperphagia and increased adiposity body weight, and display increased linear growth, as is



• **Fig. 39.2** Organization of pro-opiomelanocortin (POMC), the precursor hormone of corticotropin (ACTH, adrenocorticotropic hormone), β-lipoprotein (βLPH), and related peptides. The precursor protein contains a leader sequence (signal peptide), followed by a long fragment that includes sequence 51-62, corresponding to γ-melanocyte-stimulating hormone (γMSH). This fragment is cleaved at Lys-Arg bonds to form corticotropin 1-39, which in turn includes the sequences for αMSH (corticotropin 1-13) and corticotropin-like intermediate lobe peptide (CLIP; corticotropin 18-39) and a sequence corresponding to βLPH (1-91) that includes γLPH (1-58) and β-endorphin (61-91). The β-endorphin sequence also includes a sequence corresponding to met-enkephalin. The precursor molecule in the anterior lobe of the pituitary is processed predominantly to corticotropin and βLPH. In the intermediate pituitary lobe (in the rat), corticotropin and βLPH are further processed to αMSH and a β-endorphin–like material. In all extrapituitary tissues, post-translational processing of the prohormone resembles that in the intermediate lobe. Hypothalamic processing is similar but not identical to that in the intermediate lobe. In the latter, β-endorphin and αMSH are present predominantly in their acetylated forms. *C,* carboxy-terminal; *PC,* prohormone-converting enzyme. (Courtesy Dr. Malcolm Low, University of Michigan, Ann Arbor, MI.)

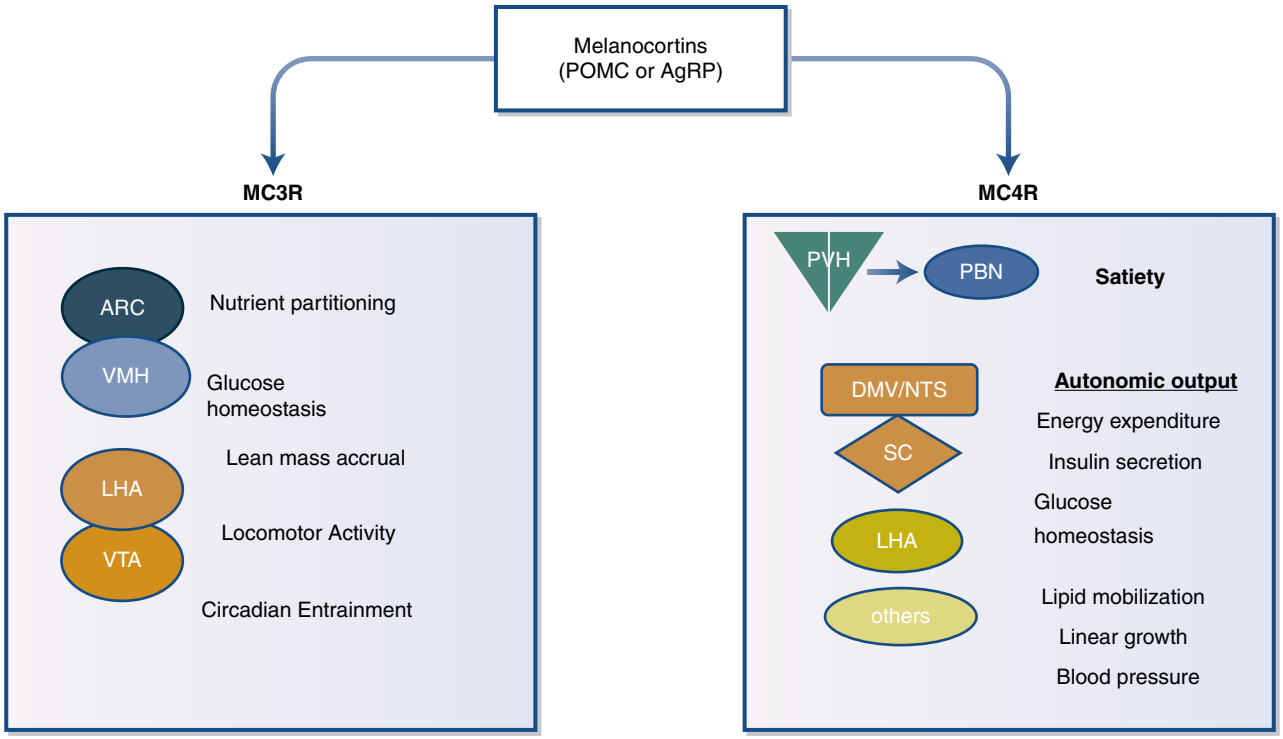
characteristic of *A^y/a* mice.[44] Mice null for *Mc3r* display a subtler obesity phenotype, as noted later in the chapter. Thus MC4R represents the major melanocortin receptor that mediates the control of food intake and body weight. MC4R is expressed in hypothalamic sites critical for feeding control, including the paraventricular nucleus, dorsomedial nucleus, ventromedial nucleus, and lateral hypothalamic area[39] as well as in the brainstem and other brain areas. While some other sites play roles in the control of energy balance, *Mc4r* expression in the paraventricular nucleus is necessary and sufficient for the control of feeding.[45,46] Thus the hypothalamic melanocortin system suppresses food intake by acting on *Mc4r*-expressing paraventricular nucleus neurons (Fig. 39.3).

Extrahypothalamic MC4Rs also contribute to melanocortin action on energy balance. For example, MC4R agonist injection into the fourth ventricle or the dorsal motor nucleus of the vagus decreases food intake, while injection of MC4R antagonists increases food intake.[47,48] Also, deleting MC4Rs in autonomic (cholinergic) neurons lowers energy expenditure and increases adiposity, especially in high-fat diet–fed animals.[49,50] Deleting MC4Rs in parasympathetic preganglionic neurons (vagal motor neurons) causes hyperinsulinemia and insulin resistance but does not significantly impact energy balance, suggesting an important role for MC4R in presympathetic cells for the control of energy expenditure.[49,50]

MC3R, which is distributed in central nervous system sites largely distinct from those that contain MC4R, also plays a role in energy homeostasis. In the mouse, this receptor is most highly expressed in arcuate nucleus POMC and NPY/AGRP neurons, the ventromedial nucleus, the ventral tegmental area, and the medial habenula.[51,52] Within the arcuate nucleus, MC3R is also expressed presynaptically on NPY/AGRP terminals and acts as an inhibitory autoreceptor on the POMC-MC4R circuitry.[53] Although *Mc3r* haploinsufficiency does not produce a phenotype, homozygous *Mc3r* deletion in mice causes increased adipose mass, decreased lean mass, reduced fast-induced refeeding,[54–56] elevated basal and fasting-induced corticosterone,[54] and defects in circadian rhythms and meal entrainment.[57]

The first evidence for a human melanocortin obesity syndrome resulted from the astute recognition of a rare agouti mouse–like syndrome in two families, resulting from null mutations in the *POMC* gene[58] (Fig. 39.4). These patients have ACTH insufficiency, red hair, and obesity, resulting from the lack of ACTH peptide in the serum and a lack of melanocortin peptides in skin and brain, respectively. This obesity syndrome demonstrated that the central melanocortin circuitry subserves energy homeostasis in humans as it does in the mouse. Shortly after the description of *POMC* mutations in humans, heterozygous frameshift mutations in the human *MC4R* locus associated with nonsyndromic obesity in two separate families were reported.[59,60] Additional reports[61–63] provide a clearer picture of the frequency and diversity of *MC4R* mutations and revealed that *MC4R* haploinsufficiency in humans represents the most common monogenic cause of severe obesity, accounting for up to 5% of cases. Remarkably, the associated physical findings are virtually identical to those reported for the mouse,[44] with increased adipose mass, increased linear growth and lean mass, hyperinsulinemia greater than that seen in matched obese control subjects, and severe hyperphagia. MC4R haploinsufficient adults also exhibit reduced sympathetic tone and mild hypotension.[64] Initial pharmacologic approaches to melanocortin obesity syndromes were complicated by blood pressure effects of MC4R agonist therapy. Recently, however, the MC4R agonist, setmelanotide (currently under clinical development), has been shown to promote significant weight loss without associated blood pressure problems in patients with either POMC deficiency or leptin receptor deficiency.[65,66] Thus central nervous system circuits that regulate energy balance may be targeted for therapeutic benefit.

While a syndrome resulting from MC3R mutations in humans has not yet been definitively identified, several other monogenetic obesity syndromes in mice and humans likely result from alterations in melanocortin signaling. The products of many of the affected genes play important roles in peptide processing. POMC is cleaved by prohormone convertase 1 (PC1, also called PCSK1 or PC1/3) and PC2 (PCSK2) to generate a precursor peptide that



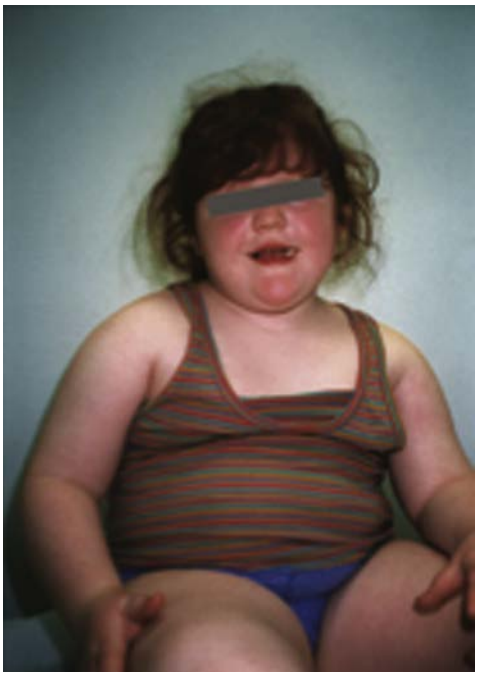
• **Fig. 39.3** Distribution of melanocortin action in the central nervous system. Pro-opiomelanocortin neurons (POMC) in the arcuate nucleus (ARC) produce melanocortin-receptor agonists, whereas agouti-related peptide (AgRP)–producing neurons antagonize melanocortin action. MC3R and MC4R are the predominant melanocortin receptors in the brain and have relatively distinct physiologic roles. MC3R is expressed at high levels in the ARC and the ventromedial nucleus (VMN), where it mainly controls nutrient partitioning, conversion of food to fat, and lean mass accrual. MC3R in the lateral hypothalamic area (LHA) and ventral tegmental area (VTA) likely control motivation and activity. MC4R is expressed in many brain regions and plays a critical role in energy balance. MC4Rs in the paraventricular hypothalamic nucleus (PVH) profoundly affect food intake through projections to the parabrachial nucleus (PBN). MC4Rs in other brain regions, including the lateral hypothalamus (LHA), dorsal motor nucleus of the vagus (DMV), nucleus of the solitary tract (NTS), and spinal cord (SC) are involved in energy expenditure, autonomic output, and glucose regulation. MC4R action in the DMV regulates insulin secretion, and MC4Rs regulate sympathetic output (and energy expenditure) through the intermediolateral cell column of the spinal cord (IML). The sites mediating melanocortin action on linear growth, lipid handling, and blood pressure are not defined.

is further cleaved by carboxypeptidase E (CPE) to generate active αMSH[67] (see Fig. 39.2). Prolyl carboxypeptidase (PRCP) acts on substrate proteins (such as αMSH) where the penultimate amino acid is a proline residue, cleaving the COOH-terminal amino acid and inactivating the peptide.[68]

The *fat* mutation, which causes diabetes and obesity in mice, results from a point mutation in the *Cpe* gene[69]; miscleavage of POMC presumably underlies the obesity in Cpe^{fat} mice. Similarly, mice and humans null for *Pcsk1* are obese with adrenal insufficiency (in addition to displaying other endocrine defects) due to impaired processing of POMC and other peptides.[70,71] Mice null for *Pcsk2* are not obese, however,[72] presumably due to the partial activity of the POMC PC1 product on MC3/4R, even in the absence of PC2-mediated processing. Conversely, mice null for *Prcp* are lean due to prolonged activity of αMSH.[68]

Hypothalamic Systems and Signals That Regulate Energy Balance

The hypothalamus, a highly conserved brain region, plays crucial roles in the maintenance of homeostasis; destruction of the hypothalamus is not compatible with life.[73] The hypothalamus receives sensory inputs from the external environment and information regarding the internal environment. In addition, several hormones crucial for the regulation of food intake and metabolism (e.g., leptin, ghrelin, insulin, estrogen) directly act on neurons in the hypothalamus. The hypothalamus integrates these inputs to modulate key outputs (including the pituitary gland, the cerebral cortex, premotor and motor neurons in the brainstem and spinal cord, and autonomic [parasympathetic and sympathetic] preganglionic neurons) to coordinate endocrine, behavioral, and autonomic responses that maintain homeostasis in several physiologic systems, including energy balance (see Fig. 39.1). Several hypothalamic sites (including the arcuate nucleus, ventromedial nucleus, dorsomedial nucleus, paraventricular nucleus, and lateral hypothalamic area) play key roles in coordinating food intake and other parameters of energy homeostasis. Information flow for the hypothalamic circuits generally begins with the arcuate nucleus, the entry point for many hormonal signals (such as insulin and leptin) (Fig. 39.5). The paraventricular nucleus mediates important outflow from the hypothalamus to brainstem centers that control food intake. In between, the dorsomedial nucleus integrates other signals and relays information to and from the arcuate and paraventricular nuclei.



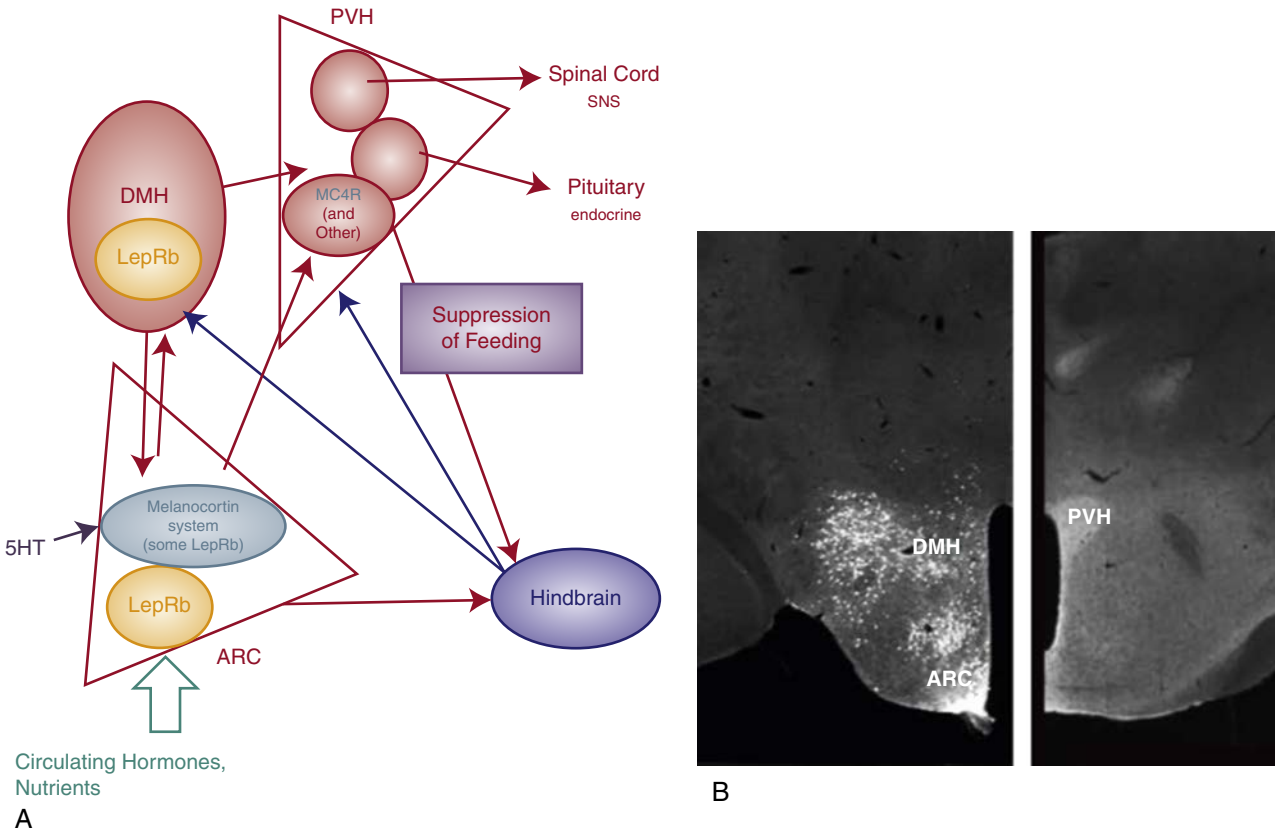
• **Fig. 39.4** A monogenic neuroendocrine obesity syndrome of adrenocorticotropic hormone insufficiency, obesity, and red hair resulting from a null mutation in the pro-opiomelanocortin gene. (Photograph courtesy Dr. A. Gruters, Charité-Universitätsmedzin, Berlin, Germany.)

Orthologs of many of the genes that encode crucial mediators of energy balance and/or that underlie rodent obesity models also cause or contribute to obesity in humans. In the past several decades, causative mutations in multiple genes have been found in human obesity disorders, including *LEP, LEPR, POMC, MC4R, PCSK1, GNAS, SH2B1, BDNF, TRKB,* and *SIM1*.[74] These mendelian disease genes appear to act primarily in the hypothalamus. Furthermore, genome-wide association studies have identified over 100 single nucleotide polymorphisms significantly associated with body mass index, and these are predominantly located in or near genes thought to act primarily in the hypothalamus.[75,76]

Leptin-Regulated Hypothalamic Circuits

Alternative splicing of the *Lepr* transcript produces multiple LepR isoforms[77]; $Lepr^{db}$ produces an mRNA splicing error that inserts the final exon of one of the short LepR isoforms (LepRa) into the mRNA, replacing the long LepRb isoform (which uniquely contains all intracellular cytokine receptor signaling motifs) with LepRa throughout the body.[78] Because the $Lepr^{db}$ allele synthesizes all LepR isoforms except LepRb, this isoform plays a crucial role in energy homeostasis.[79]

Consistent with leptin's behavioral effects (e.g., on feeding) and its effects on the neuroendocrine and autonomic systems, most LepRb-expressing cells lie in the brain,[19,80,81] suggesting the potential importance of the central nervous system as a site of leptin action. Indeed, transgenic overexpression of LepRb in the central nervous system substantially corrects the obesity syndrome



• **Fig. 39.5** Flow of information into and out of the hypothalamus. (A) Cartoon diagram of circuits. *ARC,* arcuate nucleus; *DMH,* dorsomedial nucleus; *PVH,* paraventricular nucleus; *LepRb,* leptin receptor; *SNS,* sympathetic nervous system. Core "homeostatic" hypothalamic circuits are shown in *red,* brainstem circuits are in *dark blue,* peripheral signals are *green,* leptin receptor pathways are in *orange,* and melanocortin pathways are in *light blue*. (B) Images of hypothalamic regions in question, using LepRb-GFP *(left panel)* reporter mice showing LepRb neurons in areas, including the ARC and DMH and LepRb-enhanced green fluorescent protein (EGFP) *(right panel)* reporter mice, showing projections from LepRb neurons into regions, including the PVH.

of *Lepr*$^{db\text{-}3J}$ mice (which lack all LepR isoforms).[82] Similarly, ablation of LepRb specifically in the central nervous system promotes hyperphagia, neuroendocrine failure, and obesity.[83]

Within the brain, the majority of LepRb-expressing neurons reside within the hypothalamus and brainstem, consistent with the known roles for these structures in the control of feeding and endocrine and autonomic function.[80,81,84] In the brainstem, LepRb knockdown in the nucleus of the solitary tract and surrounding regions alters the control of meal size and slightly increases long-term food intake,[85] while lateral parabrachial nucleus LepRb ablation alters the response to hypoglycemia and other metabolic emergencies but does not impact energy balance.[86,87] In contrast, pan-hypothalamic ablation of LepRb promotes a phenotype very similar in quality and magnitude to that of whole-body null *Lepr*$^{db/db}$ animals, suggesting that hypothalamic LepRb neurons mediate the majority of leptin action on energy balance.[88]

Within the hypothalamus, multiple nuclei contain populations of LepRb neurons, including the arcuate nucleus, ventromedial nucleus, dorsomedial nucleus, lateral hypothalamic area, and ventral premammillary nucleus.[80,81] Substantial subpopulations of arcuate nucleus POMC and NPY/AGRP neurons contain LepRb, although additional LepRb-expressing arcuate nucleus populations also exist.[89–91] While it was initially assumed that the absence of LepRb from POMC and/or NPY/AGRP neurons would recapitulate the obesity phenotype of *Lepr*$^{db/db}$ mice (which results from defects that exist in the early embryo), the early embryonic ablation of LepRb from these cell populations only minimally alters energy balance, suggesting important roles for LepRb in distinct hypothalamic population(s).[92–94]

Importantly, however, undefined compensatory processes attenuate phenotypes that result from the early developmental alteration of NPY/AGRP cells, and ablating LepRb from NPY/AGRP cells in adult mice produces a dramatic hyperphagic obesity.[95] Thus while LepRb in NPY/AGRP neurons contributes only minimally to the *Lepr*$^{db/db}$ phenotype, the control of NPY/AGRP neurons by LepRb plays a crucial role in energy balance in adult animals.

Roles in leptin action have been examined for other circumscribed sets of hypothalamic LepRb neurons as well. Early embryonic ablation of LepRb in the ventromedial nucleus blunts energy expenditure and thereby increases adiposity,[96] while deletion of LepRb in neurotensin-expressing lateral hypothalamic area neurons diminishes motor activity to blunt energy expenditure and increase adiposity.[96,97] LepRb in the ventral premammillary nucleus contributes to the control of reproduction[98] but not energy balance; LepRb in arcuate nucleus growth hormone–releasing hormone *(Ghrh)*–expressing cells does not contribute detectably to the control of growth or energy balance.[94]

In contrast to the minimal phenotypes observed following the early embryonic ablation of LepRb in the circumscribed sets of hypothalamic LepRb neurons tested to date, ablation of LepRb from broadly distributed hypothalamic vesicular GABA transporter (vGat)–expressing or nitric oxide synthase-1 (NOS1)–expressing neurons promotes dramatic hyperphagia and obesity.[99,100] While this might suggest that the total number of LepRb neurons affected (rather than the specific cell types) matters most for the control of energy balance, deletion of LepRb across broad populations of hypothalamic serotonin 2c receptor *(Ht2cr)*–expressing neurons largely distinct from vGat and NOS1 cells minimally impacts energy balance,[94] suggesting that specific LepRb cell types do play specialized roles in energy balance. The dorsomedial nucleus contains substantial populations of both vGat and NOS1-expressing LepRb neurons, suggesting that important sets of food intake–controlling LepRb neurons may lie in the dorsomedial nucleus. Although a subpopulation of prolactin-releasing hormone *(Prlh)*–expressing dorsomedial nucleus LepRb neurons contributes only to the control of energy expenditure,[101] ablation of dorsomedial nucleus LepRb in adults increases food intake and promotes dramatic obesity,[102] suggesting an important role for non-*Prlh* dorsomedial nucleus LepRb neurons in the control of food intake and energy balance. The vGat-containing dorsomedial nucleus LepRb neurons that play important roles in the acute regulation of NPY/AGRP neurons[103] may represent at least some of these crucial energy balance–controlling LepRb neurons.

Roles for NPY/AGRP Neurons in Energy Balance

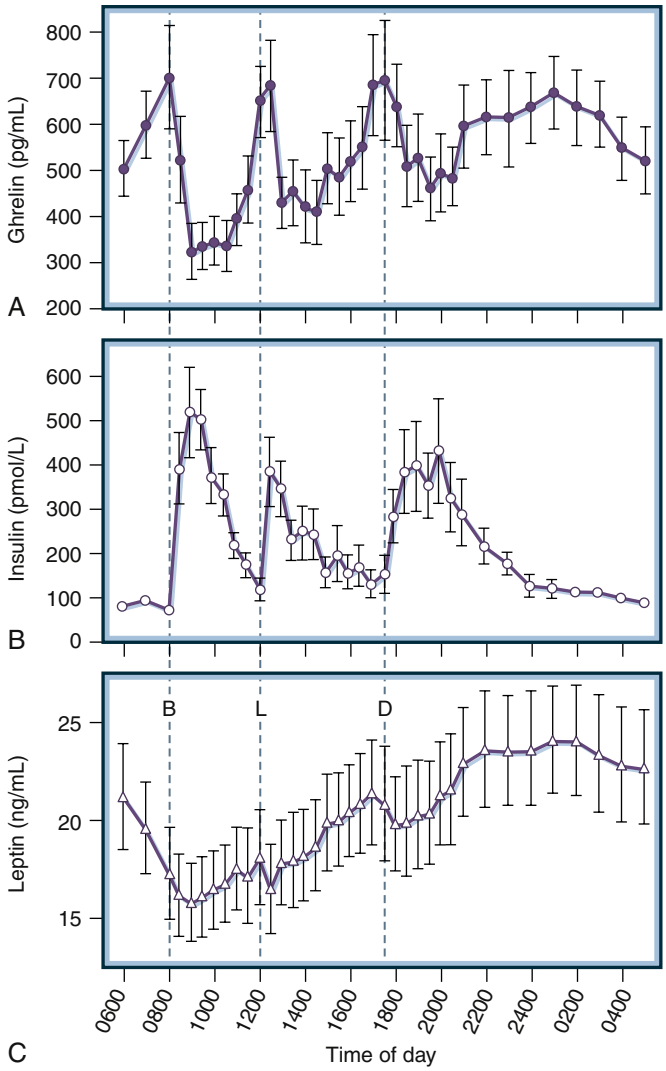
Not only does activating NPY/AGRP neurons increase food intake and body weight,[104] but ablating NPY/AGRP neurons in adult animals results in anorexia.[105–107] Thus NPY/AGRP neurons play important roles in the control of food intake and energy balance. Indeed, while leptin inhibits NPY/AGRP neurons, these cells also respond to other hormonal modulators of energy balance (including ghrelin, which activates NPY/AGRP cells to promote food intake [see upcoming discussion]), and nutrient signals (such as glucose, which inhibits NPY/AGRP cells).[108]

While early models proposed that the endogenous MC4R agonist/antagonist pair (αMSH and AgRP, respectively) might simply act competitively to determine the relative amount of activation of the MC4R, recent findings have demonstrated additional roles for AgRP.[109] AgRP not only acts via MC4R to control the activity of the G protein, GαS, but also alters membrane potential in target neurons by controlling potassium channels independently of G proteins.

Furthermore, NPY/AGRP neurons control feeding via several neuronal mechanisms. While NPY/AGRP cells contain the orexigenic peptides AgRP and NPY, early developmental ablation of these peptides minimally alters the ability of NPY/AGRP neurons to promote food intake or to control energy balance.[110] In contrast, ablation of GABA signaling by NPY/AGRP cells abrogates their orexigenic action.[111] GABA signaling by NPY/AGRP cells not only provides direct inhibitory input to arcuate nucleus POMC neurons but also is required to promote food intake by the paraventricular nucleus and to inhibit anorexigenic and aversive lateral parabrachial nucleus cells that express calcitonin gene–related peptide (CGRP) (see hindbrain section later in the chapter).[105,111,112]

Ghrelin

Ghrelin (an acylated 28–amino acid peptide that potently stimulates food intake) acts via the ghrelin receptor (known as the growth hormone secretagogue receptor [GHSR]).[113–117] Predominantly secreted by the stomach, ghrelin levels are markedly reduced with meal ingestion in both rodents and humans but rebound to baseline before the next meal or increase after an overnight fast[115–117] (Fig. 39.6). Ghrelin is unique in that it requires the addition of an eight-carbon fatty acid (octanoate) side chain to have agonist activity at the ghrelin receptor.[118] The enzyme ghrelin O-acyltransferase catalyzes the addition of the octanoyl group.[118] Unlike other signals involved with energy homeostasis, ghrelin secretion is inhibited in response to meals and stimulates appetite, leading to the notion that ghrelin participates in meal initiation.[117] Fasting ghrelin levels are inversely proportional to body weight[115] and are higher than normal in underweight subjects with anorexia nervosa and cardiac



• **Fig. 39.6** Average plasma ghrelin (A), insulin (B), and leptin (C) concentrations during a 24-hour period in 10 human subjects consuming breakfast (B), lunch (L), and dinner (D) at the times indicated (0800, 1200, and 1730 hours, respectively). (From Cummings DE, Purnell JQ, Frayo RS, et al. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes.* 2001;50:1714–1719.)

cachexia compared to control subjects.[114,119] Both of these changes presumably reflect the regulation of ghrelin by nutritional status rather than any role for ghrelin in the pathogenesis of these diseases.

Peripheral administration of ghrelin activates arcuate nucleus NPY/AGRP neurons,[120] and ablation of the arcuate nucleus blocks the actions of the ghrelin administration on feeding.[121] Furthermore, re-expression of the ghrelin receptor only in NPY/AGRP neurons in ghrelin receptor-null mice suffices to restore the orexigenic effect of exogenous ghrelin.[122]

Despite the significant amount of data suggesting that ghrelin increases food intake, mice null for ghrelin or ghrelin receptor demonstrate little alteration of food intake or energy balance. Similarly, neither genetic deletion of ghrelin O-acyltransferase[123] nor the ablation of ghrelin-producing cells[124] results in major changes in food intake and body weight. These findings have brought into question the physiologic relevance of ghrelin. They may also reflect developmental compensation of NPY/AGRP neurons to the lack of ghrelin action.

Roles for the Paraventricular Nucleus in Energy Balance

The metabolic consequences that result from abnormal paraventricular nucleus development or the interference with paraventricular nucleus function underscore the importance of the paraventricular nucleus in energy balance and autonomic function. Destruction of the paraventricular nucleus by electrolytic lesion causes hyperphagia and obesity.[125] Similarly, loss of one copy of single-minded homolog-1 (Sim1), a key transcription factor regulating paraventricular nucleus development, disrupts paraventricular nucleus development and function, resulting in hyperphagic obesity with associated glucose dysregulation in both rodents and humans.[126–128] In addition, the paraventricular nucleus serves as an important regulatory output center for stimuli that modulate food intake, including leptin, melanocortins, and dehydration.[129,130] Melanocortin agonists engage and stimulate melanocortin receptor–bearing neurons in the paraventricular nucleus to activate effector pathways that inhibit food intake and stimulate energy expenditure (see Fig. 39.5). Melanocortin action in paraventricular nucleus neurons plays crucial roles in the control of food intake, energy balance, and glucose homeostasis.[45,131] While the role for the paraventricular nucleus in regulating energy balance is clear, much less is known about the identity of the neurons and specific circuitry mediating these effects, however.

The paraventricular nucleus is composed of a heterogeneous group of glutamatergic neurons that have been classically described as parvocellular or magnocellular based on cell size and axonal projection patterns. Paraventricular nucleus parvocellular cells are neurochemically diverse and send projections within the central nervous system to three main areas: (1) the median eminence, where secreted factors (e.g., thyroid-releasing hormone [TRH] and corticotrophin-releasing hormone [CRH]) enter the portal hypophyseal circulation and affect pituitary function; (2) the brainstem, including the dorsal vagal complex (comprised of the dorsal motor nucleus of the vagus and the nucleus of the solitary tract) and the lateral parabrachial nucleus, both of which have been implicated in feeding[105,132]; and (3) the preganglionic sympathetic output centers, such as the intermediolateral column of the spinal cord.[133,134] Hypothalamic parvocellular paraventricular nucleus neurons that respond to the satiety signals are thought to regulate feeding by modulating hindbrain responses to ascending signals from the gut and periphery.[135–137] Magnocellular neurons (expressing oxytocin or vasopressin [AVP]) send their axonal projections to the posterior pituitary to release their neuropeptides and transmitters directly into the systemic circulation. Paraventricular nucleus-mediated energy balance control is thought to be regulated predominantly by parvocellular paraventricular nucleus neurons.

Specific Roles of Paraventricular Nucleus Neuronal Subsets in Metabolic Homeostasis

Oxytocin and CRH neurons within the paraventricular nucleus have garnered significant attention as potential regulators of energy balance. Both oxytocin and CRH peptides alter feeding when delivered to the brain, and oxytocin and CRH paraventricular nucleus neurons project to other brain regions that regulate feeding. Rodents lacking oxytocin or CRH have near normal energy balance under basal conditions, however.[138,139]

Moreover, direct activation of paraventricular nucleus oxytocin neurons does not alter feeding, and ablation of paraventricular nucleus oxytocin has little effect on body weight unless animals are challenged with a high-fat diet.[140,141] In addition, mice lacking oxytocin neurons demonstrate normal anorectic responses to

melanocortin agonists. Thus oxytocin neurons (and their contents) are not required for anorectic melanocortin action, and other (non-oxytocin) paraventricular nucleus neurons play crucial roles in the control of feeding (including in response to melanocortin action).

Paraventricular nucleus MC4R neurons play a critical role in energy balance control. Deletion of paraventricular nucleus *Mc4r* results in hyperphagic obesity, whereas re-expression of *Mc4r* selectively in the paraventricular nucleus in an otherwise null *Mc4r* background normalizes feeding and dramatically attenuates the obesity phenotype of these mice.[45,131] Selective manipulation of paraventricular nucleus MC4R-expressing neurons revealed that these neurons directly regulate feeding but not energy expenditure.[45] Indeed, the paraventricular nucleus MC4R → lateral parabrachial nucleus projection pathway is critical for these feeding effects.[46] Other paraventricular nucleus cell types shown to be involved in energy balance regulation include brain-derived neurotrophic factor-expressing neurons,[142] Nos1-expressing neurons,[140] AVP neurons,[143] and nesfatin-expressing cells.[144] Whether these neurochemical markers identify unique cell populations, or target a common neural circuitry for anorexia, remains unclear.

Role for the Ventromedial Nucleus in Energy Balance

As noted earlier, medial basal hypothalamus lesion studies demonstrated that combined ablation of the arcuate nucleus and ventromedial nucleus produce obesity. In addition to the roles played by arcuate nucleus (including POMC and NPY/AGRP neurons), the ventromedial nucleus contributes to the control of energy balance as well. Indeed, animals null for the transcription factor steroidogenic factor-1 (SF1), which is expressed in most if not all ventromedial nucleus neurons,[145] exhibit ventromedial nucleus dysgenesis and adrenal agenesis and develop late-onset obesity.[97,146,147] Furthermore, deletion of LepRb from SF1 neurons produces obesity due to decreased energy expenditure (especially in animals exposed to high-fat diet),[96] but without increasing food intake, suggesting a role for ventromedial nucleus neurons in the control of energy expenditure. Indeed, the ventromedial nucleus represents an important site for the modulation of sympathetic nervous system outflow.

Serotonergic Control of Energy Balance

The central serotonin (5-hydroxytryptamine [5HT]) system represents another key regulator of energy balance. This system consists of 5HT containing cell bodies that lie in several midbrain raphe nuclei and that project widely through the central nervous system; 5HT acts via 14 different 5HT receptors (HTRs).[148,149] Note also that 5HT represents a widely used and important neurotransmitter in the enteric nervous system of the gut. A variety of compounds that promote HTR signaling, including fenfluramine, promote weight loss.[150] In combination with phentermine (Fen/Phen), the clinical use of fenfluramine successfully reduced food intake and body weight in humans, prior to its removal from the market as a result of heart valve disorders. Subsequent work identified central nervous system HT_{2C}Rs[151–153] as crucial mediators of 5HT-mediated weight loss: The anorexic properties of fenfluramine depend on the presence of *Htr2c*, and mice null for *Htr2c* demonstrate hyperphagic obesity,[154] demonstrating both a physiologic and pharmacologic role for 5HT and HT_{2C}R in the control of feeding and body weight. In 2012 the US Food and Drug Administration (FDA) approved the use of the HT_{2C}R agonist lorcaserin for the treatment of obesity.[150]

The hypothalamic melanocortin pathway mediates the suppression of feeding by 5HT and HT_{2C}R.[155,156] Arcuate nucleus POMC neurons express $5HT_{2C}$Rs,[155] and 5HT directly activates POMC neurons via HT_{2C}R-mediated mechanisms.[155,157,158] Indeed, mice in which expression of the HT_{2C}R is disrupted globally but the receptor is re-expressed only in POMC neurons do not develop the hyperphagia and obesity characteristic of global $5HT_{2C}$R deficiency and respond to HT_{2C}R agonists.[156,159]

Interestingly, while POMC neurons express LepRb and HT_{2C}R, the HT_{2C}R-expressing POMC neurons are distinct from those that express LepRb,[157] suggesting that the two populations of POMC cells may suppress feeding in response to distinct stimuli. Furthermore, the regulation of the central nervous system 5HT system by feeding-relevant stimuli has only recently begun to be explored,[160] and the specific physiologic role played by the 5HT system in the control of food intake remains unclear: While the 5HT system is well known for its role in responses to a variety of psychologic (e.g., restraint, social defeat) and physiologic stressors (e.g., fasting), whether this system mediates the anorectic response to stress has not been defined.

The Role of Insulin and Glucose in the Regulation of Energy Homeostasis

In addition to the well-known role for insulin action on peripheral tissues to control glucose homeostasis, insulin acts in the central nervous system to contribute to the control of energy balance.[161] Insulin injection into the brain can reduce food intake,[161,162] and pan-neuronal deletion of insulin receptors causes mild obesity.[163] Glucose may also act in the brain to contribute to the control of energy balance. Several distinct populations of neurons in the brain sense glucose[164]: some neurons are activated by rising concentrations of glucose (glucose-excited cells), while other classes of neurons are inhibited by rising glucose (glucose-inhibited cells). While the chemical identity of many glucose-sensing cells remain to be determined, glucose-inhibited neurons include arcuate nucleus NPY/AGRP cells, lateral hypothalamic area neurons that contain orexin (OX),[165,166] lateral parabrachial nucleus LepRb cells,[86] and other cell types in the hindbrain and thalamus that likely play roles in energy homeostasis.

Estrogen

In addition to their primary reproductive effects, gonadal steroids also play a key role in regulating energy balance and glucose homeostasis.[167,168] Estrogens exert antiobesity and antidiabetic effects, and lower levels of estrogens in postmenopausal women are associated with an increased risk for developing obesity.[169–171] Ovariectomy reduces estrogen, causing obesity.[172–174] Although ovariectomy increases food intake, hyperphagia does not account for all of the ovariectomy-associated obesity.[174] Indeed, ovariectomy causes rats to gain weight to a similar extent when they are pair-fed to estradiol-treated rats,[175,176] suggesting that endogenous estrogens regulate body weight homeostasis primarily by modulating energy expenditure. Estradiol replacement decreases food intake and increases energy expenditure in rodents, however,[177] indicating that exogenous estrogens may influence both energy intake and energy expenditure.

The alpha isoform of the estrogen receptor (ERα) mediates many of estrogen's effects on body weight homeostasis. For example, female mice with a targeted deletion (knockout [KO]) in the ERα gene (ERαKO) develop obesity and hyperadiposity primarily because of decreased energy expenditure.[178–180] ERα is clearly

required to mediate the normal satiation process because estradiol-induced hypophagia and cholecystokinin (CCK)–induced satiation in wild-type mice are blocked in ERαKO mice.[180]

ERα is expressed in brain regions implicated in the regulation of energy balance, including the arcuate nucleus, paraventricular nucleus, and ventromedial nucleus in the hypothalamus, as well as key brainstem sites such as the nucleus of the solitary tract.[181,182] Consistent with the role for the ventromedial nucleus in controlling sympathetic nervous system outflow and energy expenditure, knockdown or knockout of ERα in the ventromedial nucleus produces mice that are less sensitive to estradiol-induced weight loss and develop increased visceral adipose tissue[167,183,184] due to decreased physical activity and impaired diet-induced thermogenesis; food intake of these animals is not directly affected.[183] Collectively, these results support the hypothesis that ERα signaling in ventromedial nucleus neurons plays an important role in regulating energy expenditure rather than food intake.

Arcuate nucleus POMC neurons express ERα,[167,185,186] and estrogens regulate the excitability of POMC neurons. The number of excitatory inputs to arcuate POMC neurons rises as female mice enter proestrus, when estrogen levels are high.[177,187] Further, central estradiol administration rapidly increases excitatory inputs to POMC neurons.[177,187] The synaptic changes in POMC neurons are tightly paralleled with the effects of estradiol on food intake and body weight.[177] Consistently, female mice lacking ERα only in POMC neurons develop hyperphagia.[167] These observations suggest that estrogen signals in POMC neurons are physiologically relevant in the regulation of food intake.[167] In contrast, although estradiol inhibits the activity of NPY/AGRP neurons, NPY/AGRP cells do not express ERα, suggesting that these effects are mediated indirectly.[188]

Central Nervous System Control of Thermogenesis

The systems that coordinate energy homeostasis balance energy intake with energy expenditure. Energy expenditure is often grouped into three categories: energy required for basal metabolism, energy required for physical activity, and the thermic effect of food.[189] The latter, often referred to as diet-induced thermogenesis, is estimated at 8% to 10% of total expenditure and is defined as the increased energy expenditure in response to energy intake.[189] This process is under the control of the sympathetic nervous system, and energy expenditure is increased by stimulation of β-adrenergic receptors. The importance of sympathetic output in energy balance is revealed by the profound obesity of mice lacking all β-adrenergic receptors when exposed to a high-fat diet.[190] In rodents, the major tissue mediating the energetic response to sympathetic output is brown adipose tissue, which contains adipocytes with dense collections of mitochondria.[191] In addition, brown adipocytes express uncoupling protein 1 (UCP1), which uncouples mitochondrial respiration and thus induces energy expenditure and heat. In humans the key tissue mediating energy expenditure in response to changing energy intake is likely skeletal muscle, although brown adipose tissue has been identified in humans and may play a role in energy balance control.[191,192] It is clear that the sympathetic nervous system is required for coordinated control of energy expenditure and resistance to diet-induced obesity, however.

Key mediators of central nervous system autonomic output are the parasympathetic and sympathetic preganglionic neurons in the brainstem and spinal cord.[73,193] Sympathetic preganglionic neurons are found within the interomediolateral cell column of the spinal cord and extend from the upper thoracic to the upper lumbar cord segments. Different rostral-caudal levels of the intermediolateral column provide innervation to different target organs and thus mediate distinct autonomic responses. For example, sympathetic preganglionic neurons in the upper thoracic levels of the intermediolateral column are thought to be important for control of the heart and cardiovascular system. Additionally, sympathetic preganglionic neurons in thoracic levels T6 to T12 of the intermediolateral column provide innervation of the adrenal gland and endocrine pancreas.[194–196]

Descending central nervous system input to sympathetic preganglionic neurons from key regulatory groups in the hypothalamus and brainstem play a dominant role in autonomic control.[73,197,198] The largest projection to the sympathetic preganglionic neurons in the spinal cord is composed of inputs from the arcuate nucleus, the paraventricular nucleus, and the lateral hypothalamic area,[194,198,199] although other important projections also arise from the brainstem.[200–203]

As noted earlier, the hypothalamic melanocortin system regulates energy expenditure (in addition to controlling food intake). For example, MC4R blockade in mice prevents diet-induced thermogenesis[204] and blocks the upregulation of brown adipose tissue activity.[205] In addition, blocking MC4R signaling prevents the normal induction of thermogenic proteins by high-fat diet feeding.[206] Paraventricular nucleus MC4Rs do not mediate this affect, however, because although activation of the paraventricular nucleus neurons increases energy expenditure, selective activation of MC4R paraventricular nucleus neurons does not.[46]

Sympathetic preganglionic neurons express MC4Rs[207] and receive direct inputs from leptin-responsive POMC neurons, suggesting a potential role for melanocortin action on these cells.[208] Indeed, selective deletion or re-expression of MC4R expression in cholinergic neurons produces high-fat diet sensitivity, failure to increase adipose tissue UCP1, an impairment of diet-induced thermogenesis, and increased cold tolerance.[49,209,210] Thus direct melanocortin input to sympathetic preganglionic neurons mediates important aspects of diet-induced thermogenesis and energy expenditure.

Control of Glucose Homeostasis by the Brain

While peripheral hormones (e.g., insulin) are crucial for glucose homeostasis, autonomic outputs from the central nervous system control important aspects of metabolism.[211–213] The concept that the central nervous system plays a primary role in the control of insulin action and glucose homeostasis originates from physiologist Claude Bernard's 1849 observation that physical lesions to the floor of the fourth ventricle of rabbits dramatically increased blood glucose, resulting in glucose spillover into the urine.[7,214] Bernard concluded that this effect was mediated by the stimulation of autonomic drive to increase hepatic glucose output. While the neuroanatomy and neural mechanism(s) by which this early brainstem lesion altered blood glucose remain somewhat obscure, several hypothalamic systems clearly play important roles in the control of glucose homeostasis.

A variety of nutrient and hormonal cues (e.g., glucose and leptin) act in the hypothalamus to modulate glucose homeostasis.[213,215,216] For instance, as part of the counterregulatory response (CRR) to hypoglycemia, the central nervous system activates the sympathetic nervous system to alter hormone secretion and

promote hepatic glucose output to restore normoglycemia.[217,218] Conversely, other central nervous brain systems control glucose disposal and utilization.

Leptin plays a role in glucose homeostasis as well as energy balance. For instance, *Lep*$^{ob/ob}$ mice exhibit hyperglycemia despite constitutively elevated insulin production. Similarly, lipodystrophic mice and humans exhibit severe insulin resistance that is corrected by leptin replacement.[29,30,219] Leptin action in the arcuate nucleus mediates part of this effect, since restoration of arcuate nucleus LepRb expression in otherwise LepRb-null mice improves glucose homeostasis.[220–222] Leptin mediates at least part of its effects on glucose homeostasis by promoting glucose disposal and utilization: Direct central nervous system infusion of leptin increases glucose disposal and rapidly normalizes blood glucose in *Lep*$^{ob/ob}$ mice.[223,224] Central nervous system infusion of leptin also increases glucose disposal into muscle and brown adipose tissue and normalizes blood glucose in insulin-deficient rodents.[225–227]

Ventromedial Nucleus Control of Glucose and Energy Homeostasis

The ventromedial nucleus plays important roles in the regulation of the sympathetic nervous system and contributes to both hepatic glucose output and glucose disposal.[228–231] Low glucose activates glucose-inhibited ventromedial nucleus neurons that presumably participate in augmenting hepatic glucose output (e.g., to increase blood glucose during the CRR), while glucose-excited ventromedial nucleus neurons are postulated to promote glucose disposal. Since the ventromedial nucleus contains both glucose-inhibited and glucose-excited neurons, pan-ventromedial nucleus manipulations reveal the aggregate function of all cell types within the ventromedial nucleus. For instance, activating all ventromedial nucleus neurons mimics the CRR[232] because unrestrained hepatic glucose output overwhelms the ability of peripheral tissues to dispose of glucose. Conversely, under fed conditions the neurons that promote glucose utilization play a dominant role in glycemic control; thus disrupting overall ventromedial nucleus function causes glucose intolerance and blunts energy expenditure to promote weight gain.[233] Similarly, disruption of glutamatergic neurotransmission within the ventromedial nucleus (a predominantly glutamatergic nucleus) impairs glucose tolerance and increases body weight at baseline but blunts the CRR to glucoprivation.[234]

Ventromedial nucleus LepRb neurons likely contribute to the control of glucose disposal (as well as energy expenditure), since intraventromedial nucleus leptin increases glucose uptake into muscle and brown adipose tissue, and ablation of LepRb in the ventromedial nucleus decreases glucose tolerance.[96,227] Activation of ventromedial nucleus LepRb neurons (unlike the activation of all ventromedial nucleus neurons) does not mimic the CRR, however,[232] suggesting that non-LepRb ventromedial nucleus neurons mediate the CRR and other responses that increase hepatic glucose output. The ventromedial nucleus neurons that promote hepatic glucose output and mediate the CRR have yet to be definitively identified.

POMC Neurons Sense Changes in Glucose Concentration

The hypothalamic melanocortin system, including arcuate nucleus POMC neurons, also contributes to the control of glucose homeostasis.[215,220,221] For example, mice lacking MC4Rs are hyperinsulinemic prior to the onset of obesity,[44,235] while intracerebroventricular MC4R agonists decrease plasma insulin levels in lean and obese mice.[235] Moreover, MC4R-deficient humans display greater hyperinsulinemia than would be expected from their degree of obesity alone, and children as young as 12 months old are hyperinsulinemic.[236,237] Additionally, although ablation of LepRb from arcuate nucleus POMC cells minimally impacts body weight, it promotes glucose intolerance. Conversely, restoration of POMC neuron LepRb expression on a LepRb-null background improves glucose homeostasis without substantially altering body weight.

Glucose-Inhibited Neurons of the Arcuate Nucleus and Lateral Hypothalamic Area

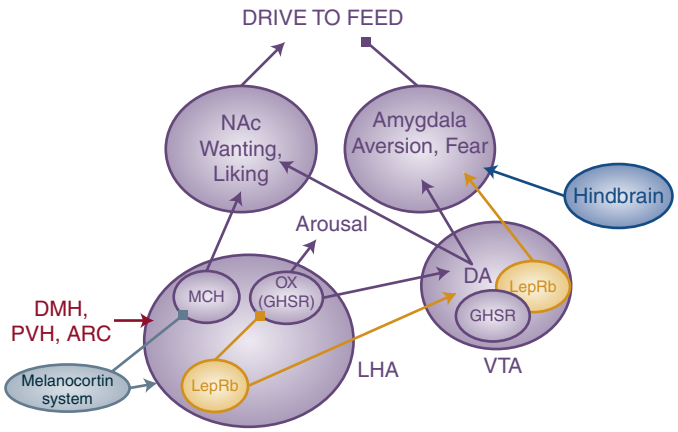
While independent effects of blood glucose, such as food intake and body weight, are less well established for arcuate nucleus NPY/AGRP cells than for POMC neurons, decreased glucose concentrations activate NPY/AGRP cells, suggesting a potential role for these cells in the response to low blood sugar (perhaps by promoting food intake during hypoglycemia). Furthermore, low glucose activates orexin-producing neurons in the lateral hypothalamic area.[165,166] Since orexin neurons play key roles in the control of arousal (see upcoming discussion), these cells could promote hypoglycemia awareness.

Intersection of Energy Balance and Reward Circuits

Behaviorally, two systems govern eating: the circuits that control the incentive and reward values (wanting and liking) of food and the satiety system, which promotes meal termination associated with the sensation of fullness.[238] Although physiologically integrated, they are often studied and described as distinct entities to reduce complexity.

Overlapping central nervous systems mediate motivation for natural and artificial rewards (e.g., food and drugs of abuse, respectively). The mesolimbic dopamine system, composed of dopaminergic neurons in the ventral tegmental area that project to the nucleus accumbens and other places, play important roles in motivation and reward.[239] For example, mice lacking the ability to produce dopamine normally die of starvation but will resume feeding after reintroduction of dopamine into the nucleus accumbens and nearby structures.[240,241] Also, lesions of the nucleus accumbens reduce food intake[242] and the desire to work for food.[243]

The neurohormonal systems that control food intake and metabolism also modulate the mesolimbic dopamine system and parameters of reward. Indeed, leptin suppresses the motivation of animals to perform rewarding brain self-stimulation,[244] and subpopulations of ventral tegmental area neurons express LepRb or ghrelin receptor.[245,246] Furthermore, ablating ventral tegmental area LepRb slightly increases the intake of rewarding foods. Ventral tegmental area LepRb neurons may also modulate anxiety-related behaviors.[247,248] Similarly, ghrelin administration increases the reward value of food, and ablation of ghrelin receptor in the ventral tegmental area increases food intake, while restoration of ventral tegmental area ghrelin receptor in ghrelin receptor-null mice increases feeding as well as partially restoring the orexigenic effects of ghrelin.[249] Taken together, these findings suggest that signals such as leptin and ghrelin not only affect hypothalamic pathways but also control reward behaviors—in part by direct action within the central nervous system reward circuitry.



• **Fig. 39.7** Reward circuits involved in feeding control. Core components of the mesolimbic dopamine system are shown in *light purple,* brainstem circuits are in *dark blue,* leptin receptor pathways are in *orange,* and melanocortin pathways are in *light gray. ARC,* arcuate nucleus; *DA,* dopamine; *DMH,* dorsomedial hypothalamic nucleus; *GHSR,* ghrelin receptor; *LepRb,* leptin receptor; *MCH,* melanin-concentrating hormone; *NAc,* nucleus accumbens; *OX,* orexin; *PVH,* paraventricular hypothalamic nucleus.

The Lateral Hypothalamic Area Links Food Intake Control and Arousal

Ablation of the lateral hypothalamic area abrogates the motivation to eat (as well as the motivation for other rewards),[250] and many types of lateral hypothalamic area neurons innervate components of the mesolimbic dopamine system. Melanin-concentrating hormone and the orexin peptides (orexin-A and orexin-B, also known as hypocretins) are uniquely expressed in distinct populations of lateral hypothalamic area neurons and contribute distinctly to the control of the mesolimbic dopamine system and feeding.[251–253] Both populations project widely throughout the brain[254,255]; the expression patterns of the receptors for these peptides are also widespread (Fig. 39.7).[256–258]

Injection of melanin-concentrating hormone into the brain increases food intake.[251] Mice lacking melanin-concentrating hormone are hypophagic and lean, while mice that overexpress melanin-concentrating hormone become obese.[259,260] In addition, mice lacking both melanin-concentrating hormone and leptin are leaner compared to mice that lack leptin but express melanin-concentrating hormone.[261] Melanin-concentrating hormone modulates the incentive value of food and drugs of abuse, at least in part via direct action in the nucleus accumbens.[262] Thus melanin-concentrating hormone contributes to the control of food intake and energy balance by modulating the reward-encoding systems of the brain.

Orexin contributes to the control of energy balance, but its role is more complex.[263,264] While initially thought to promote feeding based upon the finding that central injection of orexin peptides increases feeding and body weight,[252,265] orexin-null mice, dogs, and humans exhibit narcolepsy (a cataplectic sleep syndrome), and orexin promotes hypervigilance. Hence the control of wakefulness and vigilance represents a major function for orexin.[266,267] Indeed, mice null for orexin or its receptors exhibit obesity due to decreased activity and energy expenditure rather than changes in food intake. Thus the likely mechanism by which orexin injection promotes food intake is by the increase in wakefulness during the normal sleep cycle. Orexin neurons express ghrelin receptor, and ghrelin directly activates these cells.

While neither orexin nor melanin-concentrating hormone neurons express LepRb, leptin acts directly on a distinct set of lateral hypothalamic area LepRb neurons.[268] These lateral hypothalamic area LepRb neurons inhibit orexin neurons and also project directly to the ventral tegmental area and a few other midbrain sites and modulate the function of the mesolimbic dopamine system.[269–271] Disruption of LepRb in the neurotensin-expressing subpopulation of lateral hypothalamic area LepRb neurons alters mesolimbic dopamine function to decrease locomotor activity and promote obesity. Thus leptin action in the lateral hypothalamic area controls orexin cells and ventral tegmental area cells to modulate the mesolimbic dopamine system. Leptin likely controls melanin-concentrating hormone neurons via its action on the hypothalamic melanocortin system.[272]

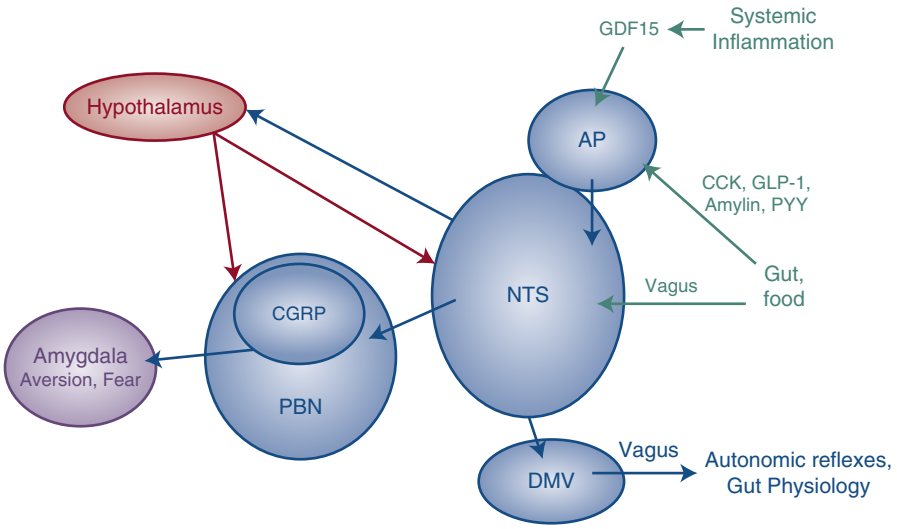
Brainstem Circuits That Regulate Energy Balance

Circuits in the brainstem play important roles in the control of food intake, as well.[273–276] The brainstem represents a crucial entry point for a wide variety of signals from visceral organs, including the gastrointestinal tract, which mediate parasympathetic reflex arcs to control gut physiology and which convey signals further into the brain to promote meal termination and to modulate emotional valence relevant to gut status (e.g., nutrients in the gut promote positive valence, while toxins in the gut promote aversion) (Fig. 39.8). These gut signals converge in the dorsal vagal complex, which contains the nucleus of the solitary tract, the dorsal motor nucleus of the vagus, and the area postrema.

The area postrema is a circumventricular organ (and thus lies outside the blood-brain barrier, facilitating the sensing of circulating factors) that lies directly above the nucleus of the solitary tract.[277] Circulating signals of gut status (including a variety of gut-derived peptides) activate area postrema neurons, which relay those signals into the nucleus of the solitary tract and the lateral parabrachial nucleus.[275]

Sensory afferent signals carried by the glossopharyngeal and vagus nerves (e.g., taste, gastric stretch, nutrients in the gut and portal vein) innervate the nucleus of the solitary tract, which represents the primary site for innervation by vagal afferents from the gut.[278–280] The cell bodies of vagal afferent nerves lie in the nodose ganglia; those deriving input from the upper gastrointestinal tract respond to three basic stimuli: gastric and duodenal distention or contraction, chemical contents of the lumen, and gut peptides and neurotransmitters released from the stomach and duodenum in response to nutrients.[278] Recent work demonstrates vagal afferent neurons that express the receptor for glucagon-like peptide-1 (GLP1; GLP1R) and convey signals of stomach distension, while those that express G protein–coupled receptor 65 (GPR65) sense nutrients in the gut.[281]

The activation of nucleus of the solitary tract neurons that receive feeding-related information from the gut suppresses food intake, consistent with a role for certain nucleus of the solitary tract neurons in the control of meal termination.[282,283] While the nucleus of the solitary tract innervates a variety of hypothalamic structures (e.g., the paraventricular nucleus and dorsomedial nucleus), other important nucleus of the solitary tract targets include brainstem sites, including the dorsal motor nucleus of the vagus and the lateral parabrachial nucleus. The dorsal motor nucleus of the vagus contains parasympathetic preganglionic neurons, which innervate and provide parasympathetic input to the



• **Fig. 39.8** Brainstem circuits involved in feeding control. Core hypothalamic circuits are shown in *red,* core components of the mesolimbic dopamine system are shown in *light purple,* brainstem circuits are in *dark blue,* peripheral signals are *green*. *AP,* area postrema; *CCK,* cholecystokinin; *DMV,* dorsal motor nucleus of the vagus; *GCRP,* calcitonin gene-related peptide; *GDF15,* growth and differentiation factor-15; *GLP1,* glucagon-like peptide-1; *NTS,* nucleus of the solitary tract; *PBN,* parabrachial nucleus; *PYY,* peptide YY.

entire gastrointestinal tract. Meal-activated nucleus of the solitary tract cells project to and modulate the activity of dorsal motor nucleus of the vagus neurons that alter parasympathetic tone to the stomach, decreasing the rate of gastric emptying as part of a meal-activated reflex arc.

Major projections of the lateral parabrachial nucleus, which receives input from the area postrema and the nucleus of the solitary tract, include the thalamus, the amygdala, and several hypothalamic sites.[284] Recent findings identified a set of calcitonin gene–related peptide (CGRP)–expressing lateral parabrachial nucleus neurons required for the suppression of food intake in response to aversive gut stimuli.[105,285,286] Stimuli that promote gut malaise (such as lithium chloride [LiCl] injection in rodents and some chemotherapeutic agents) activate these neurons. Furthermore, lateral parabrachial nucleus CGRP cells act via their projections to the amygdala to mediate both the suppression of food intake and the aversive response to such stimuli. Arcuate nucleus NPY/AGRP neurons also innervate lateral parabrachial nucleus CGRP neurons, inhibiting these cells to promote the consumption of disfavored foods when NPY/AGRP neuron activity is high (e.g., during prolonged fasting). The withdrawal of NPY/AGRP neuron-mediated inhibition of lateral parabrachial nucleus CGRP cells activates their amygdala projection to suppress food intake.[45,105,137,287–289] Other lateral parabrachial nucleus neurons appear to mediate the nonaversive suppression of food intake in response to distinct signals,[46] but the molecular identity of this putative second set of lateral parabrachial nucleus cells has yet to be determined.

Gut Peptides Involved in Satiety and Hunger

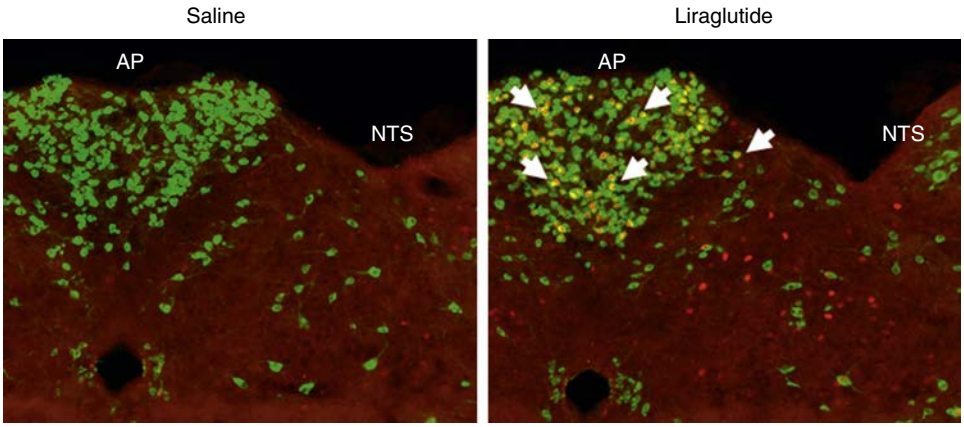
In addition to signals from gut distention, gut peptides stimulated by meal intake mediate satiety via centers in the brainstem. These gut peptide–derived brainstem signals can be modified by signals that mediate long-term energy balance, such as leptin, which acts in part via nucleus of the solitary tract LepRb neurons to increase the meal-terminating response to a number of gut peptides.[290–292] While gut-peptide signaling and brainstem signals (in general) are often considered to modulate short-term effects on feeding (e.g., decreasing meal size, which is compensated by increased meal frequency), gut peptides also interact with hypothalamic circuits that mediate long-term energy balance. Furthermore, pharmacologic agonists for many gut peptide receptors suppress food intake and can promote chronic weight loss in humans as well as in preclinical models.[293]

GLP1 Action in the Central Nervous System

Current strategies for the treatment of type 2 diabetes mellitus are not optimally effective, and even multiple drug combinations often fail to normalize glycemia in a sustained manner in many patients. Hence there remains intense interest in new therapies that safely and effectively lower blood glucose in diabetes. Over the past decade, drugs that enhance the action of incretins have seen increasing use for the treatment of type 2 diabetes and obesity.[294,295] Incretins are hormones released by the gut in response to oral ingestion of nutrients that act to increase insulin secretion. The prototypical incretin hormone is GLP1, which is derived from proglucagon; upon secretion, GLP1 undergoes rapid degradation by dipeptidyl peptidase-4 (DPP4) in the circulation.[296] While pancreatic alpha cells mainly generate glucagon from proglucagon in pancreatic alpha cells, the L cells of the intestine and the proglucagon-expressing cells in the brain mainly produce GLP1 and GLP2.[294,295]

GLP1 receptor (GLP1R) agonists induce multiple desirable antidiabetic and antiobesity actions, and protease-resistant long-acting GLP1 analogues are currently available for the treatment of type 2 diabetes and/or obesity.[294,295] In addition to enhancing glucose-dependent insulin secretion, slowing gastric emptying, and inhibiting gastric acid secretion, these compounds reduce food intake.

Given the increasing use of GLP1R analogues and DPP4 inhibitors (which block GLP1 degradation) for the treatment of diabetes and the FDA approval in 2014 of liraglutide for the treatment of obesity, understanding the central actions of GLP1 is important for predicting the biologic consequences of sustained GLP1R agonist administration. The effects of GLP1R agonists on



• **Fig. 39.9** Activation of area postrema/nucleus of the solitary tract glucagon-like peptide-1 receptor (GLP1R) neurons by peripheral administration of GLP1R agonist. *Glp1r*^{cre} mice on a green fluorescent protein (GFP) reporter background labels GLP1R expressing cells with GFP *(green)*. Neuron activation can be assayed by increased expression of the immediate early gene FOS. Treatment of fasted mice with the long-acting GLP1R agonist, liraglutide, stimulates FOS expression in GLP1R neurons *(green)* in the area postrema (AP) and nucleus of the solitary tract (NTS) 2 hours after administration. Arrows mark colocalization of FOS and GLP1R expression *(orange)*. (Images courtesy Jessica Adams, PhD, University of Michigan.)

appetite may be mediated in part via inhibition of gastric emptying and may also reflect direct effects of GLP1R agonists on satiety, including the induction of nausea and other aversive gut symptoms.[297–299]

Initial interest in the central nervous system actions of GLP1 stemmed from the observation that GLP1 inhibits food intake.[300,301] In humans, peripheral GLP1 administration to normal and diabetic subjects induces satiety and reduces food intake in short-term studies.[302–305] Chronic continuous GLP1 administration to human diabetic subjects is associated with weight loss.[306,307] The central nervous system expression of GLP1 is restricted to a population of caudal nucleus of the solitary tract neurons that receive and process viscerosensory information from the gastrointestinal tract. Thus these nucleus of the solitary tract GLP1 neurons are in a prime position to rapidly modify ingestive behavior in direct response to gut signals; these cells also express LepRb and respond to leptin. Indeed, activation of GLP1 neurons in the nucleus of the solitary tract suppresses food intake and decreases metabolic rate, and chronic activation in the setting of high-fat diet–induced obesity leads to weight loss consistent with the antiobesity effects of chronic GLP1R agonist treatment.[308]

Numerous brain areas that participate in the control of food intake receive projections from nucleus of the solitary tract GLP1 neurons and express Glp1r mRNA, including the area postrema, lateral parabrachial nucleus, paraventricular nucleus, dorsomedial nucleus, and arcuate nucleus.[281,309,310] Infusion of GLP1R agonists directly into several of these brain regions suppresses feeding consistent with the idea that GLP1R agonists exert their beneficial effects through several brain regions. Deleting *Glp1r* from specific cell types in the mouse demonstrated that the feeding effects but not the glucose-lowering effects of GLP1R agonists are located in the central nervous system.[311] Several groups have ablated *Glp1r* from POMC, paraventricular nucleus, or ventromedial nucleus cells[312] without affecting anorexic response to GLP1R agonists. Broader deletion of GLP1Rs from glutamatergic neurons dramatically attenuates the response to GLP1R agonists, however, suggesting that a crucial population of GLP1R cells may lie in the hindbrain (e.g., in the area postrema [the main site where exogenous GLP1R agonists activate GLP1R-expressing neurons]) (Fig. 39.9).[310]

Peptide YY (PYY)

Endocrine cells in the ileum and colon[313] release both a full-length (i.e., 36–amino acid) and a 34–amino acid (PYY[3-36]) form of PYY following a meal.[314] PYY is a potent agonist of both Y1 and Y2 receptors, whereas PYY(3-36) is a Y2-specific agonist, with approximately a 1000 times greater affinity for the Y2 versus Y1 receptor.[315] Y1-preferring and Y2-preferring binding sites are located in the area postrema, nucleus of the solitary tract, and dorsal motor nucleus of the vagus. Peripheral administration of PYY in the rat activates neurons in the area postrema, as well as in the nucleus of the solitary tract.[316] Infusion of PYY within the physiologic range has numerous effects, including inhibition of gastric emptying,[317] gastric acid secretion,[318] and pancreatic exocrine secretion.[318] These actions of PYY appear to be mediated by PYY action directly on the nucleus of the solitary tract and dorsal motor nucleus of the vagus as well as on gastric mucosal enterochromaffin-like cells (for review see Yang[319]). Both Y1 and Y2 receptors are found within the nucleus of the solitary tract and dorsal motor nucleus of the vagus.[320] For example, PYY appears to inhibit gastric acid secretion primarily through vagal innervation of the gastric fundus.[321] The ability of low-dose PYY and PYY(3-36) to inhibit the activity of dorsal motor nucleus of the vagus efferents appears to be Y2 mediated, whereas Y1 agonists appear to stimulate these cells.[319]

Peripheral administration of PYY(3-36) in pharmacologic doses suppresses feeding in rodents as well as humans,[276,322,323] thus suggesting a potential function as a satiety factor. The mechanisms underlying the action of PYY(3-36) in reduction of food intake have not been fully elucidated. Intraperitoneal administration of PYY(3-36) activates a small number of arcuate POMC neurons.[322,324] However, vagotomy also blocks PYY(3-36)-induced inhibition of feeding,[325] and the inhibition of feeding by PYY(3-36) persists in the MC4R knockout mouse[326] and in obese *A^{y}/a* mice.[327] Thus the release of melanocortin peptides derived from POMC and their subsequent activation of the MC4R does not appear to be required for the inhibition of feeding by PYY(3-36). Furthermore, PYY appears to induce conditioned taste aversion in

rodents and nausea in some human studies, suggesting an aversive effect of the peptide likely to involve brainstem sites such as the area postrema. Interestingly, however, PYY also contributes to the inactivation of NPY/AgRP neurons during feeding.[328]

Despite the short-acting anorexic effects of the peptide in most experimental models, two knockout studies demonstrate that removal of the gene encoding PYY produces obese hyperinsulinemic mice,[329,330] suggesting that the peptide may also play an important role in the regulation of long-term energy stores. One study shows the peptide may be specifically involved in the satiating effects of protein in the diet.[329]

Cholecystokinin (CKK)

Produced by the gastrointestinal tract in response to meal ingestion, the diverse actions of CKK include stimulation of pancreatic enzyme secretion, inhibition of gastric motility, activation of intestinal motility, and the acute suppression of feeding. Early experiments administering CCK peripherally supported a role for increased CCK levels in the early termination of a meal.[331,332] The finding that repeated injections of CCK lead to reduced meal size without a change in body weight, due to a compensatory increase in meal frequency, argued against CCK acting as a signal regulating long-term energy stores.[333,334]

Two subtypes of the CCK receptor belonging to the G protein–coupled family of receptors have been described: CCKA and CCKB. Studies using CCK receptor–specific antagonists as well as surgical or chemical vagotomy demonstrated that the satiety effects of CCK are specifically mediated via CCKA receptors on afferent vagal nerves.[335–337]

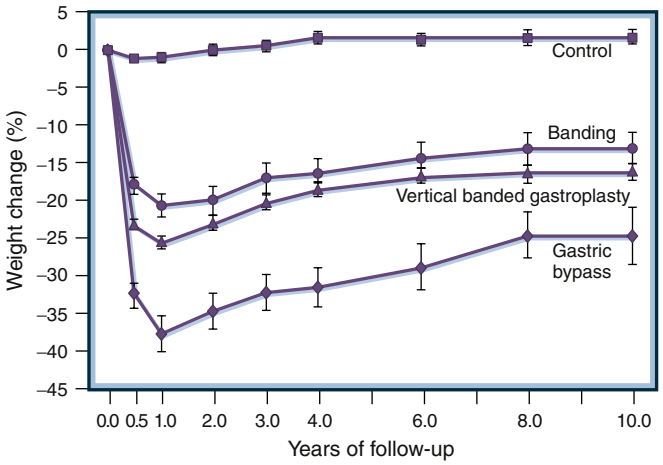
Amylin

Amylin, or islet amyloid polypeptide (IAPP), is a 37–amino acid polypeptide that is cosecreted with insulin from pancreatic beta cells in response to nutrient intake and insulin secretagogues.[338,339] In humans, pancreatic IAPP can form amyloid fibrils that are hypothesized by some to play a role in the decline in islet cell function that accompanies type 2 diabetes.[340,341] In addition, amylin has been shown to impair gastric motility and have effects independent of insulin on energy homeostasis.

Both peripheral and intracerebroventricular infusions of amylin inhibit food intake acutely and, during chronic infusion, lead to a sustained reduction in body weight.[342–344] Amylin readily enters the brain, and high-affinity amylin-binding sites have been found in several brain regions, including the hypothalamus and brainstem (including the area postrema and nucleus of the solitary tract).[342,345,346] The exact site(s) of anorectic amylin action remain unclear.

GDF15

Also known as MIC1, growth and differentiation factor-15 (GDF15) is a distant relative of the transforming growth factor-β family, most closely related to the glial-derived neurotrophic factors (GDNF).[347,348] While GDF15 is not technically a gut-derived peptide, it acts via the central nervous system to control food intake. Most tissues produce GDF15 at low levels, but a variety of cellular stressors (including hypoxia, ER stress, mitochondrial dysfunction, etc.) increase GDF15; GDF15 is not controlled by fasting, feeding, or most other nutritional interventions. Hence circulating GDF15 levels increase in disease processes that produce cellular stress, including in malignancies (most tumor cells produce GDF15), heart failure, kidney failure, and systemic infection. Exogenous GDF15 suppresses feeding (including at levels observed in the aforementioned disease states), and interference with GDF15 action provides protection from the anorexia and weight loss associated with these disease states.



• **Fig. 39.10** Apparent alteration of the adipostatic set-point after bariatric surgery. (Modified from Sjostrom L, Lindroos AK, Peltonen M, et al. Swedish obese subjects study scientific group. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med.* 2004;351:2683–2693.)

GDF15 acts via the GDNF receptor alpha-like receptor (GFRAL), which is expressed only in the area postrema (and to a lesser extent in the nearby nucleus of the solitary tract).[349–352] Mice null for *Gfral* (or *Gdf15*) display mildly increased body weight, especially on a high-fat diet, and GFRAL is required for the anorectic effects of GDF15 and (like GDF15) for weight loss in response to a variety of pathophysiologic insults. Thus GDF15 appears to act via area postrema GFRAL to limit food intake in response to pathologic conditions that cause cellular stress rather than in response to alterations in nutritional or metabolic status.

Bariatric Surgery

In contrast to most pharmacologic therapies, certain types of bariatric surgeries, such as the Roux-en-Y gastric bypass and vertical sleeve gastrectomy not only cause significant weight loss but also mediate the maintenance of reduced body weight for many years (Fig. 39.10). Furthermore, improvement in diabetes following these procedures often occurs before significant weight loss and is sustained in a significant percentage of patients. Both these findings imply that the procedures mediate a profound impact on the neuroendocrine control of long-term energy stores. That bariatric surgery appears capable of creating a new stable weight set-point suggests that hormonal or vagal and nutritional signals from the gut may indeed have a more profound impact on long-term energy homeostasis than previously thought.

Many researchers are attempting to identify the peripheral and central changes that produce weight loss and improve glucose control following these procedures, with the ultimate goal being to reproduce the response to surgery using a nonsurgical approach. Circulating GLP1 is elevated following sleeve gastrectomy[353] and gastric bypass,[353–355] as are bile acids. GLP1R deletion fails to modify weight loss or glucose responsiveness in a sleeve gastrectomy in the mouse however,[356] although alteration of the bile acid receptor (FXR) blunts the response to sleeve gastrectomy.[357] Presumably the alterations in feeding and body weight following sleeve gastrectomy result from increased activity of neurons within the area postrema and/or the dorsal vagal complex.

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The complete list of references is available online at ExpertConsult.com.

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40 Obesity

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CHAPTER OUTLINE

KEY POINTS

- Body mass index permits stratification of risk for obese individuals, but healthy ranges are specific to different ethnic groups.
- Body mass index is an imprecise measure of adiposity.
- Energy homeostasis involves complex molecular and physiologic processes that determine both food intake and energy expenditure.
- Both environmental and genetic factors contribute to energy homeostasis.
- Most human obesity is polygenic, and single gene mutations are responsible for a relatively small number of cases of obesity.
- Currently available weight-loss treatments include dietary intervention, increased physical activity, behavior modification, pharmacotherapy, and surgical approaches.

Obesity is a chronic disease and a significant risk factor for multiple serious medical problems. Obesity is also associated with impaired quality of life, and considerable economic and social burden due to increased health care costs and loss of productivity.[1,2] This chapter addresses key clinical and pathophysiologic issues in obesity.

Obesity is not a new phenomenon. It was described by Hippocrates, who made observations on the importance of diet in individuals with excess weight, also noting that the Scythians were obese because they were sedentary, and describing them as flabby and torpid.[3] Galen, writing in the second century of the common era, described obesity as an illness for which he prescribed diet and exercise and used the term *polysarkia* to refer to morbid obesity.[4] Obesity as a recognizable medical condition persists throughout history usually as a problem of the wealthy and sedentary, such as Henry VIII of England, who weighed more than 300 pounds (136 kg) and likely suffered from type 2 diabetes and gout at his death.

In the United States, rates of obesity became worrisome in the 1990s as overweight and obesity increased in the population. Until the past two decades, rates of both were higher in the United States than in other countries. Recently other nations have caught up, and prevalence is higher now in Mexico and countries in the Middle East than in the United States. Considering that the genetics of the population has not changed over the interval during which the dramatic rise in rates of obesity have occurred, the increase must result from a complex interplay of genes and environment. Many individuals have a predisposition toward eating and storing calories when they are available. The tendency to store excess fat is common in many mammals, as seen in the incidence of obesity in pets in developed countries. This may be a consequence of selection over time for effective storage, as starvation is an acute threat to survival, whereas the comorbidities of obesity take years to decades to become problematic.

Mammals have evolved to acquire food and store it as fat against times of food scarcity. Until the modern era, food scarcity had been a substantial threat to existence over excess calories. A pound of fat, containing 3500 calories, may provide 2 to 4 days of survival for an individual without any food supply. In a very calorie restricted environment, 50 pounds of extra fat would make a substantial survival difference. Lean persons will die after only approximately 60 days of starvation, when more than 35% of body weight is lost. Obese persons can tolerate longer fasts, even for more than 1 year, without major adverse effects. In the longest reported fast, a 456-pound (207-kg) man ingested only acaloric fluids, vitamins, and minerals for 382 days and lost 278 pounds (126 kg), or 61% of his initial weight.

Assuming genetic traits and a physiology favoring eating and storing excess calories dating back to antiquity, the modern obesity epidemic is more likely explained by changes in the food environment. As a consequence of modern agriculture and ease of transportation of food to the consumer, food is cheaper, more readily available, and more varied than ever before, and together with genetic predisposition much of the world population is now fighting obesity. Alternative explanations for obesity have also been proposed. For example, one posits that mammals can risk excess body weight when they are not subject to predation and that food uncertainty does not play a role in driving excess weight gain.[5]

Definition of Obesity

Body Mass Index

Obesity represents an unhealthy excess in body fat mass. The current practical definition of obesity is determined by calculating body mass index (BMI), by dividing an individual's weight in kilograms by the square of height in meters (BMI = weight [kg]/height [m^2]). Underweight, normal weight, overweight, and obesity are defined on the bases of increased risk for mortality and predisposition of comorbidity. Table 40.1 summarizes the classification of weight status by BMI proposed by the major national and international health organizations. However, BMI is an imperfect index of excess fat. BMI calculations do not factor muscle mass so that at any given weight, an individual with greater muscle mass will be less liable to comorbidities. Furthermore, individuals classified as obese may be healthy without any comorbidity. Other as yet unidentified factors predispose some individuals to metabolic consequences early in life, whereas in some, comorbidities evolve later, after decades of obesity. The relationship between BMI and comorbidity is visualized in Fig. 40.1. Increased mortality at lower BMI may represent the contribution of smoking; eating disorders; and chronic disease states such as heart failure, cancer or malabsorption. As BMI rises, mortality increases, as increased fat increases risk for diabetes, cardiovascular disease, liver disease, cancer, sleep apnea, arthritis, and other diseases.

The precise magnitude of excess weight associated with increased mortality risk remains somewhat unclear. Overweight and even class I obesity was not associated with increased mortality in a report on the National Health and Nutrition Examination Survey data collected between 1971 and 2000.[6] However, studies concur that risk is increased for class II (BMI >35 kg/m^2) or higher-magnitude obesity.

Body Fat Distribution

The relationship between BMI and health risk is not absolute but is influenced by body fat distribution, age, concomitant medical illness, weight gain, aerobic fitness, and ethnicity.

Excess body fat is not distributed equally in all people. Some obese persons develop excess visceral obesity and will be at higher risk of comorbidities than those with excess subcutaneous fat. Waist circumference correlates with abdominal fat mass and is a surrogate marker for visceral obesity, and thus is a predictor of health outcomes in adult men and women regardless of age or ethnicity. The relationship between waist circumference and comorbidities is strongest for diabetes risk, and waist circumference is an independent and better predictor of diabetes than BMI. Waist circumference thresholds for increased cardiometabolic risk are 40 inches (102 cm) in Caucasian men and 35 inches (88 cm) in women; these cutoff values were derived from waist circumference values that correlated with a BMI of 30 kg/m^2 or greater, based on populations of European origin.

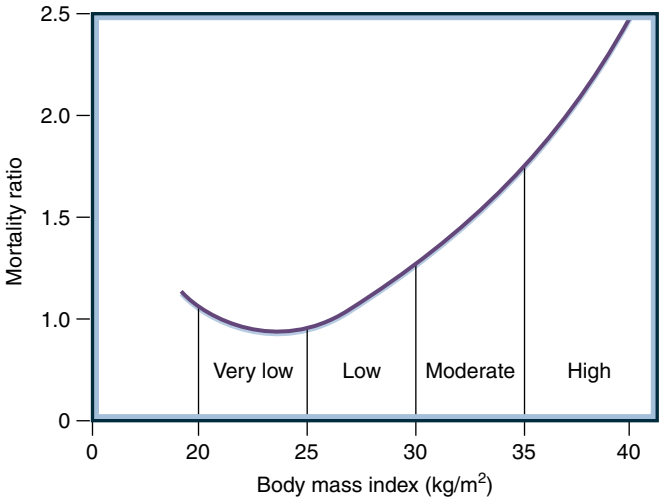
BMI-associated health risk is influenced by ethnicity. Asian-Pacific populations are at increased risk for the development of diabetes and cardiovascular disease at lower BMI ranges than other populations. Accordingly, in these populations, the World Health Organization proposed lower BMI cutoffs for public health action[7] (see Table 40.1). Asian populations also tend to have more visceral obesity at lower BMI compared with other ethnic groups, and, further, at any given waist circumference, the relative risk of mortality is higher. World Health Organization guidelines define waist circumference thresholds in the Asian population as 90 cm for men and 80 cm for women. Different levels have been suggested in Japan and China, with cutoff values of 85 cm for men and 80 cm for women, and slightly lower values have been suggested in India. Black individuals also appear to have increase disease risk at lower BMI thresholds than individuals of European descent—for example, the equivalent risk BMI for diabetes is 30 kg/m^2 in Caucasian women and 26 kg/m^2 in black women.[8] There is also a significant sex difference in comorbid disease risk, with men having higher risk than women across the range of ethnicities.[9]

The risk of developing obesity-associated diabetes or cardiovascular disease can be modified by aerobic fitness. The incidences of diabetes and cardiovascular fatality are lower in those who are physically fit, as defined by maximal ability to consume oxygen during exercise, compared with those who are unfit across a range of body adiposity. For example, in a population of middle-age men, low aerobic capacity was associated with increased mortality rates, independent of traditional risk factors, including smoking, blood pressure, and serum cholesterol, during more than 40 years of follow-up.[10]

TABLE 40.1 Weight Classification by BMI

Weight Classification	Obesity Class	BMI (kg/m^2) Europeans	BMI (kg/m^2) Asians	Risk of Obesity-Related Diseases
Underweight		<18.5	<17.5	Increased
Normal weight		18.5–24.9	17.5–22.9	Normal
Overweight		25.0–29.9	23.0–27.4	Increased
Obesity	I	30.0–34.9	27.5–32.4	High
	II	35.0–39.9	32.5–37.5	Very high
Extreme obesity (polysarkia)	III	≥40.0	≥37.5	Extremely high

BMI, Body mass index.



• **Fig. 40.1** Relative risk for morbidity and mortality in obese European populations. The thresholds for defining obesity are based on risk. Although risk rises even at fairly low body mass index, the inflection thresholds are seen in the body mass index range greater than 35 kg/m^2, especially in men.

Physiology of Energy Balance

Energy homeostasis, defined as the balance between energy intake and energy expenditure, is regulated by complex molecular and physiologic processes. Control of energy homeostasis requires physiologic integration of biologic signals from different organs including fat, muscle, liver, gut, and brain; nutrient-related signals; and postprandial neural and hormonal influences.

Regulation of energy intake is complicated because it includes both homeostatic and hedonic feeding. Homeostatic feeding is the component responding to signals aimed at maintaining weight, such as hunger occurring with long intermeal intervals, whereas hedonic feeding is defined as excess eating in the satiated state based on availability of highly palatable food or social cues that encourage eating. Eating can also be influenced by emotional moods including stress or depression, which lead to overeating in some individuals while decreasing food consumption in others.

Central Nervous System Regulation of Appetite

The hypothalamus is an important area for integrating complex signals that govern food ingestion. Pro-orexigenic neurons in the arcuate nucleus, which express neuropeptide Y (NPY) and agouti-related protein (AgRP), and appetite inhibiting neurons, which express the proopiomelanocortin (*POMC*) gene and process its product to α-melanocyte-stimulating hormone (αMSH), are two well-studied hypothalamic networks that inversely regulate weight. Key issues are summarized in the following (for additional details, see Chapter 39).

Administration of both NPY and AgRP peptides into rodent brain markedly increased food intake, even in satiated rats. Direct stimulation of these neurons also induces feeding. However, eliminating expression of NPY, AgRP, or both peptides in transgenic mice is associated with at best minimal feeding phenotypes. Full ablation of the neuronal population is required to observe decreased feeding, suggesting that the neurotransmitter expressed by theses neurons, γ-aminobutyric acid, is more important than either peptide in regulating appetite. POMC neurons, a discreet population localized to the arcuate nucleus, play a role to inhibit eating. The precursor protein POMC is processed to αMSH, which suppresses feeding. Elimination of POMC expression leads to obesity in both rodents and humans. Furthermore, a downstream receptor for αMSH in the brain, the melanocortin 4 receptor (MC4R) is critical for maintaining normal energy balance. MC4R is regulated by both αMSH and agouti, and the output of MC4R neurons in the paraventricular nucleus and other locations represents a balance of AgRP and MSH signaling. The finding that *MC4R* deletion in mice and mutations of *MC4R* in humans are associated with obesity confirms the central role of these neurons in energy balance. Indeed, in humans, *MC4R* mutations are the most common cause of monogenic obesity, accounting for about 5% of early-onset familial obesity.

Other areas in the hypothalamus contribute to feeding and weight. For example, the lateral hypothalamus contains populations of neurons expressing the neuropeptide melanin-concentrating hormone (MCH), and a distinct population expresses orexin. In rodents, MCH administration into the brain leads to an acute, robust increase in feeding, whereas genetic deletion of the neuropeptide leads to leanness.[11] MCH antagonists inhibit feeding in several species; however, they are frequently associated with undesirable side effects complicating the development of an effective therapeutic.[12] MCH neurons receive inputs from the arcuate nucleus, and MCH appears to be important in the action of leptin. However, human biology is complicated by the presence of two receptors (MCH-R1 and MCH-R2) in higher mammals. Orexin is another lateral hypothalamic peptide implicated in feeding but more important in generalized arousal, as rodents, dogs, and humans without orexin have narcolepsy.

The endocannabinoid system is also involved in the regulation of food intake, particularly the cannabinoid 1 (CB_1) receptors (encoded by *CNR1*) and their endogenous ligands, anandamide (*N*-arachidonoyl-ethanolamine) and 2-arachidonoylglycerol. Absence of CB_1 receptors in mice with a disrupted *CB_1* gene causes hypophagia and leanness. Administration of cannabinoids increases food intake and promotes body-weight gain, and treatment with selective CB_1 receptor antagonists decreases food intake and body weight in obese mice. Although randomized controlled trials in obese individuals with a CB_1 receptor antagonist found decreased body weight, administration of the antagonist was associated with depression and anhedonia,[13] which complicated attempts to target the system for weight loss.

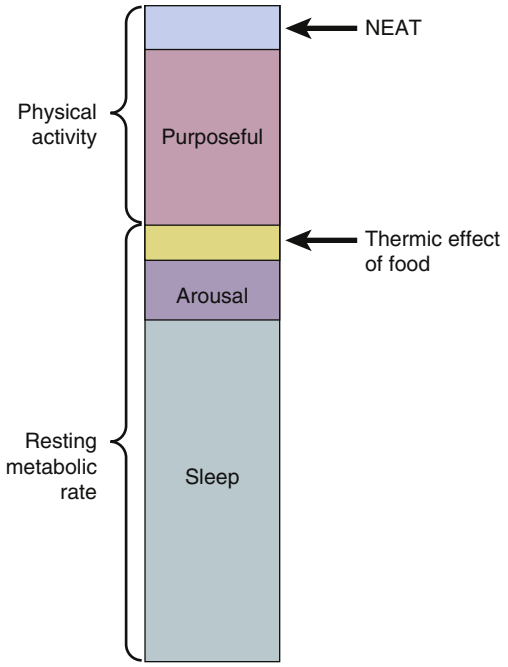
Signals From the Periphery Regulating Appetite

Multiple peripheral signals regulate energy homeostasis. Leptin, the product of the *ob* gene, is critically important for weight maintenance. Leptin absence, resulting from gene mutations, is associated with absent appetite control resulting in morbid obesity in mice, rats, and humans. Although leptin is necessary for weight maintenance, it is not sufficient. As rodents become obese, adipose tissue synthesizes increased leptin, causing increased levels in the circulation. However, the increased levels fail to decrease food intake. Likewise, most overweight and obese humans have high circulating leptin concentrations. Thus, most obesity is associated with resistance to leptin action.

Most other peripheral signals that participate in energy balance are derived from the gut. Of these, ghrelin is an orexigenic factor derived from the stomach that increases before meals and decreases after feeding. Most other gut peptides, secreted by enteroendocrine cells, inhibit appetite. Of these, glucagon-like peptide 1 (GLP1), derived from pre-proglucagon, is secreted by the L cells of the intestine that also secrete peptide YY. GLP1 and peptide YY are co-secreted after a meal and induce satiety,[14] and GLP1, when infused directly into the rat ventricle, opposes the actions of orexigenic peptides including MCH and NPY.[15] Cholecystokinin, another gut peptide, is distributed throughout the gastrointestinal tract. It stimulates bile release from the gallbladder after a meal but likely also contributes to satiety.

Energy Expenditure

Energy expenditure is a key component critical for weight homeostasis. Several factors contribute to total daily energy expenditure (Fig. 40.2). The largest component is basal or resting energy expenditure, defined as the energy required in the basal state for normal cellular and organ function; it has also been termed *basal metabolic rate*. Resting energy expenditure accounts for approximately 70% of total energy expenditure. A second significant contributor is the energy expended by physical activity, which represents a smaller contribution, about 20%, of the daily total. Voluntary activities such as exercise, and involuntary activities important in maintaining posture and fidgeting, contribute to this component of total energy expenditure. Digestion of ingested nutrients also contributes to energy expenditure and is termed the *thermic effect of food*,



• **Fig. 40.2** Components of energy expenditure. The largest component is heat generated by biochemical reactions and physiologic processes occurring at rest that are required for systemic homeostasis, including respiration, circulation, and excretion. The process of arousal increases resting energy expenditure as posture needs to be supported. Resting energy expenditure is regulated by hormones, output from the central nervous system, and sympathetic activity. Eating also requires energy, and postprandial processes involved in digestion and absorption and distribution of nutrients contribute to the thermic effect of food. Physical activity is a smaller component of energy expenditure and includes the energy expended toward purposeful activities and nonexercise activity thermogenesis (NEAT), such as fidgeting.

accounting for about 10% of the daily total and representing the energy expended in chewing, digestion, absorption, and sympathetic nervous system activation after a meal.

Resting energy expenditure is regulated by several mechanisms, particularly by thyroid hormone.[16] An excess of thyroid hormone as seen in thyrotoxicosis leads to increased resting energy expenditure that in many individuals will lead to significant weight loss, unless they experience compensatory hyperphagia. Indeed, weight loss can be a presenting symptom of hyperthyroidism. Given the effects of thyroid hormone to reduce weight, in the mid-20th century it was transiently used therapeutically in obesity. Although effective, serious side effects curtailed use.

In contrast to the clear effects of thyroid hormone to regulate resting energy expenditure, the role of the sympathetic nervous system is less clear. Epinephrine and norepinephrine are critical regulators of metabolism across multiple organs activating what is commonly referred to as the fight-or-flight response, but a precise role in regulating resting energy expenditure remains hard to define. Sympathetic activity increases after meals and contributes to the thermic effect of food. In addition, patients with tumors secreting epinephrine and norepinephrine (pheochromocytoma) manifest downstream effects of increased sympathetic activity, such as tachycardia and hypertension, but rarely present with weight loss. Although it is possible that individuals prone to obesity have lower sympathetic activity and therefore resting energy expenditure than those who maintain lower body weights, the role of sympathetic activity in energy homeostasis in humans is uncertain.

Effects of resting energy expenditure on weight gain also remain unclear. In a longitudinal study of 92 Pima Indians, the highest cumulative incidence of a 22-pound (10-kg) weight gain after 1 to 4 years was seen in those in the lowest tertile of resting energy expenditure at baseline.[17] In contrast, no relationship between initial resting energy expenditure and weight change was found in the Baltimore Longitudinal Study on Aging, which followed 775 men for 10 years.[18] However, currently available research technology is unable to detect small but chronic reductions in energy metabolism that may be clinically important over time. In addition, it is difficult to establish a causal relationship between energy expenditure and the development of obesity, because energy metabolism measurements capture only a brief point in time and therefore may not reveal abnormalities that emerge during specific life stages. Genetic or environmental differences may also impact the relationship between resting energy expenditure and weight change.

The two other components of energy expenditure—voluntary activity (exercise) and nonexercise activities—contribute to obesity. Increased food consumption is required for energy homeostasis in the setting of increased voluntary activity, as seen in athletes, people with regular exercise, or physically demanding jobs. When these activities end, as for young adults previously participating in college sports, resulting weight gain can be substantial. Likewise, nonexercise activity thermogenesis, such as fidgeting, may contribute to weight maintenance, as individuals with higher nonexercise activity thermogenesis tend to have some resistance to weight gain.

Body Weight Set-Point

Many individuals will maintain a constant weight over years and even decades, supporting the theory that there is a set-point regulating food intake and energy expenditure so that weight stays constant.[19] The set-point theory is that a complex set of physiologic adaptations maintains body weight. According to the set-point theory, body weight is predetermined such that weight loss promotes a decrease while weight gain promotes an increase in metabolic rate that acts to restore body weight to a preset level.[20] The effectiveness of the set-point has been called into question because many individuals will gain weight over time.[21]

Hypocaloric feeding reduces energy expenditure by an average of 8 kcal/kg of lean body mass in obese individuals but only 6 kcal/kg of lean body mass in normal-weight individuals, amounting to a 15% to 20% daily decrease.[22] This reduction cannot be completely accounted for by the accompanying decrease in body size, or lean or adipose body mass, and is considered a normal part of the physiologic adaptation to energy restriction.

The decrease in energy metabolism with weight loss is largely appropriate for concomitant changes in body composition, and a persistent decrease might promote weight regain. However, the reduction in resting energy expenditure that occurs during negative energy balance is transient and does not persist during maintenance of a lower body weight. Long-term maintenance of weight loss is not associated with an abnormal decrease in either resting or total energy expenditure when adjustments are made for changes in body composition,[23] although this remains controversial.[24]

When energy intake exceeds energy expenditure, weight gain occurs, but the amount of weight gained varies among individuals. Genetic factors can influence the amount of weight gained with overfeeding. Data from a study that fed monozygotic twins an extra 1000 kcal/day for 84 days found considerable variability

in weight gained among different twin pairs but that members of each twin pair gained similar amounts of weight while overfed and then lost similar amounts during the postoverfeeding recovery period.[25] The amount of weight gained may also reflect the extent of the increase in energy expenditure that is a consequence of overfeeding.[22] In another study, increase in body fat after 8 weeks of overfeeding was inversely related to changes in nonvolitional energy expenditure (e.g., fidgeting), aspects of which may be regulated.[26]

Pathogenesis of Obesity: Genes and Environment

It is challenging to identify causal factors of obesity because there are few monogenetic forms of obesity, and many single nucleotide polymorphisms (SNPs) associated with excess weight are in noncoding regions of the genome. In addition, there are substantial social and environmental determinants of weight. Food environments are complex and include not just food availability but food cost, cultural perspectives of weight, and an individual's social network.

Environmental Effects in High-Risk Populations

A striking example of the effects of modern diet on obesity is seen in Pima Indians living in Arizona. A combination of diet and lifestyle changes starting in the 1950s resulted in an epidemic of both obesity and diabetes. The modern diet is much higher in fat (50% of energy as fat) than the traditional Pima diet (15% of energy as fat). In addition, Pima Indians who became urbanized are more sedentary, especially when compared with the Pima Indian population still living in the Sierra Madre Mountains of northern Mexico. These rural Pima Indians eat a traditional diet and are physically active as farmers and sawmill workers; they have a much lower incidence of obesity and diabetes than their Arizona kindred.[27] Likewise, Aborigines of northern Australia are another high-risk population whose weight and health status has been compromised by exposure to a modern environment. Urbanized Aborigines are heavier than and have a high prevalence of type 2 diabetes mellitus compared with rural kindred, who are usually very lean (BMI <20 kg/m^2). The traditional Aboriginal diet included a low-fat, low-calorie diet of wild game, fish, and plants, as well as a high level of physical activity. Short-term (7 weeks) reexposure to the traditional lifestyle can result in weight loss and significant improvements or normalization of glucose tolerance and fasting glucose, insulin, and triglyceride concentrations in urbanized Aborigines with type 2 diabetes mellitus and hypertriglyceridemia.[28]

Influences of Childhood and Parental Obesity

Childhood obesity increases the risk of becoming an obese adult, as does having at least one obese parent. The risk of adult obesity increases with increasing age and with the severity of obesity in childhood. For example, the risk of being obese at 21 to 29 years of age ranged from 8% for persons who were obese at 1 to 2 years of age and had nonobese parents to 79% for persons who were obese at 10 to 14 years of age and had at least one obese parent, with multiple permutations between these extremes. It remains unclear how much of this is due to the genetic risk from parental obesity, including epigenetic factors, or the environmental and behavioral traits that may be shared between parent and offspring.

Genetics and Obesity

Monogenic Causes of Obesity

Only a small percentage of obese people have an identified single gene mutation that leads to obesity. The most common identified mutation associated with obesity involves MC4R, which is expressed in the central nervous system, in neurons downstream of those emanating in the arcuate nucleus. Action of αMSH from the arcuate nucleus on MC4R inhibits appetite, so a functioning MC4R is essential to maintain normal body weight. Frameshift mutations in MC4R in individuals with childhood obesity, reported by multiple groups,[29,30] may account for about 5% of early-onset obesity. These are usually heterozygous, and a gene dosage effect is seen; individuals homozygous for the same mutation or double heterozygotes for different mutations will have more severe obesity. The range of mutations is very diverse.[31] Individuals with *MC4R* mutations will respond to bariatric surgery, although the magnitude of response may vary depending on the specific mutation.[32,] In addition, MC4R agonists are currently in clinical development, as it may be possible to rescue melanocortin signaling using MC4R agonists, depending on the specific *MC4R* mutation involved.[33]

Other, much less common, mutations in the melanocortin signaling pathway involve those in the *POMC* gene, which leads to loss of all of the product peptides including adrenocorticotropic hormone and αMSH. The presentation of patients with *POMC* mutations is typically secondary to adrenal insufficiency, usually observed in early life. Consistent with absent melanocortin signaling, individuals also have red hair.

Although leptin is critical for the normal maintenance of energy balance, very few leptin-deficient humans have been identified, and leptin mutations are extremely rare.[34] The first humans with a leptin mutation,[35] which resulted in a protein that was not secreted, was found in a pair of consanguineous cousins. Shortly thereafter, three members in an obese family were reported with leptin receptor mutations.[36] There is remarkable consistency in the phenotype of mice and humans with absolute deficiency in leptin signaling, whether as a result of absent leptin or a mutation in the leptin receptor. Both mice and humans demonstrate massive early-onset obesity, hyperphagia, exaggerated food-seeking behavior, and infertility. Normal pubertal development does not occur in the absence of leptin. The handful of individuals with leptin deficiency can be treated with exogenous leptin, which normalizes weight and food intake, permits puberty,[37,38] and reestablishes insulin sensitivity.[39] As would be expected, leptin is of no use in individuals with leptin receptor deficiency.[40]

Prohormone Convertase 1 Gene Mutation

Prohormone convertase 1 (also known as proprotein convertase subtilisin/kexin type 1 [PCSK1]) cleaves POMC and is involved in processing of peptides in enteroendocrine cells in the gut. A few individuals with mutations in *PCSK1* and obesity have been identified.[41] Although the initial report involved a mutation in *PCSK1* in an adult obese woman with a history of severe childhood obesity, a significant number of individuals with this mutation present with diarrhea and failure to thrive. In some individuals, this then evolves into obesity, although the mechanisms promoting the transition remain poorly understood.

Mutation of the Neurotrophin Receptor TrkB

The neurotrophin brain-derived neurotrophic factor (BDNF) acts through a receptor kinase, TrkB, to potentiate synaptic

transmission. Heterozygous deletion of *BDNF* in mice leads to a syndrome of hyperphagia obesity and aggression. In humans, mutations in this system are very rare but include a report of a chromosomal deletion encompassing the *BDNF* gene in a mother and child pair.[42] At least one case of a mutation in the TrkB signaling pathway associated with developmental delays and obesity has been reported in an 8-year-old boy. The mutation was associated with impaired receptor autophosphorylation.[43]

Single-Minded Homolog 1 (*SIM1*) Gene Mutation

A de novo balanced translocation between chromosomes 1 and 6 was found in a severely obese girl who weighed 104 pounds (47 kg) at 5½ years of age.[44] The mutation caused a disruption in *SIM1*, the human homolog of the *Drosophila* single-minded (*sim*) gene that regulates neurogenesis. *SIM1* encodes a transcription factor involved in the formation of the paraventricular and supraoptic nuclei. It is likely that this mutation altered energy balance in this patient by stimulating food intake, because measured resting energy expenditure was normal. In addition, several chromosomal deletions involving 6q14-q21, a region that includes *SIM1*, have been identified in obese patients presenting with developmental delay and a Prader-Willi–like syndrome,[45] although the specific role of SIM1 haploinsufficiency has not been definitively established.

Src Homology 2B 1 (SH2B1) Deficiency

Src homology 2B adapter protein (SH2B1) interacts with several receptors to modulate signaling of ligands including leptin, insulin, and growth hormone. In mice, deletion of this gene results in obesity and insulin resistance when mice are exposed to a high-fat diet. In humans, this deletion is associated with a range of abnormalities including early-onset obesity and hyperphagia. Consistent with a role in insulin signaling, problems with insulin sensitivity appear disproportionate to the severity of obesity. Behavioral abnormalities have also been reported.[46] Interestingly, the result of deletions that include the *SH2B1* gene at the chromosomal 16p11.2 site may be pleiotropic and not include obesity.[47]

Obesity Syndromes

Prader-Willi Syndrome

Prader-Willi syndrome was initially described more than 60 years ago as an association of morbid obesity, short stature, hypogonadism, and cognitive deficiency.[48] Prader-Willi results from a chromosomal abnormality in which the paternal segment of chromosome 15q11.2-q12 is either deleted or absent. Interestingly, maternal deletion of the same segment is associated with a distinct phenotype known as Angelman syndrome, which is an autistic spectrum disorder.[49] Prader-Willi has a prevalence of 1 in 15,000 to 30,000 births. The molecular etiology has been linked to a deletion in the melanoma antigen gene (MAGE) family member L2 *(MAGEL2)*, a regulator of ubiquitin kinase.[50]

Bardet-Biedl Syndrome

Bardet-Biedl syndrome is rare, with a prevalence of less than 1 in 100,000, mostly seen in consanguineous populations. The disorder is clinically heterogeneous and includes obesity, hypogonadism, and abnormalities that include dysmorphic extremities, renal impairments, and retinopathies. Severe vision loss is not uncommon. Bardet-Biedl syndrome maps to multiple genes that lead to abnormal ciliary function[51,52]; how these cause obesity remains unknown.

Polygenic Causes of Obesity

Obesity results from a complex interaction of genetic predisposition and a nutrient-rich environment. More than 100 polymorphisms have been identified as potentially contributing to the predisposition to obesity.[53] However, most of these loci individually make only small weight contributions to obesity. Among hundreds of genes, the best association is with *FTO* (the fat mass and obesity associated gene).[54]

FTO was originally identified as a gene playing a role in programed cell death. In 2007, three studies demonstrated an association between variations in *FTO* and body mass. Several polymorphisms have been identified. The 16% of individuals homozygous for the risk allele SNP rs9939609 weigh about 3 kg more and have 1.67-fold increased odds of obesity when compared with those not inheriting a risk allele. This association reflects a specific increase in fat mass and can be seen in those 7 years and older.[55] A similar association with common *FTO* variants was reported in a population from Sardinia.[56] These reports were followed by numerous reports of *FTO* and excess body weight in multiple human populations. However, the mechanistic link between *FTO* and obesity has been difficult to make. The phenotype of the SNPs is difficult to identify, with some reporting increased caloric intake and some reporting decreased physical activity. Mouse studies point to a role of *FTO* in regulating body composition.[57] *FTO* has also been implicated in reward systems and methylation of proteins involved in synaptic transmission and cell signaling,[58] as *FTO* encodes a 2-oxoglutarate–dependent nucleic acid demethylase.[59] However, the causal link between *FTO* and obesity remains elusive and raises significant questions as to how to best investigate causality of human genetic variants in disease.[60]

Most individuals, perhaps up to BMIs in the high 30 range, are likely physiologically normal and become obese as they respond to the temptations of modern environments rich in high-calorie food. Given the polygenic nature of obesity, where many variants in the same or different genes contribute only modestly to body weight but may act synergistically to quantitatively affect weight, it is likely that polygenic risk scores may better estimate the overall genetic risk of obesity from common variants rather than individual risk variants considered alone.[61] Epigenetic factors may also contribute by regulating expression of obesity-associated genes. A better understanding of factors contributing to the pathogenesis of obesity might be obtained if we use the BMI range to identify the relative risk of comorbidities rather than as a guide for discovering pathology. The search for genetic variants of disease might be more fruitful if the comparisons focused on extreme leanness and extreme obesity.

Adipose Tissue as an Endocrine and Immune Organ

Until fairly recently, the traditional view of adipocytes was as a passive, efficient storage depot for triglycerides that would be released with fasting to provide fuel. This view changed as discoveries indicated adipocytes secrete multiple factors, termed *adipokines*, with both metabolic and immune functions[62] (Table 40.2). One of the first secreted factors was adipsin, a serine protease in the complement family[63] whose expression was impaired in both genetic and acquired obesity.[64] Although more than 30 years have elapsed since this initial observation, the role of adipsin in adipocyte and systemic biology remains relatively obscure and serves as an example of some of the difficulties encountered in understanding adipocyte

TABLE 40.2 Adipocyte-Secreted Factors

Category	Protein
Hormones	Leptin, resistin, angiotensinogen, adiponectin, estrogens, visfatin, glucocorticoids, angiopoietin 4, apelin
Cytokines	Interleukins 1, 6, 8, 10; monocyte chemoattractant protein 1; interferon-γ; tumor necrosis factor α
Extracellular matrix proteins	Various subtypes of collagen α1, various metalloproteinases, fibronectin, osteonectin, laminin, entactin, thrombospondin 1 and 2
Complement factors	Adipsin, complement C3, factor B
Enzymes	Cholesterol ester transfer protein, lipoprotein lipase
Acute phase response proteins	Alpha-1-acid glycoprotein, haptoglobin
Other	Fatty acids, plasminogen activator inhibitor 1, prostacyclin, vascular endothelial growth factor

biology. Understanding immune responses in obesity is further complicated by findings of increased infiltration of immune cells in fat. Initially considered pathologic, recent findings suggest that they may also play a positive role in adipose tissue metabolism by clearing lipids and senescent fat cells.[65] Different types of infiltrating immune cells may play different roles within the adipose tissue. Multiple hormones and cytokines are synthesized in fat, a selection of which are considered here.

Leptin

Potentially the most critical product of the adipocytes is the protein leptin, which is synthesized in the adipocyte and released into the circulation. Leptin is required for normal energy balance, as genetic deletion in both rodents and humans is associated with morbid obesity, as noted earlier.[66] Leptin synthesis is regulated, and levels fall dramatically with starvation, leading to many functional consequences including a decrease in fertility, which can be corrected by exogenous leptin in both mice and humans.[67,68] Leptin levels also rise with acute overfeeding.[69]

Although leptin deficiency leads to morbid obesity, most obese humans have high levels of leptin, and indeed adiposity correlates with circulating serum leptin levels in both rodents and humans, leading to the concept of leptin resistance,[70–72] which is supported by attenuated induction of downstream mediators of leptin action in obese rodents. The existence of leptin resistance, which has recently been linked to the stability of the leptin receptor,[73] also suggests that leptin does not serve as a biologic "adipostat" and that its primary function is to signal starvation.[74]

Resistin

Resistin is another signaling polypeptide secreted by adipocytes. Resistin concentrations are increased in mice with diet-induced and genetic forms of obesity and insulin resistance. Administration of recombinant resistin to normal mice leads to impaired glucose tolerance and attenuated insulin action. Based on these findings, it has been proposed that resistin is a hormone that links obesity to diabetes by inducing insulin resistance.[75]

Adiponectin

Adiponectin is a key signaling peptide produced by adipocytes; it is also the most abundant.[76] Plasma adiponectin concentrations are decreased in obesity and insulin resistance in both rodents and humans. Adiponectin acts to increase insulin sensitivity,[77] and expression increases with improved insulin sensitivity, such as when animals are treated with thiazolidinediones.[78] Weight loss[79] independent of the type of diet is also associated with increases in circulating adiponectin. However, adiponectin structure is quite complex, being composed of protein dimers and tetramers. The ratio of high-molecular-weight isoforms to low-molecular-weight isoforms is more important than total adiponectin in determining insulin sensitivity[80] and weight loss. Additionally, there are two receptors mediating its action. Administration of exogenous adiponectin lowers glucose in obese rodent models through suppression of hepatic gluconeogenesis, as well as promoting liver ceramide breakdown.[76] Unfortunately, it has not been possible to develop adiponectin-based therapies due to the complex structure. Several SNPs of adiponectin in human populations have been reported and may be linked to cardiovascular disease; however, the possible mechanism(s) remain uncertain.[81]

Estrogens

Adipose tissue contributes to total serum estrogen derived from androgens through the action of aromatase, which catalyzes formation of estrone from androstenedione. The conversion rate of androstenedione into estrone increases with age and obesity. In women, after menopause, adipose tissue becomes a significant source of estrogen biosynthesis. Increased estrogen has been implicated in the risk of breast cancer in obese women.[82] Adipose tissue also expresses estrogen receptors ERα and ERβ. Differential expression of estrogen receptors in different depots and gender differences in estrogen concentrations may explain, in part, differences in lipid accumulation in men and women.[83] Postmenopausal women who lose weight have lower breast cancer risk than those with stable weight or weight gain. It is possible that this effect is modulated through adipose estrogens, although this remains speculative.[84]

Selected Cytokines

Expansion of adipose tissue leads to a low-grade inflammatory state in fat.[65] Increased expression of cytokines from adipose tissue results in recruitment of macrophages, which may serve to aggravate inflammation and insulin resistance.[85] The precise consequences of macrophage recruitment are unclear, because while promoting inflammation, they may also have beneficial effects related to buffering of released lipids (efferocytosis) and clearance of necrotic cells.[86] Several dozen cytokines are synthesized in adipose tissue and have been reviewed extensively.[87] Two key examples are discussed here.

Tumor Necrosis Factor α

Tumor necrosis factor α (TNFα) is an inflammatory cytokine that plays a role in many disease processes. It is synthesized by multiple cell types, including adipocytes, macrophages, monocytes, and neutrophils. TNFα regulates expression of other cytokines, growth factors, and transcription factors. In adipocytes, TNFα expression increases with obesity and leads to localized inflammation that is associated with decreased insulin sensitivity. Likewise,

blocking TNFα action improves insulin sensitivity.[88] A positive correlation exists between circulating TNFα concentrations and obesity; however, it seems likely that the major effects of TNFα are local.[89] Notably, despite widespread use of anti-TNFα treatments for rheumatologic diseases,[90,91] improvements of insulin sensitivity and glycemia in patients with type 2 diabetes mellitus are not clinically evident.

Interleukin 6

Another proinflammatory cytokine increased in obesity is interleukin 6 (IL6), which may contribute to systemic inflammation and insulin resistance. Insulin sensitivity is inversely related to plasma IL6 concentrations, and IL6 directly impairs insulin signaling.[92] Administration of IL6 induces dose-dependent increases in fasting blood glucose in humans, probably by stimulating release of glucagon and other counterregulatory hormones, by inducing peripheral resistance to insulin action, or both. IL6 and other proinflammatory cytokines may also play a direct role in regulating adipocyte metabolism and vascular health.[93]

Brown Adipose Tissue

Brown adipose tissue (BAT) is a distinct fat depot that is structurally and functionally different from white adipose tissue; it contains multilocular fat vacuoles and large mitochondria and is intensively innervated by sympathetic nerves.[94] BAT contributes to energy homeostasis in rodents, where it is part of the adaptation to cold exposure[95] but also plays a role in the adaptation to very low carbohydrate diets.[96] Under these conditions, BAT is activated; as a result, there is an increase in the levels of the factor uncoupling protein 1 (UCP1), which generates a mitochondrial proton leak that results in less production of adenosine triphosphate and energy wastage through the generation of heat. Recently, BAT depots have been identified in humans, which increased interest in BAT biology. Human BAT can be activated by cold exposure[97] and increased adrenergic activity.[98] However, human BAT depots are relatively small, and it is unlikely that BAT plays a significant role in energy expenditure in otherwise healthy humans.

Metabolically Normal Obesity

Obesity is commonly associated with impaired metabolic function—namely, insulin resistance, diabetes, dyslipidemia (increased serum triglyceride and decreased serum high-density lipoprotein cholesterol), and increased blood pressure. Nevertheless, some individuals with increased weight remain metabolically normal based on measurements of lipids, blood pressure, and insulin resistance. Metabolically healthy obese individuals do not appear to have increased risk for cardiovascular mortality.[99] The concept of "metabolically healthy obese" is difficult to evaluate, and assessment of such individuals may be confounded by multiple factors, such as the presence of comorbidities, selection bias, or other factors.[100] For example, individuals most susceptible to cardiovascular disease may die earlier. It seems logical that obesity will have differential impact on individuals based on as yet undefined genetic or behavioral predispositions. It would be expected that some individuals with mild to moderate obesity would remain healthy.

Although, in general, increased BMI is associated with increased disease risk, in certain patient populations, being overweight or obese is associated with lower mortality rates than for similar patients with normal BMI values. This BMI paradox is associated with cardiovascular disease[101] (myocardial infarction, congestive heart failure, hypertension, and coronary heart disease), coronary artery bypass graft surgery, and end-stage renal disease.[102] Although this may represent an intrinsic paradox, it may also reflect that chronic disease is associated with sarcopenia, and individuals with higher BMI may be healthier as a consequence. It is also plausible that treatment of comorbidities with statins, angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, and others can provide some degree of protection when excess weight is not extreme, with differential use in the setting of excess weight compared with the same value in the normal-weight patient.

Adverse Consequences of Obesity

Obesity as a Disease Risk Factor

Metabolic Syndrome

Metabolic syndrome refers to the common co-occurrence of multiple diseases with obesity, particularly obesity with a visceral distribution. Most of these obesity-related disorders are associated with increased cardiovascular disease risk. Metabolic syndrome has multiple definitions. National Cholesterol Education Program Adult Treatment Panel III includes any three of the following five clinical measures: increased waist circumference defined on the basis of ethnic specific criteria, elevated triglyceride levels, low high-density lipoprotein cholesterol levels, increased blood pressure, or high fasting glucose. World Health Organization criteria for metabolic syndrome includes presence of insulin resistance along with the identification of at least two additional risk factors.[103] Based on meta-analysis of studies that included more than 900,000 people, metabolic syndrome led to a 2-fold increased risk of cardiovascular disease and a 1.5-fold risk of increased mortality from all causes.[104]

Obesity predisposes to cardiovascular disease through multiple mechanisms, as it increases the likelihood of type 2 diabetes mellitus, dyslipidemia, and hypertension, all of which are independent risk factors for cardiovascular morbidities. Factors secreted from the adipocyte can also promote a proinflammatory, prothrombotic state.

Type 2 Diabetes

Type 2 diabetes mellitus is a common consequence of excess adiposity.[105] Indeed, more than 90% of individuals with type 2 diabetes mellitus are obese.[106] Rising prevalence rates of type 2 diabetes mellitus coincide with rising rates of obesity. Rates of diabetes across the continental United States range from a low of 7% in Colorado to a high of 13.6% in Mississippi, and rates in the US territories of Guam and Puerto Rico are even higher.[107] Diabetes prevalence parallels the prevalence of obesity, which is lowest in Colorado, where rates range between 20% and 25%, and highest in the southeastern states, including Mississippi, with rates higher than 35%.

Risk of diabetes increases with earlier onset and more severe obesity. For example, the likelihood of developing diabetes is 70% for women with a BMI greater than 35 at age 18 years, with an expected diagnosis of diabetes by the sixth decade.[108] An increased waist to hip ratio, reflective of increased visceral obesity, is also associated with increased diabetes risk.[109]

Dyslipidemia

Primary dysfunction in the adipocyte is associated with abnormal lipid metabolism, increasing the risk of dyslipidemia. Abnormal lipid metabolism in association with genetic factors results in dyslipidemia including hypertriglyceridemia; reduced high-density

lipoprotein cholesterol levels; and an increased fraction of small, dense low-density lipoprotein particles. This association is especially strong in persons with abdominal obesity.[110]

Hypertension

There is a linear relationship between hypertension and BMI.[111] Prevalence rates are more than twice as high in obese men and women compared with lean men and women, and risk of hypertension increases with both age and weight gain. Approximately 70% of hypertension in adults is attributable to excess adiposity, especially visceral adiposity. Obesity-related hypertension has distinct genetic determinants compared with hypertension in the absence of obesity. Physiologic mechanisms of obesity-related hypertension include insulin resistance, sodium retention, increased sympathetic nervous system activity, activation of renin-angiotensin-aldosterone, and altered vascular function. Weight loss results in blood pressure reductions[112,113]; however, the effects may not be durable.

Cardiovascular Disease

Obesity is associated with a significantly increased lifetime risk of cardiovascular disease, including coronary artery disease, heart failure, and cerebrovascular disease. Risks are higher for men and for those with visceral obesity.[114] Absolute thresholds for weight-associated cardiovascular disease are hard to determine and can depend on the type of event being assessed, the subpopulation, and other confounding factors. In middle-age men, the risk of a cardiovascular event increases with increased BMI, and the risk of cardiovascular death is twofold for individuals with a BMI greater than 40 kg/m^2 compared with normal-range BMI. A similar increase in risk in cardiovascular events is seen in women, although risk of cardiovascular death does not significantly correlate with BMI. Overweight individuals have similar longevity to those with normal BMI; however, the increased risk in cardiovascular events leads to living with chronic cardiovascular morbidity.[115]

In a recent analysis of data from both the Nurses' Health Study and the Health Professionals study, no increased cardiovascular risk was seen in the overweight category. When this was adjusted for lifetime weight history, a small increase was noted in the overweight BMI range,[116] indicating that at lower excess weights, assessment of risk is complicated.

Increasing BMI also increases the risk of ischemic cerebrovascular events in both men and women. The risk of fatal and nonfatal ischemic stroke is approximately twice as great in individuals with a BMI greater than 35 kg/m^2. In intermediate BMI ranges of greater than 25 kg/m^2 and up to 32 kg/m^2, it is difficult to assess the relative increased risk in otherwise healthy individuals. At least one study reports similar survival following an ischemic event in normal-weight and overweight subjects.[117]

Obesity is also associated with an increased risk of thromboembolic disease, and risk increases with higher waist circumference.[118]

Liver Disease

As a result of the increasing prevalence of obesity, nonalcoholic fatty liver disease (NAFLD) is now the most common cause of chronic liver disease in both developed and developing countries. In the United States, NAFLD affects 30% of the obese population and 53% of obese children.[119] Risk increases with additional excess weight such that prevalence increases to 90% in morbidly obese populations.[120,121] Hepatosteatosis is diagnosed when 5% of all cells contain lipid droplets by histology or when magnetic resonance imaging reveals more than 5% fat in the liver. Diagnosis of NAFLD requires exclusion of other causes of liver pathology, including alcohol abuse, viral infections, and biliary or autoimmune disease. Although fatty liver itself is a relative benign state, it can progress unpredictably to steatosis, cirrhosis, and hepatocellular carcinoma. Progression to nonalcoholic steatohepatitis, characterized by hepatocyte apoptosis, inflammation, and fibrosis, is seen in 10% to 20% of NAFLD patients and poses a high risk for further progression to cirrhosis and hepatocellular carcinoma. Unfortunately, at present, it is not possible to identify individuals who will progress from NAFLD to nonalcoholic steatohepatitis and hepatocellular carcinoma. Effective treatments are limited to weight loss, which is problematic because few interventions lead to meaningful sustained weight loss. Thus, understanding the molecular mechanisms underlying the progression from hepatic steatosis to frank steatohepatitis is of critical importance for clinical assessments and for pharmacologic treatment.

Obstructive Sleep Apnea

Overall effects of obesity on lung function are relatively benign. However, respiratory function can be significantly affected by obstructive sleep apnea that can be severe. Obstructive sleep apnea occurs when there is either complete or partial upper airway obstruction due to mechanical pressure in the neck. Daytime sleepiness is common. Sleep apnea is also an independent risk factor for hypertension and may predispose to type 2 diabetes mellitus. Weight loss is effective in improving indices of apnea.[122]

Musculoskeletal Disease

Because overall body weight contributes to mechanical pressure at the hip and knee, obesity is a risk factor for osteoarthritis. Overweight increases the risk of knee osteoarthritis by almost twofold, whereas frank obesity is associated with additional risk. Overweight and obesity are more significant factors in new-onset knee pain than a previous knee injury. The increasing prevalence of overweight and obesity is also associated with increasing total knee and hip arthroplasty.[123] Hyperuricemia and gout are also associated with obesity.[124] The risk of gout also increases with body weight, and the relative risk in individuals with a BMI greater than 35 kg/m^2 is almost threefold higher compared with normal-weight persons.[125,126]

Cancer

Overweight and obesity increase the risk of certain cancers. Based on data from a prospective study in more than 900,000 adults in the United States, it was estimated that overweight and obesity could account for 14% of all deaths from cancer in men and 20% of such deaths in women. Obesity is associated with higher rates of death due to cancers of the gastrointestinal tract (liver, pancreas, stomach, esophagus, colon and rectum, and gallbladder) and kidney, multiple myeloma, and non-Hodgkin lymphoma, as well as prostate cancer in men and uterine, cervical, ovarian, and postmenopausal breast cancer in women. The risks of breast and endometrial cancer fatality increase with both obesity and weight gain after age 18 years. The risk of breast cancer increases with increasing BMI in postmenopausal women; in premenopausal women, increased BMI may actually protect against breast cancer. Genetic, hormonal, and metabolic factors associating adiposity to cancers remains incompletely understood.

Diagnostic and Therapeutic Approach

Height and weight measurements are intrinsic to the physical examination; most electronic medical records automatically calculate BMI, which can also be calculated manually by dividing the weight in kilograms by the height in meters squared or by using the online calculator provided by the Centers for Disease Control and Prevention. Once the current BMI is known, an essential part

of the medical history is to determine an individual's weight trend over time, often by obtaining weight at age 18 years. For normal-weight and modestly overweight individuals (i.e., BMI <27 kg/m^2 without comorbidities for those with European heritage or 25 kg/m^2 for Asian heritage), counseling with no additional intervention other than monitoring weight over time is warranted.

Beyond weight, little additional evaluation is warranted for obesity per se. In those reporting early obesity, presenting during childhood or adolescence, who also have a family history, genetic testing for a mutation in MC4R might be considered. This is the only commercially available test, and it may be helpful in a small number of individuals who test positive. However, at present, there are no specific therapies that would be more effective if the mutation were present, so pursuing the test is dependent on clinical suspicion of physician and patient preference.

Management of overweight and obesity can be difficult.[127] A conservative clinical approach is provided in Table 40.3. First, an individual must acknowledge a potential problem. At the time of annual physical examination, weight should be noted, BMI calculated, and a brief overview of BMI and risk provided. In general, mildly overweight patients need to be advised of the advantages of modest weight loss and warned against additional weight gain. For individuals with a BMI greater than 27 kg/m^2 (25 kg/m^2 for Asians) or those with comorbidities, the risk of excess weight should be addressed and patients should be encouraged to be attentive to their weight, offered access to a nutritionist, provided with dietary advice, and given an approach to behavioral modification. Using a home scale for scheduled weighing time and recording the result alone may be ineffective for achieving weight loss, but it may help an individual be attentive to risk and may prevent or slow down further weight gain.

Individuals with a BMI greater than 35 kg/m^2 should be advised of risks, encouraged to obtain a consultation from a nutritionist, and be counseled in behavioral modifications, and a repeat visit should be scheduled within 3 to 4 months of the initial visit. If the interval has passed without weight loss, pharmacotherapy should be considered. Although GLP1 receptor agonists (see later discussion) are highly effective, few individuals are willing to consider an injectable therapy at the outset; additionally, many insurance companies insist on a trial of available oral therapies to start. There is no reliable way of predicting a response to the various medications available; the initial choice needs to be individually tailored on the basis of the likelihood of compliance and risk of side effects. Metabolic surgery options should be discussed.

Once therapy is prescribed, it is critically important to evaluate weight loss within a short interval to determine effectiveness. An official weight, in a medical environment, should be recorded at 8 weeks. If no weight loss is observed, weight should be checked at 12 to 16 weeks. Weight gain should signal cessation of therapy. If weight loss is absent, or less than 5% or 3.3 pounds (1.5 kg), therapeutic dose adjustments may be considered up to maximal approved levels or therapy should be discontinued between 12 and 16 weeks. If weight loss exceeds 3.3 pounds (1.5 kg), individuals should be recalled for weight checks every 8 weeks. If weight loss is ongoing, the decision to continue medical therapy over the long term needs to be evaluated on an individualized basis that balances the risk of medication with the benefit of reduced body weight. Weight regain is common when pharmacotherapy is stopped.

TABLE 40.3 Suggested Weight-Loss Treatment Options Based on BMI and Risk Factors

BMI (kg/m^2)	Advice, Nutrition, Exercise	Pharmacotherapy[a]	Surgery[b]
25.0–26.9	Yes	No	No
27.0–29.9	Yes	Consider if there are risk factors and weight gain trajectory	No
30.0–34.9	Yes	Strongly consider with risk factors and weight gain trajectory	No
35.0–39.9	Yes	Yes	If drugs fail, with comorbidity
≥40	Yes	Yes	If drugs fail

Ethnic adjustments to BMI ranges should be considered.

[a]Pharmacotherapy should be considered in patients who are unable to achieve adequate weight loss with available conventional therapy and who do not have any absolute contraindications for drug therapy.

[b]Bariatric surgery should be considered in patients who are unable to lose weight with available conventional therapy and who do not have any absolute contraindications for surgery.

BMI, Body mass index.

Benefits of Intentional Weight Loss

Weight loss resulting from any therapeutic modality will lead to improvements in the complications of obesity and significantly lower the risk for diabetes and other comorbidities,[128] although there may be unintended risk associated with pharmacologic and surgical approaches. Diabetes remission with weight loss can be of several years duration but appears to depend on residual beta-cell function and additional potential for beta cells to recover.[129,130] However, it is interesting the lifestyle interventions promoting weight loss through decreased caloric intake and increased physical activity do not improve cardiovascular event rates in individuals with type 2 diabetes mellitus,[131] perhaps due to increased use of lipid-lowering and blood pressure medications in patients not engaged in the intervention.

Modalities that can be used to address obesity include lifestyle interventions such as diet and exercise, behavioral therapy, pharmacotherapy, bariatric surgery, and some various combinations.

Dietary Intervention

Caloric Restriction

Dietary intervention may involve decreasing food intake without making major changes in the composition of food consumed. A pound of fat contains 3500 calories, so an individual aiming to lose 1 pound per week needs a net daily calorie deficit of 500 calories. A 55-year-old, moderately active, woman weighing 300 pounds (136 kg), will need approximately 2600 calories for weight maintenance. For her, a net 500 calorie deficit will be approximately a 20% reduction in her daily food intake. If maintained, this would lead to about 50 pounds (22.5 kg) of weight loss in a year, which can seem discouragingly slow for someone aiming for ideal body weight. It is important to set realistic expectations to foster success and continued efforts.

To increase the rate of weight loss, many individuals consider restricting daily food intake by pursuing very low calorie diets using meal replacements. Very low calorie diets that limit to 800 calories per day can also be effective for weight loss, especially if coupled with either a commercial provider or physician or nutritionist support. Many commercial options providing 200 to 300 calories of meal substitutes replete with protein are available, such as Optifast, HMR, and Medifast. These are available over the counter

or may be prescribed and overseen by a physician, but they may be considered relatively expensive by the patient. Diets that limit calories to 800 calories daily are typically associated with 2 pounds of weight loss weekly. Some approaches involve a period of several weeks of meal replacements only, limiting food intake to 800 calories (3–4 meal supplements daily), whereas other approaches include a single meal of conventional food combined with two daily supplements. When initiated solely as meal replacements, very low calorie diets typically progress to modified fasts wherein a single relatively low calorie meal is consumed. A meta-analysis comparing commercial programs with simple nutritional counseling and calorie restriction reports that commercial programs fare better with more weight loss between 6 and 12 months. In general, weight loss is greater with these programs than with simple counseling, but long-term weight loss is only sustained in a relatively small number of individuals.[132]

Additional recent approaches involve limited fasting to 2 to 3 days each week. A current popular approach is the "5/2 diet," which involves limiting food intake to less than 400 calories 2 days a week and eating "normally" the other 5 days. Other approaches involve eating only during a limited number of hours daily and maintaining at least 16 hours per day without any food intake.

Macronutrient Composition

Significant attention has been given to macronutrient composition of diets in attempts to determine what proportion of nutrients might lead to greater and more sustainable weight loss. There is significant debate between those that favor low-fat diets in comparison to low-carbohydrate, Atkins-style diets. Most controlled studies show that weight loss is similar regardless of diet composition, although very low carbohydrate diets have a small early advantage.[127,133] For individuals who self-select Atkins-style diets and who also utilize a support system, weight loss can be dramatic and substantial improvements in glucose control have been observed.[134]

As there is little support for any specific type of diet, it is best to inform individuals regarding different options, recommend based on their preference, and follow closely to confirm efficacy and safety.[135] A potential exception is recommendation of Atkins-style diets in individuals with type 2 diabetes mellitus. Although still somewhat controversial, increasing evidence suggests that significant limitation of carbohydrates improves glucose control, decreases dependence on medications, and facilitates weight loss.[134]

Physical Activity

In the past half century, sedentary behavior has increased with physical labor progressively mechanized, both commercially, such as in farming operations, and in households where machines such as vacuum cleaners and dishwashers lead to less daily energy expended. Reduced energy expended on these activities is estimated to represent a 140 kcal/day decline and may contribute to population weight gain.[136] However, increasing physical activity through discretionary exercise is not particularly effective for weight loss. In a study of exercise alone, postmenopausal women were advised to exercise at moderate intensity for 45 minutes 5 days per week. Weight loss was minimal, averaging 2.8 pounds (1.3 kg) over the course of a year.[137] When coupled with diet, exercise has a modest effect to improve weight loss in men but only prevented weight gain in women.[138] The failure of exercise to have a significant impact on weight loss may reflect compensatory overeating.

Behavior Modification

Behavior modification is an unspecific term that covers approaches to weight loss and or limiting weight gain that include exercise, diet, food shopping habits, and food pantry stocking.[139] Behavioral modification strategies and guidelines should be sensitively discussed with obese and overweight individuals, especially those who are gaining weight over time. Raising awareness may slow down weight gain; prevent additional weight gain; and, under some circumstances, encourage weight loss. Recognizing and learning to avoid behaviors such as stressful eating and setting realistic and achievable goals are both important. Successful weight loss has been reported with combinations of exercise and intensive lifestyle modification that includes frequent monitoring and support. In the Look AHEAD study of individuals with type 2 diabetes mellitus and obesity, those randomized to the intensive lifestyle modification limb lost up to 17.6 pounds (8 kg) in the first year. Although, in general, individuals regained weight over time, weight loss of about 9 pounds (4 kg) persisted out to 4 years.[140]

Pharmacotherapy

Most medications currently available to treat obesity are associated with relatively modest weight loss (Table 40.4). Furthermore, nonresponder rates can be high so that for any agent, only a minority of individuals who initiate therapy in a standard outpatient setting will lose weight. It is uncertain if patients who fail one therapy are more likely to succeed with other class agents.

To date, the most tolerated and effective class of medications for weight loss are GLP1 receptor agonists. These were developed for the treatment of type 2 diabetes mellitus and fortuitously were found to have meaningful effects in reducing appetite and body weight. Effects on appetite with continuous infusion were reported early, and when the first agonist, exenetide, came to market for glucose control in type 2 diabetes mellitus, modest weight loss was noted in many patients. Subsequent studies found that weight loss effects could be extended to obese individuals without diabetes and, further, that it was possible to stratify subjects as responders or nonresponders based on weight loss after 8 weeks of treatment.[141] The GLP1-R agonist liraglutide, also used in type 2 diabetes mellitus (marketed as Victoza), and in a higher-dose formulation (Saxenda) has now been approved for obesity management in the United States, Europe, and Japan. Liraglutide provides benefit in terms of reduced rates of nonfatal myocardial infarction and stroke and cardiovascular death in patients with diabetes and established or at high risk for cardiovascular disease.[142] Semaglutide has also been recently approved for management of type 2 diabetes mellitus with once-weekly dosing. Semaglutide may also reduce the rate of nonfatal myocardial infarction or stroke or cardiovascular death in patients with type 2 diabetes mellitus at high cardiovascular risk.[143] Whether these agents will provide similar cardiovascular benefits in reducing cardiovascular risk in obese individuals is unclear. When administered at lower daily doses, it leads to weight loss in obese nondiabetic individuals. In a direct comparison of semaglutide and liraglutide, weight loss at 0.3 mg of semaglutide daily resulted in mean weight loss of 12.3% compared with mean weight loss of 8.3% in those randomized to 3.0 mg of liraglutide.[144] Currently, most GLP1 receptor agonists are administered by subcutaneous injection. Headache and gastrointestinal events such as nausea, vomiting, diarrhea, or constipation represent the major side effects that limit use of GLP1 receptor agonists.

Phentermine is a sympathomimetic that was approved for obesity treatment in 1959. It stimulates the release of norepinephrine, and to a lesser extent serotonin (5-hydroxytryptamine [5HT]) and dopamine. Weight loss with phentermine is approximately 5% at 8 to 12 weeks, and typically is administered at 15 mg to 37.5 mg orally three times daily with meals as a short-term adjunct (a few weeks) to behavioral weight loss approaches. A lower 8-mg

dose for longer-term use is available, but durability has not been reported. The clinical effect of phentermine is to diminish appetite, and it appears to work better in individuals who start with greater hunger and less cognitive restraint on their eating behavior.[145] Side effects of phentermine can include pulmonary hypertension, valvular heart disease, palpitations, increased heart rate or blood pressure, insomnia, restlessness, dry mouth, diarrhea, constipation, and changes in sexual drive.

Phentermine has been combined with topiramate into an extended-release (ER) formulation marketed as Qsymia, which is available in four doses ranging from combinations of 3.75/23 to 15/92 of phentermine to topiramate. Phentermine-topiramate ER has been evaluated in two large randomized clinical trials. Weight loss at 1 year demonstrated a dose-response relationship and was similar in both studies. In an intention-to-treat analysis after 1 year of therapy, the placebo-subtracted weight loss was approximately 9% for the top dose and approximately 6.5% for the recommended dose. Common adverse effects of phentermine-topiramate ER include dry mouth, dizziness, dysgeusia, constipation, insomnia, and paresthesia. Cognitive impairment (attention or memory deficits) is also reported and, when it occurs, leads to discontinuation of the drug.

Orlistat inhibits pancreatic lipase and thereby reduces enteric absorption of fatty acids. In turn, this leads to some degree of fat malabsorption. Excretion of about 30% of ingested triglycerides, which is near the maximum plateau value, occurs at a dose of 360 mg/day (120 mg three times daily with meals). A meta-analysis of multiple weight loss studies indicated that about 50% of individuals taking orlistat will lose weight,[146] although weight loss at 1 year is small, with an average loss of 11 pounds (5 kg).[147] Use of Orlistat is limited by the common side effect of fatty diarrhea, which leads to reduced compliance. Systemic side effects directly related to the drug are uncommon because orlistat is not absorbed.

Lorcaserin (marketed in the United States as Belviq) is a selective 5HT2c receptor agonist that decreases food intake leading to modest weight loss, which in a meta-analysis of several trials was just over 11 pounds (5 kg). In a high-risk population of overweight or obese patients, lorcaserin promoted sustained weight loss without increasing rates of major cardiovascular events.[148] Lorcaserin has greater binding affinity and activation of the 5HT2c receptor subtype over the 2a or 2b receptor subtypes, and given the tissue distribution of the receptor subtypes, it would be expected to have a reduced cardiac valve risk profile compared with earlier serotonergic agents, such as fenfluramine. Valvulopathy has not been reported. The most frequent adverse effects of lorcaserin in these studies were headache, dry mouth, dizziness, and nausea.

The naltrexone slow-release (SR)/bupropion SR combination, marketed as Contrave, is a μ-opioid receptor antagonist combined with a norepinephrine and dopamine receptor inhibitor. Bupropion has neuronal effects associated with reduced energy intake and increased energy expenditure, and naltrexone potentiates this effect such that the effects of the combination are greater than those with buproprion alone. The overall magnitude of weight loss is on the order of 5%, but in trials, more patients lose 5% of their body weight with treatment compared with placebo. There are improvements in lipid and glucose profiles and patient reported outcomes but small increases in heart rate and blood pressure.[152a]

Metformin is typically used to treat diabetes and is associated with weight loss. Data from most studies on the effect of metformin on body weight indicate that metformin does not cause weight gain, as single-agent therapy and concomitant treatment with other medications may limit the expected weight gain.[149,150] Metformin has also been reported to lead to an average net 11-pound (5-kg) weight loss after 6 months of treatment over control obese individuals without diabetes.[151] Although not approved for weight loss in nondiabetic individuals, metformin has been prescribed off label for weight loss, and because there are few side effects, therapy may be continued indefinitely if effective.

A summary of medications approved for weight loss, along with maximal placebo-adjusted maximum weight loss, is provided in Table 40.4.

Endoscopic Gastric Therapies

Endoscopic bariatric and metabolic therapies are more recently available for the treatment of obesity.[152] These include devices that are placed via flexible endoscopy and procedures that utilize flexible endoscopy instruments for weight loss indications. Currently, three intragastric balloon devices have been approved for use in the United States, including the ReShape Integrated Dual Balloon System, the ORBERA Intragastric Balloon System, and the Obalon Balloon System; others are under development. A small number of deaths have occurred in patients using these devices. Aspiration therapy using the AspireAssist device permits food to be removed from the stomach after a meal. Electrical stimulation systems place an electrical stimulator in the abdomen to block nerve activity between the brain and stomach, such as the Maestro System. Procedures include endoscopic sleeve gastrectomy, as well as POSE (Primary Obesity Surgery, Endoluminal), which is performed via endoscopy to reduce the size of the stomach and diminish hunger cravings. Currently, there are limited data to compare safety effectiveness and durability of these devices and procedures.

TABLE 40.4 Drugs Commonly Prescribed for Weight Loss[146]

Year Approved	Generic Name	Trade Name	Placebo-Corrected Anticipated Weight Loss (kg)
1959	Phentermine	Ionamin, Adipex-P, Fastin, Oby-Trim (approved only for short-term weight loss)	Approved for short-term use only
1999	Orlistat	Xenical, Ally (over the counter)	2.63
2010	Liraglutide	Victoza (approved for type 2 diabetes mellitus)	0 to 3.7[a]
2012	Phentermine-topiramate extended release	Qsymia	8.80
2013	Lorcaserin	Belviq	3.25
2014	Liraglutide	Saxenda (approved for obesity)	5.24
2014	Bupropion-naltrexone	Contrave	4.95

[a]Depending on comparator.

Note: Intensity of lifestyle interventions and maximum weight loss differs in studies. Represented values mean weight loss in excess of placebo.

Bariatric Surgery

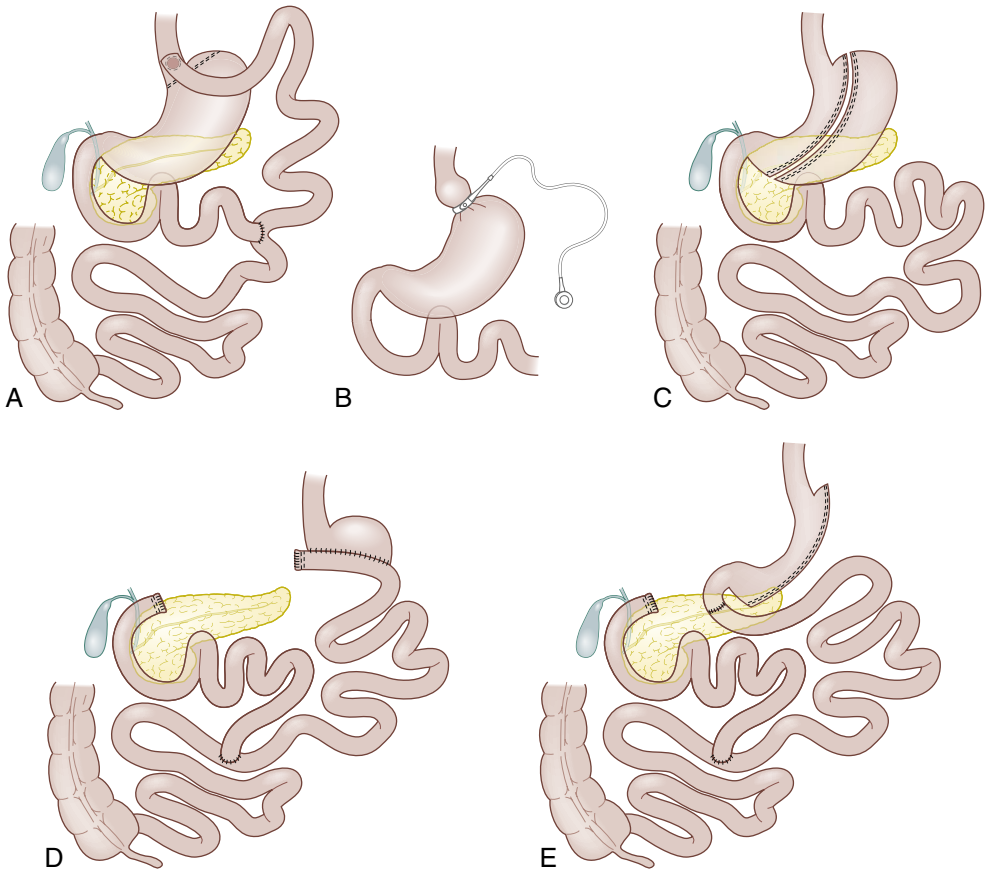
Attempts to treat obesity through surgical procedures were first attempted more than 60 years ago, with almost all initial procedures involving shunts between the jejunum and the colon. Jejunoileal bypass surgery was effective for weight loss but was associated with significant complications, including liver disease,[153] liver failure resulting in death,[154] and protein malnutrition.[155] Thus, this surgical approach was largely abandoned. A decade later, a report on the success of Roux-en-Y-gastrojejunostomy in more than 600 morbidly obese patients revealed that this surgical approach was effective with very little morbidity and mortality.[156]

Although many consider bariatric surgery as a draconian approach to obesity, weight loss is substantial and sustained, and associated with remission or improvement of type 2 diabetes mellitus, dyslipidemia, hypertension, and other weight-related comorbidities[157] that may occur early following surgery and persist for years. Not surprisingly, following the procedure, decreased levels of inflammatory markers have also been reported.[158] Surgical weight loss may reduce mortality by as much as 30% to 40% in the severely obese, although these studies were not randomized.[159,160] There is some controversy regarding whether the severity of obesity is the optimal characteristic to use when considering a patient for metabolic surgery; however, the accepted indications for surgery are a BMI greater than 40 kg/m^2 or a BMI between 35 and 40 kg/m^2 with an associated comorbidity.[161]

Four surgical procedures are offered for obesity therapy, including adjustable gastric banding, sleeve gastrectomy, Roux-en-Y gastric bypass, and biliopancreatic diversion (Fig. 40.3). Although biliopancreatic diversion is effective, few procedures are currently performed because of the higher rates of complication. Adjustable gastric banding has also become less common, with Roux-en-Y and gastric sleeve surgery now also routinely performed laparoscopically, providing more rapid recovery. In addition, gastric banding has significantly lower expected weight loss.

Roux-en-Y gastric bypass and sleeve gastrectomy are the two favored procedures. Similar weight loss is achieved, of almost 11 pounds (5 kg) per month for the first 4 months, with weight nadir between 6 and 24 months and effects that can persist over years. Perioperative mortality with bariatric surgery is low and reported as 0.04% to 0.3%, similar to that of a cholecystectomy. Potentially fatal risks include pulmonary emboli, sepsis, and bleeding. Because of the underlying increase in cardiovascular disease, the likelihood of cardiovascular risk is higher in the perioperative period. Of concern would be a leak at the anastomosis site, which can lead to sepsis and needs to be diagnosed and treated aggressively. Overall, the incidence of adverse events in the initial 28-day period was less than 5%.

Hypoglycemia can occur as a long-term complication of weight loss surgery, which may occur to some degree in up to 14% of individuals without a prior history. Severe hypoglycemia is only reported in a small number of patients[162]; it appears



• **Fig. 40.3** Schematic diagrams of Roux-en-Y gastric bypass (A), laparoscopic adjustable gastric banding (B), sleeve gastrectomy (C), biliopancreatic diversion (D), and biliopancreatic diversion with duodenal switch (E). (Modified from Bradley D, Magkos F, Klein S. Effects of bariatric surgery on glucose homeostasis and type 2 diabetes. *Gastroenterology.* 2012;143[4]:897–912.)

to be more common after the Roux-en-Y procedure[163] and may be managed by continuous blood glucose monitoring and carbohydrate support,[164] although, on occasion, somatostatin analogues or diazoxide are necessary to reduce insulin secretion following meals.

Patients having Roux-en-Y gastric bypass should be followed for development of anemia and deficiencies of fat-soluble vitamins, because the absorption of iron and fat-soluble vitamins can be impaired, leading to low levels even in the setting of standard replacement therapy with oral multivitamins. Iron infusion therapy may be needed in some patients. Bone health should be monitored. Bariatric surgery may also exacerbate depression and may increase the likelihood of this comorbidity after surgery. Rates of depression are high in individuals with morbid obesity, which is also associated with increased suicide risk.[165,166] A psychological evaluation for depression and emotional postsurgical support is an essential part of the care of individuals undergoing surgery.

Summary

Obesity results from complex interactions of individuals' risk and environmental influences. The first-order advice regarding diet and exercise is rarely successful. In the past decade, there have been some significant improvements in medical therapy, as GLP1 agonists have demonstrated effectiveness in at least 30% of individuals. Bariatric surgery has become safer and more acceptable. However, the demand for therapies persists.

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The complete list of references is available online at ExpertConsult.com.

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41

Disorders of Lipid Metabolism

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CHAPTER OUTLINE

KEY POINTS

- Abnormalities of lipid metabolism cause heart disease, pancreatitis, vitamin deficiencies, and gallstones.
- Endocrine disorders such as diabetes and obesity have protean effects on lipid metabolism.
- Lowering low-density lipoprotein decreases vascular disease, allows regression of established atherosclerotic lesions, and prolongs life.
- Substantial additional lowering of low-density lipoprotein beyond that seen with statins can be achieved by inhibiting proprotein convertase subtilisin/kexin type 9.
- Pharmacologic elevation of high-density lipoprotein has no apparent benefit.
- Extreme elevations of triglycerides should be treated to avoid pancreatitis.
- Moderate elevations of triglycerides may be associated with vascular disease, but evidence that treating this condition reduces cardiovascular events has not been established.

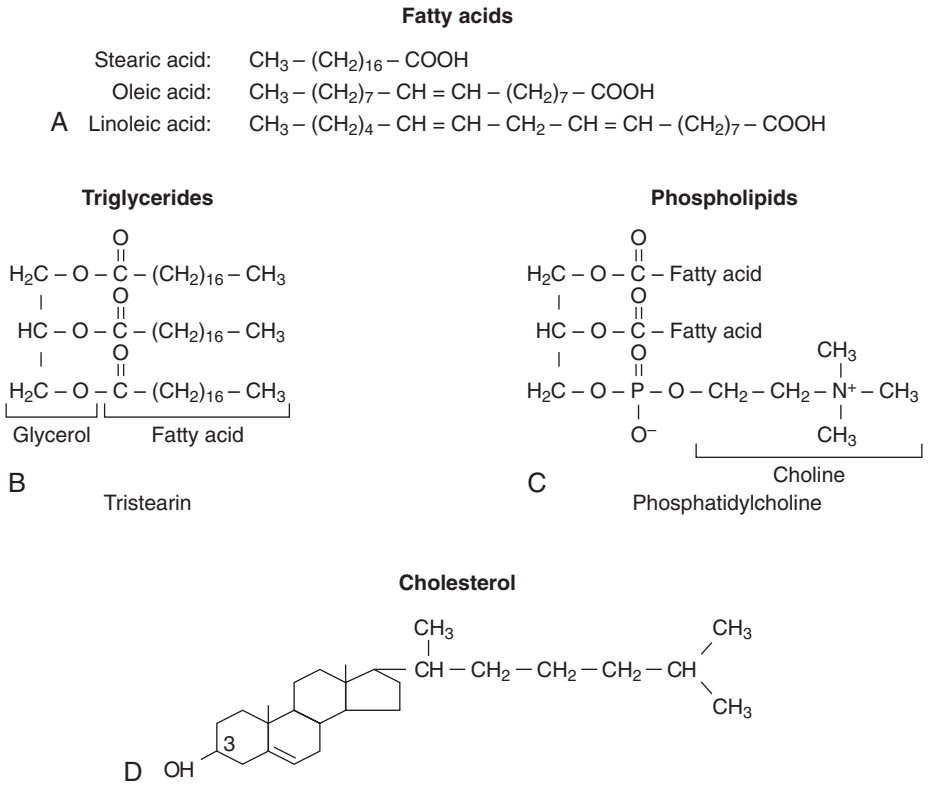
Lipid Biochemistry and Metabolism

There are multiple adverse health effects of abnormal serum or tissue lipids. Endocrine disorders impact lipids, making mechanisms underlying primary and secondary disorders of lipid metabolism relevant to both clinicians and basic scientists. Primary genetic disorders of lipid metabolism, such as familial hypercholesterolemia (FH), are relatively common, occurring in about 1 in 300 to 500 people. The low-density lipoprotein (LDL) receptor pathway is functionally impaired in FH, with higher LDL contributing to a genetic predisposition to atherosclerotic heart disease. This pathway underlies the mechanism of action of statin drugs, which decrease the risk of vascular events and prolong life,[1] and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, which lower lipid levels and reduce cardiovascular events even in the setting of statin treatment.[2] The quintessential secondary disorder of lipid metabolism is that seen in diabetes, a disease so frequently characterized by abnormalities of fat that lipids have been implicated in its pathogenesis.

Lipids are required for life, constituting the physical bilayer allowing formation of cell membranes, which compartmentalize specialized organelles inside cells and regulate transport between extracellular and intracellular environments. Lipids circulate in blood, with fatty acids and triglycerides providing an energy source to tissues such as heart and skeletal muscle, and nonnutritive sterols providing substrates for hormone production by gonads and adrenals. Specialized functions include development of surfactant in lung to maintain patency of alveoli, formation of bile to facilitate excretion of a variety of metabolites, and constitution of myelin throughout the nervous system to ensure the fidelity of nerve transmission. Lipids are also signaling molecules, serving as targets of lipid kinases that perpetuate signaling cascades, substrates for cyclooxygenases and related enzymes that generate prostaglandins, and ligands for nuclear receptors such as the peroxisome proliferator-activated receptors (PPARs). The broad spectrum of lipid functions results in part from their biophysical characteristics.

Simple and Complex Lipid Structure

The functional versatility of lipids is attributable to their hydrophobic structure. Due to the presence of fairly long carbon chains, lipids tend to associate with each other and have limited or no solubility in water. Fatty acids and cholesterol are simple lipids, whereas triglycerides and phospholipids are complex lipids (Fig. 41.1).



• **Fig. 41.1** Structures of common lipids, exemplified by the stearic, oleic, and linoleic fatty acids (A); the triglyceride tristearin (B); the phospholipid phosphatidylcholine (C); and cholesterol (D).

Fatty Acids

The chemical structure of fatty acids is determined by the number of carbon atoms and number of double bonds (see Fig. 41.1A). For example, stearic acid has 18 carbon atoms and is saturated, meaning that it has no double bonds; it is designated using the abbreviation C18:0. The 18-carbon monounsaturated fatty acid oleic acid (C18:1) has one double bond, and the polyunsaturated fatty acid linoleic acid (C18:2) has two double bonds.

Linoleic acid and arachidonic acid (C20:4) are omega-6 fatty acids, meaning that a double bond is present at the sixth carbon from the end of the molecule farthest from the carboxy (COOH)-terminus. Fish oils, which lower lipids, are omega-3 fatty acids, with a double bond present at the third carbon from the end opposite the COOH-terminus. Saturated fatty acids and some unsaturated fatty acids, such as oleic acid, are nonessential (i.e., they can be synthesized). Most omega-6 and omega-3 fatty acids are essential; they cannot be synthesized and are usually required for health, especially during development and in times of physiologic stress. Table 41.1 shows major food sources of fatty acids.

Triglycerides

The structure of tristearin, a triglyceride with three molecules of stearic acid connected to a glycerol molecule by means of ester linkages, is shown in Fig. 41.1B. Other triglycerides have a similar structure with alternative fatty acids esterified to the glycerol backbone.

Most adipose tissue mass is composed of triglycerides; triglycerides that circulate in the blood primarily reflect the fatty acid composition of adipose tissue triglycerides, and both sources reflect dietary fatty acid composition. Butter, common in Western

TABLE 41.1 Major Fatty Acids

Chemical Designation	Common Name	Common Food
Saturated Fatty Acids (No Double Bonds)		
C12:0	Lauric	Coconut oil
C14:0	Myristic	Coconut oil, butter fat
C16:0	Palmitic	Butter, cheese, meat
C18:0	Stearic	Beef, chocolate
Monounsaturated Fatty Acids (One Double Bond)		
C18:1	Oleic	Olive and canola oils
Polyunsaturated Fatty Acids (Two or More Double Bonds)		
Omega-6 Fatty Acids		
C18:2	Linoleic	Sunflower, corn, soybean, and safflower oils
C20:4	Arachidonic	Chicken, eggs
Omega-3 Fatty Acids		
C18:3	α-Linolenic	Canola, flaxseed, and soybean oils
C20:5	Eicosapentaenoic acid (EPA)	Salmon, cod, mackerel, tuna
C22:6	Docosahexaenoic acid (DHA)	Salmon, cod, mackerel, tuna

diets, consists of similar amounts of palmitate and oleate with a lesser amount of stearate; adipose tissue and circulating triglycerides in persons eating Western diets contain mostly palmitate and oleate. Olive oil, found in Mediterranean diets, is predominantly oleate with much less palmitate, so fat and circulating triglycerides in people eating a Mediterranean diet are enriched in oleic acid. Extremely high levels of triglycerides in the blood predispose to pancreatitis.

Phospholipids

The chemical structure for a generic phosphatidylcholine, a type of phospholipid, is shown in Fig. 41.1C. As with triglycerides, phospholipids have a glycerol backbone to which fatty acids are esterified at the first two alcohols. The characteristics of these fatty acids are important for determining cell membrane shape and function.[3,4] The third alcohol is esterified to a phosphate moiety linked to another molecule, such as choline, ethanolamine, or serine.

The presence of long-chain fatty acids comprising hydrophobic regions and the charged species at the end of the molecule make phospholipids ideal for generating cell membranes and lipoprotein surface components: the bilayer is oriented so that the hydrophobic regions point toward each other, and the hydrophilic regions interact with the aqueous environment. Phospholipids are distributed asymmetrically in cell membranes, with choline-containing lipids directed toward the outer surface and amine-containing lipids directed toward the cytoplasmic surface. Appearance of the aminophospholipid phosphatidylserine on the cell surface initiates blood clotting and marks apoptotic cells for phagocytosis. Several enzymes are involved in the dynamic process of remodeling cell membrane phospholipids, and some appear to be involved in metabolic disease, such as 1-acylglycerol-3-phosphate-O-acyltransferase 5 (AGPAT5), a membrane protein that converts lysophosphatidic acid to phosphatidic acid as the second step in de novo phospholipid biosynthesis, and which mediates insulin resistance in mice.[5]

Cholesterol

The structure of cholesterol is shown in Fig. 41.1D. Cholesterol in the plasma membrane is critical for maintaining membrane fluidity, probably by disrupting interactions between phosphatidylcholine and other molecules. It is also required for assembly of lipid rafts, which are ordered plasma membrane domains that mediate signal transduction.[6] The concentration of cholesterol is enriched in the plasma membrane, with much lower levels detected in the membranes of most intracellular organelles. Cholesterol is necessary for the synthesis of estrogen, progestins, androgens, aldosterone, vitamin D, glucocorticoids, and bile acids. Cholesterol deficiency is associated with severe developmental defects, as manifested in the rare Smith-Lemli-Opitz syndrome, which is likely caused by disruption of the Hedgehog signal transduction pathway.[7] Cholesterol excess is associated with gallstones and vascular disease.

Fatty Acid Metabolism

Fatty Acid Biosynthesis

In humans eating a typical Western diet, the overall contribution of de novo lipogenesis to lipid metabolism is small because the ingestion of exogenous fat is sufficient to suppress the energy-requiring process of synthesizing fats from carbohydrates. However, high-carbohydrate diets, especially those containing fructose,[8] substantially increase lipogenesis in liver and adipose tissue of humans.

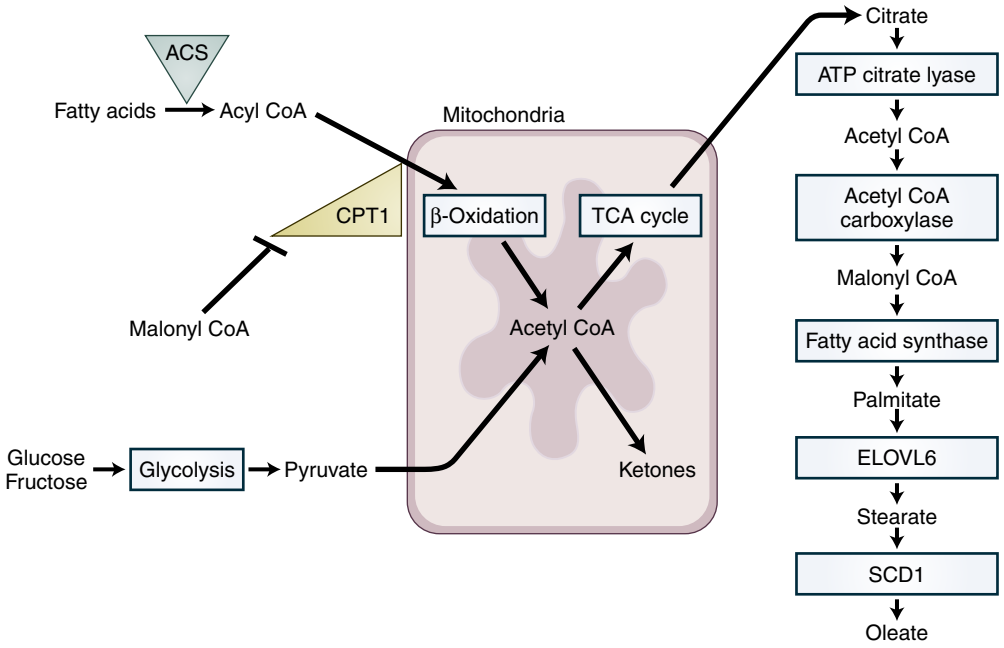
Fatty acid biosynthesis is carried out in most tissues at least minimally regardless of nutritional status. However, the liver is by far the major site of de novo fatty acid synthesis. Several key steps in fatty acid biosynthesis, presented in Fig. 41.2, also have major effects on systemic metabolism. Citrate derived from the tricarboxylic acid (TCA) cycle is converted to acetyl coenzyme A (acetyl CoA) in the cytoplasm by the action of adenosine triphosphate (ATP) citrate lyase. Acetyl CoA is then converted to malonyl CoA by acetyl CoA carboxylase (ACC), which exists in two isoforms: ACC1 (encoded by the gene *ACACA*) is cytosolic and important in liver and fat for de novo lipogenesis, and ACC2 (*ACACB*) is associated with mitochondria, also plays a role in liver metabolism, and is expressed at highest levels in muscle and heart. Antisense targeting of ACC isoforms has been shown to improve lipid metabolism and insulin sensitivity.[9]

Malonyl CoA inhibits carnitine palmitoyltransferase 1 (CPT1), which transports fatty acids into mitochondria, and thereby malonyl CoA prevents catabolism of fats under physiologic conditions in which energy is being stored as fat through fatty acid biosynthesis. Malonyl CoA also serves as substrate for fatty acid synthase, which sequentially connects two carbon fragments to generate saturated fatty acids such as palmitate. Inhibition of fatty acid synthase in the hypothalamus suppresses appetite by decreasing orexigenic peptides and increasing anorexigenic peptides in the arcuate nucleus, inducing weight loss and improving insulin sensitivity.[10] Pharmacologic inhibition of fatty acid synthase improves glucose metabolism in mice and primates,[11] and the mechanism may involve suppression of inflammatory pathways in macrophages.[12] Palmitate is converted to stearate through the action of a long-chain fatty acid elongase, which, when inactivated, promotes obesity but prevents insulin resistance.[13] Stearate is subsequently converted to oleate by stearoyl-CoA desaturase 1, which, when inactivated, increases fatty acid oxidation and protects against diet-induced obesity and insulin resistance.[14]

Fatty Acid Oxidation

Metabolism of fatty acids provides more energy per gram than metabolism of carbohydrates or proteins. Fatty acids undergo the process of β-oxidation in mitochondria (see Fig. 41.2). They are transported or diffuse across the plasma membrane, converted to acyl-CoA species by acyl-CoA synthase, then translocated to the mitochondrial matrix by CPT1 and CPT2. β-oxidation removes two carbon fragments through sequential actions of acyl-CoA dehydrogenases (e.g., medium-chain acyl-CoA dehydrogenase and very long chain acyl-CoA dehydrogenase), enoyl-CoA hydratase, hydroxy-CoA dehydrogenase, and thiolase. This process generates reduced nicotinamide adenine dinucleotide and reduced flavin adenine dinucleotide, which participate in electron transport to yield ATP. After multiple cycles, acetyl CoA is produced, which is a substrate for the tricarboxylic acid cycle and for ketogenesis.

Ketogenesis, a process restricted to the liver, is necessary for life during times of nutritional deprivation, as it can spare the use of glucose for ATP generation by the brain and other organs. Extreme production of ketones occurs in the setting of insulin deficiency usually due to autoimmune destruction of pancreatic beta cells and represents a threat to life. 3-Hydroxy-3-methylglutaryl (HMG)-CoA synthase (rate limiting in mitochondria) converts acetyl CoA to hydroxymethylglutaryl CoA, which is converted to acetoacetate by HMG-CoA lyase. Acetoacetate is either reduced to β-hydroxybutyrate or converted to acetone.



• **Fig. 41.2** Fatty acid metabolism. Fatty acids are substrates for ACS, which generates CoA moieties that are transported into mitochondria by CPT1. This process is inhibited by malonyl CoA. In mitochondria, β-oxidation generates acetyl CoA, which can also be generated from glycolysis (*bottom left*). Acetyl CoA can be used to produce ketones, or it may enter the TCA cycle, leading to production of citrate; in the cytoplasm, citrate is a substrate for ATP citrate lyase, which produces acetyl CoA. The acetyl CoA serves as a substrate for de novo synthesis of fatty acids, as depicted on the right side of the figure. *ACS,* acyl-CoA synthase; *ATP,* adenosine triphosphate; *CoA,* coenzyme A; *CPT1,* carnitine palmitoyltransferase 1; *ELOVL6,* elongation of very long chain fatty acid protein 6; *SCD1,* stearoyl-CoA desaturase 1; *TCA,* tricarboxylic acid.

Defects in fatty acid oxidation are among the most common inborn errors of metabolism. Presentations include nonketotic hypoglycemia, liver dysfunction, and cardiomyopathy.[15]

Triglyceride and Phospholipid Metabolism

Dietary fat, consisting of triglycerides and phospholipids, is digested in the stomach and proximal small intestine. Triglycerides are broken down into component fatty acids in part through the action of pancreatic lipase, which is activated by bile acids. Bile salts form micelles that acquire fatty acids and interact with the unstirred water layer of the intestine, where fatty acids are absorbed. Long-chain fatty acids are taken up by enterocytes, re-esterified into triglycerides, and exported into the lymph as lipoproteins. Medium-chain (≤C10) fatty acids directly enter the portal vein to access the liver.

Triglyceride Synthesis

Key steps in triglyceride synthesis have a major impact on systemic metabolism. Most triglycerides are synthesized through the glycerol phosphate pathway (Fig. 41.3, *top portion*) by a sequence of acylations. Another pathway, the monoacylglycerol pathway, is thought to be active only in the small intestine. Glycerol-3-phosphate is acted on by one of the glycerol-3-phosphate acyltransferases (GPATs) to generate lysophosphatidic acid. An important isoform is GPAT1, which competes with CPT1 for fatty acyl CoA molecules inside the cell, with GPAT1 prevailing when energy is to be stored and CPT1 dominant when energy is required. The next acylation is mediated by acylglycerol-3-phosphate acyltransferases (AGPATs) and generates phosphatidic acid. Human mutations in AGPAT2 are responsible for the disease called *congenital generalized lipodystrophy*.

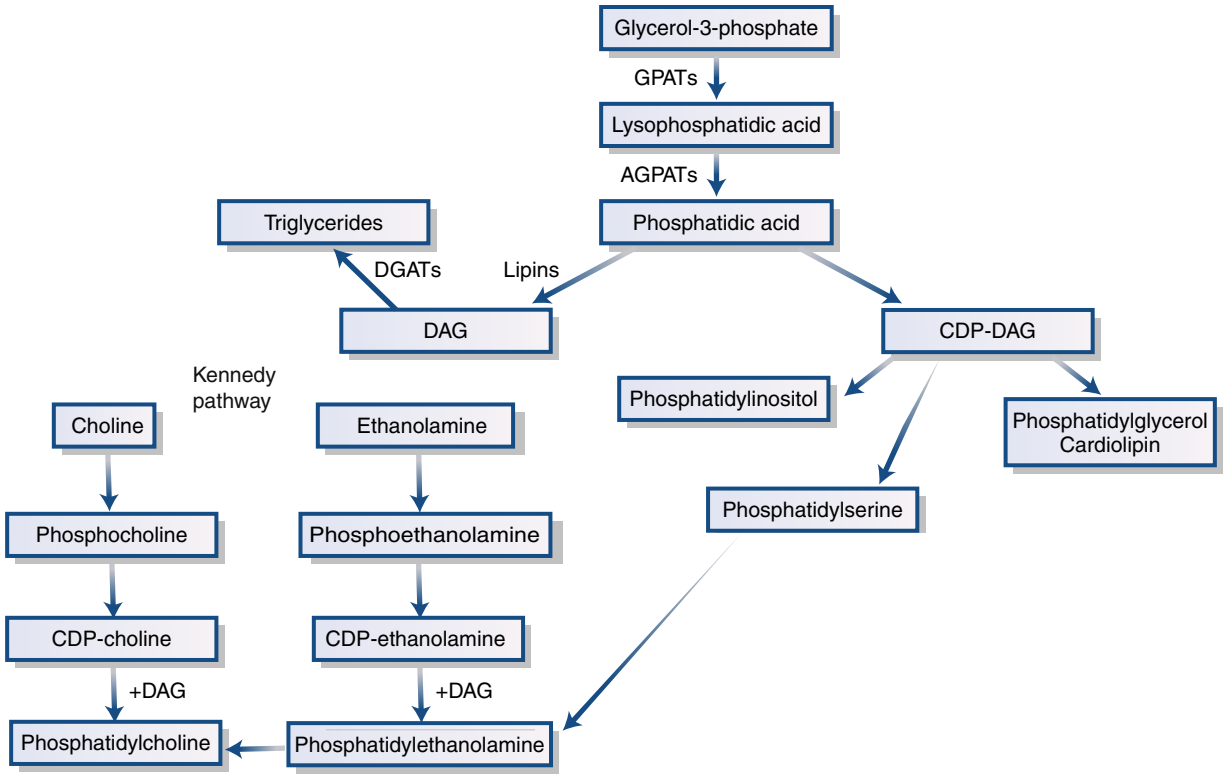
Phosphatidic acid represents an important branch point in lipid metabolism. It serves as substrate for synthesis of either cytidine diphosphate diacylglycerol (CDP-DAG, the precursor for molecules like phosphatidylinositol) or diacylglycerol (DAG). Synthesis of DAG requires a phosphatase activity provided by lipins.[16] DAG can serve as a signaling molecule and as substrate for synthesis of either triglycerides or common phospholipids. The acylation of DAG to form triglycerides is catalyzed by acyl-CoA:diacylglycerol acyltransferases (DGATs). Inactivation of DGAT1 in mice prevents diet-induced obesity,[17] but mutation of the gene in humans prevents normal fat absorption and causes steatorrhea in infants.[18]

Phospholipid Synthesis

As shown in the bottom left portion of Fig. 41.3, phospholipid synthesis is intimately related to triglyceride synthesis. Generation of the most common phospholipids, phosphatidylcholine and phosphatidylethanolamine, occurs mostly through the Kennedy pathway, which utilizes choline as an initial substrate and DAG at the final step. Mammalian liver can generate phosphatidylcholine from phosphatidylethanolamine through successive methylations. Phosphatidylserine can be converted to phosphatidylethanolamine, and other conversions between phospholipid species are possible.

Lipolysis of Triglyceride Stores in Adipose Tissue

Most of the body's triglyceride mass resides in adipose tissue, and turnover of energy stores from fat has important effects on lipid metabolism, normal physiology, and human health. Increased lipolysis in adipose tissue in obesity results in elevated circulating free fatty acids, which may cause dysfunction in pancreatic



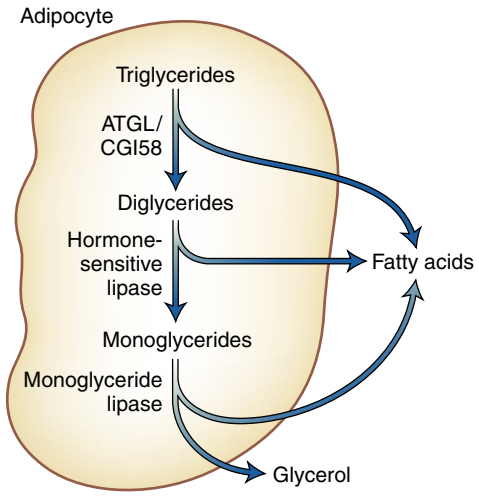
• **Fig. 41.3** Phospholipid and triglyceride synthesis. Glycerol-3-phosphate is converted by glycerol-3-phosphate acyltransferases (GPATs) to lysophosphatidic acid, which is converted to phosphatidic acid by acylglycerol-phosphate acyltransferases (AGPATs). Phosphatidic acid can be converted to cytidine diphosphate diacylglycerol (CDP-DAG), to fuel one arm of phospholipid synthesis, or to diacylglycerol (DAG), which is a substrate for another arm of phospholipid synthesis and for acyl-CoA:diacylglycerol acyltransferases (DGATs), which generate triglycerides. The Kennedy pathway, which utilizes DAG at its final steps, is depicted at the bottom left of the figure.

beta cells, liver, skeletal muscle, and heart. Healthy offspring with parents who have type 2 diabetes mellitus have impaired insulin-mediated suppression of circulating fatty acids,[19] suggesting that an early defect in adipose tissue fatty acid metabolism contributes to the evolution of diabetes.

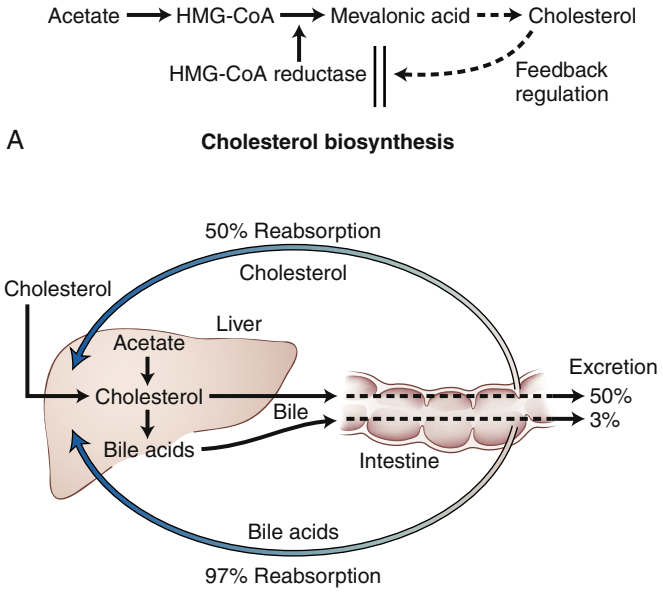
Release of free fatty acids and glycerol from adipose tissue is controlled by a variety of hormones, many of which act through G protein–coupled receptors. The most robust mediators of fatty acid release are catecholamines, which bind to β-adrenergic receptors, activating stimulatory G proteins (G_s) that prompt an increase in the activity of cyclic adenosine monophosphate and protein kinase A. Glucagon, adrenocorticotropic hormone, α-melanocyte-stimulating hormone, and thyroid-stimulating hormone also induce lipolysis through G_s proteins. Adenosine suppresses lipolysis by binding to receptors that activate inhibitory G proteins (G_i). Niacin, which also suppresses lipolysis, binds to the G protein receptor GPR109A, but this interaction does not mediate this vitamin's effects on lipid metabolism.[20] Insulin is a major lipolytic inhibitor, activating the insulin receptor–signaling cascade and suppressing lipolysis at many steps, one of which includes a decrease in protein kinase A activity. At least three enzymes and two accessory proteins are required for the normal process of hormone-induced lipolysis in adipose tissue.[21] Stored triglycerides are acted on by the enzyme, adipose triglyceride lipase (encoded by *PNPLA2*), which requires the coactivator protein comparative gene identification 58 (CGI58). Diglycerides are hydrolyzed by hormone-sensitive lipase, yielding monoglycerides that are metabolized by monoglyceride lipase. This process cannot occur unless perilipin 1, a protein that coats small lipid droplets, is phosphorylated by protein kinase A. Defects in *PLIN1*, the gene encoding perilipin 1, are associated with familial partial lipodystrophy.[22] Adipose tissue lipolysis is depicted schematically in Fig. 41.4. Human mutations in adipose triglyceride lipase or *CGI58* are responsible for two variants of neutral lipid storage disease that are characterized by hepatic steatosis; lipid accumulation in skeletal muscle; cardiomyopathy; neurologic problems; and, in one variant, skin defects.

Lipoprotein Lipase

Most lipids are delivered to peripheral tissues such as muscle and fat through the activity of lipoprotein lipase (LPL). LPL is rate limiting for clearance of plasma triglycerides and essential for generation of high-density lipoprotein (HDL) particles,[23] and hydrolyzes triglycerides (and, to a lesser extent, phospholipids) in circulating triglyceride-rich lipoproteins to allow peripheral sites access to preformed fatty acids. Much of this lipid flux is controlled by insulin, which increases LPL in fat and decreases LPL in muscle. Exercise tends to have the opposite effect,[24] ensuring appropriate energy supplies to meet metabolic demands. LPL activity requires the presence of a coenzyme, apoC-II, which primarily circulates as a component of very low density lipoprotein (VLDL) and HDL. In addition, the association of LPL with the luminal surface of endothelial cells requires the presence of its anchoring protein, glycosylphosphatidylinositol-anchored HDL-binding protein 1 (GPIHBP1).



• **Fig. 41.4** Lipolysis in adipocytes. Stored triglycerides are metabolized to yield the fatty acids that circulate in plasma through the action of three distinct lipases with separate substrate specificities. Triglycerides are acted on by adipose triglyceride lipase (ATGL) in complex with the coactivator protein CGI58 to yield diglycerides, which are acted on by hormone-sensitive lipase to yield monoglycerides. The monoglycerides, in turn, are acted on by monoglyceride lipase to yield glycerol. Lipid droplet proteins modulate this lipolytic process.



• **Fig. 41.5** (A) Cholesterol biosynthesis. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme regulating cholesterol biosynthesis. The enzyme is downregulated by excess cholesterol in the cell. (B) Enterohepatic circulation of cholesterol and bile acids. Approximately 50% of cholesterol and 97% of bile acids are reabsorbed from the intestine and recirculated to the liver. (A, Modified from Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science*. 1986;232:34–47.)

Free fatty acids released from lipoproteins by the action of LPL associated with the luminal surface of capillary endothelial cells must then cross the endothelial cell barrier to be captured by resident cells via conversion to acyl-CoA species. Tissue uptake of fatty acids at low concentrations appears mediated by cell surface transporters, whereas at high concentrations fatty acids may enter and transit the member in a nonregulated, nonsaturable process, sometimes referred to as flip-flop. Fatty acyl CoAs are then either stored as triglycerides or subjected to fatty acid oxidation. LPL is not synthesized in endothelial cells but is produced in adipocytes, cardiac myocytes, and skeletal myocytes and then secreted and targeted to the luminal surface of the endothelium. Both LPL and triglyceride-rich lipoproteins bind to endothelial GPIHBP1, which serves as a platform for lipolysis in the plasma.[25]

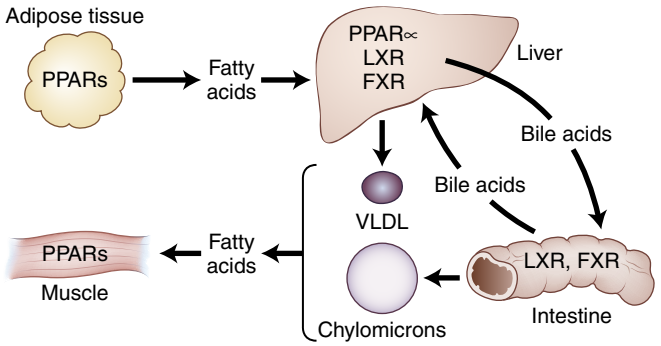
Cholesterol Metabolism

In adults, dietary cholesterol is not required because many tissues are capable of cholesterol synthesis. Most diets include animal products, the source of cholesterol. Plants do not have cholesterol, but their membranes contain phytosterols, which are structurally similar to cholesterol and are useful in the dietary treatment of hypercholesterolemia because they compete with cholesterol for absorption. The liver and intestine are quantitatively the most important sites for cholesterol metabolism in humans, although a very small amount of cholesterol is also lost through the normal turnover of skin.

Cholesterol Absorption, Synthesis, and Excretion

Cholesterol is absorbed through a process that requires formation of bile salt micelles. The efficiency of absorption varies widely in humans. There is a gradient of absorption through the intestine that is greatest in the proximal small intestine and least in the ileum. This gradient parallels the expression of Niemann-Pick C1–like 1 (NPC1L1), a transmembrane protein with a sterol-sensing domain that is involved in cholesterol absorption.[26] NPC1L1 is the target of ezetimibe, a drug that lowers cholesterol and has been shown to decrease heart disease. NPC1L1 also absorbs phytosterols such as sitosterol. The function of NPC1L1 in cholesterol absorption requires an interaction with LIM domain and actin binding 1 (LIMA1), which when mutated in humans is associated with low LDL cholesterol.[27] Sterols are pumped out of the enterocyte and into the intestinal lumen by two ATP-binding cassette (ABC) transporters: ABCG5 and ABCG8. Human mutations in these transporters cause the rare disorder sitosterolemia,[28] characterized by increased absorption and circulating levels of sitosterol and cholesterol, xanthomas, and heart disease (see later discussion).

Fig. 41.5A illustrates cholesterol synthesis. Acetate is converted to HMG-CoA. The latter is a substrate for HMG-CoA reductase, the enzyme that is rate limiting for cholesterol biosynthesis and is inhibited by statin drugs. Cells exquisitely regulate cholesterol acquisition.[29] When levels are low, the transcription factor sterol response element–binding protein (SREBP) translocates to the nucleus to activate genes that increase cholesterol biosynthesis and import cholesterol from the extracellular environment. Statins, by lowering cholesterol and preventing cholesterol biosynthesis, work predominantly by increasing liver uptake of cholesterol from the plasma through the LDL receptor (see later discussion) and promoting its excretion. Free cholesterol in cells is esterified to form cholesteryl esters for storage. This esterification reaction is carried out by acyl CoA:cholesterol acyltransferases (ACATs). These endoplasmic reticulum enzymes exist in two forms: ACAT1 is present in macrophages and has been implicated in atherosclerosis, and ACAT2 is present in the liver and intestine and is implicated in cholesterol absorption. Nonspecific ACAT inhibition in humans does not affect serum lipids and does not have beneficial effects on atherosclerosis.[30]



• **Fig. 41.6** Nuclear receptors in lipid metabolism. Peroxisome proliferator-activated receptors (PPARs) are active in adipose tissue, which is a source of fatty acids that are transported to liver, where PPARα, the liver X receptors (LXRs), and farnesoid X receptor (FXR) are active. Bile acids produced by the liver participate in an enterohepatic circulation with the intestine, another site of LXR and FXR expression. Very low density lipoprotein (VLDL), produced by the liver, and chylomicrons, from the intestine, are metabolized to release fatty acids that fuel muscle (another site of PPAR expression) and may be stored by adipose tissue.

Cholesterol, which is non-nutritive and cannot be catabolized to carbon dioxide and water, is either secreted into the bile as free cholesterol (about half of which is reabsorbed) or converted to bile acids for secretion into bile. Most bile acids are reabsorbed in the terminal ileum. This enterohepatic circulation of cholesterol and bile acids is shown in Fig. 41.5B. The rate-limiting enzyme for bile acid synthesis is cholesterol 7α-hydroxylase, which is under feedback regulation by bile acids. Interruption of the enterohepatic circulation of bile acids through the use of bile acid sequestrants reduces the farnesoid X receptor (FXR)-mediated inhibition of bile acid synthesis. The subsequent induction of bile acid synthesis is associated with a reduction in plasma cholesterol and an increase in triglyceride production, explaining triglyceride elevations seen with bile acid sequestrant therapies. At least in mice, evidence suggests that cholesterol can be excreted directly by enterocytes (independent of the biliary system) through an active metabolic process called *transintestinal cholesterol excretion*.[31]

Nuclear Receptors and Lipid Metabolism

Nuclear receptors, usually transcription factors with both ligand- and DNA-binding domains, affect lipid metabolism. Classic hormones that interact with nuclear receptors and have important lipid effects include thyroid hormone, glucocorticoids, estrogen, and testosterone.

SREBPs are a family of transcription factors that control the metabolism of both cholesterol and triglycerides.[32] In an inactive state such as occurs with excess cellular cholesterol, they are associated with the endoplasmic reticulum membrane. Reduced endoplasmic reticulum cholesterol allows proteolytic processing of SREBPs allowing translocation to the nucleus, where they regulate production of biosynthetic genes for cholesterol and the LDL receptor (SREBP2) and for triglyceride (SREBP1c) (both are discussed in more detail later).

Thyroid hormone reduces circulating cholesterol concentrations via modulation of LDL receptor expression, or through reduced hepatic synthesis of apoB lipoproteins and greater excretion of cholesterol into the bile.[33] Thus, lipid levels tend to be high in hypothyroid patients and low with hyperthyroidism. Glucocorticoids have robust effects on multiple aspects of lipid metabolism, inducing expression of HMG-CoA reductase to promote cholesterol synthesis, increasing expression of fatty acid synthase to promote fatty acid synthesis, and decreasing LPL to impair clearance of circulating lipids. Accordingly, hyperlipidemia is commonly seen in the setting of glucocorticoid treatment, and insulin resistance induced by glucocorticoids amplifies the hyperlipidemia. Estrogens and selective estrogen receptor modulators such as raloxifene lower cholesterol[34] by inducing LDL receptor activity; they tend to increase triglyceride levels, especially when higher oral doses are administered. Derivatives of cholesterol can serve as selective estrogen receptor modulators to affect the vasculature.[35] Androgens, by activating the androgen receptor, decrease HDL.[36] Changes in HDL with estrogens and androgens correlate with changes in hepatic triglyceride lipase, an enzyme that, like LPL, is released into the bloodstream after heparin injection.

Aside from classic hormones and their receptors, other nuclear receptors affect lipid metabolism after interacting with several types of metabolic by-products. These receptors include the PPARs, the liver X receptors (LXRs), and FXR. A schematic view of the roles of these receptors in lipid metabolism is presented in Fig. 41.6.

There are three known types of PPARs: α, γ, and δ. PPARα promotes fatty acid oxidation, as well as ketogenesis, and is induced by starvation. PPARα expression is highest in tissues adapted to metabolize fats, such as liver and skeletal muscle, but is also present in numerous other sites. In humans, pharmacologic activation of PPARα with fibrates lowers triglycerides and increases HDL. Fatty acids interact with the receptor, but a phosphatidylcholine species was identified as an endogenous ligand for PPARα.[37]

Whereas PPARα facilitates energy utilization, PPARγ activates genes that promote energy storage. It is expressed at the highest levels in adipose tissue and is also found in macrophages, where it may help coordinate the complex relationship between inflammation and metabolism. Ether lipids, which are phospholipids generated in peroxisomes, appear to be endogenous ligands for PPARγ.[38] Pharmacologic activation of PPARγ in humans with thiazolidinediones results in insulin sensitization and weight gain (see Chapter 35). The latter effect occurs because this nuclear receptor promotes both adipogenesis and fluid retention through effects on the kidney. Importantly, thiazolidinediones increase incident heart failure.[39] Thiazolidinedione treatment in humans tends to lower triglycerides and increase HDL, probably by modulating insulin signaling. In mice, these agents reduce atherosclerosis, and in people with metabolic syndrome with a recent stroke or transient ischemic attack, pioglitazone reduces risk of stroke and myocardial infarction.[40] Dual agonists for PPARα and PPARγ lower hemoglobin A_{1c} and serum lipids in humans but also increase the all-cause mortality rate.[41]

PPARδ promotes fatty acid oxidation in skeletal muscle, and activation of this receptor may mimic some aspects of exercise.

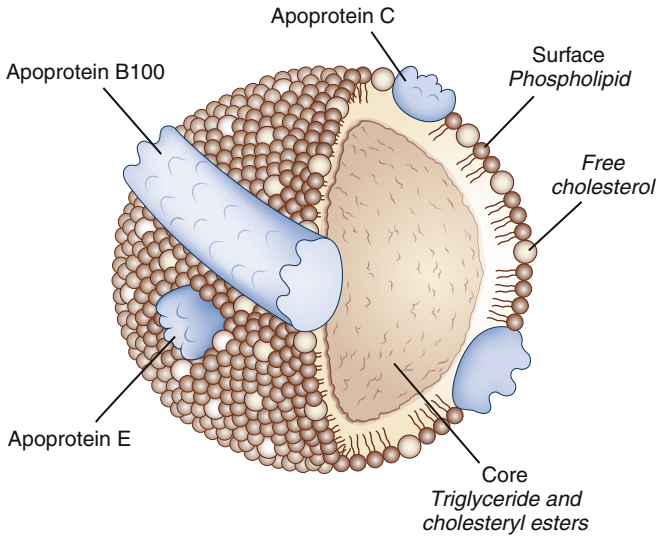
LXRs and FXR are also involved in lipid metabolism. LXRα and LXRβ are activated by oxysterols (derivatives of cholesterol) to increase conversion of cholesterol into bile acids, increase bile acid excretion, and decrease cholesterol absorption. LXR activation inhibits cholesterol uptake by inducing the degradation of the LDL receptor.[42] LXRs also induce fatty acid and triglyceride synthesis. FXR is activated by bile acids to stimulate both bile acid secretion and reabsorption. Administration of the bile acid sequestrant colesevelam to humans with diabetes lowers blood sugar, but the role of nuclear receptors in this effect is not defined.

Additional nuclear receptors also play important roles in lipogenesis—the process of converting carbohydrates to triglycerides rather than glycogen. Carbohydrate response element–binding

protein (ChREBP) may control as much as 50% of this process. It responds to carbohydrate excess by transactivating a series of glycolytic and lipogenic genes.[43] SREBP1c is also critical for this process.[44]

Plasma Lipoproteins, Apolipoproteins, Receptors, and Other Proteins

Lipoproteins, which are spherical particles that circulate in the blood, transport vital non–water-soluble nutrients, vitamins, structural components, and proteins with specialized functions from the gut through the plasma compartment to remote tissues. Appropriate concentrations of lipoproteins are essential for health, but increased circulating concentrations of certain lipoproteins are associated with increased risk for cardiovascular disease. Absent or low levels of other lipoproteins may lead to vitamin deficiency syndromes. The roles of the various lipoproteins are discussed in detail in the following sections.



• **Fig. 41.7** General structure of lipoproteins: schematic representation of a very low density lipoprotein particle.

Major Lipoproteins

A prototypical lipoprotein is shown in Fig. 41.7. The fundamental structure of a lipoprotein exploits biochemical characteristics of its components. The surface consists of charged molecules that interact with the aqueous environment, such as phospholipids and free cholesterol. Amphipathic proteins (with both hydrophilic and hydrophobic domains), called *apolipoproteins* (or simply *apoproteins*), are also present on the surface, with hydrophilic domains oriented toward the plasma and hydrophobic domains toward the core of the particle. Apolipoproteins regulate the lipoprotein interaction with metabolic enzymes and cellular receptors. The lipoprotein core consists of neutral (uncharged) lipids, such as triglycerides and cholesteryl esters.

Lipoprotein movement through the plasma compartment is dynamic. Humans spend most of their lives in the postprandial state. Eating is associated with generation of lipoproteins and the induction of enzymes that metabolize those lipoproteins. Within the circulation, lipolysis of triglyceride leads to rapid reduction in the size of chylomicrons. Along with loss of core triglyceride, excess surface components of shrinking particles are extruded, and both nonesterified fatty acids and fat-soluble vitamins are delivered into tissues.

The major classes of lipoproteins are listed in Table 41.2. They were identified based on migration in an ultracentrifuge, and classes were defined based on density assessed using salt-containing solutions. An alternative original classification scheme, which is no longer useful, involved electrophoretic mobility in agarose gels. Chylomicrons, chylomicron remnants, and VLDLs are rich in triglycerides. Intermediate-density lipoproteins (IDLs), LDLs, and lipoprotein(a) (Lp[a]), are rich in cholesterol. HDLs are enriched in phospholipids. Triglyceride-rich lipoproteins such as chylomicrons are large and generally insoluble, which accounts for the cloudy appearance of plasma when it is obtained in nonfasting subjects or in fasting subjects with some types of hyperlipidemias.

TABLE 41.2 Major Classes of Plasma Lipoproteins

Type	Density (g/mL)	Origin	Major lipids	Major Apolipoproteins	Size (nm)
Chylomicrons	<0.95	Intestine	85% Triglyceride	B48, AI, AIV, E, CI, CII, CIII	~100–500
Chylomicron remnants	<1.006	Derived from chylomicrons	60% Triglyceride 20% Cholesterol	B48, E	~80–125
VLDL	<1.006	Liver	55% Triglyceride 20% Cholesterol	B100, E, CI, CII, CIII	30–80
IDL	1.006–1.019	Derived from VLDL	35% Cholesterol 25% Triglyceride	B100, E	25–35
LDL	1.019–1.063	Derived from IDL	60% Cholesterol 5% Triglyceride	B100	18–25
HDL	1.063–1.21	Liver, intestine, plasma	25% Phospholipid 20% Cholesterol 5% Triglyceride	AI, AII, CI, CII, CIII, E	5–12
Lp(a)	1.05–1.09	Liver	60% Cholesterol 5% Triglyceride	B100, apo(a)	~30

apo, Apolipoprotein; *IDL,* intermediate-density lipoprotein; *LDL,* low-density lipoprotein; *Lp(a),* lipoprotein(a); *VLDL,* very low density lipoprotein.

Table 41.2 provides general ranges for particle size, which differ substantially among lipoproteins and within each class. The relative amount of protein versus less dense lipids correlates with the size and also the buoyancy of the lipoproteins—for example, large VLDLs contain much more lipid than the smaller HDLs. The subspecies of these particles similarly differ in size and protein/lipid content. One lipoprotein subtype, small dense LDL, is associated with cardiovascular disease, insulin resistance, and hypertriglyceridemia.

Chylomicrons originate in the gut. They are lighter than water and float to the top of a plasma sample. The particles are cleared rapidly (within minutes) after a meal and should be absent after an overnight fast. Their distinguishing apolipoprotein is apoB48, which is the only form of apolipoprotein B produced by intestinal cells in humans. B48 is so named because, as a splice variant, it is 48% of the entire B100 protein found in VLDL and LDL. Chylomicrons acquire apoC and apoE molecules by interacting with HDL particles, a process that promotes chylomicron metabolism and conversion to chylomicron remnants. Chylomicron remnants, also characterized by the presence of apoB48, are cleared rapidly from the plasma. Remnant particles, as well as LDL, promote arterial atherosclerosis.

VLDL particles are of hepatic origin. Smaller than chylomicrons, their distinguishing apolipoprotein is apoB100, the form of apoB produced by the liver. VLDLs also carry apoC molecules that modulate the conversion of VLDLs to IDLs, which are VLDL remnants and are also atherogenic. IDL particles contain apoB100 and apoE, and they are converted to LDL, which is characterized by carrying essentially only apoB100 as an apolipoprotein. LDL is the major carrier of cholesterol in most humans, and its measurement forms the basis for coronary heart disease risk stratification and treatment goals. For most clinical laboratories, LDL results represent both IDL and LDL particles.

HDL particles have a complex biology. They can be generated by the liver and intestine or assembled in the plasma as a consequence of the metabolism of other lipoproteins. They are arbitrarily divided into HDL_2 (less dense, at 1.063–1.125 g/mL), which typically contains both apoAI and apoCs, and HDL_3 (more dense, at 1.125–1.21 g/mL), which typically contains apoAI, apoAII, and apoCs. There is also a minor subclass known as HDL_1 that carries a large percentage of plasma apoE. High HDL levels are associated with low cardiovascular risk in population studies, but it is not known if HDL plays a direct role in atherosclerosis. HDL can be assessed by its cholesterol content or more recently by a functional assay of cholesterol efflux capacity. This activity, reflecting the movement of labeled cholesterol from a cultured macrophage cell line to apoB-depleted plasma, may be inversely associated with cardiovascular events (see later discussion).[45] In part, the disparity between HDL concentration and function might explain why genetic markers associated with HDL levels do not associate with cardiovascular disease risk.[46]

Lp(a), produced by the liver, consists of an LDL particle in which the apolipoprotein apo(a) has been covalently linked to apoB100. Apo(a) has substantial protein homology to plasminogen, which is required for the endogenous thrombolytic response, and it exists in isoforms based on Kringle repeats (named after a type of pastry). Isoforms with fewer repeats, and therefore lower mass, tend to circulate at higher concentrations. Higher levels increase the risk of myocardial infarction, aortic valve calcification, and aortic stenosis.[47,48]

Major Apolipoproteins

The chromosomal location, size, sites of synthesis, and major functions of important apolipoproteins are summarized in Table 41.3.

Apolipoproteins AI, AII, AIV, and AV

ApoAI is the most abundant apolipoprotein in HDL. It is synthesized by the liver and intestine and activates the enzyme lecithin:cholesterol acyltransferase (LCAT), which transfers a fatty acid from lecithin to the free hydroxyl group on cholesterol to generate cholesteryl ester. Maturation of HDL particles begins as lipid-poor discs containing apoAI which then acquire free cholesterol. Cholesterol within the nascent HDL is converted to cholesteryl ester through the activity of LCAT leading to expansion into spheres. ApoAI mediates efflux of cholesterol from peripheral tissues, an important step in the process of reverse cholesterol transport.[49] Human genetic mutations in apoAI cause low levels of HDL and corneal opacities. ApoAI is considered to be an antiatherogenic protein, but genetic defects leading to low levels of apoAI are not consistently associated with coronary artery disease.

ApoAII is present with apoAI in some HDL particles. Synthesized mostly in the liver, it has been implicated in the activation of hepatic lipase, an enzyme involved in HDL metabolism, and in the inhibition of LCAT. ApoAII disrupts the ability of HDL to promote reverse cholesterol transport, but the genetic absence of this protein in humans does not seem to be associated with a phenotype.[50]

ApoAIV originates in the gut, and its secretion is induced by consumption of a high-fat meal. It may affect food intake in mice, but information in humans is not available.

ApoAV is encoded by a locus near the apoAIV gene in the apoAI/CIII/AIV/AV gene cluster on chromosome 11. It is produced by liver and circulates at low concentrations in association with VLDL particles in humans. ApoAV is involved in the hydrolysis of triglyceride-rich lipoproteins by LPL, its expression is inversely related to triglyceride levels, and it promotes lipoprotein clearance by hepatic proteoglycans.[51] In humans, homozygous mutations in apoAV increase risk for hyperchylomicronemia and pancreatitis.[52]

Apolipoprotein B

There are two forms of this apolipoprotein, apoB100 and apoB48, which are encoded by a single gene. A unique mechanism that involves RNA editing allows translation into these two forms of apoB (Fig. 41.8). In both liver and intestinal cells, the same messenger RNA is transcribed, but an editing protein complex interacts with the message only in the intestine (in humans) to change the cytosine at nucleotide position 6666 to uracil leading to production of an intestinal protein that is approximately 48% of the length of apoB100.

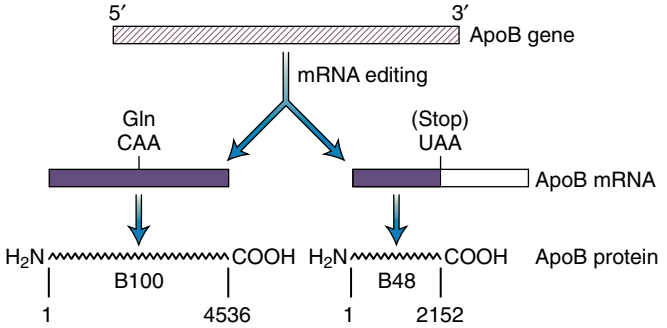
ApoB48 is important for the assembly of chylomicrons.[53] There are one or two apoB48 molecules on each chylomicron, where they provide structural support to the particle. The COOH-terminus of apoB100, missing in apoB48, determines interaction with the LDL receptor, so apoB48 does not appear to be involved in the clearance of gut-derived lipoproteins.

ApoB100 originates in the liver, where it is cotranslationally associated with lipids to coordinate the formation of VLDL particles. VLDL assembly and export, which affect the levels of circulating atherogenic lipoproteins, are determined not by transcriptional control of the apoB gene but by a mechanism involving stabilization of the apoB protein by lipid. VLDL production

TABLE 41.3 Major Apolipoproteins

Apolipoprotein (Chromosome No.)	Molecular Weight (kDa)	Synthesis	Functions
AI (11)	~29	Liver, intestine	Structural protein (HDL) Cofactor for LCAT Crucial role in reverse cholesterol transport Ligand for ABCA1 and SR-BI
AII (1)	~17 (dimer)	Liver	Inhibits apoE binding to receptors Activates hepatic lipase Inhibits LCAT
AIV (11)	~45	Intestine	Potential satiety factor Activator of LCAT Facilitates lipid secretion from intestine
AV (11)	39	Liver	Activator of LPL-mediated lipolysis Might inhibit hepatic VLDL synthesis
B100 (2)	~500	Liver	Structural protein (VLDL and LDL) Ligand for LDL receptor
B48 (2)	~200	Intestine	Structural protein (chylomicrons)
CI (19)	6.6	Liver	Modulates remnant binding to receptors Activates LCAT
CII (19)	8.9	Liver	Cofactor for LPL
CIII (11)	8.8	Liver	Modulates remnant binding to receptors Inhibitor of LPL
E (19)	~34	Liver, brain, skin, testes, spleen	Ligand for LDL and remnant receptors Local lipid redistribution Reverse cholesterol transport (HDL with apoE)
apo(a) (6)	~400–800	Liver	Modulates thrombosis/fibrinolysis

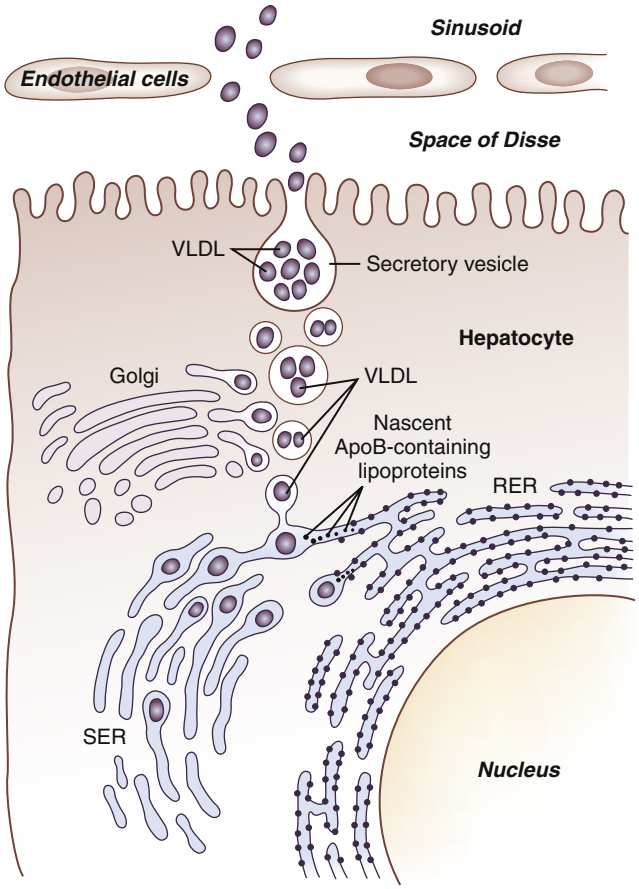
ABCA1, Adenosine triphosphate–binding cassette transporter A1; *apo,* apolipoprotein; *HDL,* high-density lipoprotein; *LCAT,* lecithin:cholesterol acyltransferase; *LDL,* low-density lipoprotein; *LPL,* lipoprotein lipase; *SR-B1,* scavenger receptor class B type 1; *VLDL,* very low density lipoprotein.



• **Fig. 41.8** Synthesis of apolipoprotein B100 and apoB48 by a messenger RNA (mRNA) editing mechanism. In the human intestine, a specific cytosine (C) is changed to a uracil (U) in the apoB mRNA. This change results in a stop codon and the formation of apoB48, which contains only the first 21^{52} amino acids of the full-length apoB100 (4536 amino acids). *COOH,* carboxy-terminus; *Gln,* glutamine; *H_2N,* amino-terminus.

is shown in Fig. 41.9. Assembly is thought to involve two distinct processes. First, as the apoB message is translated on the rough endoplasmic reticulum, it binds to lipids that are provided by microsomal triglyceride transfer protein (MTP; the target of a medication, see later discussion). This protein heterodimerizes with protein disulfide isomerase, which remodels the apoB protein by rearranging the positions of disulfide bonds in the molecule to accommodate incoming lipid. Most of this lipid originates in adipose tissue, where triglyceride lipolysis releases free fatty acids that are transported to the liver. Phospholipids and cholesterol also associate with apoB at this step. If sufficient lipids are not available in the liver, apoB (which is constitutively produced) is ubiquitinated and degraded in the proteasome. Second, maturing VLDL particles fuse with additional lipid droplets in the Golgi apparatus, a process facilitated by apoE. The triglyceride-rich particles are then secreted into the space of Disse. The discrete fates of these two potential sources of VLDL may be complex, but VLDL particles ultimately gain access to the circulation, where the transfer of apolipoproteins from other lipoproteins modifies VLDL structure to promote metabolism in the periphery.

Increased VLDL production, fueled by the increased availability of lipid, is predominantly responsible for the dyslipidemia seen with obesity and diabetes. There is one copy of apoB100 on each VLDL particle, and this relationship is retained as these lipoproteins are metabolized to IDL and then to LDL. Therefore, measurements of apoB100 in the plasma reflect the particle number, and higher levels of apoB are associated with cardiovascular disease. The complete absence of apoB, which occurs in the rare human disorder abetalipoproteinemia, is usually caused by mutations in MTP.[54] Patients with this disease can have severe neurologic deficits, probably reflecting vitamin E deficiency, because triglyceride-rich lipoproteins transport this lipid-soluble vitamin. Very low, but not absent, apoB, which occurs in the human



• **Fig. 41.9** Very low density lipoprotein (VLDL) biosynthesis by hepatocytes. The nascent apolipoprotein B (ApoB)-containing apolipoproteins synthesized by the rough endoplasmic reticulum (RER) are thought to combine with lipids in the smooth endoplasmic reticulum (SER). The VLDLs are processed in the Golgi apparatus and accumulate in large secretory vesicles. They are then released into the space of Disse and enter the plasma. (Modified from Alexander CA, Hamilton RL, Havel RJ. Subcellular localization of B apoprotein of plasma lipoproteins in rat liver. *J Cell Biol.* 1976;69:241–263; by copyright permission of the Rockefeller University Press.)

disorder hypobetalipoproteinemia, is usually caused by truncation mutations in apoB. These individuals present with low cholesterol and triglycerides and appear to be healthy. Another cause of hypobetalipoproteinemia is a defect in angiopoietin-like protein 3 (ANGPTL3), which leads to reduced hepatic secretion of lipoproteins and increased clearance without causing fatty liver.[55] A mutation at amino acid residue 3500 of the apoB100 protein, within the COOH-terminal region of the molecule that mediates binding to the LDL receptor, causes familial defective apoB100. These individuals have high LDL cholesterol, mimicking the presentation of FH.[56]

Apolipoproteins CI, CII, and CIII

These small apolipoproteins are encoded by loci residing at two different locations in the genome. ApoCI and apoCII are transcribed from a site on chromosome 19 near the apoE gene. The apoCIII gene is a component of the apoAI/CIII/AIV/AV cluster on chromosome 11. ApoCs, which can be exchanged freely among lipoprotein particles, are important for triglyceride metabolism because their presence either interferes with the recognition of apoE by lipoprotein receptors or displaces apoE from lipoproteins (both of which would increase triglycerides by impairing their clearance). The function of apoCII is more complex. High levels in mice cause elevated triglycerides by displacing apoE, but normal levels of apoCII are required for normal lipid clearance because this apolipoprotein is a cofactor for the enzyme LPL. Mutations of apoCII in humans cause severe hypertriglyceridemia, mimicking LPL deficiency.

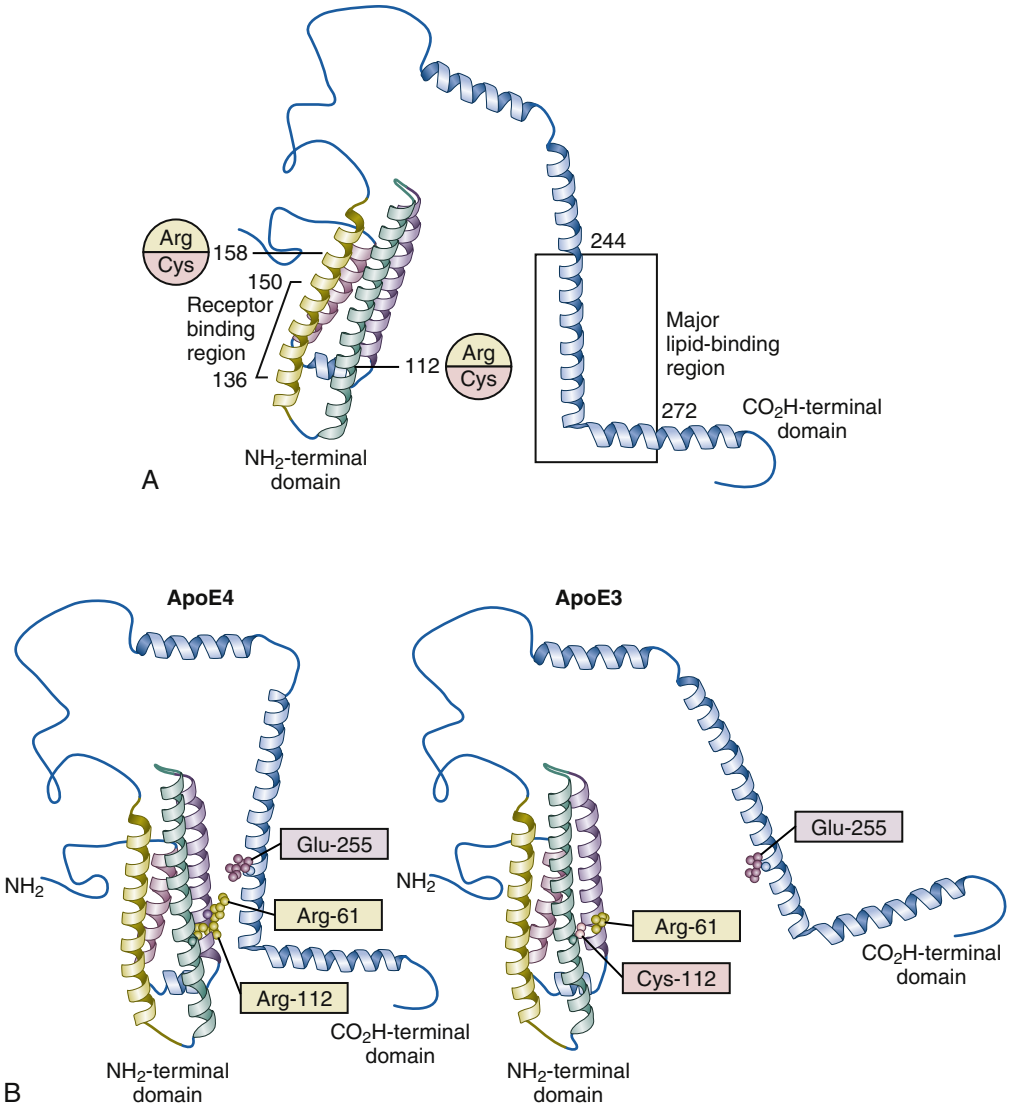
ApoCIII may be particularly relevant to human health. Levels are increased in the setting of many dyslipidemias, and most lipid-lowering medications lower apoCIII levels. A mutation in the apoCIII gene causing lower apoCIII levels is associated with an improved lipid profile and less atherosclerosis,[57] suggesting that therapies targeted at apoCIII might provide clinical benefit. In patients with extremely high triglycerides due to familial chylomicronemia syndrome (FCS; see later discussion), inhibiting the apoCIII mRNA results in substantial triglyceride lowering.[58]

Apolipoprotein E

ApoE biology is also complex. ApoE expression is highest in the liver, followed by the brain. In the brain, astrocytes and microglial cells synthesize apoE, which can also be produced by injured neurons. Many other cell types synthesize the protein, including macrophages. ApoE circulates in plasma within all lipoproteins, with the probable exception of LDL. Its principal function involves interactions with the two major receptors mediating the clearance of plasma lipoproteins: the LDL receptor and the LDL receptor–related protein (LRP1, also known as the chylomicron remnant receptor). Therefore, apoE is primarily responsible for clearance of intestinal-derived lipoproteins after a meal and for clearance of VLDL and IDL particles before they are converted to LDL.

There are three major apoE isoforms: E2, E3, and E4. They are encoded, respectively, by alleles referred to as ε2, ε3, and ε4, with charge differences caused by variations in amino acids at residues 112 and 158 in the protein. ApoE3 is considered the normal isoform; it has a cysteine at residue 112 and an arginine at 158. ApoE2 has cysteines at both 112 and 158, and apoE4 has an arginine at both 112 and 158. These variations have structural and functional consequences (Fig. 41.10). The protein has two domains: an amino (NH_2)-terminus interacts with lipoprotein receptors and a COOH-terminus interacts with lipids (see Fig. 41.10A). In apoE4, the isoform associated with disease, these domains interact, which does not occur with apoE3 (see Fig. 41.10B).

Comprehensive data (more than 86,000 individuals for lipids and more than 37,000 for coronary events) link apoE allele and genotype frequencies, lipid levels, and coronary risk.[59] Allele frequencies in healthy adults are 7% for ε2, 82% for ε3, and 11% for ε4. Genotype frequencies are 0.7% for ε2/ε2, 11.6% for ε2/ε3, 2.2% for ε2/ε4, 62.3% for ε3/ε3 (the most abundant genotype), 21.3% for ε3/ε4, and 1.9% for ε4/ε4. There is a linear relationship between the genotype and both LDL level and coronary risk, from least to most, as follows: ε2/ε2 < ε2/ε3 < ε2/ε4 < ε3/ε3 < ε3/ε4 < ε4/ε4. Compared with the reference group (ε3/ε3), the presence of the ε2 allele decreases coronary risk by about 20%, and the presence of the ε4 allele slightly increases risk. These observations are interesting for two reasons. First, ε2/ε2 individuals, although they are protected from coronary heart disease on a population basis, are at risk for dysbetalipoproteinemia, occurring in about 5% of ε2/ε2 individuals and which is associated with aggressive vascular disease. Second, the E2 protein binds less well to the LDL receptor than E3 and E4. This suggests that LDL cholesterol in patients with the E2 protein should be higher (because it is less



• **Fig. 41.10** (A) The amino (NH_2)-terminal domain of apoprotein E is composed of a four-helix bundle. A region of random structure encompassing residues 165 to 200 forms a connector or hinge region linked to the carboxy (CO_2H)-terminal domain. There are two major functional regions. Residues 136 to 150 (*yellow helix*) encompass the receptor-binding region; residues 240 to 260 in the carboxy-terminal domain encompass the lipid-binding region. (B) ApoE4 displays the unique property of domain interaction that distinguishes it from apoE3 (Arg-61 in the amino-terminal domain interacts with Glu-255 in the carboxy-terminal domain). *Arg,* arginine; *Cys,* cysteine; *Glu,* glutamic acid.

likely to be cleared by this receptor), yet the opposite is observed. These data suggest that other receptor-mediated processes, such as those mediated by heparan sulfate proteoglycans (HSPGs), may be critical for clearance of apoE-containing lipoproteins.[60]

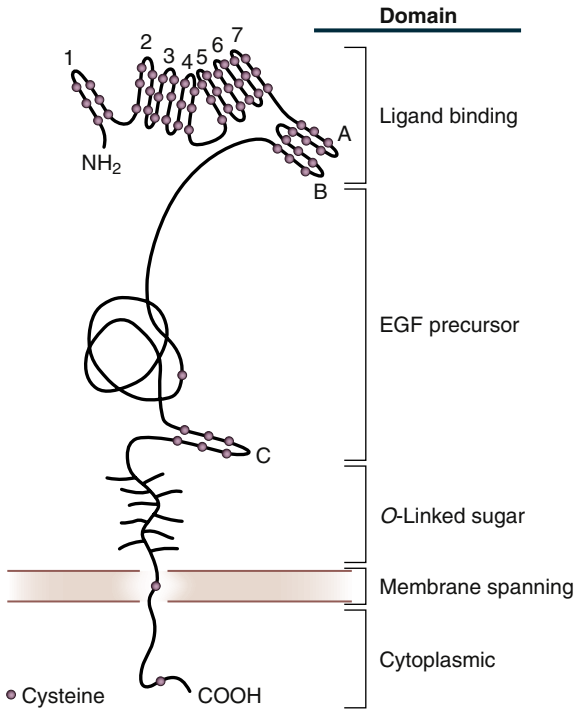
ApoE is a well-characterized genetic marker for Alzheimer disease. Risk increases approximately 3-fold in those with one ε4 allele and 12-fold in those with two ε4 alleles.[61] The presence of an ε2 allele is protective. These relationships hold for both early- and late-onset Alzheimer disease. There are HDL-like lipoproteins in the central nervous system, and apoE-mediated delivery of cholesterol is important for normal synaptic function. The relation of lipid metabolism to Alzheimer disease is incompletely understood, but evidence suggests that deposition of amyloid-β (the major constituent of the plaques that characterize the disease) begins sooner in the brains of those with the E4 protein. Because people with the ε4 allele are also more likely to have atherosclerosis, central nervous system vascular disease may help explain why this apoE variant is involved in neurodegeneration.

Major Receptors Involved in Lipid Metabolism

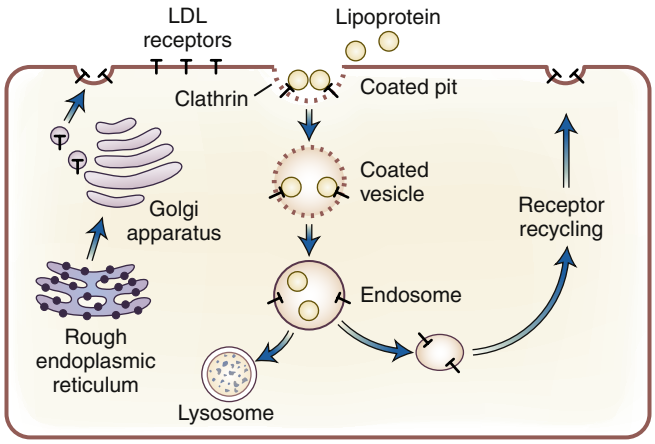
LDL Receptor Gene Family

There are at least 10 members of the LDL receptor family; LDL receptor and LRP1 are the two most important ones for systemic lipid metabolism. The LDL receptor recognizes apoB100 and apoE, whereas LRP1 recognizes only apoE. Other core family members (those that share considerable structural homology) include the VLDL receptor, the apolipoprotein E receptor 2 (apoER2 or LRP8), LRP4, LRP1B, and megalin (LRP2, also known as gp330 and as the major Heymann nephritis antigen).

Three family members lack some of the structural features of the others. They are sortilin-related receptor L1 (LR11/SORL1),



• **Fig. 41.11** Functional domains of the low-density lipoprotein receptor. Numbers 1 through 7 indicate repeats in the ligand-binding domain. A, B, and C are epidermal growth factor (EGF)-like repeats in the EGF precursor domain. See the text for a complete description. *COOH,* carboxy-terminus.



• **Fig. 41.12** Low-density lipoprotein (LDL) receptor pathway. LDL interacts with receptors on the cell surface. The complex enters the coated pit and is internalized. The coated vesicle loses its clathrin coat and becomes an endosome, the site of lipoprotein and receptor dissociation. The receptors recycle to the cell surface, and the lipoproteins are degraded. Alternatively, new receptors are synthesized in the rough endoplasmic reticulum and transported to the cell surface. (Modified from Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986;232:34–47; and Myant NB. *Cholesterol Metabolism, LDL, and the LDL Receptor*. San Diego, CA: Academic Press; 1990.)

LRP5, and LRP6. Along with the LDL receptor and LRP1, sortilin receptors participate in brain development, synaptic function, and neuroprotection, making them relevant to Alzheimer disease.[62] LRP5 and LRP6 are both coreceptors for a family of G protein–coupled receptors known as frizzled receptors. Frizzled receptors bind the Wnt molecule to induce an important signaling cascade upstream of the transcription factor β-catenin. Genetic variants in LRP5 are associated with obesity,[63] and a human mutation in LRP6 results in metabolic syndrome and coronary artery disease.[64] Loss-of-function mutations in LRP5 or LRP6 cause osteoporosis in humans, whereas gain of function in LRP5 causes osteopetrosis. These observations suggest that insulin resistance, coronary disease, and osteoporosis, which are common comorbid conditions in patients, may be related to abnormal Wnt signaling.

LDL Receptor

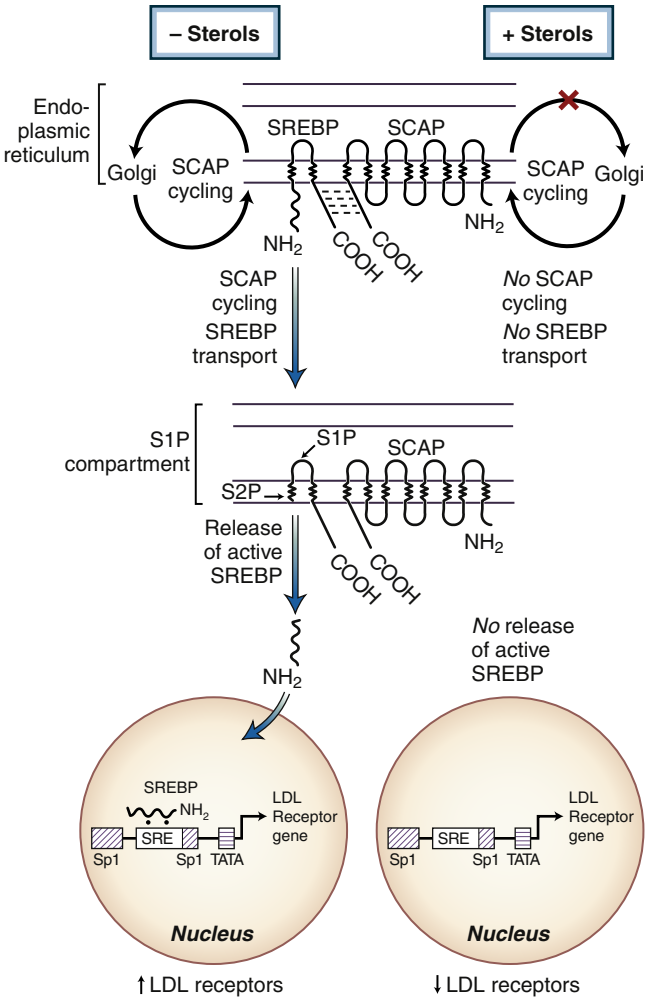
The LDL receptor is a large (160 kDa) glycoprotein expressed on most cells. Because it recognizes apoB100 as well as apoE, it is involved in the uptake of LDL, chylomicron remnants, VLDL, and IDL. Most HDL particles do not have apoE and therefore do not interact with this receptor or the LRP. The discovery of this receptor in the 1970s by Brown and Goldstein[65] explained a human disease (FH), the mechanism of action of drugs that lower cholesterol, and defined receptor-mediated endocytosis as a paradigm for providing cells with critical components from the external environment.

The LDL receptor shares functional domains with other members of this receptor family, including the ligand-binding domain, the epidermal growth factor (EGF) precursor domain, the *O*-linked sugar domain at the cell surface, the membrane-spanning domain, and the cytoplasmic domain at the COOH-terminus (Fig. 41.11). The ligand-binding domain includes seven repeats of approximately 40 amino acids, each containing six cysteines that form three disulfide bonds within the repeat to stabilize the structure. The repeat also includes negatively charged amino acids that interact with positively charged residues on the ligands apoB and apoE and with calcium ions. The EGF precursor domain consists of three EGF-like repeats (see Fig. 41.11) with a structure known as a β-propeller located between repeats B and C. The *O*-linked sugar domain is the site at which carbohydrate moieties attach to the molecule, and this is followed by a short membrane sequence. The cytoplasmic domain consists of 50 residues that include an NPXY (asparagine, proline, any amino acid, tyrosine) targeting sequence where adapter proteins dock, which leads to receptor clustering in coated pits.

Coated pits are specialized cell surface regions characterized by the presence of the protein complex clathrin. When LDL receptors bind lipoproteins, they migrate to coated pits, and clathrin directs the complex to a cell membrane region that folds inward, creating an intracellular vesicle or endosome (Fig. 41.12). Endosomes become acidic, causing the lipoprotein to be displaced from the LDL receptor by the β-propeller of the EGF precursor domain.[66] The unoccupied receptor recycles back to the cell surface. In the presence of PCSK9 (see later discussion and Fig. 41.18), the LDL receptor conformation is altered, promoting degradation and preventing recycling to the cell surface.[67]

Lipoproteins are degraded in lysosomes. Cholesterol is transported out of the lysosomes through the action of two proteins, Niemann-Pick C1 and C2 (NPC1 and NPC2), which are mutated in the human disease Niemann-Pick type C, which is characterized by intracellular accumulation of cholesterol. Intrathecal administration of cyclodextrin may confer clinical benefit.[68] NPC2, which is soluble, binds cholesterol after lipoprotein hydrolysis in the lysosome and moves this sterol to the membrane-associated NPC1 for subsequent release to the cell, where it serves structural and regulatory functions.

One key regulatory function of cholesterol is control of LDL receptor expression. Intracellular sterol concentrations are sensed



• **Fig. 41.13** Low-density lipoprotein (LDL) receptor gene regulation. *COOH,* carboxy-terminus; *S1P,* site-1 protease; *S2P,* site-2 protease; *SCAP,* SREBP cleavage–activating protein; *SRE,* sterol regulatory element; *SREBP,* sterol regulatory element–binding protein; *TATA,* the TATA box or core promoter sequence.

by SREBP cleavage–activating protein (SCAP), which binds to SREBPs in the endoplasmic reticulum. SREBP2 is most important for LDL receptor transcription; its NH_2-terminus contains a leucine zipper–type transcription factor structure that binds to a sterol regulatory element in the promoter of the LDL receptor gene. When cells are sterol depleted (Fig. 41.13, *left side*), SCAP migrates to the Golgi apparatus, where sugar moieties attached to the protein are modified. This allows SCAP to transport SREBPs to the site-1 protease (S1P) compartment. There, two proteases, S1P and site-2 protease (S2P), sequentially act on SREBPs to release their NH_2-terminus, which migrates to the nucleus and binds to the sterol regulatory element in the promoter region of lipid genes such as the LDL receptor and HMG-CoA reductase, increasing transcription and subsequent levels of functional proteins. In the presence of cholesterol (see Fig. 41.13, *right side*), SCAP does not cycle to the Golgi structure, it cannot move SREBPs to the S1P compartment, and SREBPs are not cleaved to allow their transcription factor to migrate to the nucleus. This disruption of SREBP activation in the presence of cholesterol protects cell from excessive accumulation of cholesterol.

LDL Receptor–Related Protein 1

LRP1 is also known as the apoE receptor or the chylomicron remnant receptor. LRP1 is large, containing components of four LDL receptors with a multiplicity of ligand-binding domains. Embryonic inactivation of LRP1 (but not of the LDL receptor) is lethal in mice. The major cell types expressing LRP1 include hepatocytes, neurons, and placental syncytiotrophoblasts. Multiple different ligands bind to LRP1 and participate in both nutrient flow and signaling. These ligands include amyloid precursor protein (processed to form the amyloid-β of plaques in Alzheimer disease), bacterial by-products, tissue plasminogen activator (which interacts with LRP1 to promote inflammation in the setting of brain ischemia), plasminogen activator inhibitors, and α_2-macroglobulin (which contributes to inflammation, in part by inactivating matrix metalloproteinases). LRP1 is associated with receptor-associated protein, a small protein that binds to several regions of LRP1 to chaperone this large protein during intracellular processing.

LRP1 binds apoE but not apoB100. Therefore, it mediates the metabolism of the major apoE-containing lipoproteins, including chylomicron remnants and IDL (VLDL remnants), but is not involved in LDL metabolism. The interaction between LRP1 and lipoproteins is more complex than that between LDL and the LDL receptor. Multiple apoE molecules are required for LRP1 binding, and this interaction requires an initial binding of the lipoprotein to proteoglycans on the cell surface. Other moieties on apoE-containing lipoproteins also facilitate the binding process. LPL, which metabolizes chylomicrons and VLDL particles, adheres to particles after mediating the release of fatty acids and other components at the endothelium. Lipoprotein-bound LPL molecules (as well as hepatic lipase) are thought to interact with LRP1 and to facilitate the uptake of remnants by the liver.

Pattern Recognition Receptors

The delivery of excess lipids to the blood vessel leads to atherosclerosis. The innate immune system and at least two broad types of receptors—scavenger receptors and toll-like receptors (TLRs)—that preferentially recognize ligand patterns (e.g., those associated with carbohydrates, lipids, or nucleic acids) instead of discrete features (e.g., specific amino acid sequences) are involved in the atherosclerotic process and thought to be activated by lipids.

Scavenger Receptors

The observation that macrophages can bind and internalize modified forms of LDL but not native LDL prompted the discovery of scavenger receptors. There are multiple classes of these receptors[69] that bind altered (e.g., oxidized, acetylated) LDL or other polyanionic ligands. Class A and class B receptors may be particularly important.

Class A receptors include scavenger receptor A (SR-A types 1 and 2, which consists of alternative splice variants), macrophage receptor with collagenous structure (MARCO), scavenger receptor A 5 (SCARA5), and scavenger receptor with C-type lectin domain (SRCL-I/II, also referred to as CL-P1). SR-A, the first to be discovered, binds a wide variety of ligands (including bacterial by-products), activates stress signaling pathways including mitogen-activated protein kinases, and is believed to be involved in atherosclerosis, the clearance of apoptotic cells, and Alzheimer disease.

Class B receptors include CD36 and scavenger receptor class B (SR-BI [called CLA1 in humans]). These receptors bind modified LDL, but unlike other scavenger receptors, they also bind VLDL, native LDL, and HDL. CD36 is expressed on a wide variety

of cell types, including monocytes, macrophages, adipocytes, platelets, endothelial cells, hepatocytes, microglial cells, and the tongue, where it detects dietary fat. In addition to lipoproteins, long-chain fatty acids are ligands for CD36.[70] The tissue distribution of SR-BI is more limited, with expression on hepatocytes, monocyte/macrophages, and steroidogenic tissues. A genetic deficiency of SR-BI in humans leads to increased circulating HDL but also increased risk for coronary heart disease.[71]

Toll-Like Receptors

The TLR family comprises key effectors of the innate immune system required for host defense mechanisms against pathogens. Their activation has been implicated in many chronic inflammatory diseases, including atherosclerosis, through signaling pathways that increase nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), activator protein 1 (AP1), and other transcription factors that control inflammation. Some scavenger receptors, such as CD36, may be coreceptors for TLRs. TLRs are found on myeloid cells such as monocyte/macrophages and on the gut epithelium. TRL ligands include lipopolysaccharide (TLR4) and glycolipids found in bacteria (TLR2). TLR2 mediates monocyte activation by apoCIII on triglyceride-rich lipoproteins.[72]

Other Enzymes and Transfer Proteins Mediating Lipid Metabolism

Hepatic Lipase

Heparin lipase primarily is a phospholipase with some triglyceride lipase activity. It is synthesized in hepatocytes but found mostly on endothelial cells in the liver and on HSPG in the space of Disse. It is also found in steroidogenic tissues but is not synthesized at those sites. Unlike LPL, which is mostly present at tissues remote from the liver, thereby ensuring the peripheral delivery of lipids and vitamins, hepatic lipase centrally coordinates lipoprotein metabolism. Functions include conversion of IDL to LDL, HDL_2 to HDL_3, and probably the final metabolism of chylomicron remnants to facilitate their uptake by LRP1. Unlike LPL, hepatic lipase does not require a cofactor such as apoCII, but both enzymes are displaced from their endothelial sites of activity by injection of heparin (postheparin lipase activity). High levels of hepatic lipase decrease HDL concentrations, whereas high levels of LPL increase HDL.

Endothelial Lipase

Evolutionarily related to hepatic lipase and LPL, endothelial lipase is a phospholipase with almost no triglyceride lipase activity. It is expressed at high levels in embryonic endothelial cells, with expression declining during maturation. In adults, expression is highest in endothelial cells, thyroid, lung, liver, placenta, and gonads. In mice, overexpression decreases HDL and inactivation increases HDL. Endothelial lipase is expressed in the aorta, where it may increase with atherosclerosis. Human loss-of-function mutations are associated with increased HDL.[73] Endothelial lipase does not appear to affect atherosclerotic risk.

Proprotein Convertase Subtilisin/Kexin Type 9

PCSK9 is a secreted protease that promotes the degradation of the LDL receptor by interacting with the receptor so that it is targeted to the lysosome. PCSK9 catalytic activity is not required for receptor degradation. Highest expression levels are seen in the liver, intestine, and kidney. The importance of PCSK9 in lipid metabolism was discovered when missense mutations within the gene (subsequently determined to be gain-of-function mutations) were associated with hypercholesterolemia and coronary artery disease.[74] Overexpression of PCSK9 in mice decreases LDL receptor protein. Human loss-of-function mutations in PCSK9 are associated with low LDL concentrations and decreased risk of vascular disease.[75,76] Some PCSK9 associates with circulating LDL,[77] which might assist with targeting this protein to LDL receptors. Antibodies to PCSK9 are useful for treatment in humans.

Lipoprotein-Associated Phospholipase A_2

Phospholipases hydrolyze the ester bond at the sn2 position of phospholipids, usually resulting in the release of a fatty acid and lysophosphatidylcholine, which can induce inflammation. Phospholipases were originally identified as a component of snake venom, and many distinct classes have since been characterized. Membrane phospholipids are the substrate for most phospholipases. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an exception because it can hydrolyze substrate in the aqueous phase. Lp-PLA_2 binds to both LDL and HDL lipoproteins and is a biomarker for coronary artery disease.[78] In humans, inhibition of this enzyme decreases the expansion of the lipid core of atherosclerotic plaques[79] but does not decrease cardiovascular end points.[80]

Cholesteryl Ester Transfer Protein

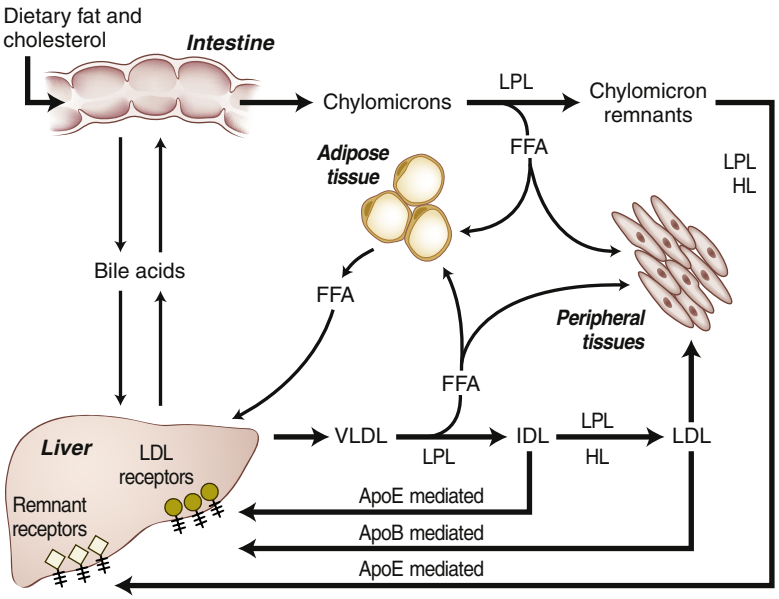
Cholesteryl ester transfer protein (CETP) promotes the exchange between lipoproteins of two classes of neutral lipids: cholesteryl esters and triglycerides. HDL cholesteryl esters are transferred to VLDL, IDL, and chylomicron remnants; in return, triglycerides from VLDL, IDL, and remnants are transferred to HDL. Humans and other primates have CETP activity; the transfer of cholesteryl ester from HDL to apoB-containing lipoproteins ultimately leads to most of their cholesterol burden being carried by LDL, and this is thought to result in atherosclerosis. Rodents and dogs do not have CETP. Most of their cholesterol is carried in HDL; LDL is low, and these animals are resistant to atherosclerosis. Such observations led to the notion of inhibiting CETP activity as a treatment for atherosclerosis in humans. In clinical trials, different inhibitors have increased HDL cholesterol and lowered LDL cholesterol but also increased mortality,[81] selectively increased HDL with no impact on cardiovascular events,[82] and reduced events perhaps due to lower LDL cholesterol.[83]

Lecithin:Cholesterol Acyltransferase

LCAT is an enzyme synthesized primarily in the liver; it circulates in the plasma associated with HDL particles and, to a lesser extent, with LDL particles. LCAT is activated by several apolipoproteins (apoAI and others) and uses the phospholipid lecithin (phosphatidylcholine) and free cholesterol as substrates to generate lysolecithin (lysophosphatidylcholine) and cholesteryl ester. Most of the cholesteryl esters in lipoproteins are derived from LCAT activity. Rare human mutations in LCAT result in low HDL levels. The role of LCAT in atherosclerosis is uncertain, and some reports suggest that humans with loss-of-function LCAT mutations do not appear to have increased risk for atherosclerosis,[84] perhaps because their residual HDL is functional in assays of reverse cholesterol transport. The other clinical manifestations of LCAT deficiency are described in the Lecithin:Cholesterol Acyltransferase Deficiency section.

Integrative Physiology of Lipid Metabolism

Lipid metabolism is characterized by a dynamic flux of multiple lipid species from the external environment to the liver, from the



• **Fig. 41.14** General scheme summarizing the major pathways involved in the metabolism of chylomicrons synthesized by the intestine and VLDL synthesized by the liver. *ApoB,* apolipoprotein B; *ApoE,* apolipoprotein E; *FFA,* free fatty acid; *HL,* hepatic lipase; *IDL,* intermediate-density lipoprotein; *LPL,* lipoprotein lipase; *VLDL,* very low density lipoprotein. (Modified from Mahley RW. Biochemistry and physiology of lipid and lipoprotein metabolism. In: Becker KL, ed. *Principles and Practice of Endocrinology and Metabolism.* 2nd ed. Philadelphia, PA: JB Lippincott; 1995:1369–1378.)

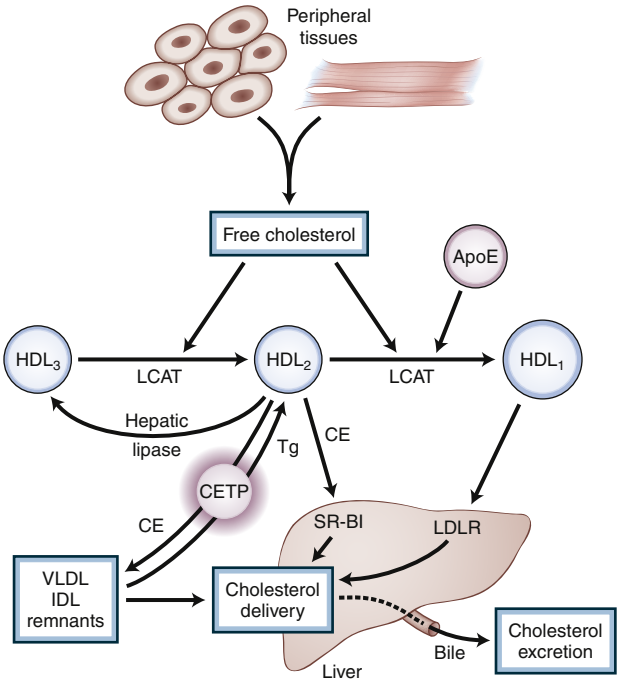
liver to peripheral tissues, from peripheral tissues back to the liver, and eventually back to the external environment through the excretion of bile acids. Integrated views of the major pathways involved are shown in Figs. 41.14 and 41.15.

Exogenous Lipid Transport

Dietary fat and cholesterol (see Fig. 41.14, *top left*) absorbed by the duodenum and proximal jejunum are used to generate chylomicrons that are secreted at the lateral borders of enterocytes and enter mesenteric lymphatics. They access the plasma via the thoracic duct and are rapidly metabolized by LPL to yield chylomicron remnants. These are taken up by remnant receptors (LRP1/HSPG) and by LDL receptors in the liver. Free fatty acids liberated by the action of LPL are available to adipose tissue for storage and to other tissues (e.g., skeletal muscle, heart) for use as energy substrates.

Endogenous Lipid Transport

Lipid derived from remnants and from lipolysis of adipose tissue is reassembled in the liver (see Fig. 41.14, *bottom left*) as VLDL particles, which are secreted into the plasma. Abnormal lipid metabolism in insulin resistance is mediated in large part by overproduction of VLDL, an event that occurs through disruption of signaling downstream of the insulin receptor and the insulin receptor substrate (IRS) adapter proteins. VLDL particles are metabolized by LPL to yield IDL particles, which are metabolized by LPL and hepatic lipase to yield LDL particles. Thus, LDL is derived from VLDL, which helps explain why treatments that increase LPL actions to lower triglycerides (carried by VLDL) are frequently associated with at least transient increases in LDL. IDL



• **Fig. 41.15** Role of HDL in the redistribution of lipids from cells with excess cholesterol to cells requiring cholesterol or to the liver for excretion. The reverse cholesterol transport pathway is indicated by *arrows* (net transfer of cholesterol from cells to HDL, then to LDL and liver). *ApoE,* apolipoprotein E; *CE,* cholesteryl ester; *CETP,* cholesteryl ester transfer protein; *HDL,* high-density lipoprotein; *IDL,* intermediate-density lipoprotein; *LCAT,* lecithin:cholesterol acyltransferase; *LDLR,* low-density lipoprotein receptor; *SR-BI,* scavenger receptor class B, type 1; *Tg,* triglyceride; *VLDL,* very low density lipoprotein.

can be taken up by the liver through an apoE-dependent process, and LDL is taken up by the liver through the binding of apoB100 to LDL receptors. Small VLDL particles, IDL particles, and LDL particles may be taken up by peripheral tissues to deliver nutrients, cholesterol, and fat-soluble vitamins. When present in excess, each of these lipoproteins may be atherogenic.

Reverse Cholesterol Transport and Dysfunctional HDL

Cholesterol cannot be metabolized by peripheral tissues and must be returned to the liver for excretion. This process, called *reverse cholesterol transport*, is dependent on HDL and its precursors and is depicted in Fig. 41.15. Excess cholesterol in tissues can be effluxed either to lipid-poor apoAI, mediated by the protein transporter ABCA1 (ATP-binding cassette transporter 1), or to nascent HDL particles, mediated by ABCG1. Efflux from cultured cells to human plasma as a biomarker of cardiovascular risk is thought to mostly reflect the activity of ABCA1. There is also evidence that cholesterol can be acquired by HDL without the assistance of transporters by following a concentration gradient at the cell surface. LCAT esterifies HDL-associated cholesterol to form cholesteryl ester and induces the maturation of HDL. HDL particles have three pathways for transporting sterols to the liver. First, they can directly bind to SR-BI (CLA1) at the liver, which induces cholesteryl ester delivery through a mechanism involving lateral lipid transfer and not receptor internalization. Second, cholesteryl esters can be transferred to apoB-containing lipoproteins by CETP, and these particles can deliver cholesterol to the liver through the LDL receptor. Third, a small portion of HDL can acquire apoE and bind to the liver LDL receptor. Once in the liver, cholesterol is converted to bile acids for excretion.

Cholesterol is the principal component of atherosclerotic plaque, and thus atherosclerosis might be treated by promoting the efflux of cholesterol from lesions. HDL participates in this process, but static levels of HDL are poor predictors of reverse cholesterol transport. Dysfunctional HDL particles incapable of mediating cholesterol efflux could explain why some HDL-elevating interventions have not been associated with decreased cardiovascular disease. Measurements of the rate of flux of cholesterol from the periphery to the liver, which may be possible in humans, would represent a better predictor of beneficial therapies. In vitro cholesterol efflux capacity might be useful for assessing risk[45] but does not appear to be universally inversely associated with disease.[85,86]

In addition to participating in reverse cholesterol transport, HDL has other properties that could be impaired by a variety of processes leading to a dysfunctional particle. They include the induction of endothelial nitric oxide synthase, the transport of proteins involved in the acute phase response and inflammation, and the suppression of thrombosis through induction of prostacyclin (which decreases thrombin production via the protein C pathway and decreases platelet activation).

Overview of Hyperlipidemia, Dyslipidemia, and Atherogenesis

Abnormal lipids can present as elevated triglycerides, elevated cholesterol, elevations of both triglycerides and cholesterol, and low HDL. A summary of the primary and secondary causes of each condition is presented in Table 41.4.

Plasma lipid levels are highly dependent on lifestyle. For example, the high-fat, high-cholesterol diets of Western societies raise plasma cholesterol, and vigorous exercise lowers both atherogenic particles and triglycerides. For this reason, normal blood concentrations—those that are within 2 standard deviations of the mean—vary among countries and over time. The overriding influences of diet and lifestyle on plasma cholesterol were illustrated by studies of ethnic Japanese populations showing that cholesterol was markedly increased in Japanese-Americans consuming a Westernized diet.[87] Because total cholesterol levels correlate with the risk for coronary heart disease over a broad range (Fig. 41.16), normal levels are preferably defined as those associated with minimal cardiovascular risk rather than population averages, suggesting that most people in the developed world have lipid levels that put them at risk for atherosclerotic cardiovascular disease. Epidemiologic studies support that total cholesterol below 150 mg/dL (3.9 mmol/L) is associated with low risk of cardiovascular disease (see Fig. 41.16). Furthermore, atherosclerosis regression, such as the reduction of the size and presumably inflammatory status of established lesions, can be seen with LDL below 70 to 80 mg/dL (<2.0 mmol/L) in patients with established atherosclerotic cardiovascular disease.[88]

Population, genetic, and therapeutic data support that atherosclerosis is frequently caused by cholesterol deposition within the arterial wall. As described earlier, several genetic hyperlipidemic disorders are associated with premature atherosclerosis. Reductions of blood cholesterol by diet, statins, and other cholesterol-lowering therapies, and even surgical ileal bypass, have convincingly shown that cholesterol reduction will reduce the incidence of major cardiovascular events, especially myocardial infarction, need for revascularization, and stroke. Most striking is the observation that the majority of patients with coronary artery disease will have reduced disease (i.e., regression) with statin therapy.[88] The cholesterol hypothesis is further supported by a wealth of animal data: altering blood lipid levels by diet or genetic modification causes atherosclerosis-free animals to develop disease, and reduction of cholesterol leads to regression.

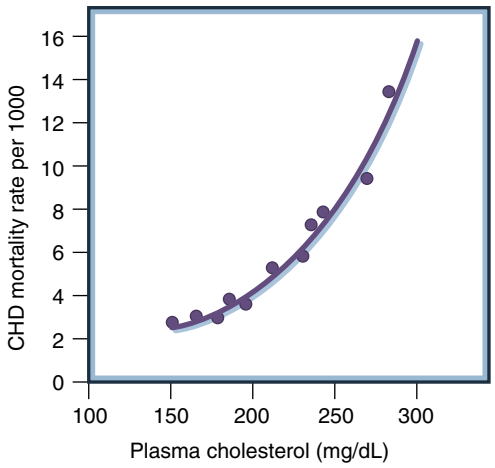
Atherosclerosis and its clinical presentations as coronary artery disease, stroke, and peripheral vascular disease are likely the product of several pathophysiologic changes. Depending on the patient and setting, arterial disease results from a varying reaction to lipid infiltration, arterial damage, and macrophage inflammation. More than 100 years ago, pathologists identified cholesterol as a major component of atherosclerosis. This poorly metabolized lipid is found to be associated with both collagen and proteoglycans (key components of connective tissue consisting of proteins attached to carbohydrates), and within arterial cells. Macrophages and smooth muscle cells are converted into foam cells, so called because of their intracellular foamy lipid. In addition, there are often acellular lipid-rich areas, a variable amount of overlying collagen-rich connective tissue, and regions where the atherosclerotic plaque has ruptured. The process begins with lipid infiltration into the arterial wall.[89] Unresolved pathogenic issues include (1) how lipid enters the artery, (2) determining which pathways lead to excess lipid uptake by foam cells, and (3) determining which processes cause rupture and thrombosis.

Lipoproteins cross the arterial barrier and infiltrate the artery wall. Larger particles, such as chylomicrons, are likely to be excluded by the endothelial barrier. LDL can leave the circulation via channels between cells, along the continuous transendothelial movement of cell-free plasma, or via interaction with specific receptors. Once in the subendothelial space, lipoproteins must

TABLE 41.4 Differential Diagnosis of Hyperlipidemia and Dyslipidemia

Hypertriglyceridemia	Hypercholesterolemia	Increased Cholesterol and Triglycerides	Low HDL
Primary Disorders			
LPL deficiency	Familial hypercholesterolemia	Familial combined hyperlipidemia	Familial hypoalphalipoproteinemia
ApoCII deficiency	Familial defective apoB100	Dysbetalipoproteinemia	ApoAI mutations
Familial hypertriglyceridemia	Polygenic hypercholesterolemia		LCAT deficiency
Dysbetalipoproteinemia	Sitosterolemia		ABCA1 deficiency
Secondary Disorders			
Diabetes mellitus	Hypothyroidism	Diabetes mellitus	Anabolic steroids
Hypothyroidism	Obstructive liver disease	Hypothyroidism	Retinoids
High-carbohydrate diets	Nephrotic syndrome	Glucocorticoids	HIV infection
Renal failure	Thiazides	Immunosuppressives	Hepatitis C infection
Obesity/insulin resistance		Protease inhibitors	
Estrogens		Nephrotic syndrome	
Ethanol		Lipodystrophies	
Beta blockers			
Protease inhibitors			
Glucocorticoids			
Retinoids			
Bile acid–binding resins			
Antipsychotics			
Lipodystrophies			
Thiazides			

ABCA1, Adenosine triphosphate–binding cassette transporter 1; *apo,* apolipoprotein; *HDL,* high-density lipoprotein; *HIV,* human immunodeficiency virus; *LCAT,* lecithin:cholesterol acyltransferase; *LPL,* lipoprotein lipase.



• **Fig. 41.16** Relation between plasma cholesterol levels and coronary heart disease (CHD) mortality rate in the Multiple Risk Factor Intervention Trial. (Modified from Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial [MRFIT]. *JAMA.* 1986;256:2823–2828; Copyright © 1986, by the American Medical Association.)

accumulate to cause disease. It is theorized that positive charges on apoB interact with negatively charged proteoglycans to promote lipoprotein retention. Both apoB100 in LDL and VLDL, apoB48 in chylomicron remnants, and apoE in several classes of lipoproteins have proteoglycan-binding sequences.[90] Another possibility is that within the artery, lipoproteins fuse to form large aggregates that are unable to disassociate and reenter the circulation. Lipoprotein uptake into cells is usually well controlled because excess cellular cholesterol downregulates LDL receptors. Therefore, aberrant lipoprotein receptor regulation may be a factor, or LDL (and remnants) may enter cells by non–cholesterol-regulated pattern recognition receptors (see earlier discussion).

Cholesterol-containing lipoproteins can become inflammatory. Enzymes within the artery might induce alterations in the protein and lipid content of LDL that convert these particles into more inflammatory oxidized LDL. Although low-dose aspirin has some anti-inflammatory properties, its reduction of cardiovascular events is most likely secondary to effects on platelet aggregation rather than direct effects on atherosclerosis. A recent clinical trial showed that blocking the interleukin 1β receptor reduced major coronary events in patients with high C-reactive protein and known vascular disease.[91] This finding supports that at least

some therapies directed to inflammation will be useful to prevent and treat residual risk for atherosclerosis even in the presence of lipid-lowering therapy.

Although the arterial lumen can be progressively narrowed by the accumulation of macrophages, the proliferation of smooth muscle cells, and the deposition of cholesterol, the truly dangerous lesion (the culprit lesion) may not cause marked luminal narrowing.[92] As atherosclerosis progresses, there is a compensatory expansion of the lumen that maintains an almost constant lumen size. As the lesion develops within the intima, the complication of rupture of the overlying intima or endothelial erosion leads to exposure of the lesional contents to platelets, initiating thrombosis. It is the acute thrombosis that is responsible for infarctions in most patients. Rupture or erosion occurs where the fibrous cap covering the underlying thrombogenic lipid is thin.

The surfaces of complicated lesions can become thrombogenic as endothelial cells are lost or the fibrous cap ruptures and the subendothelial space is exposed. Platelets can adhere to this exposed surface, promoting thrombus formation. In these unstable plaques, blood actually dissects into the artery wall, leading to the formation of a large thrombus. Calcification is also a feature of late lesions. Advanced lesions can weaken the elasticity and integrity of the artery wall, potentially creating an aneurysm of the vessel. Removal or reduction of the atherogenic stimulus can result in plaque regression and stabilization, leaving a remnant devoid of lipid that resembles a wound scar and is less likely to serve as a nidus for thrombus formation.

Observations first made in animals and now confirmed in humans indicate that the atherosclerotic process can be reversed if plasma cholesterol reduction is intensive.[93] LDL reduction to levels below 70 mg/dL (1.8 mmol/L) reduced lesions size in about two thirds of subjects studied by intravascular ultrasound.[88] Even greater regression was found with lower LDL achieved using PCSK9 inhibitors.[94]

In humans, vascular disease is not universal, even among those individuals with marked hypercholesterolemia. Similarly, patients with low cholesterol are not assured of being protected from the disease. Perhaps half of atherosclerosis is attributable to hyperlipidemia and other known cardiac risk factors.[95]

Despite the importance of lipids in atherosclerosis, there remains an unmet medical need to find additional approaches for residual risk in the lipid-lowering era. Several approaches to atherosclerosis treatment directed toward nonlipid causes have not been successful. Use of vitamin E and antibiotics has not altered cardiovascular event rates. Other inflammatory diseases (e.g., collagen vascular diseases) associated with cardiac events have not yet been shown to achieve lower cardiac event rates with cholesterol-lowering therapies. Statin-mediated cholesterol reduction appears to be ineffective in end-stage renal disease, although those patients usually do not have elevated LDL levels. In contrast, cardiovascular events in patients with less severe renal disease are reduced by treatment with statin/ezetimibe.[96]

Hypertriglyceridemia

Severe hypertriglyceridemia usually leads to pancreatitis if triglycerides exceed 2000 mg/dL (22.6 mmol/L). At approximately 500 mg/dL (5.6 mmol/L), LPL is saturated and a large bolus of fat during a dietary indiscretion may not be cleared from the bloodstream, thereby promoting severe hypertriglyceridemia and pancreatitis. Triglyceride guidelines have focused on prevention of pancreatitis and not cardiovascular disease, as there is no level 1 evidence to date that directed therapies to reduce triglyceride will affect cardiovascular events. Ongoing trials using fibrates and omega-3 fatty acids are likely to resolve this issue in the near future.

Fasting Hyperchylomicronemia

The most dramatic example of severe hypertriglyceridemia is that of fasting hyperchylomicronemia. This can result from a primary defect in chylomicron metabolism, or it can occur secondary to increased VLDL and saturation of LPL. LPL saturation occurs when triglyceride levels exceed about 500 mg/dL (5.6 mmol/L); this means that higher triglyceride levels exceed the capacity for the enzyme to act on its substrate, so dietary triglyceride is not metabolized and remains in the circulation. Therefore, familial hypertriglyceridemia, familial combined hyperlipidemia, and dysbetalipoproteinemia can be associated with fasting hyperchylomicronemia. One common cause of such exacerbations is poorly controlled diabetes leading to increased adipose intracellular lipolysis, return of fatty acids to the liver, greater secretion of VLDL triglyceride, and saturation of LPL. Several dietary and environmental factors also modulate triglyceride production. The most dramatic is alcohol, a major substrate for triglyceride production. In addition, diets that are rich in free carbohydrates, and especially simple sugars, induce triglyceride production. Fructose also increases de novo production of lipids in the liver but has less effect on circulating triglycerides.

Triglyceride levels above 500 mg/dL (5.6 mmol/L) and hypertriglyceridemic pancreatitis are relatively common; triglycerides above 1000 mg/dL (11.3 mmol/L) are found in 0.4% of a general medical population, and of these, 5.4% had a pancreatitis episode within 1 year.[97] Genetic defects leading to this condition, called *familial chylomicronemia syndrome* (or FCS), are relatively rare and estimated to occur in approximately 1 in 250,000 people.[98] Thus, the majority of patients are likely to be genetically predisposed to severe hypertriglyceridemia, with symptoms emerging due to environmental factors such as alcohol use or another underlying conditions such as diabetes. Defects in LPL most commonly cause genetic FCS; the lack of normal LPL prevents chylomicron clearance. LPL deficiency usually, but not always, manifests in childhood. The symptoms vary from difficulty feeding young infants to frank pancreatitis, which is sometimes mistaken as appendicitis. The plasma is often milky, and whole blood may have a pinkish, "cream of tomato soup" hue. The trigger level of triglyceride elevation leading to pancreatitis is variable; some patients have triglycerides in excess of 10,000 mg/dL (113 mmol/L) with no symptoms, whereas others develop pancreatitis at much lower triglyceride levels but usually in excess of 2000 mg/dL (22.6 mmol/L). However, patients presenting with pancreatitis have often avoided eating, and the first measured sample may not reflect the peak triglyceride. Several additional mutations in proteins required for normal LPL actions also cause FCS (see the LPL Deficiency section).

The pathophysiology of the relationship between hyperchylomicronemia and pancreatitis is unknown. Lipid-rich blood may sludge, leading to pancreatic ischemia. The small number of lipases that normally leak from the acinar cells may lead to exuberant local lipolysis; creation of toxic local concentrations of free fatty acids and lysolecithin, a toxic lipid produced from phosphatidylcholine; and further acinar cell damage to adjacent cells. Insults to the acinar cells such as those provided by alcohol can accelerate this process.

Although most patients with severe hyperchylomicronemia who do not develop pancreatitis are asymptomatic, a few with

extreme levels exceeding 10,000 mg/dL (113 mmol/L) develop the hyperchylomicronemia syndrome. These patients have dyspnea and confusion that may be indistinguishable from early dementia. Presumably, this is the result of reduced blood flow or defective oxygen delivery.

The marked increase in blood triglyceride concentration can lead to accumulation of triglycerides in several organs and can be observed in the blood. The latter is best appreciated by examining the blood directly, allowing the red blood cells to settle, and observing a creamy layer on the plasma, or by noting the pinkish discoloration of the blood on funduscopic examination, known as *lipemia retinalis* (Fig. 41.17B). Eruptive xanthomas, as shown in Fig. 41.17G, are 2- to 5-mm papules with a yellow center surrounded by erythema. They are caused by triglyceride-enriched skin macrophages. These lesions are sometimes confused with acne or folliculitis. For unclear reasons, eruptive xanthomas are most commonly found on the buttocks, extensor surfaces of the arms, and the back. Enlargement of the liver and spleen is not uncommon and is thought to be caused by triglyceride accumulation in these organs.

Aside from severe hypertriglyceridemia, other laboratory indices are sometimes abnormal. Plasma sodium is reduced; liver transaminases are sometimes elevated. Despite the presence of pancreatitis, amylase may be normal due to an assay artifact; serum lipase is a more reliable indicator in this setting. Often the clinical laboratory will note the severe lipemia and fail to report measurements of routine chemistries due to the turbidity of the serum. If these other measurements are required, plasma can be centrifuged, the chylomicron layer removed, and the remaining plasma examined.

LPL Deficiency

Almost every racial group has been reported to have patients with genetic defects in LPL, and a founder mutation makes the defect especially common among French Canadians. At least half of the cases of severe genetic primary hypertriglyceridemia are the result of LPL defects. Most LPL enzyme deficiencies are caused by inactive LPL protein. However, lack of protein production has also been reported, and because LPL might have receptor functions that do not require catalytic function, patients with these defects may have a more severe phenotype.

Although genetic LPL deficiency has been reported to manifest in adulthood, most cases of severe hyperchylomicronemia in adulthood are associated with partial LPL deficiency or other causes. In adults, the most important of these causes are type 2 diabetes mellitus and obesity, because insulin resistance is associated with defective clearance of lipoproteins. Postprandial lipemia is a prominent feature of diabetes. A thorough history of triglyceride-raising medications should be taken (see the Secondary Causes of Hypertriglyceridemia section).

Regulation of LPL is complicated, and defects in its actions are associated with genetic or acquired abnormalities that are exclusive of genetic defects in the LPL molecule. Defective apoCII, the obligate cofactor for LPL, leads to deficient LPL activity. Two molecular defects initially found in mice occasionally cause severe human hypertriglyceridemia. GPIHBP1 (discussed earlier) is a molecule expressed by endothelial cells whose deficiency leads to defective association of LPL with its binding site on the capillary lumen and defective intravascular lipolysis. In one report, 20% of patients with FCS had GPIHBP1 mutations.[99] Lipase maturation factor 1 (LMF1) is an intracellular protein required for correct intracellular folding and activation of LPL.[100] Mutations in LPL, GPIHBP1, and LMF1 all reduce postheparin LPL activity; mutated apoCII does not, but serum from these patients fails to maximally activate LPL. An additional genetic mutation associated with FCS involves glucokinase regulatory protein, which when dysfunctional allows excess de novo synthesis of fatty acids within the liver.[101]

Autoimmune conditions can be associated with defective triglyceride catabolism due to inhibition of LPL, apoCII, or heparin. Antibodies against heparin are thought to prevent normal LPL association with the endothelial surface. Antibodies against GPIHBP1 may be the most common causes of autoimmune chylomicronemia, as the tertiary structure of this protein appears to make it especially immunogenic.[102] Recognition of an autoimmune disorder such as concomitant presence of systemic lupus or rheumatoid arthritis is especially important because treatment with glucocorticoids, avoided in other hyperchylomicronemia syndromes, is often indicated. In addition, patients with vascular disease or generalized intravascular reactions to transfusions or chemotherapy can occasionally develop defects in LPL. Transient episodes of fasting hyperchylomicronemia have been attributed to viral infections and to excessive fat/calorie intake after fasting.

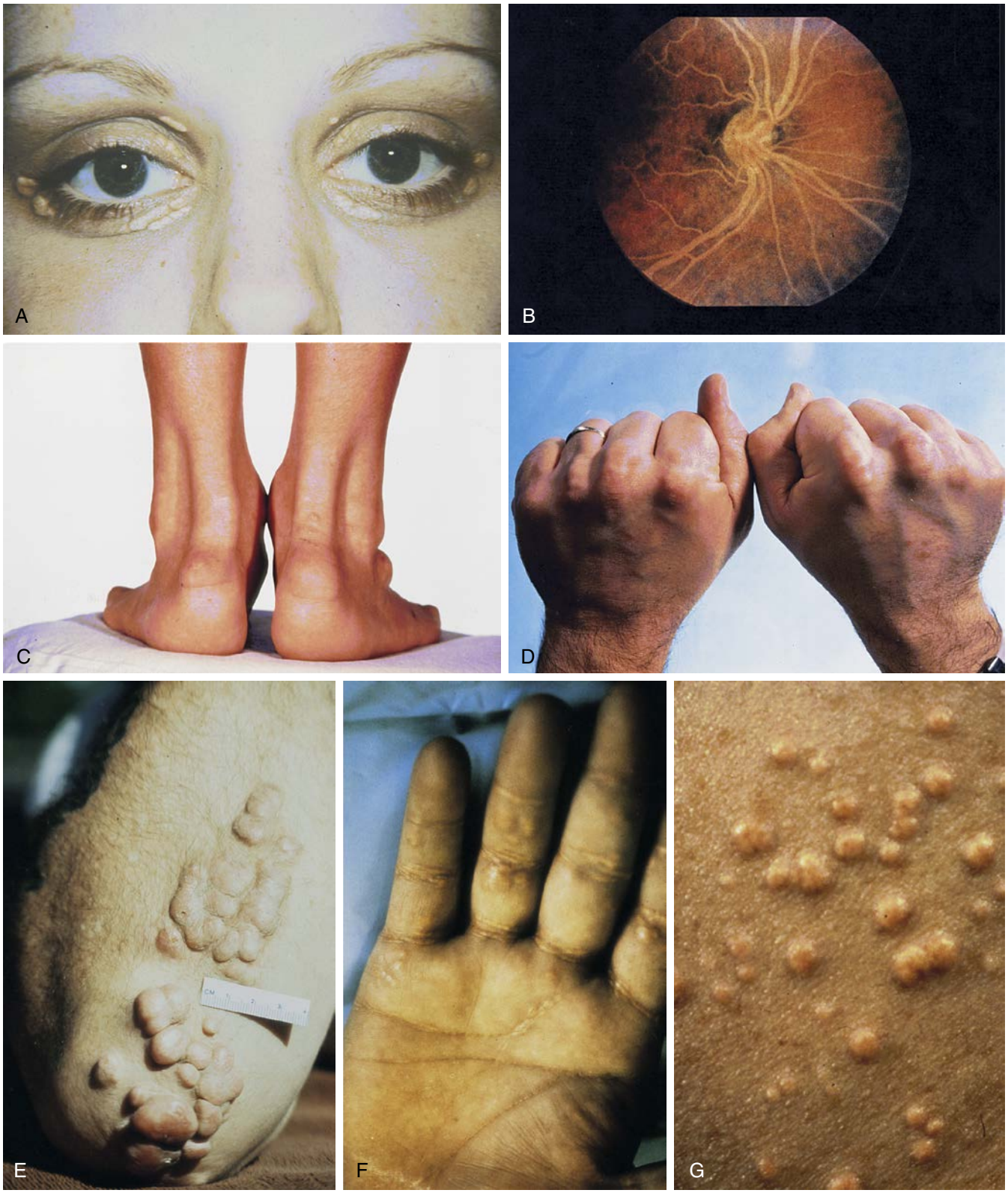
Postprandial Hyperlipidemia

Although plasma lipid levels are usually measured after an overnight fast, chylomicron remnants are associated with vascular disease in several animal models and with genetic or dietary causes of hyperlipidemia. This has led to a widely accepted hypothesis that remnant lipoproteins are an overlooked cause of human vascular disease. Postprandial lipidemia, measured as triglyceride increase, is associated with greater risk of atherosclerotic cardiovascular disease.[103] However, postprandial triglyceride elevations are also correlated with fasting triglycerides and reduced HDL, so the use of postprandial measurements in clinical practice is not currently recommended. Postprandial lipemia is a prominent feature of diabetes[104] but does not impair fasting glucose or impair glucose tolerance.[105]

Diagnostic Evaluation of Severe Hypertriglyceridemia

Assessment of underlying medical conditions and consideration of age at onset are required. If possible, biochemical and genetic evaluation of LPL and its regulating genes will refine the diagnosis. Conditions that cause fasting hypertriglyceridemia (discussed later) can lead to severe hypertriglyceridemia when exacerbated by diet, drugs, or other conditions such as diabetes or pregnancy. Genetic LPL deficiency is diagnosed by the clinical setting and either biochemical deficiency of LPL activity in postheparin blood or a genetic defect in LPL. LPL deficiency is more typically associated with younger age at onset, especially onset in childhood. A family history of low HDL is the most common lipid abnormality in heterozygous carriers. LPL variants are also a determinant of HDL levels within the general population. A family history of French Canadian ancestry is suggestive.

More than 100 mutations of the LPL gene have been described, and the diagnosis can be made through commercial DNA sequencing. Postheparin plasma can be collected for measurements of LPL activity in unusual presentations, but heparin administration is contraindicated in the setting of acute pancreatitis. This approach should be pursued in consultation with a research laboratory, because LPL activity is not measured in clinical laboratories. Deficiency of



• **Fig. 41.17** Physical examination findings associated with hyperlipidemia. (A) Xanthelasma. (B) Lipemia retinalis. (C) Achilles tendon xanthomas. Notice the marked thickening of the tendons. (D) Tendon xanthomas. (E) Tuberous xanthomas. (F) Palmar xanthomas. (G) Eruptive xanthomas. (A and B, courtesy Dr. Mark Dresner and Hospital Practice [May 1990, p. 15]; C through F, courtesy Dr. Tom Bersot; G, courtesy Dr. Alan Chait.)

apoCII, the LPL activator, and inhibitors of LPL such as antibodies can be detected in research assays by mixing the patient's serum with a standard human source of LPL and then assessing activity. Diagnosis in these rare cases can improve clinical management.[106]

Moderate Fasting Hypertriglyceridemia Due to Elevated VLDL

Triglyceride levels of 150 to 500 mg/dL (1.7–5.6 mmol/L) are considered to be abnormal. These more moderately increased levels may confer risk of pancreatitis in addition to cardiovascular disease. A study from Copenhagen of 116,550 individuals identified nonfasting triglycerides exceeding 177 mg/dL (2.0 mmol/L) as associated with a risk of acute pancreatitis that was greater than the risk for myocardial infarction.[107] Genome-wide association studies[108] and the observation that reduced triglycerides are associated with both lower atherosclerotic disease and genetic mutations in apoCIII,[57] a protein that might inhibit LPL actions and reduce uptake of triglyceride-rich lipoproteins by the liver, suggest an atherogenic role for triglycerides. Moreover, heterozygous LPL mutations also increase atherosclerosis risk,[109] even though hypertriglyceridemia is often manifested only when other conditions are present.

Several different clinical conditions lead to fasting hypertriglyceridemia. Familial combined hyperlipidemia is associated with increased apoB production, and at different times and in different family members, hypertriglyceridemia (increased VLDL), increased cholesterol (LDL), or both occur. This disorder is associated with increased risk of cardiovascular disease,[110] but its specific role is clouded by its presence in patients with other risk factors associated with metabolic syndrome. The concomitant insulin resistance, obesity, and/or overt diabetes in many hypertriglyceridemic patients make it difficult to isolate one specific cause of this metabolic disturbance. Although families with isolated triglyceride elevations and no vascular disease have been described, in the presence of metabolic syndrome, triglyceride elevations probably predispose to vascular disease through unclear mechanisms.

Some cases of isolated hypertriglyceridemia have been associated with hepatic overproduction of bile acids in the setting of impaired intestinal absorption of bile acids, analogous to the hypertriglyceridemia associated with use of bile acid–binding resins. There is uncertainty as to whether the large, triglyceride-rich VLDLs in this setting are atherogenic.

A homozygous mutation in apoE, apoE2/E2, leading to defective clearance of chylomicron and small VLDL remnants (see earlier discussion) underlies dysbetalipoproteinemia. These patients, with a prevalence of approximately 1 of every 10,000 people in the general population, present with elevated triglyceride and cholesterol due to defective clearance of remnant lipoproteins. Patients with dysbetalipoproteinemia sometimes have tuberous and palmar xanthomas and a propensity to peripheral vascular disease (see later discussion).

Genetic hypoalphalipoproteinemia syndromes are invariably associated with moderate hypertriglyceridemia. These syndromes include LCAT deficiency, Tangier disease, and apoAI Milano variant and are diagnosed as discussed later (see the Lecithin:Cholesterol Acyltransferase Deficiency, ABCA1 Deficiency, and Apolipoprotein AI Mutations sections). A reduced apoCII reservoir on HDL may be responsible.

Secondary Causes of Hypertriglyceridemia

Diabetes Mellitus

Diabetes mellitus is the most prominent cause of hypertriglyceridemia; in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, the average triglyceride was 162 mg/dL (1.8 mmol/L),[111] above the healthy ideal value. Insulin reduces fatty acid flux from adipose tissue, reduces liver apoB production, inhibits de novo triglyceride synthesis, and optimizes LPL production. Kinetic studies in humans show that both increased triglyceride secretion and reduced clearance of triglyceride from the bloodstream often occur in concert in patients with diabetes.[112] Diabetes is also associated with increased postprandial lipemia. The most common diabetic dyslipidemia is moderate hypertriglyceridemia and low HDL. Reduced HDL results from greater exchange of VLDL triglyceride for HDL cholesteryl ester, hydrolysis of the triglyceride-rich HDL by hepatic lipase, and more rapid clearance of the smaller HDL from the circulation. Defective lipolysis might also reduce the amount of cholesterol contributed to HDL from triglyceride-rich lipoproteins. CETP also enhances transfer of triglyceride to LDL, allowing these lipoproteins to be converted to smaller, denser forms that may be more atherogenic. This lipoprotein phenotype is also commonly referred to as diabetic dyslipidemia, although it is also found in nonhyperglycemic patients with metabolic syndrome. Although patients with diabetes do not have increased LDL compared with age- and sex-matched controls, improved diabetes control can be associated with reduced LDL. As stated earlier, diabetes is associated with severe fasting chylomicronemia. Many such patients also have an underlying dyslipidemia (e.g., due to heterozygous LPL deficiency), but others do not have a defined lipid disorder.

Patients with type 1 diabetes mellitus in poor control develop hypertriglyceridemia. However, because insulin stimulates HDL production, patients in good control sometimes develop high levels of HDL. Limited data suggest insulin-induced increases in HDL do not prevent cardiovascular disease.

Renal Failure

Renal failure is associated with hypertriglyceridemia and low HDL levels. The reasons for this are not clear but may reflect underlying insulin resistance and defects in lipolysis of plasma triglycerides. Hypertriglyceridemia in nephrotic syndrome has been linked to increased circulating levels of the LPL inhibitor angiopoietin-like protein 4.[113]

Drugs

Diabetes, obesity, and renal disease are common causes of fasting hypertriglyceridemia, but various drugs can also elevate triglycerides. The most common drugs associated with hypertriglyceridemia are estrogens, thiazides, beta blockers, protease inhibitors, glucocorticoids, immunosuppressives, retinoids (isotretinoin, Accutane), bile acid–binding resins, and newer antipsychotic medications.

Oral estrogen therapy increases plasma triglyceride levels as a result of greater liver production of VLDL, but combined estrogen-progestin therapy does not raise triglycerides and is sometimes associated with reduced LDL.[114] In the setting of an underlying hypertriglyceridemia, severe hyperchylomicronemia and pancreatitis can occur in patients taking oral estrogen alone or estrogen-containing birth control pills, or during oocyte induction for fertility. For this reason, triglyceride levels should be measured in women before estrogen therapy or estrogen-inducing therapy is initiated. Transdermal estrogen administration, which does not lead to high liver exposure, does not increase triglycerides.[115] Tamoxifen, a selective estrogen-receptor modulator, can cause severe hypertriglyceridemia and pancreatitis,[116] but raloxifene, another selective estrogen-receptor modulator, does not raise triglycerides.[117]

Diet and Alcohol

Diets can lead to marked changes in plasma triglyceride levels. Most lipoprotein profiles use fasting blood to avoid the postprandial increase in triglycerides that represent both dietary fat and hepatic de novo triglyceride production. Liver triglyceride production is especially robust after ingestion of simple sugars, such as those found in sweetened foods (especially beverages including high fructose corn syrup) and other carbohydrates (bread, pasta, rice, and potatoes). Excessive intake of simple carbohydrates usually leads to moderate hypertriglyceridemia but can also exacerbate underlying genetic hypertriglyceridemias. Fat intake, especially in the setting of triglyceride levels greater than 500 mg/dL (5.6 mmol/L), can cause severe hyperchylomicronemia.

Alcohol is a major clinical cause of hypertriglyceridemia. Sensitivity to the triglyceride-raising effects of alcohol is variable, but elimination of alcohol from the diet of hypertriglyceridemic patients is often curative. Alcohol has many effects on lipid metabolism, including inducing de novo fatty acid synthesis and inhibiting fatty acid oxidation in the liver. A common clinical conundrum is deciding whether hypertriglyceridemia alone is responsible for pancreatitis in an alcohol-using patient. Patients with dysbetalipoproteinemia are particularly sensitive to the effects of alcohol consumption because of alcohol-induced overproduction of VLDL and subsequent production of remnant particles in the setting of impaired remnant clearance. Because alcohol also raises HDL, the presence of elevated triglycerides without reduced HDL is a clinical clue that an alcohol effect may be contributing to the lipid disorder.

Diagnostic Evaluation of Moderate Hypertriglyceridemia

A search for associated disorders, review of medication use, and delineation of dietary choices are appropriate. If both triglycerides and cholesterol are elevated, a search for the underlying lipoprotein disorder is sometimes useful. By ultracentrifugation, dysbetalipoproteinemia due to the presence of cholesterol-enriched VLDL can be differentiated from that of familial combined hyperlipidemia: The usual ratio of VLDL triglyceride to cholesterol of approximately 5 is reduced to 3 or less in dysbetalipoproteinemia. ApoE genotyping will determine if the patient has the apoE2/E2 genotype, which can be associated with aggressive vascular disease. Cholesterol-enriched VLDL is also found in patients with hypothyroidism, renal failure, or hepatic lipase deficiency.

Hypercholesterolemia Without Hypertriglyceridemia

Because all lipoproteins contain cholesterol, dramatic increases in triglycerides will invariably also lead to elevated blood cholesterol values. However, the ratio of triglyceride to cholesterol will be greater than 5. Disorders associated with primarily increased cholesterol are discussed in this section. The clinical presentation of hypercholesterolemia is limited. Although patients with severe disease occasionally present with cosmetic concerns or orthopedic issues associated with tendon xanthomas, hypercholesterolemia is usually clinically occult and uncovered by blood testing during routine assessment or in the setting of vascular disease.

Polygenic Hypercholesterolemia

Most patients with elevated LDL cholesterol do not have FH, and even cholesterol levels above 300 mg/dL (7.8 mmol/L) are not usually associated with xanthomas or defects in the LDL receptor. If, as is conventional for other laboratory values, hypercholesterolemia is defined as a cholesterol value that exceeds the 95th percentile for the population, only 1 in 25 of these patients should have FH. Although diet and lifestyle influence LDL, the genetic and environmental factors associated with most elevated LDL levels are unknown, and therefore this type of hypercholesterolemia is referred to as polygenic. Nonetheless, the increased cholesterol is associated with a higher risk of coronary artery disease.

Increased LDL levels can be a result of defective LDL clearance with normal LDL receptors, a more subtle regulation of the receptor, or increased LDL production. This last type occurs in the setting of familial combined hyperlipoproteinemia (see later discussion), which can manifest as primary elevations of LDL. Greater LDL production due to greater absorption of gut cholesterol, abnormalities in the regulation of lipid-regulated nuclear transcription factors, or gain-of-function abnormalities in cholesterol and apoB lipoprotein assembly pathways are all possible but poorly defined.

Genetic FH

The centrality of the LDL receptor to the understanding of cholesterol metabolism was uncovered through investigations by Brown and Goldstein (see the LDL Receptor section) into the cause of heterozygous FH. This relatively common cause of severe hypercholesterolemia results from one of many defects in production of the LDL receptor leading to impaired function or production of the LDL receptor protein. Patients with FH (most with heterozygous forms of the disease) have cholesterol levels that exceed 300 mg/dL (7.8 mmol/L). Homozygous presentations of the disease include cholesterol levels that are approximately twice this value. In one health care system in the United States, the heterozygous form was detected with a prevalence of 1 in 256 in unselected participants and 1 in 118 in cardiac catheterization laboratory participants.[118] FH is a major cause of early-onset coronary artery disease. In addition, homozygous and some other severe forms of LDL receptor deficiency are associated with aortic valve calcification and stenosis. Humans can have LDL receptor defects without hypercholesterolemia, but these individuals are still at increased risk of cardiovascular disease.[119]

Patients with heterozygous FH have cholesterol deposition on tendons leading to tendon xanthomas like those seen in Fig. 41.17C and D. They are most common on the Achilles tendon. On inspection, a loss of the usual bow-like shape of the tendon occurs or a bump or generalized thickening of the tendon is apparent. The irregularity of the tendon can also be detected by palpation. This physical finding is sometimes similar to scarring that results from tendon rupture. If the tendon is abnormal, a history of athletic injury should be sought. Xanthomas also occur on extensor tendons of the hands, but less frequently, and are best appreciated on the knuckles of a clenched fist. Xanthomas of the Achilles tendon can cause recurrent episodes of Achilles tendinitis. Some xanthomas are subtle and are apparent only as a thickening of the tendon or a small bump at the insertion of the tendon into muscle. Patients with FH also have xanthelasmas (see Fig. 41.17A) and premature corneal arcus (i.e., in persons younger than 40 years), but these findings can also occur in patients without FH. Many affected subjects have no physical findings. Premature coronary artery disease is common but variable. Some FH patients develop coronary disease in the third or fourth decade of life, especially if they also have reduced HDL or associated risk due to cigarette smoking. Even before the introduction of statin therapy, some FH patients (especially women) never develop clinical vascular

disease. Incomplete penetrance or protective factors perhaps due to lifestyle may contribute to their normal life span.

Homozygosity for FH is not common but is likely to occur more frequently than previously appreciated.[120] These subjects come to clinical attention early in life because of the appearance of tendon and planar xanthomas, as well as tuberous xanthomas (see Fig. 41.17E), marked hypercholesterolemia apparent at birth, premature coronary disease, or aortic valve disease. Typical plasma cholesterol concentrations range from 600 mg/dL (15.5 mmol/L) to 1000 mg/dL (25.9 mmol/L), and LDL concentrations range from 500 to 950 mg/dL (13.0 to 24.6 mmol/L). Symptomatic coronary disease can occur before age 10 years. If not treated, these homozygous persons usually die from myocardial infarction by age 20 years. Aortic valve disease in homozygotes can be valvular or supravalvular. The diagnosis of homozygous FH should be suspected in any child with extremely high plasma cholesterol (typically >500 mg/dL [13.0 mmol/L]) or the xanthomas characteristic of FH. Both parents are obligate heterozygotes and should manifest the phenotype of heterozygous FH. These children are treated with repetitive LDL apheresis or liver transplantation.

Familial Defective Apolipoprotein B100

Population-based genotyping shows that defects in LDL binding to normal LDL receptors leads to elevated LDL cholesterol by 60 to 70 mg/dL (1.5–1.8 mmol/L).[121] Familial defective apoB100 has a phenotype that is indistinguishable from that of FH, including increased susceptibility to coronary heart disease. The substitution of glutamine for arginine at amino acid 3500, which reduces LDL binding to the LDL receptor, accounts for most cases of familial defective apoB100, although other defects have also been reported. Often the LDL elevations are less severe, such as with LDL below 200 mg/dL (5.2 mmol/L), a reflection of a partial defect in receptor binding attributed to the mutation or the continued ability of apoE to mediate lipoprotein uptake.

Rare Mutations Associated With Elevated LDL Levels

Several rare, isolated causes of hypercholesterolemia have been reported. Mutations in *LDLRAP1*, the gene encoding a putative adaptor protein (ARH) required for internalization of LDL bound by the LDL receptor on the surface of hepatocytes, cause autosomal recessive hypercholesterolemia. Mutations in ARH associated with autosomal recessive hypercholesterolemia have been reported mostly in Italians (Sardinia) and Lebanese.

Autosomal dominant hypercholesterolemia caused by a mutation in the gene encoding cholesterol 7α-hydroxylase has been reported.[122] The hypercholesterolemia is caused by defective cholesterol conversion to cholic acid.

Mutation of PCSK9 (see the LDL Receptor section) leads to alterations in LDL receptor expression because this enzyme modulates intracellular LDL degradation.[123] Gain-of-function mutations reduce LDL receptor numbers and lead to defective LDL clearance by the liver. Inhibitors of this protein are an effective therapy for hypercholesterolemia.[124] As noted earlier, loss-of-function mutations in PSCK9 are associated with low total cholesterol and LDL, and decreased risk of coronary artery disease.

Elevated Plasma Lp(a)

Apo(a), a protein of unknown function that shares high sequence homology with plasminogen, associates with apoB to produce an LDL called *Lp(a)*. Elevated levels of Lp(a) increase the risk of cardiovascular disease and calcific aortic valvular stenosis[125] and are found in approximately 20% of patients.[126] Patients with high Lp(a) are more likely to clot bypass grafts and stents. Unlike LDL, which confers risk as a continuous function of plasma level, risk is found only in individuals with the highest levels of Lp(a).

Plasma Lp(a) levels are largely determined genetically. Lp(a) particles contain a variable number of a protein repeat known as a Kringle. Smaller Lp(a) particles, with fewer Kringle repeats, are usually produced at higher levels. For this reason, some suggest Lp(a) size, rather than plasma level, that confers vascular risk. Renal failure leads to elevations of Lp(a). Niacin treatment, but not statin treatment, reduces Lp(a), whereas PCSK9 inhibitors decrease Lp(a) by increasing its plasma clearance.[127] Specific therapies to reduce Lp(a) using antisense and silencing RNA approaches are in development.

Lipoprotein(X)

Obstructive liver disease sometimes leads to a marked increase in plasma cholesterol. In part, this is the result of increased LDL, presumably due to a defect in LDL receptors. In addition, free cholesterol circulates in association with albumin, a particle referred to as Lp(X). This is caused by a deficiency in the cholesterol esterifying enzyme, LCAT. The clinical setting suggests the diagnosis. In addition, an abnormal ratio of free to total cholesterol (or cholesteryl ester) can be determined by the laboratory. The relationship of Lp(X) to vascular disease is unclear.

Sitosterolemia

In this rare disorder, dietary sitosterol and other plant sterols, which are not normally absorbed in significant quantities in the intestine, are absorbed in large amounts, resulting in their accumulation in the plasma and in peripheral tissues and causing premature atherosclerosis.[128] The molecular cause is a mutation in the genes encoding ABCG8 and ABCG5, which are responsible for resecretion of absorbed plant sterols. Patients develop tendon xanthomas in childhood and have normal to high plasma LDL; the differential diagnosis includes FH and cerebrotendinous xanthomatosis. The diagnosis can be confirmed by gas-liquid chromatography of plasma lipids, which demonstrates the high levels of plant sterols. Therapy consists of restriction of dietary plant sterols and treatment with ezetimibe (see later discussion), which inhibits absorption of dietary sterols.[129]

Cerebrotendinous Xanthomatosis

Cerebrotendinous xanthomatosis is a rare disorder of sterol metabolism associated with neurologic disease, tendon xanthomas, and cataracts in young adults. Neurologic manifestations include cerebellar ataxia, dementia, spinal cord paresis, and subnormal intelligence. Premature atherosclerosis is common. Osteoporosis has been reported and is presumably caused by alterations in vitamin D metabolism. The disorder results from mutations that cause deficiencies of 27-hydroxylase, a key enzyme in cholesterol oxidation and bile acid synthesis, leading to high plasma levels of cholesterol and cholestanol, with subsequent accumulation of these sterols in tendons and in tissues of the nervous system. Chenodeoxycholic acid is indicated for treatment.

Hypothyroidism and Elevated Cholesterol

All patients with significant hyperlipidemia should be screened for hypothyroidism, because thyroid hormone deficiency causes hypercholesterolemia, and low levels of thyroid hormone predispose to statin-induced myositis.[130] Although hypothyroidism usually increases LDL, it can also be associated with high plasma triglycerides. Levels of HDL are usually unchanged or slightly lower in hypothyroidism and may be reduced in hyperthyroidism. Subclinical hypothyroidism is a cause of hypercholesterolemia that sometimes responds to thyroid hormone replacement.[131]

Diagnostic Evaluation of Isolated Hypercholesterolemia

All adults and perhaps children should have a cholesterol level assessed at least every 5 years. In adults, cholesterol levels higher than 300 mg/dL (7.8 mmol/L) and LDL greater than 190 mg/dL (4.9 mmol/L) suggest FH. Heterozygous FH should be suspected in any person with premature atherosclerotic cardiovascular disease. The diagnosis of heterozygous FH can be confirmed clinically by the presence of tendon xanthomas and a family history of premature coronary disease. The diagnosis of FH is primarily clinical, but genetic testing is available and, although costly, may be helpful for assessing risk in family members and in genetic counseling. This is important because many patients with FH will not initially present with elevated LDL but have increased risk of cardiovascular disease, presumably due to a lifelong LDL that is higher than desirable.

Increased HDL

Occasionally, patients with hypercholesterolemia primarily have elevations of HDL with normal LDL. Usually, this pattern is found in families with little cardiovascular disease. However, genetic association studies fail to link genetic factors that elevate HDL with reduced vascular disease,[132] and no pharmacologic interventions that raise HDL in the statin era appear to provide benefit.

There are subgroups of patients with increased HDL and whose HDL does not confer a protection from cardiovascular disease. Isolation of HDL from such patients with cardiovascular disease and high levels of HDL led to the observation that these HDLs do not have anti-inflammatory properties.[133] Atherogenic HDL has been detected in patients with systemic lupus erythematosus.[134] Therefore, there are clinical circumstances in which HDL may be dysfunctional (see the Reverse Cholesterol Transport and Dysfunctional HDL section).

Genetic Disorders Causing Increased HDL

CETP deficiency is a hereditary syndrome in which plasma HDL levels are increased because of diminished activity of plasma CETP. The disorder is not uncommon in the Japanese population. Its features include marked elevations of plasma HDL in homozygotes (usually >100 mg/dL [2.6 mmol/L]). Despite the elevated HDL levels, the effect on coronary disease risk of CETP gene mutations is unclear. Heterozygotes have moderately elevated HDL. The lower activity of CETP results in diminished transfer of cholesteryl esters from HDL to apoB-containing lipoproteins. As a result, more cholesteryl esters are found in HDL, and the ratio of total cholesterol to HDL is markedly reduced. CETP is most active in the setting of hypertriglyceridemia, so, in addition to reducing HDL, it provides a mechanism for reduction of triglyceride through HDL metabolism. Perhaps for this reason, studies in mice, which normally do not have significant plasma CETP activity, have shown reduced apoB-containing lipoproteins and reduced atherosclerosis with addition of a CETP transgene. As noted, CETP inhibitors do not appear to have benefit that can be attributed to increased HDL.[83]

Several other pharmacologic agents are associated with increased plasma HDL. The antiatherogenic capacity of HDL produced in these circumstances is unknown. HDL induction occurs with the use of oral estrogens, alcohol intake, phenytoin (Dilantin), phenobarbital, and insulin therapy in some patients with type 1 diabetes mellitus.

Endothelial lipase deficiency is caused by defects in the third member of the lipase gene family—endothelial lipase. As described earlier, loss-of-function mutations in this lipase are associated with increased HDL levels but not reduced risk of coronary heart disease.

Elevated Triglycerides and Cholesterol

Elevated triglyceride and cholesterol levels can be caused by increased VLDL and LDL, combined hyperlipidemia, or the presence of increased circulating remnant lipoproteins (dysbetalipoproteinemia). Methods to diagnose dysbetalipoproteinemia are usually not fruitful unless patients present with premature vascular disease or palmar xanthomas.

Combined Hyperlipidemia

Combined hyperlipidemia is a common disorder associated with elevations of plasma cholesterol and triglyceride levels and increased susceptibility to coronary disease. Lipoprotein isolation reveals increased LDL and VLDL. Kinetic analysis has associated this pattern primarily with overproduction, rather than defective clearance, of apoB lipoproteins. When it occurs within families, it is termed *familial combined hyperlipidemia*. These individuals, who do not necessarily have other causes for lipid abnormalities, characteristically come from families with various hyperlipidemias that include increased isolated triglycerides or increased isolated LDL. Moreover, the abnormal lipoprotein pattern (increased triglycerides, cholesterol, or both) can vary over time in an individual.

Regulation of apoB production involves several steps (see the Apolipoprotein B section), and understanding this physiology explains some of the association of combined hyperlipidemia with other diseases. Increased fatty acid return to the liver and reduced insulin action prevent degradation of newly synthesized apoB. Therefore, it is not surprising that both metabolic syndrome and type 2 diabetes mellitus are commonly found with combined hyperlipidemias. Although the genetics of this disorder have been investigated, the coexistence of this lipoprotein pattern with insulin resistance and obesity syndromes has been a confounder. Therefore, although alterations in several genes associated with lipid metabolism, such as LPL and apoCIII, have been observed, a firm genetic marker is not currently available.

Familial Dysbetalipoproteinemia

Familial dysbetalipoproteinemia is an uncommon disorder of lipoprotein metabolism that is characterized by moderate to severe hypertriglyceridemia and hypercholesterolemia caused by the accumulation of cholesterol-rich remnant particles in the plasma.

Premature peripheral vascular disease and coronary artery disease are common. The cause is mutations in the apoE gene that result in defective binding of apoE to lipoprotein receptors. The disorder is associated with the apoE2 isoform and in most instances is inherited as an autosomal recessive trait. Because the phenotypic expression of the disorder is limited to approximately 5% of the patients with the apoE2/E2 phenotype, other genetic or environmental factors must also be operative. The hyperlipidemia is caused by a defect in clearance of remnant lipoproteins whose liver uptake requires apoE interaction with the LDL receptor, LRP1, and HSPG (see the Apolipoprotein E section). The remnants that accumulate have lost much of their triglyceride through LPL-mediated triglyceride hydrolysis and therefore are cholesterol rich. The predominant remnant particles, termed *β-VLDL*, can be identified by abnormal migration on gel electrophoresis or by abnormal lipid content.

Dysbetalipoproteinemia is usually diagnosed in adults and rarely detected in persons younger than 20 years. The disorder is more common in men than in women. Plasma triglyceride and cholesterol levels typically range from 300 to 400 mg/dL (3.4–45.2 mmol/L). Concentrations of HDL are normal. Xanthomas are present in more than half of affected subjects. Palmar xanthomas, which are planar xanthomas in the palmar creases (see Fig. 41.17F), are pathognomonic for this disorder. Tuberous or tuboeruptive xanthomas (see Fig. 41.17E) are also common but are less specific for this disorder. Tendon xanthomas and xanthelasma may occur. Unlike FH, in which peripheral vascular disease is uncommon, premature peripheral vascular disease occurs in addition to premature coronary artery disease in patients with dysbetalipoproteinemia. Coexisting metabolic conditions that exacerbate the phenotype of dysbetalipoproteinemia, such as obesity, alcohol consumption, diabetes mellitus, and hypothyroidism, are often present.

In addition to homozygosity for the apoE2 isoform, some mutations in the apoE gene are known to lead to the dysbetalipoproteinemia phenotype in an autosomal dominant fashion. The phenotype manifests at an early age without exacerbating factors.

Hepatic Lipase Deficiency

Hepatic lipase deficiency is a rare disorder associated with lack of heparin-releasable hepatic lipase activity in the plasma. Because this enzyme mediates the final step of conversion of IDL to LDL and is involved in chylomicron remnant clearance, its deficiency causes a phenotype that is similar to that found with dysbetalipoproteinemia—namely, elevated plasma cholesterol (250–1500 mg/dL [6.5–38.9 mmol/L]) and triglycerides (395–8200 mg/dL[4.5–92.7 mmol/L]). Patients also have palmar and tuboeruptive xanthomas, premature arcus corneae, and premature coronary artery disease. Because hepatic lipase also mediates HDL metabolism, HDL levels are not decreased. The demonstration of hepatic lipase deficiency requires in vitro assays of hepatic lipase activity in postheparin plasma or DNA analysis to identify mutations.

Nephrotic Syndrome

Hyperlipidemia almost always accompanies nephrotic syndrome. Total cholesterol, VLDL, LDL, total triglycerides, and plasma apoB may all be elevated. Nephrotic syndrome increases liver production of apoB-containing lipoproteins, leading to increased plasma LDL, VLDL, or both. This may be a response to hypoalbuminemia and an associated generalized increase in liver protein secretion.

Protease Inhibitor Use in Human Immunodeficiency Virus Infection

Treatments for human immunodeficiency virus infection often cause hyperlipidemia, lipodystrophy, and insulin resistance. Hypertriglyceridemia is the most common lipid abnormality, although increases in LDL are also found. These effects were initially thought to result from the use of protease inhibitors, but other agents, such as reverse transcriptase inhibitors, can also cause dyslipidemia. Metabolic syndrome is not uniformly induced by all drugs; older agents such as ritonavir are more likely to have metabolic side effects. The cause of this syndrome is unclear but may be related to greater liver production of apoB lipoproteins and triglycerides.

Immunosuppressive Regimens

Patients undergoing transplantation who require several medications commonly present with hypertriglyceridemia with or without hypercholesterolemia. Glucocorticoids more often raise triglycerides, cyclosporine raises cholesterol, and rapamycin increases both cholesterol and triglycerides.

Diagnostic Evaluation of Elevated Triglycerides and Cholesterol

Mixed elevations of triglycerides and cholesterol are common. Appropriate assessment usually requires that clinicians determine whether the hyperlipidemia is primarily a genetic disorder or is secondary to a systemic disorder or medications. Medications are perhaps the most common cause. Table 41.4 provides an overview of diagnostic considerations. In terms of lipid disorders, familial combined hyperlipidemia requires both medical history and fasting lipid panels for family members. This disorder is common and associated with premature vascular disease. In some situations, an evaluation for dysbetalipoproteinemia, a rare disorder, is useful. In other cases, empiric treatment is reasonable (see the Combination Therapy for Other Hyperlipidemias section).

Hypocholesterolemia

Secondary causes that can lead to very low levels of apoB-containing lipoproteins and low cholesterol levels include malabsorption, sepsis, liver failure, cachexia syndromes, and malnutrition. There are also genetic conditions that cause low cholesterol.

Familial Hypobetalipoproteinemia

Familial hypobetalipoproteinemia is defined as apoB and LDL levels below the 5th percentile. Hypobetalipoproteinemia is not associated with a phenotype and leads to a reduced risk for cardiovascular disease. Possible causes of this syndrome include mutations leading to truncation of apoB and mutations in PCSK9 presumably leading to increased numbers of LDL receptors. PCSK9 loss-of-function mutations occur in as many as 2% of African Americans but are rare in persons of European descent. Other patients have a defect in angiopoietin-like protein 3.[55] Patients with hypobetalipoproteinemia, except those due to the angiopoietin-like protein 3 defect, have an increased risk of hepatic steatosis.

Abetalipoproteinemia

Abetalipoproteinemia is a rare autosomal recessive disorder caused by a deficiency in MTP (see the Apolipoprotein B section), which results in a virtual absence of apoB-containing lipoproteins in the plasma. Abetalipoproteinemia occurs in fewer than 1 in a million persons and has the same phenotype as homozygous hypobetalipoproteinemia, including malabsorption of fat and fat-soluble vitamins from the intestine, which can lead to neurologic disease related to vitamin E deficiency. The disorder is usually detected in infancy because of fat malabsorption associated with marked decreases in plasma cholesterol and triglyceride levels.

Chylomicron Retention Syndrome

Anderson disease, or chylomicron retention syndrome, is a rare condition that is phenotypically similar to abetalipoproteinemia. Subjects with Anderson disease cannot secrete chylomicrons from the intestine. Mutations in the *SAR1B* gene (formerly *SARA2*) have been linked to Anderson disease. This gene encodes SAR1B, a protein that is important in the transport of chylomicrons through the secretory pathway in enterocytes.

Familial Hypoalphalipoproteinemia

Although low HDL in the general population is correlated with greater coronary artery disease, genetic disorders leading to very low plasma HDL have variable and sometimes undefined coronary risk. In part, this might result from the rarity of some of these diseases. For this reason, the approach to the disorders is often unclear.

Apolipoprotein AI Mutations

Mutations in the apoAI gene can decrease HDL formation and result in low plasma HDL levels. ApoAI deficiency can be caused by point mutations in the apoAI gene or by deletions or gene rearrangements at the apoAI/CIII/AIV/AV gene locus. ApoAI deficiency typically results in plasma HDL lower than 10 mg/dL (0.26 mmol/L). Some apoAI variants activate LCAT (see the next section) poorly. The molecular diagnosis is made by protein analysis showing an altered apoAI size or by genetic sequencing. Some apoAI variants are associated with amyloidosis, but there are no defined specific clinical effects of these variants.

The apoAI Milano variant ($apoAI_{Milano}$) is caused by a substitution of cysteine for arginine at amino acid 173. This results in low plasma HDL levels without premature coronary heart disease. This HDL is hypothesized to be antiatherogenic, but administration of recombinant $apoAI_{Milano}$ does not appear to induce plaque regression in acute coronary syndrome in the setting of contemporary statin therapy.[135]

Lecithin: Cholesterol Acyltransferase Deficiency

LCAT deficiency[136] leads to low HDL as a consequence of defective conversion of cholesterol to cholesteryl ester. LCAT deficiency leads to striking corneal arcus (sometimes leading to marked visual impairment), normochromic anemia, and, occasionally, renal failure in young adults. Renal biopsies show characteristic foam cells. In a phenotypically appropriate patient, measurement of the ratio of free cholesterol to cholesteryl ester is diagnostic; normally, free cholesterol is approximately 30% of the total, but this percentage increases to more than 90% in LCAT deficiency. LCAT activity measurements and sequencing can also be performed. Despite very low HDL levels, risk for coronary disease in patients with LCAT deficiency is not pronounced, which may reflect the low levels of LDL also found in many of these patients.

A rare variant of LCAT deficiency, called *fish-eye disease*, is also caused by mutations of the LCAT gene. The phenotype is less severe than that seen in complete LCAT deficiency. Fish-eye disease is characterized by low plasma HDL and corneal opacities; anemia and renal disease do not occur. The phenotypic differences between LCAT deficiency and fish-eye disease have been attributed to whether mutations in the LCAT gene encode variants that fail to esterify cholesterol of both HDL and apoB-containing lipoproteins (LCAT deficiency) or of HDL only (fish-eye disease).

ABCA1 Deficiency

The ABCA1 transporter is essential to complete synthesis of mature HDL by the liver and the small intestine. Several mutations in this receptor are associated with hypoalphalipoproteinemia. Because the number of genetic mutations is great, assays often study fibroblast "unloading" of cholesterol to apoAI to show the defect.

Tangier disease is the most flagrant example of a defect in ABCA1. These patients, originally from Tangier Island in the Chesapeake Bay, had hypocholesterolemia resulting from a marked decrease in plasma HDL, as well as low LDL and the striking physical finding of orange tonsils. The orange tonsils likely result from defective reverse cholesterol transport from macrophages of the reticuloendothelial system, with the color from carotenoids. The risk of cardiovascular disease with this syndrome is uncertain.

Treatment of Lipid Disorders

Evidence Supporting Treatment of Lipid Disorders: Cholesterol and Cardiovascular Disease

Despite a very significant decrease in the incidence of vascular disease, cardiovascular diseases, including coronary heart disease and cerebrovascular disease, remain the major causes of death in the United States for both men and women.[137] The major risk factors are age, elevated LDL, reduced HDL, smoking, hypertension, insulin resistance with or without overt diabetes mellitus, and a family history of premature cardiovascular disease. Modifiable risk factors account for most of the excess cardiovascular disease risk. Although several new risk factors have been proposed to increase the accuracy of predicting risk of cardiovascular events, four conditions—dyslipidemia, hypertension, cigarette smoking, and diabetes mellitus—account for increased cardiovascular disease risk in most patients.

Statins reduce the chances of recurrent events (secondary prevention) in patients with previous cardiovascular events.[1,138] Meta-analyses of clinical trials demonstrate that statins reduce risk of initial cardiovascular events (primary prevention) in most groups of patients, including men and women. They also reduce cardiovascular events in high risk patients with diabetes, with hypertension, and regardless of baseline LDL cholesterol.

Evidence for the benefit of lowering triglycerides or raising HDL levels, or both, is not strong. Data are most significant in studies of the fibric acid gemfibrozil. The Helsinki Heart Study[139] and the Veterans Affairs–High-Density Lipoprotein Cholesterol Intervention Trial[140] both used gemfibrozil, the former for primary prevention and the latter for secondary prevention, and in

both studies the greatest benefit was in men with high triglycerides and low HDL. Studies with other fibrates, such as fenofibrate, have yielded equivocal results.[141] In the lipid treatment arm of the ACCORD trial in type 2 diabetes, fenofibrate was added to statin therapy in patients with an average triglyceride of 162 mg/dL (1.8 mmol/L). There was no overall benefit, although a subgroup of patients with higher triglycerides and reduced HDL had reduced cardiovascular events,[142] and these observations persisted with extended follow-up.[143]

Although decreasing LDL reduces cardiovascular event rates by 30% to 50% in statin studies, there is still significant residual risk, especially in patients with established vascular disease, many of whom have events despite achieving LDL levels of 70 to 80 mg/dL (1.8–2.1 mmol/L), raising the issue of how to decrease this residual risk. Other questions include the following: How early in life should therapy be started for primary prevention? Will longer duration of therapy lead to better outcomes? What is the benefit of lowering triglycerides or raising HDL? What biomarkers are best to screen and monitor in at-risk patients? Can other mediators of atherosclerosis be manipulated? How can lifestyle changes be accomplished?

Measurement of Plasma Lipids

A complete plasma lipid profile (total cholesterol, calculated LDL, HDL, and triglycerides) should be measured in all adults 20 years of age and older at least once every 5 years. Triglycerides should be measured in all patients with pancreatitis. Ideally, plasma lipids should be measured at least twice under fasting steady-state conditions before therapeutic decisions are made. Plasma lipids are usually measured after a 12-hour fast to preclude detection of significant elevations in atherogenic remnant lipoproteins. Because cholesterol is a minor component of chylomicrons, total plasma cholesterol can be measured in either a fasting or a nonfasting state. Plasma lipid measurements are usually reliable if done within the first 24 hours after an acute myocardial infarction.[144]

Most clinical laboratories measure plasma total cholesterol, total triglycerides, and HDL; the last analysis is performed after apoB-containing lipoproteins are removed from the plasma. For many years, the plasma LDL concentration has been calculated from these measurements using the Friedewald formula:

$$\text{LDL} = \text{total cholesterol} - \text{HDL} - \text{VLDL}$$

where VLDL is calculated as triglycerides divided by 5. This formula assumes the cholesterol content of VLDL is about 20% of the plasma triglyceride level. It is reliable only when triglycerides are 400 mg/dL (4.5 mmol/L) or less. LDL concentrations calculated by this formula may be inaccurate in the presence of severe hypertriglyceridemia or when the triglyceride-to-cholesterol ratio of VLDL is altered as occurs in dysbetalipoproteinemia. Notably, the Friedewald formula may underestimate LDL levels in the low range that is now encountered with intensive statin and PCSK9 therapy.

A novel formula is now being used by commercial laboratories. The Martin-Hopkins calculation uses an adjustable factor for the triglyceride-to-cholesterol ratio of VLDL based on an array of triglyceride and non-HDL cholesterol levels from more than 1.3 million lipid samples in the United States.[145] This approach is more accurate than the Friedewald formula at determining LDL levels in certain patients, especially those with triglycerides between 100 and 400 mg/dL (0.11–4.5 mmol/L).

TABLE 41.5 Criteria for Diagnosis of Metabolic Syndrome

Measure[a]	Categorical Threshold
Waist circumference	*Whites, African Americans, Latin Americans*: Men, ≥40 in.; women, ≥35 in. *Asians*: Men, ≥35 in.; women, ≥32 in.
Elevated triglycerides	≥150 mg/dL *or* On drug treatment for elevated triglycerides
Reduced HDL	Men, <40 mg/dL; women, <50 mg/dL *or* On drug treatment for reduced HDL
Elevated blood pressure	≥130 mm Hg systolic or ≥85 mm Hg diastolic *or* On antihypertensive drug treatment
Elevated fasting glucose	≥100 mg/dL *or* On drug treatment for elevated glucose

[a]Three of the five measures are required for diagnosis.

HDL, High-density lipoprotein.

Adapted from Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Circulation*. 2005;112:2735–2752.

Specialized laboratories can directly assay different lipoproteins by ultracentrifugation or nuclear magnetic resonance techniques. Direct measurement of LDL is also now available in many clinical laboratories. Value for direct measurements in clinical practice is uncertain, as they were not utilized in large clinical trials and are not incorporated into most guidelines.

Treatment of High LDL

As noted, clinical trials clearly demonstrate the benefit of LDL reduction. Optimal risk factor management reduces clinical atherosclerotic event rates. However, in the United States, the 5-year incidence of myocardial infarction or cardiac death after a first event remains high, depending on age, race, and gender and risk factor control.[137]

The initial evaluation for all patients consists of a history and physical examination, including assessment of diet, exercise, cardiovascular risk factors, and measurement of plasma lipids. Exclusion of secondary causes of lipid disorders (see Table 41.4) is important. Obesity is an independent risk factor for cardiovascular disease, reflected in the waist circumference measurement that is used to define metabolic syndrome (see Table 41.5). Obesity aggravates dyslipidemia, hypertension, and insulin resistance, and is a target of therapy regardless of the severity of traditional cardiovascular risk factors (see Table 41.6). Particular emphasis should be placed on obtaining a detailed history of all first-degree relatives to identify cholesterol disorders or premature cardiovascular disease. In primary care, a child-parent screening strategy is effective at identifying FH with high cardiovascular risk.[146] The examination should emphasize the cardiovascular system, manifestations of hyperlipidemia, and disorders causing secondary lipid abnormalities.

Hyperchylomicronemia-Induced Pancreatitis

Markedly increased triglyceride levels are most often diagnosed by laboratory detection, including the observation of lipemia. A triglyceride higher than 1000 mg/dL (11.3 mmol/L) usually signifies

TABLE 41.6 Traditional Risk Factors for Atherosclerotic Cardiovascular Disease

- Age
- Sex
- Total cholesterol
- HDL cholesterol
- Systolic blood pressure
- Use of antihypertensive therapy
- Diabetes mellitus
- Current smoking

HDL, High-density lipoprotein.

Data from Goff DC Jr, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014;63:2935–2959.

the presence of two or more abnormalities of lipid metabolism (e.g., estrogen therapy in the presence of underlying familial hypertriglyceridemia). Elevated plasma triglyceride levels can fluctuate markedly within an individual over short periods. Fluctuation occurs because the LPL-mediated clearance mechanisms for triglyceride-rich particles becomes saturated at plasma triglyceride concentrations of approximately 500 mg/dL (5.6 mmol/L). Therefore, triglyceride levels can rise precipitously as dietary fat intake increases and can fall rapidly with dietary fat restriction. Very high carbohydrate diets, unless composed of primarily complex carbohydrate, may lead to poor glycemic control and increased triglyceride levels. Patients with the chylomicronemia syndrome may initially require a diet with less than 10% of calories from fat to decrease chylomicron production.

Visual inspection of plasma after it has been refrigerated overnight can be helpful. A cream-like layer on the top signifies chylomicrons. A turbid infranatant signifies high levels of VLDL. A cream-like top layer and turbid plasma indicates the presence of both chylomicrons and VLDL.

A history of diabetes, alcohol use, or triglyceride-elevating medications is common. Physical examination might be useful. Lipemia retinalis (see Fig. 41.17B), a condition in which lipemic blood causes opalescence of retinal arterioles, can be observed during funduscopic examination. It is typically seen only when triglycerides are 2000 mg/dL (22.6 mmol/L) or higher.

Eruptive xanthomas (see Fig. 41.17G) appear as small, yellowish, round papules that contain a pale center and an erythematous base. Their distribution includes the abdominal wall, back, buttocks, and other pressure contact areas. They are caused by accumulation of triglyceride in dermal histiocytes and typically occur when the plasma triglyceride level is 1000 to 2000 mg/mL (11.3–22.6 mmol/L) or higher. They can disappear rapidly with lowering of the plasma triglyceride concentration.

Severe hypertriglyceridemia (1000 mg/dL [>11.3 mmol/L]) should be treated aggressively because pancreatitis associated with these levels can be fatal. Lifestyle intervention includes exercise, diet (avoid high fat, simple sugars, and noncomplex carbohydrates), diabetes control, and elimination of triglyceride-raising medications if possible. Initial drug treatments are fibrates, fish oils, and/or niacin. Patients have also been treated with newer therapies that inhibit apoB production (mipomersen) or MTP (lomitapide), but increased liver accumulation of triglyceride is a concern. Antisense reduction of liver production of apoCIII effectively reduces triglyceride levels in patients with and without LPL mutations.[58] Additional approaches under development target the LPL inhibitory protein ANGPTL3.[147]

In the setting of acute pancreatitis, the approach to treatment is based on clinical impression and our understanding of the pathophysiology rather than randomized clinical trials. In the setting of diabetes, insulin therapy is essential. In nonhyperglycemic patients, low-dose insulin (1–2 units per hour) is usually sufficient to block adipose tissue lipolysis, reduce circulating free fatty acids levels, and theoretically reduce triglyceride production by the liver. LPL activity is also induced by insulin. Higher doses of insulin, such as those used to treat diabetic ketoacidosis, require large amounts of glucose infusion to prevent hypoglycemia and are likely to increase de novo triglyceride production in the liver. In most patients, triglycerides will decrease approximately 50% within 24 hours, so evidence for the lack of response requires accurate initial levels that should be obtained by plasma dilution. Plasmapheresis is rarely indicated and has not been proven to be effective,[148] despite a marked reduction in circulating triglyceride levels. For this reason, it might be most reasonable to consider this therapy only in patients not responding to conventional methods, with associated organ dysfunction, with plasma levels that are extraordinarily elevated (more than 10,000 mg/dL [259 mmol/L]), or during pregnancy when triglyceride increases are driven by estrogen induction of liver triglyceride production.

Screening for Secondary Disorders

The history and physical examination should be directed toward uncovering secondary disorders of lipid metabolism and identifying agents including medications that could cause hyperlipidemia (see Table 41.4). Minimal studies should include fasting blood glucose, glycosylated hemoglobin, renal and hepatic function tests, urinary protein, and thyroid-stimulating hormone.

Patient Selection and Treatment Goals

Many professional groups have issued and updated guidelines for management of hyperlipidemia over decades. As guidelines for treatment of lipoprotein disorders and approaches to reduce cardiovascular risk have evolved, they have engendered controversy. Early recommendations based on epidemiology, preclinical data, and limited randomized clinical data were shown to be prescient by subsequent clinical trials. Although it is widely accepted that LDL cholesterol reduction prevents atherosclerotic cardiovascular disease and reduces clinically important cardiovascular events in patients with established disease or at increased risk, who should be treated and to what lipid target has been disputed. The National Cholesterol Education Program, established by the National Institutes of Health in the late 1980s, created a standard for cholesterol levels and pioneered a practical approach to treatment by dividing the population according to cardiac risk based on lipid and other cardiac risk factors. This approach has persisted. More recent clinical trials have shown greater reductions of plaque with aggressive and safe LDL reduction. Although, in general, desirable levels of LDL for primary prevention of disease are less than 100 mg/dL (0.26 mmol/L) and for secondary prevention of disease are less than 70 mg/dL (1.8 mmol/L), others have recommended even lower targets (<55 mg/dL [1.4 mmol/L]) in the setting of documented atherosclerosis, very high risk, or evidence of continued disease progression despite LDL less than 70 mg/dL (1.8 mmol/L).[149]

The American College of Cardiology and American Heart Association (ACC/AHA) guidelines from 2013[150] (Table 41.7) were controversial because they focused not on LDL goals but on the use of high- versus low-dose statin therapies, as well as the percentage of LDL reduction achieved. Others, including the

TABLE 41.7 American College of Cardiology/American Heart Association 2013 Statin Benefit Groups

Benefit Group	Management
Clinical ASCVD	Age <75 years without contraindications: high-intensity statin Age >75 years or with contraindications: moderate-intensity statin
LDL >190 mg/dL, age >21 years	High-intensity statin Can consider nonstatin drug if further LDL reduction is desirable
Primary prevention—diabetes: age 40–75 years, LDL 70–189 mg/dL	Moderate-intensity statin If 10-year ASCVD risk is ≥7.5%, consider high-intensity statin
Primary prevention—no diabetes[a]: ≥7.5% 10-year ASCVD risk,[b] age 40–75 years, LDL 70–189 mg/dL	Moderate- to high-intensity statin

[a]Requires risk discussion between clinician and patient before statin initiation.

[b]Statin therapy may be considered if risk decision is uncertain after use of the ASCVD risk calculator.

ASCVD, Atherosclerotic cardiovascular disease; *LDL*, low-density lipoprotein.

Data from Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol.* 2014;63:2889–2934.

Canadian Cardiovascular Society and European groups have continued to include goals for treatment (Table 41.8). With evidence that all approaches to LDL reduction lead to a similar reduction in cardiovascular events and the advent of new potent therapies, such as PCSK9 inhibitors, recommended therapeutic goals will likely become more aggressive.

Risk calculators based on current ACC/AHA guidelines are available on the ACC and AHA websites. The Framingham risk calculator remains frequently used and includes age, total cholesterol, HDL, systolic blood pressure, and smoking status, and many others are available and include additional well-known risk factors such as Lp(a), C-reactive protein, and coronary calcium scores. Different risk scoring systems may be appropriate for diverse populations and regions.[151–153] In acknowledgement that lipoproteins other than LDL are also atherogenic, some guidelines use non-HDL cholesterol (total cholesterol minus the HDL) as a secondary goal that is 30 mg/dL (0.8 mmol/L) higher, accounting for the likely atherogenic risk due to triglyceride-rich lipoproteins.

Most guidelines recommend treatment for any patient with LDL greater than 190 mg/dL (4.9 mmol/L). When lipid-lowering therapy for primary prevention should be initiated is unclear. Biomarkers such as C-reactive protein, Lp(a), and imaging techniques such as coronary calcium scores are sometimes added to more precisely estimate individual risk. Guidelines for treatment of patients with type 2 diabetes mellitus consider their increased risk of cardiovascular events. Patients with established vascular disease and diabetes mellitus are considered to be at very high risk.[154] Cardiovascular disease accounts for a high percentage of deaths in the geriatric population, and there are survival benefits of treatment in elderly patients up to the age of 85 years who have known coronary heart disease.

Some patients with LDL levels below previous treatment thresholds benefit from greater LDL cholesterol reduction.[155] Therefore, treat-to-target approaches may underestimate benefits that could accrue with greater LDL reduction.[156,157] Cardiovascular outcome trials using PCSK9 antibodies, which can reduce LDL to very low levels, may lead to recommendations for LDL levels below current goals.

Specific Therapies

Specific therapies include lifestyle changes (diet, exercise, and weight management) and drug therapy.

Lifestyle Treatment

Lifestyle changes including dietary intervention, moderate exercise, and weight loss are first-line therapies for hyperlipidemia (Table 41.9) and may be sufficient for mild dyslipidemias in low-risk patients. All other treatments should build on therapeutic lifestyle changes.[158] Hypertriglyceridemia often responds to decreased intake of fat, simple sugars, alcohol, and calories. Most patients will have a 10% to 15% decrease in LDL levels with diet, which may be adequate for primary prevention in low-risk patients. In patients with established coronary heart disease, drug therapy should be instituted along with diet therapy. Response to lipid-lowering drugs may be disappointing in patients who do not follow dietary recommendations.

Daily moderate exercise, such as walking, may help reduce triglyceride levels. Exercise may be particularly useful in obese, insulin-resistant patients with high triglycerides, low HDL, and moderately elevated LDL. An exercise program combined with a moderately hypocaloric diet leading to modest weight loss may improve dyslipidemia, as well as glucose tolerance and blood pressure, especially in patients with metabolic syndrome. Team management that includes nutritionists and dietitians is likely to enhance dietary therapies. Dietary changes are best when individualized and instituted gradually. Involvement of family members is important. The DASH (Dietary Approaches to Stop Hypertension) or Mediterranean diets are appropriate. Patients with the chylomicronemia syndrome may initially require a diet with less than 10% of calories from fat to decrease chylomicron production. The response to very low fat diets may be disappointing in patients with impaired glucose tolerance unless the diet is hypocaloric.

Effects of various types of dietary fats have been studied extensively.[159] Current recommendations[160] are to restrict saturated fats and trans fats and substitute with complex carbohydrates, polyunsaturated fats, and monounsaturated fats. High cholesterol and saturated fat intake elevate plasma cholesterol by decreasing receptor-mediated clearance of LDL. High dietary cholesterol also promotes increased LDL synthesis. Trans fats are unsaturated fatty acids with at least one *trans* double bond; they are produced when liquid vegetable oils are partially hydrogenated to produce semisolid fats used in margarines and shortening. Trans fats raise LDL and reduce HDL, and they have been implicated in cardiovascular disease.

Fish oils are rich in eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) (see Table 41.1). Daily doses of 4 g of EPA plus DHA lower VLDL and treat elevated triglycerides. Observational data support that fish intake is associated with decreased cardiovascular risk in those with and without known cardiovascular disease. Many experts recommend eating two servings of oily fish weekly or taking fish oil capsules that provide 1 g/day of EPA plus DHA daily as reasonable for those not interested in consuming fish.

TABLE 41.8 Comparison of Dyslipidemia Guidelines

Risk Category	TREATMENT INTENSITY OR GOALS		
	ACC/AHA[a]	CCS[b]	Eur Soc Card+[c]
Secondary prevention/high or very high risk	Clinical ASCVD: Lifestyle + high-intensity statin (LDL reduction ≥50%)	Clinical ASCVD, most DM (age ≥40 years, age ≥30 with 15-year duration, microvascular disease), abdominal aortic aneurysm, chronic kidney disease Lifestyle + statin to goal of LDL <77 mg/dL (2.0 mmol/L) or >50% reduction or non-HDL <100 mg/dL (2.6 mmol/L)	Very high risk (documented CVD, DM with target organ damage, CKD with GFR <30 mL/min/1.73 m^2, calculated SCORE ≥10%) Lifestyle advice and concomitant drug treatment Goal of LDL <70 mg/dL (1.8 mmol/L)
Primary prevention	LDL ≥190 mg/dL (rule out secondary causes): lifestyle + high-intensity statin (add non-statin if insufficient response)	Framingham Risk Score 10% to 19% and LDL ≥135 mg/dL (3.5 mmol/L) or non-HDL ≥166 mg/dL (4.3 mmol/L) or one additional risk factor Lifestyle + statin to goal of LDL <77 mg/dL (2.0 mmol/L) or >50% reduction or non-HDL <100 mg/dL (2.6 mmol/L)	High risk: Cholesterol >310, most DM, moderate CKD with GFR 30 to 59 mL/min/1.73 m^2, calculated SCORE ≥5% to <10% Lifestyle advice with drug treatment if LDL >100 Goal of LDL <100 mg/dL (2.5 mmol/L)
	DM without ASCVD, LDL <190 mg/dL: perform risk calculation Moderate- to high-intensity statin		Moderate risk calculated SCORE level 1% to 5% Lifestyle advice, consider drug if LDL uncontrolled Goal of LDL <115 mg/dL (3.0 mmol/L)
	ASCVD risk ≥7.5% Risk discussion with patient Moderate- to high-intensity statin		
Other considerations	Evaluate triglycerides >500 mg/dL	Ezetimibe first-line add-on and PCSK9 inhibitors as second line; apoB levels for risk stratification	Triglycerides <150 desirable, physical activity and other lifestyle changes to increase HDL

[a]ACC/AHA data from American College of Cardiology/American Heart Association, Stone NJ, Robinson JG, Lichtenstein AH, et al. ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol.* 2014;63:2889–2934.

[b]Canadian Cardiovascular Society data from Anderson TJ, Grégoire J, Pearson GJ, et al. 2016 Canadian Cardiovascular Society guidelines for the management of dyslipidemia for the prevention of cardiovascular disease in the adult. *Can J Cardiol.* 2016;32(11):1263–1282.

[c]Eur Soc Card data from Piepoli MF, Hoes AW, Agewall S, et al. 2016 European guidelines on cardiovascular disease prevention in clinical practice: the Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J.* 2016;37(29):2315–2381.

ACC/AHA, American College of Cardiology/American Heart Association; *ASCVD,* atherosclerotic cardiovascular disease; *CCS,* Canadian Cardiovascular Society, *Eur Soc Card+*, European Society of Cardiology plus other societies; *CVD,* cardiovascular disease; *CKD,* chronic kidney disease; *DM,* diabetes mellitus; *GFR,* glomerular filtration rate; *HDL,* high-density lipoprotein; *LDL,* low-density lipoprotein cholesterol; *PCSK9,* proprotein convertase subtilisin/kexin type 9; *SCORE,* Systematic Coronary Risk Estimation.

It is not clear whether fish oil supplementation is effective for prevention or treatment of atherosclerotic vascular disease,[161,162] and Cochrane analysis of published reports suggests that increasing EPA and DHA has little or no effect on mortality or cardiovascular health.[163] However, a recent study using 4 grams of icosapent ethyl in hypertriglyceridemic subjects with known atherosclerosis or high risk (such as diabetes) reduced coronary events.[164]

Other dietary components can influence plasma lipids. For example, soluble fibers such as psyllium or oat bran, which can bind bile acids in the gut and promote net cholesterol excretion, decrease LDL modestly (about 5–10%). Margarines made with sitostanol or sitosterol, which are plant sterols that inhibit cholesterol absorption, reduce serum cholesterol by about 10%. Together, the combination of plant sterols, soluble fiber, and restriction of saturated fat and cholesterol can reduce LDL levels by about 30%.

Drug Treatment

Table 41.10 lists drugs that inhibit cholesterol synthesis in cells (HMG-CoA reductase inhibitors), interfere with the degradation of the LDL receptor by inducing LDL receptor expression in hepatocytes (PCSK9 inhibitors), block cholesterol absorption from the gut (ezetimibe), or interfere with bile acid absorption from the gut (bile acid sequestrants). Fibrates, and omega-3 fatty acids, and niacin either inhibit VLDL production or enhance clearance of triglyceride-rich particles. Drugs that decrease hepatic VLDL production include the apolipoprotein B antisense oligonucleotide, mipomersen, and the MTP inhibitor lomitapide.

HMG-CoA Reductase Inhibitors (Statins)

Statins inhibit cholesterol biosynthesis, upregulate LDL receptors, enhance LDL clearance, reduce lipoprotein release from the liver, and may decrease triglycerides by enhancing VLDL clearance and decreasing lipoprotein production.

Statins are useful in all types of hyperlipidemias in which LDL is elevated. They are particularly useful for patients with vascular disease and those with very high LDL (e.g., FH, combined hyperlipidemia), and they are the drugs of choice for lowering LDL as primary or secondary prevention but are less effective in homozygous LDL receptor deficiency. Several statins are approved for use in children and adolescents with FH, high LDL, or a significant family history of premature coronary artery disease.

TABLE 41.9 Lifestyle Recommendations to Reduce Cardiovascular Risk

Modality	Intervention
Diet	Follow a dietary pattern high in vegetables, fruits, whole grains, poultry, fish, low-fat dairy products, legumes, nontropical vegetable oils, and nuts Limit red meat, sweets, and sugar-sweetened beverages Limit saturated fat to 5–6% of total calories Limit calories from trans fats
Physical activity	Engage in aerobic physical activity 3–4 sessions per week of moderate- to vigorous-intensity physical activity averaging 40 minutes per session
Weight management	For obese patients (BMI ≥30) or overweight patients (BMI ≥25) who have additional risk factors, sustained weight loss of 3–5% or greater reduces ASCVD risk Consultation with a registered dietitian may be helpful to plan, start, and maintain a diet that promotes weight loss and restricts intake of saturated fats

ASCVD, Atherosclerotic cardiovascular disease; *BMI,* body mass index.

Data from Eckel RH, Jakicic JM, Ard JD, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology American/Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol.* 2014;63:2960–2984; Jensen MD, Ryan DH, Apovian CM, et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Obesity Society. *J Am Coll Cardiol.* 2014;63:2985–3023.

Table 41.11 provides the relative potency of various statins when used at different dosages, and Table 41.12 lists expected effects of various statins on LDL. They reduce LDL by 20% to 60%, increase HDL by 2% to 16%, and reduce triglycerides by 7% to 37%, depending on the drug; the dosage; and, in the case of triglycerides, baseline levels. Effects also vary among patients, with greater or lesser degrees of LDL reduction even at the same dose. For each statin, doubling of the dose typically produces an additional 6% reduction of LDL.[165] LDL lowering is seen within 1 to 2 weeks after the start of therapy and is stable in about 4 to 6 weeks. Pitavastatin, atorvastatin, and rosuvastatin have long half-lives, about 12 hours, 14 hours, and 21 hours, respectively. The other statins have half-lives of about 2 to 3 hours. First-generation statins with short half-lives should be taken in the evening. Atorvastatin and fluvastatin have minimal renal clearance and may be more suitable for patients with renal insufficiency. Several statins are available as generic drugs in the United States. Table 41.13 shows specific features of available statins.

The most common side effects of statins are abdominal pain, constipation, flatulence, nausea, headache, fatigue, diarrhea, and muscle complaints. Except for musculoskeletal symptoms, most side effects are infrequent.

Liver toxicity is not common with statin use. Hepatic aminotransferase elevation is usually mild and does not require discontinuation of the statin. It may be dose dependent, as demonstrated in clinical trials that showed rates of persistent elevations of liver aminotransferase greater than three times the upper limit of normal occurring in 0.1% to 1.9% of patients, depending on the statin and the dose. The requirement for routine monitoring of hepatic transaminases was removed from statin labeling by the US Food and Drug Administration in 2012. Hepatic transaminases should be obtained at baseline and during treatment only if there is a clinical indication for their measurement. Only about 1% of statin-using patients have aminotransferase increases to greater than three times the upper limit of normal, and the elevation often decreases even if patients continue on the statin.[166] Statins can be used cautiously in the presence of liver disease that is not decompensated, and nonalcoholic hepatic steatosis is not a contraindication.[167] If aminotransferases remain greater than three times the upper limit of normal, consider lowering the dose or changing to a different statin and identify other contributing conditions or drugs. Irreversible liver damage resulting from statins is extremely rare, with a liver failure rate of 1 case per 1 million person-years of use.[167]

About 5% of patients complain of muscle side effects, but recent randomized placebo-based trials have suggested that many of these issues are due to a nocebo effect, which is the anticipation of a side effect leading to altered perception.[168] The adverse effects associated with statin use correlate with drug dose,[169] but many large randomized, placebo-controlled clinical trials have not shown differences in rates of myalgias and creatine kinase elevations between statin-treated and placebo groups. Regardless, muscle complaints are a common reason for statin discontinuation by patients, perhaps related to misinformation about the risks of statin therapy promulgated through the internet.[170] In the proper context, it is possible to carefully reintroduce statins after a presumed myalgia.

In patients with muscle complaints after statin administration, disorders that might influence skeletal muscle or present with muscle symptoms must be excluded, such as hypothyroidism, vitamin D deficiency, rheumatologic conditions, and perhaps depression. Inhibition of statin catabolism is associated with increased myopathy risk. Drugs metabolized through the cytochrome P450 (CYP) system, such as ketoconazole, itraconazole, clarithromycin, and erythromycin, increase statin plasma levels especially with use of older statins that have a more limited hepatic disposal. Other drugs that increase the risk of statin myopathy include gemfibrozil, cyclosporine, digoxin, verapamil, diltiazem, amiodarone, colchicine, and protease inhibitors.

The most serious potential side effect of statins is rhabdomyolysis leading to myoglobinuria and renal failure. Rhabdomyolysis is rare and is more likely in patients with renal insufficiency, advanced age, other comorbid conditions, or polypharmacy and during perioperative periods. Routine surveillance of creatine kinase is not useful in most patients. Management of dyslipidemia in patients with muscle-related complaints is challenging and may include using a different statin, decreasing the dose, giving the medication less frequently, or considering other LDL-lowering drugs. Higher doses of simvastatin should be avoided. The US Food and Drug Administration recommends that simvastatin 80 mg not be used, and simvastatin should be limited to 10 mg in patients taking amiodarone, verapamil, and diltiazem, and to 20 mg in those taking amlodipine and ranolazine.

Statin therapy is associated with increased incidence of type 2 diabetes mellitus in patients with a predisposition to developing this disorder. This association was most evident with the use of the highest doses of atorvastatin (80 mg) and rosuvastatin

TABLE 41.10 Drugs used to Treat Hyperlipidemia

Class and Drugs Available	Dosage	Major Lipoprotein Decreased	Mechanism
HMG-CoA Reductase Inhibitors			
Rosuvastatin	5–40 mg qd	LDL	Decrease cholesterol synthesis; increase LDL receptor–mediated removal of LDL
Atorvastatin	10–80 mg qd		
Simvastatin	5–40 mg qd		
Lovastatin	10–80 mg qd		
Pravastatin	10–40 mg qd		
Fluvastatin	20–80 mg qd		
Pitavastatin	1–4 mg qd		
PCSK9 Inhibitors			
Evolocumab	140 mg sc q 2 weeks or 420 mg sc q month	LDL	Prevent degradation of the LDL receptor
Alirocumab	75–150 mg sc q 2 weeks		
Intestinal Cholesterol Absorption Inhibitor			
Ezetimibe	10 mg qd	LDL	Inhibits cholesterol absorption
Bile Acid Sequestrants			
Cholestyramine	4–12 g bid	LDL	Increase sterol excretion and LDL clearance
Colestipol	5–15 g bid		
Colesevelam	3.75–4.375 g qd		
Fibric Acid Derivatives			
Gemfibrozil	600 mg bid	VLDL (LDL)	Decrease VLDL production; enhance LPL action
Fenofibrate[a]	30–200 mg qd		
Omega-3 Fatty Acids			
Lovaza (1-g capsule contains EPA and DHA)	4 g qd	VLDL	Inhibit VLDL production
Vascepa (1-g capsule contains EPA)	4 g qd		
Epanova (1-g capsule contains EPA and DHA free fatty acids)	2–4 g qd		
Nicotinic Acid			
Niacin (crystalline)	1–3 g qd	VLDL (LDL)	Decrease VLDL production; enhance LPL action
Niaspan (extended-release niacin)	500–2000 mg qd		
ApoB Antisense Oligonucleotide			
Mipomersen	200 mg once a week sc injection	VLDL, LDL, Lp(a)	Inhibits synthesis of apolipoprotein B
Microsomal Triglyceride Transfer Protein Inhibitor			
Lomitapide	5–60 mg qd	VLDL, LDL, Lp(a)	Inhibits microsomal triglyceride transfer protein

[a]There are several different preparations of fenofibrate with different doses.

bid, Twice a day; *EPA*, highly concentrated ethyl esters of eicosapentaenoic acid; *DHA*, docosahexaenoic acid; *HMG-CoA*, 3-hydroxy-3-methylglutaryl coenzyme A; *LDL*, low-density lipoproteins; *Lp(a)*, lipoprotein(a); *LPL*, lipoprotein lipase; *PCSK9*, proprotein convertase subtilisin/kexin type 9; q, every; *qd*, every day; *sc*, subcutaneously; *VLDL*, very low density lipoprotein.

(40 mg).[171] Genetic studies[172,173] indicate that that variants in the LDL receptor, PCSK9, and HMG-CoA reductase (the target of statins) are associated with type 2 diabetes mellitus risk, suggesting that promoting uptake of LDL might affect diabetes pathogenesis. Nonetheless, the major adverse cardiovascular event reduction benefits of lowering LDL markedly outweigh the risks of increased glucose in all but those at lowest atherosclerotic risk. Attention to diet, exercise, and weight can mitigate the diabetes risk.

Patients have many complaints that might or might not be due to statins. Complaints about confusion or memory loss should be appropriately evaluated for causes of these symptoms. There is no evidence that statins cause direct adverse effects on renal function beyond that due to rhabdomyolysis. Statins are contraindicated during pregnancy and nursing and in patients with significant hepatic dysfunction.

Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors

This newer class of medications is fully humanized monoclonal antibodies to PCSK9 that lower LDL levels by preventing degradation of the LDL receptor, increasing recirculation of the receptor to the surface of hepatocytes, and consequent

TABLE 41.11 High-, Moderate-, and Low-Intensity Statin Therapy

High Intensity	Moderate Intensity	Low Intensity
Daily dose lowers LDL on average by approximately ≥50%*: Rosuvastatin 20–40 mg Atorvastatin 40–80 mg	Daily dose lowers LDL on average, by approximately 30% to <50%*: Rosuvastatin 5–10 mg Atorvastatin 10–20 mg Simvastatin 20–40 mg Lovastatin 40 mg Pravastatin 40–80 mg Fluvastatin 40 mg bid Pitavastatin 2–4 mg	Daily dose lowers LDL, on average, by <30%[a]: Simvastatin 10 mg Lovastatin 20 mg Pravastatin 10–20 mg Fluvastatin 20–40 mg Pitavastatin 1 mg

[a]Note that individual responses vary.

bid, Twice a day; *LDL,* low-density lipoprotein cholesterol.

Data from Robinson JG, Lichtenstein AH, et al. ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol.* 2014;63:2889–2934.

TABLE 41.12 Typical LDL Reductions (% Change From Baseline) by Statin Dose

Treatment	5 mg	10 mg	20 mg	40 mg	80 mg
Rosuvastatin	–40	–46	–52	–55	—
Atorvastatin	—	–37	–43	–48	–51
Simvastatin	–26	–30	–38	–41	–47
Lovastatin	—	–21	–27	–31	–40
Pravastatin	—	–20	–24	–30	–36
Fluvastatin	—	—	–22	–25	–35
Pitavastatin	—	—	(1 mg) –32	(2 mg) –36	(4 mg) –43
Ezetimibe 10 mg plus variable simvastatin	—	–45	–52	–55	–60

LDL, Low-density lipoprotein cholesterol; —, data not available.

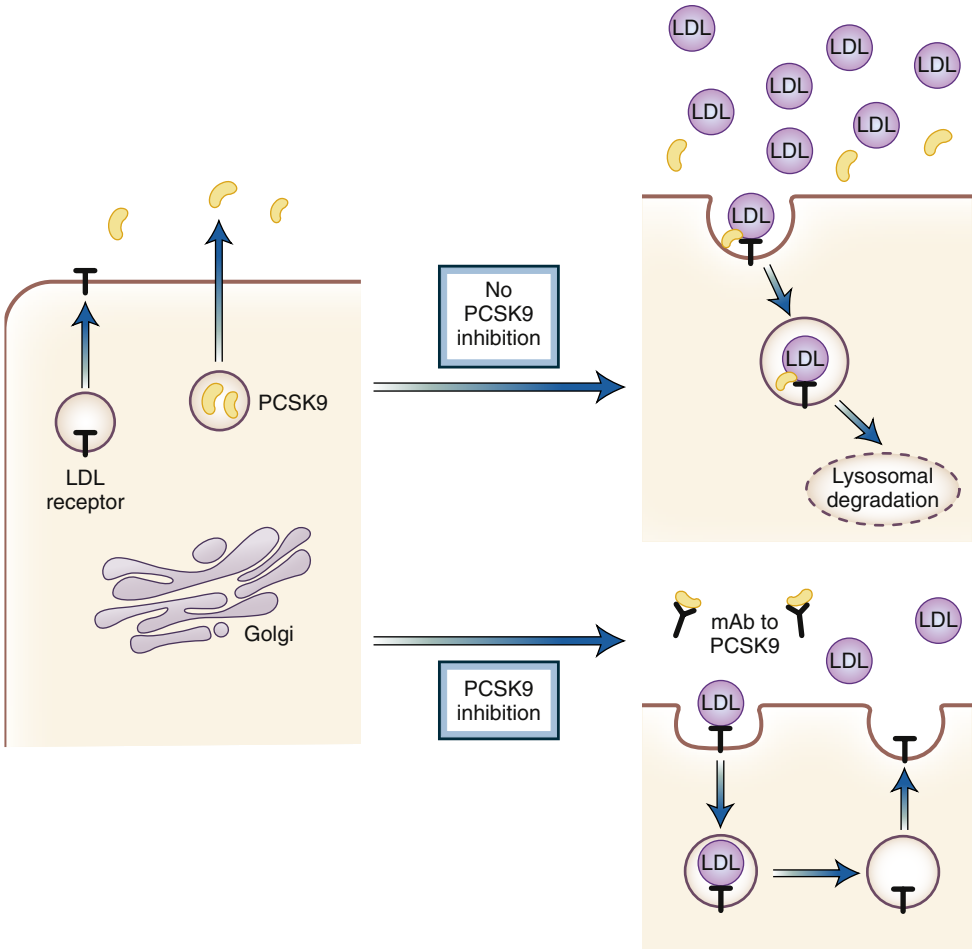
Data from Hou R, Goldberg AC. Lowering low-density lipoprotein cholesterol: statins, ezetimibe, bile acid sequestrants, and combinations—comparative efficacy and safety. *Endocrinol Metab Clin North Am.* 2009;38:79–97; Livalo package insert. Available from http://www.kowapharma.com/documents/LIVALO_PI_CURRENT.pdf.

TABLE 41.13 Features of Individual Statins

Drug	Pharmacologic Considerations	Safety Issues
Rosuvastatin	Synthetic compound; active metabolite is formed by CYP2C9	Can increase INR when used with warfarin—monitor INR; dose reduction in renal insufficiency, Asian patients, and elderly patients
Atorvastatin	Synthetic drug; <2% excreted in urine; half-life 14 hours	Interacts with CYP3A4 substrates; increases digoxin levels
Simvastatin	Synthetic derivative of fermentation product of *Aspergillus terreus*; dose reduction in severe renal insufficiency	Interactions with CYP3A4 substrates
Lovastatin	First statin marketed in United States; isolated from a strain of *A. terreus*; food intake increases absorption	Interactions with CYP3A4 substrates
Pravastatin	Derived from fermentation product of *A. terreus*; dose reduction in renal insufficiency	Drug interaction with cyclosporine
Fluvastatin	Synthetic drug; minimal renal excretion	Can interact with warfarin, phenytoin, glyburide, diclofenac, fluconazole, ketoconazole
Pitavastatin	Dose reduction in severe renal failure	Drug interaction with cyclosporine, erythromycin, rifampin

CYP2C9 and *CYP3A4,* Cytochrome P450 isoenzymes 2C9 and 3A4; *INR,* international normalized ratio.

Data from Hou R, Goldberg AC. Lowering low-density lipoprotein cholesterol: statins, ezetimibe, bile acid sequestrants, and combinations—comparative efficacy and safety. *Endocrinol Metab Clin North Am.* 2009;38:79–97; Livalo package insert. Available from http://www.kowapharma.com/documents/LIVALO_PI_CURRENT.pdf.



• **Fig. 41.18** Role of PCSK9 and its inhibition in low-density lipoprotein (LDL) metabolism. Both the LDL receptor and proprotein convertase subtilisin/kexin type 9 (PCSK9) are processed by the Golgi apparatus with PCSK9 being secreted. In the absence of PCSK9 inhibition, this protein interacts with the LDL receptor and promotes its retention in pathways that lead to lysosomal degradation of the LDL receptor protein. In the presence of PCSK9 inhibition, more of the LDL receptor recycles to the cell surface, where it clears LDL particles from the circulation to lower LDL cholesterol and decrease cardiovascular risk.

lowering of circulating LDL (Fig. 41.18). Alone or in conjunction with statins, they increase removal of LDL from the circulation, leading to nearly 60% further reduction in circulating LDL; a reduction in coronary heart disease events[2]; and, as noted earlier, atheroma regression in patients with known atherosclerosis.

Clinical trials with both PCSK9 inhibitors marketed in the United States, alirocumab and evolocumab, demonstrate reductions in atherosclerotic cardiovascular disease events, particularly in patients with recent acute coronary syndrome, multivessel coronary artery disease, or peripheral arterial disease. Reductions in LDL to levels less than 25 mg/dL (0.65 mmol/L), which occurred in more than a quarter of the patients in the clinical trials, are associated with even lower cardiovascular disease event rates, establishing no lower limit for LDL when using PCSK9 alone or in combination with a statin. PCSK9 inhibitors are recommended for high-risk patients with LDL levels of 70 mg/dL (1.8 mmol/L) or higher on maximally tolerated oral therapies including statins and/or ezetimibe. PCSK9 inhibitors are useful in homozygous FH, where carriers have a high prevalence of premature death and which can otherwise be challenging to treat. Unlike statins, PCSK9 inhibitors decrease Lp(a), although modestly. These drugs are injected every 2 to 4 weeks. As with many new biologics, insurance restrictions and expense limit clinical availability. Aside from injection site reactions, side effects of this therapy thus far appear minimal.

Ezetimibe

Ezetimibe inhibits cholesterol absorption by binding to NPCL1, the intestinal cholesterol absorption transporter. Ezetimibe lowers LDL by 14% to 25% when used alone or in combination with statins. Ezetimibe has been shown to further reduce cardiovascular events when added to statin therapy; in this study, LDL was reduced from nearly 70 to 54 mg/dL (1.8–1.4 mmol/L).[155] Ezetimibe can be useful in patients who are intolerant of statins. Ezetimibe undergoes glucuronidation, leading to extensive enterohepatic circulation. Absorption is not affected by food.

Side effects are relatively uncommon but may include diarrhea and abnormal liver functions. Myopathy is rare and, like myalgias, is not clearly related to the medication.[174] Ezetimibe can increase

cyclosporine levels. Fibrates can increase the levels of ezetimibe, a finding with unknown clinical significance.[175] Ezetimibe is contraindicated in pregnancy and in severe liver dysfunction.

Bile Acid Sequestrants

The bile acid sequestrants have been used since the 1970s but now are primarily reserved for patients unable to take statins or who require additional cholesterol reduction. Clinical trials conducted prior to widespread use of statins showed that bile acid sequestrants reduce cardiovascular events.[176,177] They work by binding negatively charged bile acids and bile salts in the small intestine to interrupt the enterohepatic circulation of bile acids and increase the conversion of cholesterol into bile. Decreased hepatocyte cholesterol content increases LDL receptors, leading to reduced circulating LDL levels. Cholesterol synthesis also increases, which promotes VLDL secretion; for this reason, these drugs are contraindicated in patients with hypertriglyceridemia.

As monotherapy, sequestrants lower LDL by 5% to 30% in a dose-dependent manner. Cholestyramine, colestipol, and colesevelam are available in the United States. Colesevelam has greater bile acid–binding capacity and affinity than cholestyramine or colestipol and is used at lower doses. LDL reduction is typically 15% at 3.8 g/day (six 625-mg tablets) and 18% at 4.3 g/day (seven 625-mg tablets).[178,179] Bile acid sequestrants lower fasting blood glucose and hemoglobin A_{1c} levels in patients with diabetes mellitus.[180] The mechanism underlying this effect is unclear but might relate to activation of a bile sensitivity receptor (TGR5) in the colon.

Gastrointestinal disturbances are common and include constipation, nausea, bloating, abdominal pain, flatulence, and aggravation of hemorrhoids. Initiation with low doses, patient education, and use of stool softeners or psyllium can increase compliance. These drugs are not absorbed, so they can be used in pregnancy. Bile acid sequestrants affect absorption of a wide variety of drugs; other medications should be taken either 1 to 2 hours before or 4 to 6 hours after the sequestrant.

Fibrates

In the Helsinki Heart Study (primary prevention) and the Veterans Affairs–High-Density Lipoprotein Cholesterol Intervention Trial (secondary prevention), the use of the fibrate gemfibrozil reduced fatal and nonfatal cardiovascular events and did not increase mortality from noncardiac causes.[139,140] In the Fenofibrate Intervention and Event Lowering in Diabetes study, although fenofibrate did not reduce the composite cardiovascular outcome of cardiovascular death, nonfatal myocardial infarction, and stroke in patients with type 2 diabetes mellitus, fewer nonfatal events were observed.[141] The addition of fenofibrate to a statin may provide benefit to diabetes patients with high triglycerides and low HDL.[142,143] Fenofibrate decreased the progression of retinopathy in patients with diabetes in the Fenofibrate Intervention and Event Lowering in Diabetes and ACCORD trials and is approved by the Australian Health Authorities for diabetic retinopathy.[181,182] The underlying mechanism is unknown and does not appear to be related to effects on circulating lipids.

Fibrates activate PPARα (see earlier discussion), which increases fatty acid oxidation, increases LPL, and increases apoAI, as well as apoAII, while lowering triglycerides (by 30–50%), decreasing apoCIII, and raising HDL (by 10–20% in those with elevated triglycerides). Fibrates may lower LDL modestly, but they are most commonly used for severe hypertriglyceridemia and combined hyperlipidemia. Fenofibrate may be taken once daily. Gemfibrozil is given twice a day with meals but is used less often due to a less favorable drug interaction profile.

Fibrates are contraindicated in patients with liver or gallbladder disease. The risk of gallstones may be increased. Liver transaminases may increase, particularly with fenofibrate. Side effects may include gastrointestinal discomfort, rash, and pruritus. Fenofibrate should be avoided in patients with renal insufficiency, which predisposes to myopathy. The combination of gemfibrozil and most statins is associated with an increased risk of myopathy due to increased statin blood levels, whereas fenofibrate does not interfere with statin metabolism and is preferred in fibrate/statin combination regimens.[183] Because of the effects on protein binding, warfarin doses may need to be adjusted when fibrate therapy is started. Gemfibrozil may be used beginning in the second trimester in pregnant women with severely elevated triglycerides who are at risk for pancreatitis.

Omega-3 Fatty Acids

Fish-derived omega-3 fatty acids (EPA and DHA) reduce circulating triglycerides, but their effects on cardiovascular events are less clear. The Japan EPA Lipid Intervention Study showed that combined treatment with a statin plus EPA (1.8 g/day) in patients with coronary heart disease reduced major coronary events by 19% compared with a statin alone.[184] This effect was similar to that found in the recent REDUCE-IT trial.[164] Other clinical outcome studies with fish oils and meta-analysis of trials with EPA and DPA do not show mortality or cardiovascular health benefits.[162,163,185] Whether the positive outcome of REDUCE-IT was due to its specific omega 3 formulation, subject selection, or dose is unclear.

Omega-3 fatty acids decrease triglyceride secretion from the liver through unclear mechanisms and are indicated for triglycerides higher than 500 mg/dL (5.6 mmol/L). EPA and DHA lower triglycerides by 20% to 50% depending on the baseline levels. There is minimal effect on HDL. LDL may increase as VLDL is converted to LDL.

Approximately 3 to 4 g/day of EPA plus DHA is used to lower triglycerides. Over-the-counter preparations have variable quantities of EPA and DHA. Prescription formulations usually contain twice the content of omega-3 fatty acids than nonprescription preparations. Preparations contain EPA and DHA, unesterified EPA and DHA, or only EPA. Unlike fibrates, EPA and DHA do not affect statin metabolism and do not increase the risk of myopathy. Side effects with omega-3 fatty acids include eructation, diarrhea, and abdominal discomfort. There is potential for increased bleeding, but this has not been seen in clinical trials.

Niacin

Nicotinic acid, or niacin, is a B-complex vitamin that was found to lower plasma cholesterol in humans in 1955. The mechanism underlying the effects of niacin are not understood beyond the physiologic observation of decreased VLDL secretion. Niacin lowers triglycerides by 10% to 30% and increases HDL by 10% to 40%. Niacin also lowers Lp(a) by up to 25%. The niacin arm of the Coronary Drug Project showed a decrease in nonfatal myocardial infarctions in men with coronary artery disease during the 6-year trial and a decrease in total mortality rate in the 9 years after the study.[186] However, more recent trials of slow-release niacin in subjects whose LDL was already markedly reduced by statins, the AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglycerides: Impact on Global Health Outcomes) and HPS2-THRIVE (Heart Protection Study 2–Treatment of HDL to Reduce the Incidence of Vascular Events) studies, failed to show additional clinical benefit with niacin[187,188]

despite improvements in HDL and triglycerides. This lack of proven efficacy and the introduction of new agents (ezetimibe and PCSK9 inhibitors) have reduced niacin use except in refractory hypercholesterolemia or hypertriglyceridemia.

Cutaneous flushing, most notable with the first doses, is the most common side effect. Tolerance can occur with repeated and consistent dosing. Flushing occurs 15 to 60 minutes after ingestion, lasts 15 to 30 minutes, and may be related to release of dermal prostaglandin D_2. Ingestion with food and taking aspirin (preferably 325 mg) 30 to 60 minutes in advance of niacin minimizes flushing,[189] whereas alcoholic beverages and spicy foods potentiate it. Prescription extended-release niacin (Niaspan) may be better tolerated than crystalline niacin. Starting low and gradually increasing the dose improves tolerability.

Niacin has additional adverse effects. Hepatotoxicity is the most serious and is seen more with crystalline niacin compared with prescription slow-release forms. Worsening of glucose tolerance and hyperuricemia may occur. Niacin is contraindicated in active peptic ulcer disease. Rare side effects include blurred vision and a reversible condition known as cystoid macular edema. Myopathy is rare with niacin alone or in combination with statins. Niacin is contraindicated during pregnancy.

Combination Therapies

Combination therapy is indicated for patients with severe lipid elevations and those who have an insufficient response to monotherapy. Patients with FH or familial combined hyperlipidemia are at particularly increased risk and may require LDL reductions that cannot be achieved with a single agent. LDL levels lower than 70 mg/dL (1.8 mmol/L) can be difficult to achieve with a single drug. Higher statin doses may be associated with increased side effects. If the highest tolerated statin dose does not produce adequate LDL reduction, adding an agent from a different class may produce the desired result. Statins, ezetimibe, PCSK9 inhibitors, and sequestrants work through different mechanisms and can be more effective in combination than when used alone. Data on clinical outcomes with combination therapy are limited. Table 41.14 presents combination therapies for LDL.

Combination Therapy for Other Hyperlipidemias

Statin Plus Fibrate

The combination of a statin plus a fibrate can be used in patients requiring treatment for elevated triglycerides and elevated LDL, which may be useful for patients with metabolic syndrome, diabetes, or other forms of mixed dyslipidemia. Risk of myopathy, including rhabdomyolysis, is increased with the combination of most statins with gemfibrozil, because the latter drug interferes with the glucuronidation of statins, leading to higher serum levels of the statin drug.[190] Rhabdomyolysis is about 15 times less likely with fenofibrate combined with statins (0.58 per 1 million prescriptions) than with gemfibrozil plus statins (8.6 per 1 million prescriptions).[183]

Statin-fibrate combinations should be avoided in patients with renal insufficiency, congestive heart failure, severe debility, or other conditions that affect medication clearance. Side effects include mild gastrointestinal discomfort, rash, and pruritus.

Other Combinations

Triple-drug therapy with a statin, ezetimibe, and fenofibrate may help to obtain adequate reductions of both triglycerides and LDL without using very high statin doses or when PCSK9 inhibitors are not covered by patient insurance. When triglycerides are decreased with a fibrate, LDL may increase. If statins are not tolerated and triglycerides well controlled, a bile acid sequestrant could be added. Ezetimibe may lower LDL in combination with a fibrate when triglyceride levels are not optimal. Adding omega-3 fatty acids can be helpful if triglycerides are not well controlled. For markedly elevated triglyceride levels, it may be necessary to combine a fibrate with niacin or with omega-3 fatty acids, or both.

TABLE 41.14 Combination Therapies for LDL

Statin plus ezetimibe	• Ezetimibe added to a statin may further reduce LDL by 20% or more and reduce triglycerides by 7–13%. • The combination provides equivalent LDL reduction to a fourfold increase in statin dose. • Daily ezetimibe added to a low-dose statin given 2–3 times per week can improve tolerance. Combination pills containing statin and ezetimibe are available. • Most common side effects reflect those of the individual drugs. • Combination ezetimibe and simvastatin has been shown to decrease cardiovascular events in patients with renal disease[96] and acute coronary syndrome.[155]
Statin and PCSK9 inhibitors	• This combination is the most effective known treatment for hypercholesterolemia. • There are no known negative interactions between these two therapies. • This combination reduces CHD events more than statin alone.
Statin plus bile acid sequestrants	• Bile acid sequestrants in combination with statins further decrease LDL from 24% to 60%. • Cholestyramine and colestipol can interfere with the absorption of statins. Colesevelam does not affect statin absorption. • The statin-colesevelam combination is not ideal for patients with high triglycerides but may be useful in type 2 diabetes mellitus because colesevelam reduces glycemia.
Statin plus niacin	• Adding niacin to a statin can lower LDL by 10% to 20% in addition to beneficial effects on triglycerides. • When used in combination with a statin, the maximum dose of niacin should be 2000 mg/day. • This combination in subjects with already low LDL levels did not reduce CHD events.
Bile acid sequestrants plus niacin	• Before the availability of statins, bile acid sequestrants plus niacin were used to lower LDL in high-risk patients. • The availability of colesevelam and extended-release niacin has made this combination tolerable for many patients who are unable to use statins.
Ezetimibe plus bile acid sequestrants	• Ezetimibe inhibits cholesterol absorption, and sequestrants enhance cholesterol excretion through conversion to bile acids. The combination can have additive effects. • This combination is useful for patients who cannot take statins.

CHD, Coronary heart disease; *LDL,* low-density lipoprotein; *PCSK9,* proprotein convertase subtilisin/kexin type 9.

Treatment for Chylomicronemia Syndrome

Patients with chylomicronemia syndrome should be treated with total fat restriction and other measures mentioned previously until the triglycerides fall to less than 1000 mg/dL (11.3 mmol/L), after which a fat-restricted diet (e.g., <10% of calories) can be instituted. The goal is to maintain triglycerides under 1000 mg/dL (11.3 mmol/L) and preferably lower if possible. Diet and modification of glycemia, alcohol consumption, or offending medications are useful. Medium-chain triglyceride oils can be provided for cooking. A fibrate or niacin is usually required to control triglycerides. Therapy with orlistat to block fat absorption may be beneficial, because it mimics a low-fat diet.[191] In the setting of pancreatitis risk, a low-fat/low-carbohydrate liquid formula diet may be indicated.

Several novel therapies for hyperchylomicronemia and recurrent pancreatitis are on the horizon. A gene therapy treatment for LPL deficiency had been available in Europe using an adeno-associated virus expressing LPL^{S447X}, an active truncated LPL. Effects were transient following multiple intramuscular injections. Additional therapies using methods to inhibit production of apoC-III or ANGPLT3 are in development.

Treatment of FH

Guidelines for FH patients are available.[192] Treatment of heterozygous FH includes a diet low in total and saturated fats and cholesterol, but effects on cholesterol are modest (5–15%). Adequate cholesterol lowering can occasionally be achieved with a single potent statin, but combinations of drugs are often needed. Many patients will achieve LDL levels below 100 mg/dL (2.59 mmol/L) with a high-intensity statin and ezetimibe. Addition of a PCSK9 inhibitor to a statin or statin plus ezetimibe can lead to even greater LDL reduction.[193] PCSK9 inhibitors have some effect in patients with mutated, but not absent, receptors.[194] LDL apheresis or liver transplantation are other options in these patients.[195]

The age at which drug treatment should begin in heterozygous FH is controversial, but starting treatment at younger ages may have a favorable impact during early stages of lesion development. Statins are approved for the treatment of children with heterozygous FH who are 8 years of age (pravastatin) or 10 years (other agents) or older. Factors such as the age at onset of coronary disease in parents and grandparents and the presence of other risk factors should be considered.

Several medical approaches for treatment of homozygous FH have been developed. Mipomersen is an apoB antisense oligonucleotide that was approved but rarely used in the United States for the treatment of homozygous FH. It prevents apoB translation, which decreases apoB levels and LDL.[196] It lowers LDL in patients with homozygous and heterozygous FH.[197,198] Pyrexia, body aches, and reactions at the injection site are the most common side effects, but potential hepatotoxicity and adverse cardiovascular effects are the most serious. Lomitapide is an inhibitor of MTP and approved in the United States and European Union for the treatment of homozygous FH. MTP (see earlier discussion) is required for VLDL assembly. Its inhibition may decrease LDL by 50% in homozygous FH.[199] Side effects include diarrhea, elevated hepatic transaminases, and increased hepatic fat. In a long-term study in homozygous FH, lomitapide decreased LDL by 50% during the initial phase of the study and by 31% at 78 weeks of treatment.[200]

Treatment of familial defective apoB100 is similar to that of heterozygous FH and consists of a low-fat, low-cholesterol diet and a combination drug regimen. Family members at risk should also be screened for the dominant mutation.

Treatment of Familial Combined Hyperlipidemia

Weight reduction and dietary treatment can help correct metabolic abnormalities such as obesity and insulin resistance that contribute to the hyperlipidemia. Drug therapy should be directed at the predominant lipid abnormality. Statins are most appropriate for most patients. Fibrates can lower triglycerides and raise HDL, and they reduce the incidence of coronary events in insulin-resistant hypertriglyceridemic patients with low HDL. Patients with low HDL should be treated with statins. Because familial combined hyperlipidemia is associated with premature coronary heart disease, affected family members should be identified.

Treatment of Metabolic Syndrome

Metabolic syndrome is an extremely common condition defined by the presence of at least three of the five features presented in Table 41.5: increased abdominal girth, hypertriglyceridemia, low HDL, hypertension, and elevated fasting glucose. Metabolic syndrome is clearly associated with increased risk of vascular disease and development of type 2 diabetes mellitus, and it is equivalent to what was previously referred to as prediabetes. Because obesity is a risk for coronary disease, as well as diabetes mellitus and dyslipidemia, a body mass index less than 25 kg/m^2 in patients of European ancestry (lower for those from southern and eastern Asia) is a goal that may require combinations of dietary, exercise, pharmacologic, and surgical therapies (see Chapter 40). Because this goal is often not achievable, these patients may also need therapies directed at hypertension, smoking, diabetes, and dyslipidemia. All patients should be assessed according to existing guidelines for treatment of high triglycerides, low HDL, hypertension, and hyperglycemia.

Treatment of Dysbetalipoproteinemia

Because dysbetalipoproteinemia is influenced by coexisting metabolic conditions, a vigorous effort should be made to identify and treat obesity, diabetes mellitus, and hypothyroidism, and to reduce alcohol consumption. Lipid abnormalities can often be resolved without the use of drug therapies. Dysbetalipoproteinemia is associated with hypothyroidism in particular and responds dramatically to thyroid hormone replacement therapy. Dietary therapy should be aimed at restricting total fat; saturated fat; cholesterol; and, if appropriate, calories. If diet and treatment of coexisting metabolic conditions are unsatisfactory, drug therapy should be initiated using statins. Combination therapy may be required. Because the disorder is associated with premature vascular disease, first-degree relatives should be screened for the presence of apoE2 (see earlier discussion).

Treatment for Elevated Plasma Lp(a)

There are no outcome trials of lowering Lp(a). Newer antisense and silencing RNA approaches are being studied in clinical trials.[201] Apheresis lowers Lp(a) and has been used in those with elevated Lp(a) and progressive coronary heart disease.[202] Evolocumab, alirocumab, niacin, mipomersen, and lomitapide each lower Lp(a), although modestly, in addition to lowering LDL.

Treatment for Low Levels of HDL

Patients with familial hypoalphalipoproteinemia can have normal or modestly increased plasma cholesterol but very low HDL, resulting in a predisposition to coronary heart disease. Such patients can have high ratios of total cholesterol to HDL (e.g., >10) despite having normal plasma cholesterol levels. Statins lower total cholesterol and represent the most efficacious way to

lower the ratio of total cholesterol to HDL. Statins decrease clinical events in patients with low HDL.[203] Fibrates do not increase HDL in patients with normal triglycerides. As noted earlier, pharmacologic therapies for raising HDL levels do not provide clinical benefits.

Summary of Treatment of Lipid Disorders

In the absence of clear demonstration of serious adverse effects, statins should be considered for most common lipid disorders. Inappropriate false claims about harms associated with statins likely contribute to discontinuation and underuse of these agents. Abundant evidence indicates that any future findings about the effects of statins will not fundamentally alter the favorable balance of benefits over risks for this class of drugs.[204]

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The complete list of references is available online at ExpertConsult.com.

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42

Multiple Endocrine Neoplasia

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CHAPTER OUTLINE

KEY POINTS

- Multiple endocrine neoplasia (MEN) syndromes, which may be inherited as autosomal dominant traits, are characterized by the occurrence of two or more tumors in a patient, and four major types (MEN1–4) are recognized.
- MEN1 is characterized by occurrence of parathyroid, anterior pituitary, and duodenopancreatic neuroendocrine tumors, and occasionally also foregut carcinoid and adrenal tumors. MEN1 is caused by mutations of the *MEN1* gene, which encodes the tumor suppressor protein menin.
- MEN2 (also referred to as MEN2A) is characterized by occurrence of medullary thyroid carcinoma (MTC), pheochromocytomas, and parathyroid tumors. MEN2 includes the variants MEN2A with Hirschsprung disease, MEN2A with cutaneous lichen amyloidosis, and familial MTC-only.
- MEN3 (also referred to as MEN2B) is characterized by occurrence of MTC and pheochromocytomas in association with a marfanoid habitus, mucosal neuromas, medullated corneal fibers, and intestinal ganglioneuromatosis.
- MEN2 and MEN3 are due to *rearranged during transfection (RET)* proto-oncogene mutations that lead to constitutive activation of the encoded receptor tyrosine kinase.
- MEN4 patients may develop parathyroid, anterior pituitary, and pancreatic neuroendocrine tumors with gonadal, adrenal, renal, and thyroid tumors due to mutations of the cyclin-dependent kinase inhibitor p27Kip1 (*CDKN1B*) gene.
- Genetic testing should be offered to MEN patients and their first-degree relatives, and individuals with a mutation, who are at risk of developing tumors, should have periodic clinical, biochemical, and radiologic screening for the early detection and treatment of tumors.
- Treatment of MEN patients, which aims to minimize the disease-associated morbidity and mortality, while maintaining quality of life, requires a multidisciplinary approach.

Introduction to Multiple Endocrine Neoplasia Syndromes

Multiple endocrine neoplasia (MEN) is characterized by the occurrence of tumors involving two or more endocrine glands within a single patient.[1–3] The disorder has previously been referred to as multiple endocrine adenopathy or pluriglandular syndrome. However, glandular hyperplasia and malignancy may also occur in some patients, and the term *multiple endocrine neoplasia* is now preferred. Four major forms of MEN—MEN types 1 through 4—are recognized, and each form is characterized by the development of tumors within specific endocrine glands[1,4] (Table 42.1). All of these forms of MEN may be inherited as autosomal dominant disorders or may occur sporadically in the absence of a family history.[1,3] However, this distinction between sporadic and familial cases may sometimes be difficult, because in some sporadic cases, a familial history may be absent because the patient with the disease may have died before symptoms developed. In addition to MEN types 1 through 4, six other syndromes, which are associated with tumors involving one or more of the endocrine glands as well as nonendocrine organs, have been reported.[5–10] These include the hyperparathyroidism-jaw tumor syndrome, von Hippel–Lindau disease, Carney complex, neurofibromatosis type 1, Cowden syndrome, and McCune-Albright syndrome; all of these may be inherited as autosomal dominant disorders, except McCune-Albright syndrome, which is due to a mosaic expression of a postzygotic somatic cell mutation. This chapter will focus on describing the major clinical and molecular aspects of MEN type 1 through 4 syndromes.

TABLE 42.1 MEN Syndromes and Their Characteristic Tumors and Associated Genetic Abnormalities

Type (Chromosome Location)	Tumors (Estimated Penetrance)	Gene; Most Frequently Mutated Codons
MEN1 (11q13)	Parathyroid adenoma (90%) Enteropancreatic tumor (30–70%) — Gastrinoma (40%) — Insulinoma (10%) — Nonfunctioning (20–55%) — Glucagonoma (<1%) — VIPoma (<1%) Pituitary adenoma (30–40%) — Prolactinoma (20%) — Somatotropinoma (10%) — Corticotropinoma (<5%) — Nonfunctioning (<5%) Associated tumors — Adrenal cortical tumor (20–40%) — Pheochromocytoma (<1%) — Bronchopulmonary NET (2%) — Thymic NET (2%) — Gastric NET (10%) — Lipomas (30%) — Angiofibromas (85%) — Collagenomas (70%) — Meningiomas (8%)	*MEN1* 83/84, 4-bp del (≈4%) 119, 3-bp del (≈3%) 209-211, 4-bp del (≈8%) 418, 3-bp del (≈4%) 514-516, del or ins (≈7%) Intron 4 ss (≈10%)
MEN2[b], also known as MEN2A (10 cen-10q11.2)	MTC (90%) Pheochromocytoma (50%) Parathyroid adenoma (20–30%)	*RET* 634, missense (e.g., Cys→Arg)
MEN3, also known as MEN2B (10 cen-10q11.2)	MTC (>90%) Pheochromocytoma (40–50%) Associated abnormalities (40–50%) Mucosal neuromas Marfanoid habitus Medullated corneal nerve fibers Megacolon	*RET* 918, Met→Thr
MEN4 (12p13)	Parathyroid adenoma[a] Pituitary adenoma[a] Reproduction organ tumors[a] (e.g., testicular cancer, neuroendocrine cervical carcinoma) ?Adrenal + renal tumors[a]	*CDKN1B*, no common mutations identified

[a]Insufficient numbers reported to provide prevalence information.

[b]MEN2 comprises variants that include familial MTC-only, MEN2A with cutaneous lichen amyloidosis, and MEN2A with Hirschsprung disease.

Autosomal-dominant inheritance of MEN1 syndrome has been established.

del, Deletion; *ins,* insertion; *MEN,* multiple endocrine neoplasia; *MEN1,* MEN type 1; *MEN2,* MEN type 2; *MEN3,* MEN type 3; *MEN4,* MEN type 4; *NET,* neuroendocrine tumor; *PPoma,* pancreatic polypeptide–secreting tumor; *VIPoma,* vasoactive intestinal polypeptide–secreting tumor; *MTC,* medullary thyroid cancer.

Modified from Thakker RV, Newey PJ, Walls GV, et al. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metab.* 2012;97(9):2990–3011.

MEN Type 1

MEN1, which has been referred to as Wermer syndrome, is an autosomal dominant disorder with an estimated prevalence of 1 in 30,000 of the population. MEN1 is characterized by the combined occurrence of parathyroid, pituitary, and duodenopancreatic neuroendocrine tumors (NETs). In addition, patients may also develop other endocrine tumors (e.g., adrenal cortical tumors, carcinoids of the thymus and bronchus) and nonendocrine tumors (e.g., meningiomas, facial angiofibromas, collagenomas, and cutaneous lipomas) (see Table 42.1). The first description of MEN1 was reported by Erdheim[11] in 1903, at autopsy in a patient with an anterior pituitary tumor and enlarged parathyroid glands. In the 1920s, the occurrence of pancreatic islet cell tumors in association with parathyroid and pituitary tumors was reported,[12,13] and throughout 1930 and 1960, the triad of parathyroid, pancreatic islet cell, and anterior pituitary tumors became recognized as characteristic of MEN1, together with the familial basis and autosomal dominant inheritance of the syndrome.[14–16] Studies throughout the 1980s and 1990s of MEN1 families and

MEN tumors led to the identification of the *MEN1* gene, which is located on chromosome 11q13.[17,18] Since then, the implementation of germline *MEN1* genetic testing of affected individuals (and their relatives) has transformed the diagnosis and management of the disorder. In addition, somatic *MEN1* mutations have been identified as major drivers of sporadic parathyroid and pancreatic NETS, and this has widened the biologic and clinical significance of the *MEN1* gene and its encoded protein, menin, which consists of 610 amino acids and is a nuclear protein that acts as a tumor suppressor by interacting with other proteins in transcription regulation, genome stability, cell division, proliferation, and epigenetic regulation.

Clinical Features and Management

The clinical manifestations of MEN1 are related to the sites of tumor development and/or consequences of hormone hypersecretion. MEN1 is highly penetrant; virtually all patients develop clinical or biochemical evidence of tumor development by age 50 years. MEN1 tumors are unusual in early childhood (i.e., ≤5 years of age) but thereafter demonstrate an increasing age-related penetrance—approximately 75% of patients will have one or more tumors by age 20 years.[19] Parathyroid tumors are typically the first manifestation of disease in 75% to 90% of MEN1 patients (see Table 42.1), although childhood presentations with pancreatic NETs (e.g., insulinoma) or pituitary tumors are not infrequent, whereas some patients may present with gastrinoma, thymic carcinoid, or adrenal tumors. Overall, clinically relevant duodenopancreatic NETs, including both hormone-secreting and nonsecreting tumors (Fig. 42.1), occur in 40% to 70% of patients, whereas anterior pituitary tumors occur in 30% to 40% of patients. The frequency of other endocrine tumors is variable: 20% to 55% of MEN1 patients have adrenal tumors, whereas fewer than 10% manifest thymic or bronchial tumors. The recognition and appropriate management of the MEN1-associated tumors is important because they are associated with high morbidity and mortality—approximately 30% to 70% of MEN1 patients will die of causes directly related to MEN1, with malignant duodenopancreatic NETs and thymic carcinoid tumors accounting for the greatest risk of premature death.[20]

A diagnosis of MEN1 may be established in an individual by one of three criteria[1,21,22]: on the basis of the occurrence of two or more primary MEN1-associated endocrine tumors (i.e., parathyroid adenoma, enteropancreatic tumor, and pituitary adenoma); the occurrence of one of the MEN1-associated tumors in a first-degree relative of a patient with a clinical diagnosis of MEN; and identification of a germline *MEN1* mutation in an individual, who may be asymptomatic and has not yet developed serum biochemical or radiologic abnormalities indicative of tumor development.

The management of each of the respective MEN1-associated tumors is broadly similar to that of their sporadic counterparts, although there are several MEN1-specific factors that require consideration. Most importantly, MEN1-associated tumors are frequently multiple, resulting in a reduced likelihood of surgical cure. For example, MEN1 patients often develop multiple small submucosal duodenal gastrinomas, and achieving biochemical remission is difficult without extensive surgical resections. In this setting, disease control with proton pump inhibitor (PPI) therapy may be considered a suitable alternative and has been associated with improved long-term outcomes. Similarly, the occurrence of synchronous pancreatic NETs may make planning of therapeutic interventions challenging (e.g., localizing functioning tumors for resection), and it is important to consider that any remnant pancreatic tissue post tumor resection will remain at risk of further tumor development. Thus, the goal of treatment in MEN1 should be to balance the potential risks and benefits of any intervention with the ultimate aim of minimizing disease-associated morbidity and mortality while preserving the patient's quality of life. In this regard, it is important that MEN1 is managed by a multidisciplinary team, and that patients play an active role in the decision-making process.

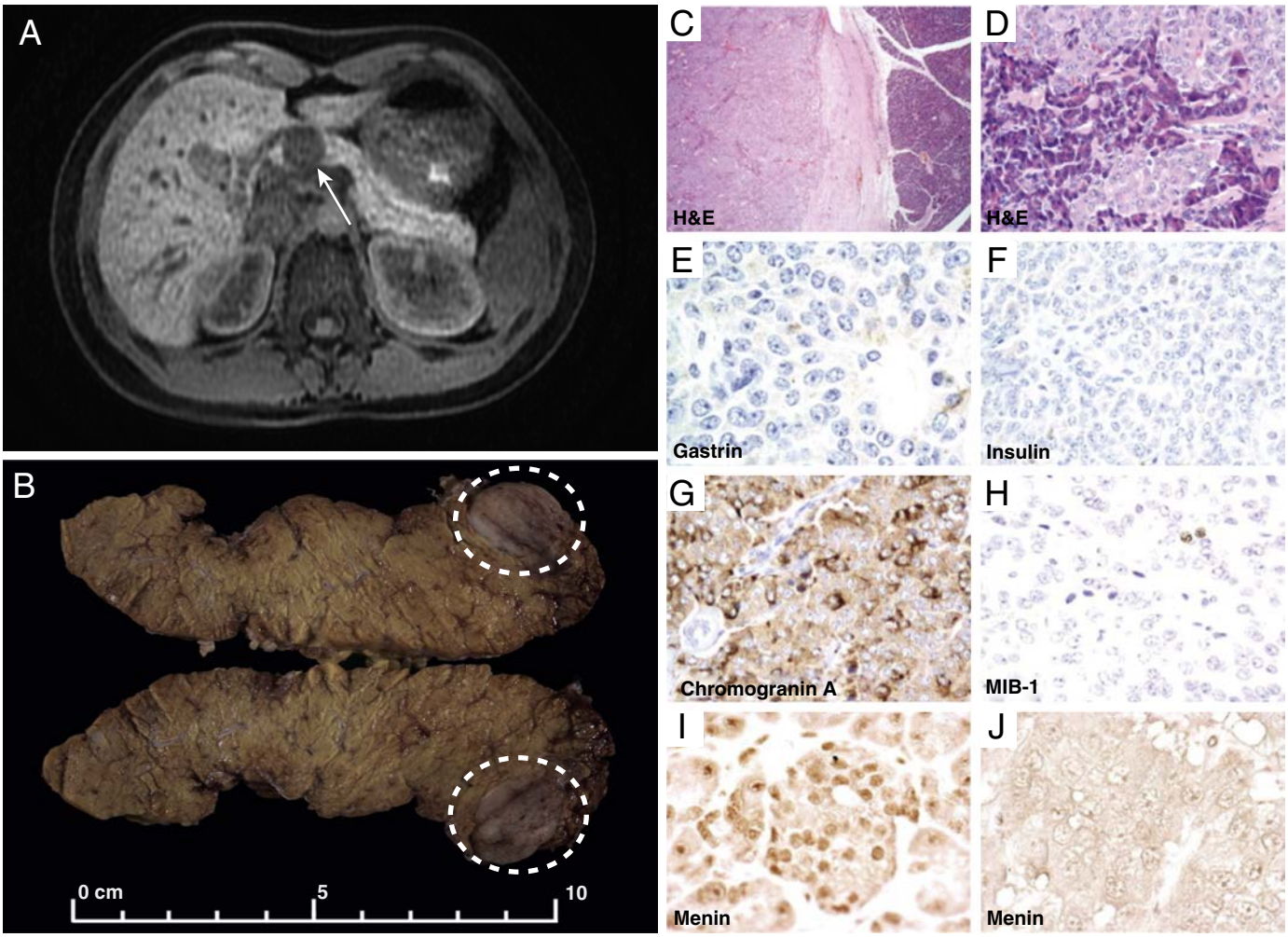
Parathyroid Tumors

Clinical Features

Primary hyperparathyroidism (PHPT) is the most common feature of MEN1. PHPT occurs in approximately 95% of all patients[1,23–25] and is the first manifestation of MEN1 in 75% to 90% of patients.[19,23] Patients are frequently asymptomatic with only biochemical evidence of disease, although symptomatic presentations due to hypercalcemia (i.e., polyuria, polydipsia, constipation, malaise) or other manifestations, including nephrolithiasis, osteitis fibrosa cystica, or peptic ulceration, may occur.[1,26] The diagnosis of PHPT is made by the demonstration of hypercalcemia in the presence of raised or inappropriately normal circulating parathyroid hormone (PTH) concentrations. The degree of hypercalcemia is usually mild, and severe hypercalcemia or parathyroid carcinoma is rare.[1] PHPT in MEN1 patients usually occurs in those older than 15 years, although the earliest symptomatic and asymptomatic presentations are reported in children of 8 and 4 years of age, respectively.[19] Biochemical evidence of PHPT has been reported in up to 75% of children and young adults with MEN1 (<21 years of age), although only a minority of these patients will have clinical features (e.g., nephrolithiasis).[19] In addition to the early age of onset, MEN1-associated PHPT has other important differences when compared with non-MEN1 PHPT, and these include an equal sex distribution (male to female, 1:1 vs. 1:3, respectively) and the synchronous or asynchronous involvement of all four parathyroid glands with tumors,[1,19,25,27] resulting from the monoclonal expansion of one or more population of cells within individuals glands due to biallelic inactivation of the *MEN1* gene.[24,28,29] MEN1-associated PHPT is reported to be associated with a greater reduction in bone mineral density than that occurring in non-MEN1 PHPT, and therefore osteoporosis and osteopenia are common in MEN1 patients.[30,31] The reduction in bone mineral density and bone demineralization in MEN1 patients is particularly evident at the lumbar spine, femoral neck, and distal radius when compared with those with equivalent sporadic PHPT.[31,32] These observations may be due to earlier age of onset and chronicity of MEN1-associated PHPT or differences in disease pathogenesis.

Treatment

Surgical removal of the overactive parathyroid glands is the treatment of choice for MEN1-associated PHPT. However, several aspects of management remain controversial, including the indications for and timing of surgery and the extent of surgery.[1] These uncertainties reflect a paucity of high-quality evidence to guide clinical recommendations.[1] Currently, surgery is recommended for MEN1-associated PHPT in those with symptomatic disease,



• **Fig. 42.1** Nonfunctioning pancreatic NET in a 14-year-old MEN1 patient. The abdominal magnetic resonance imaging scan demonstrates a low-intensity, larger than 2.0 cm (anteroposterior maximal diameter) tumor within the neck of pancreas (indicated by the *arrow*) (A). There was no evidence of invasion of adjacent structures or metastases. The pancreatic NET was removed by surgery, and macroscopic examination confirmed the location of the tumor in the neck of the pancreas (*white interrupted circles*) (B). H&E examination demonstrated a tumor that was largely well circumscribed (C), but focally the margin between tumor (paler cells) and normal pancreas was poorly defined (D). Immunostaining supported the clinical and biochemical diagnosis of a nonfunctioning pancreatic NET because the tumor did not have significant expression of gastrointestinal peptides (result for gastrin and insulin shown [E and F]) but did contain chromogranin A (G). The proliferative index measured by MIB-1 (Ki-67) was low, consistent with a low-grade tumor (H). Loss of menin expression was demonstrated in the tumor; in the adjacent nontumorous pancreatic tissue, nuclear menin expression is evident within pancreatic islets (I), whereas nuclear menin expression is lost within the tumor (J), consistent with biallelic inactivation of the *MEN1* gene. *H&E*, hematoxylin and eosin; *MEN1*, multiple endocrine neoplasia type 1; *NET*, neuroendocrine tumor. (A and C to J, modified from Newey PJ, Jeyabalan J, Walls GV, et al. Asymptomatic children with multiple endocrine neoplasia type 1 mutations may harbor nonfunctioning pancreatic neuroendocrine tumors. *J Clin Endocrinol Metab.* 2009;94[10]:3640–3646.)

severe hypercalcemia (i.e., >3.00 mmol/L), and/or evidence of end-organ damage (e.g., nephrolithiasis, hypercalciuria [>9 mmol/L per 24 hours or 400 mg per 24 hours], creatinine clearance <60 mL/minute, reduced bone mineral density [i.e., T-score <–2.5] and/or previous fragility fracture).[1] Most centers will recommend subtotal parathyroidectomy (removal of 3–3.5 glands) or total parathyroidectomy with or without autotransplantation of cryopreserved parathyroid tissue.[1,33–40] Concurrent transcervical thymectomy is also suggested at the time of neck surgery to remove parathyroid tumors that may be embedded in the thymus.[1] Minimally invasive selective parathyroidectomy, unilateral clearance, and less than subtotal parathyroidectomy (i.e., removal of <3–3.5 glands) are not recommended because all four parathyroid glands are usually affected with multiple adenomas or hyperplasia, although this histologic distinction may be difficult. The aims of parathyroid surgery in MEN1 are to maintain normocalcemia for as long as possible and to avoid iatrogenic complications of surgery, including laryngeal nerve damage and permanent

hypoparathyroidism. The lowest risk of persistent or recurrent PHPT occurs with subtotal and total parathyroidectomy, with higher rates of recurrence occurring in those with less than subtotal parathyroidectomy.[36–39] Long-term outcome data for unilateral or minimally invasive parathyroidectomy has not been reported, although recurrent disease is inevitable in this setting.[41,42] Total parathyroidectomy is reported to be associated with the highest risk of permanent hypoparathyroidism, which may occur in 13% to 67% of cases.[37,39] The development of permanent hypoparathyroidism requires lifelong therapy with active vitamin D metabolites (i.e., calcitriol or alfacalcidol), and this may be associated with significant morbidity (e.g., due to development of inadvertent significant hypo- or hypercalcemia). Total parathyroidectomy with autotransplantation of either fresh or cryopreserved parathyroid tissue into the forearm has therefore been considered as an alternate approach.[1,35,43] The use of cryopreserved tissue allows confirmation of hypoparathyroidism postoperatively but is associated with a higher graft failure rate due to reduced cell viability, as well as a higher rate of permanent hypoparathyroidism. Moreover, recurrent disease is frequently observed in the transplanted tissue,[36] which may necessitate surgical removal,[37] and the reported finding in a MEN1 patient of a metastatic thymic carcinoma within the parathyroid autotransplanted tissue highlights the need for caution with this surgical approach.[44] Thus, the majority of centers recommend either subtotal parathyroidectomy (of at least 3.5 glands) or total parathyroidectomy with long-term oral calcitriol or alfacalcidol treatment. It is recommended that the timing and extent of surgical intervention should be taken by a multidisciplinary team, which takes into account the local surgical expertise, the availability of vitamin D analogues for subsequent treatment of long-term hypoparathyroidism, and the preferences of the patient. Cinacalcet, a calcimimetic that is an allosteric modulator of the calcium-sensing receptor, has been used to reduce or normalize plasma calcium and PTH levels in MEN1 patients with PHPT in whom surgery is contraindicated because of comorbidities or where surgery has failed to cure the PHPT.[45]

Preoperative imaging (e.g., ultrasound, technetium-99m sestamibi, computed tomography [CT], magnetic resonance imaging (MRI), and fluorine-18 fluorocholine positron emission tomography [PET]-CT) is of limited value because all parathyroid glands may be affected, thereby necessitating an open bilateral neck exploration, and reducing the rationale for such preoperative localization studies. Indeed, preoperative imaging in MEN1 patients has been reported to not alter the surgical approach in more than 90% of patients; to have limited value in identifying ectopic parathyroid glands, which were correctly identified in only approximately 38% of cases; and to only correctly localize the largest parathyroid gland subsequently identified at surgery in 69% of cases while failing to identify enlarged contralateral glands in 86% of these cases.[34,46] However, the use of an intraoperative PTH assay may help identify hyperfunctioning parathyroid tissue.[34,47] For example, a reduction in PTH concentration greater than 75% is associated with a high biochemical cure rate, although it does not exclude the development of recurrent hyperparathyroidism at a later time.[47] Bilateral neck exploration, together with the use of intraoperative PTH measurement, may also indicate the likely presence of ectopic and/or supernumerary hyperfunctioning parathyroid glands, which occur predominantly within the thymus, with other locations being the mediastinum or carotid sheath. Indeed, the potential for supernumerary glands within the thymus has led to the recommendation for bilateral transcervical thymectomy at the time of parathyroid surgery.[1,34,37]

The optimal management of asymptomatic MEN1 patients, including children and young adults who manifest only mild biochemical features, remains to be defined; at present, some centers advocate early treatment to minimize impacts on bone health, whereas others favor conservative treatment involving the regular assessment of patients for the onset of symptoms and/or associated complications.[1,19]

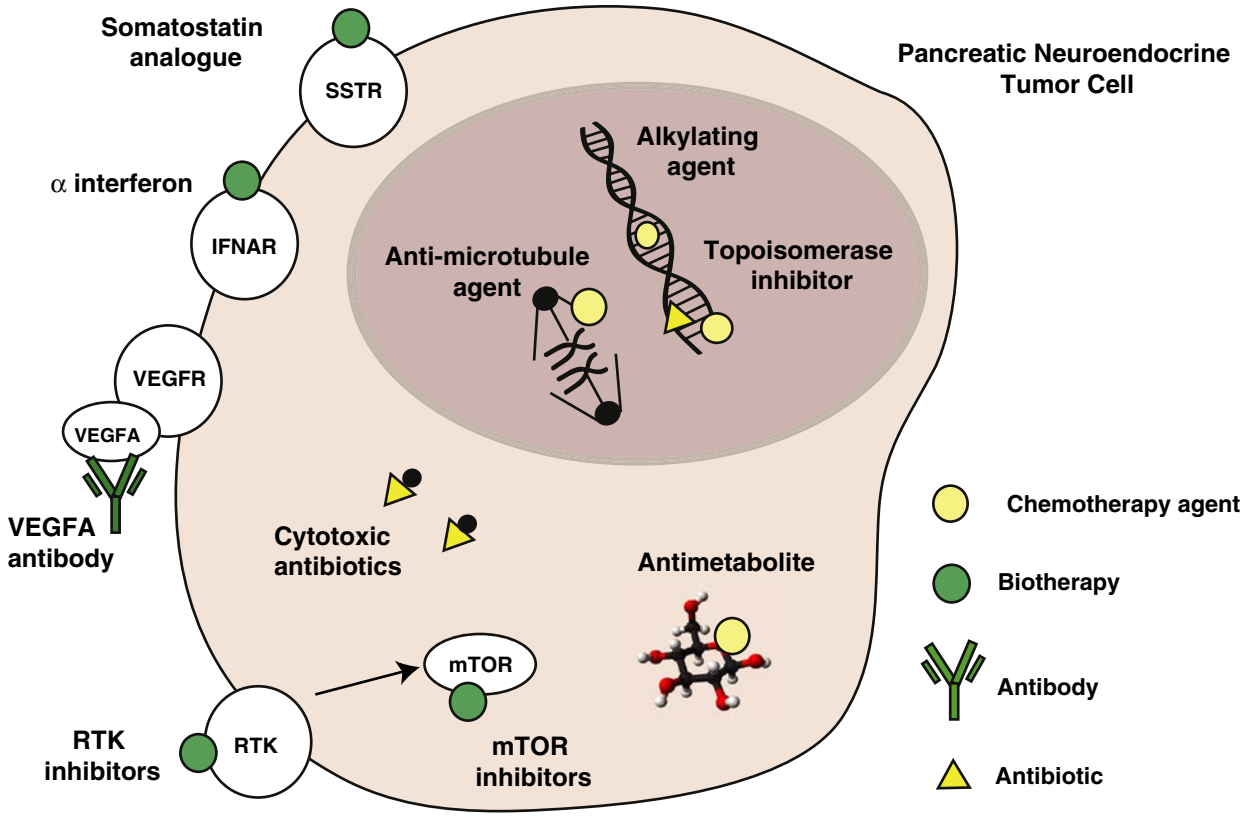
Pancreatic NETs

Pancreatic NETs remain the leading cause of premature death in MEN1 patients. Clinically apparent pancreatic NETs are reported in 30% to 80% of MEN1 patients,[1,19,48–51] although microscopic islet tumors are found in almost all MEN1 patients when evaluated histopathologically.[52] Pancreatic NETs (e.g., gastrinoma, insulinoma, and glucagonoma) may secrete excess hormones and result in relevant clinical features or may be nonsecreting (also referred to as nonfunctioning [NF]) tumors (see Fig. 42.1), and these include those producing pancreatic polypeptide (PP) that is not associated with manifestations of hormonal excess.[1] A MEN1 patient may have more than one pancreatic NET—for example, 10% of patients may have gastrinomas and insulinomas, which may arise co-temporarily or at different times.[23] The main goals of treatment for these MEN1-associated pancreatic NETs are to reduce the morbidity and mortality associated with their occurrence (i.e., relief of symptoms and risk of malignancy). However, there are many different treatments available (Fig. 42.2), and the absence of high-quality evidence for their efficacy makes it challenging to decide on correct therapy. For example, the ideal treatment of a nonmetastatic, single pancreatic NET is surgical removal, as this offers the only potentially curative treatment. However, this situation rarely arises in MEN1 patients, who will have multiple pancreatic NETs—for example, gastrinomas and insulinomas are multiple in more than 95% and approximately 40% of MEN1 patients, respectively, with sizes varying from microadenomas to larger than 4 cm. The clinical behavior of these pancreatic NETs also varies, and generally all macroscopic pancreatic NETs are potentially malignant, although the aggressiveness of an individual pancreatic NET cannot be accurately predicted by tumor size, radiologic features, or hormone production.[53] However, studies have shown that most microadenomas are stable and infrequently increase in size, 30% to 40% of macroadenomas smaller than 2 cm will increase in size over 10 years,[49] approximately 50% to 70% of pancreatic NETs between 2 and 3 cm will be associated with lymph node metastases, and approximately 25% to 40% of pancreatic NETs larger than 4 cm will be associated with hepatic metastases.[53] Survival in MEN1 patients correlates with nonmetastatic disease—for example, survival at 15 years in MEN1 patients with gastrinomas smaller than 2.5 cm in size that were associated with nonmetastatic or metastatic disease has been reported to be 100% and 50%, respectively. Thus, the occurrence of multiple pancreatic NETs and their varied and unpredictable malignant potential in MEN1 patients pose major challenges in their management.[1,53] The diagnosis and treatments for these MEN1-associated pancreatic NETs will be reviewed.

Gastrinoma

Clinical Features

Gastrin-secreting tumors are associated with a marked overproduction of gastric acid, which results in recurrent peptic ulceration, a combination referred to as Zollinger-Ellison syndrome



• **Fig. 42.2** Current and emerging medical therapies for pancreatic neuroendocrine tumors (NETs). Medical therapies for pancreatic NETs include drugs, biotherapies, and antibodies that target different pathways in cancer cells. Somatostatin analogues (e.g., octreotide and lanreotide) are used widely in the treatment of pancreatic NETs. Somatostatin analogues target members of the somatostatin receptor family on the tumor cell surface to control excess hormone secretion and inhibit growth (i.e., antiproliferative effects). Additional medical therapies include mammalian target of rapamycin (mTOR) inhibitors (e.g., everolimus) and receptor tyrosine kinase (RTK) inhibitors (e.g., sunitinib and pazopanib), which have been shown to delay pancreatic NET tumor progression. Current clinical trials are investigating the use of these agents in combination or with other therapies including monoclonal antibodies targeting the vascular endothelial growth factor receptor (VEGFR) (e.g., bevacizumab). Interferon-α (IFNα), which targets the IFNα/β-receptor (IFNAR) may also be effective in symptom and tumor control. Chemotherapeutic agents may also be effective in the treatment of metastatic pancreatic NETs and include alkylating agents (e.g., streptozocin, temozolomide, cisplatin, cyclophosphamide, procarbazine, dacarbazine, and oxabiplatin), antimicrotubule agents (e.g., docetaxel and etoposide), antimetabolites (e.g., 5-fluorouracil, capecitabine, and gemcitabine), topoisomerase inhibitors (e.g., doxorubicin, etoposide, and irinotexan), and cytotoxic antibiotics (e.g., actinomycin D, doxorubicin, mitomycin C, mitoxantrone). Combinations of chemopreventative agents that target different cellular pathways are more frequently used rather than as a monotherapy. *VEGFA,* vascular endothelial growth factor A. (Modified from Frost M, Lines KE, Thakker RV. Current and emerging therapies for PNETs in patients with or without MEN1. *Nat Rev Endocrinol.* 2018;14[4];216–227.)

(ZES).[54,55] Symptoms of ZES include those associated with peptic ulceration (i.e., abdominal pain, heartburn), as well as weight loss, diarrhea, and steatorrhea.[1] In addition, esophageal stricture and/or Barrett esophagus are also more common in patients with ZES, and acute presentations with small bowel perforation and/or hemorrhage secondary to peptic ulceration contribute to the high morbidity associated with ZES.[56] Symptomatic presentations are rare in childhood, although they have been reported in children younger than 10 years.[19] Gastrinomas occur in approximately 20% to 60% of MEN1 patients[53,57–59] and are found more frequently in adult males.[27] Approximately 20% of patients with sporadic gastrinoma will have MEN1.[1] MEN1-associated gastrinomas frequently occur as small (<5 mm in diameter), multiple nodular lesions deep within the duodenal mucosa and are only rarely observed in the pancreas,[57,60] in contrast to sporadic gastrinomas, which typically occur as solitary tumors within the pancreas or duodenum. In addition, MEN1-associated gastrinomas often occur as microscopic tumors (i.e., <1 mm), which despite their small size frequently metastasize to local lymph nodes at an early stage in the disease course.[57,60] Indeed, local lymph node metastases are found in 30% to 70% of cases at diagnosis,[53,61–63] although advanced presentations with hepatic metastases are rare in MEN1; however, when present, they are associated with a poor prognosis.[64,65] Additional poor prognostic indicators include markedly elevated gastrin levels, ectopic Cushing syndrome, and occurrence of primary pancreatic tumors.[66] Gastrinoma in MEN1

patients appears to occur rarely in the absence of PHPT,[58,67] and successful treatment of PHPT with restoration of normocalcemia is reported to result in symptomatic and biochemical improvements in approximately 20% of MEN1 patients with hypergastrinemia and ZES.[68]

The diagnosis of gastrinoma is made by demonstrating an increased fasting serum gastrin in association with increased basal gastric acid secretion.[1,61,69–71] A raised fasting serum gastrin alone is insufficient to make the diagnosis, as this may occur in achlorhydria, antral G-cell hyperplasia, *Helicobacter pylori* infection, renal failure, hypercalcemia, and PPI therapy.[69–71] An intravenous provocation test with secretin or calcium, which in patients with gastrinoma will be associated with a marked increase in gastrin, may help in the diagnosis.[1] MEN1 gastrinomas in the duodenum may be localized by endoscopic ultrasound. CT, MRI, selective angiography, and/or somatostatin receptor scintigraphy combined with selective arterial secretagogue injection (e.g., calcium) and hepatic venous gastrin measurements may help localize the tumor.[1]

Treatment

The lack of results from prospective randomized controlled trials of treatments in MEN1 patients with gastrinomas makes their management challenging and reliant on expert opinion.[1,53] The aims of treatment should be to ameliorate the symptoms and/or sequelae of the associated hypergastrinemia while reducing the likelihood of developing advanced metastatic disease. The medical treatment of gastrinoma has been transformed following the introduction of PPI therapies (e.g., omeprazole and lansoprazole), which are highly efficacious at reducing basal acid secretion to less than 10 mmol/L and reducing symptoms associated with gastrinoma.[1] H2-receptor antagonists (e.g., ranitidine) may be added if symptoms remain uncontrolled on high-dose PPI therapy. These therapies represent the mainstay of treatment for controlling symptoms and have resulted in a marked reduction in the morbidity and mortality previously associated with ZES in MEN1 patients. However, the effects on tumor growth and/or risk of developing advanced disease with this treatment are not established. In addition, the role of somatostatin analogue therapy in MEN1 gastrinomas, which may express somatostatin receptors, remains to be established.

The role of surgery in MEN1-associated gastrinoma remains controversial, which in part is due to not knowing the long-term natural course of disease in MEN1 patients.[53,72] Overall, the prognosis of gastrinoma in MEN1 patients is excellent with 5-, 10-, and 20-year survival rates of 90% to 96%, 75% to 96%, and 58% to 90%, respectively,[73] with the higher survival estimates observed following the introduction of acid-suppressive therapies. However, a minority of patients develop aggressive disease, and identifying these patients remains challenging. Poor prognostic features in MEN1-associated gastrinoma include large tumor size, a pancreatic location of the primary tumor, presence of liver metastases, early age of onset, and male gender.[65,73] The risk of hepatic metastases is higher for a pancreatic gastrinoma and correlates with tumor size, and surgery is therefore recommended for all gastrinomas larger than 2 cm in MEN1 patients.[1,73] In all other settings, the role of surgery remains controversial, with some centers advocating an initial medical approach and others pursuing an earlier surgical intervention. Centers advocating a nonsurgical approach point to the excellent long-term prognosis associated with smaller tumors, even in the presence of lymph node metastases; the low surgical cure rates in the presence of multiple small duodenal tumors; the potential high morbidity associated with pancreaticoduodenal resections; the excellent symptom control achieved with PPI therapy; and the lack of evidence demonstrating improved survival in those undergoing surgical resections.[1,53,73] In contrast, centers advocating early surgical intervention in all patients with MEN1-associated gastrinoma point to results that have achieved eugastrinemia in 30% to 75% of patients for 3 to 5 years.[74,75] Surgical approaches in these studies have included duodenotomy with excision of gastrinomas in the duodenal mucosa coupled with enucleation (if feasible) or resection of tumors in the pancreatic head, peripancreatic and lymph node removal, and corporacaudal pancreatic resection; partial pancreaticoduodenectomy; pancreas-preserving total duodenectomy; and total pancreaticoduodenectomy.[1,53,74–77] Total pancreaticoduodenectomy (i.e., Whipple procedure), which is associated with a substantially greater risk of diabetes mellitus and malabsorption, is rarely performed and is typically reserved for patients with diffuse large pancreatic tumors.[1] However, long-term remission and survival data are not known, and currently the benefits of medical therapies over such surgery have resulted in many centers and guidelines recommending nonsurgical management for gastrinomas in MEN1 patients, with surgery being reserved for patients in whom medical therapy has failed.[1,53]

The management of advanced/disseminated gastrinomas is difficult and does not differ from that of sporadic disease. Chemotherapy with streptozotocin and 5-fluorouracil, capecitabine and temozolomide, cisplatin and etoposide, hormonal therapy with octreotide or lanreotide (which are human somatostatin analogues) (see Fig. 42.2), selected internal radiation therapy, radio-frequency ablation, peptide receptor radionuclide therapy, hepatic artery embolization, administration of human leukocyte interferon, and removal of all resectable tumor and hepatic transplantation have each been employed with occasional benefit.[53,74]

Insulinoma

Clinical Features

Insulinomas, which arise from pancreatic islet beta cells, occur in approximately 10% to 30% of patients with MEN1, whereas 5% to 10% of patients presenting with insulinoma will have MEN1.[1,19,23,25] Insulinomas in MEN1 patients frequently occur, in contrast to non-MEN1 patients, before the age of 40 years and may be the first manifestation of MEN1 in approximately 10% of cases. Indeed, a recent study of 160 MEN1 patients younger than 21 years observed insulinomas to occur in 12% of the cohort, with the earliest presentation at 5 years of age.[19]

MEN1 patients with insulinomas typically present with symptoms of hypoglycemia (e.g., weakness, headaches, sweating, faintness, anxiety, altered behavior, seizures, and loss of consciousness) that develop after fasting or exercise and improve after glucose (food) intake.[19] The diagnosis is most reliably made by a supervised 72-hour fast,[1,69,78] in which hypoglycemia (i.e., glucose <2.2 mmol/L [40 mg/dL]) is documented in the presence of inappropriate elevated concentrations of insulin (together with proinsulin and C-peptide).[1] It is also important to demonstrate the absence of sulfonylureas in the plasma and urine samples obtained during the investigation of hypoglycemia. Medical therapy, which consists of frequent carbohydrate meals, diazoxide, and somatostatin analogues, is not always successful, and removal of the insulinoma by surgery is the optimal treatment. Surgical success

is improved by preoperative localization of the tumor. The majority of insulinomas are single lesions, occurring in the body or tail of the pancreas, although multiple or multicentric insulinomas may be observed in approximately 40% of MEN1 patients.[79] Furthermore, concurrent pancreatic NETs (e.g., NF tumors) are frequently present in MEN1 patients, thereby posing a challenge for the correct localization of the insulinoma. Imaging modalities routinely employed for preoperative localization include endoscopic ultrasound, CT, and MRI, whereas more specialized methods, including celiac axis angiography and selective intra-arterial calcium stimulation combined with hepatic venous insulin measurements may also be required.[80–82] Somatostatin receptor scintigraphy (SRS) with ^{111}In-octreotide has been reported to be associated with low sensitivity in insulinoma (20–60%), although ^{68}Ga-DOTATATE PET-CT may have a higher sensitivity.[83] More recently, scintigraphy based on glucagon-like peptide 1 has been reported to be highly sensitive in localizing insulinomas when compared with conventional imaging modalities and may provide a useful diagnostic modality.[83–85] The utility of ^{18}F-fluorodeoxyglucose (FDG)-PET/CT for insulinoma detection is typically limited to high-grade metastatic disease.[83] Finally, direct intraoperative pancreatic ultrasound may be used at the time of surgery to identify the likely tumor.[80,86,87]

Treatment

Surgery is the treatment of choice for those with nonmetastatic disease. Several surgical procedures have been reported to provide long-term curative outcomes, including enucleation or excision of single or multiple tumors, distal or partial pancreatectomy, and pancreatoduodenectomy.[79,88,89] In one large series of MEN1 patients undergoing surgery for insulinoma, distal pancreatectomy was associated with a lower risk of hypoglycemia recurrence than enucleation but a higher risk of surgery-related morbidity.[90] Minimally invasive approaches (e.g., laparoscopic, robot assisted) may be appropriate in selected MEN1 cases.[88,91] The surgical approach adopted will depend on the location and size of insulinoma, as well as the presence or absence of additional pancreatic tumors. In MEN1 patients, insulinomas typically occur as benign tumors, and surgical outcomes and long-term prognosis are excellent.[90] For advanced and metastatic disease, chemotherapy (using streptozotocin, 5-fluorouracil, and doxorubicin), and locoregional approaches, including hepatic artery embolization, may be employed for disease and symptom control[1,53,92] (see Fig. 42.2).

Glucagonoma

Clinical Features

Glucagonomas, which arise from the pancreatic islet α-cells and lead to excess glucagon secretion, occur in 1% to 2% of MEN1 patients.[93] However, the characteristic clinical features of skin rash (necrolytic migratory erythema), stomatitis, weight loss, venous thrombosis, and anemia may be absent.[93] Instead, glucagonomas may be detected in asymptomatic MEN1 patients following surveillance pancreatic imaging or the finding of marked hyperglucagonemia (i.e., greater than two times the upper limit of normal) with or without glucose intolerance. It should be noted that a significant proportion of NF pancreatic NETs immunostain for glucagon (either in insolation or together with additional pancreatic hormones) in the absence of hyperglucagonemia,[52,94] whereas a proportion of NF tumors will be associated with modest elevations in plasma glucagon (i.e., less than two times the upper limit of normal).

Treatment

Glucagonomas most frequently present in the tail of the pancreas, where possible surgical resection, which can be curative, is the treatment of choice. However, curative surgery may not be feasible considering that 50% to 80% of patients may have large tumors with metastases.[1] Medical treatment with somatostatin analogues (e.g., octreotide or lanreotide) or chemotherapy (with streptozotocin and 5-fluorouracil or dimethyltriazeno-imidazole carboxamide) (see Fig. 42.2) has been successful in some patients, and hepatic artery embolization has been used to treat metastatic disease.[1,53,92]

VIPoma

Clinical Features

Patients with vasoactive intestinal peptide (VIP)omas, which are VIP-secreting pancreatic tumors, develop watery diarrhea, hypokalemia, and achlorhydria.[1,93] This clinical syndrome has been referred to as Verner-Morrison syndrome, WDHA (watery diarrhea, hypokalemia, achlorhydria) syndrome, or VIPoma syndrome.[1] Only a few patients with MEN1 have been reported to have VIPomas.[93] The diagnosis is established by excluding laxative and diuretic abuse and confirming a stool volume in excess of 0.5 to 1.0 L/day during a fast, together with a markedly increased plasma VIP concentration.

Treatment

Surgical management of VIPomas, which are mostly located in the tail of the pancreas, has been curative, although approximately 50% of patients have metastases at diagnosis.[95,96] In patients with unresectable disease, treatment with somatostatin analogues such as octreotide and lanreotide, streptozotocin with 5-fluorouracil, corticosteroids, indomethacin, metoclopramide, and lithium carbonate has proved beneficial, and hepatic artery embolization has been useful for the treatment of metastases[1] (see Fig. 42.2).

NF Pancreatic NETs

Clinical Features

NF pancreatic NETs are islet-derived tumors that do not give rise to a clinical syndrome associated with excess hormone production, and hence these tumors may have presentations with symptoms related to local mass effects (i.e., pain and/or compression of adjacent structures) or metastatic disease (e.g., cachexia, jaundice, hepatomegaly, and hepatic and bone pains). However, some NF pancreatic NETs may be associated with elevations in PP and/or glucagon,[1,97] although plasma PP and glucagon, together with chromogranin A, are reported to have low sensitivity and specificity for pancreatic NET detection in MEN1 patients,[98,99] and circulating biomarkers for detection of NF pancreatic NETs are not available at this time. As a consequence, pancreatic imaging remains the mainstay of diagnosis, although the optimal modality for detection has not been established and frequently depends on local availability and expertise related to different modalities. NF pancreatic NETs in MEN1 are typically considered as a single entity (see Fig. 42.1), although they likely represent a heterogeneous group of tumors with distinct pathologic and/or molecular subtypes. The implementation of surveillance imaging programs for patients with MEN1 has shown that NF pancreatic NETs are the most prevalent pancreatic NET in MEN1, with clinically apparent tumors occurring in 15% to 55% of patients.[49,51,53,97,100] For example, a prospective endoscopic ultrasound study (EUS) demonstrated that approximately 55% of MEN1 patients had one or more NF NETs,[48] and a histopathologic study reported that almost

all MEN1 patients have small microadenoma NF tumors.[52,53] In addition, NF pancreatic NETs have been reported to occur in more than 5% to 40% of children and young adults, 12 to 20 years of age, with MEN1.[19,97,101] The current guidelines therefore recommend surveillance imaging for NF pancreatic NETs from age 10 years,[1] with the aims of detecting and monitoring clinically relevant tumors yet minimizing exposure to ionizing radiation and/or iatrogenic complications relating to the procedure. EUS has been reported to be the most sensitive method for detection of tumors smaller than 1 cm, and to provide accurate estimates of tumor growth rates, and also provides the potential opportunity to assess the mitotic count and/or Ki67 index of tumors obtained from EUS-guided fine-needle aspirates.[53,102] However, EUS is an invasive and time-consuming procedure that is dependent on user expertise, and it has been reported to overestimate the size of NF pancreatic NETs in MEN1, which has important implications considering that decisions regarding surgery are frequently based on size criteria.[103] Alternate methods of imaging include MRI, CT, SRS (i.e., based on octreotide/^{68}Gallium-DOTATATE PET), and FDG-PET, and each has been associated with high sensitivity and specificity in different series such that combinations of these modalities are often used to fully characterize tumors and for assessing the likelihood of metastatic risk.[53,104,105]

NF pancreatic NETs are the leading cause of premature mortality in patients with MEN1, and they are associated with a worse prognosis than other MEN1-associated pancreatic NETs (e.g., insulinoma, gastrinoma).[20,53,61,106] Premature morbidity and mortality typically results from the development of metastatic disease but may also arise due to complications of surgical intervention.[20,49,51,106,107] The risk of developing liver metastases correlates with primary tumor size, and synchronous liver metastases have been reported in approximately 43% of patients with NF NETs larger than 3 cm, 18% of patients with NF NETS 2 to 3 cm, 10% in those with NF NETS 1 to 2 cm, and only 4% of those with NF NETS smaller than 1 cm.[100] However, tumor size is not universally correct at predicting metastatic risk in NF NETs, as a small percentage of patients with small tumors are reported to develop advanced disease.[108] In addition, currently circulating biomarkers are not available for predicting metastatic risk in NF pancreatic NETs, and circulating biomarkers based on microRNA, circulating tumor cells, multigene signatures, and tumor DNA, which are being evaluated, may hold future promise in predicting tumor behavior.[104,109,110]

Treatment

The aims of treatment for MEN1-associated NF NETs are to minimize the risk of developing metastatic disease while avoiding unnecessary surgical interventions that are known to result in significant early and late complications.[1,53,107] However, surgical removal of NF pancreatic NETs is of benefit, as illustrated by a recent study in which only 6 of 16 (~40%) MEN1 patients with NF pancreatic NETs larger than 3 cm and who had surgery, developed hepatic metastases, or died compared with 5 of 6 (~80%) patients who did not have surgery.[51] However, the same study reported that rates of metastasis in patients undergoing surgery for NF pancreatic NETs smaller than 2 cm were not significantly different from those managed without surgery.[51] Thus, the majority of centers recommend surgery for NF pancreatic NETS larger than 2 cm, especially because smaller NF pancreatic NETS (<2 cm) have been reported to have stable appearances over an average of 10 years.[49,111] For example, in one study, 60% (28 of 46) of patients with NF pancreatic NETs smaller than 2 cm had stable disease, whereas in the remaining 40% of patients who had an increase in size or number of tumors, or developed hypersecretion syndromes, only 15% (7 of 46) required surgery and only 2% (1 of 46) of patients died from metastatic disease.[49] Moreover, another study reported that surgery in MEN1 patients with NF NETs smaller than 2 cm does not affect progression-free survival (PFS) when compared with those patients managed conservatively, as the majority of tumors displayed indolent behavior.[111] However, surgery is recommended when there is rapid tumor growth (i.e., doubling of tumor size over a 3–6 month interval) (see Fig. 42.1), and some centers will consider surgery if pancreatic NETs are 1 cm or larger in size. The decision to undertake surgery for NF pancreatic NETs in MEN1 should consider the potential presence of additional tumors within the pancreas (and elsewhere), the presence of occult metastatic disease either related to the tumor undergoing resection or from another source, and the fact that any remnant pancreatic tissue will remain a risk for the development of further tumors. Thus, all of these considerations highlight the importance of multidisciplinary working and the involvement of the patient in the decision-making process.[106]

Medical treatments for small (i.e., <2 cm) NF pancreatic NETs in MEN1 include long-acting octreotide, which has been reported to be associated with a tumor response in 10% of patients, stable disease in 80% of patients, and disease progression in 10% of patients over 12 to 15 months of treatment,[112] with reductions in PP and glucagon in those with stable tumor size.[113] For patients with advanced disease, treatments include locoregional and systemic approaches (see Fig. 42.2) and are similar to those employed for the other pancreatic NETs. However, the evidence for the use of such treatments arose from study of non-MEN1 patients, and extrapolation of this to MEN1 patients requires some caution.[53] Somatostatin analogue therapy is reported to be associated with a approximately 50% reduction in the risk of disease progression in non-MEN1 patients with well-differentiated advanced pancreatic NETs.[114,115] The receptor tyrosine kinase (RTK) inhibitor sunitinib is associated with an increase in PFS from 5.5 to 11.4 months in non-MEN1 patients with advanced well-differentiated pancreatic NETs,[116] and the mammalian target of rapamycin inhibitor everolimus increases PFS from 6 to 11 months in non-MEN1 patients with low- or intermediate-grade advanced pancreatic NETs.[117] Additional therapies for advanced NF pancreatic NETs include chemotherapy, peptide receptor radionuclide therapy, and locoregional therapies (e.g., radio-frequency ablation, transarterial chemoembolization, and selective internal radiation therapy).[53,92]

Somatostatinoma

Pancreatic tumors secreting somatostatin are associated with somatostatinoma syndrome, characterized by hyperglycemia, cholelithiasis, low acid output, steatorrhea, diarrhea, abdominal pain, anemia, and weight loss. Although elevations in somatostatin are observed in a significant proportion of MEN1-associated pancreatic NETs, somatostatinoma syndrome has not been reported.[20,93] Thus, this group of tumors is frequently considered part of the NF category.

GHRHoma

Pancreatic islet tumors secreting growth hormone–releasing hormone (GHRH) have been reported in some patients with MEN1.[1,118] Patients may present with features of acromegaly and are diagnosed by demonstrating elevations in serum growth hormone (GH), GHRH, and insulin-like growth factor 1. In the

context of MEN1, GHRHomas occur predominantly within the pancreas, although sporadic GHRHomas may arise in the lung or small intestine.[118] Surgical removal is the treatment of choice for MEN1-associated pancreatic GHRHomas.[118]

Pituitary Tumors

Clinical Features

Anterior pituitary tumors occur in 30% to 50% of patients with MEN1, although the frequency of detection has increased with the introduction of routine surveillance of MEN1 patients together with the improved sensitivity of imaging modalities.[1,23,119–121] Women are reported to be affected more frequently than men,[27,121] and one study reported an intrafamilial correlation and suggested occurrence of potential genetic modifying influences that are independent of the *MEN1* mutation.[122] Pituitary tumors typically present in early adulthood at a mean age of 30 to 40 years.[119,121] However, pituitary tumors may occur earlier; one study reported a prevalence of approximately 35% before 21 years of age, with the majority of cases presenting between 15 and 20 years of age,[19] whereas the youngest presentation reported was in a 5-year-old boy.[123] Pituitary tumors are the first manifestation of MEN1 in 10% to 20% of cases.[19,124] Initial studies reported a high prevalence (>80%) of pituitary macroadenomas (i.e., >1 cm) in MEN1 patients,[121] although more recent series indicate that microadenomas (i.e., <1 cm) occur frequently, and these differences are likely explained by the introduction of sensitive surveillance imaging.[119,124] Pituitary tumor subtypes are observed in MEN1 patients, with prolactinomas representing the most common type, accounting for 40% to 75% of MEN1-associated pituitary tumors.[19,119,120,124] Other functioning pituitary tumors include GH-secreting tumors (5–15%) and adrenocorticotropic hormone (ACTH)-secreting tumors (3–7%), whereas plurihormonal secretion (i.e., prolactin/GH and prolactin/ACTH) may be observed in a minority of tumors. The remaining tumors predominantly comprise NF pituitary tumors (15–40%),[20,119,121,124] although some of these are associated with secretion of glycoprotein subunits.[1] One recent study reported that approximately 50% of pituitary tumors detected by screening in MEN1 patients (i.e., as opposed to clinical presentations) represented NF tumors, of which the majority were microadenomas.[119] The clinical manifestations associated with MEN1-associated pituitary tumors are similar to those in patients with sporadic pituitary tumors. For example, patients with prolactinomas will have features associated with hyperprolactinemia (e.g., amenorrhea, galactorrhea, and infertility in females, and erectile dysfunction and loss of libido in males), whereas those with GH- and ACTH-secreting tumors will manifest symptoms and signs associated with acromegaly and Cushing disease, respectively. Pituitary macroadenomas typically present with local mass effects (e.g., headache, visual field defects) or features of hypopituitarism. Diagnosis and clinical investigations are similar to those for sporadic pituitary tumors and will include dynamic biochemical testing and imaging to characterize the nature of the tumor and to assess any compromise of pituitary function (e.g., hypopituitarism). Given the high risk of tumor development, *MEN1* mutation carriers are recommended to undergo periodic biochemical evaluation for prolactin and insulin-like growth factor 1, together with MRI of the pituitary fossa.

Treatment

Overall, the prognosis for patients with MEN1 pituitary tumors, the majority of which are benign neoplasms, is favorable.[20] Pituitary carcinoma is extremely rare in MEN1, with only a few cases reported.[1,125] The treatment of pituitary tumors in patients with MEN1 is similar to that employed for their sporadic counterparts and comprises appropriate medical therapy (e.g., cabergoline for prolactinoma; somatostatin analogues and/or pegvisomant for somatotropinoma) or selective transsphenoidal adenomectomy, if feasible, with radiotherapy reserved for those patients with residual and/or unresectable tumor tissue.[125] However, pituitary tumors in MEN1 patients have been reported in some studies to be larger and more invasive than their sporadic counterparts, as well as less responsive to medical treatments.[120,121,125] One study reported that pituitary tumors in young male MEN1 patients were larger than those occurring in their female counterparts, with the majority occurring as macroadenomas with invasive imaging characteristics (i.e., Hardy grade 3 or 4).[19] However, other studies of predominantly adult MEN1 patients have reported that the majority of pituitary tumors respond well to medical therapies and have a similar behavior to sporadic tumors (e.g., the response rate for prolactinomas is >90%), whereas NF tumors were frequently small and stable, not requiring surgical intervention.[72,119]

Adrenal Tumors

Clinical Features

The incidence of adrenocortical tumors in patients with MEN1 is reported to be 20% to 55%,[1,126] although a higher frequency of adrenal involvement (~75%) has been reported when highly sensitive imaging modalities have been employed, including endoscopic ultrasound.[127] Most affected patients are asymptomatic, as the majority of tumors, which may include cortical adenomas, hyperplasia, multiple adenomas, nodular hyperplasia, cysts, or carcinomas, are NF.[1,126] Indeed, fewer than 10% of patients with enlarged adrenal glands have biochemical evidence of hormonal hypersecretion, and among these, primary hyperaldosteronism and ACTH-independent Cushing syndrome are the most commonly encountered.[126] Occasionally, hyperandrogenemia may occur in association with adrenocortical carcinoma, whereas the occurrence of pheochromocytoma in MEN1 patients is rare. Although adrenal involvement is most commonly observed in adults with MEN1 (with equal sex distribution), occasional presentations in childhood are reported. For example, adrenal carcinoma has been reported in a 4-year-old boy and 16-year-old girl, each having clinical and biochemical evidence of androgen excess.[19] It is also reported that adrenal tumors demonstrate heritability in MEN1 kindreds, highlighting a need for increased vigilance in those with other affected family members.[122] Biochemical investigation (e.g., plasma renin and aldosterone concentrations, the low-dose dexamethasone suppression test, urinary catecholamines and/or metanephrines) should be undertaken for those with symptoms or signs suggestive of functioning adrenal tumors, or for those with tumors larger than 1 cm. The incidence of adrenocortical carcinoma is reported to be approximately 1% in MEN1 patients but is higher (at approximately 13%) in MEN1 patients with adrenal tumors larger than 1 cm.[126] Thus, it is important that MEN1 patients with adrenal tumors are offered annual imaging and that those displaying atypical radiologic characteristics, significant growth, or are larger than 4 cm are considered for surgical removal.[1,126]

Treatment

Consensus has not been reached about the management of MEN1-associated NF adrenal tumors, because the majority of these neoplasms are benign. However, the risk of malignancy is increased if the tumor has a diameter larger than 4 cm, although adrenocortical carcinomas have been identified in tumors smaller

than 4 cm in patients with MEN1.[1,126] Surgery is recommended for adrenal tumors that are larger than 4 cm in diameter, have atypical or suspicious radiologic features (e.g., increased Hounsfield units on an unenhanced CT scan) and are 1 to 4 cm in diameter, or show significant measurable growth over a 6-month interval.[1,127] The treatment of functioning (i.e., secreting) adrenal tumors in MEN1 patients is similar to that for tumors occurring in non-MEN1 patients.

Carcinoid Tumors

Carcinoid tumors arising in the thymus, bronchi, or gastrointestinal tract occur at varying frequencies in patients with MEN1. Thymic carcinoid tumors, although rare, present the most significant clinical challenge because they are associated with an aggressive disease course and are one of the leading causes of premature death in MEN1 patients.

Thymic Carcinoids

Thymic carcinoid tumors occur in 2% to 8% of MEN1 patients[1,128–131] and are observed predominantly in adult male patients, although gender differences appear specific to individual ethnic populations (e.g., European populations, 20 male to 1 female; Japanese populations, 2 male to 1 female; Chinese populations, 1 male to 1 female).[27,129,132] In MEN1 populations of European descent, smoking is an independent risk factor for tumor development.[129] The median age at diagnosis is 40 to 45 years,[129] although much earlier presentations have been reported, including a 16-year-old boy who died 49 months after diagnosis from local and distant metastatic disease.[19] Although the overall frequency of thymic carcinoid tumors is low in MEN1 patients, several reports highlight clustering of cases within individual families, thereby suggesting a high heritability independent of the *MEN1* mutation.[122,129] Thymic carcinoids are responsible for approximately 20% of premature deaths in MEN1 patients,[20,61,129] with the highest hazard ratio for increased mortality of all the MEN1-associated tumors (hazard ratio, 4.64; 95% confidence interval, 1.73–12.41).[20] Once diagnosed, MEN1 patients with thymic carcinoid tumors have a median survival of 8 to 10 years.[129,131] Symptomatic presentations may include pain (e.g., arising from chest, shoulder, breast) or features of vena caval obstruction,[131] whereas the features of carcinoid syndrome (i.e., flushing, diarrhea) are not typically observed.[129,131] Indeed, the majority of patients are asymptomatic at diagnosis, and most tumors are detected by radiology.[131] Biochemical markers (e.g., raised chromogranin A, urinary 5-hydroxyindoleacetic acid) are not sufficiently sensitive for tumor detection; as a consequence, diagnosis is reliant on radiologic imaging, although the optimal screening methods have not been established. CT is considered to be sensitive for tumor detection, but there is concern over the repeated exposure to ionizing radiation, particularly as the natural history of thymic carcinoid tumors in MEN1 is one of rapid development such that a frequent scanning interval (i.e., every 1–2 years) would be required.[1,130] "Low-dose" CT or MRI may be optimized for tumor detection, but further studies are required to assess their utility in MEN1. Similarly, SRS is frequently positive in thymic carcinoids, although insufficient evidence is available to recommend its use as a screening modality. FDG-PET may also be useful in the evaluation of patients with thoracic lesions in MEN1.[133] The treatment of thymic carcinoids depends on the stage of presentation. Surgical removal is recommended because it may be curative, although recurrence rates following surgery are high.[129,131] Older age of presentation, larger tumor diameter, and presence of metastases are each associated with worse clinical outcomes.[129] For those with advanced disease, chemotherapy (e.g., etoposide and cisplatin) and radiotherapy may be used.[129,131] The value of somatostatin analogue therapy in this context is not known but may improve symptoms and/or result in tumor response. It should be noted that cervical thymectomy is recommended in MEN1 patients undergoing parathyroidectomy, although there is little evidence that such prophylactic thymus removal reliably prevents thymic carcinoid development, and several cases of thymic carcinoid have been reported in patients who have undergone this procedure.[131] Similarly, although current guidelines recommend regular screening for thymic carcinoid, there is no evidence that this approach, aimed at early tumor detection, results in improved outcomes.[130]

Bronchial Carcinoids

Bronchial carcinoids, which are observed in 4% to 13% of adult patients with MEN1, are not typically associated with increased mortality,[20,128,130,134] as they frequently run a benign course, although occasional patients have developed malignant disease.[134] The incidence of bronchial carcinoids appears to be approximately equal between sexes,[128] although they were initially reported to occur predominantly in women. Bronchial carcinoids present in adulthood at a median age of approximately 40 years, and childhood presentations do not appear to be reported.[19,128,130] The majority of patients with bronchial carcinoids are asymptomatic, and features of carcinoid syndrome are typically absent.[59,128] Diagnosis is made on imaging, most frequently with CT. However, it should be noted that pulmonary lesions in MEN1 patients require careful evaluation because they may represent metastatic lesions from other tumor sites.[128,133] Surgery is typically performed for bronchial NETs, and although there is no direct evidence of improved outcomes employing this approach in MEN1 patients, in sporadic cases surgery is reported to be beneficial.[130,134,135]

Gastric Carcinoids

Type II gastric carcinoids (also referred to as enterochromaffin-like cell carcinoids [ECLomas]) are observed in approximately 15% to 70% of MEN1 patients with coexistent hypergastrinemia and are frequently detected incidentally at the time of upper gastrointestinal endoscopy.[1,136,137] The tumors are usually multiple and small (e.g., <1.5 cm), and SRS may demonstrate increased uptake in the stomach. The malignant potential of these tumors is uncertain, and, where feasible, surgical resection may be appropriate.[136,137] However, treatment with somatostatin analogue therapy has also been reported to result in regression of these tumors.[136–138]

Other Tumors

Central Nervous System Tumors

Central nervous system tumors including ependymomas, schwannomas, and meningiomas have been reported in MEN1 patients.[1] Meningiomas are reported in approximately 8% of MEN1 patients. The majority of meningiomas were not associated with symptoms, and 60% did not enlarge.[1] The treatment of MEN1-associated meningiomas is similar to that occurring in non-MEN1 patients.

Lipomas

Subcutaneous lipomas may occur in 15% to 33% of patients with MEN1, and frequently they are multiple.[1,23] In addition, visceral, pleural, or retroperitoneal lipomas may occur in patients with MEN1. Management is conservative. However, when surgically removed for cosmetic reasons, they typically do not recur.

Facial Angiofibromas and Collagenomas

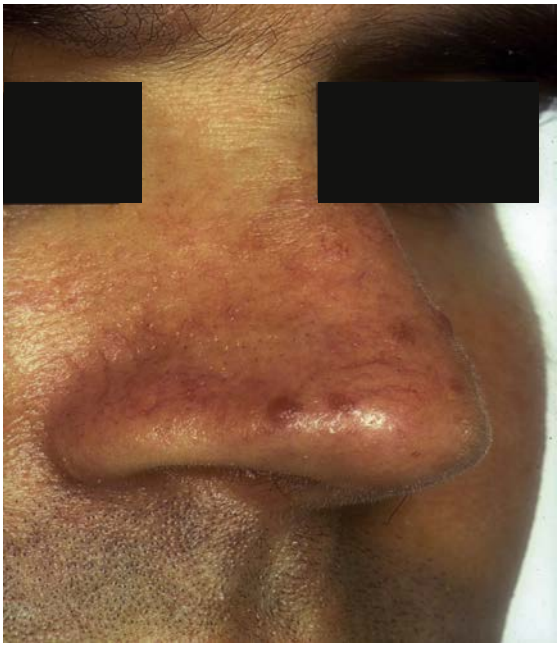
Studies have revealed that multiple facial angiofibromas occur in 22% to 88% and collagenomas in 0% to 72% of patients with MEN1[1] (Fig. 42.3). MEN1 angiofibromas are clinically and histologically identical to those observed in patients with tuberous sclerosis, with the exception that in patients with MEN1, angiofibromas were also present on the upper lip and vermilion border of the lip, which are areas not involved in tuberous sclerosis. These cutaneous findings, which occur with a higher frequency in patients with MEN1, may provide a useful means for possible presymptomatic diagnosis of MEN1 in the relatives of a patient with MEN1. Treatment for these cutaneous lesions is usually not required.

Thyroid Tumors

Thyroid tumors consisting of adenomas, colloid goiters, and carcinomas have been reported to occur in more than 25% of patients with MEN1.[1,23] However, the prevalence of thyroid disorders in the general population is high, and it has been suggested that the association of thyroid abnormalities in patients with MEN1 may be incidental.[1]

Breast Cancer

Female MEN1 patients have been reported to have an increased relative risk between 2.3 and 2.8 of developing breast cancer. The majority of breast tumors are of ductal type with mixed hormone receptor status (estrogen receptor [ER], progesterone receptor, and human epidermal growth factor receptor).[139] Some of the breast cancers had reduced menin expression and loss of heterozygosity of the *MEN1* locus, although further studies are required to confirm the association.[140] Based on these initial observations, together with subsequent studies reporting that the increased breast cancer is not related to other known risk factors,[141] some centers have advocated the introduction of screening for breast cancer in female MEN1 patients over the age of 40 years.[141] However, further evidence is required to support both the association of breast cancer with MEN1 and the value of undertaking such screening.[85]



• **Fig. 42.3** Multiple angiofibromas in a patient with multiple endocrine neoplasia type 1.

Molecular Genetics

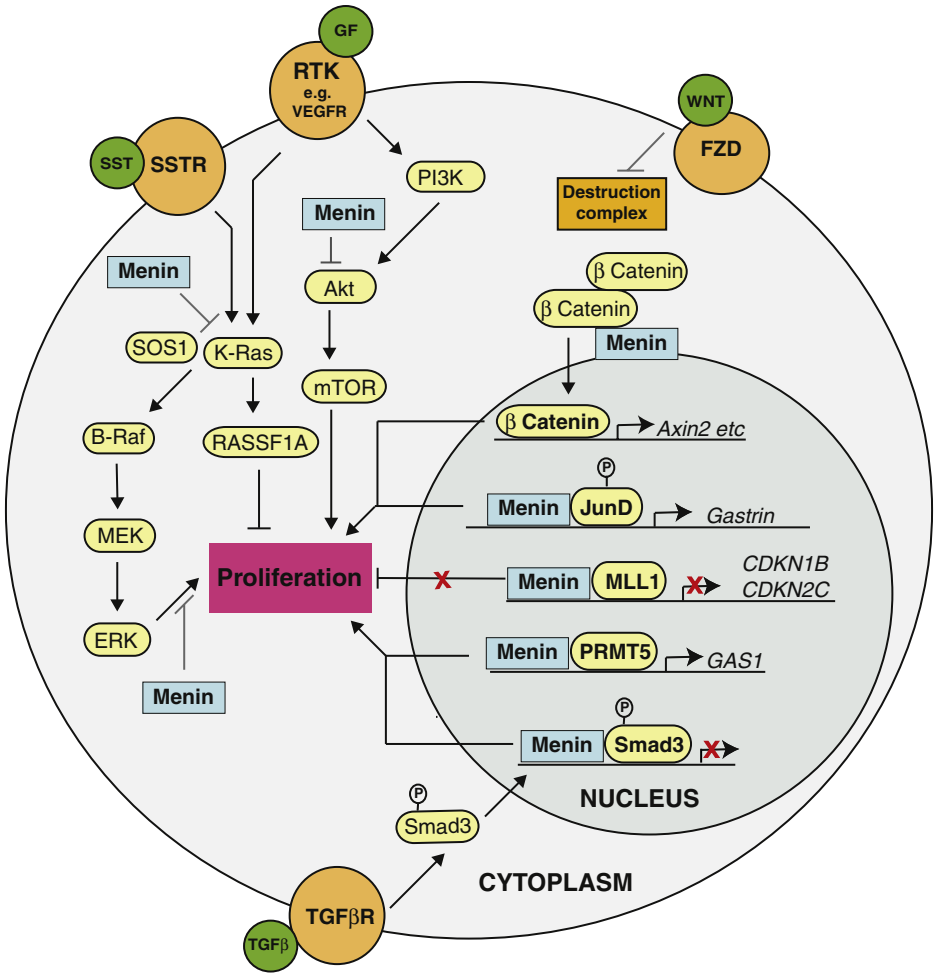
MEN1 *Gene*

The *MEN1* gene is located on chromosome 11q13 and consists of 10 exons, which encode a 610 amino acid protein, menin, that regulates transcription, chromatin structure, genome stability, and cellular proliferation through direct associations with interacting protein partners, or via the modulation of key cellular signaling pathways[18,92,142–144] (Fig. 42.4). Patients with MEN1 harbor germline heterozygous mutations of the *MEN1* gene, which predisposes them to tumor development; however, tumorigenesis requires somatic inactivation of the wild-type *MEN1* allele such that MEN1 tumors demonstrate biallelic inactivation of the *MEN1* gene. Most commonly, the inactivation of the wild-type allele occurs through a large somatic deletion (i.e., at the 11q13 locus), which manifests as loss of heterozygosity (LOH) of the tumor DNA, consistent with Knudson's "two-hit" model of inherited tumorigenesis and a tumor suppressor function for menin in endocrine tissues. However, alternate mechanisms leading to inactivation of the wild-type *MEN1* allele include point mutations (i.e., resulting in nonsense or missense amino acid substitutions) or small insertions or deletions (indels), and in such cases LOH will not be apparent.[1]

The *MEN1* gene spans 7.7 kb of genomic DNA, and at least 16 different *MEN1* transcripts have been identified. The main *MEN1* transcript is a 2.76-kb mRNA, which encodes the 610 amino acid isoform of menin, although the canonical transcript, which appears to be rare, is longer at 3.16 kb and is predicted to elongate the reading frame by 5 amino acids at the exon2/intron2 junction, giving a protein isoform of 615 amino acids. The approximately 1400-bp region upstream of exon 2 is reported to display strong promoter activity, containing both the minimal promoter region and several regulatory regions. Expression of the *MEN1* transcript is observed in all human tissues examined, although menin protein expression may not necessarily correlate with transcript levels. The *MEN1* gene and menin protein are highly conserved in mammalian species (e.g., ~89% and ~97% DNA and protein identity, respectively, with mouse and rat). Orthologues of menin are observed in evolutionary distant specifies, including zebrafish and *Drosophila*, although they are not present in yeast (e.g., *Saccharomyces cerevisiae*) or nematodes (e.g., *Caenorhabditis elegans*). Recent population-level genetic studies indicate that the coding region of *MEN1* demonstrates high levels of constraint against missense and nonsense variation, suggesting that it remains under strong evolutionary selection pressure.[145] Transcription of the *MEN1* gene is reported to be, in part, regulated by its own protein product, menin, whereby reductions in menin expression activate the *MEN1* promoter activity, and menin overexpression downregulates promoter activity.[146] More recently, the *MEN1* 3′ untranslated region has been observed to bind microRNA 24-1, which is reported to suppress menin expression.[147]

Germline MEN1 *Mutations*

To date, more than 1200 germline *MEN1* mutations have been reported in patients with MEN1 or individuals with associated tumors, and of these, approximately 600 different germline mutations are observed.[148–150] *MEN1* mutations are most frequently inherited from an affected parent, although they arise de novo in approximately 10% of cases. The majority of mutations (~70%) are predicted to result in a loss of function through premature truncation of the menin protein (i.e., frameshift deletions or insertions [40–45%], nonsense mutations [14–20%], splice-site mutations [~10%]), whereas the remainder occur as missense mutations (20–25%), in-frame deletions or insertions (~5%), or as gross deletions involving all or part of the



• **Fig. 42.4** Menin has nuclear and cytoplasmic roles. Loss of menin expression (*blue boxes*) in endocrine tissues may result in increased cell proliferation by multiple pathways. In the nucleus, the loss of menin results in disruption of its interaction with transcription factors JUND and PRMT5 that lifts the transcriptional repression of target genes *Gastrin* and *GAS1*, respectively; binding to MLL1, MLL2, and SMAD3 (a TGFβ signaling component) to promote transcription of target genes; and ability to regulate the WNT pathway because β-catenin is no longer prevented from entering the nucleus by menin, thereby enabling transcription of WNT pathway target genes. Interactions with additional transcription factors and chromatin-modifying protein complexes may further modulate oncogenic signaling pathways. In the cytoplasm, loss of menin reduces its inhibitory actions on the mammalian target of rapamycin (mTOR) pathway by binding to AKT (downstream of PI3K, part of the RTK signaling pathway) and preventing its translocation to the plasma membrane, and KRAS-induced proliferation (by possible inhibition of ERK-dependent phosphorylation (P) and prevention of the interaction between SOS1 and KRAS). All pathways affect proliferation, which involves both nuclear and cytoplasmic mechanisms (shown in the cytoplasm only). Ligands are shown as *green circles*, and receptors are *orange circles*. *Akt*, protein kinase B; *B-Raf;* serine/threonine-protein kinase B-Raf; *CDKN,* cyclin-dependent kinase inhibitor; *ERK,* extra signal-related kinase; *FZD,* frizzled; *GAS1,* growth arrest specific 1; *GF,* growth factor; *MEK,* mitogen-activated protein kinase kinase; *MLL,* mixed lineage leukemia; *PI3K,* phosphoinositide 3-kinase; *PRMT5,* protein arginine N-methytransferase 5; *RASSF1A,* Ras-associated domain family member 1 isoform A; *Smad3,* mothers against decapentaplegic hormone 3; *SOS1,* sons of sevenless 1; *SST,* somatostatin; *SSTR,* SST receptor;*TGFβ,* transforming growth factor β; *TGFβR*, TGFβ receptor; *WNT,* wingless-related integration site. (Modified from Frost M, Lines KE, Thakker RV. Current and emerging therapies for PNETs in patients with or without MEN1. *Nat Rev Endocrinol.* 2018;14[4];216–227.)

MEN1 gene (1–2.5%).[148,150] The mutations are observed throughout the entire coding region of the *MEN1* gene, although nine individual mutations have been reported to account for more than 20% of all germline *MEN1* mutations (e.g., c.249_252delGTCT, c.292C>T, c.358_360delAAG, c.628_631delACAG, c.784-9G>A, c.1243C>T, c.1378C>T, c.1546delC, c.1546_1547insC).[148] In addition, 11 individual codons (i.e., 45, 69, 70, 139, 156, 183, 220, 253, 418, 436, and 516) are reported to be affected by five or more different *MEN1* mutations.[150] Together, these studies indicate that specific regions of the *MEN1* gene may be more susceptible to mutation, and it is notable that some of these mutations occur within repetitive DNA sequences, consistent with a replication-slippage model of mutagenesis. For example, the delC and insC (c.1546-1547) in codon 516 involve a poly(C)7 tract, and a slipped-strand mispairing model has been proposed to explain the high number of insertions or deletions at this site.[148,151] An alternative proposed explanation for the occurrence of these recurrent mutations in unrelated kindreds is the presence of population-specific founder mutations, whose presence may be established by haplotype analysis.[143,152,153]

Correlations between *MEN1* mutations and clinical manifestations of the disorder appear to be absent.[4,148,151] For example, studies of several large MEN1 kindreds, each harboring the same *MEN1* mutation, have shown that members of the respective families can develop a different range of tumors.[4,154] However, it has been reported that mutations of codons 428 through 610, which disrupt menin interaction with the checkpoint kinase 1 (CHES1) protein, are associated with a higher frequency of aggressive pancreatic NETs and a higher mortality than other *MEN1* mutations,[155] and that mutations of the three menin domains (codons 1–40, 139–242, and 323–428) that affect the interaction with JunD had a higher risk of premature death when compared with other *MEN1* mutations.[156] Further studies are required to validate these findings. In addition, some kindreds with germline *MEN1* mutations have been reported to not develop the full clinical phenotype of MEN1. For example, families with the Burin or prolactinoma variant of MEN1, who harbor specific nonsense mutations (i.e., Tyr312Ter, Arg460Ter), are reported to be characterized by a high occurrence of prolactinomas but a low occurrence of gastrinoma.[157,158] Similarly, somatotropinomas were not observed in a large kindred from Tasmania carrying a splice site *MEN1* mutation (c.446-3C>G).[159] Other families with germline *MEN1* mutations only develop parathyroid tumors, a condition referred to as familial isolated hyperparathyroidism (FIHP).[148,150,160] These phenotypic variants may be due to the specific *MEN1* mutation or genetic modifiers. For example, FIHP, when compared with MEN1, is associated with a high occurrence of missense *MEN1* mutations (~38% vs. 23%, $p < .01$), and several of the FIHP missense mutations, when compared with MEN1-associated mutations, have been reported to retain menin protein stability and biologic activity, consistent with the milder phenotype.[161] However, some FIHP kindreds have the same protein-truncating mutations that occur in MEN1 families, thereby implicating a role for genetic modifiers. Germline *MEN1* mutations have also been reported in patients with apparently sporadic pancreatic NETs, although it is unclear whether all such patients were systematically evaluated for the presence of other MEN1 manifestations.[162] Independent of *MEN1* genotype, one study reported that the type O blood group was associated with an increased risk of NETs in MEN1 patients,[163] although a subsequent study found no such relationship.[164]

Finally, it should be noted that approximately 10% of patients with a clinical diagnosis of MEN1 do not harbor mutations in the *MEN1* coding region,[165] and these individuals may harbor mutations in the promoter or untranslated regions of the gene, or represent phenocopies with mutations in other genes (see later discussion).[1] In addition, some of these patients, when compared with patients with *MEN1* mutations, have been reported to present with the first endocrine tumor at a later age, to very rarely develop a third MEN1 manifestation,[165] and to have a greater life expectancy.[72,165] Thus, it seems likely that such individuals may have two coincidental sporadic endocrine tumors rather than the hereditary MEN1 syndrome.[165]

MEN1 *Polymorphisms*

At least 35 different common germline variants (i.e., polymorphisms with minor allele frequency [MAF] >0.5%) are observed in the coding and nonregions of the *MEN1* gene, comprising 5 in the coding region (3 synonymous and 2 nonsynonymous [i.e., p.Arg176Gln and p.Ala546Thr in the canonical transcript]), 20 in the intronic regions, and 10 in the untranslated regions (http://phase3browser.1000genomes.org/ and http://gnomad.broadinstitute.org/). It is important to recognize the occurrence of these polymorphisms, and especially the nonsynonymous coding region variants, as their finding in an apparently affected index case does not necessarily equate to pathogenicity, and careful evaluation of any potential novel *MEN1* mutation is required. For example, approximately 200 rare (MAF <0.5%) missense variants are observed in the GnomAD dataset (http://gnomad.broadinstitute.org/), and most of these will not be of clinical significance. The recognition of such variants as benign is becoming easier owing to the availability of large population-based databases.[145]

MEN1 Phenocopies and Mutations in Other Genes

Phenocopy refers to the development of disease manifestations, which are usually associated with a particular gene but have instead been caused by mutations in another gene or environmental factors. Phenocopies are reported in 5% to 10% of MEN1 kindreds and may occur in different clinical settings.[21,22,159,166] For example, phenocopies have been reported in the context of familial MEN1, in which patients manifesting a MEN1-associated tumor (e.g., pituitary or parathyroid tumor) do not harbor the familial *MEN1* mutation.[21,22] Phenocopies may also occur in the context of patients or kindreds presenting with an apparent clinical diagnosis of MEN1 (i.e., ≥2 MEN1-associated endocrine tumors) who do not harbor a *MEN1* mutation but instead have mutations in another gene, which is more typically associated with a different disease.[21,22] Such genes include *CDC73*, which encodes the tumor suppressor parafibromin, mutations of which result in hyperparathyroidism-jaw tumor syndrome[167]; *CASR*, which encodes the calcium-sensing receptor, mutations of which result in familial benign hypocalciuric hypercalcemia type 1 and/or FIHP[168,169]; and *AIP*, which encodes the aryl hydrocarbon receptor interacting protein, mutations of which are associated with familial isolated pituitary adenoma.[170] Finally, a small percentage of patients manifesting clinical features of MEN1 in the absence of a *MEN1* mutations may have a mutation in *CDKN1B*, which results in the associated disorder of MEN4[171,172] (see Table 42.1). Thus, the possibility of a phenocopy or an alternate genetic diagnosis should be considered in those presenting with typical or atypical manifestations of MEN1 in whom a *MEN1* mutation is not found. In addition, genetic testing should be undertaken in all kindred members where a familial *MEN1* mutation is present, irrespective of the clinical disease status of the individual.

Somatic MEN1 *Mutations*

More than 90% of tumors from MEN1 patients have LOH involving loci on chromosome 11q13, and this has generally been taken as evidence that the *MEN1* gene acts as a tumor suppressor (i.e., as a result of biallelic *MEN1* inactivation).[24,29,173] The identification of the *MEN1* gene and its encoded protein menin as a key determinant of

endocrine neoplasia has led to the investigation of its role in the development of sporadic endocrine tumors, which have been shown to have somatic inactivation of both *MEN1* alleles, which most commonly results from an inactivating point mutation or small indel affecting one allele and a large-scale deletion of the other.[148] Overall, somatic *MEN1* mutations are observed in approximately 35% of parathyroid tumors,[174,175] 40% to 45% of NF pancreatic NETs,[162,176] approximately 40% of gastrinomas,[148] 0% to 15% of insulinomas,[177] 3% to 5% of pituitary tumors,[148,178] approximately 15% to 20% of pulmonary carcinoids,[179,180] less than 3% of small intestinal NETs,[181,182] less than 3% of adrenocortical tumors, 10% of angiofibromas, and approximately 30% of lipomas.[148] The somatic mutations observed in these sporadic tumors are reported to occur throughout the *MEN1* coding region and comprise loss-of-function and missense mutations, similar to the germline mutations.[148] The identification of somatic *MEN1* mutations in sporadic tumors currently has little clinical utility, although it is possible that tumor genotyping may contribute to future personalized treatment approaches. In addition, such mutation profiling of tumors may provide prognostic information, and it has been reported that the presence of a somatic *MEN1* mutation in sporadic pancreatic NETs is associated with an improved survival when compared with those without a *MEN1* mutation.[176]

Functions of the Menin Protein and Insights Into Mechanisms of Tumorigenesis

Menin is a ubiquitously expressed protein that is located predominantly in the nucleus, and it is reported to have at least three nuclear localization signals within its C-terminus.[143,144,183] Menin is also reported to be found in the cytoplasm, where it is regulates key signaling pathways.[184] Menin, which functions as a scaffold protein, has been reported to interact with more than 20 proteins and molecules that facilitate its role in transcriptional and epigenetic regulation, genome stability, DNA repair, cell division, cell signaling, and cell motility[143,144,183] (see Fig. 42.4). The roles of menin in transcription, in which it can act as an activator and repressor, and as a component in some canonical signaling pathways, will be briefly reviewed, as these illustrate menin's ability in behaving as a tumor suppressor and oncogenic cofactor, depending on the cellular context.[183] Thus, in the majority of endocrine tissues, menin acts as a tumor suppressor, whereas in other neoplasms including leukemia, pediatric glioma, and prostate, hepatocellular, and breast cancer, it acts as an oncogene.[143,144,183,185,186]

Menin activates transcription by interacting with the mixed-lineage leukemia proteins 1 and 2 (MLL1 and MLL2) histone methyltransferase complexes,[187,188] which are responsible for histone H3 lysine 4 residue trimethylation (H3K4Me3), which causes chromatin modification and is associated with activation of transcription[187–189] (see Fig. 42.4). Menin/MLL1 occupancy has been reported to occur at the promoter regions of thousands of genes, and to generally correlate with active transcription, although menin loss is associated with changes in transcription of only a small subset of genes.[190] The menin-MLL complex is a key activator of *Hox* gene expression[187] and the p27 and p18 cyclin-dependent kinase inhibitors,[191,192] and menin physically links MLL with the lens epithelium–derived growth factor (LEDGF), which is a chromatin-associated protein required for MLL-dependent transcription that has been implicated in leukemic transformation.[193] Analysis of the crystal structure of menin in complex with MLL1 and LEDGF has demonstrated the key interacting domains, in which menin adopts a "curved left hand" structure, with the N-terminus resembling the thumb (a long β-hairpin), the central portion the palm, and the C-terminus the curved fingers.[144,194] A deep pocket within the central "palm" region forms the MLL1-binding site, whereas LEDGF is observed to simultaneously bind both menin and MLL1. Thus, menin acts as a molecular adaptor to link such proteins, and disruption to these interacting domains abrogates downstream transcriptional activity.[144,194] However, the intact menin-MLL complex enhances transcriptional activation of genes (e.g., *HOX* genes, *EZH2*) involved in leukemogenesis,[187,195,196] and small molecule inhibitors targeting this protumorigenic function of the menin-MLL interaction have been developed and are shown to be effective for the treatment of MLL-dependent leukemia in preclinical in vitro and in vivo models.[197,198] The menin-MLL complex is also reported to act as an oncogenic coactivator of androgen receptor (AR) signaling in prostate cancer, which is effectively inhibited in vivo using small molecule menin-MLL inhibitors[185]; ESR1 signaling in breast cancer, in which it recruits the transcriptional enhancers FOXA1 and GATA3 to support expression of cancer-relevant genes[183]; and platelet-derived growth factor receptor A signaling in pediatric glioma.[186] Transcriptional activation, independent of H3K4Me3 activity, has been reported to occur by a direct interaction between menin and the oncogene MYC in fibrosarcoma and hepatocellular carcinoma (HCC) cell lines.[199]

Menin regulates transcriptional repression through several direct and indirect mechanisms. Thus, menin directly interacts with JunD (see Fig. 42.4), a member of the activating protein 1 (AP1) transcription factor family,[143,144] and JunD utilizes the same menin binding pocket employed by MLL1 such that JunD and MLL1 likely compete for menin binding.[194] The binding of menin to JunD facilitates the recruitment of the Sin3 transcription regulator homolog (mSin3A) histone deacetylase (HDAC) complex to inhibit JunD-dependent transcription, and the forkhead transcription factor CHES1 associates with the menin-mSin3A transcriptional repressor complex, to regulate an S-phase checkpoint pathway related to DNA damage.[143,144,183] Menin binding to JunD blocks c-Jun N-terminal kinase (JNK)-mediated phosphorylation, thereby inhibiting post-translational modification (and subsequent activation) of JunD.[194] In addition, menin represses expression of the *PTN* gene, which encodes the pleiotrophin pro-proliferative receptor, and this increases the Polycomb gene enhancer of zeste homolog 2 (EZH2)-mediated histone H3 lysine 27 trimethylation (H3K27M3), which is a negative mark for gene transcription. Studies of HCC have revealed that elevated levels of menin, EZH2, and H3K27Me3 are associated with a poor prognosis, whereas inhibiting H3K27Me3 effectively blocked the aggressive phenotype of HCC cells.[200] Menin also recruits the transcription factor PRMT5 (see Fig. 42.4) to the promoter of the growth arrest–specific protein 1 (*GAS1*) gene, which is an important cofactor required for the binding of the Sonic Hedgehog (Shh) ligand to its cell surface receptor, and thereby acts as a negative regulator of Hedgehog signaling.[201] Menin also represses transcription mediated by members of the NFκB family, and other menin regulatory functions include modulation of gene expression through regulation of microRNA biosynthesis,[147,202] regulation of expression of nuclear receptor target genes (e.g., direct interaction with ERα), and participation in the DNA damage response in which menin undergoes phosphorylation and influences the transcriptional response through an altered affinity for RNA polymerase II.[203]

Menin modulates several canonical signaling pathways involved in endocrine and nonendocrine tumorigenesis,[92,144] which are cell- and tissue specific. For example, menin has been reported to act as a negative regulator of the Wnt/β-catenin signaling pathway (see Fig. 42.4) by regulating the phosphorylation and export of β-catenin out of the nucleus and into the cytoplasm, thereby diminishing Wnt target gene expression,[184] and loss of menin expression in human and murine pancreatic NETs has been reported to result in activation of the Wnt signaling pathway. This has provided

support for the rationale of using small molecule Wnt inhibitors for the treatment of MEN1-associated pancreatic NETs in mutant mice.[204] However, another study has reported that menin acts as a positive regulator of Wnt signaling in pancreatic endocrine cells, thereby highlighting apparent paradoxical activities dependent on the cellular environment.[205] A similar complex interplay is observed between menin and RAS signaling pathways, in which menin has been reported to determine the outcome of K-RAS signaling (see Fig. 42.4) in a cell-context specific manner within the pancreas.[206] K-RAS activation typically enhances cell proliferation, but in pancreatic endocrine cells, it suppresses proliferation; this activity is due to menin-dependent preferential activation of the antiproliferative RAS effector RASSF1A, with simultaneous suppression of the pro-proliferative RAF/MAPK pathway (see Fig. 42.4). Downregulation of menin in pancreatic endocrine cells then releases this inhibition and results in greater RAF/MAPK signaling and endocrine cell proliferation.[206] Other signaling pathways also reported to be modulated by menin expression include transforming growth factor β (TGFβ), bone morphogenetic protein (BMP), Hedgehog, AKT, and MYC pathways[92,144,189,199] (see Fig. 42.4). The better understanding of these complexities of menin function, together with the identification of key interacting proteins and alterations in major signaling pathways, has facilitated the identification of several potential novel therapeutic targets, which have been evaluated in preclinical models for both MEN1- and non–MEN1-related tumors, and these include menin-MLL inhibitors, other epigenetic-modifying agents, and Wnt inhibitors (see Fig. 42.4).

Animal Models

Menin is highly evolutionary conserved in mammalian species (i.e., 90% and 97% DNA and protein identity, respectively, between and human and mouse), whereas more evolutionary distant organisms harbor orthologues with lower degrees of similarity (e.g., zebrafish [*Danio rerio*], fly [*Drosophila melanogaster*]). Although some studies of menin function have employed these more distant model organisms (i.e., homozygote deletion of the *MEN1* orthologue *Mnn1* in *D. melanogaster* results in viable offspring but increased sensitivity to DNA damage and other environmental stressors [e.g., heat shock, hypoxia]),[207] the major insights into the in vivo function of menin have arisen from studies of conventional and conditional *Men1* mouse knockout models.[207]

Conventional Men1 *Mouse Knockout Models*

Several conventional *Men1* knockout models have been established by the targeted deletion of different exonic regions of the *Men1* gene including deletion of exons 1-2,[208] exon 2 alone,[209] exon 3,[210] and exons 3-8,[211] resulting in either the absence of *Men1* transcription (i.e., through the loss of the transcription start site) or the generation of severely truncated *Men1* transcripts.[207] Despite some subtle differences in tumor spectrum, each of these models recapitulates major features of the clinical MEN1 syndrome; thus, heterozygous ($Men1^{+/-}$) mice are observed to develop multiple tumors affecting the pancreatic islets, anterior pituitary, parathyroid, and adrenal glands.[208–211] $Men1^{+/-}$ mice also develop gonadal, thyroid, and prostate tumors that are not typically associated with MEN1 in patients.[207,208,210,211] In several instances, biochemical and/or immunohistochemical analysis have confirmed features consistent with the respective human tumors, including raised plasma insulin levels and pancreatic NETs consistent with insulinoma, raised PTH and/or hypercalcemia and parathyroid hyperplasia consistent with PHPT, plurihormonal expressing pancreatic NETs, and somatostatin receptor type 2 (SSTR2) and vascular endothelial growth factor A (VEGF-A) expression in pancreatic NETs and pituitary tumors.[208,210,211] In each of the $Men1^{+/-}$ models, the various tumors emerged in a time-dependent fashion, typically commencing at approximately 9 months, and molecular analysis of the tumors confirmed LOH at the *Men1* locus and loss of menin expression, consistent with biallelic *Men1* inactivation for tumor development, and its role as a tumor suppressor.

In each of the conventional mouse models, homozygous *Men1* ablation ($Men1^{-/-}$) is reported to result in embryonic lethality between embryonic day (E) 10.5 and 14.5 with craniofacial defects, hemorrhages, edema, and neural tube defects, as well as abnormalities of early pancreatic endocrine development.[212,213] Thus, in contrast to the situation in *D. melanogaster*, menin appears essential for viable mammalian development. However, the timing of embryonic death and the specific observed phenotypes are dependent on the background strain of mouse, indicating a possible role for genetic modifiers.[212] Furthermore, it is possible that the differences in tumor phenotype observed in each of the conventional $Men1^{+/-}$ models may also reflect similar differences in genetic background and/or influences of genetic modifiers. The potential synergy between menin and other key tumor suppressor genes has also been investigated using $Men1^{+/-}$ mice. For example, an acceleration or augmentation of tumor development was not observed in $Men1^{+/-}$ mice that also had deletion of one allele of the retinoblastoma gene ($Men1^{+/-}/Rb^{+/-}$), thereby indicating that menin and the retinoblastoma protein (pRB) act in common tumorigenic pathways.[214] This is supported by the observation that genetic ablation of the retinoblastoma binding protein 2 (RBP2; also called *JARID1A* or *KDM5A*), which is a histone demethylase that contributes to the tumor suppressor activity of pRB, decreases tumor formation, and prolongs survival of a beta-cell specific *Men1* knockout mouse model.[215] In contrast, the combined mutation of *Men1* and the cyclin-dependent kinases (CDK) inhibitor *Cdkn1b* ($Men1^{+/-}/Cdkn1b^{-/-}$) was not observed to significantly influence tumor expression, whereas disruption to *Cdkn2c* (encoding the p18 protein) accelerated endocrine tumor development in *Men1* mice ($Men1^{+/-}/Cdkn2c^{-/-}$).[216]

Conditional Men1 *Mouse Knockout Models*

The conventional *Men1* mouse models have several potential limitations to study tumor biology, and these include the incomplete penetrance of organ-specific tumor formation and the latency period required for tumorigenesis (i.e., due to the need for a somatic "second hit") such that it is difficult to study early events in tumor development. To address these deficiencies, several tissue-specific conditional knockout models have been generated, including those under temporal control, thereby enabling the consequences of controlled biallelic *Men1* inactivation to be evaluated in endocrine and nonendocrine tissues.[207] For example, a parathyroid-specific conditional mouse model, which was generated using a Cre-recombinase under the control of the PTH promoter, was found to develop hyperparathyroidism at a higher penetrance than the respective conventional $Men1^{+/-}$ model.[217] Pancreatic beta-cell specific $Men1^{-/-}$ mice, which have been generated exploiting different rat insulin promoter (RIP) Cre mouse lines, have been reported to have early-onset islet hyperplasia, followed by highly penetrant insulinoma formation, although some models also developed prolactinomas because of Rip-Cre expression in the pituitary.[207] The targeted deletion of *Men1* in both endocrine and exocrine pancreatic cells, achieved by using a Pdx1-Cre promoter, resulted in increased endocrine cell proliferation and pancreatic NET formation, but no exocrine tumor

manifestations.[218] Pancreatic α-cell-specific *Men1* knockout models surprisingly did not develop glucagonoma but instead had insulinomas, thereby suggesting the possibility of transdifferentiation of α-cells to β-cells, or the presence of modulatory paracrine effects.[219,220] Temporal regulation of beta-cell *Men1* inactivation has been achieved through the use of inducible conditional knockouts (i.e., combining established Rip-Cre *Men1* models with transgenic mice harboring an ER-Cre, thereby facilitating *Men1* ablation on exposure to tamoxifen)[207,221]; studies of these mice have revealed a rapid onset of islet cell proliferation, following *Men1* inactivation, and this mutant mouse may provide a model for investigation of early events in tumorigenesis and for assessing the effects of novel therapies on tumor development.[221]

Nonendocrine Phenotypes in Men1 *Mouse Knockout Models*

Tissue-specific inactivation of *Men1* in Pax3 or Wnt1 expressing neural crest cells resulted in mutant mice with cranial bone defects, cleft palate, and perinatal death,[222] and menin inactivation in mature osteoblasts (using an osteocalcin-cre) resulted in reduced bone mineral density, trabecular bone volume, and cortical bone thickness,[223] thereby demonstrating a critical role for menin in bone development. Hepatocyte-specific *Men1* deletion is reported to result in a high-fat diet-induced liver steatosis through a mechanism involving histone deacetylation.[224] Moreover, induction of hepatic cancers by a chemical carcinogen has been reported to be reduced in *Men1*$^{-/-}$ female mice, and studies of this model have indicated that menin plays an important epigenetic role involving H3K4Me3 in promoting liver tumorigenesis,[225]

Preclinical Evaluation of Drug Therapies in Men1 *Mouse Models*

The *Men1* knockout mouse models have been used to study several treatments, including gene therapy, new somatostatin analogues, epigenetic modulators, β-catenin antagonist, and MEK1/2 inhibitors.[92] These will be reviewed briefly.

In MEN1-associated tumors, biallelic inactivation of the *MEN1* gene is typically observed and consistent with a proposed tumor suppressor function for menin. Thus, *MEN1* gene replacement therapy offers a potential therapeutic strategy to restore menin function. To establish proof of concept, a replication-incompetent adenoviral vector containing the mouse *Men1* cDNA under a cytomegalovirus promoter was injected into the pituitary tumors of conventional *Men1*$^{+/-}$ female mice, resulting in restoration of menin expression and decreased tumor proliferation, thereby indicating the potential utility of such an approach.[226]

The efficacy of somatostatin analogue therapy has been assessed in different mouse models. Thus, pasireotide, a multiple-receptor-ligand somatostatin analogue targeting SSTR1 through SSTR3 and SSTR5, was observed to reduce proliferation and increase apoptosis of pancreatic NETs and pituitary tumors in conventional *Men1*$^{+/-}$ mice and pancreatic NETs in *Pdx1-Cre- Men1* conditional knockout mice.[227,228] Systemic pasireotide therapy was also found to reduce pancreatic NET formation in *Men1*$^{+/-}$ mice,[92,227] thereby indicating that it has chemopreventive actions. Use of a hybrid adeno-associated virus and phage displaying biologically active octreotide for targeted delivery of a tumor necrosis factor transgene to pancreatic NETs in the *Pdx1-Cre-Men1* model has shown that this can reduce tumor volume and improve survival.[229]

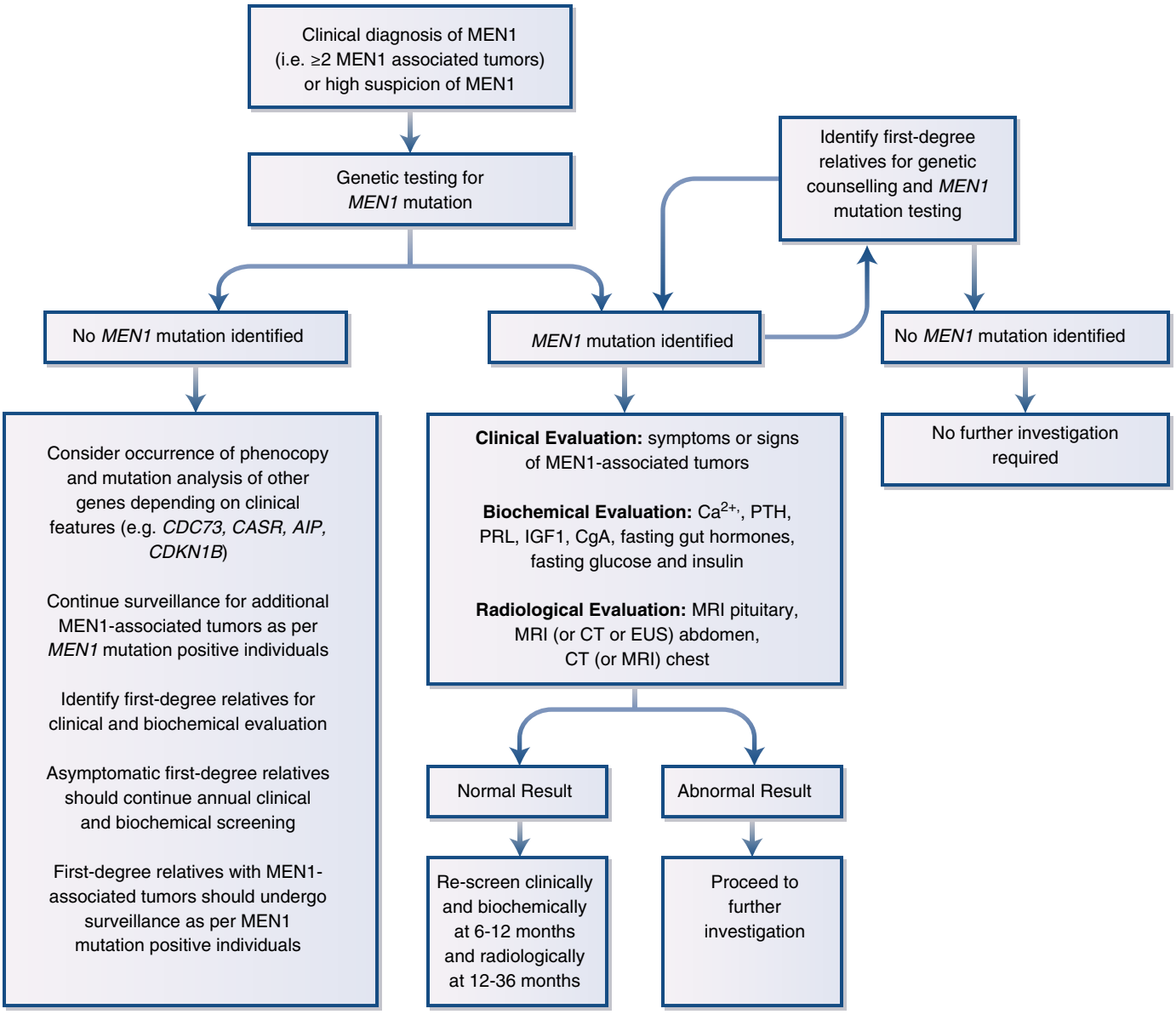
MEN1-associated tumors are reported to have alterations in epigenetic mechanisms such as histone modifications and DNA methylation, and menin interacts with several histone modifying proteins, including MLL1 and PRMT5 (see Fig. 42.4), and deacetylase complexes (e.g., histone deacetylase complex subunit MSin3A). The use of epigenetic modulators to treat pancreatic NETs, in *Men1*$^{+/-}$ mice was assessed using JQ1, an inhibitor of the bromo and extraterminal domain (BET) family of proteins that bind to acetylene residues to promote gene transcription. JQ1 reduced proliferation and increased apoptosis of pancreatic and bronchial NET cell lines in vitro, and also in pancreatic NETs, in vivo, that developed in beta-cell–specific menin knockout (*RIP-Cre-Men1*$^{-/-}$) mice.[230] Thus, inhibitors that target epigenetic mechanisms may represent a novel class of anticancer drugs in MEN1.

Other drugs that have been effective in treating pancreatic NETs in *Men1* knockout mice include WNT and K-RAS signaling modulators (see Fig. 42.4). Thus, a small molecule β-catenin antagonist (PKF115-584) reduced proliferation of the pancreatic NETs in *RIP-Cre-Men1*$^{-/-}$ mice and ameliorated the excess in insulin secretion.[204] In addition, studies of pancreatic islet cells and NETs obtained from *Ins2-Cre Men1*$^{-/-}$ mice have demonstrated that in pancreatic endocrine beta cells, K-RAS activates opposing growth pathways, but the antiproliferative pathways dominate due to menin activity, which prevents the MAPK pathway from driving growth, while leaving the RASSF1A intact (see Fig. 42.4). Thus, loss of menin in the pancreatic NETs increases proliferation due to removal of the blockage of MAPK-driven proliferation downstream of K-RAS, whereas K-RAS signaling increases proliferation by decreasing unopposed RASSF1A activity.[206] The importance of the RAF/MEK/ERK pathway in driving inappropriate growth and survival of pancreatic beta cells with reduced menin activity was further demonstrated by the use of MEK1/2 inhibitors (i.e., PD0325901 or GSK1120212) that modulate the KRAS pathway, and which revealed these inhibitors to have antiproliferative and cytotoxic actions. Thus, these may provide novel therapeutic approaches for menin-deficient tumors.

Genetic Testing, Tumor Surveillance, and Organization of Care

Clinical Utility of MEN1 *Mutational Analysis*

MEN1 mutational analysis is helpful in clinical practice in several ways that include (1) confirmation of the clinical diagnosis, (2) identification of family members who harbor the *MEN1* mutation and require screening for tumor detection and early treatment, and (3) identification of family members who do not harbor the familial germline *MEN1* mutation and can therefore be reassured[1,21] (Fig. 42.5). Current guidelines recommend that *MEN1* mutational analysis should be undertaken in (1) an index case with two or more MEN1-associated tumors (i.e., parathyroid, pancreatic islet, or pituitary tumors); (2) all first-degree relatives of a known *MEN1* mutation carrier irrespective of whether they are asymptomatic or manifest associated clinical features (i.e., having symptoms, signs, or biochemical and/or radiologic evidence of one or more MEN1-associated tumors); and (3) in patients with suspicion of MEN1 or atypical manifestations, which include those with a parathyroid adenoma below the age of 30 years and/or multigland parathyroid disease, gastrinoma, or multiple pancreatic NETs presenting at any age, or individuals who have two or more MEN1-associated tumors that are not exclusively limited to the classic triad of parathyroid, pancreatic islet, and parathyroid tumors (i.e., parathyroid plus adrenal tumor).[1,21] These recommendations are supported by results from several studies. Thus, a



• **Fig. 42.5** An approach to genetic testing and tumor screening in MEN1. Index cases, or individuals in whom there is a high suspicion of clinical MEN1, should be offered genetic counseling and *MEN1* mutation testing. The identification of a germline MEN1 mutation should prompt entry into a periodic clinical, biochemical, and radiologic screening program. At the same time, all first-degree relatives should be identified and offered genetic counseling and *MEN1* mutation testing irrespective of whether they express clinical features of MEN1 or are asymptomatic. Individuals who have inherited the *MEN1* mutation should enter periodic screening. First-degree relatives who have not inherited the *MEN1* mutation require no further follow-up and may be alleviated of the anxiety associated with the development of MEN1-associated tumors. For index cases in whom no *MEN1* mutation is identified (including the exclusion of partial or whole gene deletions [e.g., by multiplex ligation-dependent probe amplification analysis]), additional genetic testing may be indicated depending on the specific clinical features. This may include examination for mutations in genes associated with familial parathyroid syndromes including *CDC73* associated with the hyperparathyroidism-jaw tumor syndrome (HPT-JT) and the calcium-sensing receptor (*CASR*) associated with familial benign hypercalciuric hypercalcemia (FBHH), or cyclin-dependent kinase 1B (*CDKN1B*) and aryl hydrocarbon receptor interacting protein (*AIP*), which are rarely identified in those with clinical MEN1. Up to 10% of kindreds with clinical MEN1 may harbor phenocopies emphasizing the importance of accurate genetic evaluation. For MEN1 kindreds in whom no *MEN1* mutation is identified, a pragmatic approach is to offer clinical, biochemical, and radiologic screening to those with clinical manifestations of disease and to offer annual clinical and biochemical screening to asymptomatic first-degree relatives. Ca^{2+}, calcium; *CgA,* chromogranin A; *CT,* computed tomography; *EUS,* endoscopic ultrasound; *IGF1*, insulin-like growth factor 1; *MEN1,* multiple endocrine neoplasia type 1; *MRI,* magnetic resonance imaging; *PRL,* prolactin; *PTH,* parathyroid hormone. (Redrawn from Thakker RV, Newey PJ, Walls GV, et al. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metab.* 2012;97[9]:2990–3011.)

study of 200 patients with endocrine tumors reported that *MEN1* mutations occurred in more than 70% of individuals with two or more of the major MEN1-associated endocrine tumors (e.g., parathyroid, pancreatic pituitary tumors) and a family history of these tumors; approximately 60% of individuals with at least one of the major endocrine tumors and a first-degree relative with a major endocrine tumor; and 6% of patients with sporadic (i.e., nonfamilial) MEN1-associated endocrine tumors referred for testing, although the *MEN1* mutations were only observed in patients who had multiple endocrine tumors and/or were younger than 30 years of age.[231] In addition, another study has reported that the likelihood of finding a *MEN1* mutation correlates with the presence of clinical features, with 80% of index cases presenting with three MEN1-related tumors having a germline MEN1 mutation, which increased to more than 90% if family history revealed the presence of affected relatives, whereas only 15% of patients presenting with a single MEN1-associated tumor had a *MEN1* mutation, and this decreased to 0% when the family history revealed the absence of affected relatives.[232] A study of 205 patients with PHPT and a family history of PHPT revealed that approximately 45% had a *MEN1* mutation, and that multigland disease, male sex, and age younger than 45 years were independent predictors for an associated germline mutation, with odds ratios of 14, 1.7, and 8, respectively.[233] Finally, a study of 39 patients with atypical presentation (i.e., pituitary adenomas with pheochromocytoma/paraganglioma) identified two patients with germline *MEN1* mutations.[234] All of these studies indicate that failure to undertake genetic testing may result in a missed opportunity for early diagnosis.[1,235]

Following the genetic diagnosis of MEN1, predictive genetic testing should be offered to all first-degree relatives (see Fig. 42.5), and current guidelines recommend that this should be undertaken at the earliest opportunity.[1] Indeed, delays in the genetic diagnosis of first-degree relatives of an affected index case are reported to result in increased morbidity, highlighting the need for proactive cascade testing within MEN1 kindreds.[236] However, there are several ethical issues to consider, and pretest counseling and transparency are essential. Thus, patients may be concerned by the implications of finding a causative mutation on other family members, future reproductive decision making, and the potential for financial or societal discrimination. Indeed, a frequent concern of patients undergoing genetic testing is the potential implications on future employment or ability to get insurance, although many countries have legislation in place to protect individuals from such genetic discrimination. For example, in the United States, the Federal Genetic Information Nondiscrimination Act prohibits health insurance companies or employers from using genetic information to determine eligibility for health insurance coverage, or decisions in employment-related matters (i.e., hiring, promotion), respectively. With appropriate genetic counseling, most patients conclude that the potential benefits of genetic testing outweigh the potential harms. A further consideration involves the genetic testing of children, which is frequently undertaken with parental consent. Indeed, in view of the high penetrance of early-onset MEN1-associated tumors,[19] it is recommended that at-risk asymptomatic children (i.e., offspring of an affected parent) undergo genetic testing in the first decade of life and ideally before 5 years of age.[21,174]

Preconception genetic counseling, where appropriate, should be provided to individuals at risk of transmitting *MEN1* to their future offspring (i.e., symptomatic or asymptomatic *MEN1* mutation carriers), and advances in genetic testing combined with in vitro fertilization can now offer potential parents the opportunity to have preimplantation genetic diagnosis. This may be achieved by the direct sequencing of DNA from a small number of cells obtained from the early blastocyst, with subsequent implantation of only those embryos identified to not carry the mutation, thereby markedly reducing the risk of transmitting the genetic disease to the child.

Finally, it is important to emphasize that all MEN1 genetic testing should be undertaken in accredited genetics laboratories using validated testing strategies that adequately sequence all exonic and splice-site regions of the *MEN1* gene, thereby facilitating the reliable detection of all single nucleotide variants or small insertions or deletions (indels). In the absence of such mutations, multiplex ligation-dependent probe amplification should be employed to detect partial or whole gene deletions.

Surveillance of "At-Risk" Individuals

Current guidelines recommend that all individuals at high risk for MEN1 (i.e., mutant gene carriers) undergo periodic clinical, biochemical, and radiologic screening to facilitate the early detection and treatment of tumors with the aim of reducing their associated morbidity and mortality[1] (Table 42.2; see Fig. 42.5). Although it seems logical that such screening programs are likely to be beneficial, high-quality evidence supporting their effectiveness has not been established. However, most clinicians recommend the implementation of interval surveillance, although the frequency and scope of investigation is debated.[19,72,85] Two particularly controversial areas involve the detection of NF pancreatic NETs and thymic carcinoid tumors, which represent the two leading causes of premature MEN1-related death. In particular, the age to begin screening, the use of biochemical tumor markers, and the optimal radiologic approach are areas that are controversial and under debate.[19,85,98,99,237] For example, current guidelines recommend commencing pancreatic imaging from the age of 10 years, and this is supported by several reports that indicate a high penetrance of NF-NETs in the second decade of life.[19,97,101] However, some centers recommend that imaging should be delayed until at least 16 years. Similarly, for thymic carcinoid tumors, the rapid growth rates and aggressive disease course would favor at least annual screening with thoracic CT, but the high cumulative ionizing radiation dose associated with such a schedule is considered unacceptable, particularly as such tumors occur in only a minority of MEN1 patients. Another controversial topic relates to breast screening in women with MEN1, with some groups advocating screening from age 40 years because of the reported increased relative risk of breast cancer.[139,141] Thus, several uncertainties exist, and it is important to individualize these current recommendations to the patient, and to adapt them for the local availability of resources and wishes of the patient, who should be involved in the decision-making process. For example, a recent study highlights that the majority of MEN1 patients are highly fearful of disease occurrence, both for themselves and their relatives, which may be compounded by regular surveillance screening.[238] Furthermore, this psychological distress is reported to be associated with a lower health-related quality of life, and these factors should be considered.[238]

MEN Types 2 and 3

MEN2, which has been referred to as MEN2A and Sipple syndrome, is an autosomal dominant disorder with a reported incidence of 1 in 80,000 to 200,000 live births, which is characterized by the occurrence of medullary thyroid carcinoma

TABLE 42.2 Suggested Screening Guidelines for Individuals at Risk of MEN1

MEN1-Associated Tumor	Age to Begin Screening (Years)	Biochemical Screening Test (Annually)	Imaging Screening Test (Time Interval)
Parathyroid	8	Calcium, PTH	None
Pancreatic			
Gastrinoma	20	Fasting gastrin	None
Insulinoma	5	Fasting glucose (± insulin)	None
Other pancreatic NET (e.g., nonfunctioning)	10	Chromogranin A, gastrointestinal hormone profile[a] (e.g., glucagon, pancreatic polypeptide, vasoactive intestinal peptide)	MRI abdomen, EUS (annually)
Pituitary			
Prolactinoma	5	Prolactin	None
Somatotropinoma	5	Insulin-like growth factor 1	None
Other pituitary adenoma (e.g., nonfunctioning NET)	10[b]	None, unless signs or symptoms of functioning tumor (e.g., corticotroph adenoma)	MRI pituitary (every 3 years)
Adrenocortical	<10	None, unless signs or symptoms of functioning tumor or tumor >1 cm on imaging	MRI abdomen (annually)
Thymic/bronchial carcinoid	15	None	CT or MRI chest (every 1–2 years)

[a]Although chromogranin A, pancreatic polypeptide, and glucagon concentrations can be elevated with nonfunctioning PNETs, they have low sensitivity and specificity such that their value is debated.

[b]Although pituitary tumors are reported in MEN1 patients as young as 5 years of age, in the absence of symptoms, signs, or biochemical evidence of a pituitary adenoma, pituitary imaging may be delayed until after 10 years of age to coincide with pancreatic imaging.

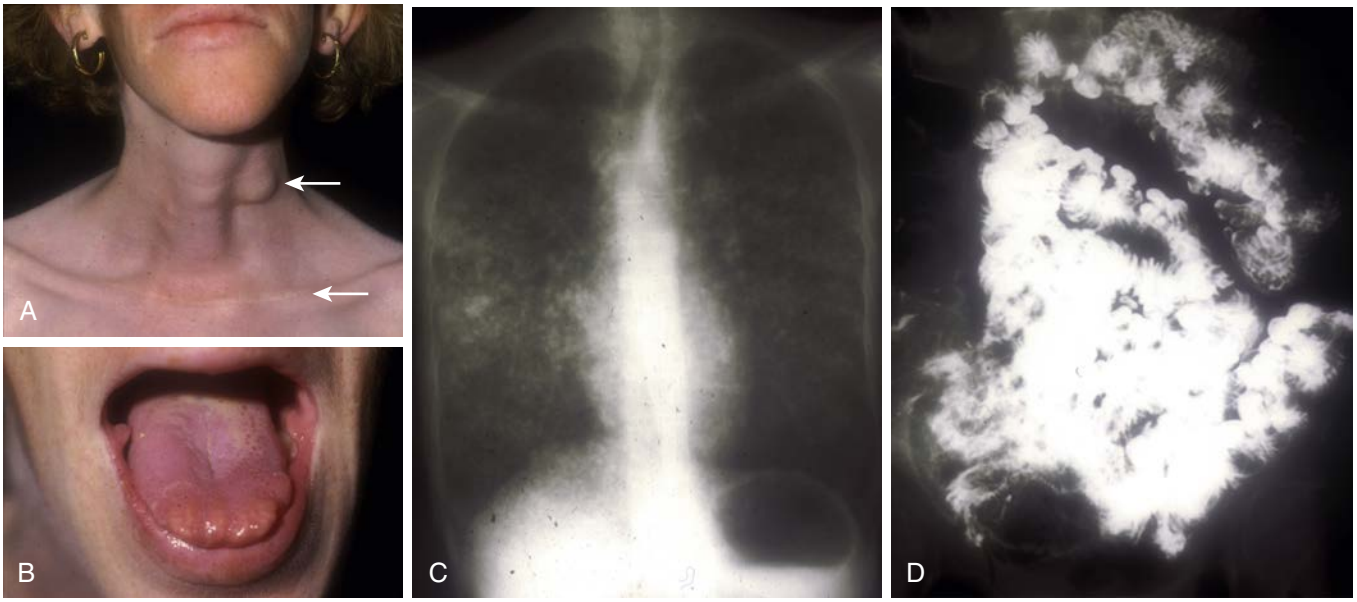
CT, Computed tomography; *EUS,* endoscopic ultrasound; *MEN1*, multiple endocrine neoplasia type 1; *MRI,* magnetic resonance imaging; *NET,* neuroendocrine tumor; *PTH,* parathyroid hormone.

Modified from Thakker RV, Newey PJ, Walls GV, et al. Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *J Clin Endocrinol Metab.* 2012;97(9):2990–3011.

(MTC) in association with pheochromocytoma and parathyroid tumors.[3,239,240] MEN2 also includes three variants, which are MEN2A with Hirschsprung disease (HSCR), MEN2A with cutaneous lichen amyloidosis (CLA), and familial MTC-only (FMTC),[3] in which MTC is the sole manifestation of the syndrome. MEN3, also referred to as MEN2B, is characterized by the occurrence of MTC and pheochromocytoma, without PHPT, but in association with a marfanoid habitus, mucosal neuromas, medullated corneal fibers, and intestinal autonomic ganglion dysfunction leading to megacolon[3] (Fig. 42.6). MEN2 is more common than MEN3, with MEN2 accounting for greater than 90% and MEN3 between 5% and 10% of patients, although MTC in MEN3 usually arises in infancy and has a more aggressive course.[3]

Historically, the association between thyroid carcinoma and pheochromocytoma was first reported by Sipple[241] in 1961, with Cushman and Rochester reporting a family with autosomal dominant inheritance of pheochromocytomas, MTC, and parathyroid adenoma,[242] and Steiner and colleagues[243] in 1968 proposing the term *MEN2* to describe a family with MTC, pheochromocytoma, PHPT, and Cushing syndrome. The association of multiple neuromas, pheochromocytoma, and MTC was reported by Williams and Pollock[244] in 1966, and Schimke and colleagues[245] in 1968, and Chong and colleagues[246] in 1975, used the term *MEN2B* to describe this disorder. Interestingly, the first descriptions of MEN2A and pheochromocytoma, in 1886, were in an 18-year-old woman who had bilateral adrenal tumors, and whose relatives, in the Black Forest in Germany, have subsequently been reported to have pheochromocytoma and MTC due to a rearranged during transfection (*RET*) mutation (Cys634Trp), indicating that this patient and family have MEN2A.[247] The first descriptions of patients with likely MEN3 were in 1922 and 1923 by Wagenmann[248] and Froboese,[249] respectively.

Genetic studies of MEN2 and MEN3 families throughout the 1980s and 1990s led to the demonstration that mutations of the RET proto-oncogene, which is located on chromosome 10q11.21 and encodes an RTK, caused the MEN2 and MEN3 syndromes. Approximately 95% of MEN2 *RET* mutations involve the cysteine-rich extracellular domain (ECD), with mutations of Cys634 accounting for approximately 85% of all MEN2 mutations, whereas approximately 95% of MEN3 *RET* mutations are of Met918, which is located in the intracellular tyrosine kinase (TK) domain (Table 42.3 and Fig. 42.7; see Table 42.1). Moreover, the identification of these *RET* mutations, which reliably predict the risk of MTC with regard to its clinical expression and age of onset, have transformed the management of patients, as it has helped to determine the timing of prophylactic thyroid surgery that is highly effective in avoiding the morbidity and mortality associated with MTC. Indeed, prophylactic thyroidectomy, with lifelong thyroxine replacement, has dramatically improved outcomes in the patients with MEN2 and MEN3 such that 90% of young patients with *RET* mutations who had a prophylactic thyroidectomy had no evidence of persistent or recurrent MTC at 7 years after surgery.[250] To facilitate management of MTC in MEN2 and MEN3 patients, the American Thyroid Association (ATA) has defined three categories for germline *RET* mutations that are based on their correlations between genotype and phenotype (e.g., aggressiveness of MTC), and these comprise highest risk (MEN3-associated Met918Thr mutation), high risk (Cys634 and Ala883Phe mutations associated with MEN2 and MEN3, respectively), and moderate risk (all other MEN2 *RET* mutations)[3] (Table 42.4; see Table 42.3). For asymptomatic individuals identified to harbor *RET* mutations, current recommendations for tumor surveillance and treatment are based on these risk categories, with the aim of identifying *RET* mutations at a sufficiently early age



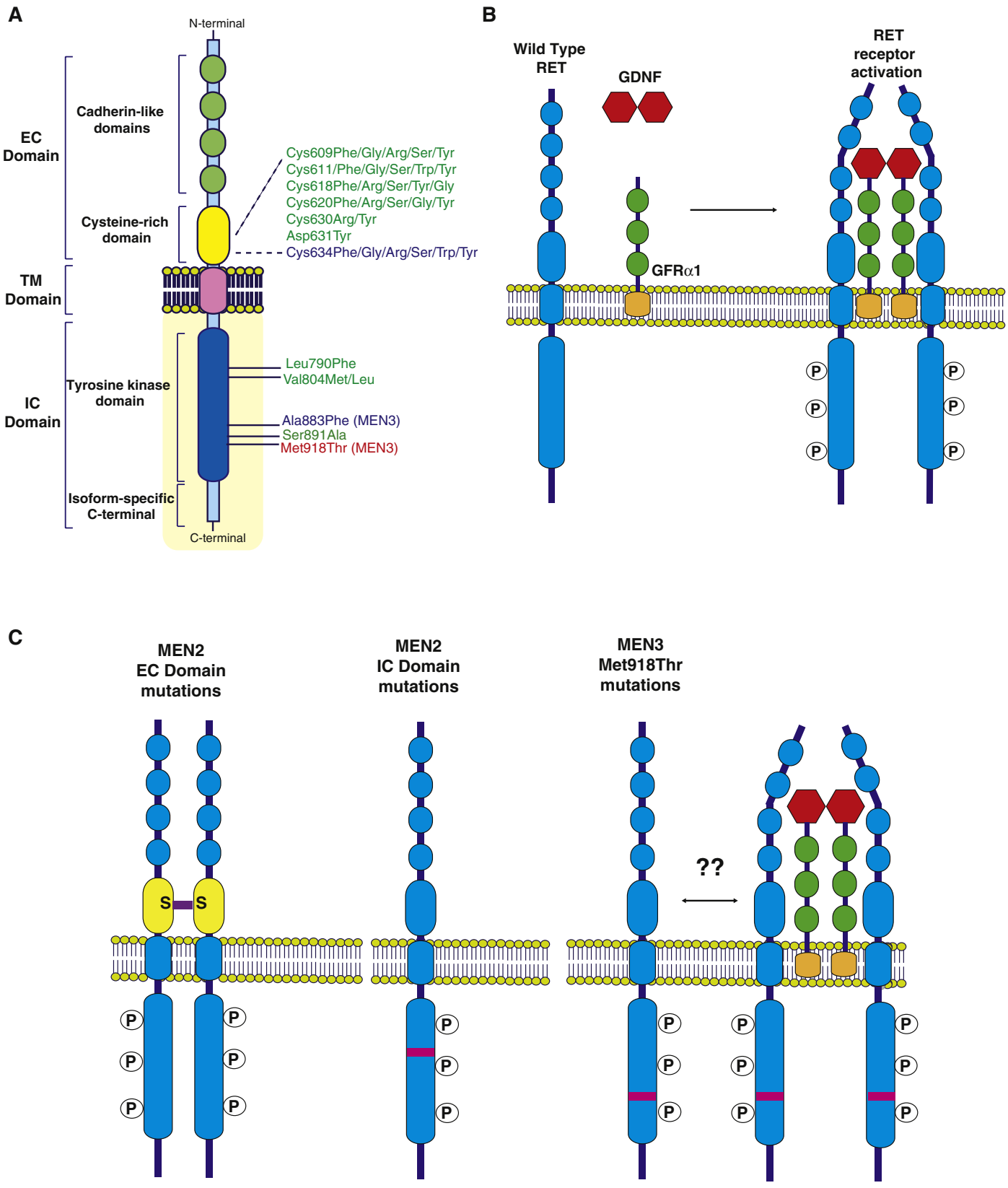
• **Fig. 42.6** Clinical features in a patient with multiple endocrine neoplasia type 3. The patient has evidence of a nodule in the left side of the neck representing metastases from medullary thyroid carcinoma (indicated by the *upper arrow*). Note the thyroidectomy scar (*lower arrow*) (A). Mucosal neuromas are evident on the tongue and lips (B). Chest x-ray demonstrating bilateral lung metastases of medullary thyroid carcinoma (C). Radiograph of barium meal and follow through demonstrating multiple intestinal diverticula, which are secondary to autonomic ganglion dysfunction (D). The patient had a history of diarrhea and malabsorption. (Modified from Thakker RV. Multiple endocrine neoplasia. *Medicine.* 2013;41[10];562–565.)

TABLE 42.3 Clinical Relationships and MTC Risk Level Associated With Common *RET* Mutations in MEN2 and MEN3

Exon	Affected Codon/Mutation	ATA MTC Risk Level	Penetrance of Pheochromocytoma	Penetrance of PHPT	Additional Reported Associations
8	Gly533Cys	Moderate	c.10%	c.10%	-
10	Cys609Phe/Gly/Arg/Ser/Tyr	Moderate	c.10–20%	c.10%	HSCR
10	Cys611Phe/Gly/Ser/Tyr/Trp	Moderate	c.10–20%	c.10%	HSCR
10	Cys618Phe/Arg/Ser	Moderate	c.10–20%	c.10%	HSCR
10	Cys620Phe/Arg/Ser	Moderate	c.10–20%	c.10%	HSCR
11	Asp631Tyr	Moderate	c.50%	—	—
11	Cys634 Phe/Gly/Arg/Ser/Trp/Tyr	High	c.50%	c.20–30%	CLA
11	Lys666Glu	Moderate	c.10%	—	—
13	Glu768Asp	Moderate	—	—	—
13	Leu790Phe	Moderate	c.10%	—	—
14	Val804Leu	Moderate	c.10%	c.10%	—
14	Val804Met	Moderate	c.10%	c.10%	CLA
15	Ala883Phe	High	c.50%	—	MEN3 clinical features
15	Ser891Ala	Moderate	c.10%	c.10%	—
16	Arg912Pro	Moderate	—	—	—
16	Met918Thr	Highest	c.50%	—	MEN3 clinical features

—, Not typically observed/associated; *ATA,* American Thyroid Association; *CLA,* cutaneous lichen amyloidosis; *HSCR,* Hirschsprung disease; *MEN2,* multiple endocrine neoplasia type 2; *MEN3,* MEN type 3; *MTC,* medullary thyroid carcinoma; *PHPT,* primary hyperparathyroidism.

Modified from Wells SA Jr, Asa SL, Dralle H, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid.* 2015;25(6):567–610.



• **Fig. 42.7** RET receptor structure highlighting the main functional domains and locations of common multiple endocrine neoplasia type 2 (MEN2)-associated *RET* mutations (A). MEN2-associated mutations arise most frequently in the cysteine-rich region of the extracellular (EC) domain, or in the intracellular (IC) tyrosine kinase domain, which are linked by the transmembrane domain (TM). The mutations shown represent those most commonly observed in MEN2 and MEN type 3 (MEN3), although additional *RET* mutations

Continued

Fig. 42.7, cont'd have been described in small numbers of kindreds. The American Thyroid Association risk category of *RET* mutation is also indicated: "highest" risk, *red*; "high" risk, *blue*; "moderate" risk, *green*. Mutations associated with MEN3 are noted in parentheses. All other mutations are associated with MEN2. RET receptor activation occurs following binding of members of the glial cell–line derived neurotropic factor (GDNF) family of ligands (GFLs), which comprise GDNF (shown), neurturin, persephin, and artemin (B). However, binding is mediated by a coreceptor, represented by members of the GDNF family receptor α group of proteins (GFRα1 shown). Thus, once formed, the GFL-GFRα complex engages RET, facilitating receptor dimerization and receptor activation, resulting in autophosphorylation of specific tyrosine residues within the tyrosine kinase domain, and subsequent recruitment and activation of downstream signaling complexes. Mutations associated with MEN2 are associated with ligand-independent receptor activation but achieve this by different mechanisms (C). MEN2 mutations in the extracellular (EC) cysteine-rich domain result in ligand-independent receptor dimerization mediated by disulphide-bond (S) formation between unpaired cysteine residues, resulting in constitutive receptor activation. In contrast, MEN2-associated mutations affecting the intracellular (IC) tyrosine kinase domain result in receptor activation in monomeric form. The MEN3 Met918Thr mutation also results in monomeric ligand-independent receptor activation but potentially may be further enhanced by the presence of RET ligand, thereby facilitating receptor dimerization and even higher levels of receptor signaling. However, the in vivo role of such ligand-enhanced activity in association with the Met918Thr mutation remains uncertain (represented by double question marks [??]). (A, modified from Newey PJ. Multiple endocrine neoplasia. *Medicine.* 2017;45[9]:538–542.)

TABLE 42.4 Recommendations for Screening and Surgery in MEN2 and MEN3

		RECOMMENDED AGE (YEARS) FOR SCREENING TEST/INTERVENTION				
ATA Risk Category[a]	Relevant *RET* Mutations	*RET* Mutational Analysis	First Serum Calcitonin and Neck US	Prophylactic Thyroidectomy	Screening for PCC[f]	Screening for PHPT
Moderate	Validated pathogenic mutations excluding those in the high and highest categories[b]	<3–5	5	>5[c]	16	16
High	Cys634Phe/Gly/Arg/Ser/Trp/Tyr Ala883Phe[d]	<3	3	5 or earlier[e]	11	11
Highest	Met918Thr[d]	ASAP and by <1	ASAP and by <0.5–1	ASAP and by <1	11	—

[a]ATA risk category as defined in the Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid.* 2015;25(6):567-561.

[b]*RET* mutations reported at ClinVar, ARUP database (arup.utah.edu/database/MEN2/MEN2_welcome.php).

[c]Timing of surgery to be based on elevation of serum calcitonin and/or the joint discussion of the pediatrician, surgeon, and parent/family. For example, later surgery may be appropriate if serum calcitonin and neck US are normal.

[d]*RET* mutation associated with MEN3.

[e]Earlier than 5 years based on elevation of serum calcitonin. The surgeon and pediatrician in consultation with the child's parent should decide the optimal timing of surgery.

[f]Individuals with MTC must have pheochromocytoma excluded prior to a surgical intervention and should be excluded in all at-risk individuals who are planning pregnancy or are pregnant.

—, Not required, as not part of MEN3; *ASAP,* as soon as possible; *ATA,* American Thyroid Association; *MEN2,* multiple endocrine neoplasia type 2; *MEN3,* MEN type 3; *MTC,* medullary thyroid carcinoma; *PCC,* pheochromocytoma; *PHPT,* primary hyperparathyroidism; *US,* ultrasound.

Data from American Thyroid Association Guidelines Taskforce, Kloos RT, Eng C, et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid.* 2009;19(6):565-612 and from Wells SA Jr, Asa SL, Dralle H, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid.* 2015;25(6):567–610.

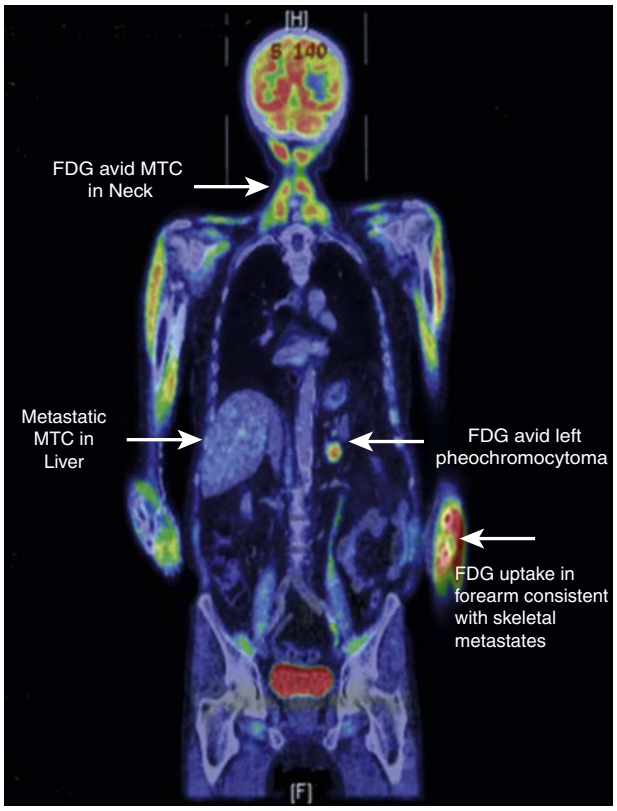
(i.e., often in the first months or years of life) to provide a window of opportunity for "prophylactic" thyroidectomy. In addition, the identification of a MEN2-associated *RET* mutation in an affected patient should prompt screening of all first-degree relatives, and the possibility of MEN2 or MEN3 should be considered in all patients presenting with MTC or pheochromocytoma, and germline *RET* genetic testing undertaken. However, it is important to note that de novo *RET* mutations are observed in 5% to 10% and approximately 75% of individuals with MEN2 and MEN3, respectively. These advances in our understanding of RET and RTKs have also resulted in the use of RTK inhibitors for the treatment of advanced and metastatic MTC. The treatment of patients presenting with one or more of the clinical manifestations of MEN2 and MEN3 require a multidisciplinary approach involving surgeons, oncologists, endocrinologists, and geneticists to ensure the optimal therapeutic approach.

Clinical Features and Management

Medullary Thyroid Carcinoma

Clinical Features

MTC is the most common and often the first manifestation of MEN2 and MEN3, and it occurs in almost all affected individuals (Fig. 42.8; see Fig. 42.6). MTC, which also represents the major cause of premature morbidity and mortality, frequently presents in children. Thus, early detection and treatment of MTC is important, and widespread implementation of *RET* mutation testing has transformed the management of patients from MEN2 and MEN3 families in whom the causative *RET* mutation is known. Indeed, the identification of *RET* as the causative gene for MEN2 and the subsequent ability to undertake genetic testing of "at-risk" first-degree relatives has resulted in a shift in clinical presentation from those presenting with a neck mass and advanced disease to



• **Fig. 42.8** ^{18}FDG-PET scan showing MTC with liver and skeletal metastasis and PET-avid uptake by the left adrenal pheochromocytoma in a patient with MEN2 due to a Cys634Arg *RET* mutation. *FDG*, fluorodeoxyglucose; *MEN2*, multiple endocrine neoplasia type 2; *MTC*, medullary thyroid carcinoma; *PET*, positron emission tomography (From Naziat A, Karavitaki N, Thakker R, et al. Confusing genes: a patient with MEN2A and Cushing's disease. *Clin Endocrinol.* 2013;78[6]:966–968.)

otherwise asymptomatic *RET* mutation carriers, who are recommended to have prophylactic thyroidectomy that substantially reduces their likelihood of developing advanced MTC.[250,251] Furthermore, widespread availability of *RET* genetic testing that identifies presymptomatic individuals who then undergo a prophylactic thyroidectomy has resulted in a significant fall in the proportion of MEN2 cases who present as index cases with MTC.[252] However, in the absence of a relevant family history (and/or prior knowledge of *RET* genetic status), the presentation of MTC is typically with a palpable neck mass, which may be asymptomatic or associated with symptoms of pressure or dysphagia in more than 15% of patients, although it should be noted that only a small minority (0.3–1.4%) of the population presenting with thyroid nodules harbor an underlying MTC.[3] For such index cases presenting with MTC (i.e., with a neck lump), regional and/or distant metastases are frequently evident at the point of diagnosis, and surgical cure is rarely achieved. MTC and pheochromocytoma may be present synchronously, and this possibility should be considered prior to any thyroid surgical intervention, particularly if the results of *RET* genetic testing are unavailable. Additional symptoms associated with MTC include diarrhea, which is reported in approximately 30% of patients, and/or flushing, each reflecting high circulating concentrations of calcitonin or other tumor-secreted hormones (e.g., serotonin, prostaglandins). Diarrhea occurs most frequently in those with advanced disease and most often in the presence of liver metastases.[3] The ectopic production of ACTH or corticotropin-releasing hormone from MTC may give rise to Cushing syndrome, and 1% to 3% of all cases of ectopic Cushing syndrome are due to MTC.[253] In those presenting with a neck mass, lymph node and distant metastases are frequently present at the time of diagnosis, and the extent of lymph node involvement provides important prognostic information.[3,254] The cervical and mediastinal nodes are the most common sites of local metastases, whereas distant spread typically involves bone, liver, lung, or brain.

MTC occurs following the malignant transformation of parafollicular C cells, which are concentrated in the middle and upper regions of the thyroid gland. The embryologic origins of the parafollicular C cells are considered to be of neuroectodermal origin, although there may be species-specific differences.[255] In mammalian species, C cells are reported to be derived from a subpopulation of ectodermal-derived neural crest cells that migrate to the most inferior pharyngeal arch (i.e., fourth arch), whereby they merge with a pair of endoderm-derived ultimobranchial bodies and become part of the developing thyroid gland.[255] C cells secrete several biogenic amines and peptides, including calcitonin, an evolutionary conserved hormone that is involved in calcium homeostasis. However, the physiologic function of calcitonin in mammalian species is unknown, as an absence of the hormone either by genetic knockout of the calcitonin gene in mice, or thyroid dysgenesis in humans, does not affect viability or bone development and indeed is associated with a normal development and a normal phenotype.[255] The transformation of C cells to MTC occurs along a multistep pathway, which initially involves C-cell hyperplasia (CCH), followed by noninvasive microscopic MTC (i.e., <1 cm), that progresses to invasive carcinoma with lymph node and distant metastases.[239] The molecular basis of each of these stages remains ill defined, although aberrant *RET* signaling almost certainly represents the key initiating event in tumor formation. In the context of MEN2, MTC is frequently multifocal and bilateral, occurring in the upper two thirds of the thyroid corresponding to the highest concentration of C cells, and it is likely that the CCH observed early in the disease course represents monoclonal proliferation of transformed progenitor cells rather than hyperplasia per se.[3] The secretory capacity for calcitonin is usually retained throughout each stage of tumor development, thereby providing a valuable tumor marker, with clinical utility for both MTC diagnosis and subsequent tumor surveillance (i.e., detecting residual, recurrent, or progressive disease).[3] Thus, the diagnosis of MTC relies on the demonstration of high basal serum calcitonin concentrations together with supporting cytologic/histopathologic evidence.[3]

Neck ultrasound with fine-needle aspiration (FNA) is the initial investigation of choice for individuals presenting with a solitary thyroid nodule, whereas radionuclide thyroid scans may reveal MTC tumors as "cold" nodules. MTC may display variable cytologic appearances and on occasion may be misdiagnosed as other tumor types.[3] Typical cytologic appearances include the presence of discohesive or weakly cohesive cells, which may be spindle shaped or have plasmacytoid or epithelioid appearances.[3] Important cytologic criteria for MTC are reported to include a dispersed pattern of polygonal or triangular cells, azurophilic cytoplasmic granules, extremely eccentrically placed nuclei with coarse granular chromatin, and amyloid.[3] Some studies have reported a high diagnostic accuracy of FNA in MTC (i.e., >80%), but a meta-analysis of 15 studies and 641 MTC cases reported a detection rate of approximately 55%, with several specimens that were initially classified as indeterminate or benign being subsequently found to be MTC; however, many specimens that were not identified as MTC were found to be malignancies, thereby indicating a need for surgery.[3,256] For aspirate

results that are suspicious for MTC or indeterminate, the diagnostic accuracy may be improved by measuring calcitonin levels in the FNA washout fluid and/or undertaking additional immunohistochemical evaluation for neuroendocrine markers including calcitonin, chromogranin, and carcinoembryonic antigen (CEA).[3,257]

Once MTC is suspected, the measurement of basal serum calcitonin levels should be undertaken. Modern commercial immunochemiluminometric assays are highly sensitive and specific for monomeric calcitonin and do not typically display cross reactivity for procalcitonin or other calcitonin-related peptides. However, elevated serum calcitonin concentrations may be observed in other conditions, such as chronic renal failure, parathyroid disease, thyroiditis, lung and prostate cancers, and other NETs, thereby diminishing the specificity of basal calcitonin levels for diagnosis of MTC.[3] Elevated (or occasionally inappropriately low) calcitonin levels may also occur in the presence of heterophile antibodies, whereas false-negative or inappropriately low calcitonin levels may occasionally be observed due to the "Hook effect," in which very high levels of serum calcitonin saturate the binding capacity of the antibody in the immunoassay, although the likelihood of this is reduced with modern immunochemiluminometric assays. Inappropriately low serum calcitonin concentrations (i.e., relative to disease stage) may also rarely occur in the setting of advanced MTC, in which tumor dedifferentiation results in reduced calcitonin secretion.[3] Finally, occasional case reports of calcitonin-negative MTC presenting with a solitary thyroid nodule have been reported.[258] Reference ranges for basal serum calcitonin levels are assay specific, which must also take into account age-related and sex-dependent factors. For example, serum calcitonin concentrations are typically higher in males than females, reflecting a higher total C-cell mass. In addition, serum calcitonin levels are elevated in infants and young children, and age-specific reference ranges are required until 2 to 3 years of age, after which serum calcitonin concentrations are indistinguishable from those of adults.[3,259] Establishing a serum calcitonin concentration below which the diagnosis of MTC can be confidently excluded in a patient presenting with a thyroid nodule remains challenging, as false positives occur frequently.[260] Thus, routine measurement of basal serum calcitonin concentrations in all patients presenting with thyroid nodules and before an FNA is controversial. Advocates of calcitonin measurement pre-FNA cite the need for early diagnosis given the low cure rates observed once MTC spreads beyond the thyroid. However, the very low overall frequency of MTC in this setting (i.e., <1%) raises concerns over cost effectiveness, whereas the potential for iatrogenic morbidity in those with raised basal serum calcitonin levels, but no subsequent MTC, has not been fully evaluated.[261] Thus, current guidelines recognize differences in clinical practice and do not make any clear recommendation.[3] Previously, provocation tests with potent secretogogues including calcium and pentagastrin, which were commonly used to improve the diagnostic value of serum calcitonin measurements, are now employed less frequently.[3] In addition to calcitonin, other biomarkers that may be associated with MTC include CEA, although this is not specific for MTC and has little role in establishing the diagnosis but may be useful for monitoring disease progression,[3] and others such as serum carbohydrate antigen 19.9, which may be associated with MTC but is not routinely employed for diagnosis.[262]

Once the diagnosis of MTC has been made by FNA of a thyroid nodule and demonstration of an elevated basal serum calcitonin concentration, further investigations are required to determine the likely extent of disease. Thus, preoperative staging with neck ultrasound is mandatory, whereas cross-sectional imaging, using CT or MRI, should be undertaken in those in whom metastatic disease is suspected, which is usually based on extensive neck disease and/or very high serum calcitonin levels (e.g., >500 pg/mL). CT is used widely to detect lung and mediastinal lymph node involvement, whereas complementary additional modalities (e.g., MRI, bone scintigraphy) are employed to detect metastases at other locations. FDG-PET/CT or F-DOPA-PET/CT, which may be helpful (see Fig. 42.8), is not readily available in many centers. Finally, for the investigation of MTC, it is important to undertake *RET* germline genetic testing in all patients, and for those who have a mutation (or for those in whom there is likely to be a significant delay in testing), the presence of pheochromocytoma and PHPT should be excluded prior to surgery.[3]

MTC is highly penetrant in MEN2, with 70% to 100% of affected individuals developing disease by the age of 70 years. There is a strong genotype-phenotype correlation such that the timing of MTC is predicted (at least in part) by the specific *RET* mutation, and the current ATA risk categorizations of germline *RET* mutations are based on the potential "aggressiveness" of MTC, which in turn is based on the age of onset rather than tumor behavior[3,263] (see Table 42.4). Thus, the ATA highest risk category, represented by the Met918Thr mutation in association with MEN3, is invariably associated with MTC initiation during the first few years of life, and macroscopic disease may occur before the age of 1 year. Indeed, the earliest reported case of MTC in MEN3 is 9 weeks of age, and lymph node metastases have been identified in the first year of life.[264,265] However, the high frequency of de novo mutations in MEN3 patients frequently results in a delayed diagnosis of the associated MTC until the second decade of life (i.e., mean age of diagnosis ~14 years), by which time spread beyond the thyroid is invariably present and the opportunity for curative treatment missed.[264–266] Indeed, MTC is the leading cause of death in MEN3 and is associated with an apparent aggressive disease course such that it has a worse 10-year survival than the MTC occurring in MEN2, although this may, in part, reflect the earlier age of onset and typical later stage of diagnosis.[265]

MTC occurs in MEN2 and MEN3 patients with ATA high-risk *RET* mutations (i.e., codon Cys634 mutations and the Ala883Phe mutation, respectively), with a median age of diagnosis of 20 to 25 years, although it has been reported in those younger than 5 years of age.[263,267] However, in these high-risk *RET* mutation carriers, lymph node metastases are unusual in those younger than 10 years of age,[3,268] and different codon 634 mutations may be associated with subtle differences in MTC expression. For example, the penetrance of MTC is reported to be higher in carriers of the Cys634Arg mutation when compared with other codon 634 substitutions.[269,270] MEN2 patients with ATA moderate-risk *RET* mutations (i.e., all other *RET* mutations excluding Met918Thr, those of Cys634 and Ala883Phe) have a median age of MTC onset that is typically later than those with higher-risk mutations, but this may be highly variable. For example, in a series of 127 patients with moderate-risk *RET* mutations, the median age of MTC diagnosis was 42 years, with a range of 6 to 86 years.[263] Furthermore, despite the later age of MTC diagnosis in moderate-risk compared with high-risk mutation carriers, no difference in clinical course was observed, suggesting that the *RET* mutations predominantly influence age of onset rather disease aggressiveness per se.[263] In addition, recent studies indicate that it may be possible to further subdivide the ATA moderate-risk category to include moderate-high risk groups, such as mutations affecting codons Cys611, Cys618, and Cys620, and moderate-low risk groups, such as mutations affecting codons Leu790, Val804,

and Ser891, according to the age-related progression of MTC.[267] The penetrance of MTC associated with certain moderate-risk *RET* mutations (e.g., Val804Met) is also likely to be significantly reduced, as indicated by a higher than anticipated frequency of individuals harboring the variant in the background population.[145,271] Thus, the risk and age-related penetrance of MTC is strongly determined by codon-specific *RET* mutations, although additional genetic and/or environmental modifying factors are likely to influence disease expression. For example, the risk of MTC (and other MEN2 clinical features) in individuals with a specific *RET* mutation may also be influenced by additional *RET* coding variants and/or the background *RET* haplotype.[272,273]

Treatment

Surgery, comprising total thyroidectomy with dissection of cervical lymph node compartments, is the recommended treatment for sporadic and hereditary MTC, as it offers the best chance of achieving cure. For recurrent or metastatic MTC, several different treatment strategies (e.g., locoregional cytoreductive surgery, external beam radiotherapy, radio-frequency ablation, chemoembolization, and systemic targeted therapies such as RTK inhibitors) are available.

The majority of patients presenting with MTC in the context of a thyroid nodule will have evidence of lymph node metastases at diagnosis, and the extent of surgery remains controversial. In addition, index cases with MEN2 may have multifocal and/or bilateral disease, and the degree of cervical lymph node involvement and/or presence of distant metastases will influence the extent of surgery. Preoperative basal serum calcitonin concentrations have been reported to be associated with the degree of cervical lymph node involvement and are used in some centers to make decisions on the extent of lymph node resection. For example, a study of 300 treatment-naive MTC patients reported that the presence of lymph node metastases in the ipsilateral central and lateral neck, contralateral central neck, contralateral lateral neck, and upper mediastinum were associated with basal serum calcitonin concentrations above 20, 50, 200, and 500 pg/mL, respectively,[274] and that bilateral compartment-oriented neck surgery could achieve biochemical cure in more than 50% of patients with pretreatment calcitonin levels of 1000 pg/mL or higher, whereas such outcomes were not achieved in patients with preoperative calcitonin levels of 10,000 pg/mL or higher.[274] Postoperative biochemical remission or cure is important, as it is associated with low recurrence rates and excellent long-term survival rates of 98% at 10 years.[275,276] Prognostic information on lymph node involvement and surgical cure rates has also been reported for CEA.[277] For example, preoperative serum CEA concentrations greater than 30 ng/mL are reported to indicate central and ipsilateral lateral lymph node metastases, whereas serum CEA concentrations greater than 100 ng/mL are reported to signify the presence of contralateral lateral lymph node involvement and/or distant metastases.[277] However, consensus has not been reached on the optimal surgical approach for MTC with regard to the extent of lymph node resection, and the current guidelines suggest total thyroidectomy and central compartment lymph node clearance in patients with MTC and no evidence of cervical lymph node metastases on ultrasound, whereas removal of lymph nodes in the lateral compartments may be considered based on calcitonin levels.[3] If there is cervical lymph node involvement, then dissection of the central and involved lateral compartment is recommended, although if the lateral compartment on the opposite side to that of the MTC is observed to be free of disease, then decisions on lymph node removal in this area may be based on calcitonin levels.[3]

In patients with regional lymph node involvement, total thyroidectomy and bilateral lymph node dissection frequently fails to achieve biochemical cure. For example, in those with involvement of 1 to 10 cervical lymph nodes involved, immediate postoperative normalization of serum calcitonin is achieved in only 31% to 57% of patients, whereas in those with involvement of more than 10 cervical nodes, biochemical remission is observed in 0% to 4%.[278,279] Thus, the presence of lymph node metastases is a poor prognostic factor, and the number of involved lymph nodes has also been reported to provide prognostic information on the likelihood of developing distant metastases.[254,280] In addition, it has been reported that in patients undergoing thyroidectomy and lymphadenectomy for MTC that a higher number of lymph nodes harvested at surgery (i.e., lymph node yield)—a surrogate for adequate staging—and the metastatic lymph node ratio (i.e., number of metastatic lymph nodes/lymph node yield) correlated with a poorer survival.[280] The American Joint Committee for Cancer tumor, node, metastasis staging system also provides prognostic utility, and increased recurrence rates are reported for a primary tumor that extends beyond the thyroid capsule to invade local structures (i.e., pT4 tumors).[3] However, the American Joint Committee for Cancer system does not incorporate age, pre- and postoperative serum calcitonin levels, or quantification of the number of affected lymph nodes, which each also provide additional prognostic information.

Thyroidectomy together with lymph node clearance may be associated with several postoperative complications, including lymphatic leakage, transient (and occasionally permanent) hypoparathyroidism, and transient or longer-term damage to the recurrent laryngeal nerves and/or spinal accessory nerve. Following surgery, basal or stimulated calcitonin levels should be ascertained to assess disease status, although typically this is delayed by approximately 3 months to allow sufficient time for levels to reach a nadir.[3,281] Following total thyroidectomy, all patents require lifelong thyroid hormone replacement and will also require monitoring of plasma calcium concentrations with appropriate treatment, where necessary. In patients with known advanced disease (i.e., extensive regional or metastatic disease), less aggressive surgery may be appropriate because cure will not be achieved and the aims in these patients will be to maintain the voice and swallowing, preserve the quality of life, and avoid local complications.[3]

Prophylactic Thyroidectomy in MEN2

The ability to identify individuals at high-risk of developing hereditary MTC through germline *RET* mutation testing provides a window of opportunity to undertake preventative or curative surgery in otherwise asymptomatic individuals.[3,239,268] Indeed, "prophylactic" thyroidectomy has become the mainstay of treatment for children at risk of hereditary MTC, and when undertaken at a sufficiently early stage, it has the potential to avoid the morbidity and mortality associated with MTC development.[3,239,250–252,268,282,283] For example, in 2005, a study reported that prophylactic thyroidectomy in 50 patients who were younger than 19 years of age with MEN2-associated *RET* mutations resulted in no evidence of residual or recurrent disease in approximately 90% of cases at a mean follow-up period of 7 years. More recently, a study reporting the outcomes of prophylactic thyroidectomy in 167 children with MEN2- and MEN3-associated *RET* mutations reported no instances of recurrent or residual disease after a mean of 7 years in any of the 149 patients for whom follow-up data were available. In addition, normalization of postoperative serum calcitonin was observed in 99% of patients who had

raised preoperative calcitonin levels.[251] However, it is important to note that the aim of such preventative surgery is not necessarily to remove the thyroid before any abnormality develops but rather to do so before there is a significant risk of metastatic disease. In this regard, the advent of highly sensitive serum calcitonin assays now provides additional information that may help inform surgical decisions, although it should be noted that serum calcitonin concentrations in young children, and especially in the first few months of life, are frequently high and do not accurately reflect disease status. Thus, although prior recommendations on the timing of prophylactic thyroidectomy were based on the risk category of the *RET* mutation alone,[2] more recent guidelines suggest that in some instances it is reasonable to take into account basal or stimulated serum calcitonin levels, which provide a reliable indicator of MTC status and disease risk.[3] This represents an important change and recognizes that there remains considerable heterogeneity in the age of MTC onset in those carrying the same *RET* mutation who may be even from the same kindred. Furthermore, it suggests that although the risk category of *RET* mutation is the major determinant of the age of onset of CCH and subsequent transformation to MTC, additional genetic and/or environmental factors may influence disease expression.

Thyroidectomy in early childhood is associated with a higher complication rate than that observed in older children or adults. Such surgery should only be undertaken at a center with appropriate experience. Complications associated with surgery include a higher likelihood of developing hypoparathyroidism, as frequently the parathyroid glands are hard to identify in very young children. Complications in early childhood are also increased if central lymph node dissection is performed, and this includes the higher risks of transient and permanent hypoparathyroidism, as well as the likelihood of transient recurrent laryngeal nerve palsy.[268,284] As a consequence, the timing of prophylactic surgery aims to strike an important balance between the risks associated with early surgery and those associated with more extensive surgical procedures, which may be necessary when intervention is delayed. Current guidelines attempt to address this balance, offering some flexibility in the timing of prophylactic thyroidectomy in children with germline *RET* mutations associated with a later age of MTC onset while recommending early surgery in those deemed to be at highest risk (see Table 42.4). Thus, children identified to harbor the ATA highest-risk *RET* mutations (i.e., Met918Thr) are recommended to undergo total thyroidectomy in the first year of life, and the higher potential complication rate of hypoparathyroidism in this age group is considered acceptable given the potential for metastatic disease by delaying treatment. In children with ATA high-risk mutations (i.e., codon Cys634 and Ala883Phe mutations), prophylactic thyroidectomy is typically recommended before 5 years of age, with the exact timing based on annual clinical examination, neck ultrasound, and serum calcitonin levels starting from the age of 3 years. For those children with ATA moderate-risk *RET* mutations, the timing of prophylactic thyroidectomy should be based on the findings of clinical examination, neck ultrasound, and serum calcitonin concentrations commencing at age 5 years.[3] Thyroidectomy is indicated once serum calcitonin concentrations are elevated, although it may also be appropriate in children with normal calcitonin levels in whom such long-term monitoring is not feasible or desired. The window of "safe" serum calcitonin concentrations in which curative surgery is feasible has not been precisely defined, although once basal serum calcitonin levels exceed the upper end of the normal range (e.g., ~10 pg/mL), this may herald the early stages of MTC development and a suitable time for surgical intervention. Serum calcitonin concentrations between 10 and 30 pg/mL may represent an optimal window for intervention, as nodal metastases were not observed to occur in children with *RET* mutations who had serum calcitonin concentrations of 30 pg/mL or less.[268,283] Once calcitonin levels are greater than 30 pg/mL, the likelihood of nodal metastases increases, and this will often necessitate central node dissection, which is associated with increased operative morbidity and a reduction in duration of long-term remission.[268,285]

Postoperative Evaluation and Management of Patients With MTC

Following surgery, the normalization of serum calcitonin levels is associated with very favorable long-term outcomes, although a small proportion of these patients may develop recurrent disease. For example, patients with postoperative basal serum calcitonin concentrations less than 10 pg/mL are reported to have 3- and 5-year relapse-free survival of 95% and 90%, respectively,[276] with only approximately 4% of patients with recurrent disease found to have had normal postoperative serum calcitonin levels.[286] Thus, postoperative normalization of serum calcitonin levels cannot be considered curative in all patients and highlights the need for long-term clinical and biochemical follow-up. Patients who have postoperative basal serum calcitonin levels above the reference range will require further investigation, and this will depend on the severity the hypercalcitonemia. Thus, serum calcitonin levels that are greater than 10 pg/mL but less than 150 pg/mL indicate localized residual disease within the neck that should be evaluated by ultrasound, whereas serum calcitonin concentrations greater than 150 pg/mL indicate the possibility of more extensive disease that will require additional imaging (e.g., CT, MRI, bone scintigraphy). The extent and location of the residual/recurrent disease with the rate of tumor progression determined both by imaging and calcitonin and/or CEA doubling time will determine which of the different local or systemic treatment options (see the following discussion) should be pursued. Calcitonin doubling time is an important guide of prognosis,[281] and calcitonin doubling times of less than 6 months and 6 to 24 months have been reported by one study to be associated with 10-year survivals of approximately 10% and approximately 40%, respectively, whereas all patients with a doubling time of greater than 24 months were alive at the end of the study.[287] In another study, both calcitonin and CEA doubling times were also strongly related to disease progression, with approximately 95% of those with doubling times of 2 years or less observed to have progressive disease, whereas approximately 85% of those with doubling times greater than 2 years had stable disease.[288]

Management of Advanced Disease

The management of advanced MTC in patients with MEN2 or MEN3 does not differ from that in patients with sporadic MTC.[3] In MEN2 and MEN3 patients with significant elevations of serum calcitonin preoperatively (e.g., >500 pg/mL), postoperatively (e.g., >150 pg/mL), or during the subsequent follow-up periods, the possibility of locally advanced and/or metastatic disease should be investigated, as this will influence the choice of downstream therapeutic approach.[3]

In patients with residual or recurrent disease that is limited to the neck, reoperation with compartmental dissection of central and/or lateral regions based on preoperative investigation (i.e., imaging and/or biopsy) may be appropriate, with the aim of normalizing or reducing serum calcitonin levels, although these potential benefits must be balanced against the increased

morbidity that may be associated with further neck surgery. Postoperative radioiodine is not recommended for patients with MTC unless there is evidence that the recurrent MTC contains elements of papillary or follicular thyroid cancer. External beam radiotherapy (EBRT) to the neck has been used widely for locoregional disease control, although there is little evidence of survival benefit when other prognostic factors are taken into account.[289] Current guidelines suggest considering EBRT in those patients at high risk for local disease recurrence and those at risk of airway obstruction. However, the decision to use EBRT in these situations should take into account the potential acute and chronic toxicities associated with the treatment.[3]

Once distant metastases have developed, treatments are aimed at disease control and/or symptomatic benefit, as curative therapies are not available. Determining the optimal therapy will depend on several factors, including the site(s) and extent of metastases, the rate of tumor progression, the performance status of the patient, the treatment modalities available to the patient, and the wishes of the patient. MTC most commonly metastasizes to the liver and axial skeleton (see Fig. 42.8), and to a lesser extent the lungs (see Fig. 42.6), brain, and skin, and in these settings, several treatment approaches may be employed to achieve symptom and/or disease control.[3] Treatments for metastatic disease are broadly categorized into local and systemic therapies. Local treatments, which are typically used for disease or symptom control at specific sites related to tumor spread, include surgical resection/debulking, chemoembolization, radio-frequency ablation, and EBRT.[3] Symptoms related to aberrant hormonal secretion may also require treatment. For example, antimotility agents (e.g., loperamide) and/or somatostatin analogues may improve diarrhea in those with hormonally active hepatic metastases and may be used prior to surgical debulking and/or selective arterial chemoembolization.[3] Similarly, biochemical control of Cushing syndrome (i.e., due to the ectopic production of corticotropin-releasing hormone or ACTH) may be required to minimize the associated morbidity (e.g., hyperglycemia, osteoporosis, hypertension, hypokalemia, gastritis), and this may be achieved by several approaches, including medical therapies (e.g., ketoconazole, metyrapone, mitotane), bilateral adrenalectomy, or debulking of hepatic metastases. Remission of ectopic Cushing syndrome due to MTC has also been observed in case reports of patients receiving the RTK inhibitor vandetanib (see the following discussion).[290]

Systemic antitumor therapies are typically reserved for patients with metastatic MTC in whom there is evidence of significant disease burden and/or evidence of disease progression. The advent of targeted therapy with RTK inhibitors has provided a significant advance in the field, and these drugs now represent first-line treatment for the majority of patients with advanced progressive disease (see the following discussion). In contrast, conventional cytotoxic chemotherapeutic regimens, either as single agents or in combination, have been associated with low response rates and are not routinely recommended. Alternate systemic therapies, including radionuclide therapies (e.g., [^{90}Y-DOTA]-TOC), have also been employed in early-phase clinical trials, but these treatments require further evaluation to support their wider clinical use.[3]

Targeted Therapies for MEN2-Associated MTC

Currently, two RTK inhibitors are approved for use in advanced progressive MTC: vandetanib, which targets RET, endothelial growth factor receptor (EGFR), and vascular EGFR (VEGFR) kinases, and cabozantinib, which targets RET, c-Met, and VEGFR kinases.[3,266,291–293] The anti-RET activity of these drugs is considered to be the determinant of their clinical efficacy in MTC, but it is important to note that the targeting of alternate TKs, including VEGFR, by these drugs may also contribute substantially to their antitumor activity.

The safety and efficacy of vandetanib in advanced MTC was initially evaluated in clinical trials of MEN2 and MEN3 patients, which demonstrated partial response rates of 15% to 20% and stable disease persisting for 24 weeks or more in an additional approximately 50% of patients.[294,295] In a subsequent phase III trial of patients with advanced MTC due to sporadic of hereditary disease, vandetanib demonstrated increased PFS when compared with placebo (median PFS 30.5 months vs. 19.5 months, respectively), whereas approximately 45% of patients in the treatment group demonstrated a partial tumor response.[291] Vandetanib treatment in 28 patients with germline *RET* mutations revealed approximately 45% to have an objective tumor response,[291] and tumors harboring a somatic Met918Thr *RET* mutation also had an enhanced response.[291] A subsequent study of vandetanib in children and adolescents with MEN3 and locally advanced or metastatic MTC reported an objective partial response rate of 47%, whereas all 15 MEN3 patients harboring the germline Met918Thr mutation experienced some reduction in tumor size.[296]

Cabozantinib, in a phase III clinical trial, resulted in a partial tumor response in 28% of patients with locally advanced or metastatic MTC compared with 0% in the placebo group, whereas PFS was increased in those receiving treatment compared with placebo (11.2 vs. 4.0 months, respectively).[297] However, no overall survival benefit was demonstrated between treatment and placebo arms.[297] Subsequent analysis of this trial reported an increased median PFS in patients with a *RET* or *RAS* mutation receiving cabozantinib compared with placebo (13.8 months vs. 4.6 months, and 10.8 months vs. 1.8 months, respectively). In contrast, no such benefit was observed in patients without *RET* or *RAS* mutations (median PFS in treatment and placebo groups of 5.6 months vs. 5.3 months, respectively).[298] In addition, patients with tumors harboring the Met918Thr *RET* mutation were also found to have the greatest median PFS benefit with cabozantinib treatment (14.1 months), and, importantly, these patients were the only group to have increased overall survival.[298–300] Moreover, objective response rates of 32% and 22% were observed in patients with and without *RET* mutations, respectively, indicating some antitumor activity in patients without *RET* mutations.[298,299]

These studies reveal that treatment with RTK inhibitors of patients with advanced MTC resulted in objective tumor responses in more than 50% of patients. In addition, the majority of patients developed drug resistance, thereby resulting in only short- to medium-term improvements in disease control, and long-term disease remission and major survival benefits were not demonstrated.[266,299,300] Furthermore, the adverse effects associated with these drugs, which included diarrhea, rash, fatigue, hypertension, abdominal pain, photosensitivity, prolonged QT interval, and gastrointestinal fistulas, resulted in either drug discontinuation or the requirement for a dose reduction in 12% to 16% and 35% to 79%, respectively.[3] There was also marked heterogeneity in drug response even between individuals harboring similar *RET* mutations, and improved molecular understanding of these agents is required to establish their optimal use.[266,296] For example, determining the clinical activities in the presence of different *RET* mutations and establishing the relative contribution of their different anti-TK activities, such as anti-VEGFR, would be important, especially because cabozantinib was demonstrated by using in vitro and in vivo tumor models to be active against several

mutated forms of *RET*, such as Met918Thr and Tyr791Phe, whereas other mutated forms of RET, including those harboring substitutions at Val804, appeared to be resistant to certain RTK inhibitors, including vandetanib.[301,302]

Other multi-TK and RET inhibitors have also been evaluated in early-phase clinical trials for advanced MTC, including sorafenib, which demonstrated partial responses in 6% to 25% of patients[303,304]; lenvatinib, which demonstrated objective tumor responses in approximately 35% of MTC patients[305]; and sunitinib, which demonstrated an objective response rate of 38%.[306] However, these and several additional TK inhibitors are not currently approved for use, and larger studies are required to determine the effectiveness of these treatments in MEN2 and MEN3 patients with MTC. Preclinical studies have evaluated other RTK and RET inhibitors with activity against specific TK receptor mutations. For example, alectinib and ponatinib demonstrated significant anti-RET activity, including effective targeting of the Val804 "gatekeeper" mutants (e.g., Val804Met and Val804Leu),[292,299,307] and several RET-specific inhibitors are in development.[292]

Finally, it is likely that either sequential or combination systemic therapy will be required to achieve longer-term treatment responses, and early phase studies are in progress.[292] Furthermore, an improved understanding of the mechanisms of acquired resistance is required to determine the optimal use of emerging agents.

Pheochromocytoma

Clinical Features

Pheochromocytomas are the second most frequent neoplasms in MEN2 and MEN3, with an overall penetrance of 40% to 50%. Pheochromocytoma may be the first manifestation in a minority (<10%) of cases and may also occur synchronously with MTC in approximately 35% of cases[308–310] (see Fig. 42.8). Childhood presentations of pheochromocytoma are rare in MEN2, although the earliest ages of onset related to codon Cys634 and Met918Thr mutations are 8 and 12 years, respectively.[3,311] A key characteristic of MEN2-associated pheochromocytoma is that in 50% of patients, it occurs as a bilateral disease, which can occur synchronously or metachronously.[308,312–315] Such bilateral disease has a higher frequency in individuals with specific genotypes including the Cys634Arg *RET* mutation.[269,270,315] The majority (>95%) of MEN2- and MEN3-associated pheochromocytomas arise within the adrenal gland and occur on the background of adrenal medullary hyperplasia.[239,316] However, more recent studies indicate that regions of adrenal medullary hyperplasia share molecular characteristics with pheochromocytoma and should be more appropriately considered as micropheochromocytomas.[317] The overwhelming majority (>95%) of MEN2- and MEN3-associated pheochromocytomas are benign, and only 0% to 4% are reported to progress to malignancy in large series, although individual cases reports highlight occasional instances of metastatic spread.[308,315,318–320] Extra-adrenal pheochromocytoma/paraganglioma is extremely rare in MEN2 and MEN3.[321]

The clinical signs and symptoms of pheochromocytoma in MEN2 and MEN3 do not differ from those occurring in patients with nonsyndromic and nonfamilial pheochromocytomas. Thus, MEN2 and MEN3 patients with pheochromocytoma may present with features associated with excess catecholamine secretion, and these include episodic headaches, sweating, palpitations, anxiety, and hypertension. However, 30% to 50% of MEN2 and MEN3 patients are asymptomatic, and the pheochromocytoma is diagnosed during a screening program.[315,322] Thus, the possibility of pheochromocytoma due to MEN2 should be considered in all patients diagnosed with MTC prior to surgery (irrespective of signs or symptoms), as failure to diagnose and treat a concurrent pheochromocytoma may result in catastrophic outcomes due to an intraoperative adrenergic crisis.[3] Furthermore, it is important to exclude pheochromocytoma in all "at-risk" MEN2 and MEN3 patients prior to pregnancy.[3]

The diagnosis of pheochromocytoma is confirmed by demonstrating elevated concentrations of plasma and/or urinary free fractionated metanephrines. MEN2-associated pheochromocytomas are typically adrenergic and are reported to secrete disproportionate amounts of epinephrine,[239,315] and elevated epinephrine (and associated metanephrine) concentrations may help distinguish MEN2 from other hereditary pheochromocytoma/paraganglioma syndromes, such as von Hippel–Lindau disease and familial paraganglioma syndromes, in which norepinephrine or other metabolites predominate. Preoperative tumor localization studies are essential because there is a high probability of bilateral disease, and the imaging should evaluate both adrenal glands. Cross-sectional imaging with CT or MRI is recommended, and some centers will prefer CT because it provides a higher resolution with the pheochromocytoma appearing as a dense, hypervascular mass.[312,323] Several functional imaging modalities (e.g., ^{123}I-metaiodobenzylguanidine [^{123}I-MIBG], ^{18}F-FDOPA PET/CT, ^{18}F-FDG PET, 68Ga-DOTA) are also associated with high sensitivity and specificity for disease localization and detection of metastatic and extra-adrenal disease, although their usefulness in MEN2 and MEN3 may not be great considering that the majority of patients have benign disease limited to the adrenal medulla.[324,325]

The occurrence of pheochromocytomas in patients with MEN2 and MEN3 is dependent on genotype.[239,268,270,312,314,322] Thus, the highest prevalence of pheochromocytoma is observed in those with ATA high (codon Cys634 and Ala883Phe)- and highest (Met918Thr)-risk *RET* mutations, whereas a lower disease frequency is observed in those with moderate-risk *RET* mutations (see Table 42.3 and Fig. 42.7). To date, most estimates of pheochromocytoma penetrance are age related, and the absolute lifetime risk for several *RET* mutations is unknown. For example, 30% and 100% of patients with the Met918Thr mutation developed pheochromocytoma by the age of 27 and 56 years, respectively; 25%, 30% to 60%, 52%, and 88% of codon Cys634 *RET* mutation carriers developed disease by age 30, 35, 50, and 77 years, respectively; and fewer than 20% of patients carrying moderate-risk exon 10 mutations (e.g., those affecting codons 609–620) had pheochromocytomas by 35 years of age.[268,270,309,312,314,322] Different mutations affecting the same Cys634 residue have been reported to give rise to different tumor penetrances, with the Cys634Arg mutation being associated with the highest incidence of pheochromocytoma in MEN2 patients.[269,270,310] In addition, recent studies also indicate the presence of potential genetic or environmental disease modifiers, and a different natural history of pheochromocytoma in MEN2 is reported in individuals with the same *RET* mutations from different geographic regions. For example, MEN2 patients from South America with exon 11 mutations (e.g., affecting codon Cys634) appear to have a lower disease penetrance and/or later age of onset than those from European locations (i.e., Southern, Central, Western Europe).[326] Further support for the influence of genetic modifiers is provided by the observation that even within the same kindred, the penetrance and expression of pheochromocytoma varies. Potential insights into such modifying influences include the observation that certain haplotypes containing *RET* mutations alongside additional

RET rare variants (e.g., Tyr791Phe) or polymorphisms (e.g., Leu769Leu, Ser836Ser, and Gly691Ser/Ser904Ser) result in an increased age-related risk of pheochromocytoma.[273,327]

Treatment

Surgical removal of the pheochromocytoma(s) is the treatment of choice. However, pre- and perioperative α- and β-blockade must be instituted, and in patients having bilateral adrenalectomy, who are at risk of postoperative steroid and mineralocorticoid deficiency, perioperative steroids should be administered. For unilateral or bilateral adrenalectomy, open (i.e., retroperitoneoscopic) and laparoscopic approaches are appropriate and reported to be associated with similar outcomes.[3] However, several additional factors should be considered specifically in MEN2 and MEN3 patients when compared with patients with nonsyndromic and nonfamilial pheochromocytomas in planning the surgical strategy. For example, in patients with synchronous bilateral pheochromocytomas, the simultaneous removal of both adrenal glands results in postoperative adrenal insufficiency, with all patients requiring lifelong glucocorticoid and mineralocorticoid replacement.[268,322] For this reason, bilateral adrenalectomy is not recommended in those with unilateral disease despite the potential for tumor development in the contralateral gland.[3] To reduce the risk of adrenal insufficiency in patients requiring bilateral adrenalectomy, some centers have advocated adrenal-sparing surgery (i.e., subtotal adrenalectomy) despite the potential risk for tumor recurrence in the remnant tissue.[268,312,322,328] Adrenal-sparing surgery involves removal of the pheochromocytoma while aiming to leave 10% to 30% residual adrenal cortical tissue to provide sufficient adrenal reserve for glucocorticoid and mineralocorticoid function. Supporters of this approach cite the low risk of malignancy, a relatively low tumor recurrence rate with substantial disease-free intervals, and a low frequency of adrenal insufficiency associated with adrenal-sparing surgery. Several retrospective series have compared clinical outcomes between conventional and adrenal-sparing adrenalectomy. For example, in one series of 552 MEN2 patients undergoing surgery for pheochromocytoma, approximately 20% were treated by adrenal-sparing surgery and approximately 60% of those undergoing bilateral tumor resections were demonstrated to have preserved glucocorticoid production.[322,328] Furthermore, following either unilateral or bilateral adrenal-sparing surgery, a low tumor recurrence rate of approximately 3% was observed in the remnant operated gland after 10 years of follow-up.[312,322] Although higher recurrence rates are reported in other series, and are likely to rise with increasingly lengths of follow-up, current guidelines suggest adrenal-sparing surgery as an alternative to adrenalectomy, although this approach may not be technically feasible in MEN2 and MEN3 patients with multifocal disease.[3,268,312,314,328–330] Finally, in patients who have pheochromocytoma concurrently with MTC, the usual practice is to remove the adrenal tumor(s) prior to thyroidectomy.[3] The decisions regarding surgical strategy should balance the relative risks and benefits of the planned approach, as well as take into account the wishes of the patient. In addition, it is important to note that the improvements in the screening programs and treatment approaches have reduced the morbidity and mortality associated with MEN2- and MEN3-associated pheochromocytoma, which have a very low frequency of malignant disease; thus, current outcomes generally are very favorable.[312,322] Following surgery, lifelong follow-up is required for all patients to ensure appropriate compliance and monitoring of glucocorticoid and mineralocorticoid replacement therapy in those with adrenal insufficiency, and to monitor for development of additional pheochromocytoma in the contralateral adrenal gland following unilateral resections, or in remnant adrenal tissue following adrenal-sparing surgery, so that appropriate surgery (i.e., adrenalectomy) can be planned and provided.

Primary Hyperparathyroidism

Clinical Features

PHPT, which occurs in approximately 30% of patients with MEN2, typically presents in the third to fourth decade of life.[3,268,270,312,314,331] The risk of PHPT is related to *RET* genotype, and patients with the Met918Thr *RET* mutation do not develop PHPT, whereas approximately 10% to 20% of patients with codon Cys634 mutations will develop PHPT by 35 to 40 years of age[268,269,314]; among these, carriers of the Cys634Arg *RET* mutation are reported to be at the highest risk of having PHPT.[269,270] Patients with other *RET* mutations (e.g., of exon 10 involving codons Cys609, Cys611, Cys618, and Cys620) have less than 5% risk of having PHPT.[3,332] However, in some MEN2 patients with codon Cys634 mutations, PHPT has been reported to occur as early as 2 years of age.[3,333,334] In MEN2 patients with PHPT, multiple parathyroid glands may be affected with hyperplasia and/or adenoma formation, and frequently this is asynchronous such that a dominant single-gland parathyroid tumor is often observed.[312]

The diagnosis of PHPT in MEN2 does not differ from patients with nonsyndromic and nonfamilial presentations and is reliant on demonstrating hypercalcemia with elevated or inappropriately normal plasma PTH concentrations. However, MEN2 patients with PHPT are frequently asymptomatic and only have mild hypercalcemia[3,268] such that the diagnosis is often made following routine biochemical testing or during screening for other MEN2 manifestations. Preoperative imaging using sestamibi, ultrasound, and four-dimensional CT scans to localize the parathyroids is useful only in patients who have had prior neck surgery (i.e., thyroidectomy and/or central node neck dissection) for MTC, as this may help to guide the surgical approach. However, in patients in whom the diagnosis of PHPT is made concurrently with that of MTC, neck ultrasound or MRI for MTC staging may help identify enlarged parathyroid glands, but there is little added value of further parathyroid imaging because surgical exploration will allow evaluation of all four parathyroid glands.

Treatment

The current recommendations for treatment of PHPT in MEN2 favor the removal of only enlarged/diseased parathyroid glands rather than the more substantial operations recommended for MEN1.[3,268] However, the surgical approach will depend on the timing of the diagnosis relative to MTC. Thus, in patients who have synchronous MTC and PHPT and are undergoing thyroidectomy for the MTC, the recommendation is to remove only the enlarged parathyroid glands, with the success of parathyroidectomy being monitored by intraoperative PTH assays.[268] However, in patients with four-gland involvement, it is recommended that subtotal parathyroidectomy that leaves a remnant in situ on a vascular pedicle or total parathyroidectomy with autotransplantation should be undertaken.[3] In patients who have development of PHPT subsequent to thyroidectomy, the goals of parathyroid surgery should be tailored to the removal of only enlarged parathyroid glands, which may have been identified by preoperative imaging studies,[3] and intra-operative PTH assays used to monitor for successful removal of the enlarged glands, with the normal parathyroid glands being left in situ. Finally, in patients undergoing thyroidectomy for MTC with normal calcium and PTH, it

recommended that prophylactic parathyroidectomy is not undertaken, but that normal-appearing and viable parathyroid glands should be left in situ.[331]

Additional Clinical Features Associated With MEN2A Variant Disorders

Familial MTC-Only

FMTC is characterized by MTC being the sole manifestation of MEN2. However, distinction between FMTC and MEN2A is difficult because of the low penetrance of pheochromocytoma in some patients, and FMTC should be considered only if there are at least four family members older than 50 years who are affected by MTC but not pheochromocytomas or PHPT.[3,335,336] FMTC, when strict diagnostic criteria are applied, is found to be very rare.

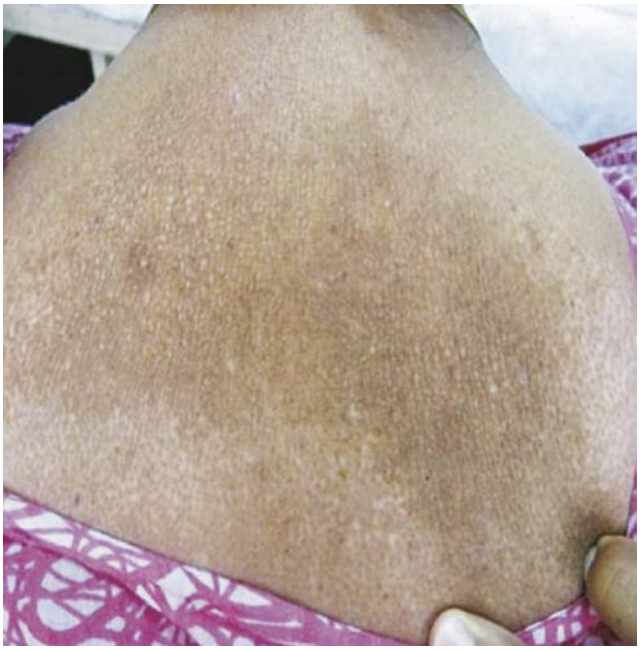
MEN2 With CLA

CLA has been reported to occur in up to 35% of MEN2 patients harboring codon Cys634 mutations[337] (Fig. 42.9) but is very rarely observed in MEN2 patients with other *RET* mutations. CLA typically presents with intense pruritus and a rash in the interscapular area of the T2-6 dermatome region[3] (see Fig. 42.9). The lesions improve with sunlight and may worsen at times of stress. CLA may predate the other clinical manifestations of MEN2. Treatment includes the use of topical creams including corticosteroids, systemic antihistamines, and/or phototherapy, which may provide partial symptom relief.[3]

MEN2 With HSCR

Approximately 7% of patients with MEN2 manifest features of HSCR and typically present shortly after birth with the inability to pass stool and the development of megacolon, resulting from an absence of enteric ganglia along a variable length of the intestine. The phenotype is only observed in MEN2 patients harboring activating *RET* mutations in exon 10 that affect cysteine residues at positions 609, 611, 618, and 620. Heterozygous loss-of-function *RET* mutations are observed in approximately 50% of patients with sporadic HSCR, and thus the co-occurrence of MEN2 with HSCR appears paradoxical (i.e., HSCR occurring in the context of both activating and loss-of-function *RET* mutations).[338,339] Knowledge of receptor structure/function (see the following discussion) has provided potential insights into the molecular basis of these paradoxical observations, with the hypothesis that these specific exon 10 mutations result in constitutive receptor activation sufficient for oncogenic signaling (i.e., resulting in the MEN2 phenotype), but diminishing cell surface receptor expression (e.g., through reduced stability), thereby resulting in inadequate ligand-dependent RET signaling during enteric nervous system development.[340]

Very occasionally, dermatologic features more typical of MEN3 have been reported in patients with MEN2 including dermal hyperneury (i.e., hypertrophic myelinated and unmyelinated nerve fibers in the skin). In addition, multiple sclerotic fibromas have also been reported, more reminiscent of changes observed in the PTEN hamartoma tumor syndrome.[341]



• **Fig. 42.9** Cutaneous lichen amyloidosis in a patient with multiple endocrine neoplasia type 2 harboring a codon Cys634 mutation. (From Birla S, Singla R, Sharma A, Tandon N. Rare manifestation of multiple endocrine neoplasia type 2A & cutaneous lichen amyloidosis in a family with *RET* gene mutation. *Indian J Med Res.* 2014;139[5];779–781.)

Additional Clinical Manifestations Associated With MEN3

Extraendocrine features occur in virtually all MEN3 patients, who typically have the endocrine manifestations of early-onset aggressive MTC together with pheochromocytoma[265]. However, the penetrance and/or severity of these extraendocrine features may be variable, although some may be evident very early in life; when recognized, they may facilitate early diagnosis. However, such features frequently are overlooked, resulting in delays in treatment.[342] These extra- (or non)endocrine features include a skeletal phenotype giving rise to a marfanoid habitus with tall stature, long limbs, narrow elongated face, high arched palate, chest wall abnormalities (e.g., pes excavatum, pes cavus), scoliosis, arachnodactyly, and increased risk of slipped capital femoral epiphyses[3,265]; multiple mucosal neuromas, typically presenting as soft papules in and around the oral cavity (e.g., affecting tongue, lips), as well as in the nasal and laryngeal mucosa (see Fig. 42.6); ocular manifestations such as alacrima, mild ptosis, conjunctival neuromas, and prominent corneal nerve fibers[265]; upper gastrointestinal symptoms such as swallowing difficulties and vomiting, likely due to esophageal abnormalities; lower gastrointestinal symptoms such as altered bowel habit (most commonly constipation) and early feeding intolerance due to diffuse intestinal ganglioneuromatosis (see Fig. 42.6) and impaired colonic motility resulting in megacolon[265,343]; tooth malposition; and dermal hyperneury.

Molecular Genetics

The RET Proto-Oncogene

The human *RET* proto-oncogene, which encodes a single-pass transmembrane receptor of the TK family, is located in the pericentromeric region of chromosome 10q11.2 and comprises 21 coding exons that span approximately 55 kb of genomic DNA.[293,307,344–348] The RET receptor, which is an RTK, consists of an ECD containing four cadherin-like repeats and a cysteine-rich region, a transmembrane domain, and an intracellular domain (ICD) that comprises the TK domain and an isoform specific C-terminal (see Fig. 42.7). *RET* transcription is regulated by several DNA-binding factors that act on upstream promoter and enhancer elements, and the transcript levels are further controlled by regulatory elements within intronic and 3′ untranslated regions.[307] Alternate splicing

of *RET* results in several highly conserved protein isoforms, and the two predominant isoforms are RET9 ("short") and RET51 ("long"), which differ only by 9 and 51 C-terminal amino acids, respectively, in their extreme C-terminal regions[307] (see Fig. 42.7).

Germline RET *Mutations*

More than 50 different germline heterozygous *RET* mutations, which result in receptor activation, have been reported in association with MEN2 and MEN3.[240,340] The majority of MEN2-associated *RET* mutations involve heterozygous nonsynonymous amino acid substitutions of cysteine residues within the cysteine-rich ECD of the receptor, or of noncysteine residues within the intracellular TK domain (see Fig. 42.7). However, several additional MEN2-associated *RET* mutations, the majority of which affect a relatively small number of codons, are reported and include either nonsynonymous amino acid substitutions outside of these regions or small duplications, insertions, and deletions (see Table 42.3). Thus, MEN2 is most frequently associated with amino acid substitutions of codon Cys634 in the cysteine-rich ECD (see Fig. 42.7), with mutations of cysteine residues at codons 609, 611, 618, or 620 in this domain also accounting for a significant proportion of the remaining MEN2 mutations.[240,270,340] MEN2 mutations also occur in the intracellular TK domain, and these involve substitutions of Leucine 790 (i.e., Leu790Phe), Valine 804 (i.e., Val804Met, Val804Leu), and Serine 891 (i.e., Ser891Ala). Additional variants in the cysteine-rich and the TK domains have also been reported in some patients. However, some variants previously reported as pathogenic (i.e., mutations) may have been misclassified due to ascertainment and/or reporting biases, and these likely represent benign variants (e.g., Ser649Leu, Tyr791Phe, and Ile852Met).[267,340,349] Approximately 95% of MEN3 mutations involve a methionine to threonine substitution of codon 918 (Met918Thr) of the intracellular TK domain, and fewer than 5% are represented by the Ala883Phe mutation, which is typically associated with a less aggressive disease course.[350]

A paradigmatic feature of MEN2 and MEN3, which has transformed the clinical management of patients, is the strong genotype-phenotype correlation that predicts the potential disease spectrum and age of MTC onset associated with a specific *RET* mutation. However, the disease phenotype and its severity may vary between individuals harboring the same mutation, and even within the same kindred, thereby implicating a role for genetic modifiers, which may include the co-occurrence of *cis*-acting *RET* coding region variants alongside the pathogenic mutations.[307,340] For example, several *RET* variants (e.g., Glu805Lys, Tyr806Cys, and Ser904Phe) have been reported in individuals harboring the Val804Met mutation, and these are associated with enhanced receptor signaling, which results in a more severe disease phenotype that resembles MEN3.[351,352] In addition, several noncoding and synonymous *RET* polymorphisms, identified by haplotype analysis, have been reported to modify disease expression and clinical course.[272]

The overall prevalence of MEN2-associated germline *RET* mutations in European populations has been estimated to be approximately 1 in 80 to 100,000,[240] although recent evaluation of the Exome Aggregation Consortium cohort revealed a much higher frequency at approximately 1 in 2000.[145] In particular, a high frequency of the ATA moderate-risk Val804Met *RET* variant was observed, indicating that this mutation is likely associated with a lower disease penetrance than reported previously.[145] This observation is supported by a further bioinformatic study in which the Val804Met was estimated to have a disease penetrance for MTC of less than 5%.[271] Together, these studies highlight the need for further unbiased population-based studies to refine current estimates of disease prevalence in *RET* variant carriers, as has been performed for other hereditary endocrine tumor genes.[353] However, it is also important to note that the incidence and spectrum of *RET* mutations may vary by geographic area, reflecting possible population-specific factors and the presence of potential founder mutations. For example, a study of approximately 500 MEN2 families from Germany, Italy, and France demonstrated that approximately 34% of kindreds harbored codon Cys634 *RET* mutations, with approximately 17%, approximately 10%, and 7.6% having codon Val804, Met918, and Leu790 mutations, respectively.[240] In contrast, another study from Denmark reported that codon Cys611 *RET* mutations were the most prevalent, likely reflecting a founder mutation (e.g., Cys611Tyr) in this population.[354]

More than 50% of patients with FMTC have *RET* mutations of Cys618, with Cys618Arg being the most common. However, a small number of families with FMTC do not have *RET* mutations, and recently a germline *ESR2* mutation was reported to cosegregate with MTC in one family,[355] thereby indicating the occurrence of genetic heterogeneity and roles for other genes in the etiology of MTC.

Germline loss of function *RET* mutations are the most common cause of isolated HSCR,[356] and more than 200 different heterozygous *RET* mutations, which are predicted to result in diminished RET receptor signaling and a failure to transmit key developmental signals required for enteric nervous system development, have been reported in HSCR patients.

Somatic RET *Mutations and Rearrangements*

Somatic *RET* mutations are the most common recurrent mutation in sporadic MTC.[357] Overall, 40% to 50% of sporadic MTCs are reported to harbor somatic *RET* mutations, although this frequency varies by tumor size and stage of disease.[340,357,358] Thus, the frequency of *RET* mutations is increased in tumors larger than 2 cm and may exceed 80% in those with advanced or metastatic presentations.[358,359] The somatic *RET* mutations occurring in sporadic MTC typically affect the same residues as those disrupted in MEN2 and MEN3, and 60% to 80% are represented by the MEN3-associated Met918Thr substitution.[358,359] In MTC without *RET* mutations, somatic *RAS* mutations (predominantly of *HRAS* and *KRAS*) represent the second most common genetic abnormality and occur in approximately 10% to 30% of sporadic MTCs, thereby supporting the role for activation of the RET-RAS-MAPK kinase pathway as a central feature of MTC development.[357] Testing for somatic *RET* mutations may be of future clinical use, as a recent meta-analysis has reported that patients with somatic *RET* mutations have an increased risk of lymph node metastases, distant metastases, tumor recurrence, and higher mortality,[360] and another study has reported that advanced MTCs harboring double somatic *RET* mutations were associated with unfavorable outcomes.[359]

Somatic rearrangements of the *RET* locus, giving rise to chimeric fusion proteins with constitutive TK activity, have been reported in approximately 20% to 40% of sporadic papillary thyroid carcinomas (PTCs), and these different RET fusion partners are collectively referred to as RET/PTC rearrangements. Each RET/PTC rearrangement is characterized by the placement of the *RET* TK domain genetic locus adjacent to a ubiquitously expressed donor gene containing a coiled-coil dimerization domain that facilitates constitutive receptor function and

activation of downstream oncogenic signaling pathways.[340] For example, the RET/PTC1 and RET/PTC3 rearrangements, which are most commonly observed, are the result of intrachromosomal inversions on the long arm of chromosome 10 that bring the RET TK domain adjacent to the coiled-coil domain containing 6 (*CCDC6*) or nuclear receptor coactivator 4 (*NCOA4*) genes, respectively. The RET/PTC chimeric proteins have a higher prevalence in radiation-induced PTC, indicating that ionizing radiation likely is a risk factor for their occurrence, although they may also occur without exposure to radiation and are often observed in children with PTC.[345] In addition, RET/PTC fusions have been reported in benign thyroid nodules and in patients with Hashimoto thyroiditis.[340] Somatic RET rearrangements resulting in chimeric proteins have also been reported in approximately 1% of lung adenocarcinomas (e.g., predominantly involving fusions with *KIF5B*) and in case reports of hematopoietic malignancies including chronic myelomonocytic leukemia.[345]

RET *Polymorphisms*

Common polymorphisms and nonpathogenic rare sequence variants within the *RET* gene need to be distinguished from pathogenic *RET* mutations. Common polymorphisms (global MAF >0.5%) within the coding region include 2 missense (p.Gly691Ser, p.Arg982Cys) and 6 synonymous variants. However, many additional missense variants are observed at MAF ≥0.5% in specific ethnic groups (e.g., p.Leu56Met in the European population; p.Tyr791Phe in the Finnish European and Ashkenazi Jewish populations; p.Asp489Asn, pThr278Asn, p.Arg67His, p.Arg114His in East Asian populations; and p.Gly446Arg in African populations), although many of these are also observed at lower frequencies in other ethnic populations. At least 15 common noncoding variants are observed in the *RET* untranslated regions, whereas the large *RET* intronic regions harbor more than 300 additional noncoding common polymorphisms (http://phase3browser.1000genomes.org/ and http://gnomad.broadinstitute.org/). Thus, it is important to recognize the occurrence of these polymorphisms, and caution is required when *RET* sequence variants are identified during genetic testing. This is particularly important for novel missense variants occurring in proximity to known functional RET domains, where ascribing pathogenicity may not be possible until there is clear evidence of variant segregation with the disease phenotype and/or abnormalities in cellular function established. The potential challenge of variant interpretation is highlighted by the observation that approximately 600 different rare (MAF <0.5%) missense *RET* variants have been reported in the GnomAD dataset (http://gnomad.broadinstitute.org/), whereas a recent study evaluating the Exome Aggregation Consortium cohort estimated that approximately 1 in every 35 individuals in the background population will harbor a rare (MAF <0.5%) nonsynonymous *RET* variant.[145] In each setting, most individual *RET* coding variants will not be of clinical significance.

RET Structure and Function

The RET receptor comprises several specific regions: a large ECD containing four cadherin-like repeats and a cysteine-rich region, a hydrophobic single-pass transmembrane domain, a juxtamembrane segment, an intracellular TK domain, and an isoform-specific C-terminal tail[2,307,344–346,348] (see Fig. 42.7). RET signaling is activated by the binding of members of the glial cell–line derived neurotropic factor (GDNF) family of ligands (GFLs), which comprise GDNF, neurturin, persephin, and artemin[344] (see Fig. 42.7). However, for receptor engagement, each of these ligands requires the presence of a ligand-binding coreceptor, which comprises members of the GDNF family receptor α (GFRα) group of proteins.[307,344,347] Four different GDFα coreceptors are described (GFRα1–4), with apparent preferential selectivity for each of the four GFLs. Although the precise sequence of events leading to receptor activation is debated, the formation of the GFL-GFRα complex allows RET recruitment and the formation of heterodimers in which the RET-GFL-GFRα complex has a 2:2:2 stoichiometry.[344] The formation of this ternary complex results in activation of the TK domain and autophosphorylation of multiple intracellular tyrosine residues, which in turn form docking sites for signaling proteins such as those with SRC homology 2 (SH2) or phosphotyrosine-binding domains that ultimately facilitate downstream transmission of signals within the cell via a large number of effector pathways.[293,344] Several conserved residues have been found to have key functions in catalytic activation and subsequent signal transduction.[293,344] For example, autophosphorylated tyrosine residues at Tyr1062 and Tyr1096 in the RET51 isoform appear to form key signaling hubs that facilitate activation of multiple distinct signaling pathways (e.g., RAS-MAPK and PI3K-AKT),[307,346] and autophosphorylation of the juxtamembrane residue Tyr687 has been shown to increase RET catalytic activity. Receptor function is also reported to be dependent on a phosphoserine residue (Ser909) within the activation loop indicating that RET acts as a dual-specific kinase.[361]

The activation of multiple downstream pathways (e.g., Ras/MAPK, PI3K/Akt, phospholipase C-γ, JNK, JAK-STAT, FAK, and β-catenin/Wnt) modulates cellular processes that include differentiation, proliferation, migration, and cytokine production.[348] The ability of RET to direct specific cellular effects is likely to be achieved by several mechanisms. For example, the binding of different RET ligands (i.e., GFLs) may result in differences in receptor activation,[348] whereas the expression of different RET isoforms (e.g., RET9 and RET51) may result in the differential deployment of individual intracellular signaling pathways.[362] Furthermore, the availability of each of the various intracellular signaling partner complexes in different cells may also contribute to RET signaling specificity.

Under physiologic conditions, the RET protein is expressed at its highest levels during embryonic development, where it plays an essential role in the development of multiple tissues. For example, RET is essential for the normal development of the kidneys and urogenital tract, where it plays a key role in modulating wolffian duct patterning and branching morphogenesis of the ureteric bud.[307,363,364] In addition, RET is predominantly expressed in cell lineages derived from the neural crest, including neuroendocrine and neuronal lineages, and it is therefore not surprising that several of these relate to the diseases observed in patients with *RET* mutations. Thus, RET is expressed in the thyroid parafollicular cells, adrenal chromaffin cells, and neurons, where it plays an essential role in the migration and establishment of the enteric nervous system.[293,365] RET expression also occurs in neural crest–derived peripheral and central neuronal populations, where it regulates axonal growth and cell survival.[307] For example, RET is expressed in spinal cord motor neurons during early development, where it mediates potent prosurvival activities, and similar protective effects have been reported in adult ventral midbrain dopaminergic neurons.[347] Finally, RET is expressed at low levels in hematopoietic and immune cell precursors, and it is reported to be required for the formation of gut-associated lymphoid tissue and Peyer patches.[366]

RET *Mutations and Receptor Function*

Mechanistic molecular insights into RET receptor function have been provided by studies of disease-associated *RET* mutations or translocations that result in abnormalities of receptor signaling. Thus, studies of heterozygous *RET* mutations, which cause loss of function and are associated with HSCR or gain of function (i.e., enhanced receptor signaling and are associated with MEN2 and MEN3), have increased our understanding of the structure-function relationships of this RTK. For example, MEN2 mutations involving substitutions of cysteine residues in the ECD (e.g., Cys609, Cys611, Cys18, Cys620, Cys630, Cys634) result in an unpaired cysteine residue that can form a disulfide bond with a similarly unpaired residue on a neighboring mutant RET receptor, thereby resulting in ligand-independent dimerization and constitutive receptor activation (see Fig. 42.7). Interestingly, some of these mutations (e.g., affecting Cys611, Cys18, Cys620) can result in RET receptors with apparent paradoxical gain- and loss-of-function activity. This occurs because the mutant RET receptors, although having constitutive activity, have poor maturation that results in their decreased cell surface expression, which will have detrimental effects on the development of tissues dependent on GFLs. Thus, the mutant RET receptors, although activated, will be fewer, and this provides an explanation for the coexistence of MEN2 due to enhanced RET signaling, and HSCR, which is associated with RET haploinsufficiency and loss-of-function mutations.[293,307,338] The RET receptor mutations of the ECD may also alter downstream receptor signaling. For example, the ligand-independent dimerization associated with cysteine-rich domain mutations may be associated with different cellular localizations and result in altered access to downstream signaling complexes and preferential activation of individual pathways such as the PI3K/AKT pathway[307,348] or otherwise altered ICD function that results in different patterns of receptor autophosphorylation and recruitment of adaptor proteins. These different effects on RET signaling may, in turn, contribute to the observed differences in phenotypes in patients.

The MEN2-associated *RET* mutations of the ICD (e.g., affecting Leu790, Tyr791, Val804, Ser891) result in mutant receptors that are active as monomers in the absence of ligand, and this autonomous signaling is not further enhanced in the presence of ligand. This contrasts to the MEN3-associated *RET* mutation, Met918Thr, which also resides in the ICD TK domain but results in a receptor conformation change that increases affinity for adenosine triphosphate and ligand-independent kinase activity in both monomeric and dimeric forms, which is enhanced further by the presence of ligand.[293,344] This enhanced function is more pronounced for the RET9 isoform when compared with RET51, and this greater signaling activity may contribute to the aggressive clinical phenotype observed in MEN3 patients. In addition, mutations in the RET ICD are reported to result in phosphorylation of substrates more commonly associated with cytoplasmic TKs (e.g., SRC and FAK), and this may also contribute to enhanced tumorigenic activity and more aggressive tumor manifestations in patients.[344]

Animal Models

The *RET* gene is highly evolutionary and conserved in mammalian and vertebrate species, with all higher organisms having a single copy of a RET orthologue. Furthermore, all vertebrates have been observed to harbor GDNF family ligands and respective GFRα proteins. RET orthologues are also observed in more evolutionary distant organisms, including *D. melanogaster* and *Amphioxus*.[347] Although some insight into RET function has come from the study of nonmammalian models, including zebrafish (*D. rerio*) and *Drosophila*, the predominant model organism for evaluating *RET* mutations that are relevant to MEN2 and MEN3 has been the mouse. Several mouse models have been generated by the transgenic expression of mutant RET proteins, or knock-in methods in which specific mutations have been introduced into the *Ret* gene.[367,368] Mice that are homozygous for an inactivating *Ret* mutation die soon after birth and have renal agenesis and a lack of enteric neurons in the gastrointestinal tract.[340,363]

For MEN2, two transgenic models have been established in which the human Cys634Arg mutation is expressed in a tissue-restricted manner. In one model, expression of the human mutant RET9 isoform under the control of the rat calcitonin-gene related peptide/calcitonin promoter was reported to result in overt CCH followed by bilateral MTC with morphologic features similar to human MTC.[369] In the other model, expression of the human mutant RET51 isoform under control of the human calcitonin promoter resulted in mice with early-onset CCH and subsequent bilateral MTC, and one founder line also developed tumors resembling papillary thyroid cancer, pancreatic cystadenomas, and cystoadenocarcinomas.[368,370] In addition, a transgenic mouse expressing the human *RET* gene with the Cys634Arg mutation in multiple tissues (e.g., thyroid, heart, liver, colon, brain) developed CCH and/or MTC that was accompanied by high serum calcitonin concentrations, and the tumors, but not normal tissues, from these mice had RET dimerization and complex formation with Shc and Grb2 adaptor proteins.[371] A knock-in mouse model with the Cys620Arg mutation has also been established for MEN2, and aged heterozygous ($Ret^{+/620R}$) mice were found to develop CCH and adrenal gland hyperplasia, although no overt MTC or pheochromocytoma developed. However, homozygote mice ($Ret^{620R/620R}$) were observed to die on the first postnatal day with renal agenesis and intestinal aganglionosis, consistent with the phenotype observed in conventional *Ret* knockout mice, and these observations further demonstrate the apparent paradoxical activity of certain *RET* mutations that exhibit both loss- and gain-of-function activity.[372]

For MEN3, transgenic and knock-in mouse models have been generated. Transgenic expression of the MEN3-associated Met918Thr mutation under the human calcitonin promoter in one mouse model resulted in the development of nodular CCH from 8 months of age and progression to MTC in 13% of mice after 11 months.[373] Transgenic expression of the same mutation in another mouse model using the dopamine β-hydroxylase promoter that facilitated expression in the developing sympathetic nervous system, enteric nervous system, and adrenal medulla resulted in neuroglial tumors, which were histologically indistinct from human ganglioneuromas, in the adrenals and sympathetic nervous system.[374] A knock-in mouse model harboring the murine equivalent of the Met918Thr mutation (i.e., Met919Thr) has been generated in which heterozygous ($Ret^{+/MEN3}$) and homozygous ($Ret^{MEN3/MEN3}$) mice develop CCH and adrenal chromaffin cell hyperplasia.[375] Progression to pheochromocytoma and a more severe thyroid phenotype were observed in homozygote mice only, thereby indicating a potential dosage effect of the activating mutation on disease expression. However, none of the mouse models developed mucosal neuromas or gastrointestinal ganglioneuromas, which are found in MEN3 patients.[367,368,375]

Genetic Testing, Tumor Surveillance, and Organization of Care

Clinical Utility of Genetic Testing for MEN2 and MEN3

The widespread implementation of *RET* genetic testing has transformed the management of patients with MEN2 and MEN3, as it enables the identification of those individuals who are at a higher risk of developing the disease, and very often this is now in early childhood and before the onset of clinical features (i.e., presymptomatic diagnosis), and therefore appropriate monitoring and early treatment can be implemented. Indeed, the effectiveness of *RET* genetic testing in MEN2 has been demonstrated in studies from Germany, which reveal a fall in the percentage of index cases in the population, owing to the increased detection of at-risk individuals through cascade genetic testing, together with a marked fall in MTC occurrence in nonindex *RET* mutation carriers, owing to prophylactic thyroidectomy.[252] The situation for MEN3 is more difficult due to the high percentage (~75%) of cases presenting with de novo mutations, and thus opportunities for early detection and prophylactic treatment are often missed.[342]

The potential for improved clinical outcomes within kindreds following a genetic diagnosis of MEN2 highlights the need to identify potential index cases harboring germline *RET* mutations. Thus, genetic testing should be considered in several settings to maximize the likelihood of successfully establishing the diagnosis in an index case. Thus, currently, *RET* genetic testing is indicated in all patients presenting with a clinical diagnosis of MEN2 (e.g., MTC and pheochromocytoma), as well as those with clinical features of an associated MEN2 syndrome (e.g., FMTC, CLA, HSCR) or phenotypic manifestations of MEN3. Similarly, those presenting with MEN2-associated clinical features and a relevant family history require testing. In addition, all patients presenting with apparently sporadic MTC, or unilateral or bilateral adrenal pheochromocytoma, should undergo *RET* mutational analysis.[3,340] For patients in whom the *RET* mutation status is unknown, either a prioritized DNA sequencing approach of the *RET* gene that incorporates recurrently affected exons (e.g., exons 10, 11, 13–16) or sequencing of the entire *RET* coding region should be undertaken. In patients in whom the clinical phenotype is highly suggestive of a specific genotype (e.g., typical MEN3 extraendocrine features with Met918Thr mutations), a more limited testing of the *RET* gene sequence may be appropriate. However, if *RET* testing is limited to specific exons, it is possible that additional sequence variants may be overlooked, and this may have clinical implications for the patient.[3] *RET* sequence variants that have not been reported previously in MEN2 and MEN3 patients will need careful evaluation by the clinical genetics team for the likely pathogenicity of the variant, and decisions on the role of cascade genetic testing of family members will have to be taken based on the clinical picture.

Once an established pathogenic *RET* mutation has been identified in an index case, genetic counseling and appropriate genetic testing for the specific sequence variant should be offered to all at-risk first-degree relatives. Cooperation of the patient will be required to contact the relatives, and occasionally complex ethical issues may arise (e.g., if the patient is reluctant to disclose their health status to family members). In the management of children, similar ethical challenges may arise if an affected parent refuses to allow appropriate investigation and testing of an at-risk child. However, such situations are rare. *RET* genetic testing of at-risk individuals should be performed at an early time point, and in children who may harbor the ATA highest- and high-risk categories of *RET* mutations, this should be in the first months or years of life, respectively, due to the early onset of disease in these groups. *RET* gene testing of the first-degree relatives of a presumed affected index case whose mutational status is unknown or unavailable is also indicated. Preconception genetic counseling, where appropriate, should be provided to all individuals at risk of transmitting *RET* mutations to their future offspring. Prenatal genetic testing using invasive (e.g., amniocentesis) or noninvasive approaches (e.g., by evaluating cell-free fetal DNA from maternal blood) may inform prospective parents regarding the disease status of the developing fetus. Recent advances in genetic testing combined with in vitro fertilization also offer prospective parents the opportunity to have a preimplantation genetic diagnosis.

Screening and Intervention in RET *Mutation Carriers*

The ability to make a genetic diagnosis of MEN2 or MEN3 in at-risk individuals, often in early childhood and before the onset of clinical features, enables the appropriate monitoring and treatment of asymptomatic individuals. Recommendations for the respective schedule of tumor surveillance and timing of potential prophylactic thyroidectomy is determined by the ATA risk category of *RET* mutation (see Table 42.4). Thus, for those with the highest- and high-risk categories of *RET* mutations, prophylactic thyroidectomy is typically recommended by the ages of 1 and 5 years, respectively (although earlier in some instances based on clinical, biochemical, and/or radiologic features). The timing of prophylactic surgery in those with moderate-risk *RET* mutations is now based on the serial measurement of basal serum calcitonin levels in combination with neck imaging studies.[3] Appropriate annual screening for pheochromocytoma and PHPT, based on genotype-phenotype correlations, is also recommended and should commence at an age commensurate with the risk profile of *RET* mutation (see Table 42.4). In adult patients identified to harbor MEN2-associated *RET* mutations through cascade genetic testing, serum calcitonin concentrations and plasma/urinary free fractionated metanephrines should be determined immediately. In individuals with normal serum calcitonin concentrations and/or normal metanephrines, annual monitoring of serum calcitonin and plasma/urinary metanephrines should commence. This situation is most frequently observed for moderate-risk *RET* mutations that may result in late-onset disease or reduced tumor penetrance. In those identified to have elevated serum calcitonin concentrations or elevated plasma or urinary metanephrines, full evaluations to determine the stages of the MTC and/or pheochromocytoma should be performed prior to appropriate treatment. Furthermore, it is imperative that coexistent pathologies such as pheochromocytoma should be excluded prior to any intervention in all patients with a *RET* mutation or who has MTC, MEN2, or MEN3.

MEN Type 4

MEN4, an autosomal dominant disorder due to mutation of the *CDKN1B* gene located on chromosome 12p13, has been reported in fewer than 20 index cases,[172,376,377] and patients had clinical features that are similar to those patients with MEN1. Indeed, among the 5% to 10% of patients with a clinical diagnosis of MEN1 who do not have *MEN1* gene mutations, approximately 3% will have *CDKN1B* mutations.

Clinical Features and Management

MEN4 patients predominantly have parathyroid, pituitary, and pancreatic tumors in association with other tumors, which

include gastrointestinal, bronchial, and cervical NETs; NF adrenal tumors; PTC; lipoma; and breast cancer.[5,172,376–379] Parathyroid tumors associated with PHPT have been reported in approximately 80% of MEN4 cases, although PHPT is observed to occur at a later age than MEN1. Pituitary tumors (including somatotropinoma, corticotropinoma, prolactinoma, and NF adenoma) occur in approximately 40% of cases,[172] and duodenopancreatic NETs occur in approximately 35% of cases. The investigation and treatments for these MEN4-associated tumors are similar to those for MEN1 and non-MEN1 tumors.

Molecular Genetics and Animal Models

The identification of the gene responsible for MEN4 resulted from observations in a rat model of endocrine neoplasia (termed *MENX*),[380] in which the disease locus was mapped to the distal part of chromosome 4,[381] a region that contained the *Cdkn1b* gene. *Cdkn1b* encodes the CDKI $p27^{kip1}$, also referred to as p27.[171,172,377,379] A homozygous frameshifting insertion of 8 bp in the rat *Cdkn1b* gene at codon 179, which resulted in a missense peptide and termination at codon 218 and a highly unstable mutant $p27^{kip1}$ protein, was identified in the MENX rats.[171,172] Subsequent studies of a patient with a MEN1 phenotype (pituitary and parathyroid disease) but absence of the *MEN1* mutation identified a nonsense *CDKN1B* mutation.[171] The identification of further patients harboring *CDKN1B* mutations followed, and MENX was renamed *MEN4*.[172,382,383] To date, fewer than 20 MEN4 index cases with *CDKN1B* mutations have been reported, and the majority of these mutations are associated with reduced levels of $p27^{Kip1}$ or altered protein function, consistent with a tumor suppressor role. The mutations reported include nonsense, frameshift, splice-site, and missense mutations.[171,172,376,377,382–385] Germline *CDNK1B* mutations have also been reported in patients with apparently sporadic (i.e., nonfamilial) PHPT, although further studies are required to confirm the clinical significance of the variants and whether such individuals develop additional MEN-associated tumors.[386,387] It is notable that that some rare missense variants in *CDKN1B* and other CDK inhibitor genes are observed at relative high frequency in the background population,[145] thereby suggesting that some *CDKN1B* variants that are benign polymorphisms may have been misclassified as mutations.

$p27^{kip1}$ participates in cell cycle regulation through its interaction with several cyclins and their respective CDKs. These include interactions with cyclin E/cdk2, cyclin A/cdk2, and cyclin D/cdk4.[388–390] The major function of the $p27^{kip1}$ protein appears to be the control of G1 to S phase during cell replication, where the binding of $p27^{kip1}$ to cyclin E/cdk2 arrests cell cycle progression.[388] The cellular $p27^{kip1}$ levels are tightly regulated at transcriptional, translational, and post-translational levels, and the transcription of *CDKN1B* is under the regulation of menin through its interaction with MLL.[191,192] However, post-translational regulation facilitates rapid changes in $p27^{kip1}$ expression. For example, on receipt of a mitogenic signal, at least two pathways in the cytoplasm and nucleus are activated, which result in phosphorylation of specific residues on $p27^{kip1}$ leading to proteosomal-mediated degradation and a derepression of cell cycle progression.[388,391,392] Furthermore, downregulation of $p27^{kip1}$ is observed in many cancers, including those of the colon, breast, stomach, and prostate.[388] The reported mechanisms for reduced levels of $p27^{kip1}$ protein may include decreased transcription, reduced translation, increased protein degradation, reduced binding to key interacting partners, or cellular mislocalization.[376,382–384,388]

Genetic Testing, Tumor Surveillance, and Organization of Care

Genetic analysis for *CDKN1B* mutations is indicated in individuals who have clinical evidence of typical or atypical MEN1 but without *MEN1* mutations or those who are first-degree relatives of patients with a known *CDKN1B* mutation.[1,172,376] The few numbers of patients reported with MEN4 make it difficult to provide guidelines specifically for the management of MEN4, and currently it would appear reasonable to follow a similar protocol of surveillance to that of MEN1.[1,172,376,377]

Future Directions and Concluding Remarks

Significant progress has been made in the diagnosis and management of the MEN syndromes. In particular, the widespread implementation of *MEN1*, *RET*, and *CDKN1B* genetic testing has facilitated the identification of individuals at risk of developing MEN1, MEN2 and MEN3, and MEN4, respectively. This approach has facilitated the periodic screening of mutation carriers, thereby enabling the early and/or presymptomatic detection of tumors, as well as leading to treatments that aim to reduce disease-associated morbidity and mortality. For example, the implementation of "prophylactic" thyroidectomy in MEN2 has resulted in significant improvements in clinical outcomes. Furthermore, an improved understanding of the natural history of the MEN syndromes has led to the formulation of clinical guidelines, but these are often based on a consensus among experts rather than evidence from clinical trials, which are required to address key issues such as the optimal treatment of MEN1-associated pancreatic NETs and parathyroid tumors. Reliable biomarkers and diagnostic tools that accurately predict the behavior of MEN1-associated pancreatic NETs and thymic carcinoids are also required, as is the evaluation of novel therapeutic approaches that have emerged from an understanding of menin biology, which have been demonstrated to be effective in preclinical models. In addition, the genetic and environmental factors that influence MEN2 disease expression and penetrance remain to be elucidated, and improved screening programs that enable the detection of de novo mutations associated with MEN3 are needed to reduce the number of children presenting with advanced MTC. More effective targeted treatments for advanced MTC are also required, as well as establishing the optimal use of existing therapies, including RTK inhibitors. This is likely to be aided by further mechanistic insights into RET receptor structure/function and the development of personalized therapies targeting individual *RET* mutations. Finally, the natural history of MEN4 and its clinical expression needs to be characterized by the continued identification and careful clinical evaluation of individuals and kindreds with *CDKN1B* mutations.

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43

The Immunoendocrinopathy Syndromes

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CHAPTER OUTLINE

KEY POINTS

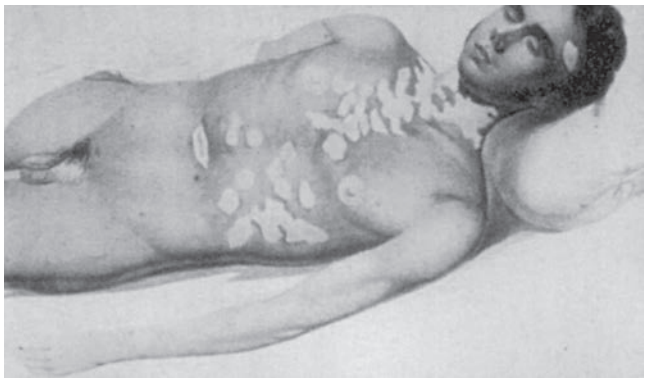
- Endocrine diseases may occur together, and understanding of these associations can lead to earlier diagnosis of additional disorders.
- Many autoimmune endocrine diseases have genetic risk at overlapping genetic loci, explaining, in part, their concurrence in individuals.
- Elucidation of the causes of these rare disorders has led to fundamental insights into the functioning of the immune system in autoimmunity.
- Studies of these disorders have uncovered the genetic basis for these rare syndromes and have helped to define important immune pathways.
- The search for means to define endocrine autoimmunity and disease states has led to the development of new assays that have become the cornerstone of endocrine autoimmune testing.
- Recommended testing for these related disorders is discussed in this chapter.

Since Addison's initial description of primary adrenal insufficiency in a patient with two autoimmune disorders (vitiligo and the hyperpigmentation of Addison disease), the immunoendocrinopathy syndromes have contributed to the understanding of both endocrinology and immunology (Fig. 43.1). Understanding the pathogenesis of the polyendocrine syndromes continues to expand. In particular, shared genetic loci underlying disease susceptibility, potential environmental factors, and organ-specific autoantigens targeted by the immune system are being defined. Recent advances include the development of more reliable T-cell and other immunologic assays, further refinement in predictive models of disease, and continued unraveling of the genetic factors underlying disease susceptibility.

Most autoimmune endocrine disorders (e.g., type 1 diabetes, autoimmune thyroid disease) occur in isolation. Two distinct autoimmune polyendocrine syndromes with characteristic groupings of manifestations are readily recognized. *Autoimmune polyendocrine syndrome type I* (APS-I) is a rare disorder with autosomal recessive inheritance that is caused by defects in the *autoimmune regulator* (*AIRE*) gene. In contrast, *autoimmune polyendocrine syndrome type II* (APS-II) is more common but less well defined and includes overlapping groups of disorders. A unifying characteristic within APS-II is the strong association with polymorphic genes of the human leukocyte antigen (HLA) region located on the short arm of chromosome 6 (band 6p21.3). In addition to HLA, many other genetic loci are likely to contribute to susceptibility to APS-II. For purposes of simplicity in this chapter, APS-II encompasses what some clinicians divide into APS-II (Addison disease plus type 1 diabetes or thyroid autoimmunity), APS-III (thyroid autoimmunity plus other autoimmune diseases, not Addison disease or type 1 diabetes), and APS-IV (two or more other organ-specific autoimmune disorders).

APS-II has also been known by various other names, including Schmidt syndrome, polyglandular autoimmune disease, polyglandular failure syndrome, organ-specific autoimmune disease, and polyendocrinopathy diabetes. Such diverse names reflect the large number of studies and case reports of this syndrome and its historical importance. Each of these other names has some shortcomings, such as failure to include the fact that both hyperfunction and hypofunction of endocrine glands can occur, or failure to recognize that nonendocrine disorders such as pernicious anemia and celiac disease are parts of the syndrome. Studies of patients with APS-II were instrumental in identifying the autoimmune bases of several diseases and developing autoantibody assays such as those for type 1 diabetes and cytoplasmic islet cell antibodies.

Other rare autoimmune endocrine disorders have contributed to an understanding of the development of autoimmunity. For example, the rare disorder called *immunodysregulation polyendocrinopathy enteropathy X-linked syndrome* (IPEX) is caused by a mutation of the *forkhead box P3* (*FOXP3*) gene. *FOXP3* plays a central role in the development and function of regulatory $CD4^+$ T cells that function to maintain tolerance to self. It has become increasingly



• **Fig. 43.1** This illustration accompanied Addison's initial description of primary adrenal insufficiency (Addison disease). (From Addison T. *On the Constitutional and Local Effects of Disease of the Supra-renal Capsules*. London, UK: Samuel Highley; 1855.)

recognized that these T cells play a key role in the pathogenesis of many autoimmune diseases, and therapies targeting these cells will likely be developed and tested. A thorough understanding of these rare and often genetically simple disorders provides insight into the development of syndromes that are characterized by polygenic inheritance and that affect a larger group of patients.

Autoimmunity Primer

An understanding of the pathophysiology of autoimmune disease requires a basic knowledge of the immunologic mechanisms that underlie tolerance (the ability to differentiate self from nonself). Autoimmunity develops when the mechanisms of immune tolerance break down. It can occur centrally at the level of the generative organs (e.g., thymus, bone marrow) or peripherally in the target organs or lymphoid tissues. T lymphocytes and autoantibodies produced by B cells are two arms of the immune system that differ fundamentally in their recognition of target antigens. Autoantibodies react with intact molecules (including both soluble and cell surface molecules) and usually interact with conformational determinants of the autoantigen. In contrast, T lymphocytes recognize peptide fragments of autoantigens, often 8 to 12 amino acids in length, that are presented on the surface of another cell by molecules of the major histocompatibility complex (MHC).

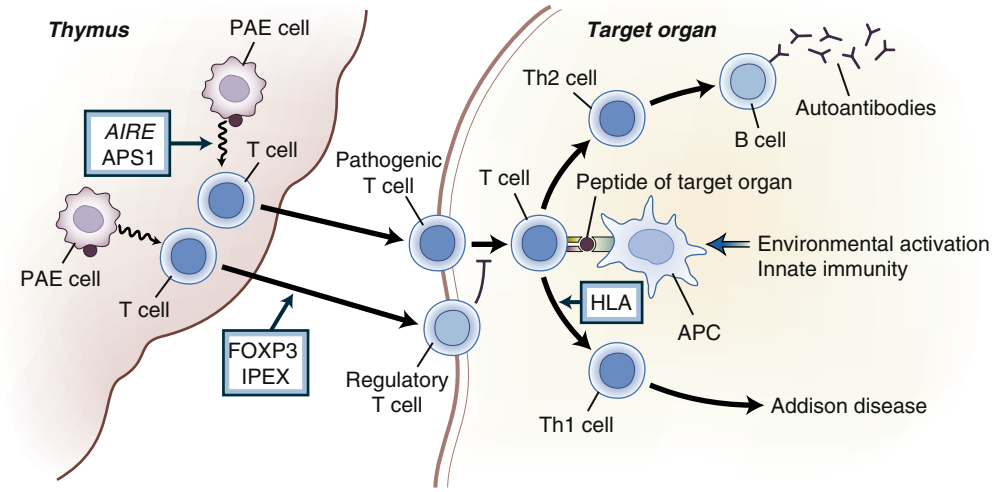
Histocompatibility molecules interact with T-cell receptors when bound with an antigenic peptide. These molecules resemble a hot dog in a bun, with the antigenic peptide (the hot dog) bound in the groove of the histocompatibility molecule (the bun). Histocompatibility molecules are extremely polymorphic, with different amino acids lining the peptide-binding groove. These variable amino acids determine which peptides are bound and presented to T lymphocytes.

T cells differ based on multiple cell surface molecules, and these molecules determine their function in the immune system. T cells interact with other cells within and outside the immune system. $CD4^+$ T cells typically react with peptides that are derived from proteins in extracellular compartments that are bound and acquired by class II histocompatibility molecules (HLA-DP, HLA-DQ, or HLA-DR in humans), expressed on antigen-presenting cells (APCs) such as macrophages, dendritic cells, and B lymphocytes. $CD8^+$ T cells react with peptides bound by class I histocompatibility molecules (HLA-A, HLA-B, and HLA-C). Class I molecules are present on the surface of almost all nucleated cells. The antigen peptide in this case is derived from proteins expressed endogenously and is presented in a complex by class I HLA by the target cell itself. Recognition of the antigenic peptide by $CD8^+$ T cells typically leads to the release of cytotoxic chemicals that kill the target cell.

The T-cell response depends on the context in which the antigen is presented. The simple expression of histocompatibility molecules and recognition of antigen by a T cell are not sufficient for T-cell activation. This context is at least partially determined by the interaction of cell surface molecules on both the T cell and the APC. Interaction among the MHC, the peptide, and the T-cell receptor (*signal one*) is critical to the activation process; other co-stimulatory molecules then help to define the nature of the immune response (*signal two*). The context in which the antigens are presented is critical for the determination of this response. Cell surface molecules and receptors, cytokines, and chemokines form the context in which the antigen is presented. Based on this context, the cell can become activated, tolerized, or anergic (immune unresponsive). For example, the APC cell surface molecule CD80 or CD86 engages the CD28 receptor on the T cell and amplifies signal one, which leads to T-cell activation. When a T cell recognizes an antigen in the context of the MHC and does not receive the appropriate second signal, anergy results.

Tolerance induction is a staged process that begins in the thymus during T-cell maturation. This process depends in part on the presence of *peripheral antigens* in the thymus. Peripheral antigens are self-antigens (e.g., insulin) normally expressed in tissues outside the immune system that are expressed at low levels in the thymus. Developing T cells that react strongly to these peripheral molecules in the context of the MHC are deleted in the thymus and are thus removed from the T-cell repertoire in a process known as negative selection. Study of *AIRE* gene knockout mice has supported the importance of these phenomena in the development of autoimmunity. These mice have low levels of expression of peripheral antigens in the thymus and develop lymphocytic infiltrates in multiple organs (see later discussion).

Peripheral tolerance is an important mechanism for tolerance induction after T cells have matured in the thymus. Anergic and regulatory T cells are integral in the development of tolerance for naive T cells. A major population of T-regulatory cells carry the cell surface markers CD4 and CD25 and express FOXP3. The function of the population of $CD4^+/CD25^{high}$ cells involves an active suppressive activity and relies on the transcription factor FOXP3. Deletion of this transcription factor leads to fulminant autoimmunity in neonates (e.g., neonatal type 1 diabetes and enteropathy), often resulting in death within the first year of life (IPEX syndrome; see later discussion). Another set of key molecules that help control peripheral T-cell tolerance are cytotoxic T-lymphocyte antigen 4 (CTLA4) and programmed death 1 (PD1).[1] CTLA4 is expressed in T cells and acts as a negative regulator of T-cell signaling by competing with the T-cell activator CD28 (described earlier). CTLA4 outcompetes CD28 for binding to its ligands CD80 and CD86 due to its higher affinity for ligand. In addition, CTLA4 is broadly expressed on the surface of FOXP3-expressing CD4-positive T-regulatory cells, where it likely plays a role in blocking CD28 interactions with CD80 and CD86. PD1 is yet another co-stimulatory molecule that becomes upregulated on T cells that have been chronically activated, and it confers negative signals through inhibitory signaling domains in its intracytoplasmic tail. The importance of both PD1 and CTLA4 in peripheral tolerance is underscored in knockout mouse models that develop spontaneous multiorgan autoimmunity and in cancer patients treated with antibodies that block their activity where many of these patients develop autoimmune complications (see later discussion).



• **Fig. 43.2** Model of the pathogenesis of autoimmunity in polyendocrine disorders. The development of autoimmune disease is determined by a group of T cells that recognize one or more organ-specific epitomes. Peptides are presented in the human leukocyte antigen (HLA) molecule and are recognized by the T-cell receptor. Recognition of self molecules depends on the maturation of the T cell, a process that begins in the thymus and continues in the periphery. The transcription factor FOXP3 stimulates the development of $CD4^+/CD25^+$ regulatory T cells. B cells produce autoantibodies under the stimulation of T cells. *AIRE*, autoimmune regulator; *APC*, antigen-presenting cell; *APS1*, autoimmune polyendocrine syndrome 1; *IPEX*, immunodysregulation polyendocrinopathy enteropathy X-linked; *PAE*, peripheral antigen-expressing cell; *Th1*, type 1 helper T cell; *Th2*, type 2 helper T cell. (From Eisenbarth GS, Gottlieb PA. Autoimmune polyendocrine syndromes. *N Engl J Med*. 2004;350:2068–2079.)

Cognate help is the process by which B cells are activated by $CD4^+$ T cells that are responding to the same antigen. $CD4^+$ T cells activate B cells to produce the humoral immune response. This occurs after the $CD4^+$ T cell engages an antigen in the context of the MHC on the cell surface of a B cell. The cytokines (interleukin [IL] 4, IL5, and IL6) produced by the $CD4^+$ T cells induce the maturation of a B cell. Depending on the cytokine milieu, the B cell will switch from producing immunoglobulin M (IgM) to IgG, IgE, or IgA. The development of B-cell tolerance is partially dependent on this linked recognition: autoreactive B-cell clones that do not have a $CD4^+$ T cell that can bind with the antigen in its MHC groove will not normally be activated. Thus, in most cases, the generation of autoantibodies by B cells is also linked to an autoreactive T cell specific for the same self-antigen. Growing evidence supports the role of autoreactive B cells as critical APCs to autoreactive T cells, creating a positive feedback loop in the expansion and maintenance of the autoimmune process.

Natural History of Autoimmune Disorders

The natural history of autoimmune disorders can be divided into a series of stages beginning with genetic susceptibility, followed by triggering of autoimmunity (e.g., dietary gliadin exposure in celiac disease) and active autoimmunity preceding clinical manifestations (e.g., progressive glandular destruction), and, finally, overt disease. This is a theoretical construct that may be useful for understanding factors involved with the development of autoimmunity and disease, but of necessity it is simplified and does not reflect the potential relapsing-remitting nature of autoimmunity (Fig. 43.2).

Genetic Associations

Although there is familial aggregation of APS-II and its component disorders, there is no simple pattern of inheritance (Table 43.1). Susceptibility is probably determined by multiple genetic loci (with HLA having the strongest effect) interacting with environmental factors. Autoimmune diseases share common genetic risk factors, including HLA, the MHC class I–related gene A (*MICA*), the gene for lymphoid tyrosine phosphatase (*PTPN22*), the cytotoxic T lymphocyte–associated antigen 4 (CTLA4), and the gene for NACHT leucine-rich repeat protein 1 (NLRP1, or NALP1).[2] In addition, genetic susceptibility for some autoimmune diseases has been linked to polymorphisms that are organ specific—for example, polymorphisms in the variable nucleotide tandem repeat upstream from the insulin gene have been associated with risk for type 1 diabetes.[3]

Genes located on the MHC found on chromosome 6 have been implicated in the pathogenesis of organ-specific autoimmune diseases. These genes are in strong linkage disequilibrium with each other and encode proteins that are important in the function of the immune system. Foremost in importance for the genetics of organ-specific autoimmune diseases are class I and class II HLA genes. Molecular HLA genotyping has revealed many subtypes of the older, serologically defined alleles, and the unique genetic sequence encoding each polymorphic chain of the histocompatibility molecules is now given a unique identifying number. A case in point is the DQ molecule, which is the histocompatibility molecule most strongly associated with endocrine autoimmunity. A number is assigned for each unique α- and β-chain sequence. Examples are DQA1*0501 for the α chain and DQB1*0201 for the β chain of the DQ molecule (also termed *DQ2*) commonly encoded on DR3 (DRB1*0301) haplotypes. A haplotype consists of a series of alleles of different genes on a contiguous region of a chromosome (e.g., DQA1 and DQB1 alleles) that are inherited together. A genotype is the combination of the haplotypes of both chromosomes. Fine mapping of the HLA has shown remarkable conservation of the HLA-A1/B8/DR3 haplotype such that a region of approximately 2.9 megabases is invariable. Conservation of large areas

TABLE 43.1 Genetic Associations With Autoimmune Disease

Gene	Proposed Mechanism of Action	Polymorphism/Mutation	Disease	Inheritance
HLA	Antigen presentation	DR3-DQ2/DR4-DQ8 DR3-DQ2 DR3-DQ2/DRB1*0404-DQ8 DR3-DQ2/DR4-DQ8 DR3 DR5	Type 1 diabetes Celiac disease Addison disease Graves disease Hypothyroidism	Multigenic
MICA	Priming of naive T cells	5, 5.1 4, 5.1 5.1	Type 1 diabetes Celiac disease Addison disease	Multigenic
PTPN22	T-cell receptor signaling pathway through interaction with regulatory kinases	Tryptophan substitution for arginine at position 620	Type 1 diabetes SLE RA Graves disease Hypothyroidism Vitiligo	Multigenic
CTLA4	Receptor on activated $CD4^+$ and $CD8^+$ T cells; decreases T-cell activation	CT60 CT60; +49A/G CT60; +49A/G ++49A/G ++49A/G	Type 1 diabetes Graves disease Hypothyroidism Celiac disease Addison disease	Multigenic
AIRE	"Peripheral" antigen presentation in the thymus	Multiple reported mutations	APS-I	Autosomal recessive
FOXP3	Transcription factor important for maturation of $CD4^+$/$CD25^+$ regulatory T cells	Multiple reported mutations	IPEX	X-linked

AIRE, Autoimmune regulator; *APS-I*, autoimmune polyendocrine syndrome type I; *CTLA4*, cytotoxic T lymphocyte–associated antigen 4; *FOXP3*, forkhead box protein 3; *HLA*, human leukocyte antigen; *IPEX*, immunodysregulation polyendocrinopathy enteropathy X-linked; *MICA*, major histocompatibility complex class I–related gene A; *PTPN22*, the gene for lymphoid tyrosine phosphatase; *RA*, rheumatoid arthritis; *SLE*, systemic lupus erythematosus.

suggests that these areas of the genome have been inherited without recombination and are in very tight linkage disequilibrium. This greatly complicates the ability to identify which, if any, of the genes within the area of conservation are associated with disease and must be accounted for when assessing susceptibility to disease in this region.

Part of the overlapping risk for autoimmune disease is related to shared genetic susceptibility, especially within the HLA. For example, the highest-risk HLA genotype for type 1 diabetes is DR3-DQ2, DR4-DQ8 (DQ8 = DQA1*0301-DQB1*0302). The importance of this HLA genotype in the development of type 1 diabetes is highlighted by the observation that children who inherited the same DR3-DQ2, DR4-DQ8 as a sibling with type 1 diabetes are at greater than 75% risk for development of autoimmunity by age 12 years and at greater than 50% risk of developing diabetes by 12 years.[4] A specific DR4 subtype of this gene, DRB1*0404, shows a strong association with Addison disease.[5,6] The DR3-DQ2 haplotype is associated with celiac disease both in the presence[7] and absence[8] of type 1 diabetes. This haplotype has been associated with autoimmune thyroid disease,[9] although conflicting reports exist.[10]

Whereas some HLA alleles increase disease risk, others are associated with protection from disease. For example, the DQ alleles DQA1*0102-DQB1*0602 (usually associated with DR2) not only confer strong protection from type 1A diabetes in a dominant fashion[11] but also confer susceptibility to another autoimmune disorder—multiple sclerosis. Furthermore, this protection is not general to endocrine autoimmunity, because no protection from Addison disease is afforded by DQB1*0602. DP is another gene within the MHC, and its 0402 polymorphism has been shown to be associated with a decreased risk for type 1 diabetes in subjects with the highest-risk HLA genotype for type 1 diabetes (DR3/DR4).[12] Observations such as these suggest that as more is learned about the genetic influence of disease, researchers will be able to combine different genotypes and refine prediction of autoimmune disease.

MICA produces a protein that is expressed in the thymus and in naive $CD8^+$ T cells. Polymorphisms of *MICA* have been associated with type 1 diabetes,[13] celiac disease,[14] and Addison disease.[15] A particular polymorphism of *MICA*, denoted 5.1, results from the insertion of a single base pair. This insertion produces a premature stop codon and a truncated protein. This particular polymorphism has been shown to influence the risk for Addison disease in subjects with autoimmunity associated with Addison disease.

Genes outside the MHC have also been implicated in the pathogenesis of autoimmune disease. For example, the *PTPN22* gene encodes lymphoid tyrosine phosphatase (LYP) protein. LYP, through interactions with regulatory kinases such as CSK, appears to act as an inhibitor of the signal cascade downstream from the T-cell receptor. A specific polymorphism associated with a tryptophan substitution for arginine at position 620 (R620W) blocks LYP's interaction with CSK.[16] Recent work has suggested that this allele may increase the development of autoreactive B cells[16,17] and affect intracellular signaling pathways in both T and B cells.[18] This polymorphism has been associated

with type 1 diabetes,[19] rheumatoid arthritis,[20] systemic lupus erythematosus (SLE),[21] Graves disease,[22] and vitiligo,[23] and is weakly associated with Addison disease.[24] Furthermore, this disease-associated allele has also been associated with SLE, rheumatoid arthritis, type 1 diabetes, and autoimmune hypothyroidism in families with several members affected by more than one autoimmune disease.[24]

CTLA4 is expressed on activated $CD4^+$ and $CD8^+$ T cells, where it is hypothesized to act as a negative regulator as outlined earlier.[25] Several polymorphisms within the CTLA4 gene have been associated with autoimmune diseases. One polymorphism associated with AT repeats has been shown to reduce the inhibitory function of CTLA4 in subjects with Graves disease.[26] A single nucleotide polymorphism in the 3′ untranslated region, denoted CT60, has been associated with Graves disease[27] and autoimmune hypothyroidism.[28] An additional polymorphism, denoted +49A/G, has been associated with celiac disease in the Dutch population,[29] with autoimmune thyroid disease,[30] and with Addison disease.[31]

NALP1 regulates the innate immune system. After the initial observation that this gene was associated with the risk of vitiligo[32] and other related autoimmune diseases, it was associated with Addison disease and type 1 diabetes.[33]

Organ-specific genetic polymorphisms have been associated with the development of specific autoimmune diseases. For example, polymorphisms of the variable number of tandem repeats upstream of the insulin gene have been associated with the development of type 1 diabetes. Higher numbers of tandem repeats are associated with increased production of insulin in the thymus and protection from type 1 diabetes presumably due to improved negative selection of insulin-reactive T cells.[3] Similarly, polymorphisms of the thyroglobulin gene are associated with autoimmune thyroid disease.[34]

Single-gene defects such as *AIRE* and *FOXP3* cause multiorgan autoimmunity and are discussed in sections devoted to those topics. Analysis of mutations of the *AIRE* gene indicates that it generally does not play a role in APS-II or sporadic Addison disease, with 1 (1.1%) in 90 patients with Addison disease (non–APS-I) and 1 (0.2%) in 576 control subjects having *AIRE* mutations.[31]

Environmental Triggers

Although genetics is known to play an important role in the development of autoimmunity, it does not tell the whole story. For example, the highest-risk HLA genotype for type 1 diabetes (DR3-DQ2, DR4-DQ8) is associated with a risk of 1 in 20 for the development of diabetes.[35] Although this is greater than the general population prevalence rate of 1 in 200 by the age of 20 years, it is certainly not a 100% risk. Therefore, other factors (genetic and environmental) must be involved in the initiation of autoimmunity. Some theorize that these factors may be environmental triggers.

For one disease, celiac disease, the underlying environmental trigger has been identified: gluten. Through studies such as DAISY (the Diabetes Autoimmunity Study in the Young), BabyDiab, and CEDAR (Celiac Disease Autoimmunity Research), the timing of first exposure to cereal has been identified as a risk factor for the development of diabetes and celiac disease autoimmunity. Infants exposed at a very young age to cereal developed diabetes and celiac-associated autoimmunity at a greater rate than those who had cereal introduced at a later date.[36–38] In epidemiologic studies, cod liver oil consumption has been associated with a decreased risk for type 1 diabetes. Cod liver oil contains omega-3 polyunsaturated fats and vitamin D. In prospective studies, there is some suggestion that lower consumption of omega-3 polyunsaturated fats is associated with an increased risk for autoimmunity associated with type 1 diabetes.[39] Further investigation may identify additional environmental associations. Changes in our diet, our food composition, and use of medicines such as antibiotics have the potential to change our gut flora and microbiome. Animal studies suggest that changes in the gut microbiome can affect disease in these models of autoimmunity.[40,41] Human evidence is being sought to determine whether the interaction between the microbiome and innate immunity is leading to increased inflammation and setting the stage for increased frequencies of all autoimmune disorders that have recently been observed.[42,43]

Virus and other infections have been considered as a cause of autoimmunity and have been shown to cause the development of type 1 diabetes when infection occurs in utero for rubella, for example. Examination of pancreata from individuals with prediabetes (autoantibodies) or those with diabetes has provided evidence consistent with viral infection of the organ.[44,45] Association is not causality, and further experiments are needed to determine whether this finding is an important component of how either autoimmunity is initiated or propagated.

Targeted immunologic therapies are also associated with the induction of autoimmunity. A remarkable example is the treatment of patients with multiple sclerosis with an anti-CD52 monoclonal antibody. One-third of 27 patients given the monoclonal antibody developed antithyrotropin receptor autoantibodies and hyperthyroidism.[46] Interferon-α (IFNα) therapy for hepatitis has been associated with thyroid autoimmunity[47] and type 1 diabetes.[48] Severe hypoglycemia associated with insulin autoantibodies in the absence of insulin administration, termed *Hirata disease*, is associated with methimazole treatment of Graves disease. The development of Hirata disease in these patients is associated with HLA-Bw62/Cw4/DR4 with a specific DRB1 allele (DRB1*0406).[49] Immune checkpoint blockade is now an approved treatment approach for several cancers, and these antibodies that block CTLA4 or PD1 on T cells have both been associated with the induction of autoimmune complications in a subset of patients. These autoimmune complications are broad but now include the induction of endocrine-related autoimmunity including thyroiditis, type 1 diabetes, lymphocytic hypophysitis, and adrenalitis.[50]

Development of Organ-Specific Autoimmunity

Autoantibodies highly specific for a given disorder are present before disease onset. Each specific autoantibody reacts with only a single autoantigen, although autoantigens may be present in multiple tissues. The targets of autoantibodies appear to be unrelated; however, for organ-specific autoimmunity, they are usually expressed in specific cells and cellular sites. Anti-islet antibodies include antibodies to glutamic acid decarboxylase (GAD); islet cell antibody (ICA) 512 (also termed *insulinoma antigen 2* [IA2]); insulin; and, the most recently discovered, ZnT8.[51] Celiac disease is associated with antibodies against tissue transglutaminase (tTG). Addison disease is associated with antibodies against 21-hydroxylase.

Given that the antibodies can be identified before the development of organ dysfunction, they can be used to screen subjects who are at high risk for development of autoimmune disease to identify risk for additional autoimmune diseases. This approach has been employed in studies such as TrialNet for type 1 diabetes to screen first-degree relatives of patients with type 1 diabetes for diabetes-related autoantibodies. In this and other cohorts, risk for development of diabetes increases with the number of autoantibodies and their persistence.

Organ-specific autoantibodies (identified with appropriate assays) are rarely present (approximately 1 in 100) in the general population and identify a subset of people who are at greater risk for clinical disease. These autoantibodies may be expressed for years before the disease develops, and additional autoantibodies can develop over time. The pace at which disease develops is highly variable. For example, children as young as 1 year can present with type 1 diabetes. In contrast, a subset of subjects (5–10%) with type 2 diabetes diagnosed in adulthood have autoimmunity as the underlying cause. This may be due in part to genetics, because subjects who develop autoimmune diabetes at an older age have a higher proportion of the protective diabetes allele DQB1*0602, although even in adults, DQB1*0602 provides dramatic protection.[52]

In contrast, less is known concerning the specificity of pathogenic T cells. Given the observation that cross-reactive recognition by pathogenic T-cell clones may be determined by as few as four properly spaced amino acids of a nonapeptide and the estimate that each T-cell receptor might react with a million different peptides, there is considerable potential for patterns of autoimmunity to be determined by cross-reactive T cells. An important development has been the discovery in the thymus and other lymphoid tissues of peripheral antigen-expressing cells that express autoantigens such as insulin. Minute quantities of such molecules in the thymus can contribute to tolerance. Insulin messenger RNA in the thymus is regulated by genetic polymorphisms of the insulin gene associated with diabetes risk.[3] There is also evidence that stromal and lymphoid cells ($CD11c^+$) in the spleen, lymph nodes, and circulation express multiple similar antigens.[53]

Failure of Gland

Organ dysfunction develops over time and can include a period of intermediate function that may be characterized by increased levels of the stimulatory hormones (e.g., thyroid-stimulating hormone, corticotropin [ACTH]) with normal levels of certain hormones (triiodothyronine, thyroxine, and cortisol). Once a significant portion of the gland has been destroyed, overt disease is then present.

Autoimmune Polyendocrine Syndrome Type I

Clinical Features

Table 43.2 compares the features of APS-I with those of APS-II. Table 43.3 shows the clinical features and recommended follow-up for patients with APS-I. Note some of the distinctions in the pattern of disease features of the two syndromes, particularly the propensity for hypoparathyroidism and candidiasis in APS-I, which is virtually absent in APS-II. Likewise, celiac disease is frequently observed in APS-II but is not seen in APS-I.

APS-I (Mendelian Inheritance in Man [MIM] 240300), also known as *autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy*, or APECED, is characterized by the classic triad of mucocutaneous candidiasis, autoimmune hypoparathyroidism, and Addison disease, which form three of the most common components of the disorder. Patients with APS-I are at risk for the development of autoimmune diseases affecting almost every organ. Multiple patient series have been reported, including subjects in two large series from both Finland[54–56] and the United States.[57,58]

TABLE 43.2 Contrasting Features of Autoimmune Polyendocrine Syndrome

Feature	APS-I	APS-II
Inheritance pattern	Autosomal recessive (only siblings affected)	Polygenic (multiple generations affected)
Associated gene	*AIRE* gene mutation	*HLA-DR3* and *DR4* associated
Gender association	Equal gender incidence	Female preponderance
Age at onset	Onset in infancy	Peak onset 20–60 years
Clinical features	Mucocutaneous candidiasis Hypoparathyroidism Addison disease	Type 1 diabetes Autoimmune thyroid disease Addison disease
Diagnostic antibodies	Anti-interferon	

APS, Autoimmune polyendocrine syndrome.

In a series of 89 Finnish patients described by Perheentupa,[54] all had chronic candidiasis at some time, 86% had hypoparathyroidism, and 79% had Addison disease. Gonadal failure (72% in women, 26% in men) and hypoplasia of the dental enamel (77% of patients) were also frequent findings. Other manifestations that occurred less often included alopecia (40%), vitiligo (26%), intestinal malabsorption (18%), type 1 diabetes (23%), pernicious anemia (31%), chronic active hepatitis (17%), and hypothyroidism (18%). The incidence rates for many of these disorders peak in the first or second decade of life, and the disease continues to develop over decades (Fig. 43.3). Therefore, reported prevalence rates of component disorders are highly dependent on the age at which follow-up ended.

APS-I is characteristically recognized in early childhood. Infants can present with chronic or recurrent mucocutaneous candidiasis in the first year of life, followed by hypoparathyroidism and Addison disease, but new components can develop at any age. Decades can elapse between the diagnosis of one disorder and the onset of another in the same patient. Consequently, lifelong follow-up is important to allow early detection of additional components.

Recurrent candidiasis commonly affects the mouth and nails and, less frequently, the skin and esophagus.[54] Chronic oral candidiasis can result in atrophic disease with areas suggestive of leukoplakia. If this develops, the patient is at significant risk for carcinoma of the oral mucosa (with its high mortality rate).

Ectodermal dystrophy is another component of the syndrome (manifested by pitted nails, keratopathy, and enamel hypoplasia) and cannot be attributed to hypoparathyroidism. Enamel hypoplasia can precede the onset of hypoparathyroidism and, despite adequate replacement therapy, can also affect teeth forming after the onset of hypoparathyroidism.[59]

TABLE 43.3 Clinical Features and Recommended Follow-Up for APS-I and APS-II

Component Disease	Frequency at Age 40 Years (%)	Recommended Evaluation
Autoimmune Polyendocrine Syndrome Type I		
Addison disease	79	Sodium, potassium, ACTH, cortisol, plasma renin activity, 21-hydroxylase autoantibodies
Diarrhea	18	History
Ectodermal dysplasia	50-75	Physical examination
Hypoparathyroidism	86	Serum calcium, phosphate, PTH
Hepatitis	17	Liver function test
Hypothyroidism	18	TSH; thyroid peroxidase and/or thyroglobulin autoantibodies
Male hypogonadism	26	FSH/LH
Mucocutaneous candidiasis	100	Physical examination
Obstipation	21	History
Ovarian failure	72	FSH/LH
Pernicious anemia	31	CBC, vitamin B_{12} levels
Splenic atrophy	15	Blood smear for Howell-Jolly bodies; platelet count; ultrasound if positive
Type 1 diabetes	23	Glucose, hemoglobin A_{1c}, diabetes-associated autoantibodies (insulin, GAD65, IA2)
Autoimmune Polyendocrine Syndrome Type II[a]		
Addison disease	0.5	21-Hydroxylase autoantibodies ACTH stimulation testing if positive
Alopecia		Physical examination
Autoimmune hypothyroidism	15–30	TSH; thyroid peroxidase and/or thyroglobulin autoantibodies
Celiac disease	5–10	Transglutaminase autoantibodies; small intestine biopsy if positive
Cerebellar ataxia	Rare[b]	Dictated by signs and symptoms of disease
Chronic inflammatory demyelinating polyneuropathy	Rare[b]	Dictated by signs and symptoms of disease
Hypophysitis	Rare[b]	Dictated by signs and symptoms of disease
Idiopathic heart block	Rare[b]	Dictated by signs and symptoms of disease
IgA deficiency	0.5	IgA level
Myasthenia gravis	Rare[b]	Dictated by signs and symptoms of disease
Myocarditis	Rare[b]	Dictated by signs and symptoms of disease
Pernicious anemia	0.5–5	Anti–parietal cell autoantibodies CBC, vitamin B_{12} levels if positive
Serositis	Rare[b]	Dictated by signs and symptoms of disease
Stiff-man syndrome	Rare[b]	Dictated by signs and symptoms of disease
Vitiligo	1–9	Physical examination

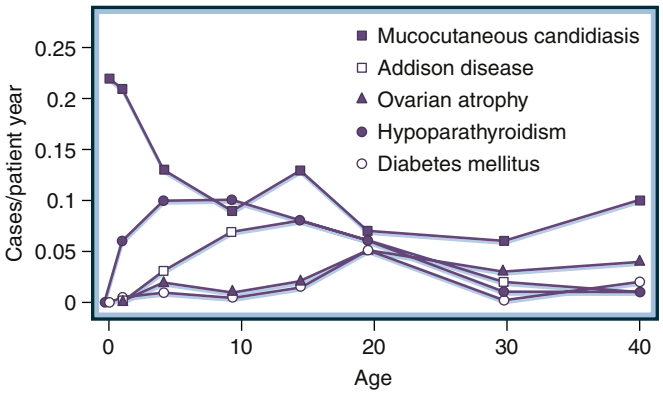
[a]In the population with type 1 diabetes.

[b]Rare reported disorders in subjects with APS-II.

ACTH, Adrenocorticotropic hormone; *APS*, autoimmune polyendocrine syndrome; *CBC*, complete blood count; *FSH*, follicle-stimulating hormone; *GAD*, glutamic acid decarboxylase; *IA2*, insulinoma antigen 2; *IgA*, immunoglobulin A; *LH*, luteinizing hormone; *PTH*, parathyroid hormone; *TSH*, thyroid-stimulating hormone.

Friedman and colleagues[60] reported the frequent occurrence of asplenism and cholelithiasis as additional features of APS-I. Splenic atrophy may also cause immune deficiency. Although the cause of this part of the disorder is unknown, it is relatively common: up to 15% of patients are asplenic.[54] The presence of Howell-Jolly bodies on peripheral blood smear is suggestive of asplenia. If asplenia is identified, immunization with polyvalent pneumococcal vaccine should be administered, and follow-up antibody titers should be obtained. If an adequate response is not produced, daily prophylactic antibiotics may be necessary.

Malabsorption with steatorrhea is of uncertain origin, is usually intermittent, and may be exacerbated by hypocalcemia. Bereket and associates[61] reported a case in which patchy intestinal lymphangiectasia was discovered by endoscopically directed biopsy. Pancreatic insufficiency has been treated with cyclosporine.[62] Autoantibodies (e.g., tryptophan hydroxylase, histidine decarboxylase) reacting with intestinal endocrine cells (enterochromaffin, cholecystokinin, and enterochromaffin like) occur and are associated with loss of endocrine cells on biopsy and with gastrointestinal dysfunction.[63,64]



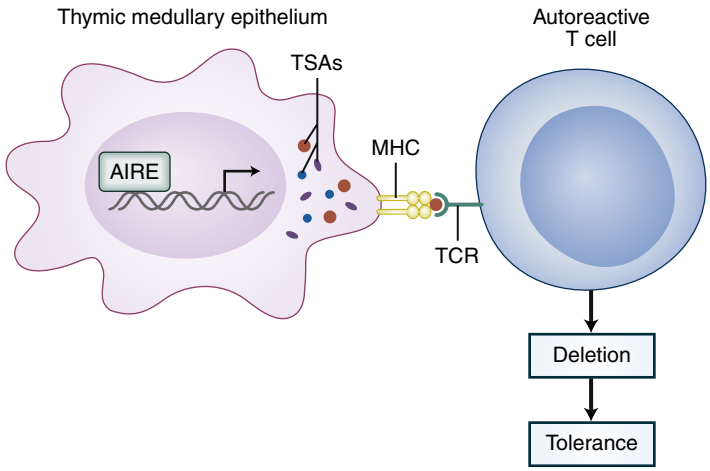
• **Fig. 43.3** Incidence of disease development by age in patients with autoimmune polyendocrine syndrome type I (APS-I). (From Perheentupa J. APS-I/APECED: the clinical disease and therapy. *Endocrinol Metab Clin North Am*. 2002;31:295–320.)

Genetics

APS-I is inherited in a classic mendelian autosomal recessive fashion and is caused by mutations in the *AIRE* gene, located on the short arm of chromosome 21 (near markers D21s49 and D21s171 on 21p22.3).[65] The gene encodes a transcriptional regulator protein that is highly expressed in antigen-presenting epithelial cells in the thymus and in a small subset of cells in lymphoid tissues.[66,67] It has been localized to the nucleus, and mutations have been demonstrated to be associated with decreased transcription of reporter products.[68,69] A mouse model of APS-I has been generated, and *Aire* knockout mice also spontaneously develop autoimmune features. Detailed analyses of antigen-presenting epithelial cells in the thymus of *Aire* knockout mice has shown that these cells have decreased expression of peripheral *tissue-specific self-antigens* (TSAs) and that Aire promotes the expression of thousands of such self-antigens in these cells.[66] Furthermore, autoreactive T cells with specificity to these TSAs can escape thymic deletion when Aire is defective and promote autoimmunity.[70–72] Thus, Aire appears to act as a transcriptional activator that promotes the expression of a wide array of TSAs in the thymus to promote central tolerance (Fig. 43.4).

Multiple mutations of *AIRE* have been identified in subjects with APS-I. The frequency of specific mutations varies in different populations. For example, in Sardinia, a deletion of amino acid 257 is present in 90% of mutated alleles. A 136–base pair deletion in exon 8 is present in 71% of British alleles and in 56% of alleles in the United States. Analysis of haplotypes indicates that this deletion has arisen on many occasions.

Most cases of APS-I are associated with autosomal recessive inheritance patterns; however, there are now reports of autosomal dominant inheritance. A rare kindred of patients from Italy have a mutation in the SAND domain of the AIRE protein (G228W)



• **Fig. 43.4** The autoimmune regulator (AIRE) gene functions to maintain tolerance by promoting the display of self-antigens in the thymus. An AIRE-expressing thymic medullary epithelial cell is shown (*left*). AIRE operates in the nucleus to promote the expression of thousands of different tissue-specific self-antigens (TSAs) that include many endocrine organ-specific proteins such as insulin. Peptide fragments of these TSAs are displayed on major histocompatibility complex (MHC) molecules to help promote the deletion of autoreactive T cells (*right*) that can be generated in the thymus from random gene rearrangement of the T-cell receptor (TCR). Such T cells are deleted when they encounter their antigen-MHC in the thymus, and thus immune tolerance is maintained.

that when present in the heterozygous state is associated with autoimmunity, thus fitting an autosomal dominant inheritance pattern.[73] In a mouse model of this mutation, the expression of TSAs in the thymus was decreased compared with that inthe control model, suggesting a mechanism for this observed inheritance pattern.[74] In addition, point mutations in the PHD1 domain of AIRE have now been associated with a dominant inheritance pattern.[75] In all cases, the molecular explanation could be related to the observation that AIRE forms multimers through its N-terminal domain, and thus mutations in other domains could still allow for multimerization with a wild-type copy of AIRE and a dominant-negative effect in AIRE activity. The autoimmune phenotype in this group of patients appears to be milder with the development of fewer autoimmune features or even a single disease feature that likely reflects partial AIRE activity in these patients.

Diagnosis

The diagnosis of APS-I is highly likely when two or more of the primary component disorders (i.e., mucocutaneous candidiasis, hypoparathyroidism, and Addison disease) are present. Siblings of an affected patient should be considered affected even if only one of these disorders is present. Sequence analysis of *AIRE* for mutations may be helpful in identifying subjects with APS-I. Any patient with any of the component disorders deserves careful follow-up to watch for the development of additional disease.

Autoantibodies against IFNα and IFNω have been identified in almost 100% of subjects with APS-I, regardless of age at screening.[76] The autoantibody has been found in subjects with many different mutations of the *AIRE* gene; it is not present in other autoimmune diseases, and it has been proposed to use this autoantibody to screen subjects with multiple autoimmune diseases for APS-I. It is unclear if antibodies to these type 1 IFNs are of any clinical consequence, as APS-I subjects do not have an increased propensity for viral infections.[54] In contrast, it has also been shown that many APS-I subjects also harbor autoantibodies to IL17A, IL17F, and IL22.[77,78] These are key cytokines for the function of the Th17 T-cell subset, and recent work has demonstrated that loss of function of these cytokines, as in patients with mutations in the IL17 receptor, is associated with increased susceptibility to *Candida albicans* infections.[79] Thus, it appears that the *Candida* susceptibility in APS-I may be due to an autoimmune response against this effector cytokine family.

Therapy and Follow-Up

The treatment of adrenal insufficiency and hypoparathyroidism is the same as that discussed in other chapters with the caveat that malabsorption can complicate treatment. The therapy for mucocutaneous candidiasis has been improved with the use of orally active antifungal drugs such as fluconazole and ketoconazole. Infection often recurs when the drug is discontinued or the dosage is decreased. Patients must be monitored carefully, because ketoconazole can inhibit adrenal and gonadal steroid synthesis and can precipitate adrenal failure. It is also associated with transient elevation of liver enzyme levels and, occasionally, with hepatitis. Fluconazole is associated with a lower frequency of hepatitis and does not inhibit steroidogenesis when given in the recommended doses.

Autoantibodies associated with multiple autoimmune diseases have been reported. Antiparathyroid and antiadrenal antibodies have been reported. Whereas 21-hydroxylase appears to be the major autoantigen in isolated Addison disease and in Addison disease associated with APS-II, autoantibodies against 17α-hydroxylase and cytochrome P450 side-chain cleavage enzyme (CYP11A1) have also been reported in Addison disease associated with APS-I.[80] There have been reports of antibodies to tryptophan hydroxylase in intestinal disease, tyrosine hydroxylase in alopecia areata, L-amino acid decarboxylase in hepatitis and vitiligo, and phenylalanine hydroxylase,[81,82] and antibodies reacting with hair follicles.[83] Tuomi and coworkers[84] originally observed that many more APS-I patients (41%) express anti-GAD65 autoantibodies than become diabetic, suggesting that reactivity to this single autoantigen has low predictive value in this population. Recently, antibodies against NALP5 were identified in 49% of patients with APS-I and hypoparathyroidism compared with none of those with APS-I but no hypoparathyroidism.[85] Screening for autoantibodies associated with additional autoimmune diseases may be useful in patients with APS-I.

Screening to allow the early detection of new disorders before overt symptoms and signs develop is recommended, including autoantibody studies, electrolytes, calcium and phosphorus levels, thyroid and liver function tests, blood smear, and plasma vitamin B_{12}. Patients at risk for adrenal failure can be screened by measurement of basal ACTH and supine plasma renin activity, followed by dynamic testing as appropriate. Evaluation for asplenism[60] with abdominal ultrasonography and blood smear examination for Howell-Jolly bodies is warranted, with pneumococcal vaccination and appropriate antibiotic coverage for affected patients.

There are case reports of severely affected patients who have benefited from immunosuppressive therapy. For example, Ward and colleagues[62] treated a 13-year-old patient who had keratoconjunctivitis, hepatitis, and severe pancreatic insufficiency. Treatment with cyclosporine was associated with normalization of stool fat (from 31.5 to 2.5 g/day).

Autoimmune Polyendocrine Syndrome Type II

Clinical Features

APS-II (MIM 269200) is defined by the occurrence in the same patient of two or more of the following: primary adrenal insufficiency (Addison disease), Graves disease, autoimmune thyroiditis, type 1A diabetes, primary hypogonadism, myasthenia gravis, and celiac disease. Vitiligo, alopecia, serositis, and pernicious anemia also occur with increased frequency in patients with this syndrome and in their family members (see Table 43.3). APS-II is more common than APS-I. It occurs more often in female than in male patients, often has its onset in adulthood, and exhibits familial aggregation (see Table 43.2).

When one of the component disorders is present, an associated disorder occurs more commonly than in the general population. Furthermore, circulating organ-specific autoantibodies are often present even in the absence of overt clinical disease. For example, in subjects with type 1 diabetes, there is a 15% to 20% risk of hypothyroidism, a 5% to 10% risk of celiac-related autoimmunity, and a 1.5% risk of adrenal autoimmunity. The risk of autoimmunity is greater in relatives of patients with APS-II. In our assessment of APS-II families with Addison disease, up to 15% of relatives were found to have 21-hydroxylase autoantibodies (Addison disease–associated autoantibodies), anti-islet autoantibodies, or tTG autoantibodies (celiac disease–associated autoantibodies). The initial lesion and precipitating events that result in the syndrome are unknown, but immunogenetic and immunologic similarities are present with regard to both the time course and the pathogenesis of each of the component disorders.

Because of the chronic development of organ-specific autoimmunity, patients with the syndrome and their families should have repeated endocrinologic evaluations over time. In a family in which the syndrome has been documented, relatives should be advised of the early symptoms and signs of the principal component diseases (a list is available from the Barbara Davis Center for Diabetes website[86]). Relatives of patients with multiple disorders should have a medical history, physical examination, and screening every 3 to 5 years, with measurement of anti-islet autoantibodies, a sensitive thyrotropin assay, and measurement of serum vitamin B_{12} levels. If there are any symptoms or signs, or if 21-hydroxylase autoantibodies are present, the patient should have annual assays of basal ACTH levels with Cortrosyn stimulation testing.

Among 224 patients with Addison disease and APS-II reported by Neufeld and colleagues,[57] type 1 diabetes and autoimmune thyroid disease were the most common coexisting conditions (52% and 69% of patients, respectively). Other components were less common, including vitiligo (5%) and gonadal failure (4%). In an Italian cohort of more than 600 patients, the majority of patients with Addison disease (86.7%, after exclusion of subjects with APS-I) had additional autoimmunity diseases, most frequently thyroid disease (56%) and diabetes (12%). A significant proportion of subjects exhibited thyroid- (16%) and diabetes- (8.8%) related autoimmunity without yet developing disease over average follow-up of 10 years.[87] Therefore, careful monitoring of patients with Addison disease for additional autoimmune diseases including thyroid, type 1A diabetes, and premature ovarian insufficiency is warranted.

Among patients with type 1A diabetes, thyroid autoimmunity and celiac disease coexist with sufficient frequency to justify screening. Thyroid peroxidase autoantibodies are present in 10% to 20% of children with type 1 diabetes; this incidence is higher in female patients and increases in all patients with age and with diabetes duration. A significant fraction of patients with type 1 diabetes and thyroid peroxidase autoantibodies develop thyroid disease. One study showed that after follow-up for more than 15 years, 80% of patients with thyroid peroxidase autoantibodies and type 1 diabetes became hypothyroid.[88] However, several studies have shown that a subset of patients with negative autoantibodies develop thyroid disease. Therefore, patients with type 1 diabetes should be screened annually for thyrotropin levels, which is a cost-effective approach.

With the identification of tTG as the major endomysial autoantigen of celiac disease, radioimmunoassays were developed and demonstrated that 10% to 12% of patients with type 1 diabetes have tTG autoantibodies.[7] The prevalence of tTG autoantibodies was higher in diabetic patients with HLA-DQ2; one-third of DQ2-homozygous subjects were found to express anti-tTG antibody, reflective of the influence of HLA-DQ2 in the development of both type 1 diabetes and celiac disease. Seventy percent of those with high-titer antibody who underwent biopsy were subsequently found to have the disease.[89] Celiac disease may be identified at onset of type 1 diabetes. However, it can develop in patients with long-standing diabetes as well and in at least one cohort has been shown to be increased in patients with adult-onset type 1 diabetes compared with childhood onset.[90] If left untreated, symptomatic celiac disease is also associated with an increased risk of gastrointestinal malignancy, especially lymphoma. Survival analysis in patients with type 1 diabetes and celiac disease shows an increase in mortality rate in patients with type 1 diabetes and celiac disease duration of greater than 15 years.[91] Frequency and method of screening remain areas of debate. At the very least, providers should be aware of the association and have a low threshold for screening with tTG antibodies. If the results are positive and are confirmed on repeat assay, small bowel biopsy to document celiac disease is warranted, with institution of a gluten-free diet if the disease is present. Many patients have asymptomatic celiac disease that is nevertheless associated with osteopenia and impaired growth.

Diagnosis

The diagnosis of APS-II requires an understanding of the risk for additional autoimmune diseases in patients with a single autoimmune endocrine disorder. A thorough history and physical examination may identify symptoms or signs of an additional autoimmune disorder. Screening for disease can include screening for markers of the autoimmune diseases (organ-specific autoantibodies) and assays of glandular function (e.g., thyrotropin levels).

Improved assays for several organ-specific autoantibodies have been developed since the cloning of specific autoantigens and the development of assays that use recombinant antigens. These radioimmunoassays are superior to assays based on immunofluorescence with tissue sections, such as ICA testing. The most notable finding is the identification of multiple autoantigens targeted even in single autoimmune disorders. Most of the endocrine autoantigens are hormones (e.g., insulin) or enzymes associated with differentiated endocrine function: thyroid peroxidase in thyroiditis; GAD, carboxypeptidase H, and ICA 512/IA2 in type 1 diabetes; 17α-hydroxylase and 21-hydroxylase in Addison disease; and the parietal cell enzyme H^+/K^+-adenosine triphosphatase in pernicious anemia.

In type 1 diabetes, the four most informative assays identify autoantibodies reacting with insulin, GAD65, ICA512/IA2, and ZnT8.[51] Similarly, a radioassay for autoantibodies against the enzyme 21-hydroxylase in Addison disease has been developed and provides excellent disease specificity and sensitivity. Adrenal autoantibodies reacting with recombinant 21-hydroxylase usually precede the development of Addison disease. Screening with 21-hydroxylase autoantibodies may identify patients with antibodies but have normal production of cortisol in response to ACTH. Annual screening with a basal level of corticotropin with follow-up cosyntropin stimulation testing is an effective strategy for identifying adrenal insufficiency in patients with 21-hydroxylase autoantibodies.

Typically, autoantibodies mark the presence of or risk for autoimmune disease caused by T-cell–mediated glandular destruction. However, autoantibodies may also be pathogenic. A hallmark of pathogenic autoantibodies is the existence of a neonatal form of the disorder, secondary to transplacental passage of the autoantibody. Examples include neonatal Graves disease (anti–thyrotropin-receptor autoantibodies) and neonatal myasthenia gravis (anti–acetylcholine-receptor autoantibodies).

Therapy

Treatment of the individual diseases of the polyendocrine autoimmune syndrome is discussed in other chapters. Therapeutic considerations related specifically to APS-II are discussed here.

Many of the component disorders of the syndrome have a long prodromal phase and are associated with the expression of autoantibodies before the manifestation of overt disease. Therefore, people at risk for autoimmune diseases can be identified prior to the clinical onset of disease, and if the right treatment were

identified, disease may be preventable. This is particularly important for type 1A diabetes but is also likely to apply to Addison disease and hypogonadism.

Prevention strategies have been extensively evaluated in patients with type 1 diabetes. Prevention of disease can occur at multiple time points along the natural history of evolving beta-cell dysfunction. Treatments targeted at patients with genetic risk without evidence for autoimmunity will expose people who will never get disease to an intervention (primary prevention). Therefore, any intervention must be safe and easily administered. Prevention trials in patients with diabetes-related autoantibodies prior to abnormalities of glucose metabolism (secondary prevention) can employ agents with higher risks of adverse events. Tertiary prevention of diabetes employs immune-modulating agents for the preservation of the beta-cell mass with the hopes of inducing a prolonged C-peptide production.

Primary prevention of type 1 diabetes targets infants at the highest risk for the development of type 1 diabetes on the basis of family history or specific HLA genotypes. Infants must be negative for diabetes-related autoantibodies. Interventions have focused on dietary manipulations including gluten-free diets, docosahexaenoic acid (DHA) supplementation, and elemental formulas lacking bovine insulin. To date, the interventions have not prevented the development of diabetes-related autoantibodies or diabetes in large-scale clinical trials.[92]

Secondary prevention of type 1 diabetes has been antigen specific (insulin and GAD65) and antigen nonspecific (nicotinamide). These studies have targeted subjects with positive diabetes-related autoantibodies prior to the development of diabetes. Some studies have included patients with abnormalities of glucose metabolism such as impaired first-phase insulin response and impaired glucose tolerance.

A large National Institutes of Health trial—the Diabetes Prevention Trial–Type 1 (DPT-1)—directly tested oral and parenteral insulin for prevention of diabetes. DPT-1 had two arms: intravenous-subcutaneous for those at high risk (risk of diabetes >50% within 5 years) and oral insulin for those at moderate risk (risk of diabetes 25–50% within 5 years). Neither parenteral[93] nor oral[94] insulin slowed progression to diabetes. However, in a subgroup analysis of subjects in the DPT-1 oral trial, a treatment effect was noted for those who had the higher insulin autoantibody levels at diagnosis,[94] and further trials are under way. In preclinical Addison disease, a short course of glucocorticoids appeared to suppress the expression of adrenal autoantibodies and prevent progressive adrenal destruction.[95] Studies of immune-modulating agents (abatacept and teplizumab) in patients with diabetes-related autoantibodies and abnormalities of glucose metabolism are currently under way.

Because of the autoimmune nature of these disorders, several studies have evaluated the use of immunosuppressive and immune-modulating drugs. Drugs such as cyclosporine have preserved some residual insulin secretion. However, because cyclosporine is nephrotoxic and potentially oncogenic, more generalized use is precluded. Newer immunosuppressive agents (e.g., sirolimus) are being studied, and biologics such as anti-CD20 antibody (rituximab), abatacept, and nonmitogenic CD3 antibodies have been shown to prolong C-peptide production and to result in a decreased insulin dose through the first year of diabetes when compared with control subjects.[96–100]

Thyroxine therapy can precipitate a life-threatening addisonian crisis in a patient with untreated adrenal insufficiency and hypothyroidism. Therefore, it is necessary to evaluate adrenal function in all hypothyroid patients in whom the syndrome is suspected before instituting such therapy. A decreasing insulin requirement in a patient with insulin-dependent diabetes can be one of the earliest indications of adrenal insufficiency, occurring before the development of hyperpigmentation or electrolyte abnormalities.

Other Polyendocrine Deficiency Autoimmune Syndromes

Rare polyendocrine syndromes are listed in Table 43.4.

Immunodysregulation Polyendocrinopathy Enteropathy X-Linked Syndrome

IPEX (MIM 340790, MIM 300292), first described in 1982, is a rare, X-linked recessive disorder that is characterized by immune dysregulation and results in multiple autoimmune diseases and early death (see Fig. 43.4). It is caused by mutations of the *FOXP3* gene. Clinical characteristics include very early onset type 1A diabetes, severe enteropathy resulting in failure to thrive, and dermatitis, generally resulting in death within the first couple of years of life unless definitive treatment is pursued. Other reported abnormalities include atopy, thrombocytopenia, hemolytic anemia, hypothyroidism, lymphadenopathy, nephropathy, and alopecia (2012 review).[101] Immunologic evaluation shows elevated IgE and eosinophilia in addition to the abnormalities associated with the component disorders.

The identification of *FOXP3* as the gene associated with IPEX was aided by the scurfy mouse model. The scurfy mouse exhibits characteristics similar to those seen in children affected with IPEX. The gene associated with this disorder in the scurfy mouse was found to be a DNA-binding protein with characteristics of a transcription factor.[102] Clues about the function of this gene included the observations that implantation of thymus from a scurfy mouse into immune-incompetent mice transfers the disease, but transplantation of thymus into immune-competent mice does not transfer disease, and injections of normal T cells can rescue the phenotype, suggesting that a regulatory cell is involved in the pathogenesis of this disorder. Linkage analysis demonstrated that a 17-centimorgan stretch of the X chromosome (Xp11.1-q13.3) is associated with IPEX, and mutations within the *FOXP3* gene have been identified in most of the families studied thus far.[103] *FOXP3* encodes a protein that is a member of the forkhead class of winged helix transcription factors (forkhead box protein 3). *FOXP3* has been shown to be expressed in $CD4^+/CD25^+$ regulatory T cells.[104] These T cells can suppress activation of other T cells.[104] Therefore, mutations in *FOXP3* result in inability to generate regulatory T cells and the development of IPEX. Patients with mutations throughout the *FOXP3* gene have been described. In general, milder phenotypes are associated with point mutations or small deletions that do not result in a total lack of *FOXP3*. However, significant variability in phenotype within a single mutation suggests that other genes or environmental exposures may play an important role in the development of the disorder.

Treatment options are targeted to the underlying disorders. At the time of diagnosis, infants may be so affected by the enteropathy that they require bowel rest and parenteral nutrition. Diabetes is managed with insulin therapy. Immunosuppression with calcineurin inhibitors has been used in the initial,

TABLE 43.4 Rare Polyendocrine Disorders

Disorder	Clinical Features	Cause
Hirata disease (insulin resistance syndrome)	Hypoglycemia	Insulin autoantibodies Associated with methimazole
IPEX or CD25 deficiency	Type 1 diabetes Enteropathy	Mutations of FOXP3 for IPEX Mutations of IL2RA for CD25-deficiency
Autoimmune lymphoproliferative syndrome type V	Autoimmune thyroiditis Autoimmune cytopenias Hypogammaglobulinemia	Mutations of CTLA4 resulting in haploinsufficiency
Infantile-onset autoimmune disease 1	Autoimmune cytopenias Autoimmune thyroiditis Type 1 diabetes Short stature	Dominant activating mutations of STAT3 in heterozygosity
Kearns-Sayre syndrome	Hypoparathyroidism Primary gonadal failure Nonautoimmune diabetes Hypopituitarism	Deletions of mitochondrial DNA
POEMS	Polyneuropathy Organomegaly Diabetes Primary gonadal failure	Plasma cell dyscrasia with production of M protein and cytokines
Thymic tumors	Myasthenia gravis Red blood cell hypoglobulinemia Autoimmune thyroid disease Adrenal insufficiency	Thymomas
Type B insulin resistance	Severe insulin resistance	Insulin receptor autoantibodies
Wolfram syndrome	Diabetes insipidus Nonautoimmune diabetes Bilateral optic atrophy Sensorineural deafness	Mutations of WSF1, which encodes wolframin

IPEX, Immunodysregulation polyendocrinopathy enteropathy X-linked; *POEMS,* plasma cell dyscrasia with *p*olyneuropathy, *o*rganomegaly, *e*ndocrinopathy, *m* protein, and *s*kin changes.

stabilization phase of treatment. Definitive treatment is with hematopoietic stem cell transplantation (HSCT). HSCT results in reversal of the enteropathy and other autoimmune components.[105] Generally, established type 1 diabetes and thyroid disease do not resolve. However, some reports document reversal of type 1 diabetes in patients who have received the conditioning immunotherapy and HSCT.[106] An additional case report shows that reconstitution of the gut immune system may occur later than reconstitution of the bone marrow.[107] Novel approaches to insert a functional *FOXP3* gene into lymphocytes as gene therapy in addition to or eventually in place of HSCT are being developed as well.[108,109]

Over the past several years, disorders with similar clinical characteristics as IPEX but without mutations in *FOXP3* have been identified. In these disorders, mutations in *IL2RA*,[110,111] *STAT5b*,[112] *STAT-1*,[113] and *ITCH*[114] result in abnormalities of regulatory T-cell function, emphasizing the important role of these cell populations in the maintenance of immune tolerance.

CTLA4, STAT3, and LRBA Mutations

With recent advances in whole genome sequencing, there has been rapid progress in associating rare genetic variants in isolated patients or kindreds with multiorgan autoimmunity that often involves endocrinopathy. Loss of function mutations in heterozygosity in the CTLA4 gene have now been associated with autoimmunity in a few isolated kindreds and appears to be associated with a defect in T-regulatory cell function.[115,116] However, it should be noted that the autoimmunity associated with these mutations is much milder than IPEX. A similar syndrome has also been recently described in patients with heterozygous mutations in the LRBA gene.[117] Here, the mechanism is less clear but may involve proper intracellular trafficking of CTLA4 in T cells. Finally, dominant activating mutations in the STAT3 gene have now been associated with an autoimmune syndrome in isolated cases and several families.[118,119] Here, patients frequently develop thyroiditis, cytopenias, and type 1 diabetes, along with other unusual features including short stature. The exact

mechanism of how these mutations lead to autoimmunity in these subjects is not clear but could involve improper activation of T cells, as STAT3 is a known transducer of effector cytokines.

Anti–Insulin Receptor Autoantibodies

In this rarely reported disorder (<100 patients), also called *type B insulin resistance* or *acanthosis nigricans*, insulin resistance is caused by the presence of anti–insulin receptor antibodies and anti-insulin antibodies.[120] Approximately one-third of patients with these antibodies have an associated autoimmune illness such as SLE or Sjögren syndrome. Arthralgia, vitiligo, alopecia, autoimmune thyroid disease, secondary amenorrhea, and family history of autoimmunity have also been reported. Autoimmune thyroid disease has been described in two such patients, one with hypothyroidism and the other with antithyroid antibodies. Antinuclear antibodies, an elevated erythrocyte sedimentation rate, hyperglobulinemia, leukopenia, and hypocomplementemia are common.[121]

The major clinical manifestations are related to the anti–insulin receptor antibodies. Insulin resistance is profound, and up to 175,000 U of insulin given intravenously per day may be ineffective in lowering the elevated glucose. Despite hyperglycemia and marked insulin resistance, ketoacidosis is uncommon. The course of the diabetes is variable, and several patients have had spontaneous remissions. Other patients have had severe hypoglycemia (perhaps related to the insulin-like effects of anti–insulin receptor antibodies demonstrable in vitro).[122] The acanthosis nigricans, which is caused by hypertrophy and folding of otherwise histologically normal skin, appears to be related to the insulin-resistant state. Other forms of marked insulin resistance in the absence of antireceptor antibodies are also associated with acanthosis nigricans. A treatment regimen developed at the National Institutes of Health that includes rituximab that targets B lymphocytes with cyclophosphamide and pulse corticosteroids has been used successfully to treat this rare disorder.[122,123]

POEMS Syndrome

The components of the multisystem disorder POEMS (*p*lasma cell dyscrasia with polyneuropathy, *o*rganomegaly, *e*ndocrinopathy, *m*onoclonal plasma cell disorder, and *s*kin changes), also known as Crow-Fukase syndrome (MIM 192240), consist of diabetes mellitus (3–36% of patients), primary gonadal failure (55–89% of patients), plasma cell dyscrasia, sclerotic bone lesions, and neuropathy.[124] Patients usually present with severe progressive sensorimotor polyneuropathy, hepatosplenomegaly, lymphadenopathy, and hyperpigmentation. On evaluation, they are found to have plasma cell dyscrasia and sclerotic bone lesions. Patients present in the fifth to sixth decades of life and have a median survival time after diagnosis of 14 years.[124,125]

The pathophysiology of POEMS is poorly understood. There is evidence implicating cytokines such as IL1A, IL6, and TNFα in addition to the M protein in the pathogenesis of this disorder. In several studies, elevated levels of vascular endothelial growth factor (VEGF) correlated with the disease state, and treatment with immunosuppressive agents reduced the symptoms of the disease and the levels of VEGF, suggesting that this growth factor plays a role in the disease.[126,127] A therapeutic trial of an anti-VEGF antibody would provide more definitive evidence for this hypothesis.

Algorithms have been proposed for the treatment of POEMS.[125] Important features of treatment include extensive baseline evaluation, ongoing monitoring for component disorders, systemic therapy for the plasma cell disorder, and radiation at the site of identified bone lesions. The diabetes mellitus responds to small, subcutaneous doses of insulin.

Kearns-Sayre Syndrome

The rare Kearns-Sayre syndrome (MIM 530000), also known as oculocraniosomatic disease or oculocraniosomatic neuromuscular disease with ragged red fibers, is characterized by myopathic abnormalities leading to ophthalmoplegia and progressive weakness in association with several endocrine abnormalities, including hypoparathyroidism, primary gonadal failure, diabetes mellitus, and hypopituitarism.[128] Crystalline mitochondrial inclusions are found in muscle biopsy specimens, and such inclusions have also been observed in the cerebellum. The relation between the mitochondrial disorders and endocrinologic abnormalities is not known. Antiparathyroid antibodies have not been described; however, antibodies to the anterior pituitary gland and striated muscle have been found, and the disease may have autoimmune components. Other abnormalities include retinitis pigmentosa and heart block. Deletions in mitochondrial DNA have been associated with Kearns-Sayre syndrome.[129] These mutations usually occur sporadically and are not associated with a familial syndrome.

Thymic Tumors

The thymus is a complex tissue with a specialized endocrine epithelium that synthesizes a variety of biologically active peptides involved in the control of T-cell maturation. This epithelium is derived from the neural crest and contains complex gangliosides that react with monoclonal antibody (A2B5) and tetanus toxin in a manner similar to that of pancreatic islets.

The illnesses associated with thymomas are similar to those seen in APS-II,[130] although the incidence of specific disorders is different. In one review of patients with thymoma, myasthenia gravis was found to occur in 44% of the patients, red blood cell aplasia in approximately 20%, hypoglobulinemia in 6%, autoimmune thyroid disease in 2%, and adrenal insufficiency in 1 of 423 patients (0.24%). The incidence of autoimmune thyroid disease reported in patients with thymoma is probably an underestimate, given the incidence of unsuspected thyroid disease in patients with myasthenia gravis. Mucocutaneous candidiasis in adults is also associated with thymomas. Interestingly, recent work suggests that an alteration in the proper expression of AIRE and peripheral antigens may be part of the explanation of the autoimmunity that arises in these patients.[131]

Wolfram Syndrome

Wolfram syndrome (MIM 222300, chromosome 4; MIM 598500, mitochondrial) is a rare autosomal recessive disease that is also called *DIDMOAD* (*d*iabetes *i*nsipidus, *d*iabetes *m*ellitus, progressive bilateral *o*ptic *a*trophy, and sensorineural *d*eafness). In addition, neurologic and psychiatric disturbances are prominent in most patients and can cause severe disability. Atrophic changes in the brain have been found on magnetic resonance imaging.[132] Segregation analysis of the mutations found in familial and sporadic cases of Wolfram syndrome led to the identification of wolframin, a 100-kDa transmembrane protein encoded by *WFS1*, a gene located at 4p16.1.116. Genotype and phenotype analyses have identified the severe phenotype (defined as the development of neurologic disease within the

first decade) in patients with truncated proteins and mutations in the carboxy-terminus of the protein.[133]

Wolframin has been localized to the endoplasmic reticulum[134] and is found in neuronal and neuroendocrine tissue.[135] Its expression induces ion channel activity with a resultant increase in intracellular calcium and may play an important role in intracellular calcium homeostasis.[136] Functional studies have shown that reported *WFS1* mutations lead to decreased stability of the protein wolframin.[137] Linkage to other loci in addition to *WFS1* may explain the variability in phenotype seen in this disorder.

Wolfram syndrome appears to be a slowly progressive neurodegenerative process, and there is also (nonautoimmune) selective destruction of the pancreatic beta cells, recently associated in a stem cell model with increased endoplasmic reticulum stress in the pancreatic beta cell.[138] This association is probably a result of the expression pattern of *WFS1.* Diabetes mellitus with an onset in childhood is usually the first manifestation. Diabetes mellitus and optic atrophy are present in all reported cases, but expression of the other features is variable. Duration of diabetes is linked to the development of microvascular complications.[137] Additional endocrinologic diseases, such as ACTH deficiency and growth hormone deficiency, have been reported.[137] In one case report, two related children with Wolfram syndrome had megaloblastic and sideroblastic anemia that responded to treatment with thiamine. Furthermore, thiamine treatment was associated with a marked decrease in insulin requirements.[139]

Omenn Syndrome

Omenn syndrome (MIM 603554) is a primary immunodeficiency syndrome with autoimmune manifestations affecting mainly the skin and gastrointestinal tract. Mutations associated with decreased recombination of the T-cell receptor have been described. One study showed that the levels of *AIRE* gene expression were decreased in the thymus of two affected patients and that this was associated with decreased expression of peripheral antigens compared with control subjects.[125]

Chromosomal Disorders

Down syndrome, or trisomy 21 (MIM 190685), is associated with the development of type 1 diabetes, thyroiditis, and celiac disease. Patients with Turner syndrome are at increased risk for the development of thyroid disease and celiac disease. It is recommended to screen patients with trisomy 21 and Turner syndrome for associated autoimmune diseases on a regular basis.

Conclusion

Through the study of rare disorders such as APS-I and IPEX, the processes of thymic expression of peripheral antigens and development of regulatory T cells are beginning to be defined. This understanding provides invaluable insight into the development of the normal immune system and the mistakes that can occur and lead to autoimmunity. Lessons learned from these rare diseases will help to better define the pathophysiology of more common autoimmune endocrine disorders, possibly leading to the development of immunologic methods for the prevention and treatment of these disorders.

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The complete list of references is available online at ExpertConsult.com.

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Endocrinology of HIV/AIDS

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CHAPTER OUTLINE

KEY POINTS

- In patients infected with human immunodeficiency virus (HIV) adrenal dysfunction is common. Specific protease inhibitors, including ritonavir, and the P450CYP3A4 inhibitor cobicistat may reduce metabolism of inhaled and injected steroids and increase systemic exposure.
- Hypogonadism is often seen among HIV-infected patients, is frequently associated with low or normal gonadotropins, and should be assessed using a specific free testosterone assay, given increases in sex hormone–binding globulin in this population.
- Fracture incidence is increased among HIV-infected patients and is multifactorial in nature, potentially related to increases in bone turnover, certain antiretroviral medications, immune activation, vitamin D deficiency, and gonadal dysfunction.
- Cardiovascular disease is increased among HIV-infected patients and relates to increases in traditional risk factors and nontraditional risk factors including immunologic and inflammatory factors.
- Endocrine management of insulin resistance, diabetes mellitus, and dyslipidemia, common among HIV-infected patients, may reduce cardiovascular disease risk in this population.
- Acquired immunodeficiency syndrome wasting, characterized by sarcopenia, should be distinguished from HIV lipodystrophy, characterized by subcutaneous fat loss.
- Endocrine strategies may be used to treat sarcopenia in acquired immunodeficiency syndrome wasting (testosterone and growth hormone) or to reduce visceral fat (growth hormone–releasing hormone) in HIV lipodystrophy.
- Medications used in the treatment of HIV disease have numerous endocrine and metabolic effects, including effects on steroid metabolism, gonadal function, vitamin D synthesis, renal phosphate excretion, glucose uptake, and very low density lipoprotein metabolism.

Human immunodeficiency virus (HIV) disease affects up to 36.7 million patients worldwide and more than 1.1 million in the United States. In many parts of the world, such as in sub-Saharan Africa, HIV remains epidemic, with an estimated 25.6 million people infected.[1] In addition, the number of HIV-infected patients is growing rapidly in Asia and other parts of the world. Endocrine dysfunction is common among HIV-infected patients. Adrenal, gonadal, thyroid, bone, and metabolic abnormalities have all been reported. HIV itself, related infectious organisms, immune activation, cytokines, and antiretroviral medications may all affect endocrine function. Endocrine disorders in HIV disease, such as hypogonadism, adrenal insufficiency, diabetes mellitus (DM), and bone loss, may cause significant morbidity and are thus important to diagnose. Interactions between antiretroviral therapy (ART) and specific medication may also contribute to endocrine disturbances. Endocrine strategies may improve quality of life and long-term survival through effects on critical metabolic and body composition parameters, including loss of muscle mass (sarcopenia) in acquired immunodeficiency syndrome (AIDS) wasting, fat redistribution with loss of peripheral and abdominal subcutaneous fat, and relative or absolute gains in central (visceral) adiposity among some patients. However, diagnosis and treatment may be difficult because of varying nutritional conditions and effects of the varied medications used to treat HIV disease. As HIV patients live longer as a result of the success of antiretroviral medications, adverse effects resulting from these very medications and HIV-related immune dysfunction have resulted in increased cardiovascular risk and metabolic changes that require intervention and long-term management by the endocrine specialist. This chapter will review the prevalence,

mechanisms, and optimal treatment strategies for endocrine abnormalities in HIV-infected patients.

Adrenal Function

Adrenal dysfunction may be suspected in the patient with advanced HIV disease because of fatigue, hyponatremia, and other features of adrenal insufficiency. Although clinical adrenal dysfunction is relatively rare among patients with AIDS, subtle impairments in adrenal reserve may be seen in this population. Adrenal dysfunction most often is caused by destruction of adrenal tissue by cytomegalovirus (CMV) in patients with advanced HIV disease but may also be caused by medications, hypothalamic/pituitary disease from opportunistic infection; idiopathic inflammation or tissue destruction; or, in rare cases, by cortisol resistance. In addition, some features of Cushing syndrome may be seen among HIV-infected patients with fat redistribution, but true Cushing disease is rare.

Adrenal Insufficiency

Biochemical evidence of adrenal insufficiency is relatively common among hospitalized AIDS patients, with 17% of 74 hospitalized AIDS patients screened by a cosyntropin test demonstrating inadequate adrenal stimulation (1-hour cortisol <18 μg/dL) in an early study. In contrast, fewer patients, 4%, demonstrate clinical symptoms of adrenal insufficiency.[2] Among patients with clinical symptoms and signs of adrenal insufficiency, including hyponatremia, a higher percentage, up to 30%, may demonstrate inadequate reserve using cosyntropin testing.[3]

Adrenal insufficiency occurring in the context of advanced HIV disease is most often caused by tissue destruction of the adrenal glands from opportunistic infections. CMV adrenalitis is the most common cause, seen in approximately 40% to 90% of patients with CMV infections at autopsy. However, adrenocortical destruction caused by CMV is usually less than 50% and therefore unlikely to cause adrenal insufficiency,[4] and CMV disease is rare with well-preserved immune function in patients on newer potent ARTs. Other organisms and processes that have been associated with adrenal destruction in HIV disease include *Mycobacterium tuberculosis*, *Mycobacterium avium-intracellulare* (MAI), *Cryptococcus*, and hemorrhage. Additionally, pituitary/hypothalamic destruction resulting in secondary adrenal insufficiency may be caused in rare instances by opportunistic infection (e.g., toxoplasmosis, *Cryptococcus*, and CMV). Idiopathic adenohypophyseal necrosis is also observed in a minority of patients, approximately 10% at autopsy, and may be related to a direct effect of HIV.[5]

Glucocorticoid Excess: Adrenal Shunting and Cortisol Resistance

Increased cortisol levels may also be seen in HIV-infected patients. More commonly, increased cortisol levels are seen as a stress response, in association with low weight or increasing degree of illness. Intra-adrenal shunting toward cortisol synthesis, potentially as a result of 17,20-lyase dysfunction, has been suggested by studies demonstrating a reduced dehydroepiandrosterone (DHEA) to cortisol ratio on cosyntropin testing.[6] Cytokine modulation of the hypothalamic-pituitary-adrenal (HPA) axis may also contribute to increased cortisol levels. Interleukin 1 (IL1), produced in the median eminence, has been shown to increase corticotropin-releasing hormone and adrenocorticotropic hormone (ACTH) secretion in vitro and in animal studies. Increased IL1 secretion from infected monocytes in the median eminence is thus another possible cause of increased cortisol secretion in HIV-infected patients. In turn, higher cortisol levels and greater diurnal variation may reduce T-cell immune activation.[7] Glucocorticoid resistance has also been shown in rare patients with advanced HIV disease, who demonstrate addisonian symptoms, including hyperpigmentation, in the setting of hypercortisolism and increased ACTH.

Medication Effects

Medications may contribute to adrenal insufficiency in HIV patients (Table 44.1). Ketoconazole, an antifungal agent, inhibits side-chain cleavage enzyme and 11β-hydroxylase. These effects are not generally seen with fluconazole, itraconazole, and more recently introduced imidazole derivatives. Phenytoin, opiates, and rifampin, among other drugs, affect cortisol metabolism. For example, adrenal insufficiency may be precipitated by the use of rifampin for treatment of tuberculosis in patients with reduced adrenal reserve. Megestrol acetate, a potent synthetic progestational derivative used as an appetite stimulant, has glucocorticoid properties and decreases ACTH. Abrupt withdrawal of megestrol acetate may precipitate adrenal insufficiency, and such patients should be tested for adrenal insufficiency and receive physiologic glucocorticoid administration as needed after megestrol withdrawal. In addition, megestrol acetate can decrease gonadal function, which should also be monitored during and after therapy. Cases of Cushing syndrome have been described with the concomitant use of fluticasone and ritonavir, via inhibition of CYP3A4 by ritonavir and resultant reduction in metabolism of fluticasone. This combination of medications can result in symptoms of severe cortisol excess and potential severe adrenal insufficiency with discontinuation of fluticasone.[8] Such patients demonstrate very low measured cortisol and ACTH levels, despite symptoms of hypercortisolemia, due to suppression of the endogenous HPA axis by increased concentrations of circulating fluticasone. After discontinuation of the fluticasone, long-term physiologic steroid replacement is necessary until the HPA axis recovers. In addition, adrenal insufficiency has been reported in approximately 5% of patients receiving intra-articular steroids while on protease inhibitors (PIs), particularly ritonavir, with increasing risk seen among those with more than two injections within the prior 6 months.[9] Similar interactions with intra-articular steroids have been seen with cobicistat, another potent CYP3A4 inhibitor used in combination with certain antiretroviral medications.[10]

Clinical Assessment

HIV-infected patients with symptoms of adrenal insufficiency, and particularly those with hyponatremia and risk factors for adrenal insufficiency, such as known disseminated CMV or recent use of megestrol acetate, should be evaluated. Evaluation of the cortisol axis should proceed as in other patients with suspected adrenal dysfunction. Cosyntropin testing is usually an adequate first step, except in those patients in whom hypothalamic or pituitary insufficiency of recent onset is suspected. In such patients, use of morning cortisol levels, metyrapone, or insulin tolerance testing may be necessary, if there are no contraindications. After adrenal insufficiency is documented, ACTH testing and appropriate imaging are used to localize the defect. In patients with clinical symptoms of adrenal insufficiency and elevated cortisol

TABLE 44.1 Major Endocrine and Metabolic Effects of Medications Used in the Treatment of HIV

Endocrine/Metabolic System	Interacting Medication	Mechanism	Effect
Adrenal	Ritonavir Cobicistat	CYP3A4 inhibitor that decreases metabolism of fluticasone and potentially other steroids	Cushing stigmata with reduced ACTH/cortisol
	Ketoconazole	11β-Hydroxylase inhibition	Adrenal insufficiency
	Megestrol acetate	Synthetic progestational agent with glucocorticoid properties	Adrenal insufficiency
	Rifampin	Increased cortisol metabolism	Adrenal insufficiency
Gonadal	Megestrol acetate	Decreased gonadotropins	Hypogonadism
	Ketoconazole	Inhibits side-chain cleavage enzyme	Hypogonadism
	Protease inhibitors	Variable effects to cause hyperprolactinemia directly or via dopamine antagonism	Potential hypogonadism
	Anabolic steroids	Decreased gonadotropins	Hypogonadism
Thyroid	Rifampin	Increased hepatic clearance of thyroid hormone	Reduced thyroxine levels
	Interferon	Autoimmune thyroiditis	Variable effects on thyroid function
Fluid and electrolytes	Trimethoprim	Structural similarities to amiloride with inhibition of tubular potassium secretion	Hyperkalemia
	Tenofovir disoproxil fumarate	Fanconi-like syndrome, phosphate wasting	Hypokalemia Hypophosphatemia
Calcium	Protease inhibitors	Variable effects to inhibit 1α-hydroxylation of 25-hydroxyvitamin D	Hypocalcemia
	Efavirenz	Increased catabolism of 25-hydroxyvitamin D through induction of CYP24A	Vitamin D deficiency
	Rifabutin	Induces cytochrome P450 and alters vitamin D metabolism	Vitamin D deficiency
	Foscarnet	Complexes with calcium	Decreased ionized calcium, magnesium
	Pentamidine	Renal magnesium wasting	Hypocalcemia
	Ketoconazole	Inhibits 1,25-dihydroxyvitamin D synthesis	Hypocalcemia
Bone	Tenofovir	Phosphate wasting and tubulopathy, possible direct effect on osteoclast function	Bone loss
	Protease inhibitors	Unclear	Bone loss
Glucose	Protease inhibitors	Variable effects to inhibit GLUT4-mediated glucose transport	Hyperglycemia
	NRTIs	Variable effects to inhibit mitochondrial DNA polymerase gamma	Hyperglycemia
	Pentamidine	Islet cell inflammation and insulin release	Hypoglycemia and subsequent hyperglycemia
Body composition	Protease inhibitors	Variable effects to inhibit SREBP1 and PPARγ signaling in subcutaneous fat	Subcutaneous fat loss
	NRTIs	Variable effects to inhibit mitochondrial DNA polymerase gamma in subcutaneous fat	Subcutaneous fat loss
Lipids	Protease inhibitors	Variable effects to decrease lipoprotein lipase-mediated clearance of VLDL and TG	Hypertriglyceridemia
	Anabolic steroids	Possible effects on hepatic lipase and LCAT	Low HDL

ACTH, Adrenocorticotropic hormone (corticotropin); *GLUT4,* glucose transporter type 4; *HDL,* high-density lipoprotein; *HIV,* human immunodeficiency virus; *LCAT,* lecithin:cholesterol acyltransferase; *NRTIs,* nucleoside reverse transcriptase inhibitors; *PPAR,* peroxisome proliferator-activated receptor; *SREB1,* sterol regulatory element–binding protein 1; *TG,* triglyceride; *VLDL,* very low density lipoprotein.

levels, cortisol resistance may be present and the diagnosis may be made by glucocorticoid receptor studies in blood monocytes. Conversely, in patients with symptoms of adrenal excess and low cortisol and ACTH levels, exogenous steroid use or interactions with ART should be suspected.

Gonadal Function

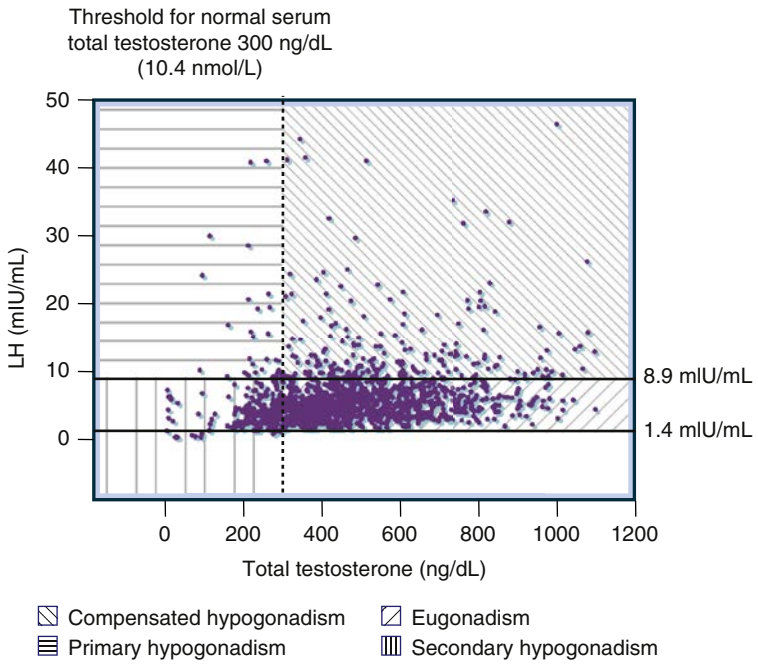
Male Gonadal Dysfunction

Gonadal dysfunction is common among HIV-infected men with weight loss and advanced illness. Initial studies indicated biochemical hypogonadism in approximately 50% of men with AIDS, in association with increased disease severity. Among HIV-infected men with low weight, hypogonadism was seen in 20%.[11] More recent studies suggest a lower prevalence of hypogonadism of approximately 9% to 16%.[12–15] The mechanisms of hypogonadism in HIV-infected patients may relate to severe illness or effects of undernutrition on gonadotropin secretion; medication effects; or, more rarely, tissue destruction from opportunistic infections. Most often, hypogonadism is secondary in nature, with low or inappropriately normal gonadotropin levels, as seen in 91% of patients with reduced free testosterone levels during initiation of highly active antiretroviral therapy (HAART) in the Swiss HIV cohort.[16] Primary hypogonadism is seen less often and may be caused by cytokine effects on the testes, including effects of tumor necrosis factor (TNF) to inhibit steroidogenesis via effects on the side-chain cleavage enzyme and of IL1 to inhibit Leydig cell steroidogenesis and luteinizing hormone binding to the Leydig cell. Indeed, among young men, median age 45 years, using an early morning total testosterone as a test to define hypogonadism in a large Italian cohort, Rochira and associates[13] demonstrated that gonadotropins were elevated in 16% of patients studied (Fig. 44.1). In addition, opportunistic infections of the testes have rarely been reported, and up to 25% of HIV-infected patients with AIDS will demonstrate testicular involvement of widespread opportunistic infection or systemic neoplasms, including CMV, toxoplasmosis, Kaposi sarcoma (KS), and testicular lymphoma, although there are few data to suggest that primary hypogonadism develops in all such cases.

In addition, several medications may affect the hypothalamic-pituitary-gonadal axis. Ketoconazole inhibits side-chain cleavage enzyme and other critical enzymes in testicular steroidogenesis. Megestrol acetate is used to increase appetite, but as a synthetic progestational agent it suppresses gonadotropin secretion and results in hypogonadism. Opiate therapy affects gonadotropin-releasing hormone secretion and may result in hypogonadotropic hypogonadism.

More recently, increased prolactin levels and gynecomastia have been demonstrated among HIV-infected patients. In a case-control study, gynecomastia was seen in 1.8% of 2275 consecutively screened HIV-infected patients and was associated with hypogonadism, hepatitis C, and degree of lipoatrophy (subcutaneous fat loss associated with potent ART). Thyroid-stimulating hormone (TSH, thyrotropin) levels were increased, although the proportion with hypothyroidism was not different.[17] Of ART medications, efavirenz is most closely associated with gynecomastia,[18] which is due to direct activation of the estrogen receptor.[19] Hyperprolactinemia has been reported in 21% of HIV-infected men with stable HIV disease and was significantly associated with opioid use and increased CD4 count but not with changes in body composition or gynecomastia.[20] Increased prolactin levels in association with galactorrhea have also been described among patients treated with PIs. The mechanism of this effect is unclear and may relate to a direct stimulation of prolactin secretion by specific PIs or effects on the P450 system to potentiate the dopamine antagonistic effect of other drugs.[21] Dopamine agonists should be used cautiously among HIV-infected men receiving PIs because of the potential for interactions.

Sex hormone–binding globulin levels are increased in 30% to 55% of HIV-infected patients. Therefore, use of bioavailable



• **Fig. 44.1** Gonadal status according to serum total testosterone threshold of 300 ng/dL and luteinizing hormone (LH) normal range. (From Rochira V, Zirilli L, Orlando G, et al. Premature decline of serum total testosterone in HIV-infected men in the HAART-era. *PLoS One.* 2011;6:e28512, Fig. 2.)

or free testosterone is recommended to diagnose hypogonadism, because use of total testosterone assays may underestimate the prevalence of true hypogonadism in this population. As a caveat, analogue assays of free testosterone may not be independent of sex hormone–binding globulin and should not be used. In contrast, determination of free testosterone by equilibrium dialysis or calculation by an accepted equation are more useful strategies to detect hypogonadism among the HIV population. For example, Monroe and colleagues[12] conducted a large study in the Multicenter AIDS Cohort Study, comparing normal-weight HIV-infected and well-matched uninfected patients in the current era of ART. In this study, total testosterone levels were similar in those who were HIV infected versus those who were not HIV infected, but free testosterone calculated using the Vermeulen equation was significantly lower in the HIV-infected patients. Reliance on total testosterone alone would have missed 33% of patients with hypogonadism.[12] Use of total testosterone or free testosterone analogue radioimmunoassay to diagnose hypogonadism among HIV-infected patients is associated with poor sensitivities of 25% and 33%, respectively.[14]

In patients in whom acute and chronic illness may contribute to hypogonadism, retesting of gonadal function by measuring an early morning free testosterone level is recommended on resolution of the illness, as endogenous function may return with improved health. In patients who remain hypogonadal, administration of physiologic testosterone replacement after appropriate diagnostic workup for the cause of hypogonadism is appropriate. In contrast to the potential utility of specific anabolic steroids in the treatment of weight loss in AIDS wasting (see the Treatment of AIDS Wasting and Loss of Lean Body Mass section), no role has been demonstrated for the use of anabolic steroids alone or in combination with testosterone for the treatment of hypogonadism per se in HIV-infected men.

Female Gonadal Dysfunction

Amenorrhea is seen in approximately 25% of HIV-infected women and may be caused by the reduction of gonadotropin production associated with the stress of illness. In contrast, anovulation may be seen in up to 50% of HIV-infected women in association with reduced CD4 counts. Among anovulatory HIV-infected women, changes in menstrual function are three times as likely compared with normally ovulating patients. Early menopause has been reported in up to 8% of HIV-infected women.[22]

Androgen levels are often reduced in HIV-infected women. In one study, androgen levels, assessed with the use of a free testosterone assay, were reduced below the level seen in age-matched healthy women in more than 50% of HIV-infected women with significant weight loss and in more than one third of HIV-infected women without weight loss.[23] The mechanisms of androgen deficiency in HIV disease may be caused in part by intra-adrenal shunting toward cortisol production and away from androgen production, particularly in women with significant weight loss[6] (see the Adrenal Function section).

Thyroid Function

Altered thyroid function tests are common in HIV-infected patients. Thyroid-binding globulin (TBG) levels are increased in HIV-infected patients and correlate inversely with CD4 counts.[24] Abnormal thyroid function test results may be caused by the stress of illness in patients with advanced disease or concomitant disorders, as found in other patients with euthyroid sick syndrome. However, among adults, some studies have shown that reverse triiodothyronine (rT_3) levels do not rise in association with decreasing T_3 levels, as one would expect in nonthyroidal illness.[3] Patients with progressive HIV disease therefore exhibit decreased T_3 levels, increased TBG, and decreased rT_3 levels with increasing illness.

In addition to the euthyroid sick syndrome, large screening studies have demonstrated an increased prevalence of primary hypothyroidism in HIV-infected patients. Recent studies have investigated the prevalence of thyroid dysfunction in the current era of HAART. In one study of 1565 HIV-infected patients, the prevalence of overt hypothyroidism was 2.5% and of overt hyperthyroidism 1%. A higher percentage of patients demonstrated subclinical hypothyroidism (4%) and abnormal thyroid test results associated with nonthyroidal illness (17%). Conversely, 76% of patients demonstrated normal thyroid function tests. HIV therapy and specific antiretroviral medications were not associated with thyroid dysfunction.[25] Among 2437 HIV-infected patients, Nelson and coworkers[26] demonstrated prevalence rates of hyperthyroidism and hypothyroidism each to be 1%. Use of specific antiretroviral agents, including PIs and efavirenz, were associated with thyroid dysfunction, but this has not been a consistent finding among studies.

Increased TSH has been demonstrated in young children with an average age of 1.5 years and failure to thrive. Thyroxine (T_4) levels were normal, but thyrotropin-releasing hormone testing showed exaggerated TSH responses, and growth rates increased in response to thyroid hormone.[27] Fundaro and associates[28] demonstrated increased antithyroglobulin antibodies in 34% of symptomatic HIV-infected children. Increased TSH levels were found in 28% of HIV-infected children, particularly those with severe immunosuppression. In contrast, a larger study in perinatally infected children demonstrated reduced total T_3, total T_4, and free T_4 and increased rT_3, TBG, and TSH, with negative autoantibodies, suggesting a euthyroid sick pattern, particularly in those with severe immunosuppression. HIV-infected children with failure to thrive should be screened for true hypothyroidism, but more often the thyroid function tests will reflect nonthyroidal illness and the severity of immune compromise.[29]

Recently, thyroid dysfunction has been described with an immune reconstitution syndrome in which autoimmune thyroid disease occurs in association with use of potent ART and improved immune function, typically 12 to 36 months after ART is initiated.[30] Graves disease is most often reported in this context. The estimated prevalence for immune reconstitution thyroid disease with initiation of HAART was 3% for women and 0.2% for men.[31] Graves disease has also been described after IL2 therapy in HIV-infected patients.[32]

In addition to autoimmune causes, thyroid disease related to anatomic replacement and infection of the thyroid has been reported in HIV-infected patients. *Pneumocystis* thyroiditis has been reported to cause a painful thyroiditis-like picture, with hyperthyroidism followed by hypothyroidism, decreased uptake on scanning, and a firm but tender gland. *Pneumocystis* thyroiditis may result from the use of inhaled pentamidine, which is associated with extrapulmonary *Pneumocystis* infections.

CMV, MAI, *Cryptococcus*, and KS have been demonstrated in the thyroid on autopsy but have not been related to clinical thyroid disease among patients with AIDS. Clinically apparent thyroidal abscesses from *Aspergillus* and *Rhodococcus equi* have been reported. Hypothalamic/pituitary replacement from

opportunistic infections, such as toxoplasmosis and CMV, has also been reported to cause secondary hypothyroidism.

Medications may affect thyroid function. Rifampin influences hepatic clearance of T_4, and interferon is associated with an increased incidence of autoimmune hypothyroidism.

Fluid Balance and Electrolytes

Disorders of fluid balance and electrolytes are common among patients with AIDS. Hyponatremia may be seen in upward of 50% of patients and is most often related to the syndrome of inappropriate secretion of antidiuretic hormone (SIADH). Hyperkalemia is also frequently reported and may be seen in association with various drugs, such as trimethoprim. More rarely, hyperkalemia may be associated with adrenal insufficiency.

Sodium

Hyponatremia (sodium <130 mmol/L) is seen in 40% to 60% of hospitalized patients with AIDS and 20% of outpatients. SIADH (volume-replete patients with low levels of serum sodium, and inappropriately elevated levels of urine osmolarity) is seen in 23% to 47% of hyponatremic patients. SIADH may be caused by various infections and tumors and is treated with fluid restriction and hypertonic saline if severe. The vasopressin receptor 2 antagonist, tolvaptan, has also been used in the treatment of hyponatremia in patients with advanced HIV disease.[33]

Adrenal insufficiency is documented in 30% of volume-depleted, hyponatremic HIV-infected patients.[34] Volume depletion (diarrhea, vomiting) with excessive free water and impaired water clearance (HIV nephropathy) may cause hyponatremia among ill HIV-infected patients, especially those in the hospital. Volume repletion is the treatment. Hyporeninemic hypoaldosteronism,[35] more typically associated with hyperkalemia, may be another cause of hyponatremia and is treated with mineralocorticoids. The use of medications such as vidarabine, miconazole, and pentamidine is associated with hyponatremia of unknown cause. Hypernatremia may be caused by foscarnet-induced nephrogenic diabetes insipidus.

Potassium

Hyperkalemia occurs in 20% to 53% of AIDS patients on trimethoprim because of structural similarities to amiloride and inhibition of tubular potassium excretion.[36] Other potential causes include pentamidine-associated tubular nephropathy; HIV nephropathy (glomerular sclerosis); primary adrenal insufficiency; and, rarely, hyporeninemic hypoaldosteronism. Physiologic studies investigating potassium balance in HIV-infected patients also suggest an inadequate aldosterone response to hyperkalemia in HIV-infected patients. A Fanconi-like syndrome with tubular dysfunction, phosphate wasting, and hypokalemia has been described with the use of tenofovir disoproxil fumarate (TDF) and more rarely with adefovir, cidofovir, and didanosine.[37]

Calcium Homeostasis and Bone Changes

Calcium Homeostasis

Hypocalcemia is common in advanced HIV disease. Hypocalcemia, based on albumin-adjusted total calcium levels, was demonstrated in 6.5% of a large cohort of HIV-infected patients with AIDS. Calcium levels decreased progressively with stage of disease. Among patients with hypocalcemia, 48% were vitamin D deficient, and the expected increase in parathyroid hormone (PTH) levels was lacking in the majority.[38] Jaeger and colleagues[39] also demonstrated decreased PTH secretion in severely immunocompromised patients with AIDS, but the mechanism is unknown. In addition, decreased PTH levels may occur in the setting of hypomagnesemia during severe illness or in association with renal magnesium wasting. Vitamin D deficiency may be caused by malabsorption from AIDS enteropathy or by specific effects of antiretroviral drugs (e.g., inhibition of 1 α-hydroxylation of 25-hydroxyvitamin D by PIs or increased conversion of 25-hydroxyvitamin D to 24,25-dihydroxyvitamin D by efavirenz).[40–42]

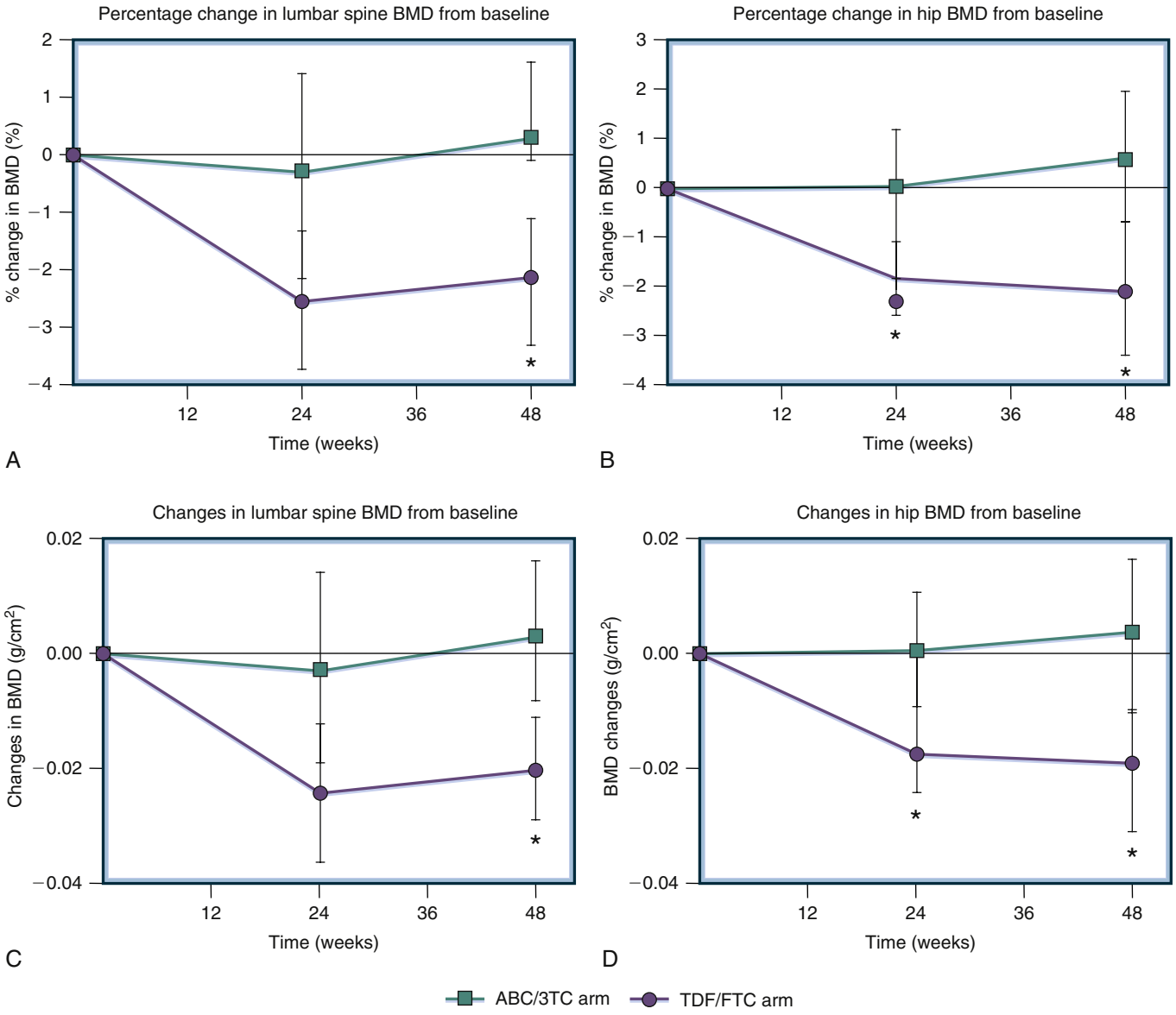
Severe vitamin D deficiency of nutritional origin has also been described in HIV-infected children. Osteomalacia has been associated with use of rifabutin for MAI in an HIV-infected patient. Rifabutin induces cytochrome P450, which may affect vitamin D metabolism. Other drugs that induce P450 could have a similar effect. Several drugs may affect calcium homeostasis (see Table 44.1). Foscarnet complexes with calcium to decrease ionized calcium levels and may also induce severe hypomagnesemia. Pentamidine therapy has been associated with renal magnesium wasting and severe hypomagnesemia, which may, in turn, cause hypocalcemia, through decreased PTH release and resistance to circulating PTH. Ketoconazole inhibits 1,25-dihydroxyvitamin D synthesis.

Among patients with HIV disease, hypercalcemia can be caused by excessive 1,25-dihydroxyvitamin D production in the setting of granulomatous disease (tuberculosis) or lymphoma, by local osteoclastic bone resorption from disseminated CMV, or by human T-cell lymphotropic virus 1–related activation of PTH-related protein. Immune reconstitution–associated hypercalcemia has been described in the setting of ART initiation among patients with known tuberculosis, in whom immune reconstitution results in increased granulomatous activity and 1,25-dihydroxyvitamin D production.[43]

Bone Loss: Prevalence, Etiologic Factors, and Treatment Strategies

Studies performed recently in the current era of ART give an estimate of the prevalence and risk factors for progression of bone loss among HIV-infected patients. Bonjoch and associates[44] investigated 671 HIV-infected patients and demonstrated osteopenia in 47.5% and osteoporosis in 23%. Progression rates to osteopenia and osteoporosis were 12.5% and 15.6% over 2.5 years of follow-up and 18% and 29% over 5 years of follow-up. In this large cohort, factors associated with progression of bone loss were age, male sex, lower body mass index (BMI), PI use, and TDF use.[44] Significant bone loss has also been shown among postmenopausal HIV-infected women, with time since menopause and traditional risk factors most significantly associated with bone loss. In a longitudinal analysis of postmenopausal HIV-infected women, Yin and coworkers[45] demonstrated increased markers of bone resorption and annualized bone loss in HIV-infected women versus non–HIV-infected control subjects.

Specific antiretroviral strategies may affect bone in the HIV population. Studies demonstrate that switching to a TDF-based regimen is associated with bone loss and increased bone turnover[46,47] (Fig. 44.2). Strong evidence for an effect of TDF is shown in studies in which patients at risk for HIV are given single-agent



• **Fig. 44.2** Changes from baseline in hip and lumbar spine BMD. Percentage and absolute mean changes from baseline in lumbar spine (A, C) and hip (B, D) bone mass density as measured by dual-energy x-ray absorptiometry. *ABC/3TC,* abacavir/lamivudine; *BMD,* bone mineral density; *TDF/FTC,* tenofovir/emtricitabine. Error bars show 95% confidence intervals. (From Rasmussen TA, Jensen D, Tolstrup M, et al. Comparison of bone and renal effects in HIV-infected adults switching to abacavir or tenofovir based therapy in a randomized trial. *PLoS One.* 2012;7:e32445, Fig. 2.)

tenofovir for preexposure prophylaxis. In these patients, bone loss averaged approximately 1% compared with control subjects over 2 years of follow-up. Larger effects might be seen in less healthy HIV-infected patients with other comorbid contributors.[48]

Current recommendations suggest that TDF should be avoided for the initial or continued treatment of HIV-infected persons at risk for fracture.[49] ART switching from TDF to abacavir, raltegravir, or the tenofovir prodrug, tenofovir alafenamide are associated with improvements in bone mineral density (BMD) in HIV-infected persons on effective ART.[50–52] In addition, because of their negative effects on BMD, PIs should be avoided in HIV-infected persons at risk for fracture.

Recent data suggest that immunologic factors including T-cell activation,[53] low CD4 cell count,[54] and coinfection with hepatitis B and C are strongly related to reduced bone density, particularly among women.[55] These data suggest the need to assess and follow bone density in HIV-infected patients with specific risk factors, such as older age, long-term ART use, low BMI, hypogonadism, hepatitis, low CD4 count, and TDF use.

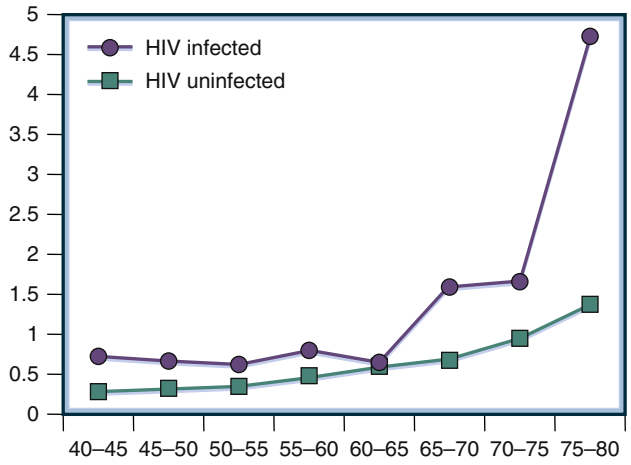
Endocrine factors may contribute to reduced bone density in HIV-infected patients, including hypogonadism, relative growth hormone (GH) deficiency, and vitamin D deficiency. In one study, GH pulse area determined from overnight frequent sampling was reduced in patients with central fat accumulation and correlated significantly with vertebral bone density.[56] This relationship may explain, in part, the inverse association between increased visceral fat and reduced bone density in HIV-infected patients.[57] Vitamin D deficiency is common among HIV-infected patients.

In a study of ambulatory clinic patients in London, the prevalence of 25-hydroxyvitamin D deficiency (<20 ng/mL) and severe 25-hydroxyvitamin D deficiency (<10 ng/mL) was 58.5% and 12.6%, respectively.[58] In a large cohort of 2044 consecutively followed HIV patients in Brussels, the prevalence of severe vitamin D deficiency using the 10-ng/mL cutoff was 32.4%.[59] Among HIV-infected patients in the study, advanced disease (CD4 <200 cells/mm^3) and current use of efavirenz were significantly associated with severe vitamin D deficiency.[59] In carefully conducted case matching studies, the odds ratio for vitamin D deficiency in HIV versus non–HIV-infected patients tended to be increased at 1.46, although this did not reach statistical significance.[60] TDF may increase PTH and bone turnover, potentially related to effects on proximal tubule phosphate reabsorption or other effects. PIs may also inhibit 1α-hydroxylase and result in vitamin D deficiency.[40]

Studies in HIV-infected patients receiving HAART have investigated markers of bone resorption and formation and found evidence of increased bone turnover.[61] Tebas and colleagues[62] evaluated serum and urine bone markers in 73 HIV-positive patients receiving PI therapy. Increased serum bone alkaline phosphatase and urine *N*-telopeptide were found to be inversely correlated with BMD T- and Z-scores measured by dual-energy x-ray absorptiometry, suggesting an increased rate of bone turnover among HIV-infected patients receiving PI therapy.[62] Recent data investigating the receptor activator for nuclear factor κB ligand (RANKL) system demonstrate reduced soluble RANKL in HIV-infected patients,[63,64] arguing against activation of this system as a mechanism for increased bone turnover in HIV-infected patients. In contrast, increased osteoprotegerin was shown among HIV-infected women with low bone density, suggesting a compensatory increase in the context of low bone density and high bone resorption rates.[65]

Several recent studies have examined fracture rates among HIV-infected patients. In a US-based data registry study, the overall fracture prevalence was 2.87 versus 1.77 per 100 persons for HIV versus non–HIV-infected patients.[66] In a large cohort study performed in Europe, significantly increased adjusted hazard ratios for hip and major fracture were 4.7 and 1.8, with an increased relative risk (RR) of fracture among older HIV-infected patients in the study[67] (Fig. 44.3). In a Veterans Administration cohort of men, Womack and coworkers[68] demonstrated that increased fragility fractures were strongly associated with a higher frailty score, low BMI, alcohol-related diagnoses, white race, proton pump inhibitor use, and PI use. In a study limited to women, lifetime fragility fractures were significantly more common in HIV-infected versus non–HIV-infected women (odds ratio, 1.7; 95% confidence interval, 1.1–2.6).[69] Use of the FRAX (Fracture Risk Assessment Tool) score to predict fragility fracture has shown relative poor sensitivity but good specificity among HIV-infected patients.[70] In partial contrast to the data on bone density, data from nonrandomized longitudinal cohorts demonstrate that overall use of PIs, nucleoside reverse transcriptase inhibitors (NRTIs), and non-NRTIs (NNRTIs) is associated with reduced fracture rates, whereas the effects of individual drugs vary. Low CD4 count, hepatitis, and DM are independently related to increased fracture risk.[71] These data suggest that overall effects of ART may be to improve bone through effects on improving immune function and virologic factors, but adverse effects of specific ART agents on bone may also be important.[72]

Limited data are available on treatment strategies for bone loss in HIV-infected patients. Among patients with idiopathic bone loss and high bone turnover, recent studies suggest that alendronate is effective in increasing bone density among HIV-infected patients, with studies demonstrating increases in lumbar spine bone density from 3.4% to 5.2% over 42 weeks and good safety tolerability, albeit in relatively small numbers of patients.[73,74] Use of longer acting bisphosphonates (e.g., zoledronate) showed even greater changes in spinal bone density of 8.9% over 2 years in a randomized, placebo-controlled study.[75] Importantly, significant differences persisted for bone density and bone turnover between groups 5 years after the final dose, suggesting potent, persistent effects after short-term dosing.[76] To date, there have been no studies examining the effect of teriparatide, abaloparatide, or denosumab in the HIV-infected population. Among men with AIDS wasting, testosterone at high doses (200 mg/week) has been shown to increase bone density.[77]

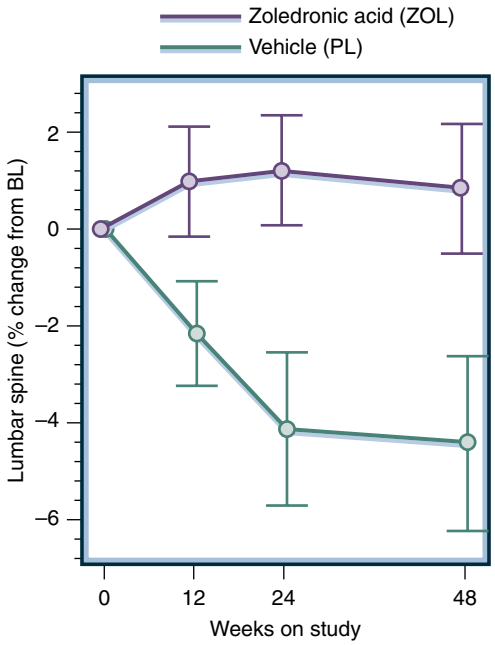


• **Fig. 44.3** Age-specific fracture incidence rates (per 100 person-years) in human immunodeficiency virus–infected versus uninfected patients. (From Guerri-Fernandez R, Vestergaard P, Carbonell C, et al. HIV infection is strongly associated with hip fracture risk, independently of age, gender, and comorbidities: a population-based cohort study. *J Bone Miner Res.* 2013;28:1259–1263, Fig. 1, used with permission.)

The first 2 years after the initiation of ART are associated with a 2% to 6% decrease in BMD, with the larger decreases in this range observed in those receiving TDF or PIs.[78–81] Advanced HIV disease at the time of ART initiation has also been associated with greater loss of BMD in this period.[82] In addition to avoiding TDF and PIs, as well as starting ART at a higher CD4 cell count, several strategies have been tested to mitigate bone loss during ART initiation. In a randomized controlled trial, high-dose vitamin D_3 (4000 IU/day) and calcium (1000 mg/day) at the time of ART initiation with TDF/emtricitabine/efavirenz was shown to attenuate ART-initiation–associated bone loss by 50%.[83] In another randomized trial, a single dose of zoledronic acid (5 mg) prior to ART initiation prevented bone loss over 96 weeks[84]; this may be an important strategy to maintain bone health in those HIV-infected patients initiating ART who are at risk for fracture because of older age, lower BMD, or the presence of other fracture risk factors (Fig. 44.4).

Bone Metabolism in HIV-Infected Children

Reduced bone density has also been reported in HIV-infected children. O'Brien and associates[85] demonstrated reduced total-body bone density in perinatally infected girls at age 9 years in association with increased *N*-telopeptide and PTH levels. In a 2013 study, HIV-infected children ages 12 to 13 years had a greater



• **Fig. 44.4** Longitudinal percentage change over 48 weeks in lumbar spine bone mineral density (BMD) with initiation of antiretroviral therapy (ART) with concomitant zoledronic acid (5 mg intravenous; ZOL) or placebo (PL). *BL,* baseline. (Redrawn from Ofotokun I, Titanji K, Lahiri CD, et al. A single-dose zoledronic acid infusion prevents antiretroviral therapy-induced bone loss in treatment-naive HIV-infected patients: a phase IIb trial. *Clin Infect Dis.* 2016;63[5]:663–671; Fig. 3B.)

prevalence of low bone density than HIV-exposed but uninfected children, although these differences were attenuated after height and weight adjustment, suggesting that delays in growth may account for differences in bone density.[86] Among young HIV-infected adults ages 20 to 25 years, Tanner stage 5, infected either perinatally or during adolescence, both volumetric bone density and cortical and trabecular thickness on high-resolution peripheral quantitative computed tomography was reduced, suggesting lower peak bone mass among HIV-infected patients.[87] In contrast to increased markers of resorption, reduced osteocalcin levels have been reported in HIV-infected children, suggesting reduced bone formation and a relative discrepancy between increased resorption and reduced formation in this group.[88] Similar to data in adults, bone density is related to insulin-like growth factor 1 (IGF1) in HIV-infected children, suggesting a potential effect of low GH on bone.[89] Mora and colleagues[90] followed bone density longitudinally over 1 year to compare changes in HIV-infected children and control subjects and demonstrated relative reductions in total-body but not spinal bone density accrual and relative increases in bone turnover. ART initiation with TDF has been associated with small decreases in BMD in children but does not appear to affect accrual of bone over 10 years in small studies.[91,92] There is conflicting evidence whether TDF exposure affects bone health or linear growth in HIV-exposed uninfected infants.[93,94]

Vitamin D supplementation has been tested as a strategy to improve BMD in HIV-infected children and adolescents. Among children, supplementation with vitamin D (100,000 IU of cholecalciferol every 2 months and calcium [1 g/day]) did not increase bone density over 2 years.[95] In another study of adolescents taking ART containing TDF, vitamin D_3 (50,000 IU/monthly) was associated with modest increases in lumbar spine BMD, which was not observed in the placebo comparison group.[96] Another randomized, placebo-controlled study over 24 months showed no differences in BMD change between vitamin D_3 dosing of 18,000 IU/month, 60,000 IU/month, and 120,000 IU/month.[97] Taken together, these finding suggest that the effect of vitamin D supplementation on BMD in children and adolescents may be minimal.

Avascular Necrosis of Bone

Miller and coworkers[98] demonstrated a 4.4% prevalence of avascular necrosis (AVN) among 339 asymptomatic HIV-infected individuals. A significant relationship was reported between AVN and prior use of systemic corticosteroids. Other potential factors significantly associated with an increased risk of AVN included the presence of anticardiolipin antibodies, as well as routine bodybuilding and its associated mechanical stress. Alcohol use may also be a factor associated with AVN among HIV-infected patients.[99] In an analysis among HIV-infected patients, osteonecrosis of the bone was associated with exposure to one or more ART drugs and high triglyceride and cholesterol levels, as well as high serum immunoglobulin E.[100] A role for altered coagulation and inflammation was also suggested by the observation of increased D-dimer among patients with osteonecrosis[101] and that past history of immunosuppression was a major risk factor for AVN incidence.[102]

The GH/IGF1 Axis

Significant abnormalities in the GH/IGF1 axis occur in HIV-infected patients. Among patients with AIDS wasting and significant weight loss, GH levels are increased in association with reduced IGF1 levels, a pattern typical of GH resistance seen with malnutrition. In contrast, in the setting of visceral fat accumulation, frequent sampling of GH levels over 24 hours has suggested a different pattern.[103] Mean overnight GH and GH pulse amplitude were decreased in this setting, whereas pulse frequency was not different compared with age-matched and BMI-matched non-lipodystrophic HIV- and non–HIV-infected patients. Reduced GH levels were strongly predicted by increased visceral fat in the patients. A role for suppression of GH release by free fatty acids (FFAs) was suggested by experiments in which acipimox, a nicotinic acid derivative that blocks peripheral tissue lipolysis and lowers FFA levels, was administered. Peak GH response to GH–releasing hormone (GHRH) was increased in response to acipimox, in inverse association to the change in FFA.[104] Physiologic studies of GH in HIV-infected patients suggest a schema whereby increased somatostatin tone, reduced ghrelin, and increased lipolysis contribute to reduced GH secretion in viscerally obese HIV-infected patients with lipodystrophic changes in fat distribution.[104]

GH deficiency is a potential cause of growth failure in HIV-infected children, and treatment with GH results in improved auxologic parameters in such children.[105] Increased IGF-binding protein 3 (IGFBP3) proteolysis and reduced IGF1, IGFBP3, and acid-labile subunit of the IGFBP3 ternary complex are demonstrated among HIV-infected children with failure to thrive.[106] IGF1 and IGFBP3 responses to GH may be impaired in HIV-infected children, suggesting a degree of GH insensitivity in this population. These responses may improve with weight gain and improved immune function in response to HAART.[107] GH deficiency may also disrupt normal thymic development in

HIV-infected children. GH has been used to increase height in HIV-infected children with normal GH responses to stimulatory testing.[105] Among HIV-infected children, reduced GH secretion is associated with excess visceral adiposity. Among adults, GH is approved by the US Food and Drug Administration (FDA) for the treatment of AIDS wasting. In addition, a GHRH analogue, tesamorelin, is FDA approved to reduce excess visceral fat accumulation in HIV lipodystrophy (see the discussion in the Treatment of Metabolic and Body Composition Changes in HIV-Infected Patients section).

Glucose Homeostasis and Pancreatic Function

Disorders of glucose homeostasis were relatively infrequent prior to the institution of potent ART but may result from use of specific antiretroviral agents and are commonly seen in association with dyslipidemia and fat redistribution in the current era of HAART (see the Metabolic and Body Composition Changes in HIV-Infected Patients section). The pancreas is a frequent target of opportunistic infections and malignancies in patients with advanced HIV disease. However, clinical endocrine dysfunction rarely results, except in cases of massive pancreatic replacement from lymphoma or KS. For example, opportunistic infections of the pancreas are seen on postmortem examination but are rarely clinically relevant. More commonly, pancreatitis and hypoglycemia follow use of certain drugs, such as pentamidine, didanosine, or zalcitabine. Hypoglycemia can result from pentamidine administration secondary to islet cell inflammation and insulin release, especially in the context of high-dose therapy and azotemia. Subsequently, chronic hyperglycemia from pancreatic B-cell destruction may follow pentamidine use. Megestrol acetate use has been associated with new-onset DM because of its potent glucocorticoid action. Pancreatitis is common among patients with HIV and most often related to a drug effect (i.e., pentamidine, trimethoprim-sulfamethoxazole, didanosine, and zalcitabine). In a large study of almost 6000 patients followed for 23,460 person-years at the Johns Hopkins HIV Clinic, the incidence of acute pancreatitis was 5.1 in 1000 person-years between 2001 and 2006. Low CD4 count, aerosolized pentamidine, and female gender were associated with pancreatitis. In contrast, specific antiretroviral medications were not.[108] In the EuroSIDA study, a lower incidence of pancreatitis was seen since 2001—1.27 in 1000 person-years—and, again, low CD4 count was predictive. Similar to the data from the Hopkins cohort, no association was seen between cumulative exposure to ART and exposure to didanosine and stavudine—NRTIs that have been associated with pancreatitis.[109] Amylase levels may also be elevated in HIV-infected patients secondary to macroamylasemia and salivary amylase.

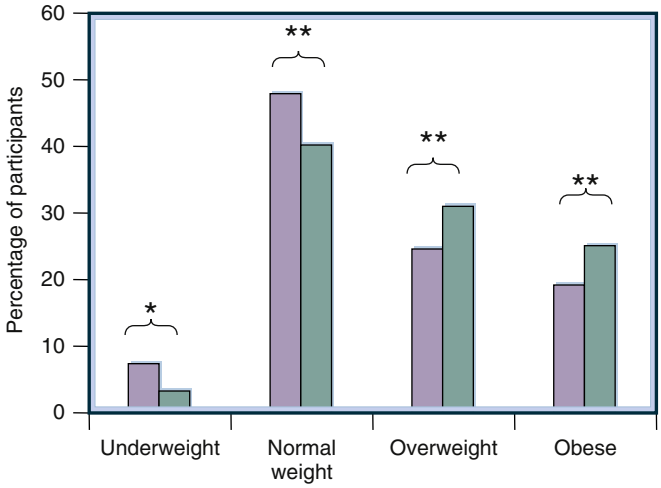
Metabolic and Body Composition Changes in HIV-Infected Patients

HIV-infected patients demonstrate several changes in body composition and metabolism. These changes are present to varying degrees among HIV-infected patients and are multifactorial in nature, related, in part, to the HIV virus itself, inflammation, and specific antiretroviral drugs, as well as the interplay of these factors.[110,111] Of importance, some of these changes may contribute to increased cardiovascular disease (CVD) and respond to changes in ART, lifestyle modification (LSM), and specific pharmacologic strategies (e.g., to improve lipids or insulin sensitivity).

The AIDS Wasting Syndrome and Loss of Lean Body Mass

Wasting was initially a common feature of progressive HIV disease, which was known originally as "slim disease." The AIDS wasting syndrome is currently defined by weight less than 90% ideal body weight or weight loss greater than 10% over 3 months. It is characterized by a disproportionate loss of lean body mass, with a relative sparing of body fat, particularly in men. In women, fat mass may be lost disproportionately with disease progression. The loss of lean body mass occurs early and may antedate weight loss. Muscle wasting, weakness, and increased resting energy expenditure of 8% to 9% are also features of this disease. Macallan and associates[112] demonstrated that energy expenditure fell during periods of rapid weight loss but less than the decrease in caloric intake. Cytokines associated with severe illness may increase energy expenditure and decrease appetite. In addition, chronic weight loss may be associated with gastrointestinal disease, including malabsorption. Weight loss is a significant predictor of mortality rate in HIV infection, with BMI less than 18.4 kg/m^2 associated with a 2.2-fold increase in mortality rate and BMI less than 16.0 kg/m^2 associated with a 4.4-fold increase in mortality rate.[113] Hypogonadism is observed in 30% to 50% of men with AIDS wasting and may contribute to loss of lean body mass[114] (see the Gonadal Function section).

In contrast to the traditional paradigm of wasting, HIV disease may be associated with overweight and obesity, particularly in developed countries with emerging obesity epidemics. In one study, performed at a large urban clinic for HIV-infected patients located in the southern United States, the prevalence of underweight was less than 10% and the prevalence of overweight and obesity was 44% despite high viral load at ART initiation.[115] With ART, 20% of patients increased from normal to overweight or overweight to obese[115] (Fig. 44.5). Subjects with more severe



• **Fig. 44.5** Proportion of study sample by body mass index category at antiretroviral therapy initiation versus 2 years on therapy among treatment-naive human immunodeficiency virus–infected patients at the University of Alabama at Birmingham 1917 HIV/AIDS Clinic, 2000-2008. *Purple* bars = therapy initiation; *teal* bars = 24 months. *, $p < .05$; **, $p < .01$. (From Tate T, Willig AL, Willig JH, et al. HIV infection and obesity: where did all the wasting go? *Antivir Ther.* 2012;17:1281–1289, Fig. 2A, used with permission.)

immune dysfunction and using boosted PIs tended to gain the most weight with ART.

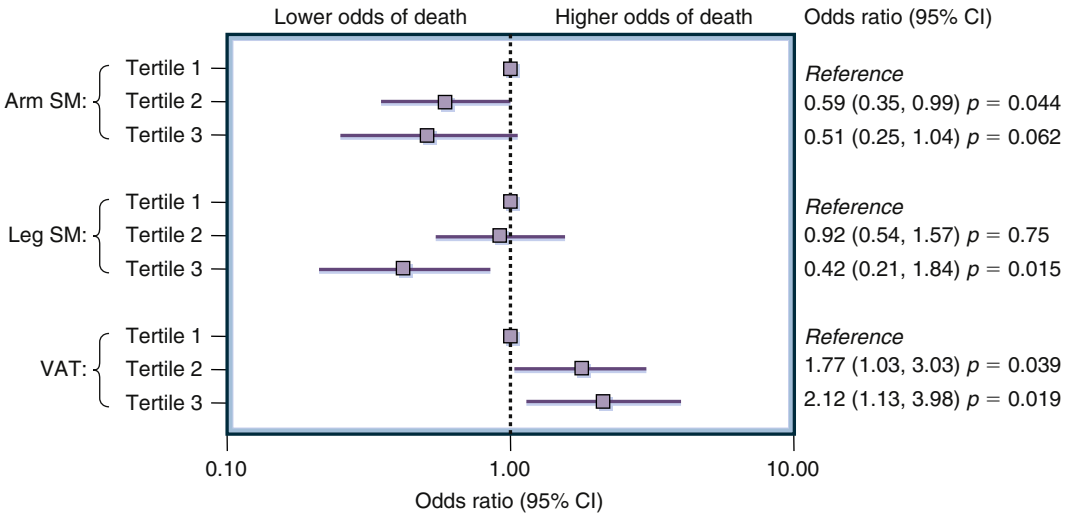
Changes in Fat Mass and Distribution

The most commonly seen change in body composition among HIV-infected patients is loss of abdominal and peripheral subcutaneous fat, including loss of subcutaneous fat in the face.[116] Other changes that may also be seen include relative preservation of central fat, with relative and absolute accumulation of excess visceral fat and excess upper trunk fat.[116,117] In addition, ectopic fat collections can be seen, including in the dorsocervical area. Increased fat deposition in the liver and muscle is also seen and is associated with insulin resistance. In prospective studies of antiretroviral-naive patients beginning treatment in the early 2000s, including an NRTI and PI, initial gains in both peripheral subcutaneous and central fat depots are seen with reversal of the catabolic wasting state in association with control of viral infection. These changes are followed by subsequent decreases in peripheral fat and relative preservation and even absolute gains in central fat. More recent data of ART-naive persons initiating treatment with more modern regimens suggest increase in both subcutaneous and visceral fat over 96 weeks.[118] Data from the Study of Fat Redistribution and Metabolic Change in HIV Infection (FRAM study) demonstrate that increases in visceral fat and reductions in limb fat are independently associated with increased mortality rate in HIV-infected patients[119] (Fig. 44.6).

The cause of relative central fat accumulation remains unknown. For example, it remains unknown whether this is a direct effect of specific antiretroviral drugs and whether abnormal nutrient partitioning to relatively preserved central adipose stores, less affected by NRTI administration and mitochondrial toxicity, may contribute. In contrast, several mechanisms have been shown to contribute to the fat loss seen peripherally. NRTIs can inhibit mitochondrial DNA polymerase gamma and contribute to mitochondrial dysfunction. Use of specific NRTIs, including older NRTIs such as stavudine, has been associated with apoptosis of fat and reduced mitochondrial DNA in vitro and in vivo, as well as reduced expression of lipid metabolism genes, and clinically with reduced subcutaneous fat and lipoatrophy.[120,121] PIs may have direct effects on adipogenesis (via inhibition of nuclear localization of sterol regulatory element–binding protein 1 [SREBP1] and reduction in peroxisome proliferator-activated receptor γ [PPARγ] expression).[122] NRTIs and PIs have been associated with increased lipolysis in vivo and in vitro.

Genetic polymorphisms may predispose to changes in body composition and metabolic alterations in HIV-infected patients receiving ART. Such findings could indicate a gene-environment interaction contributing to such changes. For example, single-nucleotide polymorphisms in the resistin gene were seen to predict the development of dyslipidemia, insulin resistance, and limb fat loss in response to a specific antiretroviral regimen.[123] In the same study, polymorphisms in the hemochromatosis gene and specific mitochondrial gene haplotypes were also associated with increased limb fat loss.[124] Specific haplotypes of the Fas gene (*APOC3*), PPAR gene, and adrenergic receptor have also been associated with the development of lipoatrophy.[125]

Other studies suggest early molecular changes in the fat of patients with subsequent peripheral adipose tissue loss. Kratz and colleagues[126] showed that reduced expression of messenger RNA (mRNA) encoding lipoprotein lipase and the transcription factors SREBP1, PPARγ, and CCAAT/enhancer binding protein C/EBPα in thigh subcutaneous fat were associated with the loss of fat, before it became evident clinically, whereas increased levels of mRNA encoding hydroxysteroid 11β-dehydrogenase 1 and the transcription factor C/EBPβ were found in adipose tissue from patients with preservation of subcutaneous fat mass. The expression of endoribonuclease Dicer, which modulates brown and white adipocyte differentiation, was found to be reduced in subcutaneous fat of HIV-infected persons with lipodystrophy, providing a



• **Fig. 44.6** Multivariable adjusted associations of magnetic resonance imaging–measured skeletal muscle and adipose tissue with 5-year mortality rate in human immunodeficiency virus–infected FRAM participants. The x-axis is on $\log_{10}$ scale. Estimates from multivariable adjusted models controlling for age, sex, race, traditional cardiovascular disease risk factors, HIV-related factors, C-reactive protein, fibrinogen, estimated glomerular filtration rate using cystatin C, albuminuria, arm SM, leg SM, and VAT. Reference category is tertile 1, those with the lowest amount of muscle or adipose tissue. *CI,* confidence interval; *SM,* skeletal muscle; *VAT,* visceral adipose tissue. (From Scherzer R, Heymsfield SB, Lee D, et al. Decreased limb muscle and increased central adiposity are associated with 5-year all-cause mortality in HIV infection. *AIDS.* 2011;25[11]:1405–1414, Fig. 1, used with permission.)

novel mechanisms for adipose dysfunction in HIV.[127] In addition, studies suggest increased activation of the renin-angiotensin-aldosterone system associated with visceral adipose accumulation in HIV. Recent data suggest that use of the mineralocorticoid receptor antagonist eplerenone in HIV-infected patients may reduce immune activation and improve select metabolic indices.[128]

Changes in fat distribution have been most widely recognized since the introduction of HAART, but abnormal fat distribution can be seen in antiretroviral-naive patients, suggesting that viral factors also may contribute. In this regard, recent data suggest that adipose tissue dysfunction may be related to a viral accessory protein, Vpr, which may simultaneously coactivate the glucocorticoid receptor and repress PPARγ.[129]

The changes in fat distribution seen among HIV-infected patients bear some similarities to those in Cushing syndrome, with dorsocervical fat accumulation and centripetal fat distribution. However, more specific stigmata of true Cushing syndrome, including proximal muscle weakness, facial plethora, thin skin, bruising, and violaceous striae, have not been seen, and thus these changes constitute a pseudo-Cushing syndrome.[130] Miller and coworkers[130] observed normal cortisol levels and adequate suppression in response to dexamethasone among HIV-infected patients with cushingoid features. Yanovski and associates[131] compared HIV-infected patients with PI-associated lipodystrophic changes in fat to control patients and those with true Cushing syndrome. In contrast to patients with true Cushing syndrome, patients with PI-associated lipodystrophy demonstrated normal diurnal variation in cortisol levels. The 24-hour urine free cortisol levels were reduced and 17-hydroxysteroid levels were increased compared with levels in control subjects. Among HIV-infected patients with changes in fat distribution, increased 11β-hydroxysteroid dehydrogenase expression in subcutaneous adipose tissue (SAT) has been demonstrated in association with an increased ratio of urinary cortisol to cortisone metabolites and may also contribute to increased cortisol production.[132] In addition, as noted earlier, viral proteins may activate the glucocorticoid receptor in specific fat depots.[129]

Other abnormalities of steroid metabolism have been noted in association with changes in fat distribution. In a longitudinal evaluation, the development of lipodystrophy was associated with reduced DHEA, increased cortisol/DHEA ratio, and increased interferon α.[133] Increased cortisol regeneration from affected fat depots may contribute to insulin resistance and further fat redistribution.

Lipid Abnormalities

Lipid abnormalities are highly prevalent among HIV-infected patients, particularly those with changes in fat distribution and increased visceral fat and upper trunk fat. Hypertriglyceridemia has long been associated with HIV infection; was observed prior to the introduction of potent ART; and is related, in part, to increased very low density lipoprotein (VLDL) secretion and decreased clearance.[134] The cause of these changes is not known but may relate to the effects of viral infection itself, microbial translocation via lipopolysaccharide,[135] altered cytokines including interferon α,[136] or increased apolipoprotein E.[137] In longitudinal studies, reduction in high-density lipoprotein (HDL), total cholesterol, and LDL cholesterol has been observed with seroconversion. With antiretroviral treatment, cholesterol and LDL rise to preinfection levels but low HDL levels persist.[138]

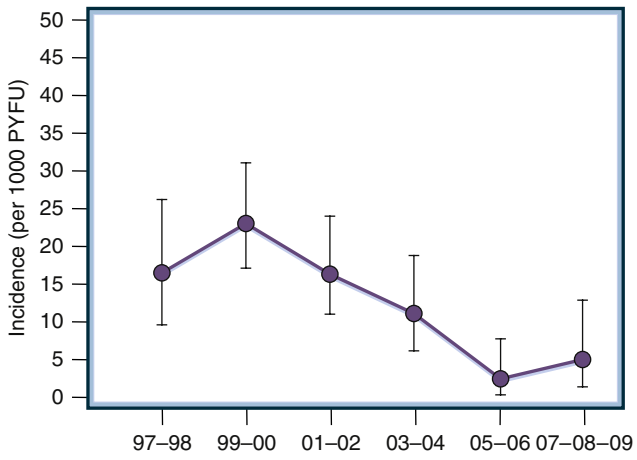
Among HIV-infected patients receiving combination ART including a PI, hypercholesterolemia (>240 mg/dL), hypertriglyceridemia (>200 mg/dL), and low HDL (<35 mg/dL) were reported in 27%, 40%, and 27%, respectively, compared with corresponding percentages of 8%, 15%, and 26% in previously untreated patients.[139] Among patients with changes in fat distribution, 57% demonstrated hypertriglyceridemia and 46% low HDL in comparison to an age- and BMI-matched cohort from the Framingham Offspring Study.[140] A phenotype of atherogenic small, dense LDL particles among HIV-infected patients with lipodystrophy has been described.[141] In addition, investigators have demonstrated increased triglyceride enrichment of LDL and impaired hepatic lipase, suggesting impaired lipoprotein processing, further contributing to atherogenic dyslipidemia.[142]

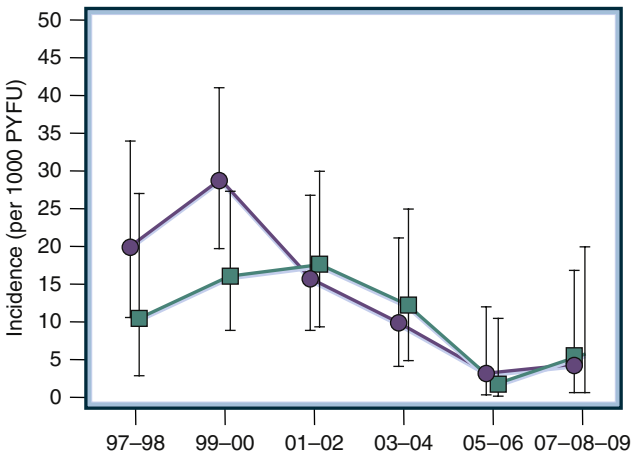
Dyslipidemia among HIV-infected patients may result from the effects of antiretroviral drugs, including specific PIs, which have been shown to increase triglyceride levels. Use of PIs may also be associated with an atherogenic dyslipidemia and an increase in small, dense LDL[143]; increased apolipoprotein C-III and apolipoprotein E; and decreased proteasomal degradation of apolipoprotein B.[144,145] Mulligan and colleagues[146] demonstrated that changes in lipid levels occur within 3 months of PI therapy. Hypertriglyceridemia is most severe among patients treated with ritonavir or the ritonavir/saquinavir combination. Among the currently approved PIs, atazanavir is least often associated with hyperlipidemia.[147] Lipid changes appear to be less and improve with use of atazanavir in contrast to other PIs.[148] Some NNRTIs, such as efavirenz, may raise total cholesterol and non-HDL cholesterol but also raise HDL cholesterol.[149] Newer NNRTIs, such as rilpivirine and etravirine, have less pronounced effects on the lipid profile than efavirenz.[150–152] Integrase strand transfer inhibitors, such as raltegravir, dolutegravir, elvitegravir, and bictegravir, have a more favorable lipid profile than PIs.[153–155] These agents are preferred as initial management of HIV infection in combination with NRTIs.[156]

Hyperglycemia and Insulin Resistance

Insulin resistance and DM are relatively common among HIV-infected patients. In an early longitudinal study, DM was 3.1 times more likely to develop in HIV-infected patients receiving combination ART than in control subjects.[157] More recent studies confirm increased risk of DM prior to 2000 but found equivalent rates among HIV-infected and non–HIV-infected patients in the era of modern ART, with agents less likely to contribute to glucose abnormalities.[158] These estimates suggest a peak incidence of 23.2 in 1000 patient-years immediately prior to 2000 that has decreased to less than 10 in 1000 patient-years, with no difference between subjects naive to and those using ART[159] (Fig. 44.7). However, a recent analysis comparing a large nationally representative population of HIV-infected persons had an adjusted DM prevalence of 11.8%, which was significantly higher than 8.0% DM prevalence in the National Health and Nutrition Examination Survey.[160] BMI, lipodystrophy, low CD4 counts, and exposure to specific, older ART medications including stavudine and indinavir are predictive of DM in the HIV population.[158] HIV-infected patients with impaired glucose tolerance demonstrate hyperinsulinemia, suggesting that insulin resistance contributes to the development of impaired glucose homeostasis in this population.

Hemoglobin A_{1c} has been shown to underestimate levels of blood glucose in HIV-infected patients, particularly those with increased mean corpuscular volume resulting from specific ART agents. In one study, use of an A_{1c} cutoff of 5.8% optimized the area under the curve for the diagnosis of DM and increased





ART regimen (%)						
PI	100	89	59	50	53	55
PI/r	9	21	27	30	41	47
NNRTI	10	43	49	47	47	45
Stavudine	69	66	46	25	13	7
Indinavir	47	35	19	12	5	3
Didanosine	31	36	35	31	24	15

A

100	100	88	91	61	57	55	43	62	43	63	45
11	7	23	19	31	22	35	25	49	31	55	37
13	6	47	39	50	48	45	50	43	51	41	49
77	59	73	57	53	37	30	19	16	11	8	6
49	43	37	34	20	18	14	9	6	5	3	3
32	30	41	31	40	29	35	26	26	21	17	13

B

• **Fig. 44.7** Calendar incidence of new-onset diabetes and antiretroviral exposure in the ANRS CO8 APROCO-COPILOTE cohort. Diabetes incidence per 1000 PYFU over the period from 1997 to 2009 is indicated for two groups: (A) all included patients, and (B) patients who had never previously been treated with ART prior to PI initiation (ART-naive, *teal line*) or patients who had previously been treated with ART (non-naive, *purple line*). The percentages of patients receiving various drugs during follow-up are presented below the graphs (in graph B, *gray lettering* corresponds to ART-naive patients). *Vertical lines* indicate 95% confidence intervals. *ART,* antiretroviral therapy; *NNRTI,* non-nucleoside analogue reverse transcriptase inhibitor; *PI,* protease inhibitor; *PI/r,* ritonavir-boosted PI; *PYFU,* person-years of follow-up. (From Capeau J, Bouteloup V, Katlama C, et al. Ten-year diabetes incidence in 1046 HIV-infected patients started on a combination antiretroviral treatment. *AIDS.* 2012;26:303–314, Fig. 1, used with permission.)

sensitivity from 40.9% to 88.8% while simultaneously decreasing specificity from 97.5% to 77.5% compared with use of a cutoff of 6.5%.[161]

Insulin resistance among HIV-infected patients may be caused by the abnormal fat distribution itself (e.g., increased central adiposity, loss of peripheral subcutaneous fat, and associated molecular changes). These changes include altered cytokines (e.g., low adiponectin, increased resistin, or elevated TNF) or changes in other processes such as mitochondrial dysfunction, increased lipolysis, increased proteolysis, increased expression of suppressor of cytokine signaling 1 (SOCS1),[162] and increased accumulation of fat in the muscle and liver. Increased inflammation after initiation of ART is associated with development of DM among HIV-infected patients,[163] and metabolic changes may result from persistent microbial translocation across the gastrointestinal barrier, resulting in increased levels of lipopolysaccharide in the blood despite viral suppression.[164] Metabolic abnormalities in HIV have been shown to be associated with increased levels of retinol-binding protein 4 (RB4), which may further contribute to insulin resistance.[165] Rarely, insulin receptor autoantibodies may develop among HIV-infected patients experiencing an immune reconstitution syndrome during ART initiation.[166] In addition, significant evidence suggests direct effects of specific antiretrovirals to reduce insulin sensitivity. PIs have been shown to decrease glucose uptake by inhibiting the transport function of glucose transporter type 4 (GLUT4) in vitro[167] and have been shown to reduce insulin sensitivity in vivo.[168] Effects to decrease beta-cell apoptosis and compromise insulin secretion have also been postulated.[169] NRTIs are associated with insulin resistance, which may be a direct effect, potentially related to mitochondrial toxicity[170] or through effects on subcutaneous fat.[121] For more details on the metabolic effects of specific ART agents, see Table 44.1. The specific manifestations and course of DM in HIV patients have not been well studied, but initial studies suggest an increased risk of albuminuria in HIV-infected patients, particularly those with increased viral load, independent of blood pressure.[171]

Treatment of Metabolic and Body Composition Changes in HIV-Infected Patients

There are several options available for the treatment of body composition and metabolic abnormalities in HIV-infected patients.

Treatment of AIDS Wasting and Loss of Lean Body Mass

Testosterone has been used successfully to increase lean body mass in men with AIDS wasting. Randomized studies of intramuscular testosterone for hypogonadal men with AIDS wasting suggest a beneficial effect of testosterone administration on lean body mass (2.0 kg over 6 months) and improved quality of life.[172] Although a limited number of studies have shown a benefit of anabolic steroids in HIV-infected patients with wasting, these agents potently suppress endogenous gonadal function and may

thus cause hypogonadism. Methyltestosterone and anabolic steroids may cause problems with the liver, including peliosis hepatis, worsening liver function, and potentially malignancy. In addition, high-dose oxandrolone suppresses endogenous testosterone levels, significantly increases transaminase levels, and increases LDLs.[173] Nandrolone (100 mg intramuscularly every other week) was shown to be effective in increasing weight and lean body mass in HIV-infected women with weight loss.[174] Anabolic steroids are associated with decreased HDL and other side effects, including effects on the liver, and hold no advantage over natural testosterone in the treatment of hypogonadism associated with AIDS-related weight loss. Short-term use of anabolic steroids may be considered in eugonadal patients with severe wasting, but they may be associated with adverse effects and should generally be avoided. DHEA may be used to improve mood and depression among HIV-infected patients with subsyndromal depression and dysthymia,[175] but the use of DHEA has not been standardized particularly with respect to dose, duration of treatment, and clinical end points, and thus it remains investigational.

Several studies have investigated androgen administration to HIV-infected women with low weight. These studies have investigated testosterone using a transdermal patch designed to deliver low physiologic doses of 150 to 300 μg/day. Among studies using the 150-μg/day dose, functional capacity and strength significantly improved with a trend toward increased lean body mass over 6 months. Hirsutism was not seen, and virilization did not occur. An 18-month randomized, placebo-controlled study conducted among relatively androgen-deficient HIV-infected women demonstrated that 300 μg/day increased lean body mass and bone density at the hip and improved depression indices without aggravation of lipid or glucose parameters.[176] Preliminary studies have investigated the effects of DHEA on androgen levels in HIV-infected premenopausal women, but the clinical utility of this strategy in HIV-infected women remains unknown.

Megestrol acetate is a synthetic progestational agent with glucocorticoid-like properties. Randomized studies in the literature show that megestrol acetate increases weight 3 to 4 kg over 12 weeks with an increase in caloric intake.[177,178] However, the change in weight is almost entirely fat mass without an increase in lean body mass. In addition, megestrol acetate, because of its glucocorticoid-like properties, is associated with several side effects, including hypogonadism and hyperglycemia, and abrupt withdrawal can precipitate adrenal crisis. In children, megestrol acetate promotes weight gain without improving linear growth.[179]

Several other agents have been used in the setting of AIDS wasting. Thalidomide blocks the action of TNFα and decreases esophageal ulcers in AIDS patients. Clinical studies demonstrate a modest beneficial short-term effect of thalidomide on weight indices but significant associated adverse effects, including rash and fever. Human chorionic gonadotropin results in increased testosterone levels and may have independent effects to inhibit KS. No data are available from randomized controlled studies to determine effects on wasting in humans.

In addition, patients with AIDS wasting may demonstrate a typical pattern of nutrition-related GH resistance (see The GH/IGF1 Axis section). These patients exhibit elevated GH levels but decreased IGF1, the primary hormone mediating the action of GH on muscle, suggesting GH resistance.[180,181] GH has been used in HIV-infected patients to increase lean body mass in sarcopenic patients with AIDS wasting. Among patients with AIDS wasting, high-dose, supraphysiologic GH (0.1 mg/kg per day) has been investigated. Relatively small but significant effects on weight (1.6 kg) were seen over 3 months in a placebo-controlled study.[182] GH administration at 0.1 mg/kg has also been shown to increase work output during treadmill exercise[182] and quality of life, as well as peripheral muscle oxygen extraction and utilization, in patients with AIDS wasting.[183] These studies suggest that large doses of GH may be necessary to significantly increase lean mass in patients with AIDS wasting and nutritionally mediated resistance to GH. However, high-dose GH is associated with side effects including hyperglycemia and fluid retention[184] and is not well tolerated in the long term. For these reasons, caution should be used with respect to long-term high doses of GH for treatment of AIDS wasting, because this treatment may cause acute and chronic side effects of GH excess. At high doses, GH was shown to increase thymic mass and circulating CD4 cells in one study[185] and to induce HIV1-specific T-cell responses at low doses (0.7 mg/day) in patients on effective ART,[186] but further studies are needed to investigate the effects of GH on the immune axis.

A multidisciplinary approach to AIDS wasting syndrome is most useful. Optimization of ART is paramount in conjunction with provision of adequate nutrition and protein intake. However, even in this context, weight and muscle loss may occur because of the highly catabolic nature of the disease. In such cases, endocrine evaluation should include assessment of gonadal function, which will often be reduced. GH levels are increased in AIDS wasting, but supraphysiologic administration, as approved by the FDA, can further increase lean body mass. This strategy is best reserved for severe wasting refractory to other treatments. Other therapeutic strategies that increase weight by stimulating appetite, including megestrol acetate, are not associated with gain in lean body mass and may be associated with side effects. In the current era of HAART, lipoatrophy, usually associated with antiretroviral use, should be distinguished from traditional wasting. Whereas true AIDS wasting involves sarcopenia and requires anabolic strategies to increase muscle mass, the presence of severe lipoatrophy suggests the need for strategies to spare fat loss.

Strategies for Treating Lipoatrophy and Subcutaneous Fat Loss

Antiretroviral switching to less toxic NRTIs or PIs may be useful for improving changes in fat distribution and hyperlipidemia.[187] For example, one study demonstrated that switching off lopinavir/ritonavir to atazanavir/ritonavir improved glucose trafficking into muscle, reduced triglyceride levels (by 182 mg/dL on average), and decreased visceral fat by 25%.[148] Other studies have demonstrated significant increases in SAT and reductions in visceral adipose tissue (VAT) after switching to a non-thymidine analogue–based ART strategy.[188] LSM and resistance training are unlikely to reverse the loss of subcutaneous fat often seen in HIV-infected patients receiving ART.

Treatments for Visceral Fat Accumulation

LSM, in combination with exercise, may improve lipid levels and visceral adiposity among HIV-infected patients.[189] Progressive resistance training (PRT) may also significantly improve glucose homeostasis in HIV-infected patients through a reduction in muscle adiposity.[190] Studies of LSM in HIV-infected patients modeled after the Diabetes Prevention Program demonstrate effects of LSM to improve cardiorespiratory fitness and raise levels of HDL and C-reactive protein (CRP)[191] but do not show significant effects on body composition.

Limited data are available on the use of testosterone among relatively androgen-deficient HIV-infected men with abdominal

fat accumulation (waist-to-hip ratio >0.95 or waist circumference >100 cm).[192] Ten grams of topical testosterone daily did not improve visceral fat mass but reduced overall trunk fat by 15% relative to placebo over 24 weeks. Significant effects on glucose and insulin were not seen. Effects of high-dose testosterone and anabolic steroids on lipid parameters may limit utility of this class of drugs to reduce visceral fat in HIV-infected patients. Moreover, studies among older, male, non–HIV-infected patients suggest that testosterone use may be associated with increased CVD events.[193,194] Use of testosterone among HIV-infected men should be limited to those with documented hypogonadism, among whom treatment may improve lean body mass and bone.

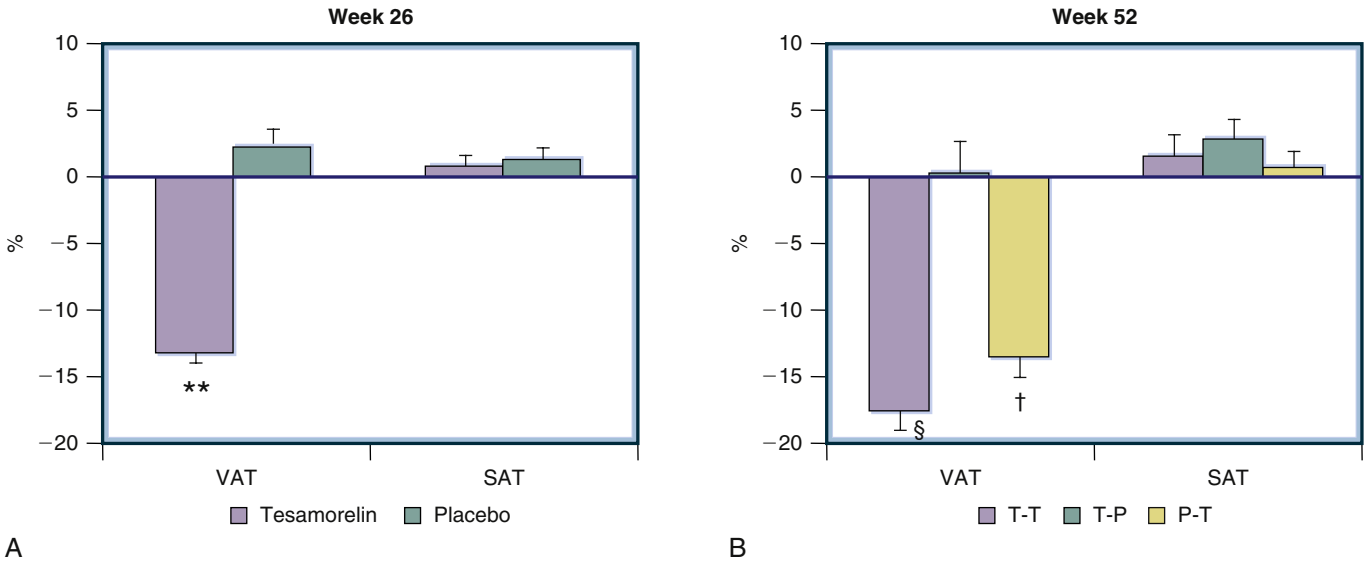
GH has also been used to reduce visceral fat in HIV-infected patients with central fat accumulation. Lo and coworkers[195] investigated low-dose, long-term GH over 18 months in HIV-infected patients with central fat accumulation and deficient responses to GHRH-arginine testing. Low-dose GH was shown to reduce VAT by 9% over 18 months, simultaneously lowering blood pressure and triglyceride level. Even with low-dose GH, however, 2-hour glucose increased, demonstrating that GH is potentially useful in decreasing central fat accumulation but is hard to titrate in a population with significant insulin resistance. Largely because of its ability to aggravate glucose, GH was not approved by the FDA for HIV lipodystrophy.

In contrast, GH secretagogues have been used successfully to increase lean body mass and reduce visceral and truncal fat, without significant effects on glucose. In the combined analysis of two large, randomized, placebo-controlled phase III trials of more than 800 patients, GHRH(1-44) (tesamorelin) reduced VAT by 15.4% relative to placebo in HIV-infected patients with central fat accumulation. Improvements were noted in triglyceride level (equal to 43 mg/dL), total cholesterol, and cholesterol-to-HDL ratio. Further improvements in body composition were seen over 12 months[196] (Fig. 44.8). Interestingly, no clinically significant effect to reduce SAT was seen, and thus GHRH(1-44) was selective for VAT. Use of GHRH(1-44) was associated with physiologic increases in IGF1 levels and was not associated with increased glucose or insulin levels, in contrast to low-dose GH. Larger reductions in visceral fat were associated with improvements in lipid and glucose levels, as well as adiponectin, suggesting metabolic improvements with VAT reduction.[197] Recently, GHRH(1-44) was also shown to improve liver fat in association with visceral fat, suggesting beneficial effects on other ectopic fat depots.[198] Discontinuation of both GH and GHRH resulted in reaccumulation of visceral fat back to baseline levels, demonstrating that the effects of these agents do not outlast the treatment period.[199–201] GHRH(1-44) was approved by the FDA for the treatment of central fat accumulation in HIV-infected patients in 2010.

Insulin-Sensitizing Strategies

Significant insulin resistance occurs among HIV-infected patients, in association with use of specific antiretroviral agents, changes in fat distribution, and other factors. Metformin is particularly appropriate for use in patients with significant truncal adiposity and increased FFA concentrations, in whom insulin resistance is, in part, attributable to increased hepatic glucose production. In addition, metformin has a modestly favorable effect on lipids. In patients with hyperlipidemia, metformin has been demonstrated to decrease triglyceride and LDL levels without adversely affecting other parameters. The modest 10% to 20% reduction in plasma triglyceride levels is thought to be caused by decreased hepatic VLDL production.

The effects of metformin have been investigated in HIV-infected patients with central fat accumulation and insulin resistance. Using a dose of 500 mg orally twice a day in a randomized, placebo-controlled 12-week study,[202] Hadigan and associates[202,203]



• **Fig. 44.8** Percentage change from baseline in VAT and SAT at 26 (A) and 52 (B) weeks. Data are mean ± SEM. **, $p < .001$ versus placebo; §, $p < .001$ versus baseline and versus T-P; †, $p < .001$ versus baseline; *P-T,* placebo for initial 26 weeks and tesamorelin for subsequent 26 weeks; *SAT,* subcutaneous adipose tissue; *T-T,* tesamorelin for initial 26 weeks and subsequent 26 weeks; *T-P,* tesamorelin for initial 26 weeks and placebo for subsequent 26 weeks; *VAT,* visceral adipose tissue. (From Falutz J, Mamputu JC, Potvin D, et al. Effects of tesamorelin (TH9507), a growth hormone-releasing factor analog, in human immunodeficiency virus-infected patients with excess abdominal fat: a pooled analysis of two multicenter, double-blind placebo-controlled phase 3 trials with safety extension data. *J Clin Endocrinol Metab.* 2010;95:4291–4304, Fig. 2, used with permission.)

demonstrated that administration of low-dose metformin over 12 weeks significantly reduced insulin resistance, diastolic blood pressure, tissue plasminogen activator, and plasminogen activator inhibitor concentrations and tended to reduce abdominal visceral fat, thus improving the CVD risk profile of such patients. The effects of metformin were sustained over 9 months.[204] Other studies of metformin in HIV-infected patients also demonstrate general improvements in markers of insulin sensitivity but less consistent effects on abdominal fat.[205] In a randomized trial, the effects of metformin and PRT were compared with metformin alone, and the addition of PRT to metformin further improved central adiposity, blood pressure, and insulin resistance.[206] Compared with LSM, metformin was shown to significantly reduce coronary artery calcium in HIV-infected patients.[191]

The loss of subcutaneous fat in HIV-infected patients may further contribute to insulin resistance by limiting peripheral glucose and triglyceride uptake. Therefore, attention has focused on insulin-sensitizing strategies that may act to increase subcutaneous adipogenesis. The thiazolidinediones (TZDs) have been shown to promote adipogenesis, primarily through activation of PPARγ. Although the TZDs have effects on both hepatic and peripheral insulin resistance, the dominant effect is to improve peripheral glucose uptake. The therapeutic efficacy of the TZDs has been questioned among non–HIV-infected patients, in whom rosiglitazone, but not pioglitazone, has been associated with an increased mortality rate.[207] Weight has reported to increase in response to TZDs, in contrast to metformin, which is associated with weight loss. Nonetheless, based on the known effects of TZDs to stimulate insulin sensitivity through PPARγ and promote adipogenesis, this strategy has been investigated in HIV-infected patients with subcutaneous fat loss and insulin resistance.

In a 3-month randomized, placebo-controlled study involving 28 patients selected based on insulin resistance, rosiglitazone was shown to improve insulin sensitivity, adiponectin, FFA, and subcutaneous fat mass.[208] In contrast, an effect of rosiglitazone on subcutaneous fat was not shown over 48 weeks in patients with lipoatrophy, selected for fat loss but not for insulin resistance.[209] Adiponectin levels significantly increased and resisting levels decreased in response to rosiglitazone among HIV-infected patients. Rosiglitazone may be effective in increasing subcutaneous fat in selected subpopulations of HIV-infected patients, particularly those with insulin resistance,[210] but significant adverse effects on lipid levels, particularly LDL, suggest that pioglitazone may be better in this regard. Slama and colleagues[211] investigated pioglitazone among HIV-infected patients with confirmed lipoatrophy in a large, randomized, placebo-controlled trial. Pioglitazone increased peripheral fat and improved HDL levels, suggesting the potential utility among HIV-infected patients with insulin resistance and fat atrophy. Overall, the TZDs contrast with metformin in terms of effects on lipid and body composition but demonstrate similar effects to improve glucose in HIV-infected patients.[212]

Mitochondrial function is significantly affected by NRTI therapy in HIV-infected patients. Mallon and associates[213] have shown that simultaneous use of NRTIs may reduce effects of TZD agents to promote subcutaneous adipogenesis in HIV-infected patients with lipoatrophy by limiting effects of rosiglitazone to increase adipose expression of PPARγ. In addition, therapies for mitochondrial dysfunction, including increasing glutathione with cysteine and glycine supplementation, have been shown to improve mitochondrial fat and carbohydrate oxidation and insulin sensitivity in small studies among HIV-infected patients.[214] L-Acetylcarnitine was shown to decrease intramyocellular lipid accumulation and to increase percentage of leg fat while decreasing FFA in an open label study[215] and then was shown to increase T-cell mitochondrial DNA but to have no effect on body composition and metabolic indices in a second randomized study.[216] Studies to date using strategies to improve mitochondrial function are small, and further research is needed in this area.

Leptin Treatment for Metabolic Dysregulation Among HIV-Infected Patients With Lipoatrophy

Leptin levels are low in association with fat loss and lipoatrophy in HIV-infected patients. Initial small studies of leptin administration to HIV-infected patients with lipoatrophy and reduced leptin levels demonstrated significant improvements in insulin sensitivity, HDL, and triglyceride, as well as reduced truncal and visceral fat.[217,218] Among patients with mixed lipoatrophy and lipohypertrophy but low leptin levels, leptin administration improved total cholesterol and non-HDL cholesterol, as well as glucose parameters, but not HDL or triglyceride or lipid kinetics.[219] Because leptin reduces appetite, administration may be associated with weight loss. Metreleptin is FDA approved for treatment of complications related to congenital and acquired lipodystrophy, not related to HIV infection or its treatment. Further studies are needed to understand the clinical potential of leptin to improve metabolic parameters and fat redistribution among HIV-infected patients.

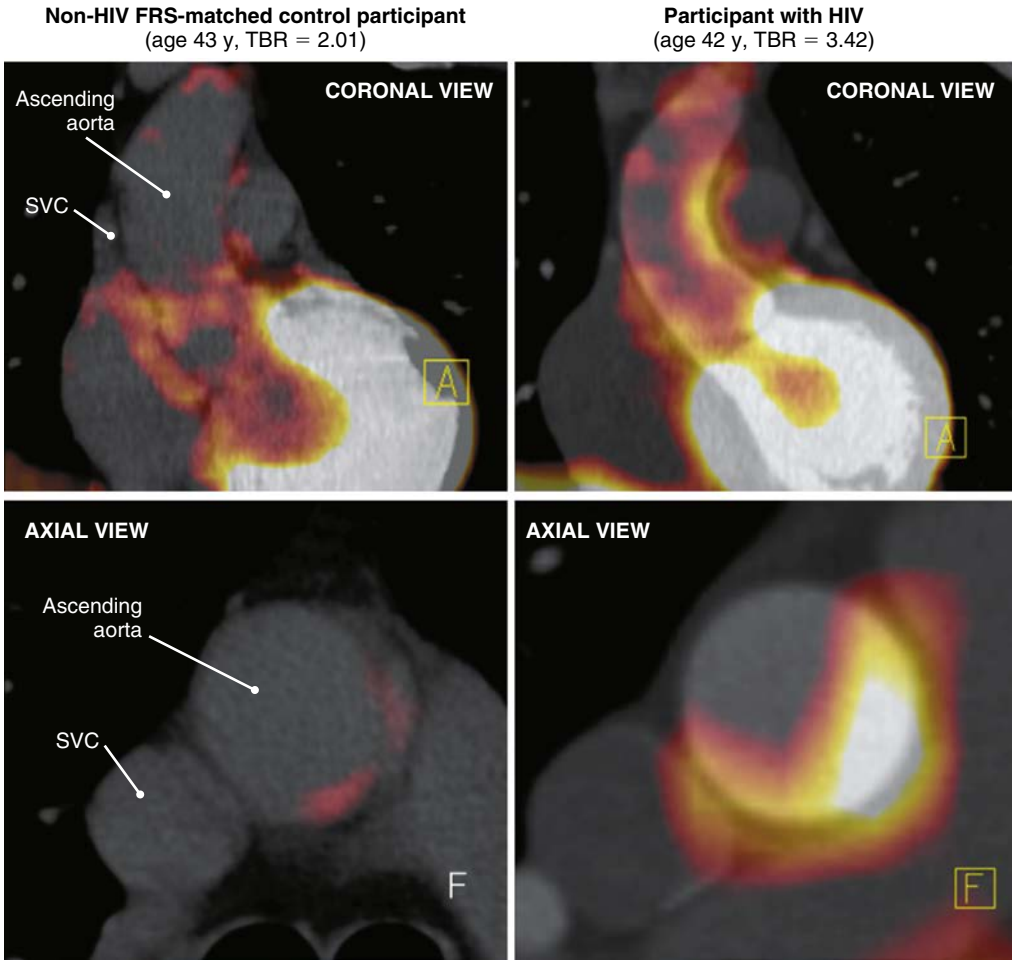
Lipid Management

See the Strategies for Hyperlipidemia Among HIV-Infected Patients section.

CVD in HIV-Infected Patients

CVD is increased among HIV-infected patients. Traditional risk factors for CVD in HIV-infected patients include increased smoking rates, insulin resistance, atherogenic dyslipidemia, truncal adiposity, hypertension (HTN), impaired fibrinolysis, and increased plasminogen activator inhibitor I and tissue plasminogen activator levels, as well as reduced adiponectin and increased CRP levels. DM is a risk equivalent in HIV-infected patients, conferring an increase in the RR of coronary heart disease equivalent to 2.41.[220] More than 40% of HIV-infected subjects meet the definition of the metabolic syndrome, and predicted myocardial infarction (MI) rates are increased in this subgroup.[221]

Abnormalities in surrogate markers, including carotid intima-media thickness and endothelial function, suggest increased CVD in HIV-infected patients. Abnormal endothelial function correlates with dyslipidemia, including increased chylomicrons, VLDLs, and intermediate-density lipoproteins and reduced HDL. Evidence for subclinical atherosclerosis has been demonstrated using coronary computed tomography in HIV-infected patients. Lo and coworkers[222] demonstrated an increased prevalence of coronary plaque among asymptomatic HIV-infected patients compared with well-matched non–HIV-infected patients (59% vs. 34%). Follow-up studies confirmed that the plaque in HIV-infected patients is more often noncalcified,[223] with vulnerable plaque features, including low-attenuation fatty lesions and eccentric positive remodeling.[224] Using fluorodeoxyglucose positron emission tomography to assess the degree of inflammation at the vascular surface, Subramanian and associates[225] demonstrated increased arterial inflammation (Fig. 44.9), even among those with minimal traditional cardiovascular risk indices. Increased immune activation indices correlated with the arterial inflammation, independent of traditional risk factors,

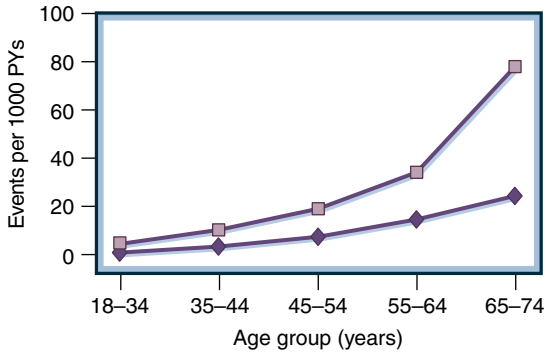


• **Fig. 44.9** Representative ^{18}F-FDG-PET/CT imaging of the aorta. There is increased aortic PET-FDG uptake (*red coloration*) in a participant infected with HIV compared with a non-HIV FRS-matched control participant. Neither participant had known heart disease. For each participant, the FRS was low with a score of 2, and calcium was not present on the cardiac CT scan. Neither participant was receiving a statin. *A* indicates anterior-posterior orientation, and *F* indicates foot-head orientation. *CT,* computed tomography; *^{18}F-FDG-PET,* [^{18}F]fluoro-2-deoxy-D-glucose positron emission tomography; *FRS,* Framingham risk score; *HIV,* human immunodeficiency virus; *SVC,* superior vena cava; *TBR,* target-to-background ratio. (From Subramanian S, Tawakol A, Burdo TH, et al. Arterial inflammation in patients with HIV. *JAMA.* 2012;308:379–386, Fig. 2, used with permission.)

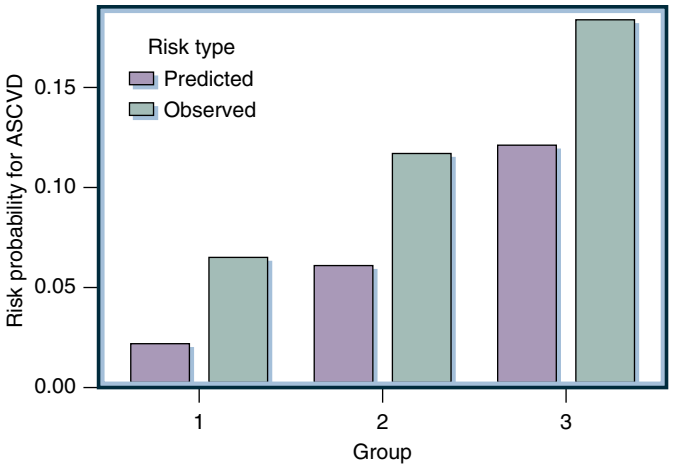
suggesting an important role for nontraditional risk factors, including immune activation, in the accelerated atherogenesis observed in HIV-infected patients. In particular, markers of monocyte activation, including soluble CD163 and CD14,[226,227] and bacterial translocation[228] have been associated with atherosclerotic indices, including intima-media thickness and noncalcified coronary plaque among HIV-infected patients. In contrast, traditional risk factors cluster with coronary calcium among such patients[226] and may underestimate atherosclerotic burden.[229] Genetic polymorphisms and mRNA expression of chemokine receptor type 5 (CCR5) and oxidative stress markers may also be associated with increased atherosclerosis among HIV-infected patients.[230,231] Immune activation may contribute to the increased prevalence of inflamed vulnerable plaque with high-risk morphologic findings, which in turn may contribute to the increased risk of sudden cardiac death among HIV-infected patients.[232]

Several studies suggest increased MI rates in HIV patients. Initial studies suggested dyslipidemia from ART was a major factor contributing to increased CVD risk in HIV-infected patients. In a large prospective study of 23,468 patients, the covariate-adjusted risk was 1.26 per each additional year of antiretroviral exposure. Other risk factors included male sex, DM, older age, previous MI, HTN, and dyslipidemia.[233] Controlling for dyslipidemia significantly reduced the effects of HAART exposure, suggesting that dyslipidemia may contribute to excess CVD in HIV-infected patients.

In contrast, more contemporary studies suggest that other factors beyond traditional risks may contribute to increased CVD rates in HIV. In a large study of patients in a major US health care center, Triant and colleagues[234] demonstrated an RR of 1.75 (95% confidence interval, 1.51–2.02; $p < .0001$) for increased MI in HIV versus non–HIV-infected patients in a model accounting for age, gender, and race (Fig. 44.10). Increased rates of traditional risk factors, including DM (11.5% vs. 6.6%, HIV infected vs. non–HIV infected), HTN (21.2% vs. 15.9%), and dyslipidemia (23.3% vs. 17.6%) were seen, and each contributed to the increase in MI rates (RRs of 1.62, 1.98, and 3.03, respectively, for each risk factor). However, regression modeling, accounting for HTN, DM, and dyslipidemia, demonstrated that these three risk factors contributed to only 25% of the excess risk in HIV- versus non–HIV-infected patients, suggesting that other factors might contribute. Freiberg and associates[235] demonstrated an increased hazard ratio of 1.48 for MI among a large cohort of HIV-infected



• **Fig. 44.10** Myocardial infarction rates by age group. *Top line* (*squares*) indicates patients diagnosed with human immunodeficiency virus disease. *Bottom line* (*diamonds*) indicates patients not diagnosed with human immunodeficiency virus disease. Data shown include both genders. Rates represent number of events per 1000 person-years (PYs) as determined by International Classification of Diseases coding. (From Triant VA, Lee H, Hadigan C, Grinspoon SK. Increased acute myocardial infarction rates and cardiovascular risk factors among patients with human immunodeficiency virus disease. *J Clin Endocrinol Metab.* 2007;92:2506–2512, Fig. 1B, used with permission.)



• **Fig. 44.11** Observed and predicted 5-year risk of atherosclerotic cardiovascular disease (ASCVD) risk by predicted risk group in persons living with human immunodeficiency virus (group 1: <5%; group 2 5–7.5%; group 3: >7.5%). (Redrawn from Triant VA, Perez J, Regan S, et al. Cardiovascular risk prediction functions underestimate risk in HIV infection. *Circulation.* 2018;137[21]:2203–2214.)

men in the Veterans Administration system, controlling for Framingham risk factors. Smoking rates are increased and contribute to increased CVD risk in HIV patients.

Inflammation may play an important role in increased CVD risk among HIV-infected patients. Increased CRP levels are observed among HIV-infected patients and predict increased MI rates.[236] The SMART (Strategies for Management of Antiretroviral Therapy) Study Group demonstrated an increased rate of cardiovascular events among patients randomized to interrupted therapy with less strict goals for control of immune function.[237] Indeed, a 2014 study suggests that low CD4 and nadir CD4 are important contributing factors to CVD among HIV-infected patients, suggesting an important role for immune dysfunction.[238]

HIV-infected women are also at risk for increased CVD. In large cohort studies, the RR of MI and stroke is more increased in HIV-infected women compared with non–HIV-infected female control subjects (RR 2.98 for acute MI) than among HIV-infected men compared with non–HIV-infected male control subjects (RR 1.40 for acute MI).[234] Increased waist-to-hip ratio, visceral adiposity, CRP, IL6, triglyceride, and LDL, as well as reduced HDL and adiponectin, have been demonstrated in HIV-infected women compared with age- and BMI-matched control subjects. Central adiposity was significantly predictive of abnormal CVD risk indices, including newer inflammatory indices.[239] In addition, studies suggest a marked increase in immune activation indices among HIV-infected female patients, including markers of monocyte activation, in association with noncalcified plaque.[240]

Strategies for Hyperlipidemia Among HIV-Infected Patients

Treatment for hyperlipidemia among HIV-infected patients is indicated to reduce cardiovascular risk and should proceed according to current guidelines. It should be noted that CVD risk prediction equations established in the general population, such as Framingham coronary heart disease or American College of Cardiology/American Heart Association scores, perform poorly in HIV-infected persons and tend to underestimate true risk.[241] (Fig. 44.11). An HIV-specific risk prediction equation established in the Data Collection on Adverse Effects of Anti-HIV Drugs Study, which includes HIV-specific variables, may improve risk prediction compared with the Framingham CHD score[242] although this has not been seen in all populations.[243]

Severe hypertriglyceridemia should be treated to reduce the risk of pancreatitis. An initial option in patients with severe elevations in triglyceride levels is to switch treatment to a PI less likely to cause dyslipidemia. Triglyceride levels are reduced approximately 20% by diet[244] and 30% by exercise[245] in HIV-infected patients. Neither diet nor exercise is likely to normalize triglyceride levels in the HIV population with severe dyslipidemia. Fenofibrate resulted in a 40% reduction in triglyceride levels and a 14% reduction in total cholesterol levels over 3 months in HIV-infected patients with hypertriglyceridemia.[246] Lesser, but nonetheless beneficial, effects may be seen with gemfibrozil or other fibrate derivatives. Niacin also significantly reduces the triglyceride level but may worsen glucose tolerance in HIV-infected patients, although such changes in glucose may only be transient.[247] In addition, niacin may be difficult to use because of its associated flushing and potential liver abnormalities. In a large randomized 24-week controlled trial of diet and exercise with either fenofibrate, niacin, or both, maximal reduction in triglyceride (–52%) was seen with combination drug therapy plus diet and exercise, whereas HDL increased most with niacin and diet and exercise. Flushing was reported by 35% to 40% of niacin-treated subjects.[248] Recent studies also suggest the efficacy of omega-3 polyunsaturated fatty acids, which were shown to reduce triglyceride levels by 25.5% and to be well tolerated in a randomized, placebo-controlled study.[249] In a study investigating combined therapy, triglyceride levels were reduced by 65.5% among hypertriglyceridemic HIV-infected patients receiving simultaneous fenofibrate and fish oil.[250] Comparing fish oil with gemfibrozil, fibrates, and atorvastatin in hypertriglyceridemic HIV-infected patients, fish oil was shown to decrease triglyceride by 45 mg/dL, whereas fibrates (–66 mg/dL), but not atorvastatin (–39 mg/dL), were more effective in this regard.[251]

The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are most useful for lowering cholesterol levels in HIV-infected patients but are less effective in lowering triglyceride levels. For example, pravastatin combined with dietary advice

reduced total cholesterol levels by 17% over 24 weeks without effects on triglyceride levels.[252] The combination of gemfibrozil and atorvastatin resulted in a 30% reduction in cholesterol and 60% reduction in triglyceride in HIV-infected patients[244] and may be useful in HIV-infected patients with combined hyperlipidemia. When using the combination of HMG-CoA reductase inhibitors and fibric acid derivatives, the risk of rhabdomyolysis increases. In addition, PIs can themselves affect metabolism of the HMG-CoA reductase inhibitors. In this regard, ritonavir was shown to increase simvastatin levels by 3059% and atorvastatin levels by 79%.[253] Use of simvastatin should be avoided in patients receiving PI therapy. In an analysis of almost 900 HIV-infected patients in the Kaiser Permanente system, use of statins was shown to lower LDL by 25.6% versus 28.3% in HIV-versus non–HIV-infected patients, whereas use of gemfibrozil lowered triglyceride by 44.2% versus 59.3% in HIV- and non–HIV-infected patients. Effects of statins on LDL did not vary by antiretroviral treatment class, whereas effects of gemfibrozil were less in HIV-infected patients receiving PIs and best among those patients receiving therapy with NNRTIs. Safety of the statins, including atorvastatin, pravastatin, and lovastatin, were good, with few cases of myositis. The relative potency of each statin on LDL was generally similar, with reductions ranging from nearly 26.4% with atorvastatin to nearly 23.6% with pravastatin.[254] Rosuvastatin must be used at lower doses due to potential interaction with darunavir but has been shown to lower LDL by 28% at 10 mg/day in placebo-controlled trials.[255] New drugs, such as pitavastatin, may have the fewest interactions with ART, because they are glucuronidated and do not affect CYP3A metabolism. Ezetimibe is well tolerated in combination with statins in patients with HIV and further lowers LDL (additional 14% reduction).[256] Effects of statins on clinical events have not yet been reported in large randomized controlled trials, but cohort studies suggest a potential benefit on overall mortality rate among HIV-infected patients treated with ART with virologic suppression,[257] and preliminary studies suggest effects to improve atherosclerotic indices, such as carotid intima-media thickness and coronary plaque.[258,259] A large, placebo-controlled randomized trial examining the effect of pitavastatin on clinical events, called *REPRIEVE*, is under way in HIV-infected persons who do not have an indication for statin therapy.[260] Inhibition of proprotein convertase subtilisin kexin 9 (PCSK9) offers an additional treatment strategy for HIV-infected persons at high CVD risk.[261] PCSK9 levels were found to be higher in HIV-infected persons compared with matched HIV-uninfected controls and were related to markers of monocyte activation.[262]

Anti-Inflammatory Strategies for CVD in HIV

Initial studies are now investigating effects of anti-inflammatory strategies to improve endothelial function and reduce arterial inflammation. Such strategies include use of pentoxifylline, canakinumab, and methotrexate, but safety and efficacy of these agents in the HIV population remain unclear. Use of statins may also be beneficial as an anti-inflammatory strategy, as they may lower CRP and have pleiotropic effects on monocyte activation.

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45

Neuroendocrine Tumors and Related Disorders

KJELL ÖBERG

CHAPTER OUTLINE

KEY POINTS

- The World Health Organization (WHO) classification system (2017) in neuroendocrine tumors (NET-G1, NET-G2, NET-G3) and neuroendocrine carcinoma (NEC-G3) is informative for the clinical management of neuroendocrine intestinal tumors (carcinoids).
- The most important circulating biomarkers are chromogranin A (general), urinary 5-hydroxyindoleacetic acid (5HIAA), and neuron-specific enolase (NSE) for lung tumors.
- The carcinoid syndrome includes flushing, diarrhea, right-sided heart fibrosis, and bronchial wheezing.
- Molecular imaging ^{111}In-DTPA-Phe octreotide (Octreoscan) and more recently ^{68}Ga-DOTA-octreotate positron emission tomography (PET) scanning are important procedures for staging of the disease as well determining possible therapy with peptide receptor radiotherapy (PRRT).
- First-line therapy for low proliferating small intestinal neuroendocrine tumors (e.g., NET-G1) are somatostatin analogues.
- Chemotherapy is reserved for NET-G2 mostly in the lung (atypical carcinoids).
- Peptide receptor radiotherapy could be considered for both NET-G1 and NET-G2.

The first clinical and histopathologic description of carcinoid tumor was made by Otto Lubarsch in 1888.[1] He was impressed by the multicentric origin of carcinoid tumors of the gastrointestinal (GI) tract, their lack of gland formation, and their lack of similarity with the usual adenocarcinoma of the alimentary system.

The term *Karzinoide* was introduced in 1907 by pathologist Siegfried Oberndorfer[2] as a descriptive name for what he considered to be a benign type of neoplasm of the ileum, which could nevertheless behave like a carcinoma. This myth of benignity has survived to the present, even though in 1949 Pearson and Fitzgerald[3] described a large series of metastasizing carcinoid tumors. The term *carcinoid* is nowadays less used and the preferred recommended term is *neuroendocrine tumor*. The typical midgut carcinoid is called *small intestinal neuroendocrine tumor* (SI-NET). Lung-neuroendocrine tumors are still usually called carcinoids. In this chapter, the term *carcinoid* will be used also for the gastrointestinal NETs.

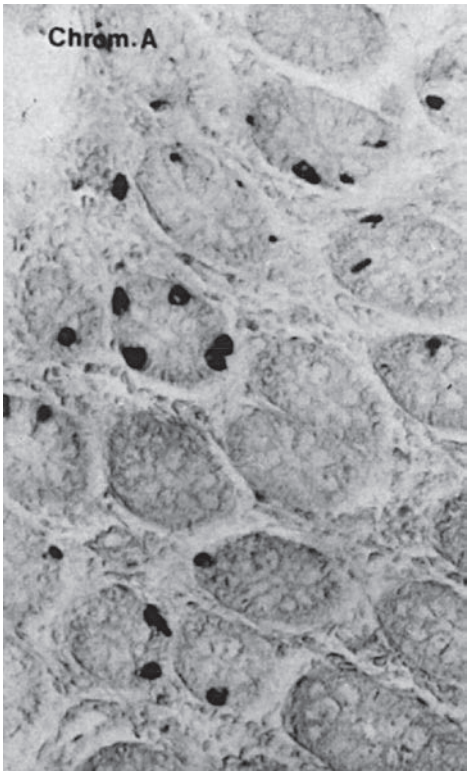
Neuroendocrine tumors have subsequently been reported in a wide range of organs, but they most commonly involve the lungs and GI tract. Carcinoid tumors of the thymus, ovaries, testes, heart, and middle ear have also been described. The clinically well-known *carcinoid syndrome* was described by Thorson and associates[4] in 1954; 1 year earlier, Lembeck[5] had extracted serotonin from a carcinoid tumor.

Phylogenesis and Embryology

Carcinoid tumors are derived from neuroendocrine cells, and Gosset and Masson[6] in 1914 were the first to point out the neuroendocrine properties of carcinoid tumors. Masson[7] later described the remarkable affinity for silver salts displayed by intracytoplasmic granules in tumor cells and noted that carcinoid tumors originate from enterochromaffin cells, the Kulchitsky cells in the crypts of Lieberkühn in the intestinal epithelium. Furthermore, he suggested that the tumors were of endocrine origin (Fig. 45.1).

The mammalian GI tract and pancreas contain 14 endocrine cell types, which initially were believed to originate from the neuroectoderm. This observation gave rise to the APUD (*a*mine *p*recursor *u*ptake and *d*ecarboxylation) concept because of the ability of these cells to take up and decarboxylate amino acid precursors of biogenic amines such as serotonin and catecholamines.[8] The APUD concept was later revised by others, who postulated that these endocrine cells might also be derived from mesoderm and endoderm.[9] The neuronal phenotype is clearly seen when culturing carcinoid tumor cells in vitro. The enterochromaffin cells, from which many carcinoid tumors derive, have the property of producing and secreting amines (such as serotonin) and polypeptides (such as neurokinin A and substance P) (Fig. 45.2).

Carcinoid tumors might also originate from other neuroendocrine cells, such as the enterochromaffin-like (ECL) cells of the gut and endocrine cells in the bronchi. The tumors derived from these cells can produce a wide range of hormones, such as gastrin, gastrin-releasing peptide (GRP), ghrelin, calcitonin, pancreatic polypeptide, adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone (CRH), and growth hormone–releasing hormone (GHRH), as well as somatostatin, glucagon, and calcitonin gene–related peptide (CGRP).[10] A common secretory product from all types of carcinoid tumors is the glycoprotein chromogranin A (CgA)—the most important general tumor marker in these patients (see later discussion).



• **Fig. 45.1** Normal human intestine stained with chromogranin A (Chrom. A) to delineate neuroendocrine cells. The cells are scattered in the intestinal mucosa.

Molecular Genetics

Despite advances in the diagnosis, localization, and treatment of carcinoid tumors, no etiologic factor associated with the development of these tumors has been identified. Little is known about molecular genetic changes underlying tumorigenesis. Sporadic foregut carcinoids as well as the familial-type multiple endocrine neoplasia type 1 (MEN1) often display allelic losses at chromosome 11q13, and somatic *MEN1* gene mutations have been reported in one-third of sporadic foregut tumors.[11] In contrast with foregut carcinoids, molecular and cytogenetic data for midgut carcinoids are quite limited, and these tumors are not included in MEN1 syndrome. Deletions of chromosomes 18q and 18p have been reported in 38% and 33%, respectively, of GI carcinoids.[12]

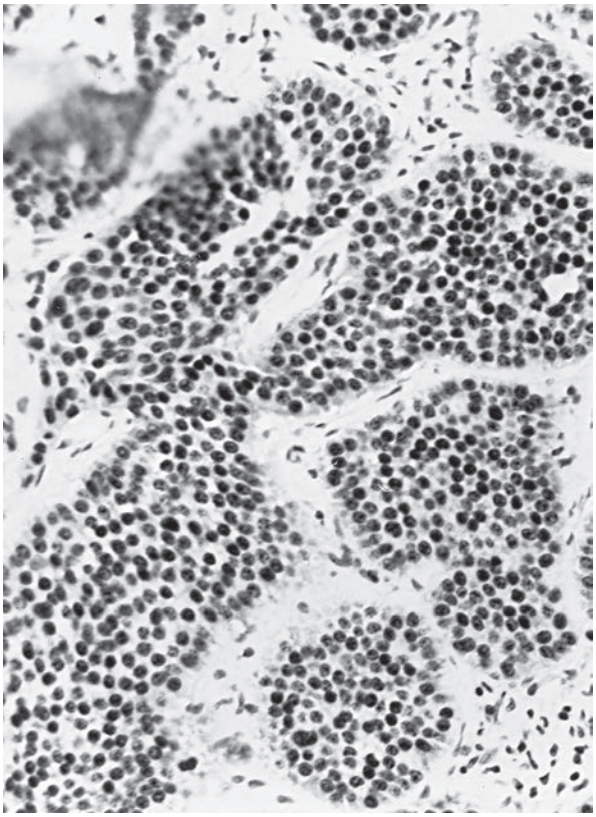
In one publication, deletions on chromosome 18 were found in 88% of midgut carcinoid tumors, but the *SMAD4/DPC4* locus was not deleted.[13] In addition to the consistent finding of deletions on chromosome 18, multiple deletions on other chromosomes (4, 5, 7, 9, 14, 20) were noticed in single tumors. The region telomeric to *SMAD4/DPC4/DCC* loci must be further explored for possible losses of a tumor suppressor gene in this area. Gene expression arrays in carcinoid tumors have demonstrated upregulation of the *RET* proto-oncogene, but no mutations have been detected so far. Reports indicate that the Notch signaling pathway is a significant regulator of neuroendocrine differentiation and serotonin production in GI carcinoid tumors.[14,15] The Wnt signaling pathway as well as transforming growth factor-β (TGFβ) signaling are upregulated in carcinoid tumors.[16] CDKN1B, a tumor suppressor in small intestinal nets, encodes a cyclin-dependent kinase inhibitor p27Kip1.[17] Mutations in the *MUTYH* gene involved in DNA repair have recently been reported.[18]

Classification

In 1963, Williams and Sandler reported a relationship between the embryonic origin of carcinoid tumors and the histologic, biochemical, and (to some extent) clinical features of the tumors.[19] Three distinct groups were formed (Table 45.1): foregut carcinoids (i.e., intrathoracic, gastric, and duodenal carcinoids), midgut carcinoids (carcinoids of the small intestine, appendix, and proximal colon), and hindgut carcinoids (carcinoid tumors of the distal colon and rectum).

Although this original classification has been useful in the clinical assessment of patients with carcinoid tumors, it has demonstrated significant shortcomings. As a result, a new classification system (World Health Organization [WHO] classification of 2010) has emerged that takes into account not only the site of origin but also variations in the histopathologic characteristics of carcinoid tumors.[20] In this revised system, typical tumors are classified as NET-G1 tumors, which show a characteristic growth pattern. Most recently, the WHO classification system has been updated (2017). This includes a new group NET-G3, which are well-differentiated tumors with higher proliferation (Ki67 >20%) but not as aggressive as NEC-G3.[21] Typical carcinoid tumors are usually slow growing, with low proliferation capacity (proliferation index <2%). They are usually confined to the mucosa and submucosa and are less than 1 to 2 cm in diameter (classical

midgut carcinoid). NET-G2 tumors show widely invasive growth and a high proliferation index (PI 2–20%). Poorly differentiated carcinomas (NEC-G3) are large tumors with metastases and a proliferation index greater than 20% (Fig. 45.3). The European Neuroendocrine Tumor Society (ENETS) has proposed a new tumor-node-metastasis (TNM) classification and grading system, which is widely used in the clinic.[22]



• **Fig. 45.2** Histopathology of a classic well-differentiated midgut carcinoid tumor.

The neuroendocrine lung tumors are divided into typical carcinoids, atypical carcinoids, large cell neuroendocrine carcinomas, and small cell lung carcinomas. The difference between the typical and atypical carcinoid is based on histopathologic features, with higher proliferation and necrosis found in the atypical carcinoid[23] (Fig. 45.4). The incidence of carcinoid tumors is similar in Western countries and is estimated to be 2.8 to 4.5 per 100,000 people (Surveillance, Epidemiology, and End Results [SEER] database).[24] Because many carcinoid tumors are indolent, the true incidence may be higher. In particular, appendiceal carcinoids have not been included in many studies, but a higher incidence of 8.2 per 100,000 was found in an autopsy study when appendiceal carcinoids were included.[25] The incidence of patients with a carcinoid syndrome is about 0.5 per 100,000.[26] Data from the United States, based on results from the End Results Group and the Third National Cancer Survey, 1950 to 1969 and 1969 to 1971, respectively, found that the stomach was the most common site of carcinoid tumors, followed by the rectum, ileum, lungs, and bronchi.[27]

An analysis done in the SEER program of the National Cancer Institute between 1973 and 2000 reported an increase in the percentage of pulmonary and gastric carcinoids and a decrease in the percentage of appendiceal carcinoids.[24] Age-specific incidence rates showed a peak between 65 and 75 years (7.5–9.5/100,000), with a male predominance. In persons younger than 50 years, a female predominance has been observed both for appendiceal and lung carcinoids.[28] Analyses of the SEER database (SEER 18) show an incidence of 6.98/100,000 in 2012 and a prevalence of 48/100,000 indicating a significant increase since 1993[24,27,29] (Fig. 45.5).

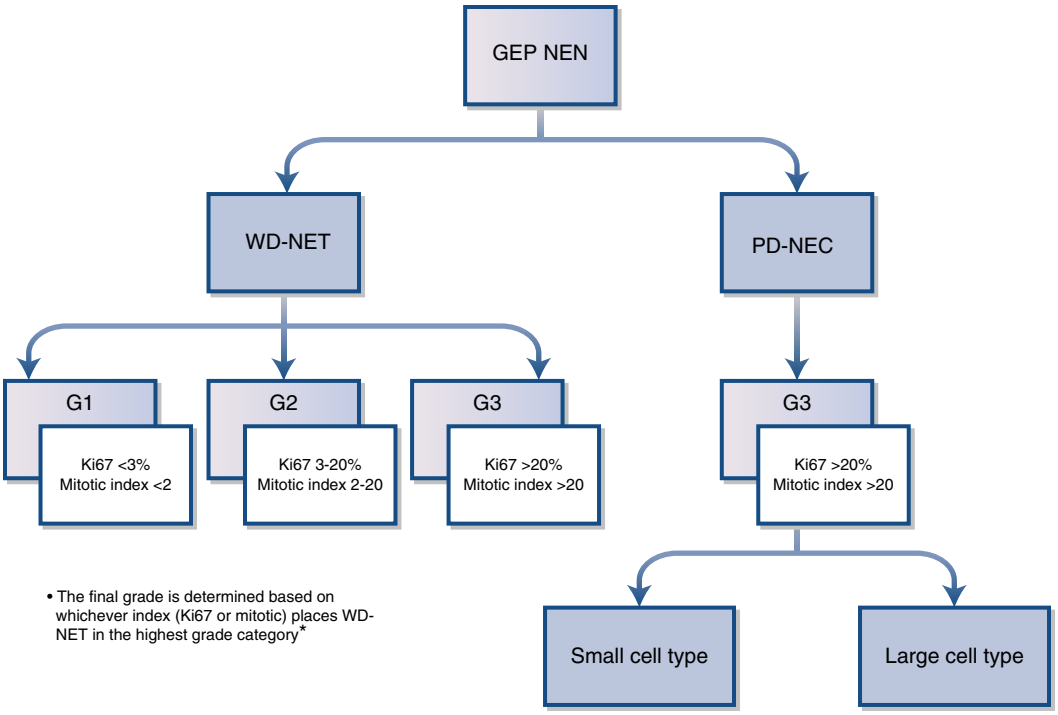
Biochemistry

The production of hormones is a highly organized function of carcinoid cells. In 1953, Lembeck isolated serotonin from a carcinoid tumor; since then, the carcinoid syndrome has been related to serotonin overproduction.[5] The biosynthesis of serotonin and its metabolic degradation are outlined in Fig. 45.6.

TABLE 45.1 Classification of Carcinoid Tumors

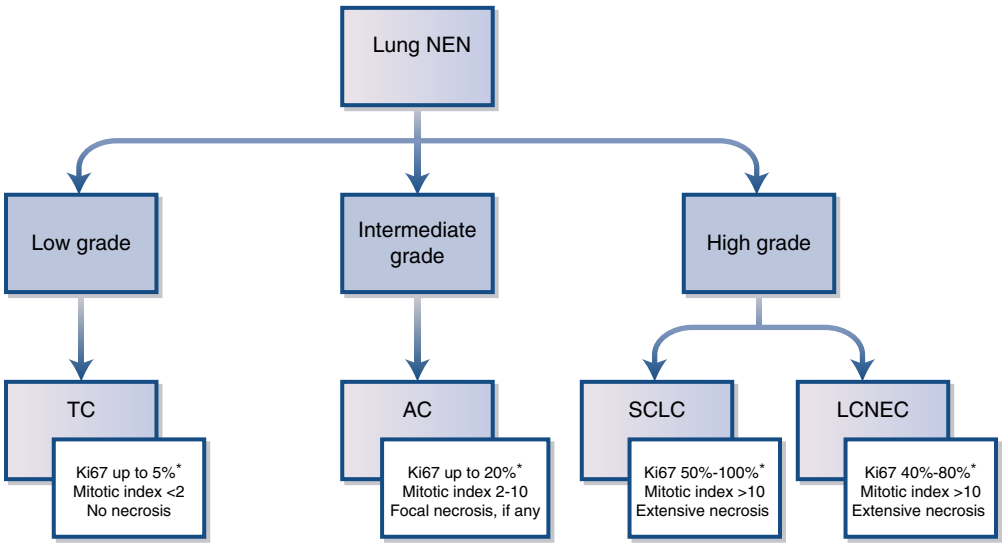
Foregut	Midgut	Hindgut
Histopathology		
Argyrophilic	Argentaffin positive	Argyrophilic
CgA positive	CgA positive	SVP2 positive
NSE positive	NSE positive	CgA positive, NSE positive
Molecular Genetics		
Chromosome 11q13 deletion	Chromosome 18q, 18p deletion	Unknown
Secretory Products		
CgA, 5HT, 5HTP, histamine, ACTH, GHRH, CGRP, somatostatin, AVP, glucagon, gastrin, NKA, substance P, neurotensin, GRP	CgA, 5HT, NKA, substance P, prostaglandin E_1 and F_2, bradykinin	PP, YY, somatostatin
Carcinoid Syndrome		
Present (30%)	Present (70%)	Absent

ACTH, Adrenocorticotropic hormone; *AVP,* arginine vasopressin; *CgA,* chromogranin A; *CGRP,* calcitonin gene–releasing peptide; *GHRH,* growth hormone–releasing hormone; *GRP,* gastrin-releasing peptide; *5HT,* 5-hydroxytryptamine; *5HTP,* 5-hydroxytryptophan; *NKA,* neurokinin; *NSE,* neuron-specific enolase; *PP,* pancreatic peptide; *YY,* peptide YY; *SVP2,* synaptic vesicle protein 2.



*Klöppel G, Couvelard A, Hruban RH et al. Neoplasms of the neuroendocrine pancreas. In: Lloyd RV, Osamura RY, Klöppel G et al, eds. WHO classification of tumours of endocrine organs. 4th ed. Lyon, France: IARC Press, 2017:209–239.

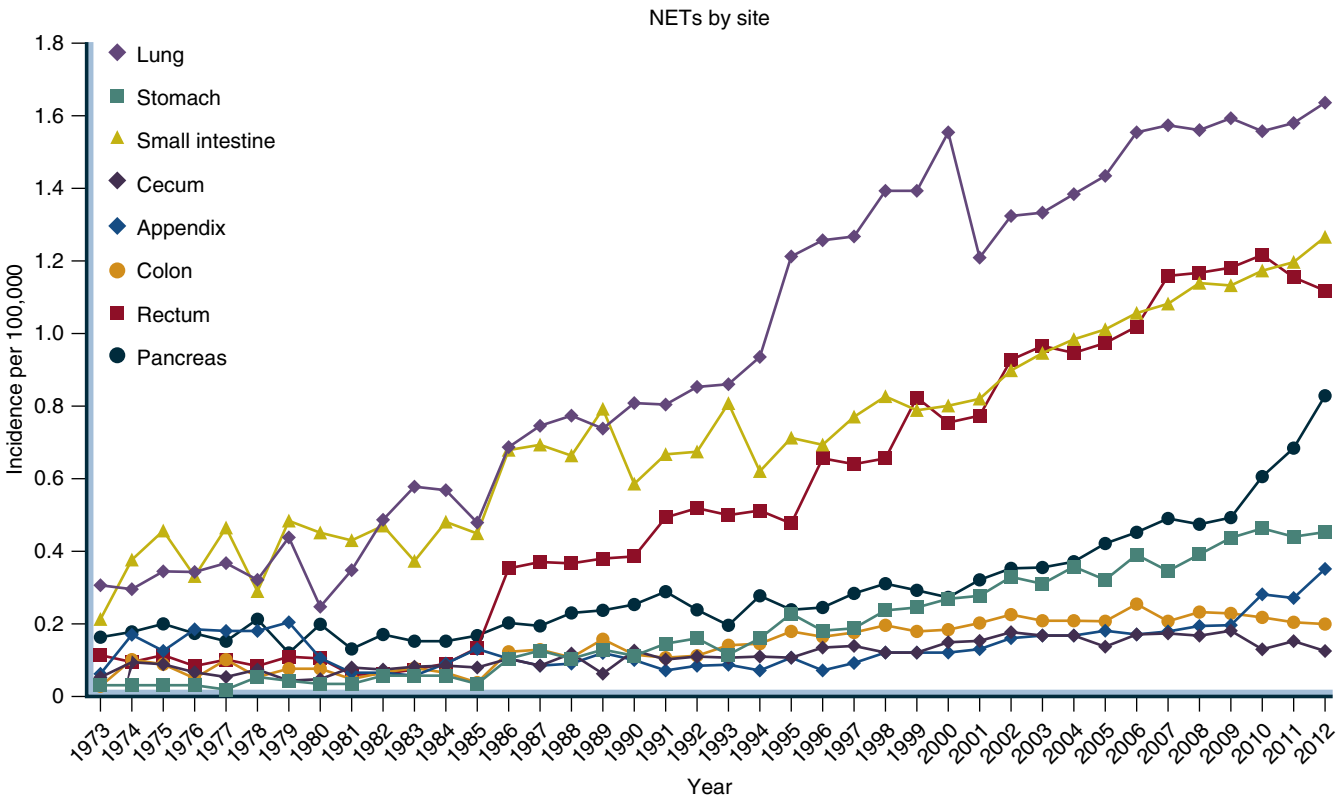
• **Fig. 45.3** Pathologic classification of gastroenteropancreatic neuroendocrine neoplasms, GEP-NEN (from WHO in 2017). Mitotic index is per 10 high-power fields. *G,* grade; *GEP,* gastroenteropancreatic; *HPF,* high-powered fields; *NEC,* neuroendocrine carcinoma; *PD,* poorly differentiated; *WD,* well differentiated. (Data from de Mestier L, Cros J, Neuzillet C, et al. Digestive system mixed neuroendocrine-non-neuroendocrine neoplasms. *Neuroendocrinology*. 2017;105:412–425.)



• A tumor with carcinoid-like morphology and mitotic rate >10 is likely to be aggressive and is best classified as LCNEC*

*Travis WD, Brambilla E, Burke AP et al, eds. WHO classification of tumours of the lung, pleura, thymus and heart. 4th ed. Lyon, France: International Agency for Research on Cancer, 2015.

• **Fig. 45.4** Pathologic classification of lung neuroendocrine neoplasms, Lung-NEN (from WHO in 2015). Mitotic index is per 2 mm^2. *AC,* atypical carcinoid; *LCNEC,* large cell neuroendocrine carcinoma; *SCLC,* small cell lung carcinoma; *TC,* typical carcinoid. (Modified from Travis WD. Pathology and diagnosis of neuroendocrine tumors: lung neuroendocrine. *Thorac Surg Clin*. 2014;24:257–266.)



• **Fig. 45.5** Age-adjusted incidence of gastroenteropancreatic neuroendocrine tumors by primary site (SEER 18). Notice the significant increase during the past decades. *NET*, neuroendocrine tumor; *SEER*, Surveillance, Epidemiology, and End Results. (From Dasari A, Shen C, Halperin D, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. *JAMA Oncol*. 2017;3:1335–1342.)

Neuroendocrine tumors of the midgut and foregut regions with metastatic disease secrete serotonin and show elevated urinary excretion of 5-hydroxyindoleacetic acid (5HIAA) in 76% and 30%, respectively.[30] Carcinoid tumors arising from the foregut, however, commonly have low levels of L-amino acid decarboxylase, which converts 5-hydroxytryptophan (5HTP) to serotonin. Thus these tumors secrete primarily 5HTP.[31,32]

For many years, it was believed that the entire carcinoid syndrome could be explained by the secretion of these biologically active amines. However, further studies have indicated that serotonin is mainly involved in the pathogenesis of diarrhea and that other biologically active substances play a more important part in the carcinoid flush and bronchoconstriction.

Oates and associates[33] proposed that kallikrein, an enzyme found in carcinoid tumors, is released in association with flush and stimulates plasma kininogen to liberate lysyl-bradykinin and bradykinin. These biologically active substances cause vasodilation, hypotension, tachycardia, and edema.[33–35] Furthermore, prostaglandins (E_1, E_2, F_1, F_2) might also play a role in the carcinoid syndrome.[36] Gastric carcinoids and lung carcinoids have been found to contain and secrete histamine, which might be responsible for the characteristic bright red flush seen in these patients.[37–39] Metabolites of histamine are often present in high concentration in the urine from these patients. Dopamine and norepinephrine have also been found in carcinoid tumors.[40]

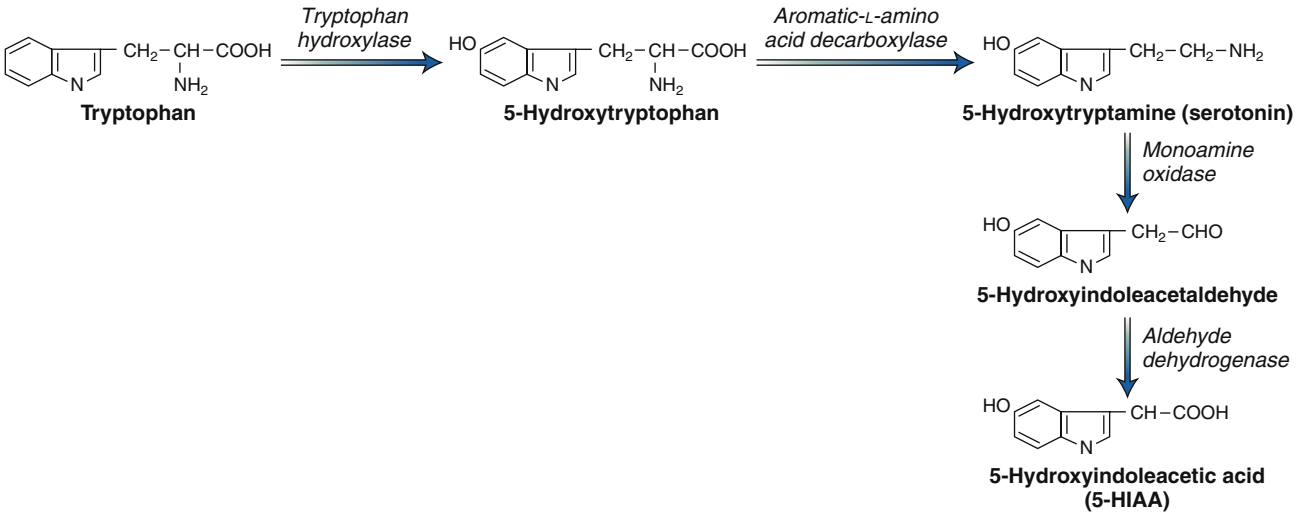
The occurrence of substance P in carcinoid tumors was first demonstrated by Håkansson and coworkers in 1977.[41] Substance P belongs to a family of polypeptides that share the same carboxy-terminus and are called *tachykinins* (Fig. 45.7). A number of tachykinin-related peptides have been isolated from carcinoid tumors, such as neurokinin A, neuropeptide K, and eledoisin. During stimulation of flush in patients with midgut carcinoids, multiple forms of tachykinins are released to the circulation (Fig. 45.8).[42–44]

Many different polypeptides (e.g., insulin, gastrin, somatostatin, S100 protein, polypeptide YY, pancreatic polypeptide, human chorionic gonadotropin α-subunit [hCGα], motilin, calcitonin, vasoactive intestinal polypeptide [VIP], and endorphins) have been demonstrated in carcinoid tumors by immunohistochemical staining and sometimes in tumor extracts.[10] Ectopic ACTH or CRH production may be found in foregut carcinoids; in particular, patients with bronchial or thymic carcinoids seem susceptible to Cushing syndrome.[45] Patients with carcinoid tumors of the foregut type might also present with acromegaly due to ectopic secretion of GHRH from the tumor.[46] Duodenal carcinoids as part of von Recklinghausen disease can secrete somatostatin.[47]

The chromogranin/secretogranin family consists of CgA, CgB (sometimes called *secretogranin I*), secretogranin II (sometimes called *CgC*), and some other members. CgA was first isolated in 1965 as a water-soluble protein present in chromaffin cells from bovine adrenal medulla.[48] Its immunoreactivity has been found in all parts of the GI tract and pancreas and has also been isolated from all endocrine glands.[49]

CgA is an acidic glycoprotein of 439 amino acids with a molecular weight of 48 kDa. It can be spliced into smaller fragments at dibasic cleavage sites, generating multiple bioactive fragments such as vasostatins, chromostatins, and pancreastatins[49–53] (Fig. 45.9).

Amines and hormones are stored intracellularly in two types of vesicles: large dense-core vesicles and small synaptic-like vesicles.



• **Fig. 45.6** Biosynthesis and metabolism of 5-hydroxytryptamine (5HT) (serotonin).

```
Substance P            Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH2
Neurokinin A               His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH2
Neurokinin B                   Asp-Met-His-Asp-Phe-Val-Gly-Leu-Met-NH2
Eledoisin              Pyr-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH2
Kassinin          Asp-Val-Pro-Lys-Ser-Asp-Glu-Phe-Val-Gly-Leu-Met-NH2
Physalemin             Pyr-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH2
Neuropeptide K         Arg-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH2
                     [1]-Lys-His-Ser-Ile-Gln-Gly-His-Gly-Tyr-Leu-Ala-Lys
               Asp-Ala-Asp-Ser-Ser-Ile-Glu-Lys-Gln-Val-Ala-Leu-Leu[1]
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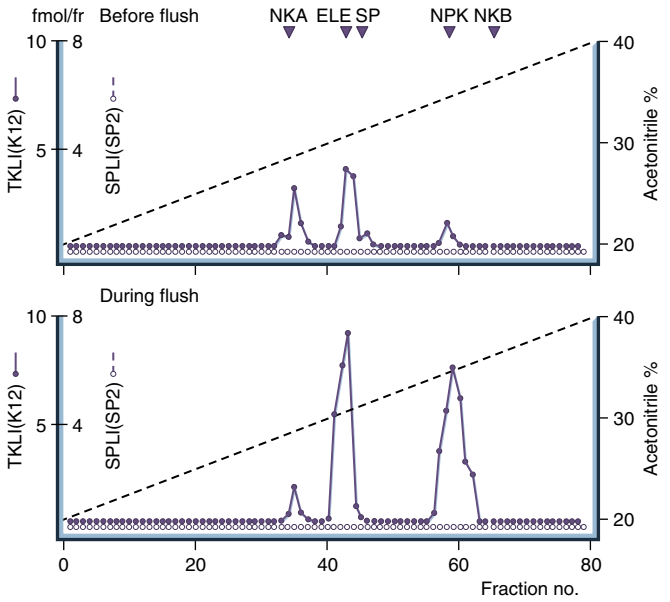
• **Fig. 45.7** The tachykinin family of peptides shares the same carboxy-terminus. Neuropeptide K is a prohormone containing neurokinin A, which can be spliced off.



• **Fig. 45.8** Chromatography samples of plasma from a patient with carcinoid before flush *(upper panel)* and during flush *(lower panel)*. Note the significant increase in eledoisin-like peptide as well as in neuropeptide K. *ELE,* eledoisin; *NKA,* neurokinin A; *NKB,* neurokinin B; *NPK,* neuropeptide K; *SP,* substance P; *SPLI(SP2),* substance P–like immunoreactivity; *TKLI(K12),* tachykinin-like immunoreactivity.

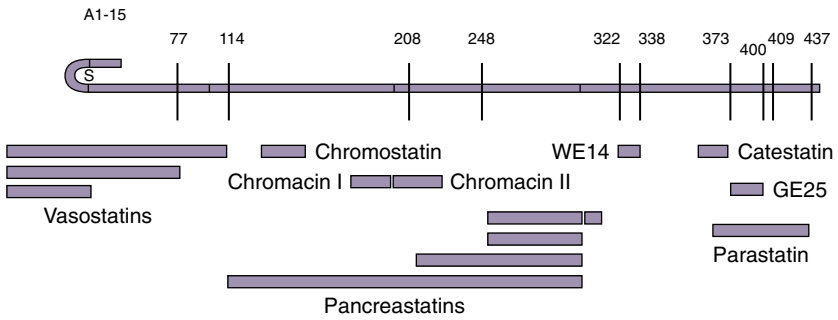
These vesicles release amines and hormones on stimulation. Large dense-core vesicles contain the hormones and one or more members of the chromogranin/secretogranin family of proteins.[50,54] Both amines and peptides are co-released (Fig. 45.10).

The physiologic function of CgA is not fully elucidated. Its ubiquitous presence in neuroendocrine tissues and its co-secretion with peptide hormones and amines indicate a storage role of the peptide within the secretory granule.[49,50,54] It also acts as a prohormone that can generate bioactive smaller fragments. CgA is an important tissue and serum marker for different types of neuroendocrine tumors, including those of the foregut, midgut, and hindgut (see Table 45.1 and later discussion).

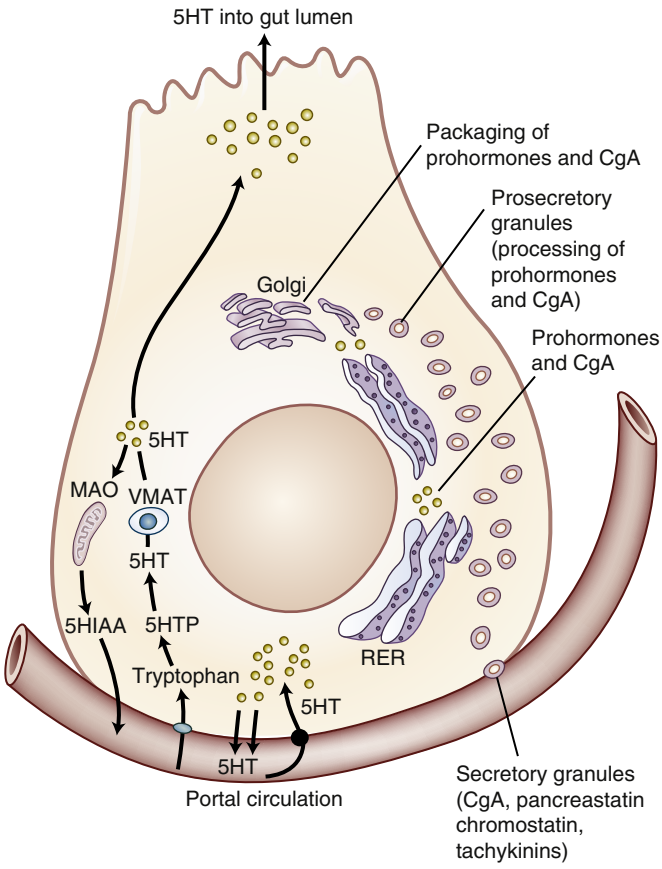
Clinical Presentation

The clinical presentation of neuroendocrine tumors depends on localization, hormone production, and extent of the disease. Usually a lung carcinoid is diagnosed incidentally on routine pulmonary radiography, whereas a midgut carcinoid may be identified as a bowel obstruction or as a cause of abdominal discomfort or pain. Rectal carcinoids might cause bleeding or obstruction. However, lung carcinoids can also manifest clinically with Cushing syndrome, due to secretion of CRH or ACTH, or with the carcinoid syndrome, due to production of serotonin, 5HTP, or histamine.[55] A midgut carcinoid often manifests with the carcinoid syndrome due to production of serotonin and tachykinins.

The clinical manifestations at referral depend on the type of referral center. At our institution, which cares for patients with malignant tumors, 74% of the patients present with the carcinoid syndrome, 13% with abdominal pain, 12% with carcinoid heart disease, and 2% with bronchial constriction.[27] When unbiased material is analyzed, bowel obstruction is the most common problem leading to the diagnosis of ileal carcinoid tumor. The second most common symptom is abdominal pain. Flushing and diarrhea, which are components of the carcinoid



• **Fig. 45.9** The glycoprotein chromogranin A and related peptides, including GE25 and WE14.



• **Fig. 45.10** Schematic of an enterochromaffin cell. The initial step in 5-hydroxytryptamine (5HT) synthesis is carrier transport of the amino acid tryptophan from blood into the cell across the cell membrane. Intracellular tryptophan is first converted to 5-hydroxytryptophan (5HTP), which in turn is converted to 5HT and stored in secretory granules. The transport of 5HT into granules requires vesicular monoamine transporters (VMATs). Via the basal lateral membrane, 5HT can be released into the circulation. There is also a membrane pump mechanism in the cell membrane responsible for amine reuptake. A minor part of 5HT can also be released into the gut lumen. Monoamine oxidase (MAO) degrades 5HT to 5-hydroxyindoleacetic acid (5HIAA). Peptide prohormones are synthesized in the rough endoplasmic reticulum (RER) together with chromogranin A (CgA) and other granule proteins. The products are transported to the Golgi apparatus for packaging into prosecretory granules. On stimulation, the secretory products are released from the granules by exocytosis.

syndrome, constitute only the third most common presentation.[56–58] Because many patients have vague symptoms, however, diagnosis of the tumor may be delayed by approximately 2 to 3 years.[26]

The Carcinoid Syndrome

In 1954, Thorson and coworkers for the first time described the carcinoid syndrome as having the following features: malignant carcinoid of the small intestine with metastases to the liver, valvular disease of the right side of the heart (pulmonary stenosis and tricuspid insufficiency without septal defect), peripheral vasomotor symptoms, bronchial constriction, and an unusual type of cyanosis.[4] One year later, Dr. William Bean[59] gave this colorful description of the carcinoid syndrome: "This witch's brew of unlikely signs and symptoms, intriguing to the most fastidious connoisseur of clinical esoterica—the skin underwent rapid and extreme changes—resembling in clinical miniature the fecal phantasmagoria of the aurora borealis."

The syndrome is thus well characterized and includes flushing, diarrhea, right-sided heart failure, and sometimes bronchial constriction and increased urinary levels of 5HIAA.[60,61] This is the classic carcinoid syndrome, but some patients display only one or two of the features. Other symptoms related to the syndrome are weight loss, sweating, and pellagra-like skin lesions.

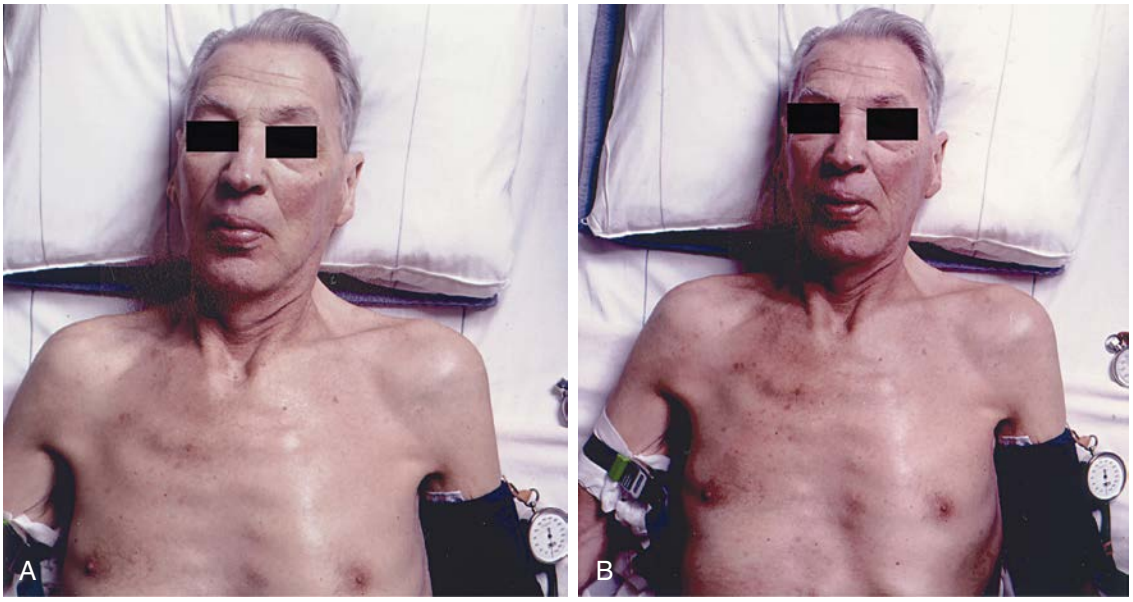
Development of the carcinoid syndrome is a function of tumor mass, extent and localization of metastases, and localization of the primary tumor. The syndrome is most common in tumors originating in the small intestine and proximal colon; 40% to 60% of patients with these tumors experience the syndrome.[27,57,60,61] The disorders are less common in patients with bronchial carcinoids and do not occur in patients with rectal carcinoids.[55,62,63] The syndrome rarely occurs in patients with midgut carcinoids and a small tumor burden, such as only regional lymph node metastases.[58] Patients with the full syndrome usually have multiple liver metastases. The association with hepatic metastases is due to efficient inactivation by the liver of amines and peptides released into the portal circulation. The venous drainage of liver metastases is directly into the systemic circulation and bypasses hepatic inactivation.[64]

Other carcinoid tumors likely to be associated with the carcinoid syndrome in the absence of liver metastases are ovarian carcinoids and bronchial carcinoids, which release mediators directly into the systemic rather than the portal circulation. Retroperitoneal metastases from classic midgut carcinoids also release mediators directly into the circulation and might cause the carcinoid syndrome without any liver metastases.[60,61]

Flushing

Four types of flushing have been described in the literature: erythematous, violaceous, prolonged, and bright red.[60,61]

The first and best known type is the sudden, diffuse, erythematous flush, usually affecting the face, neck, and upper chest (i.e., the normal flushing area) (Fig. 45.11). This type of flush is



• **Fig. 45.11** Carcinoid syndrome before and after provocation. (A) Before flush provocation. (B) The same patient after pentagastrin-stimulated flush.

commonly of short duration, lasting from 1 to 5 minutes, and is related to early-stage midgut carcinoids. Patients usually experience a sensation of warmth during flushing and sometimes heart palpitations. This type of flushing is reported in 20% to 70% of patients with midgut carcinoid at onset of the disease.[26,60,62]

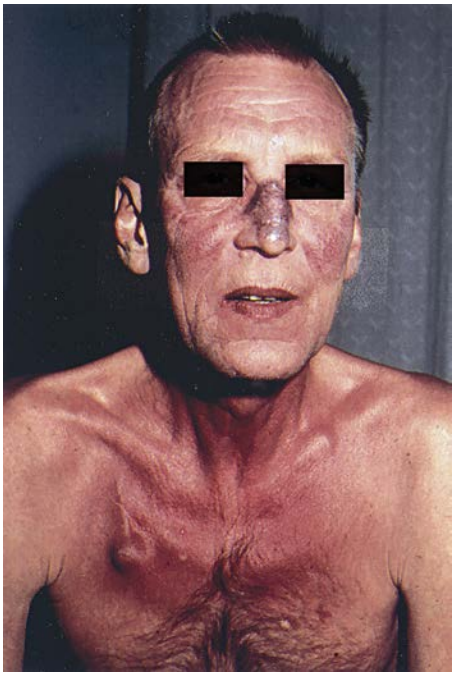
The second type is the violaceous flush, which affects the same area of the body. It has roughly the same time course or sometimes lasts a little longer. Patients may also have facial telangiectasia. This flush occurs during the later stages of midgut carcinoid (Fig. 45.12) and is normally not felt by patients because they have become accustomed to the flushing reaction.

The third type is prolonged flushing that usually lasts a couple of hours but can last up to several days. This flush sometimes involves the whole body and is associated with profuse lacrimation, swelling of the salivary gland, hypotension, and facial edema (Fig. 45.13). These symptoms are usually associated with malignant bronchial carcinoids.

The fourth type of flushing is a bright red, patchy flush seen in patients with chronic atrophic gastritis and ECL cell hyperplasia, or ECLoma (derived from ECL cells). This type of flushing is related to an increased release of histamine and histamine metabolites.

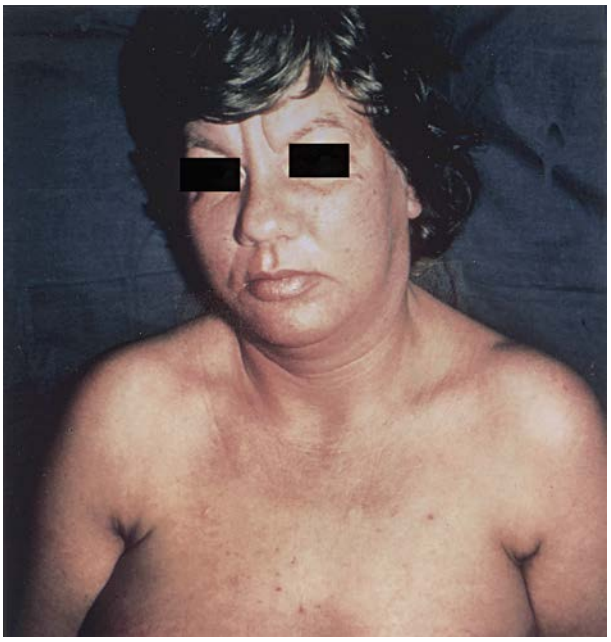
Flushes may be spontaneous or may be precipitated by stress (physical and mental); infection; alcohol; certain foods (spicy); or drugs, such as by injections of catecholamines, calcium, or pentagastrin (see later discussion). The pathophysiology of flushing in the carcinoid syndrome is not yet elucidated.[65–67] It was previously believed to be totally related to excess production of serotonin or serotonin metabolites.[66] However, several patients with high levels of plasma serotonin did not have any flushing, nor did a serotonin antagonist (e.g., methysergide, cyproheptadine, or ketanserin) have any effect on the flushing.[65,68]

In a study from our own group in which we measured the release of tachykinins, neuropeptide K, and substance P during flushing provoked by pentagastrin or alcohol, a clear correlation was found between the onset and intensity of the flushing reaction and the release of tachykinins (see Fig. 45.6). Furthermore, when the release of tachykinins was blocked by prestimulatory administration of octreotide, little or no flushing was observed in the same patient (Fig. 45.14).[42–44] Other mediators of the flushing reaction may be kallikrein and bradykinins, which are released during provoked flushing.[33–35]

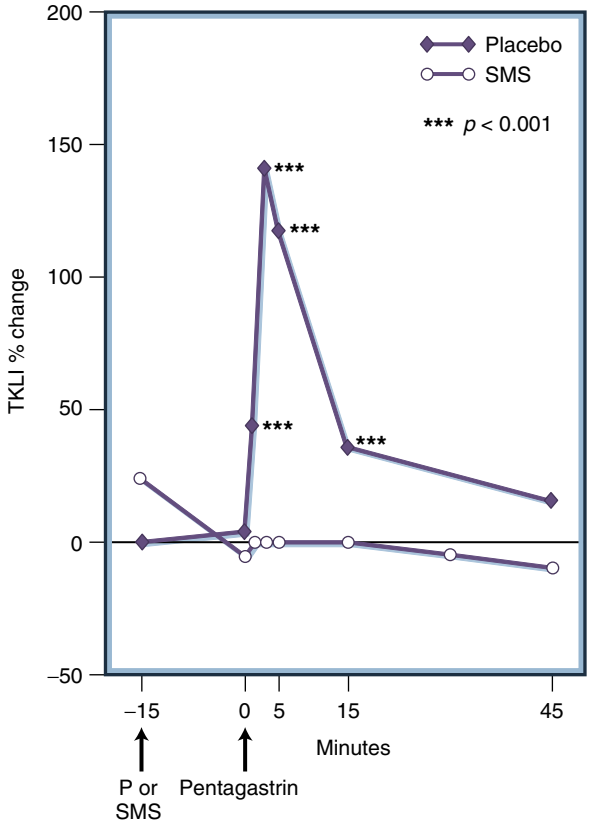


• **Fig. 45.12** Long-lasting chronic flushing in a patient with long-standing carcinoid disease. Note the telangiectases.

Histamine may be a mediator of the flushes seen in lung carcinoids and in gastric carcinoids (ECLomas).[37–39] Tachykinins, bradykinins, and histamines are well-known vasodilators, and somatostatin analogues might alleviate flushing by reducing circulating levels of these agents (see later discussion).[42–44,67–72] Furschgott and Zawadski have suggested that flushing is caused by an indirect vasodilation mediated by endothelium-derived relaxing factor (EDRF) or by nitric oxide released by 5HTP during platelet activation.[73]



• **Fig. 45.13** This patient has lung carcinoid and carcinoid syndrome with severe, long-standing flushing, lacrimation, and a swollen face.



• **Fig. 45.14** Tachykinin levels (TKLI) after stimulation with pentagastrin in patients with classic midgut carcinoids. Pretreatment for 15 minutes with somatostatin (SMS) causes inhibition of tachykinin release and inhibition of the flush reaction (*open circles*). *P,* placebo.

The facial flushing associated with carcinoid tumors should be distinguished from idiopathic flushing and menopausal hot flushes. Patients with idiopathic flushes usually have a long history of flushing starting early in life and sometimes with a family history without occurrence of a tumor. Menopausal hot flushes usually involve the whole body and are accompanied by intense sweating. Postmenopausal women in whom a true carcinoid syndrome is developing can differentiate between the two types of flushes.

Diarrhea

Diarrhea occurs in 30% to 80% of patients with the carcinoid syndrome.[26,27,60,61] Its pathophysiology is poorly understood but is probably multifactorial. The diarrhea is often accompanied by abdominal cramping, and endocrine, paracrine, and mechanical factors contribute to this condition. A variety of tumor products, including serotonin, tachykinins, histamines, kallikrein, and prostaglandins, can stimulate peristalsis, electromechanical activity, and tone in the intestine.[67,74–76] Secretory diarrhea can occur with fluid and electrolyte imbalance. Malabsorption can result from intestinal resections, from lymphangiectasia, secondary to mesenteric fibrosis, from bacterial overgrowth, and secondary to a tumor partially obstructing the small bowel or rapid intestinal transit. Increased secretion by the small bowel, malabsorption, or accelerated transit can overwhelm the normal storage and absorptive capacity of proximal colon and result in diarrhea, which may be aggravated if the reabsorptive function of the colon is impaired.

In a study of patients with elevated serotonin levels and the carcinoid syndrome, transit time in the small bowel and colon was significantly decreased in comparison with that of normal subjects.[77] The volume of the ascending colon was significantly smaller than that in normal subjects, and the postprandial colonic tone was markedly increased. This indicates that in patients in whom the carcinoid syndrome is associated with diarrhea, major alterations in gut motor function occur that affect both the small intestine and the colon. Many patients with carcinoid tumors have undergone wide resection of the small intestine, and they may be affected by the symptoms of short-bowel syndrome.

Serotonin is believed to be responsible for the diarrhea in the carcinoid syndrome by its effects on gut motility and intestinal electrolyte and fluid secretion.[61,74–76] Serotonin receptor antagonists, such as ondansetron and ketanserin, relieve the diarrhea to a certain degree.[74,78–80] Recently an inhibitor of the enzyme tryptophan-hydroxylase, telotristat ethyl, was shown to reduce serotonin levels and concomitantly the number of bowel movements.[81]

Carcinoid Heart Disease

A unique endocrine effect of carcinoid tumors is the development of plaque-like thickenings of the endocardium, valve leaflets, atria, and ventricles in 10% to 20% of the patients.[82,83] This fibrotic involvement causes stenosis and regurgitation of the blood flow. Findings of new collagen beneath the endothelium of the endocardium is almost pathognomonic for carcinoid heart disease.[82–84] The incidence of these lesions depends on the diagnostic methodology. Echocardiography can demonstrate early lesions in about 70% of patients with the carcinoid syndrome, whereas routine clinical examinations detect them in only 30% to 40%.[82,83,85] These figures have significantly dropped to 5% to 10%, probably because of earlier diagnosis and the use of biologic antitumor treatments such as somatostatin analogues and α-interferons. Both of these agents control the hormonal release and excess that are thought to be involved in the fibrotic process.

In a study performed in 1987,[26] 40% of patients with carcinoid tumors died of cardiac complications related to the carcinoid disease. Data from 2008 reveal that this complication is a rare event and that patients usually die of the effects of a progressive tumor.[27]

The precise mechanism behind the fibrosis in the right side of the heart has not been determined yet, but it occurs mainly

in patients with liver metastases who usually also have the carcinoid syndrome.[82,83] Substances inducing fibrosis are believed to be released directly into the right side of the heart and are then neutralized or degraded as they pass through the pulmonary circulation, because few patients present with similar lesions of the left side of the heart.[82,83] However, patients with lung carcinoids occasionally display the same fibrotic changes in the left side of the heart. Histologically, the plaque-like thickenings in the endocardium consist of myofibroblasts and fibroblasts embedded in a stroma that is rich in mucopolysaccharides and collagen.[82]

We have previously shown that the TGFβ family of growth factors is upregulated in carcinoid fibrous plaques on the right side of the heart.[86] The TGFβ family of growth factors is known to stimulate matrix formation and collagen deposition. The substances that induce TGFβ locally in the heart are not known, but serotonin, tachykinins, and insulin-like growth factor-1 (IGF1) may be mediators.[82,87]

A correlation has been found between circulating levels of serotonin and tachykinins and the degree and frequency of carcinoid heart lesions. The weight-reducing drugs fenfluramine and dexfenfluramine appear to interfere with normal serotonin metabolism and have been associated with valvular lesions identical to those seen in carcinoid heart disease.[88,89] However, treatment resulting in decreased urinary 5HIAA excretion does not result in regression of cardiac lesions.[90] Two animal studies indicate that serotonin might play a significant role in the development of carcinoid heart disease. Serotonin is also known to induce $TGF\beta_1$ in in vitro experiments.[91–93] Connective tissue growth factor (CTGF) is produced by carcinoid tumor cells, and increased expression is found in patients with advanced fibrosis. CTGF is known to stimulate TGFβ.[94] For early detection of carcinoid heart disease, measurement of NT-proBNP is the best diagnostic and prognostic marker.[95]

Bronchial Constriction

A true asthma episode is rare in patients with the carcinoid syndrome.[26,60,61] The causative agents of bronchial constriction are not known, but tachykinins and bradykinins have been suggested as mediators.[96,97] These agents can constrict smooth muscle in the respiratory tract and can also cause local edema in the airways.

Other Manifestations of the Carcinoid Syndrome

Fibrotic complications other than heart lesions may be found in patients with carcinoid tumors. They include intra-abdominal and retroperitoneal fibroses, occlusion of the mesenteric arteries and veins, Peyronie disease, and carcinoid arthropathy.[60,61]

Intra-abdominal fibrosis can lead to intestinal adhesions and bowel obstruction and is a more common cause of bowel obstruction than is the primary carcinoid tumor itself.[58,98,99] Retroperitoneal fibrosis can result in obstruction of the ureter that impairs kidney function, which sometimes requires treatment with ureteral stents.

Narrowing and occlusion of arteries and veins by fibrosis are potentially life threatening. Ischemic loops of the bowel might have to be removed, and this procedure ultimately causes short-bowel syndrome.[58,99]

Other rare features of the syndrome are pellagra-like skin lesions with hyperkeratosis and pigmentation, myopathy, and sexual dysfunction.[61]

Carcinoid Crisis

Carcinoid crisis has become rare since the introduction of treatment with somatostatin analogues.[100] It might occur spontaneously or during induction of anesthesia, embolization procedures, chemotherapy, or infection. Carcinoid crisis is a clinical condition characterized by severe flushing, diarrhea, hypotension, hyperthermia, and tachycardia. Without treatment, patients might die during the crisis.[100–102]

Intravenous (IV) or subcutaneous somatostatin analogues (or both) are given before, during, and after surgery to prevent the development of carcinoid crisis.[100,102–104] Patients with metastatic lung carcinoids are particularly difficult to treat during crisis. IV infusions of octreotide at doses of 50 to 100 μg/hour, supplemented with histamine H_1-receptor and H_2-receptor blockers and IV sodium chloride, are recommended.[105]

Other Clinical Manifestations of Carcinoid Tumors

Ectopic secretion of CRH and ACTH from pulmonary carcinoid tumors and thymic carcinoids accounts for 1% of all cases of Cushing syndrome.[45,106] Acromegaly due to ectopic secretion of GHRH has also been reported in foregut carcinoids.[46,107] Gastric carcinoid tumors make up less than 1% of gastric neoplasms. They can be separated into three distinct groups or types on the basis of clinical and histologic characteristics and originate from gastric ECL cells.[108] Type 1 is associated with chronic atrophic gastritis type A (80%). Type 2 is associated with Zollinger-Ellison syndrome as part of MEN1 syndrome (6%). Type 3 represents sporadic gastric carcinoids occurring without hypergastrinemia and pursue a more malignant course, with 50% to 60% developing metastases.[108,109]

About 80% of gastric carcinoids are associated with chronic atrophic gastritis type A, and more than 50% of patients with these carcinoids also have pernicious anemia. These tumors are more common in women than in men and are usually identified endoscopically during diagnostic evaluation for anemia or abdominal pain.[108,110] They are often multifocal and localized in the gastric fundus area, and they are derived from ECL cells. Patients have hypochlorhydria and hypergastrinemia. Gastrin hypersecretion has been postulated to result in hyperplasia of the ECL cells, which might later develop into carcinoid tumors.[111,112] Hyperplasia of ECL cells has been noticed in patients on long-standing proton-pump inhibitor therapy.[113,114]

Diagnosis

The diagnosis of a suspected carcinoid tumor must take into consideration molecular genetics, tumor biology, histopathology, biochemistry, and localization. The diagnosis of a carcinoid tumor may be suspected from clinical symptoms suggesting the carcinoid syndrome or from the presence of other clinical symptoms, or it can be made in relatively asymptomatic patients from the histopathologic findings at surgery or after liver biopsy for unknown hepatic lesions.

In one study involving 154 consecutive patients with GI carcinoids found at surgery, 60% were asymptomatic.[115] In patients with symptomatic tumors, the time from onset of symptoms until diagnosis is often delayed 1 to 2 years.[26] The current tumor biology program includes growth factors (platelet-derived growth factor, epidermal growth factor, IGF1, TGF)[116] and proliferation factors (measurements of the nuclear antigen Ki67) as a proliferation index. Such an index correlates with tumor aggressiveness and survival.[117,118] Adhesion molecules such as CD44, particularly exon-V6 and exon-V9, have been related to improved survival.[119] Determination of the expression of angiogenic factors basic fibroblast growth factor (bFGF) and vascular endothelial growth factor

(VEGF) should also be included in a tumor biology program. Somatostatin analogues are cornerstones in the treatment of the carcinoid syndrome; therefore determination of the different subtypes of somatostatin receptors (SSTR1–SSTR5) with specific antibodies is warranted.[120,121] Rare cases with familial carcinoids should be analyzed with respect to loss of heterozygosity on chromosome 11q13 and chromosome 18. Integrated genome-wide analysis, including exome and whole-genome sequencing, gene expression, DNA methylation, and copy number analysis, has identified three novel molecular subtypes of small intestinal NETs (carcinoids) with different clinical outcome. Epigenetic dysregulation is common in small intestinal NET.[17] Recently we identified mutations in the *MUTYH* gene involved in protein of DNA from oxidative stress.[18]

Histopathologic Diagnosis

The histopathologic diagnosis of carcinoids is based on immunohistochemistry using antibodies against CgA, synaptophysin, and neuron-specific enolase. These immunohistochemical stains have replaced the old silver stains, the argyrophil stains by Grimelius and Sevier-Munger. The argentaffin stain by Masson to demonstrate content of serotonin has also been replaced by immunocytochemistry with serotonin antibodies.[10] These neuroendocrine markers can be supplemented by specific immunocytochemistry to different hormones such as substance P, gastrin, and ACTH. The WHO classification forms the basis for therapeutic decisions (see earlier discussion) and therefore determination of Ki67 (MIB1) for analysis of cell proliferation is mandatory. Antibodies to TTF1 and CDX2 give good information on the localization of the primary tumor in patients with unknown primary tumors.[122]

Biochemical Diagnosis

In patients with flushing and other manifestations of the carcinoid syndrome, the diagnosis can be established by measuring the urinary excretion of 5HIAA because levels are invariably elevated under these circumstances.[123] Patients with carcinoid tumors usually have urinary 5HIAA levels of 100 to 3000 µmol/24 hours (15–60 mg/24 hours) (reference range: <50 µmol/24 hours [10 mg/24 hours]). Assays for urinary 5HIAA include high-pressure liquid chromatography (HPLC) with electrochemical detection and colorimetric and fluorescence methods.[124] Various foods and drugs can interfere with the measurement of urinary 5HIAA, and patients should avoid these agents during the 24-hour sampling (Table 45.2).[125] Normally two 24-hour urine collections are recommended. In a study of patients with malignant midgut carcinoid tumors, 60% to 73% presented with increased urinary 5HIAA levels,[27,60,61] with a specificity of almost 100%.

Today, measurement of urinary 5HIAA for diagnosis of carcinoid tumor is the predominant biochemical analytic procedure. However, urinary and platelet measurements of serotonin itself can give additional information. In some studies, platelet serotonin levels were more sensitive than urinary 5HIAA and urinary serotonin levels and were not affected by the patient's diet, as are 5HIAA levels.

In a comparative study of 44 consecutive patients with carcinoid tumor, the platelet serotonin, urinary 5HIAA, and urinary serotonin levels were measured. In foregut carcinoids the sensitivities were 50%, 29%, and 55%, respectively. For midgut carcinoids, the sensitivities were 100%, 92%, and 82%, respectively, and for hindgut carcinoids they were 20%, 0%, and 60%, respectively.[126]

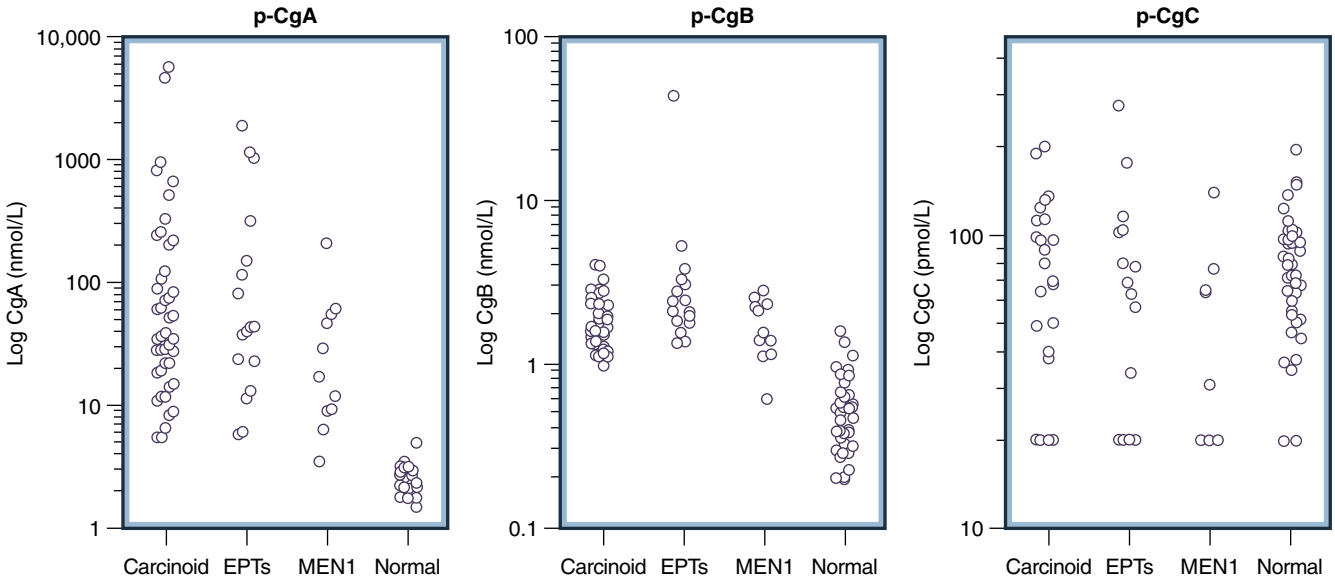
A method to determine 5HIAA in serum has been developed and will replace the urine 5HIAA.[127] Elevations of 5HIAA can occur in malabsorption states and a number of other conditions. Foregut carcinoids tend to produce an atypical carcinoid syndrome with increased plasma 5HTP, but not serotonin, because they lack the appropriate decarboxylase that results in normal urinary 5HIAA.[31,40] However, some of the 5HTP is decarboxylated in the intestine and other tissues, and many of these patients have slightly elevated urinary 5HT or 5HIAA levels.

Attempts have been made to identify more specific and sensitive serum markers for carcinoid tumors that might allow earlier diagnosis. One such marker is CgA. It has been shown that CgA and CgB are more abundant than CgC in human neuroendocrine tissues.[49,50,128] In 44 patients with carcinoid tumors, CgA was increased in 99%, CgB in 88%, and CgC in only 6% (Fig. 45.15).[129] It has been proposed that CgA levels in plasma might reflect tumor size. In a study of 75 patients with midgut carcinoids and the carcinoid syndrome,

TABLE 45.2 Factors That Interfere With Urinary 5HIAA Measurement

Foods	Drugs
Factors That Produce False-Positive Results	
Avocado	Acetaminophen
Banana	Acetanilide
Chocolate	Caffeine
Coffee	Fluorouracil
Eggplant	Guaifenesin
Pecan	L-Dopa
Pineapple	Melphalan
Plum	Mephenesin
Tea	Methamphetamine
Walnuts	Methocarbamol
	Methysergide maleate
	Phenmetrazine
	Reserpine
	Salicylates
Factors That Cause False-Negative Results	
None	Corticotropin
	p-Chlorophenylalanine
	Chlorpromazine
	Heparin
	Imipramine
	Isoniazid
	Methenamine mandelate
	Methyldopa
	Monoamine oxidase inhibitors
	Phenothiazine
	Promethazine

5HIAA, 5-Hydroxyindoleacetic acid.



• **Fig. 45.15** Plasma (*p*) levels of chromogranin A (CgA), CgB, and CgC in patients with various neuroendocrine tumors. *EPTs,* endocrine pancreatic tumors; *MEN1,* multiple endocrine neoplasia type 1.

CgA was elevated in 87% of carcinoid patients. Furthermore, a correlation between levels of plasma chromogranin and extent of disease was found ($p < 0.0001$).[27] In the same study, urinary 5HIAA was elevated in 76% of midgut carcinoids, and there were no correlations with tumor size or extent of disease.

CgA is a more sensitive marker than urinary 5HIAA in detecting carcinoid tumors, but because CgA is released and secreted from various types of neuroendocrine tumors, the specificity is lower.[129–132] Therefore in a workup of patients with the carcinoid syndrome, one should combine the determination of plasma CgA with urinary 5HIAA or serotonin. Importantly, plasma CgA might be elevated in other conditions such as chronic atrophic gastritis, treatment with proton-pump inhibitors, or impaired kidney function.[133] Plasma neuron-specific enolase (NSE) shows a lower sensitivity and specificity than does plasma CgA.[129] Plasma NSE is particularly helpful in patients with lung NETs and other NETs with higher proliferation.[134] Serum hCGα has been reported to be increased in 60% of patients with foregut carcinoid tumors and in 50% of those with hindgut carcinoids but only 11% in those patients with midgut carcinoids and the carcinoid syndrome. Plasma neuropeptide K levels have been reported to be elevated in 46% of patients with midgut carcinoids, whereas only 9% of patients with foregut carcinoids displayed elevated levels.[130,135] Plasma substance P has a sensitivity of 32% and a specificity of 85%.[27,42–44] Neurokinin A is reported to be a sensitive marker for carcinoids of the small intestine both for diagnosis and prognostication.[136] Pancreatic polypeptide levels are also elevated in about one-third of patients with midgut carcinoids and in as many with foregut carcinoids.[137] Recently a gene transcript test (51 genes) analyzing circulating mRNA presented a sensitivity/specificity of 98/97% to detect neuroendocrine tumors and predicted outcome of therapy.[137,138]

During therapy with somatostatin analogues, neither plasma CgA nor urinary 5HIAA is a reliable marker of tumor size because somatostatin inhibits the synthesis and release of the hormones without changes in tumor size.

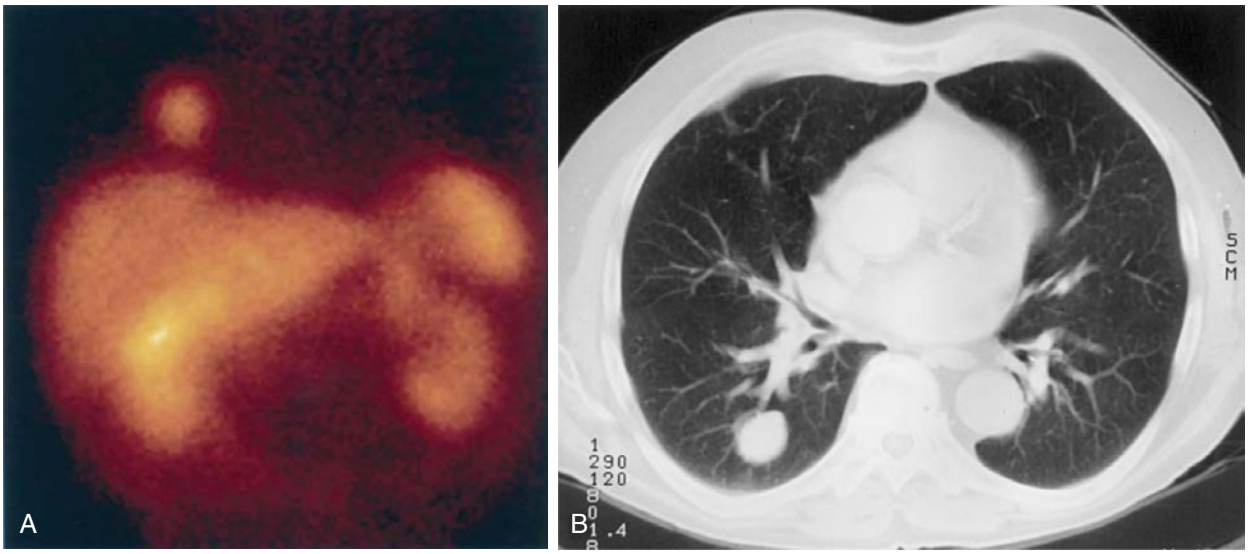
Localization Procedures

Numerous imaging techniques, including endoscopy, barium enema, chest radiography, ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and angiography, have been used to determine the location of the primary tumor as well as the metastases in patients with carcinoid tumors. In more recent years, somatostatin-receptor scintigraphy (SRS) and iodinated meta-iodobenzylguanidine (^{131}I-MIBG) scanning have been used to localize and stage the disease.[139–142] Bronchial carcinoids are usually detected by chest radiography, CT, or (occasionally) bronchoscopy.[143] The primary midgut tumor is usually small and difficult to localize with traditional diagnostic methods such as barium enema, CT scan, or MRI. Some of these tumors can be localized by angiography, capsule endoscopy, or SRS. Liver metastases are usually detected by CT or MRI. At present, CT or MRI and SRS are the primary diagnostic modalities for tumor staging (Fig. 45.16). SRS is nowadays replaced by ^{68}Ga-DOTATATE/TOC positron emission tomography (PET), which shows significantly higher sensitivity and specificity.[144,145]

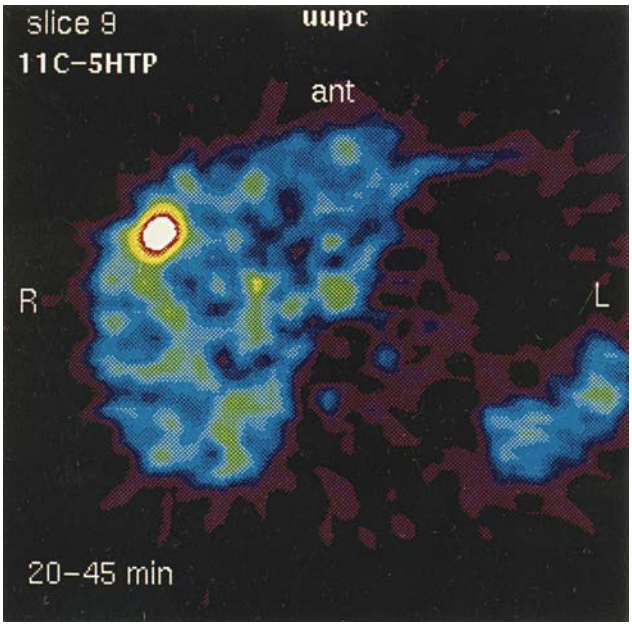
Other sensitive methods are PET or ^{18}F-DOPA-PET scanning[146] using ^{11}C5HTP, the precursor of serotonin synthesis (Fig. 45.17).[147,148] These isotopes accumulate in carcinoid tumors (APUD mechanism); with the development of PET cameras, tumors as small as 0.5 cm in diameter can be detected.[147] During treatment, a close relation has been found among changes in the PET scan, transport rate constant, and urinary 5HIAA, suggesting that PET scanning may be useful in monitoring the results of therapy. PET scanning using ^{18}F-fluorodeoxyglucose (FDG-PET) has been considered not useful in detecting low-proliferating neuroendocrine tumors, but recent data indicate it to be beneficial in identifying poorly differentiated anaplastic tumors but also well-differentiated tumors.[149]

Carcinoid tumors contain high-affinity receptors for somatostatin in 80% to 100% of cases.[120,121,150] The receptors are present in both the primary tumor and metastases. Five subtypes of somatostatin receptors have been cloned (SSTR1–SSTR5), and somatostatin receptor type 2 is the predominant subtype expressed in carcinoid tumors.

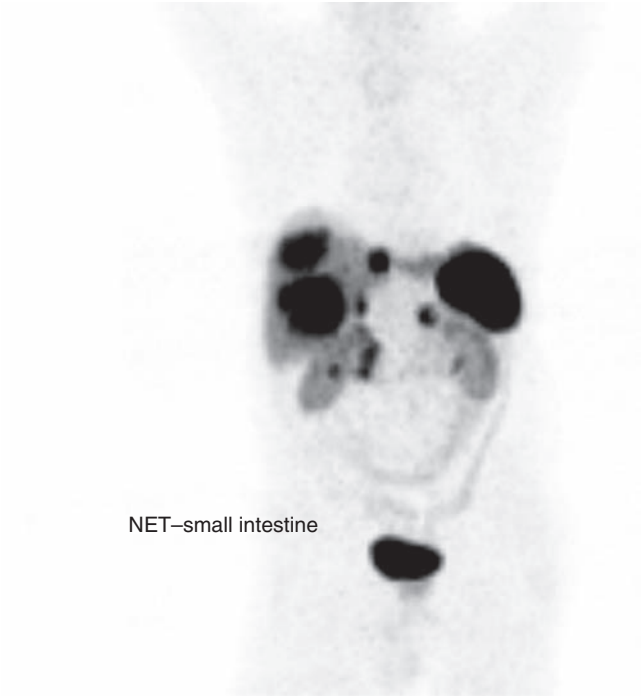
The most commonly available somatostatin analogue, octreotide, binds with high affinity to SSTR2 and with lower affinity to SSTR3 and SSTR5.[151–153] SRS with ^{111}In-DTPA-Phe-octreotide (Octreoscan) has been reported to detect carcinoids with a



• **Fig. 45.16** Bronchial carcinoid. (A) Somatostatin-receptor scintigraphy in a patient with a bronchial carcinoid. (B) Computed tomography scan in the same patient.



• **Fig. 45.17** Positron emission tomography (PET) scan with ^{11}C-5-hydroxytryptophan. Note the metastasis in the liver.



• **Fig. 45.18** ^{68}Ga-DOTA-octreotate positron emission tomography/computed tomography (PET/CT) scan of a patient with carcinoid tumors. Notice liver and lymph node metastases. *NET,* neuroendocrine tumor.

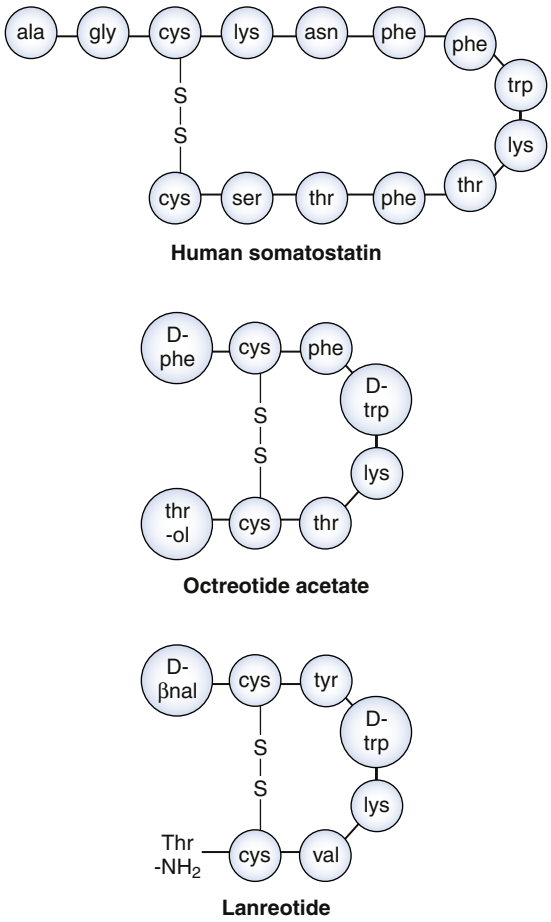
sensitivity of 80% to 90% in patients.[153,154] Many studies have demonstrated that SRS has greater sensitivity for localizing carcinoids compared with conventional imaging studies.[154–157] False-positive scans can be encountered in patients with granulomas (e.g., sarcoidosis, tuberculosis), activated lymphocytes (lymphomas, chronic infection), thyroid diseases (goiter, thyroiditis), endocrine pancreatic tumors, and other endocrine tumors. Because of its high sensitivity and ability to image, whole-body SRS should be the initial imaging procedure to localize and establish the stage of the disease. Bone metastases, which are common with carcinoid tumors, are efficiently detected by SRS, which is as sensitive as traditional bone scanning with technetium.[144,158–161] ^{68}Ga-DOTATATE/TOC-PET scanning has now been developed, showing higher sensitivity than Octreoscan and replacing it worldwide (Fig. 45.18).

Scintigraphy with ^{123}I-MIBG has been applied in patients with midgut carcinoids with a sensitivity of about 50%, which is lower than that for SRS (80–90%). However, it can pick up carcinoids in patients who are sensitive to therapy with ^{123}I-MIBG.[162] The current imaging workup is summarized by Van Essen and coworkers.[163]

A diagnostic algorithm is outlined in Fig. 45.19.

Treatment

Treatment of carcinoid tumors with the carcinoid syndrome requires a multimodal approach, including symptomatic control



• **Fig. 45.19** Molecular structure of human somatostatin-14, octreotide acetate, and lanreotide.

as well as tumor reduction. Most patients with the carcinoid syndrome have metastatic disease. The therapeutic goals are to ameliorate and improve clinical symptoms, abrogate the tumor growth, improve quality of life, and (if possible) prolong overall survival.

Symptomatic control of the carcinoid syndrome includes lifestyle changes, diet supplementation, and specific medical treatment that reduces the clinical symptoms related to the different components of the carcinoid syndrome. Avoiding stress, both psychologic and physical, as well as substances such as alcohol, spicy foods, and medications that precipitate a flushing reaction might be sufficient in early cases.[61]

Production of serotonin by the tumor consumes tryptophan. Normally, about 1% of body tryptophan is used for production of serotonin; in carcinoid tumors, however, as much as 60% of the available tryptophan may be consumed for the synthesis of serotonin, and this can result in tryptophan and niacin deficiencies. Therefore supplemental niacin to prevent the development of pellagra has been recommended over the years. Many patients have undergone resection of the terminal ileum, which can result in vitamin B_{12} and folic acid deficiencies. Supplementation is needed in these patients.

Heart failure due to carcinoid heart disease can require diuretics or angiotensin-converting enzyme (ACE) inhibitors. A few patients need bronchodilators such as salbutamol, which interacts with α-adrenergic receptors and does not induce flushing. The diarrhea seen in the carcinoid syndrome might be controlled by loperamide or diphenoxylate.[164] If patients still have the carcinoid syndrome, they receive somatostatin analogue treatment, which has replaced most of the earlier types of serotonin and serotonin receptor inhibitors. Serotonin inhibitors (e.g., parachlorophenylalanine and α-methyldopa), which inhibit serotonin synthesis, and serotonin receptor antagonists (e.g., cyproheptadine, methysergide, and ketanserin) are not used routinely clinically.[164]

These earlier treatments had limited efficacy in terms of inhibiting flushing and diarrhea and were accompanied by significant side effects. Telotristat ethyl, an inhibitor of the enzyme tryptophan hydroxylase, reduces the serotonin levels. In one study the compound reduced bowel movements by 44% and urinary 5HIAA levels by 75%. The patients experienced a significant improvement.[165] A combination of histamine H_1 and H_2 receptor antagonists is effective in the carcinoid syndrome that is caused by foregut carcinoids due to concomitant secretion of histamine and serotonin. Prednisolone in doses of 15 to 30 mg/day gives occasional relief in some cases with severe flushing and diarrhea.[164]

Somatostatin Analogues

Although natural somatostatin-14 reduces symptoms in patients with the carcinoid syndrome,[166] its use is limited by its short half-life (~2.5 minutes). During the past 30 years, synthetic somatostatin analogues (octapeptides) have been developed for clinical use. Octreotide is the most commonly available drug; other analogues are lanreotide and vapreotide.[167–172]

The somatostatin analogues used in clinical practice (octreotide, lanreotide) (Fig. 45.20) bind to receptors SSTR1 and SSTR5 and, with lower affinity, to SSTR3. They exert their cellular action through interaction with specific cell and transmembrane receptors belonging to the superfamily of G protein–coupled membrane receptors. They inhibit adenylate cyclase activity, activate phosphotyrosine phosphatases (PTPs), and modulate mitogen-activated protein kinases (MAPKs).[153,155,173–175] Receptor subtypes 2 and 5 modulate K^+ and Ca^{2+} fluxes in the cell.[173] Activation of all these pathways results in inhibition of known growth factor production and release and has antiproliferative effects.[154,155,168–171,173,174]

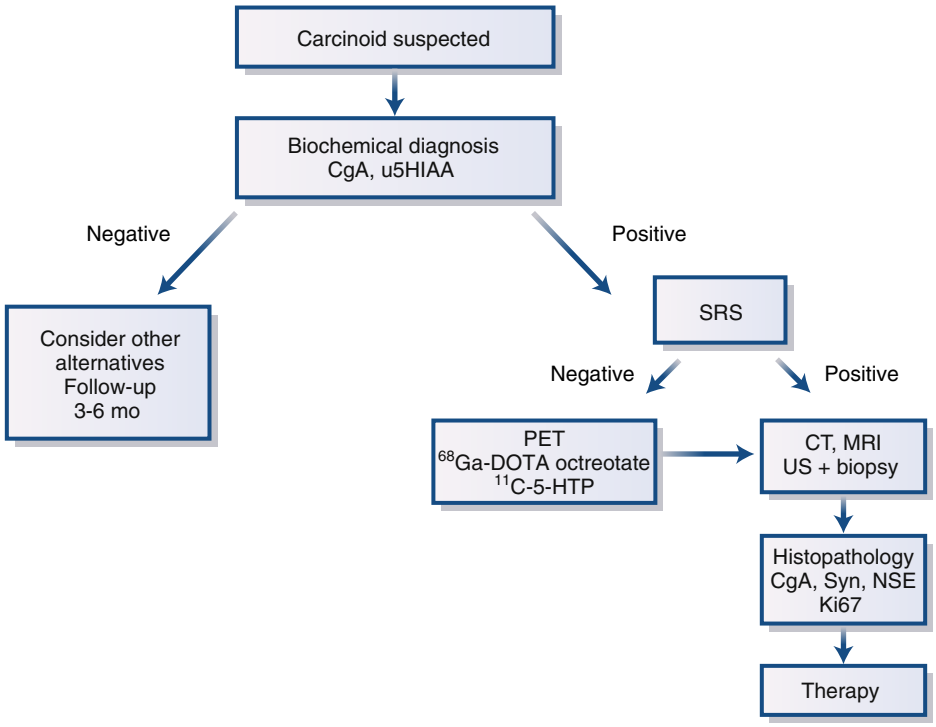
SSTR3 is known to mediate PTP-dependent apoptosis accompanied by activation of TP53 and BAX.[175] Four of the five somatostatin receptor subtypes (SSTR2–SSTR5) undergo rapid internalization after ligand binding, which has been explored by tumor-targeted radioactive somatostatin analogue therapy.[176–179]

An antiproliferative effect has been reported, probably through a combination of SSTR2 and SSTR5 activities, which inhibits MAPK and K^+ and Ca^{2+} fluxes leading to cell cycle arrest[173–175]; the precise antitumor mechanism, however, is not known.

It is now known that different subtypes of somatostatin receptors (SSTR1 and SSTR5) form heterodimers with dopamine receptor D_2. This cross-talk modulates the intracellular signal and gives a fine tuning of the mediated effects.[177]

All five subtypes of somatostatin receptors are expressed in carcinoid tumors; they are expressed in various combinations, although some tumors express all five subtypes.[177–179] The receptors are expressed not only on tumor cells but also in peritumoral veins.[180] Antiangiogenesis might be another antitumor mechanism of somatostatin analogues.[181]

Subcutaneous administration of octreotide and lanreotide every 8 to 12 hours can control the clinical symptoms in about 60% to 70% of patients with the carcinoid syndrome; these agents are considered the drugs of choice.[182–187] Octreotide and lanreotide decrease serotonin and urinary 5HIAA levels as well as plasma tachykinin and CgA



• **Fig. 45.20** Diagnostic algorithm for small intestinal neuroendocrine tumors (carcinoids). *CgA,* chromogranin A; *^{11}C-5-HTP,* ^{11}C-5-hydroxytryptophan; *CT,* computed tomography; *5HIAA,* 5-hydroxyindoleacetic acid; *MRI,* magnetic resonance imaging; *NSE,* neuron-specific enolase; *PET,* positron emission tomography; *SRS,* somatostatin-receptor scintigraphy; *Syn,* synaptophysin; *US,* ultrasound.

levels. The recommended dose for octreotide is 100 to 150 µg two or three times a day, a standard treatment for controlling clinical symptoms.[186] However, some patients require higher doses, up to a total of 3000 µg/day, to control the clinical symptoms and tumor growth, particularly during long-term therapy.[188]

Tachyphylaxis (reduced sensitivity) to somatostatin analogues can develop during long-term therapy.[189] Long-acting, slow-release formulations of octreotide acetate and lanreotide have been developed, and doses of octreotide LAR of 20 to 30 mg given once a month or lanreotide Autogel 90 to 120 mg once a month control clinical symptoms and hormone levels in 50% to 60% of patients with the carcinoid syndrome.[189,190] The long-acting formulations of somatostatin analogues have clearly improved the quality of life of patients by reducing the number of injections and provide more stable control of clinical symptoms.[189] A newer somatostatin analogue, pasireotide, binding to SSTR1, SSTR2, SSTR3, and SSTR5, has come into some clinical trials in carcinoid tumors. In a phase II study of carcinoid patients refractory to octreotide, the number of bowel movements and flushing episodes was reduced in one-third of the patients.[190,191] However, the drug did not exhibit superiority to standard octreotide in a randomized trial in small intestinal NETs.[189]

High-dose therapy with lanreotide (12 mg/day) and octreotide (3 mg/day) has increased the percentage of patients demonstrating a significant reduction in tumor size (12% vs. 5% for the standard dose).[192–195] Induction of apoptosis has been reported during high-dose therapy,[196] possibly mediated through activation of SSTR3. Ultrahigh-dose octreotide has generated significant antitumor responses in patients resistant to standard dose therapy.[195,196] A prospective randomized study in midgut carcinoids (PROMID) demonstrated a significantly longer time to progression for octreotide LAR compared to placebo.[197] These data have changed the US National Comprehensive Cancer Network (NCCN) guidelines for treatment of carcinoid tumors. All types of carcinoid tumors, irrespective of functionality, can now be treated with octreotide. The early results indicating an antitumor effect of somatostatin analogues is further supported by a 2014 study (CLARINET).[198] Lanreotide significantly prolongs progression-free survival time in nonfunctioning neuroendocrine tumors.[198]

For patients at risk for carcinoid crisis, somatostatin analogue therapy is the treatment of choice. Carcinoid crisis is a life-threatening complication of the carcinoid syndrome and can occur spontaneously or may be associated with stress and anesthesia, chemotherapy, and infections (see earlier discussion). Patients usually experience severe flushing, diarrhea, abdominal pain, and hypotension. Continuous infusion with somatostatin analogues, 50 to 100 µg/hour, is recommended and usually alters the life-threatening condition. It is also recommended that patients be given subcutaneous somatostatin analogues before surgery or other stressful situations.

Side effects of somatostatin analogue therapy have generally not been serious and occur in 20% to 40% of patients. They include pain at the injection site, gas formation, diarrhea, and abdominal cramping. Significant long-term side effects include gallstone formation, sludge in the gallbladder, steatorrhea, deterioration of glucose tolerance, and hypocalcemia.[103,184,185,188,189,195] The incidence of gallstones in patients treated over the long term has varied from 5% to 70%, and the incidence of symptomatic gallstones requiring surgical treatment is less than 10%.[189]

Interferons

Interferon-α (IFNα)—alone or in combination with a somatostatin analogue—is effective in the treatment of the carcinoid

syndrome. Symptomatic and biochemical control may be obtained in 40% to 50% of patients with the recommended doses of 3 to 5 million units of recombinant interferon alfa-2a or interferon alfa-2b three to five times per week subcutaneously.[184,199,200–204] Significant tumor reduction is reported in 10% to 20% of the patients.[204–206]

IFNα exerts a direct effect on the tumor cells by blocking cell division in the G_1/S phase, by inhibiting protein and hormone synthesis, and by reducing angiogenesis through inhibition of angiogenic factors bFGF and VEGF. It has also an indirect effect through stimulation of the immune system, particularly T cells and natural killer cells.[207,208] Response to IFNα can be predicted by analyzing induction of 2′,5′-oligoadenylate synthetase or protein kinase p68 (PKR), enzymes involved in cell cycle regulation and protein synthesis.[208–212] Long-acting formulations of IFNα are now available (pegylated interferons) that can be applied at doses of 80 to 150 μg/week subcutaneously.

Treatment with IFNα induces an intratumoral fibrosis that is not picked up by regular CT scanning or ultrasonography; therefore tumor size may remain unchanged.[213] The side effects of α-interferons are more pronounced than with somatostatin analogues and include chronic fatigue syndrome, anemia, leukopenia, and thrombocytopenia as well as the development of autoimmune reactions in 10% to 15% of the patients.[208,214] Most of the side effects are dose dependent and can be managed by individualizing the dose.

Patients with the carcinoid syndrome who have not responded to octreotide or IFNα alone may be given a combination of both agents. Such combinations have generated symptomatic control in 70% of patients and stabilization of tumor growth in 40% to 50% of patients.[215–217] The combination also offers better tolerance of α-interferons when somatostatin analogues are added. Moreover, somatostatin analogue treatment is hampered by development of tachyphylaxis with time, which means less sensitivity to the somatostatin analogue, necessitating escalating doses and, finally, withdrawal of the compound for several months, when IFNα therapy can continue. Conversely, IFNα can be withdrawn and the somatostatin analogue can be continued if severe side effects to IFNα (mainly chronic fatigue syndrome or mental depression) develop.[215–217] Recently, a prospective randomized phase III study between IFNα2b and bevacizumab showed similar antitumor activity.[218]

Chemotherapy

Most agree that patients with classic midgut carcinoids and the carcinoid syndrome, in which tumors show low proliferation capacity, should not receive chemotherapy. The results in various studies have been disappointing: Response rates are not more than 5% to 10%, are short lived, and are accompanied by considerable side effects.[219,220] The combination of streptozotocin and 5-fluorouracil, which has demonstrated antitumor effect in endocrine pancreatic tumors, has not shown similar effects in classic midgut carcinoids.[220] In foregut carcinoids, which usually manifest a more malignant behavior, however, cytotoxic treatment may be attempted. Such combinations include streptozotocin plus 5-fluorouracil, doxorubicin, cisplatin plus etoposide, and dacarbazine plus 5-fluorouracil.[221–223] Temozolomide has significant efficacy in foregut carcinoids.[224] All of these cytotoxic treatments can be combined with a somatostatin analogue.

Other Agents

Tyrosine kinase receptors (platelet-derived growth factor receptor [PDGFR] α/β, epidermal growth factor receptor [EGFR], VEGFR) are expressed in carcinoid tumor cells as well as in the stroma cells. Therefore treatment with tyrosine kinase receptor inhibitors has been attempted with objective response rates of about 10% to 15%.[225] Mammalian target of rapamycin (mTOR) inhibitors are new drugs that block the mTOR signaling pathway, which is activated in many tumors. Everolimus alone or in combination with octreotide has generated 15% to 20% objective responses.[226–228] Everolimus was evaluated in a randomized placebo-controlled study in nonfunctional lung or gastrointestinal NETs (RADIANT4). Treatment with everolimus was associated with significant improvement in progression-free survival.[225] However, in another trial in patients with carcinoid syndrome, no difference in overall survival was noted in patients receiving everolimus versus placebo (RADIANT2).[228]

Surgery

Because most tumors in patients with the carcinoid syndrome are malignant at the time of clinical presentation, surgical cure is seldom obtained. Resection of local disease or regional nodular metastatic disease can cure some patients; however, even if radical surgery cannot be performed, debulking procedures and bypass should always be considered and can be performed at any time during the course of treatment.[58,99,229]

A more proactive attitude among surgeons has emerged, and currently wider resections and debulking procedures are being performed.[229,230] Resection of primary tumor and locoregional metastases in patients has recently been debated. The final decision needs a prospective randomized trial.[231] In contrast to other metastatic tumors to the liver, in which liver transplantation has generally given poor results, an interest in liver transplantation is increasing for patients with metastatic carcinoids.[232–234] In a review of 103 patients with malignant neuroendocrine tumors, including both carcinoids and pancreatic endocrine tumors, 5-year and 2-year survival rates were 16% and 47%, respectively; however, recurrence-free survival rate was less than 24%.[234] Liver transplantation might be considered in younger patients (<50 years of age) with a life-threatening uncontrolled carcinoid syndrome during medical therapy or tumor-targeted radioactive treatment without known metastatic spread outside the liver (Milan criteria). However, a study from our group challenges the results of liver transplantation, with a 5-year survival time in patients younger than 55 years without surgery of 92 ± 9 months compared with transplanted patients fulfilling the Milan criteria showing a 5-year survival time of 97 ± 6 months.[232]

Another means of tumor reduction is hepatic artery embolization, which not only improves the carcinoid syndrome in about 50% of the patients but also reduces the tumor size in as many. The therapeutic effect may last for 9 to 12 months, and the procedure can be repeated.[235,236] Chemoembolization, simultaneous embolization with surgical gel (Gelfoam), and chemotherapy (doxorubicin, mitomycin C, cisplatin, 5-fluorouracil), or IFNα has resulted in symptomatic improvement in a significant number of patients with the carcinoid syndrome.[237,238] However, hepatic artery occlusion or embolization can result in serious side effects (nausea, vomiting, liver pain, fever) and major complications (hepatorenal syndrome, sepsis, gallbladder perforation, and intestinal necrosis). Complications are seen in 5% to 7% of patients.[236–238]

Other cytoreductive treatments include cryotherapy and radiofrequency ablation.[239] However, these procedures are limited to patients with smaller tumor burden, tumors less than 5 cm in diameter, and a limited number of metastases.

Irradiation

External irradiation has demonstrated limited efficacy and is used mainly to palliate symptoms related to bone and brain metastases.[240,241] MIBG is taken up by carcinoids and is concentrated. The possibility of radiolabeled MIBG therapy has been evaluated in a limited number of patients. The response rate has been reported to be about 30% with ^{125}I-MIBG or ^{131}I-MIBG.[242,243]

Somatostatin analogue–based tumor-targeted radioactive treatment has been applied using ^{111}In-DTPA-octreotide. Symptomatic improvement is reported in about 40% of the patients and tumor stabilization in about 30%.[244] Indium-111 (^{111}In) is a weak irradiator (Auger electrons) and has been replaced by yttrium-90 (^{90}Y) and lutetium-177 (^{177}Lu) (β-emitters).[245,247]

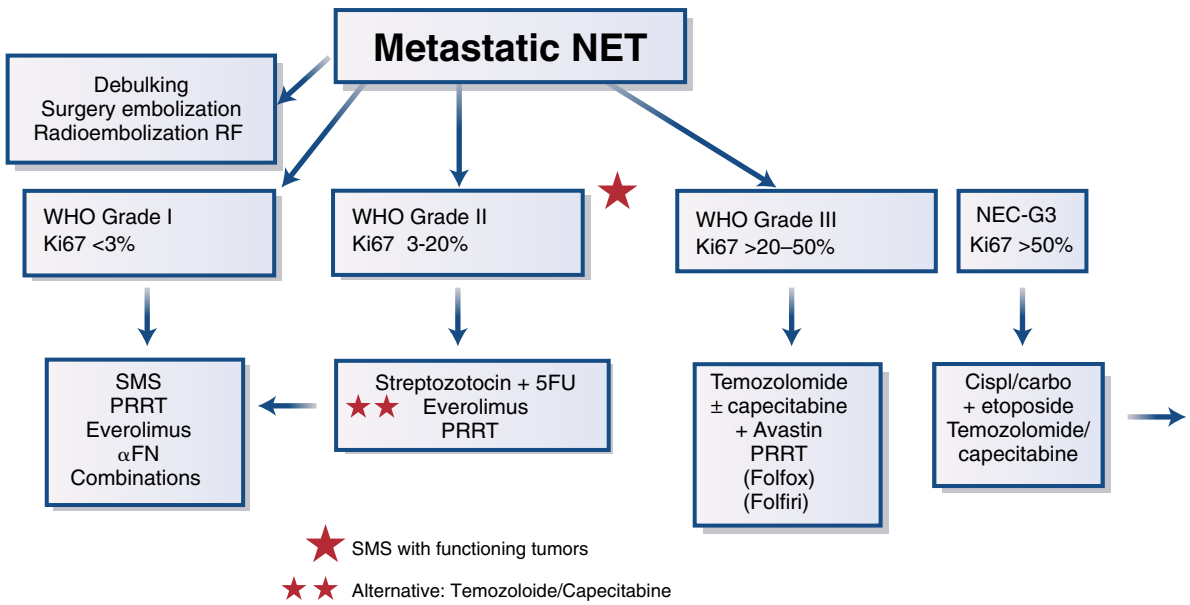
Studies with ^{90}Y-DOTA-octreotide have been reported with promising results.[245] The relatively new isotope, ^{177}Lu-DOTA-octreotate, has come into clinical use with further improved results. Significant tumor reduction occurs in 30% to 40% of patients with advanced disease. However, it is more effective for small tumors. It is an attractive mode of treatment because the radioactive ligand, after binding to the receptor, is internalized and transported to the cell nucleus, causing DNA damage.[245] Because tumor cells usually have higher-density somatostatin receptors (SSTR2 and SSTR5) than do surrounding normal tissues, the treatment might be better tolerated. A pivotal trial was recently published where small intestinal NETs were treated with either four cycles of ^{177}Lu-DOTATATE or 60 mg octreotide LAR every 4 weeks (NETTER1). Patients undergoing radioactive treatment showed a significant longer progression-free survival.[248] This trial has now been the basis for registration by EMA and FDA for treatment of patients with nonsurgical metastatic gastrointestinal NETs. Most recently, a genomic signature in blood was shown to predict response of PRRT with ^{177}Lu octreotide.[249]

In liver-dominant disease, radioembolization might be a valid alternative using ^{90}Y microspheres in the form of glass or resin beads injected into the hepatic artery.[250] The current treatment of metastatic carcinoid tumor is summarized in an algorithm (Fig. 45.21).

Prognosis

Clinically, the carcinoid syndrome is generally a manifestation of advanced disease. Carcinoids from various sites differ not only in the percentage developing the carcinoid syndrome but also in their aggressiveness. Survival rates for patients with various carcinoids depend on the site, the extent of the tumor, and tumor biology. In patients with only localized disease, the 5-year survival rate for midgut carcinoids is about 65%, not essentially higher than that for patients with regional metastases. In patients with distant metastases, the 5-year survival rate is reduced to 39%.[26–28,251,252] The relative 5-year, 10-year, and 15-year survival rates for midgut carcinoids were 67%, 54%, and 44%, respectively.[251] The 5-year and 10-year survival rates for typical bronchial carcinoid are 95% and 80%. Atypical lung carcinoids have significantly shorter survival, only 50% 5-year survival rate.[23]

One of the main determinants of survival in carcinoid patients is the presence of metastases. Female gender and a younger age are associated with a better prognosis. Other factors that correlate with lower survival rates are high CgA level at diagnosis and high proliferation index (Ki67).[38,117] During the 1990s there was a reduced incidence of death from carcinoid heart disease, possibly a result of earlier diagnosis, active surgery, and the introduction of somatostatin analogues and α-interferons. In an earlier study performed by our group, 30% of the patients died of carcinoid heart complications.[25] In a more recent 2008 study, this rate was reduced to less than 10%.[27] Clinically significant carcinoid heart disease is now rare. Between 5% and 10% of patients with



• **Fig. 45.21** Treatment algorithm for metastatic neuroendocrine tumor (NET). *5FU,* 5-Fluorouracil; *cispl/carbo,* cisplatin/carboplatin; *FN,* fibronectin; *IFN,* interferon; *NEC,* neuroendocrine carcinoma; *PNET,* primitive neuroectodermal tumor; *PRRT,* peptide receptor radiotherapy; *SMS,* somatostatin; *RF,* radiofrequency; *WHO,* World Health Organization.

carcinoids are at an increased risk for developing simultaneous adenocarcinoma of the large intestine. The occurrence of a second malignancy is associated with a worse prognosis.[23,26] In two studies (in 2011 and 2014) comparing almost 900 patients with midgut or hindgut tumors, survival correlated with the WHO grading system as well with the TNM staging. For grade 1 (G1) the 10-year survival rate was 80%, for G2 tumor 50% to 66%, and for G3 tumor 35%; for stage I and II tumors survival rate was 100%, stage III 85%, and stage IV 35%.[251]

Significant negative prognostic factors for overall survival were old age at diagnosis, carcinoid heart disease, liver tumor load, high WHO grade, and peritoneal carcinomatosis.[250,252] Locoregional surgery had a positive impact on survival.[252]

Other Flushing Disorders

Medullary Thyroid Carcinoma and VIPoma

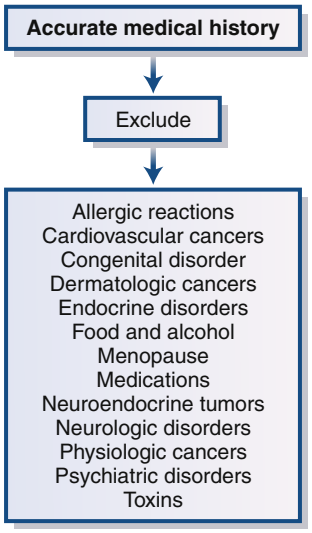
Other neuroendocrine tumors, such as medullary thyroid carcinoma (MTC) and VIP-producing tumors (ganglioneuroma, endocrine pancreatic tumors), can manifest with flushing syndromes (Fig. 45.22).[253,254] Patients might also have diarrhea, particularly those with VIP-producing tumors, which are accompanied by a severe secretory diarrhea. In patients with MTC, flushing and diarrhea are infrequent symptoms and are seen mainly in patients with high circulating levels of calcitonin and CGRP.

The mechanism behind the flushing and diarrhea is unknown, but it has been postulated to be mediated through prostaglandins stimulated by calcitonin. The frequency of flushing and diarrhea is usually less than 5% in patients with advanced metastatic MTC.[253,255] Treatment is directed against tumor growth and can consist of surgical resection, embolization of liver metastases, and cytotoxic treatment (doxorubicin-based combination therapies). Somatostatin analogue therapy can alleviate the diarrhea. Therapy with tyrosine kinase inhibitor vandetanib can reduce these symptoms and present an antitumor effect.[255]

VIPoma or WDHA (*w*atery *d*iarrhea, *h*ypokalemia, and *a*chlorhydria) syndrome (Verner-Morrison syndrome) is associated with severe secretory diarrhea (up to 15 L/day), and some patients also display a continuous whole-body violaceous flushing and hypotension.[254,256,257] The syndrome also includes achlorhydria, hypokalemia, and metabolic acidosis and is related to overproduction of VIP and a related peptide-peptide histidine methionine. These patients have tumors in the pancreas, lung, or sympathetic ganglia.[256,257]

The diagnosis is confirmed by measuring plasma VIP, which usually exceeds 70 pmol/L.[257]

Treatment is directed against the tumor and hormone excess. Administration of somatostatin analogues by either subcutaneous or IV infusion in the worst cases can control clinical symptoms.[258] Cytotoxic treatment with streptozotocin-based combinations, 5-fluorouracil, or doxorubicin is recommended for malignant cases.[256] Inhibitors, such as mTOR (everolimus), demonstrate an antitumor effect in VIPomas with reduction of clinical symptoms.[225]



• **Fig. 45.22** Evaluation of flushing disorders. (From Yale SH, Vasudeva S, Mazza JJ, et al. Disorders of flushing. *Compr Ther.* 2005;31:59–71.)

Mastocytosis and Related Disorders

Mastocytosis, as well as other systemic mast cell activation, is clinically related to flushing disorders. Most patients with mastocytosis have an indolent course, but some forms of mastocytosis are aggressive. Symptoms are attributed primarily to paroxysmal mast cell activation.[259,260]

Most patients with mastocytosis have evidence of cutaneous involvement, the most common being multiple, small pigmented lesions that produce urticaria on stroking with a blunt object (Darier sign); this condition is called *urticaria pigmentosa*.[261,262] Another form of cutaneous mastocytosis is a more telangiectatic form called *telangiectasia macularis eruptiva persistans.* Hepatomegaly and splenomegaly can be due to infiltration of mast cells, and hepatic fibrosis is also common.[262,263]

Bone involvement can be manifested by either osteoporosis or osteosclerosis.[264] Systemic mastocytosis can also involve the GI tract with mucosal nodules in the ileum, stomach, and large bowel.[265]

Hematologic abnormalities are nonspecific, with marked mast cell infiltration of the bone, anemia, leukocytosis, sometimes lymphadenopathy, and eosinophilia.[266] In a subgroup of patients, the mastocytosis is secondary to primary hematologic disorders, usually myeloproliferative or myelodysplastic disease.[266–268] Mast cell leukemia has been reported in rare cases.[269] Some cases with FIP1-like 1/PDGFRα *(FIP1L1-PDGFRA)* gene arrangement overlap to chronic eosinophilic leukemia. They also manifest elevated serum tryptase levels.[270] A majority (>80%) of patients with systemic mastocytosis have an activating c-kit mutation in codon 816 in the mast cell.[270]

Clinical signs of systemic mastocytosis include flushing, tachycardia, hypotension, and sometimes nausea, vomiting, and diarrhea. This syndrome resembles the carcinoid syndrome. Histamine is a potent vasodilator and is released from mast cells. Other mediators of the syndrome are the release of prostaglandin D_2, tryptase, and heparin.[270–273] Prostaglandin D_2 is a more potent mediator than is histamine.

The diagnosis is made by measurement of histamine and histamine metabolites in the urine.[272] Quantification of histamine metabolites (*N*-methylhistamine and methylimidazole acetic acid) appears to be more sensitive for overproduction of histamine in patients with mastocytosis.[273] Measurement of endogenous production of prostaglandin D_2 can be assessed by quantifying the major urinary metabolite (9α-hydroxy-11,15-dioxo-2,3,18,19-tet-ranorprost-5-ene-1,20-dioic acid).[273] However, these measurements can be done only in specialized laboratories. Measurement of the tryptase release is easier to perform, and increased quantities

of this granule-associated enzyme tryptase (>20 ng/mL) can be detected by immunoassay.[274] Bone marrow analysis of CD25 cells might support the diagnosis of mast cell disease.[275]

Treatment depends on the severity of the disease. As in the treatment of allergic anaphylaxis, epinephrine is effective in reversing the hypotension associated with mast cell mediator release[275]; thus these patients should have constant access to epinephrine in the form of subcutaneous injection or inhalation. Chronic therapy to prevent acute attacks includes antihistamine therapy combined with inhibition of prostaglandin biosynthesis. Blockade of both histamine H_1 and H_2 receptors is required to prevent the vasodilator effect of histamine.[246,275,276]

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the cyclooxygenase enzyme that catalyzes the formation of prostaglandins. Aspirin has been used, but some patients cannot tolerate it because of side effects in the gut and allergic reactions.[277] In patients resistant to both antihistamines and NSAIDs, IFNα has been attempted with a reduction in mast cell numbers as well as excretion of mast cell mediators. Treatment with IFNα is still considered experimental.[278] A subset of patients who carry the *FIP1L1-PDGFRA* oncogene will achieve complete clinical, histologic, and molecular remission with imatinib mesylate therapy, in contrast to those with c-kit D816V mutation.[277]

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The complete list of references is available online at ExpertConsult.com.

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Managing Reproductive Disorders in Cancer

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CHAPTER OUTLINE

KEY POINTS

- With increasing cancer survival the adverse sequelae of the tumor, and treatment thereof, on multiple organ systems have increasingly been recognized. The endocrine system is one of the most frequent organ systems to be affected, with greater than 40% of childhood cancer survivors showing abnormalities.
- Oncofertility is a relatively new interdisciplinary field at the intersection of oncology and reproductive medicine that expands fertility options for young cancer patients.
- Aggressive gonadotoxic anticancer regimens including alkylating chemotherapy and total-body irradiation are used often in treating young cancer patients. The risks of gonadotoxicity and subsequent iatrogenic fertility loss depend mainly on the type and stage of the disease, dose and dosage of anticancer therapy, and the age of the patient at the beginning of treatment.
- If the risk of gonadotoxicity and subsequent iatrogenic fertility loss is greater than 50% and the patient desires to have children in the future, a fertility preservation and restoration strategy should be initiated before, during, and after chemotherapy and radiotherapy.
- Fertility preservation and restoration strategy should be individualized and tailored to the patient's circumstances after obtaining patient informed consent or that of the legal guardians for children.
- Fertility preservation and restoration options include gamete and gonadal tissue freezing and further autotransplantation, gonadal protection, gonadal tissue bioengineering, stem cells reproductive technology, and neoadjuvant cytoprotective pharmacotherapy.
- A multidisciplinary oncofertility approach involving strong coordination among oncologists, gynecologists, endocrinologists, surgeons, reproductive biologists, research scientists, and patient navigators is essential to ensure a high standard of care.

What Is Oncofertility, and Why Is It Important?

Scope of the Problem

Cancer affects more than 14 million individuals annually worldwide, of whom 10% are of reproductive age.[1] Among children and adolescents, cancer survival rates range from 71% to 86%, mostly due to successful early detection and effective cancer therapies. However, up to 80% of children, adolescents, and adults with cancer receive treatment that may temporarily or permanently affect their reproductive health, including fertility, gonadal function, and psychosexual well-being.[2] At the heart of the field of oncofertility is the consideration of a patient's reproductive health at the same time that decisions are being made about life-preserving but fertility-threatening cancer therapy. Thus, a comprehensive discussion of the endocrine consequences of cancer treatment, including fertility loss, dysfunction of steroid hormone–dependent tissues (e.g., bone and heart), and impaired psychosexual health, as well as a discussion of fertility preservation and contraception options, is critical for optimizing the future quality of life (QoL) of cancer survivors.[3]

Current oncology clinical practice guidelines recommend that health care providers engage in an active discussion about the potential effects of cancer therapy on future reproductive health prior to, during, and after treatment.[4] Although there is growing awareness of the impact of cancer treatment on reproductive health among health care providers, up to 50% of patients with cancer are not offered counseling on reproductive health prior to starting cancer treatment.[2,5,6] Cancer patients are deeply

interested in engaging in a discussion with their health care providers about reproductive health throughout the course of their cancer diagnosis, treatment, and follow-up. Among young women with cancer, 62% express having had plans for children before receiving their cancer diagnosis.[4,7] In fact, concern about future fertility is a source of distress among women of reproductive age who are undergoing cancer therapy, with one-third reporting symptoms of depression and/or anxiety that interfere with daily functioning and 15% requiring psychotropic medications.[8] Even among those cancer patients who do receive counseling regarding reproductive health prior to initiation of cancer therapy, many report dissatisfaction with the quantity and quality of the information provided.[4] For example, adult women with breast cancer report overall satisfaction with the fact that fertility preservation was mentioned prior to initiation of treatment, but many also note frustration with the focus on infertility as opposed to the variety of fertility preservation options and success rates, the cost of fertility preservation procedures, and experimental protocols. Importantly, adult women who felt they had received adequate and high-quality information about fertility preservation prior to initiation of cancer therapy were five times more likely to pursue fertility preservation than women who did not receive counseling. These women also report less regret and better QoL even if they chose not to pursue fertility preservation prior to starting treatment.[4,7] Among adult men with cancer, counseling regarding fertility preservation prior to cancer therapy is felt to be particularly important, as fertility status is interwoven with their sense of masculinity and their intimate relationships.[9] Men pursuing fertility preservation report reassurance about the prospect of biologic fatherhood as a reaffirmation of their masculine identity.[9] However, male cancer patients are less likely to be informed about potentially compromised fertility than their female counterparts.[5]

Furthermore, adolescent cancer patients face unique additional challenges in navigating issues related to personal body image, intimacy, relationships, and sexuality during and after cancer treatment. Both adolescents with cancer and their parents, who frequently serve as primary decision makers, express concern about future fertility. However, parents of adolescents with cancer prioritize initiation of therapy over fertility preservation and underestimate their children's concerns about future fertility.[10] Parents of pediatric cancer patients sometimes regulate the information presented to younger cancer patients (12–15 years of age) to reduce distress during a particularly emotional time. Consequently, some adults with a history of cancer in childhood and adolescence convey dissatisfaction about having been fully or partially excluded from conversations regarding the impact of cancer treatment on their fertility by both their parents and their health care providers.[11] Furthermore, many of these adults report having received insufficient information about reassessing fertility potential after completion of cancer treatment, including counseling regarding safe sex practices and contraception, family planning, and nonbiologic parenthood options.[11] In fact, both adult survivors of pediatric cancer and their parents retrospectively express regret over inadequate allotment of time and attention to discussions about fertility prior to initiation of cancer therapy and attribute responsibility to the health care providers for insufficient counseling.[12] Parents of adult survivors of pediatric cancer also retrospectively report that their children should have been involved in discussions regarding future fertility, regardless of their age at cancer diagnosis. For many adult survivors and their parents, discussion of future fertility prior to cancer therapy signifies hope for the future.[12]

Reproductive health counseling should also include discussion of chronic comorbidities associated with cancer treatment and subsequent endocrine dysfunction, including osteoporosis, cardiovascular disease, cognitive dysfunction, and mental health issues.[13] Not only are certain cancer treatments (anthracyclines, trastuzumab, chest irradiation) associated with cardiac toxicity,[14,15] but specific chemotherapeutic and immunologic agents, cranial irradiation, surgical tumor removal, and antihormone therapies are also linked with adverse metabolic parameters such as obesity, glucose intolerance, insulin resistance, hypertension, atherosclerosis, and dyslipidemia that increase the risk of cardiovascular disease.[16,17] In addition, many cancer treatments result in estrogen and testosterone deficiency by damaging the organs that produce these hormones, thereby inhibiting estrogen receptor–mediated signaling and aromatization of testosterone to estrogen and downregulating the hypothalamic-pituitary-gonadal axis. Considering that these steroid hormones are essential for bone health, cancer treatments that deplete estrogen and testosterone are associated with altered bone physiology, a rapid decline in bone mineral density, and a twofold increased risk of fracture in both men and women with cancer. In fact, more than 80% of men with prostate cancer experience a decline in bone mineral density after medical or surgical castration, and women with breast cancer experience an accelerated decline in bone density after onset of therapy-induced ovarian insufficiency. Cancer therapy is also associated with chronic nutritional deficiencies that impede normal bone building and with chronic deconditioning and treatment-related neuropathies that increase the risk of falls and subsequent fractures.[18] Last, cancer survivors also experience greater mental health morbidity than the general population,[19] with greater reported emotional and social distress among older, single, unemployed female cancer survivors of bone and central nervous system (CNS) tumors.[20] Sexuality, fertility, and parenthood are particularly important factors in psychosocial well-being among adult cancer survivors and are major contributors to the long-term psychological sequelae of cancer diagnosis and treatment.[21]

Reproductive Health Concerns During Cancer Treatment

Fertility

Fertility is a critical component of reproductive health and plays a significant role in health-related QoL among cancer patients, especially women. In fact, 60% of women and more than 50% of men with cancer report a desire for parenthood.[22] Temporary or permanent infertility after cancer treatment may affect up to 60% of cancer survivors. The impact of cancer treatment on fertility is often unpredictable and is influenced by the type of chemotherapy agent, dose of radiation, patient age, and gonadal reserve prior to therapy. For women with cancer, alkylating agents and pelvic irradiation are particularly toxic to primordial follicles, and these therapies deplete the ovarian reserve and shorten the reproductive window.[23] For men with cancer, chemotherapy and pelvic irradiation are associated with azoospermia within 3 months of the start of treatment, and almost a quarter of male cancer survivors experience persistent azoospermia or oligospermia. Chemotherapy and radiation may also result in unrepaired DNA damage in spermatogonia, which compromises fertility and/or increases the risk of congenital abnormalities in offspring.[24]

Biologic parenthood is central to cancer patients' sense of adult and gender identity, existential purpose, and perception of social acceptance. Female cancer survivors view gestation as a marker

of femininity, whereas male cancer survivors are more focused on biologic fatherhood as a marker of masculinity. Unknown fertility status further augments reproductive health-related anxiety among cancer survivors, especially those who are young and single and may fear that potential infertility will adversely affect their ability to find a partner. Thus, reassessment of reproductive function after completion of cancer therapy is an important aspect of the comprehensive care of cancer patients. Notably, cancer survivors who are fertile may choose not to pursue conception due to concerns about cancer recurrence, transmission of cancer to offspring, and the effect of their own morbidity and mortality on their future child.[22] Thus, health care providers involved in the care of cancer patients must not only address the logistics of fertility preservation and initiation of cancer treatment but also provide compassionate support related to the emotional aspects of oncofertility that are important for patients' overall and long-term well-being.

Contraception During Cancer Therapy

Despite the gonadotoxic effects of cancer therapy, many cancer patients remain fertile during treatment. Up to 10% of women undergoing cancer treatment do not consistently use a reliable contraceptive method,[25] and 3% of women with cancer experience an unplanned pregnancy.[26] Selection of an appropriate method of female contraception is guided by the type of cancer (e.g., a nonhormonal copper intrauterine device in women with breast cancer vs. a contraceptive implant in women with cervical cancer), functional status and comorbidities (e.g., immunocompetence, thromboembolic risk, liver dysfunction), and personal preference.[27] Barrier contraception provides protection against sexually transmitted infections[22] but is not as effective as intrauterine devices and contraceptive implants in preventing pregnancy due to high user failure rates. Emergency contraception, including a short course of oral agents, is considered safe for women with cancer. Options for male contraception are being expanded rapidly, and many have been shown to be acceptable among men. Methods that induce azoospermia include androgens alone or in combination with progestins, gonadotropin-releasing hormone (GnRH) agonists, GnRH antagonists, and vasectomy. Reversible inhibition of sperm under guidance using a polymer gel causes reversible sperm damage, whereas vaso-occlusive devices prevent transmission of sperm through the vas deferens.[28] However, use of these male contraceptive methods is limited by the discomfort of administration, cost, stigma against male contraception, concerns regarding effects on libido and sexual function, and ease of reversibility.[29] Selective androgen receptor modulators and nonhormonal agents are currently being investigated as contraceptive options in men.[29] Among women with cancer, contraceptive use is discordant with the desire for pregnancy. Many women with cancer indicate that they would opt for abortion if they became pregnant during cancer treatment, although abortion rates among pregnant women with cancer are not well reported. Cancer patients prioritize contraception as an important aspect of their care, but only a few studies have adequately documented the quantity and quality of contraception counseling in cancer patients. Notably, 65% of women who participate in contraception counseling report using an effective method of contraception.[25]

Appropriate timing of conception after completion of cancer treatment is also an important aspect of reproductive health in cancer survivors. Conception should not be attempted sooner than 6 months after the completion of chemotherapy and 12 months after completion of radiation therapy in women with cancer,[23] and no sooner than 12 to 24 months after completion of therapy in men given the risk of unrepaired DNA damage in ejaculated spermatozoa.[24] Additionally, the risk of cancer recurrence must also be considered prior to attempting conception. Appropriately planned contraception allows cancer patients to time pregnancy to a stage of better maternal health and to optimize fertilization, embryonic development, and early pregnancy outcomes.[24,30]

Gonadal Dysfunction

Women With Cancer

Women undergoing surgery, chemotherapy, or radiation therapy for the treatment of cancer are at an increased risk of premature ovarian insufficiency (POI). POI is characterized by amenorrhea and gonadal steroid hormone deficiency with concurrent elevation in serum follicle-stimulating hormone on at least two occasions at least 1 month apart in women younger than 40 years. The prevalence of POI among young female cancer survivors ranges widely at between 2% and 82%. Age at diagnosis, type of chemotherapy (e.g., alkylating agents and alkylating-like agents), dose and target area of radiation, and a diagnosis of Hodgkin lymphoma are risk factors for POI. Additionally, oophorectomy is associated with a rapid decline in estrogens and androgens that precipitates symptoms of POI, including hot flashes, night sweats, vaginal dryness, and decreased sexual desire. Vulvectomy and radical hysterectomy add to scarring and pain of the perineum, further augmenting sexual dysfunction. Importantly, ovarian hormone deficiency is associated with increased morbidity (e.g., osteoporosis and fracture risk, cardiovascular disease, mental illness, and cognitive decline) and mortality. Recovery of ovarian function is variable, somewhat unpredictable, and dependent on baseline ovarian reserve. Methods for better predicting ovarian reserve in female cancer survivors, including measurement of serum antimüllerian hormone, antral follicle count, and ovarian volume and surface area, are still being optimized. Standardized methods for assessing ovarian reserve (rather than or in addition to symptoms of POI) among female cancer survivors could be used to direct counseling on contraception and guide screening for long-term complications of POI.[31,32]

Several strategies have been developed to address the symptoms of POI. Hot flashes may be managed with lifestyle changes such as weight management and avoidance of triggers; cognitive behavioral therapy and hypnosis; and specific, nonhormonal medications.[14] Incontinence, vaginal dryness, and dyspareunia associated with estrogen deficiency may be improved with pelvic floor physical therapy, clitoral therapy devices, avoidance of irritants, lubricants/moisturizers, topical anesthetics, topical and intravaginal estrogen, testosterone, or dehydroepiandrosterone, and psychotherapy.[14] Although hormone replacement therapy is sometimes unfavorable in women with hormone-sensitive cancers, such as breast, endometrial, cervical, and some ovarian cancers, a few studies have demonstrated that hormone replacement therapy in female survivors of early gynecologic cancer is not associated with an increased risk of cancer recurrence.[14,33] However, alleviation of menopausal symptoms in female cancer survivors is not always associated with better QoL; thus, the psychosocial aspects of cancer survivorship must also be addressed.[32]

Men With Cancer

Hypogonadism in men with cancer can result from direct damage to the testes, such as a consequence of surgical resection of gonadal tumors or exposure to pelvic irradiation or systemic chemotherapy. Testicular dysfunction can also occur secondary to pituitary or hypothalamic damage, as in the case of cranial radiotherapy

or resection of tumors of the CNS. Men with hypogonadism due to cancer therapy may present with ambiguous symptoms of decreased sexual desire or function, hot flashes, fatigue, slowed mental processing, and dysthymia (persistent depressive disorder), which may be difficult to distinguish from the common side effects of cancer treatment. They may also present with objective changes in body habitus (e.g., decreased muscle tone, gynecomastia, weight gain, redistribution of fat, loss of body hair), reduced testicular size, and normochromic normocytic anemia. Men undergoing bilateral orchidectomy, hypophysectomy, or pelvic or cranial radiotherapy may develop symptoms of hypogonadism soon after treatment, whereas those undergoing systemic chemotherapy may experience a slower onset of hypogonadal symptoms. Many men treated with chemotherapy or radiation to the retroperitoneum will experience some manifestations of testosterone deficiency over the long term, with a higher risk among men older than 45 years.[34] The effect of chemotherapy on testicular function is agent specific, even within the same chemotherapeutic category.[31] Baseline testicular volume is inversely associated with risk of hypogonadism after cancer treatment.[34]

Testosterone deficiency is linked to decreased sexual desire, erectile dysfunction, and orgasmic disorders that develop across the spectrum of subnormal and low testosterone concentrations. Symptoms of hypogonadism are associated with worse health-related QoL. Testosterone deficiency is also associated with osteoporosis, depression, metabolic dysfunction, cardiovascular disease, and mortality. Thus, it is important to assess for hypogonadism in men undergoing cancer treatment.[34] Testosterone replacement is readily available but may not be an option for men with advanced prostate cancer or breast cancer.[34]

Physical Changes Related to Cancer Therapy

Anatomic changes after mastectomy for the treatment of breast cancer or hysterectomy for the treatment of cervical or endometrial cancer adversely affect self-esteem, decrease sexual desire, and negatively affect a woman's sense of sexuality and intimacy.[22,35,36] Nipple-sparing mastectomy has evolved as a surgical alternative that is associated with better cosmetic outcome, greater patient satisfaction, psychosocial and sexual well-being, and higher health-related QoL.[37,38] Considering that few surgical alternatives are available for women with gynecologic cancer, the impact of gynecologic surgery on psychosocial well-being must be identified and addressed in this cohort of cancer patients. Men with prostate and testicular cancer also suffer from changes to body image, self-perceived masculinity, and QoL after surgery.[39] Among testicular cancer survivors who undergo surgical removal of testis (orchiectomy) for cancer treatment, 7% report problems with ejaculation and erection, 17% report negative body image, 25% report decreased sexual interest, and 40% report reduced sexual activity 3 years after completion of therapy. Sexual dysfunction is particularly prevalent among men who undergo retroperitoneal lymph node dissection. Men who experience these unfavorable outcomes after cancer surgery also report lower marital satisfaction and compromised long-term relationships.[40] More than a quarter of men who undergo orchiectomy for unilateral testicular cancer opt for a testicular prosthesis, noting reasons related to regaining accepted male appearance, and more than 80% note long-term satisfaction with the prosthesis.[41] It is clear that anatomic changes related to cancer therapy have significant implications for self-image, sexual health, and QoL among men and women with cancer and must be appropriately addressed in the comprehensive care of cancer survivors.

Logistics of Fertility Preservation

Importance of Fertility Preservation and Restoration

When aggressive chemotherapy and radiotherapy are used, gonadotoxicity may occur as a side effect, leading to impairment of reproductive function, impairment of gonadal steroid hormone–dependent tissues (e.g., bone and heart), and fertility loss. To avoid or at least mitigate these devastating complications, effective strategies for preserving and restoring fertility in young patients with cancer should be offered before, during, and after chemotherapy and radiotherapy.[42–46]

As the numbers of young cancer patients surviving into adulthood have increased due to advances in early diagnosis and treatment, long-term health issues such as fertility have gained growing attention. To date, assisted reproduction technologies (ARTs), cryobiology, and experimental translational medicine protocols have been utilized to prevent or overcome chemotherapy- and radiotherapy-induced gonadotoxicity in patients with cancer to preserve and restore fertility. Each fertility preservation option has advantages and disadvantages and may not be feasible for all patients. A growing recognition of the importance of fertility preservation for patients with cancer is underscored by the numerous international fertility preservation and restoration guidelines that have been published in the past years by the American Society of Clinical Oncology (ASCO),[47–49] the American Society for Reproductive Medicine (ASRM),[50,51] the European Society for Medical Oncology (ESMO),[52,53] the American Oncofertility Consortium,[54,55] the International Society for Fertility Preservation,[56–59] the National Comprehensive Cancer Network,[60] the American Academy of Pediatrics,[61] the Association of Pediatric Hematology/Oncology Nurses,[62] and FertiPROTEKT (the German fertility preservation network).[63–65]

Fertility Loss, Preservation, and Restoration in Female Cancer Patients

Epidemiology of Cancer in Young Female Patients

According to statistics from the United Kingdom (2013–2015), the most common cancers in prepubertal girls (age 0–14 years) are leukemias (29%), CNS malignancies (28%), lymphomas (7%), and renal (6%) cancers. The most common cancers in female adolescents and young adults (AYA) (age 15–24 years) are lymphomas (20%), melanoma (16%), CNS (14%), and leukemias (7%). The most common cancers in adult females (age 25–49 years) are breast (44%), melanoma (9%), cervical (8%), CNS (6%), and ovarian (5%). The most common cancers that occur during the female reproductive years and may require aggressive gonadotoxic chemotherapy and radiotherapy are breast, cervical, leukemia, lymphoma, CNS, renal, and bone[64] (Table 46.1).

Risks of Gonadotoxicity and Fertility Loss in Young Female Patients

In young female patients undergoing cancer treatment, gonadotoxicity, POI, premature ovarian failure (POF), and fertility loss are more likely to occur when the ovaries are exposed to alkylating chemotherapies such as cyclophosphamide, ifosfamide, and busulfan; after ionizing radiotherapy to the pelvis and abdomen; or following cranial or total-body irradiation. The degree of gonadotoxicity depends mainly on the type, dose, and duration of cancer therapy, as well as the age of the patient and the type and

TABLE 46.1 Common Cancers Across the Life Span According to Reproductive Age in the United Kingdom (2013–2015)

	Female	Male
Prepubertal (0–14 years)	Leukemias (29%), central nervous system (28%), lymphoma (7%), and renal (6%)	Leukemias (31%), central nervous system (26%), and lymphoma (13%)
Adolescents and young adults (15–24 years)	Lymphomas (20%), melanoma (16%), central nervous system (14%), and leukemias (7%)	Germ cell tumors (27%), lymphomas (22%), central nervous system (13%), and leukemias (10%)
Adults (25–49 years)	Breast (44%), melanoma (9%), cervix (8%), central nervous system (6%), and ovary (5%)	Testis (14%), melanoma (11%), central nervous system (10%), bowel (10%), and head and neck (8%)
Most common types of cancer that may require aggressive (gonadotoxic) anticancer chemotherapy and radiotherapy and necessitate prior fertility preservation measures	Breast, cervical, leukemia, lymphoma, central nervous system	Testicular, germ cell tumor, leukemia, lymphoma, central nervous system

Data from Cancer Research UK. Cancer incidence by age. Available at http://www.cancerresearchuk.org/health-professional/cancer-statistics/incidence/age. Accessed February 8, 2019.

stage of cancer. Some guidelines have attempted to classify cancer treatments according to their gonadotoxicity and associated risks of POF and fertility loss in women[47–49,66,67] (Table 46.2).

Fertility Preservation and Restoration Options for Young Female Patients

When the estimated risk of gonadotoxicity from cancer therapy is greater than 50%, fertility preservation strategies should be offered to young patients (<40 years of age) prior to starting treatment. Several established, debatable, and experimental options are available for fertility preservation for young women and girls with cancer. Established options include embryo freezing and egg freezing. Debatable options include ovarian protection techniques such as treatment with GnRH analogues and hormonal suppression, surgical ovarian transposition (oophoropexy), gonadal shielding, and the use of fractionated chemotherapy and radiotherapy. Experimental options include ovarian tissue freezing for use in future autotransplantation, oocyte in vitro maturation (IVM), artificial ovary systems, stem cell transplantations, and neoadjuvant cytoprotective pharmacotherapy, among others[68–74] (Table 46.3 and Fig. 46.1).

Established Options for Fertility Preservation in Women and Girls

Embryo Freezing

Embryo freezing was the first established method for preserving female fertility and is still considered the gold standard. It involves cryopreservation of in vitro–fertilized mature oocytes via slow freezing or vitrification; the latter is now preferred due to a better post-thaw survival rate.[75–77] Embryo freezing requires prior ovarian stimulation, mature oocyte retrieval, and sperm for in vitro fertilization (IVF).[78–83] Therefore, it is not suitable for prepubertal girls who have an inactive hypothalamic-pituitary-ovarian axis and for single women who do not wish to use donated sperm for personal, ethical, or religious reasons. It is also not suitable for women with estrogen-sensitive cancers, such as breast and endometrial cancers, as conventional ovarian stimulation may lead to high serum estrogen levels. Alternative ovarian stimulation protocols to minimize the effects of elevated estrogen use either tamoxifen (a selective estrogen receptor modulator)[84,85] or letrozole (an aromatase inhibitor).[86–91] In addition, conventional ovarian stimulation may take up to several weeks and carries a risk of ovarian hyperstimulation syndrome); thus, it may not be a suitable option for women with highly aggressive malignancies (e.g., hematologic) that require immediate cancer treatment. For these women, random-start ovarian stimulation for emergency fertility preservation may be an option.[92–99] The standard storage period for frozen embryos, eggs, and sperm is 10 years, although longer storage periods may be possible. In healthy women, the live birth rate per frozen embryo transfer is nearly 30%.[100–102] However, in women with cancer, the live birth rate per frozen embryo transfer is reduced to approximately 15%, without any increased risk for congenital abnormalities.[103]

Egg Freezing

In 2012, the ASRM approved egg freezing as an established option for female fertility preservation.[104] Egg freezing involves cryopreservation of mature oocytes via slow freezing or vitrification.[105–108] Like embryo freezing, egg freezing requires prior ovarian stimulation for mature oocyte retrieval but does not involve IVF. Thus, egg freezing is not suitable for prepubertal girls; however, it is an option for single women who do not want to use donor sperm. Egg freezing carries the same disadvantages related to ovarian stimulation mentioned earlier for embryo freezing. In healthy women, the live birth rate per frozen oocyte is approximately 6% but continues to improve steadily due to advances in vitrification protocols and egg donation programs.[109–113] However, in women with cancer, there is not enough data on egg freezing outcomes to estimate the live birth rate per frozen oocyte. To date, only a few live births have been reported after oocyte vitrification in women with cancer.[113–115] Until more data become available, extrapolating the outcomes of egg freezing to female cancer patients should be done with caution during oncofertility counseling.

Debatable Options for Fertility Preservation in Women and Girls

GnRH Analogues and Hormonal Suppression

GnRH analogues are commonly prescribed in the fields of gynecologic endocrinology and reproductive medicine; however, the role of GnRH analogue treatment before and during chemotherapy to protect the ovaries from damage is widely debated.[116–120] Some randomized trials, systematic reviews, and meta-analyses

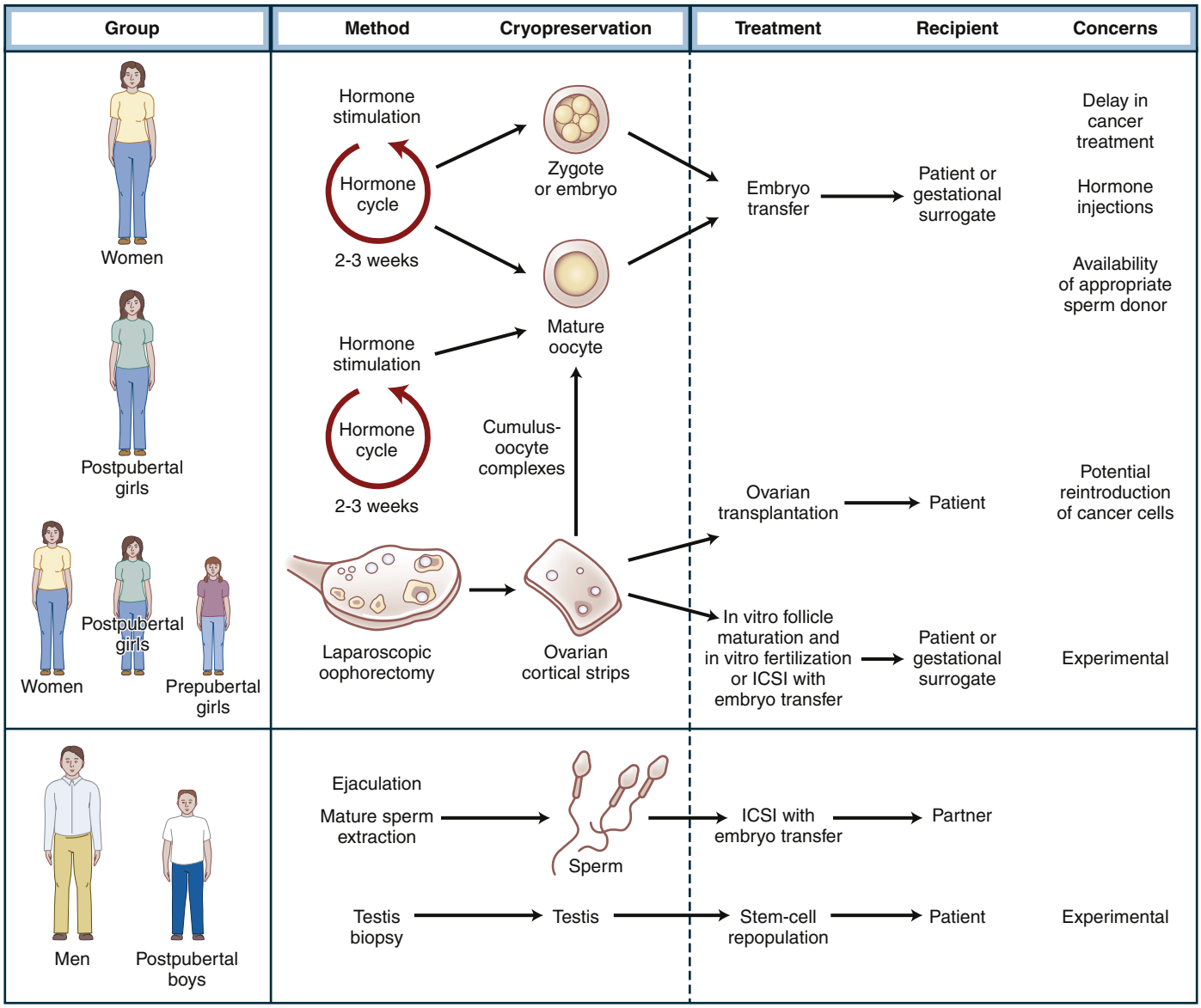
TABLE 46.2 Anticancer Therapy and Related Risks of Gonadotoxicity and Fertility Loss

Risk Level	Risk in Females	Risk in Males
High	• Hematopoietic stem cell transplantation with cyclophosphamide/total-body irradiation (TBI) or cyclophosphamide/busulfan • External beam radiation to a field that includes the ovaries. • CMF, CEF, CAF × 6 cycles in women 40 years and older (adjuvant breast cancer therapy) • Surgical removal of one or both ovaries or the pituitary gland	• TBI • Testicular radiation dose >2.5 Gy in men • Testicular radiation dose >6 Gy in boys • Cranial radiation >40 Gy • Protocols containing procarbazine: COPP, MOPP, MVPP, ChlVPP, ChlVPP/EVA, MOPP/ABVD, COPP/ABVD • Alkylating chemotherapy for transplant conditioning (cyclophosphamide, busulfan, melphalan) • Any alkylating agent (e.g., procarbazine, nitrogen mustard, cyclophosphamide) + TBI, pelvic radiation, or testicular radiation • Total cyclophosphamide dose >7.5 g/m^2 • Surgical removal of one or both testicles or the pituitary gland
Intermediate	• CMF, CEF, CAF × 6 cycles in women age 30–39 years (adjuvant breast cancer therapy) • AC × 4 cycles in women 40 years and older (adjuvant breast cancer therapy)	• Testicular radiation dose 1–6 Gy (due to scatter from abdominal/pelvic radiation) • BEP x 2–4 cycles • Cumulative cisplatin dose >400 mg/m^2 • Cumulative carboplatin dose ≥2 g/m^2 • Hormone treatments (prostate cancer) • Surgical procedures within in the pelvis (prostate, bladder, lower large intestine, rectum) • CHOP/COP
Low	• ABVD • CHOP × 4–6 cycles • CVP • Acute myeloid leukemia therapy (anthracycline/cytarabine) • Acute lymphoblastic leukemia therapy (multiagent) • CMF, CEF, CAF × 6 cycles in women younger than 30 years (adjuvant breast cancer therapy) • AC × 4 cycles in women younger than 40 years (adjuvant breast cancer therapy)	• Testicular radiation dose 0.2–0.7 Gy • Nonalkylating agents: ABVD, multiagent therapies for leukemia • Anthracycline + cytarabine • Bevacizumab (Avastin)
Very low or none	• Methotrexate, fluorouracil, vincristine, bleomycin, dactinomycin.	• Testicular radiation dose <0.2 Gy • Radioactive iodine • Multiagent therapies using vincristine
Unknown	• Taxanes, oxaliplatin, irinotecan, monoclonal antibodies, tyrosine kinase inhibitors.	• Irinotecan • Monoclonal antibodies such as cetuximab (Erbitux) • Tyrosine kinase inhibitors such as erlotinib (Tarceva), imatinib (Gleevec)

ABVD, Doxorubicin (Adriamycin), bleomycin, vinblastine, dacarbazine; *AC,* doxorubicin (Adriamycin), cyclophosphamide; *BEP,* bleomycin, etoposide, cisplatin; *CAF,* cyclophosphamide, doxorubicin (Adriamycin), fluorouracil; *CEF,* cyclophosphamide, epirubicin, fluorouracil; *ChlVPP,* chlorambucil, vinblastine, procarbazine, prednisolone; *CHOP,* cyclophosphamide, hydroxydaunomycin, Oncovin, prednisone; *CMF,* cyclophosphamide, methotrexate, fluorouracil; *COP,* cyclophosphamide, Oncovin, prednisone; *COPP,* cyclophosphamide, Oncovin, procarbazine, prednisone; *CVP,* cyclophosphamide, vincristine, prednisone; *EVA,* etoposide, vinblastine, Adriamycin (doxorubicin); *MOPP,* mechlorethamine, Oncovin (vincristine), procarbazine, prednisone; *MVPP,* mechlorethamine, vinblastine, procarbazine, prednisolone; *NOVP,* Novantrone (mitoxantrone), Oncovin, vinblastine, prednisone; *OEPA,* Oncovin, etoposide, prednisone, Adriamycin (doxorubicin).

TABLE 46.3 Fertility Preservation Options for Patients Undergoing Gonadotoxic Anticancer Therapy

Option	Females	Males
Established	• Embryo freezing • Egg freezing	• Sperm freezing
Debatable	• Gonadotropin-releasing hormone (GnRH) analogues and hormonal suppression • Oophoropexy • Gonadal shielding • Fractionated chemotherapy and radiotherapy	• GnRH analogues and hormonal suppression • Gonadal shielding • Fractionated chemotherapy and radiotherapy
Experimental	• Ovarian tissue freezing and autotransplantation • In vitro maturation of oocytes and vitrification • Artificial ovary • Stem cells • Neoadjuvant cytoprotective pharmacotherapy • Others	• Testicular tissue freezing • Stem cells • Neoadjuvant cytoprotective pharmacotherapy • Others



• **Fig. 46.1** Options for fertility preservation in patients with cancer. ICSI, intracytoplasmic sperm injection. (Redrawn from Jeruss JS, Woodruff TK. Preservation of fertility in patients with cancer. *N Engl J Med*. 2009;360[9]:902–911.)

have shown a correlation between administration of GnRH analogues before and during chemotherapy and lower rates of POF in female cancer survivors.[119,121–126] The mechanism of action of GnRH analogues and their direct and indirect effects on ovaries are not fully understood. It is known that GnRH analogues suppress gonadotropin secretion from the pituitary gland and hence suppress ovarian function indirectly.[127,128] Some theories suggest that administration of GnRH analogues before and during chemotherapy suppresses the ovaries, decreasing the number of primordial follicles entering the growing pool; these dormant follicles are thought to be less sensitive to the gonadotoxic effects of chemotherapeutic agents. Other theories suggest a direct protective effect of GnRH analogues on the ovaries, including via upregulation of intraovarian antiapoptotic molecules and protection of ovarian germline stem cells.[129,130] In most patients, GnRH analogues have not been shown to protect ovaries from radiotherapy-induced gonadotoxicity. For this reason, GnRH analogues are not recommended for fertility preservation in female patients with cancer scheduled to undergo pelvic irradiation. According to ASCO, European Society for Medical Oncology, and ASRM guidelines,[47–53] GnRH analogues and other hormonal suppression methods (e.g., oral contraceptives) should not be relied on as female fertility preservation methods. Nevertheless, several recent publications have encouraged the use of GnRH analogues to preserve female fertility during chemotherapy.[128,131–139]

Oophoropexy

Oophoropexy is the surgical transposition of ovaries away from the field of pelvic irradiation, which is often used to treat pelvic malignancies such as Hodgkin lymphoma, cervical carcinoma, vaginal carcinoma, and pelvic sarcoma. During oophoropexy, ovaries can be transposed either laterally toward the pelvic wall or medially behind the uterus. Although underutilized, oophoropexy can be carried out via mini-laparotomy, laparoscopy, or even robotic surgery. The simplest and most successful technique is laparoscopic lateral oophoropexy. Oophoropexy has been

performed on one ovary while the other ovary is excised for ovarian tissue freezing.[140–146] According to ASCO guidelines, the success of oophoropexy in protecting ovaries and preserving fertility is debatable and varies according to the dose, site, and type of pelvic irradiation; the age of the patient; and whether radiation is combined with chemotherapy. There is also the probability of remigration of the transposed ovaries to their original positions during the course of radiotherapy. Following successful oophoropexy, spontaneous pregnancy may be possible without the need for ART. Oophoropexy does not protect ovaries from chemotherapy induced-gonadotoxicity. For this reason, it is not recommended for female patients with cancer scheduled to receive concomitant chemotherapeutic treatments.[47,48]

Gonadal Shielding

Gonadal shielding should be routinely used during pelvic irradiation to provide some ovarian protection, especially in young patients. Similar to oophoropexy, gonadal shielding does not protect ovaries from chemotherapy induced-gonadotoxicity and therefore has a limited role when chemotherapy is combined with radiation therapy.[47–63]

Fractionated Chemotherapy and Radiotherapy

Fractionation of chemotherapy and radiotherapy doses may reduce gonadotoxicity and gonadal damage and should be attempted whenever possible.[47–63,65]

Experimental Options for Fertility Preservation in Women and Girls

Ovarian Tissue Freezing and Autotransplantation

Ovarian tissue freezing is still considered an experimental method for female fertility preservation. It involves cryopreservation of surgically excised cortical ovarian tissues for later use in autotransplantation or in vitro oocyte maturation.[147–151] Additionally, immature oocytes could be retrieved directly from the extracted ovarian tissue for IVM and vitrification.[152–157] At least half of one ovary is excised via laparoscopy or laparotomy before the initiation of cancer treatment. The extracted ovarian tissue is transported within 24 hours under special conditions to central cryobanks to be processed by experienced oncofertility teams.[158–160] Ovarian tissue freezing is performed via slow freezing[161–163]; however, vitrification protocols have been attempted in numerous research trials with promising results.[164–168]

After recovery from cancer and when pregnancy is desired, the frozen ovarian tissue can be thawed and transplanted back into the same patient (autotransplantation), typically onto the remaining ovary or ovarian fossa. In the case of severe pelvic adhesions or poor pelvic vasculature due to previous irradiation, frozen-thawed ovarian tissue can be autotransplanted heterotopically to other sites, such as the subcutaneous space of abdominal wall or forearm. Following successful ovarian tissue freezing and autotransplantation, ovarian function may resume between 2 and 9 months postoperatively and may last for several years. After orthotopic autotransplantation, spontaneous pregnancy can be expected or patients with either orthotopic or heterotopic transplants can undergo ovarian stimulation, oocyte retrieval, and IVF. Although an international registry is still needed, at least 120 healthy babies have been born worldwide after ovarian tissue slow freezing and orthotopic autotransplantation, without any increased risk for miscarriage or congenital abnormalities. In such cases, the live birth rate per transplant was roughly estimated to be 25%. Ovarian tissue vitrification and heterotopic autotransplantation have resulted in few reported live births.[169–173]

Ovarian tissue freezing followed by autotransplantation may be the only suitable fertility preservation option for prepubertal girls, although few babies have been born in women whose ovarian tissue was frozen before puberty.[174,175] Compared with the established female fertility preservation options (embryo and egg freezing), ovarian tissue freezing and autotransplantation does not need prior ovarian stimulation and therefore does not delay cancer treatments. Moreover, this option can also restore both endocrine and reproductive ovarian function for several years.[147–151]

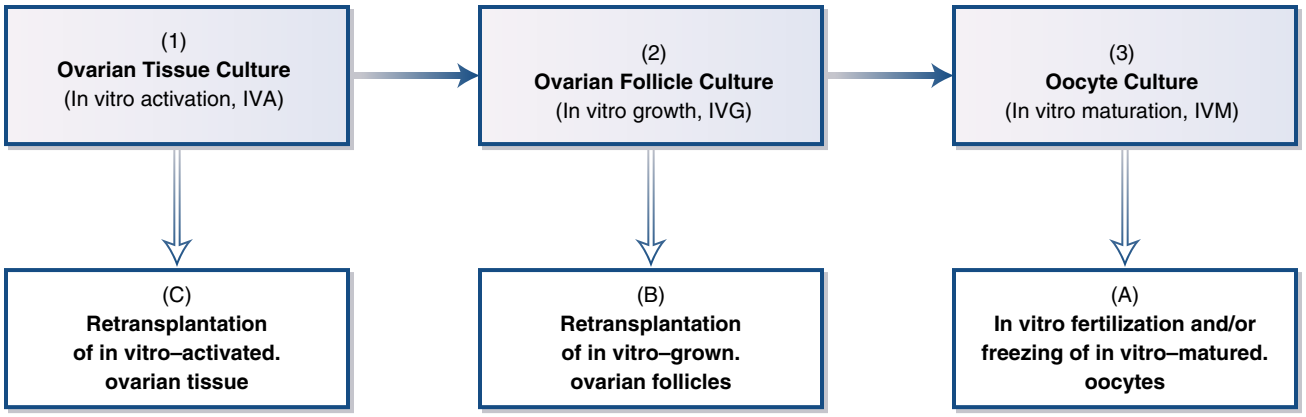
Transplantation of fresh and frozen-thawed ovarian tissue between monozygotic twin sisters has been successful, resulting in healthy live births.[176–178] Whole ovary cryopreservation via slow freezing or vitrification has been studied in human and several animal species.[179–182] However, the major challenges associated with cryopreservation and autotransplantation of the whole ovary are the very high follicular loss due to related cryoinjury and post-transplantation vascular complications and ischemia.[183–187]

Although promising, the use of autotransplantation of frozen-thawed ovarian tissue for fertility preservation in patients with cancer presents a significant and serious concern of possible contamination of ovarian tissue with malignant cells, or the presence of minimal residual disease in the case of ovarian carcinomas or in malignancies that may metastasize in ovaries. According to several studies, systematic reviews, and meta-analyses assessing the risk of reintroducing malignant cells, autotransplantation of frozen-thawed ovarian tissue should be absolutely contraindicated for women with any type of ovarian carcinoma or leukemia (high risk). It may be an option in women with Hodgkin lymphoma, and breast, bone, and connective tissue malignancies (mild risk), or with non-Hodgkin lymphoma and gastrointestinal cancers (moderate risk).[150,188–191] To assess the risk of reintroducing malignant cells, several tests can be performed on the excised tissue prior to autotransplantation, including histologic examination, in vitro culture, immunohistochemistry, polymerase chain reaction, multicolor flow cytometry, and long-term xenografting of ovarian tissue into immunodeficient mice.[159,192–200] The first delivery in a leukemia survivor after autotransplantation of cryopreserved ovarian tissue evaluated for leukemia cells contamination prior to reimplantation has recently been reported.[201] Although remarkable, it is not clear whether the risk of contamination is the same for all patients with leukemia; rather, it appears to be case dependent, and the results should therefore be extrapolated to oncofertility care with caution.

IVM of Oocytes

Considering that autotransplantation of frozen-thawed ovarian tissue should be absolutely contraindicated in some patients due to a high risk of ovarian tissue contamination with malignant cells or because of the presence of minimal residual disease, the safest way to restore fertility may be IVM of oocytes and artificial ovary technology.

In vitro, immature oocytes can be retrieved directly from extracted ovarian tissue and from unstimulated ovaries through a routine ultrasound-guided transvaginal approach. The retrieved immature oocytes are cultured in vitro for 24 to 48 hours to mature into metaphase II oocytes ready for either IVF or vitrification.[202–205] Although IVM is technically challenging, it is feasible for all patients, including prepubertal girls. Recently, the success rates of oocyte IVM have improved,[206–209] and a few new reports have shown comparable results to traditional IVF.[210,211]



• **Fig. 46.2** Artificial human ovary: the concept (*blue boxes*) and the applications (*white boxes*). (Redrawn from Salama M, Isachenko V, Isachenko E, Rahimi G, Mallmann P. Advances in fertility preservation of female patients with hematological malignancies. *Expert Rev Hematol*. 2017;10[11]:951–960.)

Artificial Ovary

The ovarian bioprosthetic, or artificial ovary, is a novel experimental technology that aims to produce mature oocytes ready for IVF through an ex vivo multistep strategy that includes sequential in vitro culture of ovarian tissue, follicles, and oocytes.[212–214] Although successful only in mice,[215,216] research is under way to improve artificial ovary methodology and outcomes and help to establish this technology for translation to clinical care[217] (Fig. 46.2).

Ovarian tissue culture (encapsulated in vitro follicle growth) involves in vitro culture of fresh or frozen-thawed human cortical ovarian tissue pieces from 6 to 10 days to activate the growth of primordial follicles within the tissue to reach preantral stages.[218,219] Preantral follicles are then isolated enzymatically and/or mechanically from the cultured ovarian tissue and cultured individually for up to 4 weeks in a biodegradable three-dimensional (3D) microenvironment made of alginate, matrigel, fibrin, or other biomaterials.[220] The 3D in vitro follicle culture supports preantral follicle growth to the early antral stage.[221–225] These in vitro–grown early antral follicles are then released enzymatically and/or mechanically from the surrounding 3D microenvironment and punctured to release the enclosed oocytes.[226] Finally, the released immature oocytes are cultured in vitro for 24 to 48 hours to mature into metaphase II oocytes ready for IVF or vitrification.[152–157]

Additional applications of the artificial ovary have included (1) IVF and/or vitrification of in vitro–matured oocytes,[152–157] (2) retransplantation of in vitro–grown ovarian follicles within a 3D biodegradable microenvironment with potential success in a xenotransplantation mouse model,[227–231] and (3) retransplantation of in vitro–activated ovarian tissue with reported live births in patients with POF[232–234] (see Fig. 46.2). Additional emerging technologies with potential applications in the field of female fertility preservation and restoration include tissue engineering[235] and 3D-printed organs.[236] The 3D-printed bioprosthetic ovary is a novel experimental technology in which isolated ovarian follicles are embedded in 3D-printed microporous hydrogel scaffolds to support follicle growth after transplantation in vivo. Full restoration of ovarian function after transplantation of a bioprosthetic ovary in surgically sterilized mice has been accomplished. After natural mating, mice with 3D-printed ovaries produced healthy pups.[236] These findings open a new horizon for creating a 3D-printed artificial human ovary to restore fertility in cancer patients.

Stem Cells

Due to recent advances in stem cell research over the past decades, many studies have examined potential applications in the field of female reproductive endocrinology and infertility. The recent discovery of ovarian (oogonial) stem cells and the creation of induced pluripotent stem cells has revealed new opportunities to maintain ovarian follicle and oocyte production, which normally halts just after birth. Stem cell–based therapies could potentially treat various conditions leading to infertility, including POF following aggressive gonadotoxic chemotherapy and radiotherapy.[237–245] Although promising, there is still a long way to go to translate stem cell science into routine clinical practice.

Neoadjuvant Cytoprotective Pharmacotherapy

The development of mitigation strategies to reduce chemotherapy- and radiotherapy-induced gonadotoxicity is another approach being taken in fertility preservation research. Recently, several strategies have been attempted to reduce the gonadotoxic effects of specific chemotherapeutic agents in animal models, with promising results. Examples of such strategies include the use of nanoencapsulated arsenic trioxide,[246] c-Abl-TAp63 pathway inactivation by imatinib,[247,248] and use of the GnRH analogue triptorelin.[123,249,250] S1P,[251] AS101,[252] and FTY720[253] treatments have also been investigated as approaches to reduce radiotherapy-induced gonadotoxicity.

Fertility Loss, Preservation, and Restoration in Male Patients With Cancer

Epidemiology of Cancer in Young Male Patients

According to statistics from the United Kingdom (2013–2015), the most common cancers in prepubertal boys (age 0–14 years) are leukemias (31%), CNS cancers (26%), and lymphomas (13%). The most common cancers in AYA males (age 15–24 years) are germ cell tumors (27%), lymphomas (22%), CNS cancers (13%), and leukemias (10%). The most common cancers in adult males (age 25–49 years) are testicular (14%), melanoma (11%), CNS (10%), bowel (10%), and head and neck cancers (8%). The most common cancers that arise during the male reproductive years that may require aggressive gonadotoxic chemotherapy and radiotherapy are testicular cancer, germ cell tumors, leukemia, lymphoma, and CNS cancer[64] (see Table 46.1).

Risks of Gonadotoxicity and Fertility Loss in Young Male Patients

In young male patients undergoing cancer treatment, gonadotoxicity, azoospermia, and subsequent fertility loss usually occur when the testes are exposed to alkylating chemotherapies such as chlorambucil, nitrogen mustard, and cyclophosphamide, or to ionizing radiotherapy to the pelvis and abdomen or cranial or total-body irradiation. The degree of gonadotoxicity depends mainly on the type, dose, and dosage of cancer therapy, as well as the age of the patient and the type and stage of cancer. Cancer treatments have been classified according to their risk of gonadotoxicity, azoospermia, and subsequent fertility loss in men[24,254–259] (see Table 46.2).

Fertility Preservation and Restoration Options in Young Male Patients

As in female patients with cancer, when the estimated risk of gonadotoxicity is greater than 50%, fertility preservation strategies should be offered to young male patients (<40 years of age) prior to cancer treatment. To preserve the fertility of young men and boys with cancer, a few established, debatable, and experimental options can be offered. The established option is sperm freezing. Debatable options include the use of GnRH analogues and hormonal suppression, gonadal shielding, and fractionated chemotherapy and radiotherapy. Experimental options include testicular tissue freezing, stem cells, neoadjuvant cytoprotective pharmacotherapy, and others[260–263] (see Table 46.3 and Fig. 46.1).

Established Options for Fertility Preservation in Men and Boys

Sperm Freezing

Sperm freezing is the first and the only established method for male fertility cryopreservation; since the 1950s, sperm freezing has been considered the gold standard option.[264,265] It involves sperm retrieval and cryopreservation of spermatozoa via slow freezing or vitrification; the latter approach is now preferred due to a better post-thaw survival rate.[266,267] Recently, cryoprotectant-free vitrification of human spermatozoa has been attempted and resulted in healthy live births.[268–270] Under normal conditions, sperm samples are obtained from adults and adolescents males by masturbation. However, in case of ejaculation disorders, vibratory or electrical stimulation can be used to obtain sperm. In patients with retrograde ejaculation, spermatozoa can be isolated from urine. When obstructive or nonobstructive azoospermia is diagnosed, testicular sperm aspiration or extraction can be performed to obtain sperm.[271–273] In clinical practice, frozen and fresh sperm have comparable live birth rates, and according to some reports, frozen sperm can be stored for more than 20 years without negative side effects. Healthy live births have been reported from IVF with semen stored for 21 years,[274] from intrauterine insemination with semen stored for 28 years,[275] and from intracytoplasmic sperm injection with semen stored for 40 years.[276]

Debatable Options for Fertility Preservation in Men and Boys

GnRH Analogues and Hormonal Suppression

The use of GnRH analogues before and during chemotherapy in males has been shown to have a gonadoprotective effect in mouse and rat models, but it does not appear to have the same effect in human and nonhuman primates.[277,278] In most cases, GnRH analogues do not protect the testes from radiotherapy-induced gonadotoxicity. Other hormonal methods to suppress testes with androgens and antiandrogens have not shown promising gonadoprotective effects in adult men. For these reasons, ASCO does not recommend the use of GnRH analogues and other hormonal suppression methods for male fertility preservation.[47–49]

Gonadal Shielding

Gonadal shielding should be routinely used during pelvic irradiation to provide some testicular protection, especially in young patients. However, gonadal shielding does not protect testes from chemotherapy-induced gonadotoxicity and therefore has a limited role when chemotherapy is combined with radiation.[47–63,65]

Fractionated Chemotherapy and Radiotherapy

As in women and girls with cancer, fractionation of chemotherapy and radiotherapy doses may reduce gonadotoxicity and gonadal damage and should be attempted whenever possible.[47–63,65]

Experimental Options for Fertility Preservation in Men and Boys

Testicular Tissue Freezing

Testicular tissue freezing is an experimental option that may be offered when sperm production is not possible, such as in prepubertal boys or in adults with azoospermia. In this technique, spermatogonial stem cells, either within the testicular tissue or as a cell suspension, are frozen via slow freezing or vitrification and stored for future autotransplantation, in vitro culture, or in vitro spermatogenesis.[279] As with ovarian tissue, autotransplantation of frozen-thawed testicular tissue may carry the risk of reintroducing malignant cells back into the patient, especially in patients with testicular cancer or malignancies that metastasize to the testes, such as leukemia. To date, testicular tissue freezing and spermatogonial stem cell autotransplantation, xenografting, in vitro culture, and in vitro spermatogenesis have proven more promising in mice than in other animal species and humans.[271,279–285]

Stem Cells

The recent discovery of spermatogonial stem cells and creation of induced pluripotent stem cells have opened new horizons for the recovery and maintenance of spermatogenesis after aggressive gonadotoxic chemotherapy and radiotherapy.[286–292] Although promising, much work still needs to be done to realize the promise of stem cell research for the clinical practice of fertility preservation.

Neoadjuvant Cytoprotective Pharmacotherapy

Although the development of neoadjuvant cytoprotective pharmacotherapy strategies to reduce chemotherapy- and radiotherapy-induced gonadotoxicity is an important topic of interest in fertility preservation research, few studies have examined the use of these strategies in men. Partial success has been observed with the immunomodulatory AS101 and carnitine in rodents.[293,294]

Other Experimental Options

Recently, several research technologies have emerged with potential applications in the field of male fertility preservation and restoration. Examples of such technologies include testicular tissue culture, in vitro spermatogenesis, and gene therapy.[295–302]

TABLE 46.4 Plausible Fertility Preservation and Restoration Strategies for Female Patients With Cancer

Age	Before Anticancer Therapy (Fertility Preservation)	During Anticancer Therapy (Fertility Preservation)	After Anticancer Therapy (Fertility Restoration)
Prepubertal (0–14 years)	• Ovarian tissue freezing • IVM of oocytes and vitrification • Oophoropexy	• Gonadal shielding • Fractionation of chemotherapy and radiotherapy • Neoadjuvant cytoprotective pharmacotherapy	• IVF/ICSI of frozen oocytes • Autotransplantation of frozen ovarian tissue • Artificial ovary • Stem cells
Adolescent & young adult (15–24 years)	• Egg freezing • Embryo freezing • Ovarian tissue freezing • IVM of oocytes and vitrification • Oophoropexy	• GnRH analogues and hormonal suppression • Gonadal shielding • Fractionation of chemotherapy and radiotherapy • Neoadjuvant cytoprotective pharmacotherapy	• IVF/ICSI of frozen oocytes • Intrauterine transfer of frozen embryo • Autotransplantation of frozen ovarian tissue • Artificial ovary • Stem cells
Adult (25–40 years)	• Embryo freezing • Egg freezing • Ovarian tissue freezing • IVM of oocytes and vitrification • Oophoropexy	• GnRH analogues and hormonal suppression • Gonadal shielding • Fractionation of chemotherapy and radiotherapy • Neoadjuvant cytoprotective pharmacotherapy	• Intrauterine transfer of frozen embryo • IVF/ICSI of frozen oocytes • Autotransplantation of frozen ovarian tissue • Artificial ovary • Stem cells

GnRH, Gonadotropin-releasing hormone; *ICSI,* intracytoplasmic sperm injection; *IVF,* in vitro fertilization; *IVM,* in vitro maturation.

Decision-Making Strategies for Young Female and Male Patients With Cancer

To provide fertility preservation and restoration strategies to young patients with cancer, the treating center should be properly equipped, with a highly skilled team of oncologists, gynecologists, andrologists, reproductive biologists, transplantation surgeons, and research scientists. Referring patients from oncology clinics, small medical centers, or general hospitals to highly specialized oncofertility centers is strongly encouraged to guarantee a high standard of care.[1,303,304]

For young patients with cancer, it is recommended that oncofertility counseling starts immediately after diagnosis. If the patient is younger than 40 years and has a reasonable chance of survival, is in good health, and has satisfactory reproductive function, the cancer treatment plan should be reviewed by the oncofertility team and the risk of gonadotoxicity and subsequent fertility loss should be assessed. If the risk is greater than 50% and the patient wishes to have children in the future, a fertility preservation strategy should be pursued before and during chemotherapy and radiotherapy. After the patient completes cancer therapy and crosses into the realm of survivorship, a new assessment of endocrine and reproductive function should be performed. If it is determined that the patient suffers from cancer therapy–induced fertility loss, the frozen gametes, gonadal tissue, or stem cells can be used to restore fertility. From a clinical perspective, fertility preservation and restoration strategies should be tailored to each patient's circumstances and wishes, and may involve a combination of established, debatable, and experimental options (after obtaining informed consent from the patient or legal guardians in case of a child).[1,305–309] As shown in Tables 46.4 and 46.5, plausible strategies according to patient's gender and age can be attempted—when feasible and not contraindicated—before, during, and after cancer therapy.

If fertility preservation options are rejected, contraindicated, or unavailable, adoption and third-party reproduction (gamete donation and surrogacy) can be offered as alternatives when possible.[310,311] If third-party reproduction services are not available or not legally allowed, patients may consider traveling abroad to pursue these options. This growing phenomenon has been called *cross-border reproductive care* or *fertility tourism* in the media.[312–319]

Challenges of Fertility Preservation

Factors That Prohibit Oncofertility Support

Only about one third of AYA cancer survivors pursue fertility preservation.[21] Barriers to oncofertility support include both patient- and provider-related factors.[25] Female sex, age, ethnicity, relationship status and current parenthood, religious beliefs, economic concerns, self-perceived lack of time, and emotional distress, as well as functional status, type of malignancy, and prognosis at the time of diagnosis, are important contributors to the provision of and participation in adequate counseling on reproductive health.[25,26,320] Some cancer patients hesitate to initiate conversations about reproductive health with their health care team due to the assumption that the topic should first be introduced by their providers.[321] Furthermore, many patients making fertility preservation decisions are in the early stages of coping with their cancer diagnosis and are overwhelmed by considerations of personal mortality, cancer recurrence, and comorbidities related to cancer therapy, in addition to evaluating the financial and ethical implications of fertility preservation.[29]

Although more than 80% of cancer health care providers believe that reproductive health is important for cancer patients, more than 70% of providers feel unprepared to formally assess and address these concerns.[321] Provider age, practice setting, perception

TABLE 46.5 Plausible Fertility Preservation and Restoration Strategies for Male Patients With Cancer

Age	Before Anticancer Therapy (Fertility Preservation)	During Anticancer Therapy (Fertility Preservation)	After Anticancer Therapy (Fertility Restoration)
Prepubertal (0–14 years)	• Testicular tissue freezing	• Gonadal shielding • Fractionation of chemotherapy and radiotherapy • Neoadjuvant cytoprotective pharmacotherapy	• Autotransplantation of frozen testicular tissue • Stem cells
Adolescent & young adult (15–24 years)	• Sperm freezing • Testicular tissue freezing	• GnRH analogues and hormonal suppression • Gonadal shielding • Fractionation of chemotherapy and radiotherapy • Neoadjuvant cytoprotective pharmacotherapy	• IUI/IVF/ICSI using frozen sperm • Autotransplantation of frozen testicular tissue • Stem cells
Adult (25–40 years)	• Sperm freezing • Testicular tissue freezing	• GnRH analogues and hormonal suppression • Gonadal shielding • Fractionation of chemotherapy and radiotherapy • Neoadjuvant cytoprotective pharmacotherapy	• IUI/IVF/ICSI using frozen sperm • Autotransplantation of frozen testicular tissue • Stem cells

GnRH, Gonadotropin-releasing hormone; *ICSI*, intracytoplasmic sperm injection; *IVF*, in vitro fertilization; *IUI*, intrauterine insemination.

of inadequate training, time, and resources, and personal view of the patient's desire and comfort with discussing reproductive health also influence whether oncofertility is included in comprehensive cancer care.[25,321] Among providers caring for pediatric and adolescent cancer patients, discomfort with discussing reproductive health with sexually immature patients and their parents influences the extent to which reproductive health is addressed. In particular, providers express concerns regarding patients' decision-making capacity on issues pertaining to their future fertility.[322]

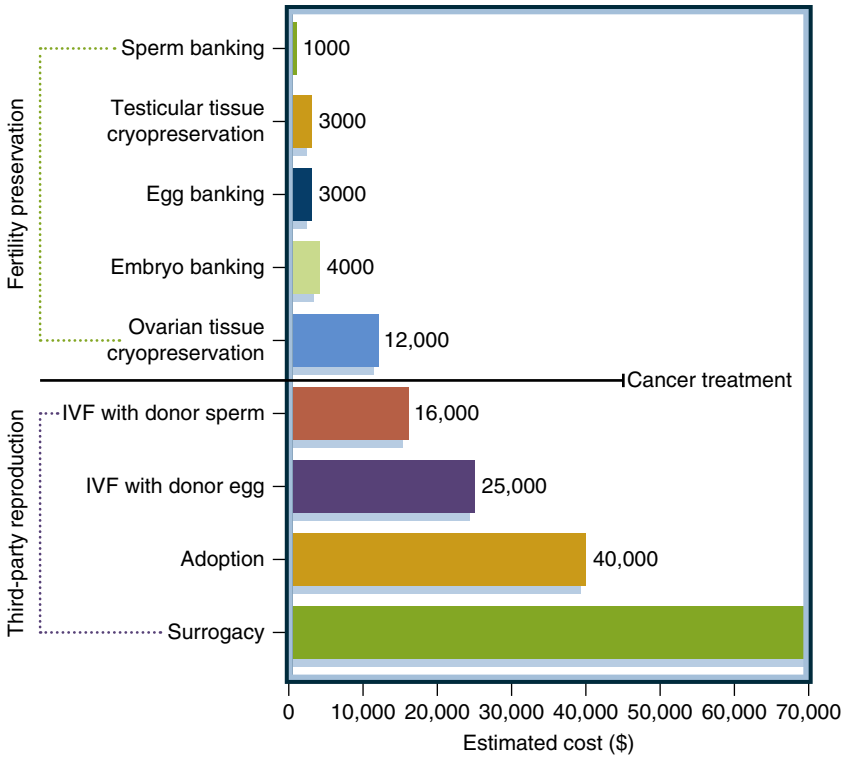
Both male and female cancer patients identify fertility as a high-priority element of their psychological and social well-being. Although an increasing number of cancer patients are receiving information about fertility preservation prior to treatment, fertility preservation options are highly underutilized,[21] partly due to insufficient referral to reproductive specialists after initial counseling.[323] Patients who undergo evaluation by a reproductive specialist are more likely to report better understanding of fertility preservation options prior to starting treatment, regardless of whether they pursue these options in the future.[323] Thus, effective execution of fertility preservation counseling requires an intricate level of communication and coordination and a shared sense of responsibility among those treating the cancer (oncologists, surgeons, radiologists) and fertility specialists and scientists.[324]

Special Patient Populations

The relationship between reproductive health and cancer diagnosis and treatment is particularly multifaceted for ethnic, cultural, and sexual minorities. Provision of oncofertility counseling varies by ethnicity.[320] Culturally specific beliefs about illness, spirituality, and mortality,[325] trust in the medical system[326] and medical decision making,[327] sexuality,[328,329] self-identity and autonomy,[330] and QoL[331] impact cancer patients' perspectives on the role that reproductive health plays in their overall well-being. Furthermore, patients' religious and cultural beliefs, specifically regarding gamete collection and storage, fertilization, procreation, and surrogacy, guide their selection among different fertility preservation options.[332] Thus, culturally competent oncofertility care is important, as it can improve patient-provider communication, reduce decision regret, and improve health-related QoL.[326]

Sexual minorities encounter distinct challenges relating to intimacy, fertility, and parenthood prior to, during, and after cancer therapy. On the one hand, members of the lesbian, gay, bisexual, transgender, or queer (LGBTQ) community who have cancer experience similar concerns regarding relationships, fertility, and body image as their heterosexual counterparts. On the other hand, LGBTQ cancer patients also uniquely express beliefs in the "chosen family," which is a family founded on love and kinship and not necessarily blood or biologic relation; they are more open to alternative parenting options, such as adoption, gamete donation, and surrogacy, and report less impact of fertility on their relationships compared with heterosexual cancer patients. Because many providers may not appropriately address these unique aspects of intimacy, relationships, and family building, almost one-third of LGBTQ cancer patients report unsatisfactory counseling about reproductive health prior to cancer treatment.[333]

Decisions regarding fertility and family building are also challenging for pregnant women with cancer, who comprise about 0.1% of the population of all pregnant women.[334] Although rare, the common cancers diagnosed during pregnancy include breast (the most common) and cervical cancers, Hodgkin lymphoma, non-Hodgkin lymphoma, ovarian cancer, malignant melanoma, leukemia, and thyroid and colorectal cancers.[335,336] Pregnant women with cancer may struggle with distinguishing between cancer- and pregnancy-related symptoms, are hesitant to undergo certain imaging or other diagnostic procedures, and are concerned about the possibility of missing clinically obscure cancers in the setting of pregnancy-related changes to their bodies. As such, they are at higher risk of pregnancy termination.[337] Because of the complexity of cancer during pregnancy, guidelines for the prenatal care for women with cancer during pregnancy have been established.[338] Treatment requiring surgery, radiotherapy, or chemotherapy necessitates close monitoring by a multidisciplinary team of oncologists, obstetricians, neonatologists, cardiologists, pharmacologists, surgeons, radiation oncologists, ethicists, and mental health providers. Live birth rates as high as 86% have been reported among pregnant women with cancer, whereas the rates of preterm delivery, small for gestational age birth weight, and neonatal intensive care unit admission are 48%, 21%, and 41%, respectively. Maternal age, type of cancer, extent of disease (systemic vs. localized), and type of chemotherapeutic agent (e.g.,



• **Fig. 46.3** Cost of fertility preservation versus third-party reproduction. Potential costs vary by state, institution, and insurance status. Fertility preservation costs do not include annual storage fees of reproductive tissues (approximately $250/year) or cost of in vitro fertilization (IVF; approximately $12,000). (Courtesy the Oncofertility Consortium and Teresa K. Woodruff.)

platinum based, taxanes) are risk factors for certain adverse obstetric and neonatal outcomes in pregnant women with cancer. Thus, changes to administration standards for chemotherapy (e.g., after the first trimester and no later than the 35th week of gestation) have been implemented to decrease the risk of congenital malformations and myelosuppression, respectively.[339] Appropriate timing and perioperative care prior to surgery for cancer treatment have reduced the risk of preterm delivery among women with cancer.[340] Nonetheless, women who undergo radiation therapy as part of their cancer treatment experience a dose-dependent increase in the risk of miscarriage, preterm delivery, abnormal placentation, fetal malposition, and low fetal birth weight in subsequent pregnancies.[31,341] The ultimate long-term impact of concurrent pregnancy and cancer treatment on cancer recurrence and overall maternal health is still unknown.[342] Notably, growth, cardiac function, neuropsychological capacity, and overall health in kids of mothers who had cancer during pregnancy are comparable to controls.[343]

Financial and Ethical Considerations

About 50% of cancer survivors experience financial hardship 1 year after diagnosis.[344] In fact, cancer patients are more than 2.6 times more likely to file for bankruptcy within 5 years of diagnosis, with patients 21 to 44 years of age bearing the greatest risk compared with matched controls.[345] "Financial toxicity" encompasses all of the financial considerations that patients with cancer experience throughout the course of their diagnosis, treatment, and follow-up. Financial toxicity is particularly burdensome for younger cancer patients and plays a significant role in personal choice about pursuing fertility preservation.[345] Out-of-pocket costs of fertility preservation are shown in Fig. 46.3. Many health insurance plans do not cover costs associated with fertility preservation, and only two states mandate insurance coverage for costs associated with iatrogenic infertility. Furthermore, some plans selectively cover the cost of ovarian stimulation but do not cover egg/embryo storage fees. Thus, fertility preservation may be an untenable option for many cancer patients of reproductive age.[346] In fact, more than 20% of cancer patients cite cost as the primary reason for not pursuing fertility preservation.[320] Disparities in financial hardship contribute to decreased health care–related QoL after cancer treatment, especially among certain ethnic minorities.[344,347] As such, patient financial literacy and navigation programs are making strides toward attenuating the financial burden of fertility preservation.[348]

The provision of adequate counseling on reproductive health prior to cancer treatment and the timely implementation of cancer treatment are commonly at odds at the time of cancer diagnosis, calling forth complex ethical questions. Specific ethical concerns include purposeful disclosure of fertility preservation options despite the potential delay to initiation of cancer therapy; ongoing awareness of patient confidentiality and privacy; procurement of assent and consent for fertility preservation in pediatric and adolescent patients; allocation of rights for future use, disposal, and donation of preserved tissue (including posthumous reproduction); utilization of preimplantation genetic diagnosis in future reproduction; provision of the costs associated with fertility preservation between the patient and involved partners; potential impact on QoL of subsequent offspring of cancer survivors; and avoidance of false hope in discussions of future fertility in patients with poor prognosis.[349] In prepubertal cancer patients, conversations about reproductive health, including

current contraception, sexuality, and future fertility, as well as the logistics of acquiring semen from boys and ovarian tissue from girls, are largely shaped by parents' and providers' beliefs about the child's sexual maturity.[332] Adult survivors of cancer who underwent fertility preservation counseling at a young age may disagree with decisions made by their parents at the time of cancer diagnosis.[350] Many cancer survivors experience social and emotional pressure to use previously stored gonadal tissue or gametes for achievement of genetic parenthood, especially in the context of socially constructed ideals of femininity, masculinity, and parenthood.[351] Furthermore, bioethical, legal, and regulatory concerns are particularly pertinent for young cancer patients considering experimental fertility preservation options and for cancer survivors considering reproductive tissue allografting or isografting or gestational surrogacy as ways to achieve parenthood.[352] Thus, many institutions have implemented task forces to help navigate the ethical issues that individual cancer patients face throughout the course of their cancer diagnosis, treatment, and follow-up.

Methods for Addressing Challenges

Providing Personalized Risk Assessment

The availability of information about the infertility risk posed by cancer or a particular cancer treatment is of the utmost importance for making an informed decision to undergo fertility preservation treatment. This is especially true for women, as the available fertility preservation techniques are costly and require several days or weeks to be completed. Several cohort studies have demonstrated the reduction of female and male fertility after cancer treatment; the probability of having children was found to be reduced by half in the Scandinavian Cohort Study and the Childhood Cancer Survivor Study.[353–355] However, prospective studies are still lacking or are of inadequate size, and randomized controlled trials are difficult to implement in this population of patients due to the amount of time required to assess fertility status after treatment. The sample size in particular is a difficult hurdle to overcome due to the heterogeneity of patient age, tumor stage, and treatment. Prospective or observational studies must last several years or decades to evaluate whether a particular cancer type or a cancer treatment prematurely reduces the ovarian reserve and fertility potential. Patient selection may also be biased, as the patients who enroll in these studies are highly interested in fertility. Despite these challenges, efforts have been made to identify individual predictors of infertility risk among patients with cancer, the most important of which are age in women and the type of cancer treatment in both men and women. Pretreatment evaluation of the ovarian reserve or sperm production is an important first step in estimating infertility risk, as reproductive function may already be compromised in patients with cancer. After cancer treatment, the recovery of ovarian or testicular function may occur months to years after radiation or chemotherapy. In men, semen analysis should be performed repeatedly to evaluate the recovery of sperm production, which can be delayed years after treatment because stem cells of the testicle reinitiate spermatogenesis. Although most studies have used amenorrhea as a surrogate marker for fertility loss in women, it is not accurate; in fact, resumption of regular menses does not always signal fertility. New and more accurate markers of ovarian function are needed to counsel patients about infertility risk and fertility status before and after cancer treatment.

Practice Management, Knowledge, and Access Barriers to Clinical Care

Oncofertility is a field that bridges reproductive science and oncology in an effort to preserve reproductive function for patients diagnosed with cancer. Achieving this goal requires a close collaboration of specialists involved in the treatment of cancer and infertility, who often have competing priorities. Discussions with patients about the possible risks posed by cancer and its treatment on fertility and the options for fertility preservation are necessarily complex—not only because multiple perspectives are in play but also because timing is crucial, particularly for patients with aggressive forms of cancer. This task can be particularly difficult in children with cancer, requiring practitioners to evaluate long-term infertility risks and offer appropriate fertility preservation techniques as soon as the urgent need for gonadotoxic treatment is established. Fertility preservation options for the youngest patients (immature gamete retrieval and cryopreservation) remain largely experimental, and the availability of this procedure is limited to only a few centers. Indeed, one of the major threats to this field is the need to have professional societies in many disciplines embrace fertility in their clinical setting—oncology, urology, allied health professionals, and reproductive endocrinologists (adult and pediatric) all need to be part of the equation.

Although the need for oncofertility and the collaboration between oncology and reproductive endocrinology is becoming globally recognized, with several scientific societies around the world establishing guidelines for fertility preservation in cancer patients,[50,356] there is still a gap in access—to knowledge, to the procedures themselves, and to support. To more fully address the issue of iatrogenic infertility after cancer treatment, it is essential to share information about oncofertility with individual cancer treatment centers, reproductive endocrinology and infertility practices, and infertility clinics. Updates in the field should be sent regularly to each of these stakeholders. Social media and traditional media must be engaged. Cross-platform resources should be widely distributed and made available online. Mobile applications linking practitioners and patients to oncofertility care must be advertised extensively.

Inclusion of Psychological Support Is Critical to Oncofertility Clinical Care Models

Psychological support during the oncofertility decision-making process is essential, especially for pediatric oncologic patients, who are presented with very specific challenges.[357] They (or their parents) may be overwhelmed by the cancer diagnosis and focused on what is necessary to survive cancer rather than a discussion of possible future infertility. Even when a patient decides to undergo fertility preservation, they are more likely to select a quick "one-stop" strategy, such as ovarian tissue cryopreservation, that does not delay the initiation of cancer treatment and does not require the patient to be actively engaged in the fertility preservation treatment, as would be necessary for embryo or oocyte cryopreservation. This raises some concern, because in many cases patients may be choosing to undergo an experimental, but quicker, procedure when an established but slower one could be at least as effective and certainly less invasive. It is important that practices understand the value of specialized oncofertility support personnel and models that train psychologists in oncofertility navigation and counseling who can provide psychological and decision-making support to patients at single centers or between centers. Building this capacity into oncofertility care is an important strategy that could be implemented on a macro (state, region, or country) scale.

Access and Affordability

The cost of fertility preservation is well beyond the reach of most people; in the United States, the average upfront cost of ART to cryopreserve oocytes is $9200, plus annual storage fees of approximately $300, and $4400 for thawing, fertilizing, and implanting the frozen eggs.[358] Although insurance, reimbursement, and specific cost issues vary in Europe, Asia, and North America, the primary reason for the underuse of ART, which has been available for the past 35 years around the world, is the cost of the procedures and medications, both inside and outside the cancer setting. In many places in the world, including in countries in the global north (like the United States), infertility treatments are frequently not covered by health insurance.[359] In the global south, infertility treatments are commonly seen as luxury items, given the lack of resources and the need to prioritize basic, lifesaving health care.[360] In Portugal, considerable effort has been made to improve financial support programs for ART, and today, public ART centers offer fertility preservation for men and women, with 69% of medication costs covered by social security.[361] Country-by-country assessments of the costs of oncofertility are ongoing and will provide insight into future approaches to reduce cost as a barrier to access for patients with cancer. The ability to have genetic children is important to many women and men throughout the world,[360] and the World Health Organization considers infertility to be a global health issue.[362] The significance of genetic parenthood and the public health perspective are important to factor into the arguments for pursuing fertility preservation, particularly in resource-limited environments. Ultimately, costs and priorities are intertwined and should be considered in equal measure.

Public Awareness

Although public awareness about oncofertility has increased dramatically in the past decade, there is still an overall lack of knowledge and understanding of the importance of fertility preservation in the cancer setting. The media is a powerful tool for disseminating health-related news and has played an important role in educating the public about oncofertility. However, awareness remains low among individuals who are less likely to be reached via the news media, those who are less educated, those who have lower health literacy, and those who are from lower socioeconomic backgrounds. Public awareness is the first step in creating public support for a given cause. Without widespread public support, it is difficult for a movement to gain momentum and engender real change. One of the barriers preventing oncofertility from accumulating more public support is the perception that ART is an elective procedure that is not medically necessary. Yet several professional medical organizations categorize infertility as a disease, and there is substantial evidence demonstrating the physical, psychological, social, and economic impact of infertility and its treatment. Correcting this misperception and illustrating the health benefits of oncofertility, beyond fertility preservation, for patients with cancer is a major focus in the field.

Distinguishing Between Oncofertility, Infertility, and Social Egg Freezing

Although oncofertility involves the same ART procedures used for infertility treatment, oncofertility is specifically focused on the needs young patients with cancer whose future fertility is threatened by the cancer or its treatment. It is important to recognize the differences between oncofertility patients and patients with traditional infertility. Unlike patients who seek treatment for infertility, oncofertility patients have *anticipated* iatrogenic infertility that is directly related to their lifesaving cancer treatment. Unfortunately, these two categories are often conflated, leading to a similar exclusion of oncofertility procedures from insurance coverage as a form of infertility treatment.[363] Clearly classifying oncofertility as part of the cancer treatment plan would help establish the difference between oncofertility and infertility treatments, as well as improve access to and insurance coverage for ART procedures specifically in the oncofertility setting.[364]

Many in the public also have difficulty distinguishing between the use of ART for fertility preservation in cancer patients and the use of ART for fertility preservation to avoid age-related infertility—what has been called *social egg freezing*.[365] In the past few years, social egg freezing by women who want to delay childbearing has received a great deal of news media attention.[366] Given low health and science literacy rates among the public, people may not be able to immediately understand the different reasons for fertility preservation in cancer patients versus healthy women who are concerned about age-related infertility, especially since the same technologies are used in each setting. With the increasing demand for social egg freezing, there is the concern that the cost of fertility preservation will increase for all patients, including oncofertility patients. More work must be done to more clearly illustrate in plain language the differences in the use of ART in the oncofertility setting (e.g., for infertility) versus social egg freezing.

Summary and Next Steps

Oncofertility sits at the fulcrum of disciplines and requires an integration of medical specialties—oncology, urology, and reproductive endocrinology—both with each other and with the emerging technologies that will be tomorrow's breakthroughs. The loss of reproductive function due to cancer has a profound impact on endocrine health, fertility, and psychosocial well-being, and attention to this rapidly moving field is crucial for ensuring a high QoL among cancer survivors.

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